Growth Factors in the Collateral Circulation of Chronic Total Coronary Occlusions

Relation to Duration of Occlusion and Collateral Function

Gerald S. Werner, MD; Enrico Jandt, PhD; Andreas Krack, MD; Gero Schwarz, MD; Oliver Mutschke, MD; Friedhelm Kuethe, MD; Markus Ferrari, MD; Hans R. Figulla, MD

Background—Despite extensive animal experimental evidence, there are few data on the relation of growth factors and collateral function in humans.

Methods and Results—In 104 patients with a chronic total coronary occlusion (CTO; >2 weeks’ duration), collateral function was assessed invasively during recanalization by intracoronary Doppler and pressure recordings. A collateral resistance index, $R_{coll}$, was calculated. Blood samples were drawn from the distal coronary bed supplied by the collaterals and from the aortic root to measure basic fibroblast growth factor (bFGF), monocyte chemotactic protein-1 (MCP-1), transforming growth factor-β (TGF-β), placenta growth factor (PlGF), and tumor necrosis factor-α (TNF-α). The bFGF concentration in the collateralized artery was higher than in the aortic root (34±20 versus 18±14 pg/mL; $P<0.001$). bFGF was highest in recent occlusions (2 to 12 weeks) with the highest $R_{coll}$. Higher collateral concentrations were also observed for MCP-1, TGF-β, and PlGF, but without a close relation to the duration of occlusion. TNF-α was not increased in collaterals compared with the systemic circulation. MCP-1, PlGF, and TGF-β were significantly increased in small collaterals with the highest shear stress. Diabetic patients had lower bFGF and higher MCP-1 levels than nondiabetics.

Conclusions—In CTOs, the continuous release of bFGF into collaterals showed a close relation to the duration of occlusion and collateral function, which underscores its therapeutic potential. Other factors influencing growth factor release appeared to be shear stress for MCP-1, TGF-β, and PlGF and the presence of diabetes. (Circulation. 2004;110:1940-1945.)

Key Words: collateral circulation • growth substances • angiogenesis • coronary disease

Collateral development is regulated by the interaction of locally generated growth factors in ischemic myocardium leading to the recruitment and differentiation of preformed interarterial connections.1,2 The transfer of these observations from animal models to humans is limited,3,4 but there is also clinical evidence that these cytokines have a pathophysiological role in acute coronary syndromes and myocardial infarction (MI).5-8 A profound understanding of their role in collateral development in humans has potential impact on the choice of therapeutic interventions in ischemic heart disease.9,10

We recently showed the feasibility of accessing a collateralized vascular segment before antegrade blood flow had been restored in patients with chronic total coronary occlusions (CTOs).11,12 In the present study, we used this in vivo approach to test the hypothesis that local release of growth factors would be related to the duration of the occlusion and collateral function in human coronary circulation. We assessed cytokines (basic fibroblast growth factor [bFGF], monocyte chemotactic protein-1 [MCP-1], transforming growth factor-β [TGF-β], placenta growth factor [PlGF], and tumor necrosis factor-α [TNF-α]) with an established role in collateral development1,2,13,14 and with potential for the therapeutic promotion of collaterals.15

Methods

Patients

In 104 consecutive patients with a successful percutaneous coronary intervention (PCI) of a CTO in a major coronary branch, collateral flow was measured before reopening of the artery. The inclusion criteria were (1) duration of the occlusion >2 weeks on the basis of a previous angiogram, the date of a prior MI, or the onset of symptoms; (2) TIMI 0 coronary flow; and (3) evidence of ischemia related to the occlusion or viable myocardium in case of akinesia. The study protocol had been approved by the university ethics committee, and written informed consent was obtained.

