Promotion of Collateral Growth by Granulocyte-Macrophage Colony-Stimulating Factor in Patients With Coronary Artery Disease

A Randomized, Double-Blind, Placebo-Controlled Study

Christian Seiler, MD; Tilmann Pohl, MD; Kerstin Wustmann, MD; Damian Hutter, MD; Pierre-Alain Nicolet, MD; Stephan Windecker, MD; Franz R. Eberli, MD; Bernhard Meier, MD

Background—Experimentally, activated macrophages have been documented to induce vascular proliferation.

Methods and Results—In 21 patients (age 74±9 years) with extensive coronary artery disease not eligible for coronary artery bypass surgery, the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF, Molgramostim) on quantitatively assessed collateral flow was tested in a randomized, double-blind, placebo-controlled fashion. The study protocol consisted of an invasive collateral flow index (CFI) measurement immediately before intracoronary injection of 40 μg of GM-CSF (n=10) or placebo (n=11) and after a 2-week period with subcutaneous GM-CSF (10 μg/kg) or placebo, respectively. CFI was determined by simultaneous measurement of mean aortic pressure (Pao, mm Hg), distal coronary occlusive pressure (Poccl, mm Hg; using intracoronary sensor guidewires), and central venous pressure (CVP, mm Hg): CFI=(Poccl−CVP)/(Pao−CVP). CFI, expressing collateral flow during coronary occlusion relative to normal antegrade flow during vessel patency, changed from 0.21±0.14 to 0.31±0.23 in the GM-CSF group (P<0.05) and from 0.30±0.16 to 0.23±0.11 in the placebo group (P=NS). The treatment-induced difference in CFI was +0.11±0.12 in the GM-CSF group and -0.07±0.12 in the placebo group (P=0.01). ECG signs of myocardial ischemia during coronary balloon occlusion occurred in 9 of 10 patients before and 5 of 10 patients after GM-CSF treatment (P=0.04), whereas they were observed in 5 of 11 patients before and 8 of 11 patients after placebo (P=NS).

Conclusions—This first clinical study investigating the potential of GM-CSF to improve collateral flow in patients with coronary artery disease documents its efficacy in a short-term administration protocol. (Circulation. 2001;104:2012-2017.)

Key Words: coronary disease ■ collateral circulation ■ growth substances ■ granulocyte-macrophage colony-stimulating factor

Cardiovascular diseases, in particular coronary artery disease (CAD), are the leading cause of death in adults in industrialized countries. Established options for revascularization include angioplasty and surgical bypass, both of which are not suitable in 1 of 5 to 1 of 3 patients in whom the extent of coronary atherosclerosis is especially severe. An alternative treatment strategy is therefore warranted both to control symptoms as well as to alter the course of advanced CAD. Therapeutic angiogenesis is a new and promising strategy for revascularizing ischemic myocardial tissue by formation of natural bypasses, ie, collaterals. Angiogenesis is induced by surgical or catheter-based delivery of various promoters, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), angiogenic agents most often used in present clinical studies.1–4 Although animal studies have established the principle that collateral function improves after delivering angiogenic growth factors,5 and although first uncontrolled clinical studies have demonstrated safety and feasibility of VEGF and bFGF,5 efficacy data of angiogenic therapy have been scarce and controversial, which is partly attributable to the lack of controlled investigations.

See p 1994

In addition, controversy on the ability of angiogenic growth factors to promote coronary collaterals may be related to the use of endpoints for their assessment that are too blunt to discern subtle changes in collateral flow. Also, angiogenic factors may have been used that induce the formation of small, high-resistance capillaries (termed angiogenesis) rather than large interconnecting arterioles (termed arteriogenesis), which are required for the salvage of myocardium in the...
presence of occlusive CAD. Experimentally, arteriogenesis has been shown to be induced by activated macrophages, lipopolysaccharide, monocyte chemotactic protein-1, tumor necrosis factor-α (TNF-α), bFGF, and also granulocyte-macrophage colony-stimulating factor (recombinant human GM-CSF; Molgramostim). Therefore, the purpose of this study was to test the hypothesis that short-term intracoronary and subcutaneous administration of GM-CSF improves recruitable collateral flow.