Study Protocol

The PCI was performed via the femoral approach with 6F guiding catheters as described previously.11 All patients received a bolus of...
heparin (100 IU/kg), and they were taking aspirin (100 mg) and clopidogrel (75 mg). Intracoronary nitroglycerin (0.1 to 0.2 mg) was administered before subsequent measurements. After the lesion was crossed by a 0.014-in guidewire, an over-the-wire exchange catheter was advanced distal to the occlusion. The wire was removed, and 4 to 6 mL of blood was drawn with moderate suction within 20 seconds through the exchange catheter. Immediately thereafter, 4 to 6 mL of blood was drawn from the guiding catheter in the aortic root. Then a Doppler wire (FlouWireJ, Volcano Therapeutics), followed by a pressure wire (PressureWireJ, RADI Medical Systems), was advanced distal to the occluded segment. After the Doppler and pressure recordings, the angioplasty was continued.

**Intracoronary Doppler and Pressure Recordings**
Baseline measurement of collateral flow was done before antegrade flow to the collateralized vascular bed could have occurred. A leakage along the exchange catheter was excluded by the absence of an alteration of the Doppler signal after proximal contrast injection. Positioning of the Doppler wire in a segment at least 5 mm from major side branches was done on the basis of the diagnostic cine images via filling from the collateral donor artery. The Doppler wire was moved within a range of 10 mm to obtain the recording with the maximum flow velocity integral. Average peak velocity was calculated from the Doppler flow signals distal to the occlusion.13

The pressure transducer was positioned exactly at the previous Doppler transducer position. Mean distal coronary pressure (Pd) was recorded together with mean aortic pressure (Pa) from the guiding catheter. A collateral pressure index (CPI) was calculated as (Pd−CVP)/(Pa−CVP), where CVP is the central venous pressure.16 CVP was assessed in 65 of the 104 patients and substituted for in the remaining patients by the average of invasive recordings (9±4 mm Hg; range 1 to 15). The collateral resistance index (Rcoll) was calculated as ((Pa−Pd)/APVd), where APVd indicates Doppler-derived average peak velocity; because flow velocity was measured instead of flow volume, the dimension was mm Hg · cm−1 · s−1.

**Angiographic Assessment of Collaterals**
Collateral supply was graded angiographically according to Rentrop et al.18 The angiographically visible collateral diameter was graded as described previously: CC0 (no continuous visible connection between donor and recipient branch; CC1 (continuous threadlike connection); or CC2 (continuous small side-branch–like connection). Angiograms were assessed independently by 2 blinded investigators, and in case of discordance, a consensus was obtained.

**Growth Factor Assays**
Blood samples were obtained and transferred into sterile serum vials and then immediately stored on ice. Within 30 minutes, they were centrifuged at 1000 g for 15 minutes and then stored in aliquots of 100 μL at −80°C. The concentration of bFGF was measured by a commercially available and standardized ELISA with a specific monoclonal antibody for bFGF (Quantikine High Sensitivity, R&D Systems). The assay was performed according to the manufacturer’s instructions. Two aliquots of each blood sample were assessed in duplicates simultaneously in each assay with a variation of 8.9±8.6%. MCP-1, TGF-β, TNF-α, and PIGF were also measured by ELISA kits (Quantikine, R&D Systems). The variations of measuring 2 aliquots within 1 assay were 8.3±9.5%, 17.6±16.4%, 11.1±13.5%, and 15.4±17.9%, respectively. Because of the limited amount of blood samples, PIGF and TNF-α could be measured only in 52 of 104 patients.

**Study Groups**
Patients were divided into subgroups according to the duration of occlusion, with a cutoff at 12 weeks to discriminate between recent and long-standing occlusions. Further subgroups were patients with and without diabetes and patients with normal left ventricular (LV) function (wall motion severity index ≥1 SD/chord) versus patients with impaired regional LV function and recent or long-standing occlusion.

**Statistical Analysis**
Data are given as mean±SD. Differences between the 2 groups were analyzed by a Student’s t test or a Fisher’s exact test when appropriate. Differences within 1 group were evaluated by a paired t test. ANOVA was applied for multiple comparisons, and the Scheffé test was used for post hoc analysis. A level of P<0.05 was considered significant. All calculations were done with SPSS for Windows (version 11.5, SPSS Inc.).

**Results**
The median duration of occlusion was 3.8 months (range 0.5 to 338 months). Fifty-seven patients with a CTO of >12 weeks’ duration were compared with 47 patients with a recent occlusion of 2 to 12 weeks’ duration. Patients with recent occlusions more often had a prior MI and had more severely impaired regional wall motion than patients with long-standing CTOs, but no other clinical differences were noted (Table 1).

**Systemic and Collateral Growth Factor Concentrations in CTOs**
In the entire group of 104 patients, bFGF in the collateralized segment distal to the CTO was more than double the concentration in the aortic root. A significant but smaller distal-to-aortic gradient was observed for MCP-1, PIGF, and TNF-α, whereas there was no difference for TGF-β (Figure 1).

In patients with a recent CTO, the distal collateral pressure was lower and the Rcoll higher than in patients with a CTO >12 weeks (Table 2). The distal-to-aortic gradient of bFGF was higher in recent CTOs. Moderate differences were observed for MCP-1, with a lower aortic concentration in recent CTOs, and for TGF-β, with a trend for a higher distal concentration in recent CTOs (Table 2). TNF-α and PIGF were not related to the duration of occlusion.

**Collateral Development and Growth Factor Release**
In patients with impaired LV function and an occlusion of >12 weeks’ duration, the collaterals were developed to a similar functional level, indicated by a similar Rcoll, as in patients with well-preserved LV function without a history of a prior MI (Figure 2). In analogy to the functional parameters, bFGF concentrations were similar in these 2 latter groups, whereas distal and aortic bFGF were higher in CTOs with impaired LV function and 2 to 12 weeks’ duration. A significant gradient for bFGF was observed in all groups. For the other cytokines, no significant differences were observed (Figure 2). There was a significant inverse correlation between CPI and bFGF (distal: r = −0.24, aortic: r = −0.26, P<0.05). CTOs with less developed collaterals (CPI <0.25) had a higher distal and aortic bFGF concentration than CTOs with a CPI ≥0.25 (distal: 43±24 versus 32±19 pg/mL, P=0.09; aortic: 28±20 versus 17±13 pg/mL, P=0.01). Such a relation was not observed for any of the other cytokines.

Collateral anatomic development can be evaluated by assessing the size of the collateral connection. The highest Rcoll was observed in collaterals without visible continuous collateral connections and decreased with increasing collateral diameters (Figure 3). The distal-to-aortic gradient of bFGF was present in all groups but was not related to
collateral size, whereas distal MCP-1 and PlGF were highest in CC0 collaterals, and TGF-β showed the largest gradient.

**Diabetes Mellitus and Growth Factor Release**

Thirty-eight patients with diabetes had a similar R Coll as 64 patients without diabetes (7.05 ± 4.44 versus 7.20 ± 4.54 mm Hg · cm⁻¹ · s⁻¹; P = 0.87). Both distal and aortic bFGF tended to be lower in diabetic patients (distal: 30 ± 17 versus 36 ± 21 pg/mL, P = 0.11; aortic: 14 ± 12 versus 20 ± 15 pg/mL, P = 0.06), whereas MCP-1 tended to be higher (distal: 163 ± 139 versus 111 ± 99 pg/mL, P = 0.06; aortic: 139 ± 88 versus 107 ± 95 pg/mL, P = 0.16). No differences were observed for TGF-β, TNF-α, or PlGF.

**Discussion**

In this study in patients with coronary artery disease and a collateralized CTO, we investigated growth factors with a role in experimental models of collateral development. We detected release of bFGF into the collateral circulation with a specific relation to the duration of the occlusion and the quality of collateral function, whereas the release of MCP-1, PlGF, and TGF-β appeared to be related to increased shear stress.

**Time Course of Collateral Development**

Immediately after occlusion, some collaterals may already be present because they started to develop before the acute thrombotic occlusion, but generally, newly developing coronary collaterals become angiographically visible within 10 to 14 days after an acute MI. In the rat hind-limb occlusion model, collateral development from preformed arteriolar anastomosis starts within 24 hours, becoming almost complete after 1 week, but other animal models suggest a period of 8 to 12 weeks for full functional maturation, which is similar to our human data.