Methods

Patients

Twenty-one patients (age 75 ± 10 years, 10 men and 11 women) with two- (n = 7) to three-vessel (n = 14) CAD not eligible for or unwilling to undergo (n = 3) coronary artery bypass surgery and eligible for percutaneous tranluminal coronary angioplasty (PTCA) of at least one stenotic lesion were included in the study. All underwent diagnostic coronary angiography because of symptoms related to stable CAD. Patients were prospectively selected on the basis of the following criteria: (1) no previous transmural infarction in the myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive heart failure; (4) no myocardial area assessed for coronary collaterals

Cardiac Catheterization and Coronary Angiography

Patients underwent left heart catheterization for diagnostic purposes from the right femoral approach. Aortic pressure was measured using a 6F PTCA guiding catheter. Central venous pressure was obtained via the right femoral vein. Left ventricular end-diastolic pressure was determined before PTCA. Biplane left ventriculography was performed followed by biplane coronary angiography. Coronary artery stenoses were estimated quantitatively as percent diameter narrowing.

Figure 1. Determination of collateral flow in a patient receiving GM-CSF before (left) and after (right) therapy. Top, Surface (I, III, and avF) and intracoronary (i.c.) ECG lead recordings are shown. After 1 minute of vessel occlusion before GM-CSF CFI amounts to (18–8)/[(84–8)/(84–8)]=0.13. CFI increases to 0.23 after GM-CSF treatment (right).
vessel occlusion, simultaneous $P_{o}c$, $P_{ao}$, and CVP were obtained for the calculation of CFI (Figure 1). During the entire procedure, an intracoronary ECG obtained from the guidewire and a 3-lead surface ECG were recorded. After initial CFI determination, the angioplasty balloon was deflated, the pressure guidewire was removed, and, during a 2- to 3-minute, low-pressure reinflation, the study drug was injected through a millipore filter via the angioplasty catheter. It was prepared a few minutes before the first balloon inflation by the laboratory personnel according to the randomization table. The study drug consisted of 40 mg of GM-CSF in 5 mL of saline 0.9% or of 0.1% albumin in 5 mL of saline 0.9% (placebo). After intracoronary injection and removal of the balloon catheter, 10 mL of blood was obtained from the guiding catheter at the end of the procedure for determination of intracoronary TNF-α concentration (enzyme-linked immunosorbent assay with a monoclonal antibody specific for TNF-α; Biosource International). The initial invasive procedure was followed by a 2-week, out-of-hospital period with subcutaneous injections of GM-CSF (10 µg/kg in 0.27 mL aqua ad injectionem) or placebo (0.1% albumin in 0.27 mL aqua ad injectionem) every other day. The subcutaneous study drug was prepared by the hospital pharmacy. The investigators were blinded to both the study and placebo and died 6 days later because of extensive CAD was not included in the study. All patients in the GM-CSF group

### Statistical Analysis

Power analysis before the study hypothesizing a relative change of CFI in the treatment group of $\geq 50\%$ (SD 0.10) at a significance level of $<0.05$ provided a sample size of 20 patients. Between-group comparisons of continuous clinical, hemodynamic, angiographic, and collateral flow data were performed by a Mann Withney test. A $\chi^2$ test was used for comparison of categorical variables among the two study groups. Intraindividual comparisons of baseline versus follow-up data were performed using Wilcoxon signed rank test.

### Results

#### Patient Characteristics and Clinical Data at Baseline

There were no statistically significant differences between the two groups regarding age of the patients, sex, body mass index, heart rate, and severity and duration of angina pectoris. There were no statistical differences either in the frequency of cardiovascular risk factors or the use of acetylsalicylic acid or vasoactive drugs.

#### Hemodynamic, Angiographic, and Collateral Data at Baseline

Mean blood pressure during vessel occlusion, left ventricular ejection fraction, end-diastolic pressure, distal coronary occlusive pressure, and central venous pressure during coronary occlusion were similar between the study groups at baseline (Table 1). In the group receiving GM-CSF, the number of vessels affected by CAD tended to be higher than in the control group. There were no statistical differences among the groups in the total number of hemodynamically relevant stenoses in the vessel selected for injection of the study drug and for PTCA or in the severity of the treated and untreated stenoses. Qualitative and quantitative variables for the assessment of the collateral circulation were similar among the groups (Table 1).

### Side Effects

A patient who had undergone the baseline examination with placebo and died 6 days later because of extensive CAD was not included in the study. All patients in the GM-CSF group

<table>
<thead>
<tr>
<th>TABLE 1. Hemodynamic, Coronary Angiographic, and Collateral Data at Baseline</th>
<th>GM-CSF (n=10)</th>
<th>Placebo (n=11)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemodynamic data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean blood pressure during occlusion, mm Hg</td>
<td>96±17</td>
<td>91±18</td>
<td>NS</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>65±10</td>
<td>58±18</td>
<td>NS</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure, mm Hg</td>
<td>11±6</td>
<td>10±8</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary occlusive pressure, mm Hg</td>
<td>28±14</td>
<td>34±14</td>
<td>NS</td>
</tr>
<tr>
<td>CVP during occlusion, mm Hg</td>
<td>10±5</td>
<td>8±4</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary angiography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of diseased vessels</td>
<td>2.9±0.3</td>
<td>2.4±0.7</td>
<td>0.05</td>
</tr>
<tr>
<td>No. of stenoses $&gt;50%$ in diameter</td>
<td>4.7±2.1</td>
<td>4.8±2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Vessel (PTCA), LAD/LCX/RCA</td>
<td>5/2/3</td>
<td>4/4/3</td>
<td>NS</td>
</tr>
<tr>
<td>Diameter stenosis of treated lesion, %</td>
<td>73±12</td>
<td>75±9</td>
<td>NS</td>
</tr>
<tr>
<td>Diameter stenosis of untreated lesions, %</td>
<td>71±10</td>
<td>74±8</td>
<td>NS</td>
</tr>
<tr>
<td>Collateral assessment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiographic degree before occlusion (0–3)</td>
<td>1.0±0.8</td>
<td>1.3±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Angina pectoris during occlusion, %</td>
<td>7 (70)</td>
<td>6 (65)</td>
<td>NS</td>
</tr>
<tr>
<td>ECG ST changes $&gt;1$ mm during occlusion, %</td>
<td>9 (90)</td>
<td>5 (45)</td>
<td>NS</td>
</tr>
<tr>
<td>Collateral flow index, no unit</td>
<td>0.21±0.14</td>
<td>0.30±0.16</td>
<td>NS</td>
</tr>
</tbody>
</table>

LAD indicates left anterior descending coronary artery; LCX, left circumflex coronary artery; PTCA, percutaneous transluminal coronary angioplasty; and RCA, right coronary artery.
took the study medication until the follow-up invasive examination except for one who refused to continue the subcutaneous injections without specified reason. Side effects of the study medication included low fever or sweating, skin rashes at the site of injection, and, in <3 patients each, malaise, headache, nausea, paraesthesia, loss of appetite, and muscle pain. Patients in the GM-CSF group complained about any side effect in 6 of 10 instances, and those of the placebo group did so in 2 of 11 cases (P<0.05). Low-fever temperatures occurred in 3 of 10 patients in the GM-CSF group and in 1 of 11 individuals in the placebo group (P=NS). Skin rashes during treatment appeared in 7 of 11 cases in the GM-CSF group and in 2 of 11 placebo cases (P=0.01).