**Collateral Development and Growth Factors in CTOs**

We observed a higher concentration of bFGF in the effluent from collaterals compared with the systemic arterial concentration in all patient subgroups, indicating a continuous release. The duration of the occlusion was the major trigger for bFGF generation, with the highest collateral and systemic concentrations in recent CTOs 2 to 12 weeks after an acute MI, which decreased to a level in CTOs >12 weeks' duration similar to that in patients who had fully developed collaterals and no history of MI. This time-dependent relation indicates increased endothelial and monocyte activation as a potential source of bFGF in the first weeks of collateral development. This could be due to ischemia-independent mechanisms of arteriogenesis, with flow-mediated remodeling of preformed arterioles, as in experimental animal models. But additional ischemia-related mecha-

---

**TABLE 1. Clinical Characteristics of Patients With CTOs**

<table>
<thead>
<tr>
<th>Duration of Occlusion</th>
<th>3–12 Weeks (n=47)</th>
<th>&gt;12 Weeks (n=57)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>62.6 ± 10.2</td>
<td>62.7 ± 10.8</td>
<td>0.96</td>
</tr>
<tr>
<td>Male, %</td>
<td>77</td>
<td>72</td>
<td>0.66</td>
</tr>
<tr>
<td>1/2/3 Diseased arteries, %</td>
<td>51/34/15</td>
<td>39/39/23</td>
<td>0.39</td>
</tr>
<tr>
<td>Occluded artery, right/LAD/LCx, %</td>
<td>64/36/0</td>
<td>61/37/2</td>
<td>0.65</td>
</tr>
<tr>
<td>Previous Q-wave myocardial infarction, %</td>
<td>70</td>
<td>49</td>
<td>0.030</td>
</tr>
<tr>
<td>Angina pectoris, CCS 0/1/2/3/4, %</td>
<td>0/9/42/45/4</td>
<td>2/3/33/58/4</td>
<td>0.49</td>
</tr>
<tr>
<td>Heart failure, NYHA 0/1/2/3/4, %</td>
<td>0/43/34/23/0</td>
<td>2/51/28/19/0</td>
<td>0.64</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>30</td>
<td>42</td>
<td>0.20</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>81</td>
<td>89</td>
<td>0.22</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>66</td>
<td>77</td>
<td>0.21</td>
</tr>
<tr>
<td>History of smoking, %</td>
<td>57</td>
<td>60</td>
<td>0.82</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>0.59 ± 0.18</td>
<td>0.64 ± 0.20</td>
<td>0.26</td>
</tr>
<tr>
<td>Wall-motion severity index, SD/chord</td>
<td>2.16 ± 1.37</td>
<td>1.49 ± 1.31</td>
<td>0.014</td>
</tr>
<tr>
<td>Wall-motion extension, No. of chords</td>
<td>14.3 ± 11.7</td>
<td>9.8 ± 11.6</td>
<td>0.057</td>
</tr>
</tbody>
</table>

LAD indicates left anterior descending artery; LCx, left circumflex; CCS, Canadian Cardiovascular Society classification of chest pain; NYHA, New York Heart Association classification of heart failure; and SD, standard deviation.
Despite a significant release into the collateral circulation of MCP-1 and a moderately higher distal (collateral) concentration of PlGF and TGF-β, but not of TNF-α, only MCP-1 showed a trend for a similar relation to the time course of occlusion as bFGF. In view of the experimental evidence, the lack of a close relation to collateral development and function in the experimental setup in the present study could indicate that PlGF and TGF-β were no longer upregulated beyond 2 weeks after occlusion or that the spillover into the collateral circulation would not adequately represent their interstitial concentrations. Furthermore, the generation of these cytokines is determined not only by collateral development but also by the process of atherosclerosis itself, which leads to a wide scatter of individual values within the study groups.