**Treatment-Induced Laboratory Parameters and Collateral Flow Changes**

Total leukocyte count, neutrophils, and monocytes increased significantly in the GM-CSF group, whereas they remained statistically unchanged in the placebo group (Table 2). After treatment, those parameters plus the eosinophil count were significantly higher in the GM-CSF than in the placebo group. C-reactive protein and serum creatinine tended to increase in the GM-CSF group, whereas they remained statistically unaltered in the placebo group, patients tended to experience less chest pain during balloon occlusion. ECG signs of myocardial ischemia during vessel occlusion were reduced among patients receiving GM-CSF (Figure 1) but not in the placebo group (Table 2). Continuous values of CFI significantly increased during treatment in the GM-CSF group, and they remained statistically unaltered in the placebo group (Table 2; Figures 1 and 2). CFI change during the treatment period was positive in the GM-CSF and negative in the placebo group. There was a significant direct and linear correlation between TNF-α concentration (at detectable values >2pg/mL) at follow-up (TNF-α<sub>2</sub>) and the treatment-induced CFI change, as follows: CFI change=0.003TNF-α<sub>2</sub>−0.058, r=0.56, P=0.04.

**Discussion**

This first study investigating the potential of Molgramostim (recombinant human GM-CSF, an established drug used in oncology for the last 10 years<sup>12</sup>) to improve quantitatively
determined coronary collateral flow in patients with advanced CAD documents its efficacy in a short-term administration protocol using the local intracoronary and systemic delivery routes.

**Clinical Trials on Angiogenesis**

Acidic FGF was the agent first chosen for angiogenic therapy in 40 patients undergoing coronary artery bypass surgery, whereby they received intramyocardial injections with active or heat-denatured protein in the supply area of the distal left anterior descending coronary artery.1 Twelve weeks later, patients having received the active growth factor appeared angiographically to accumulate more contrast dye than the control group. Except for one controlled phase I study using bFGF heparin-alginate microcapsules implanted surgically,13 subsequently performed phase I trials using VEGF or bFGF were designed as uncontrolled, open-label investigations.3,4,14,15 Although they all have documented safety and feasibility of VEGF and bFGF, some preliminary evidence for possible efficacy in the case of bFGF has been provided only in the mentioned controlled work by Laham et al,13 in which an improvement of regional myocardial perfusion was demonstrable by nuclear stress testing 16 months after treatment. Although the ultimate goal of angiogenic therapy in CAD is to reduce adverse cardiac events by collateralizing ischemic myocardium, presently surrogate endpoints for death, infarction, unstable angina pectoris, etc, have to suffice. Patient numbers are small related to the ongoing selection process among numerous angiogenic factor candidates. Growth factors assumed to promote collateral growth should be clearly shown to increase collateral flow. The requirement of collateral flow measurement is not fulfilled using as study endpoint noninvasively obtained myocardial perfusion during vessel patency, because respective increases can be attributable to augmented native vessel flow, collateral flow, or both. Our study used repetitive invasive measurements during vascular occlusion, which is the only possibility to reliably gauge collateral flow.

The only preliminary controlled phase II trial of a growth factor, the VEGF to Improve left Ventricle Function and Angiogenesis (VIVA) trial, has included 178 patients receiving intracoronary and intravenous VEGF165.6 Improvements in treadmill time and angina pectoris at 60 and 120 days were noted in all groups without statistical difference among patients receiving VEGF165 or placebo. The VIVA-preceding, uncontrolled phase I trial had found an improvement of resting nuclear perfusion in the high-dose group.3 Pathophysiologically, the lacking efficacy of VEGF may be interpreted on the basis of its ability to promote the growth of small rather than large, conductive collateral vessels.6,10