Collateral Function and bFGF

We observed an inverse correlation of systemic and collateral bFGF concentrations with collateral function, which suggests that with the improvement of blood flow through collaterals, the trigger for bFGF generation was decreased. This inverse relation is in contrast to a previous study in which collateral function was studied during balloon occlusion and blood was retrieved through a balloon catheter. Only 10 of 76 patients had well-developed collaterals (CPI >0.3). Because these patients had higher bFGF than patients with insufficient collaterals (CPI <0.3), a positive relation between bFGF and collateral function was suggested. But the latter patients had less severe angiographic stenosis and no trigger for collateral development, and that study had compared patients with and without collaterals but not with different stages of collateral development, as in the present study. A recent study observed a higher level of bFGF in the coronary sinus in patients with collaterals (CPI >0.25), none of whom had a CTO, than in those without collaterals. This also indicates that studies during brief balloon occlusion assessing acutely recruited collaterals are not comparable to studies in CTOs in which collaterals are assessed in a baseline state.

Figure 2. Rcoll and bFGF, MCP-1, TGF-β, TNF-α, and PlGF in CTOs with either normal regional LV function or impaired function and occlusion duration of either 2 to 12 weeks or >12 weeks. Values are mean±SEM. Comparison within groups: *P<0.05; H, P<0.01.

TABLE 2. Collateral Hemodynamics and Growth Factor Concentrations in CTOs

<table>
<thead>
<tr>
<th>Duration of Occlusion</th>
<th>3–12 Weeks (n=47)</th>
<th>&gt;12 Weeks (n=57)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>66±11</td>
<td>66±10</td>
<td>0.85</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>19.7±7.6</td>
<td>19.3±8.8</td>
<td>0.78</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>9±4</td>
<td>10±6</td>
<td>0.26</td>
</tr>
<tr>
<td>APVb, cm/s</td>
<td>11.3±7.2</td>
<td>11.6±5.6</td>
<td>0.85</td>
</tr>
<tr>
<td>Mean Pao, mm Hg</td>
<td>103±15</td>
<td>101±16</td>
<td>0.56</td>
</tr>
<tr>
<td>Mean Pd, mm Hg</td>
<td>38±12</td>
<td>46±12</td>
<td>0.001</td>
</tr>
<tr>
<td>CPI</td>
<td>0.34±0.11</td>
<td>0.44±0.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rcoll, mm Hg·cm⁻¹·s⁻¹</td>
<td>7.94±4.79</td>
<td>5.86±3.55</td>
<td>0.02</td>
</tr>
<tr>
<td>bFGF aortic, pg/mL</td>
<td>21±15</td>
<td>15±13</td>
<td>0.01</td>
</tr>
<tr>
<td>bFGF distal, pg/mL</td>
<td>40±20</td>
<td>29±18</td>
<td>0.003</td>
</tr>
<tr>
<td>MCP-1 aortic, pg/mL</td>
<td>87±67</td>
<td>139±102</td>
<td>0.02</td>
</tr>
<tr>
<td>MCP-1 distal, pg/mL</td>
<td>117±137</td>
<td>135±106</td>
<td>0.51</td>
</tr>
<tr>
<td>TGF-β aortic, ng/mL</td>
<td>4.4±2.7</td>
<td>3.6±2.5</td>
<td>0.22</td>
</tr>
<tr>
<td>TGF-β distal, ng/mL</td>
<td>4.6±2.7</td>
<td>3.8±2.0</td>
<td>0.10</td>
</tr>
<tr>
<td>TNF-α aortic, pg/mL*</td>
<td>1.1±0.5</td>
<td>1.2±1.1</td>
<td>0.69</td>
</tr>
<tr>
<td>TNF-α distal, pg/mL*</td>
<td>1.2±0.5</td>
<td>1.3±0.9</td>
<td>0.72</td>
</tr>
<tr>
<td>PlGF aortic, pg/mL*</td>
<td>51±29</td>
<td>53±39</td>
<td>0.56</td>
</tr>
<tr>
<td>PlGF distal, pg/mL*</td>
<td>47±32</td>
<td>49±30</td>
<td>0.54</td>
</tr>
</tbody>
</table>

APV indicates average peak velocity; LVEDP, LV end-diastolic pressure; MV, maximum velocity; Pao, aortic pressure; and Pd, distal pressure.

* n=22 (2–12 weeks); n=30 (>12 weeks).

Collaterals may play a role, especially in those patients studied in the first few weeks after an acute MI.

Acute ischemia leads to increased levels of vascular endothelial growth factor (VEGF) as the major cytokine of hypoxemia-induced angiogenesis, but experimental studies of the coronary circulation suggest that bFGF is superior in enhancing collateral function. In the pericardial fluid of patients undergoing bypass surgery, bFGF, but not VEGF, was highest when collaterals were present.
Shear Stress and Growth Factors in Chronic Coronary Occlusions

Arteriogenesis is triggered by shear stress–induced activation of monocytes that involves various cytokines. When we compared the concentrations among groups with different angiographic stages of collateral development, the largest gradient and highest distal concentration of MCP-1 were detected in collaterals with no visible direct connection between donor and recipient segments. These collaterals with the narrowest arterial connections will have the highest shear stress. A similar relation with collateral size was observed for PlGF, which is also involved in the attraction of monocytes. TGF-β, which interacts with MCP-1 in collateral development, also showed a higher concentration gradient in these less developed collaterals.

Growth Factors in Diabetic Patients

In diabetic patients, a paradox of increased angiogenesis leading to retinopathy and impaired collateral development is observed. There is experimental evidence that some growth factors may be downregulated and others upregulated in diabetes and that the crucial monocyte activation is impaired. We observed a downregulation for bFGF and higher levels of MCP-1 in diabetics. Despite these differences in cytokine levels, the invasively assessed collateral function before recanalization was similar in diabetic and nondiabetic patients. However, during balloon reocclusion of a recanalized CTO, we previously showed that the acute recruitment of collaterals is impaired in diabetic patients with a recent CTO of 2 to 12 weeks' duration.

Study Limitations

Flow velocity and pressure recorded distal to an occlusion are approximations of collateral function, but this is the only direct approach to collateral assessment in humans that has a higher spatial resolution than noninvasive techniques. The limitations of this technique have been addressed in detail. Collateral function was related to growth factor concentrations in the coronary segment distal to the CTO and the aortic root. Limitations to the interpretation of these data include that the main effect of the growth factors is located in the interstitium around a collateral, whereas we detected a spillover into the collateral and systemic circulation. The role of interstitially released cytokines could be assessed by sampling from the coronary sinus, but this is difficult in the setting of an already time-consuming therapeutic procedure of recanalizing a CTO. Second, bFGF is known to interact with heparin, and its level may increase by the displacement of bFGF from its binding sites by heparin. Heparin is mandatory during PCI, but to minimize its effect, it was administered on a weight-adjusted basis. We did not measure VEGF because of an even greater interference of heparin administration with the assay for VEGF.

Clinical Implications

bFGF is closely related to collateral development and function in patients with CTOs. It is continuously released into the collateral circulation, with the highest concentrations 2 to 12 weeks after the occlusion. In view of ongoing attempts to promote collateral development using various exogenously administered growth factors, the present data support bFGF as a principal candidate to promote collaterals in the human coronary circulation. Its detectable release into the collateral circulation beyond the acute phase of occlusion might support a prolonged therapeutic regimen.

Acknowledgments

The authors are indebted to Elisabeth Frommhold and Juliane Gebhardt and her colleagues of the cardiac catheterization laboratory of our institution for their dedicated technical assistance in conducting this study, and the authors thank Annette Schmidt for her assistance with the cytokine assays.

References

3. Maxwell MP, Hearse DJ, Yellon DM. Species variation in the coronary collateral circulation during regional myocardial ischaemia: a critical determinant of the rate
Growth Factors in the Collateral Circulation of Chronic Total Coronary Occlusions: Relation to Duration of Occlusion and Collateral Function

Gerald S. Werner, Enrico Jandt, Andreas Krack, Gero Schwarz, Oliver Mutschke, Friedhelm Kuethe, Markus Ferrari and Hans R. Figulla

_Circulation._ 2004;110:1940-1945; originally published online September 27, 2004; doi: 10.1161/01.CIR.0000143624.72027.11

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/14/1940

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at: http://circ.ahajournals.org/subscriptions/