**Arteriogenesis and GM-CSF: Data From the Literature**

In patients with severe, advanced CAD, having experienced sufficient myocardial ischemic stimuli to incite the sprouting of small collateral vessels, their development into large, conductive collaterals is warranted to augment perfusion in the stenotic vascular area to such an extent that parameters such as exercise time or even cardiac mortality will improve. So far, promotion of large, conductive collaterals (ie, arteriogenesis8) has been investigated only experimentally. Monocyte chemotactant protein-1, a regulator for monocyte trafficking to sites of inflammation, has been found to be a very potent enhancer of angiogenesis and arteriogenesis in rabbits.16 Arras et al12 documented a similar effect by an intravenous infusion of the endotoxin lipopolysaccharide, the strongest activator of TNF-α in monocytes and macrophages. TNF-α is responsible for adhesion and activation of additional monocytes via upregulation of cell adhesion molecules and by upregulation of GM-CSF.10 Apart from these recent investigations, there was also an early study by Polverini et al7 documenting in guinea pig corneas that activated macrophages induce vascular proliferation. Our clinical study provides proof of principle of the coronary arteriogenic potential of GM-CSF by verifying the hypothesis that a short-term local and systemic application protocol augments collateral flow. However, collateral flow index responses in the GM-CSF group were quite variable, including 4 good responders, 3 moderate responders, and 3 nonresponders (Figure 2). Speculation on reasons for the changeable effect of the study drug include the fact that one patient, a nonresponder, did not complete the drug administration phase, and that sedentary patients might not have experienced enough ischemic stimuli to induce angiogenesis. The observation of a direct relation between therapy-induced collateral flow augmentation and TNF-α at follow-up raises the possibility that TNF-α itself was the decisive arteriogenic factor. A reflection of the cytokine increase accompanying collateral flow augmentation in the GM-CSF group is the tendency to elevated C-reactive protein and the more frequent episodes of subfebrile temperatures than in the placebo group.

**GM-CSF and Atherogenesis**

Aside from the role of those changes as indicators of ongoing coronary arteriogenesis, they may be interpreted as a possible sign of a proatherogenic effect of GM-CSF. Because angiogenesis may also involve vasa vasorum of atherosclerotic plaques, such an interpretation would be reasonable, and proatherogenic effects of angiogenic factors have been documented in the case of VEGF.17 Follow-up in our study was too short to detect progression of coronary atherosclerotic lesions. The atherogenic risk factor profile altered significantly in response to GM-CSF, showing a reduction in both total and HDL cholesterol. To speculate that these changes may have even caused regression of atherosclerosis would be unjustifiable, because the cholesterol-to-HDL ratio remained unchanged. Documentation of a potential atherogenic effect of GM-CSF has been controversial. It includes experimental work by Shindo et al,18 who found a reduced surface area of atheromatous plaques in the aortic arch of hyperlipidemic rabbits treated with GM-CSF–treated versus placebo-treated animals. Conversely, recent clinical studies have revealed a direct association between atherosclerotic progression of CAD (ie, unstable angina pectoris) and M-CSF plasma concentration, with elevated M-CSF levels even predicting future cardiac events during a 20-month follow-up.19 Because patients with unstable angina pectoris suffer more cardiac events than those with stable CAD, the
above-mentioned association may be interpreted as a sign of augmented collateral growth activity instead of atherosclerotic progression among the unstable CAD patients.

**Study Limitations**

Because theoretically the manifestation of side-effects would have carried a certain risk of uncovering the randomization table, the investigator recording the side-effects and the one performing collateral measurements and data analysis were different.

**Acknowledgments**

This work was supported by Grant 32-58945.99 from the Swiss National Science Foundation.

**References**

Promotion of Collateral Growth by Granulocyte-Macrophage Colony-Stimulating Factor in Patients With Coronary Artery Disease: A Randomized, Double-Blind, Placebo-Controlled Study
Christian Seiler, Tilmann Pohl, Kerstin Wustmann, Damian Hutter, Pierre-Alain Nicolet, Stephan Windecker, Franz R. Eberli and Bernhard Meier

*Circulation*. 2001;104:2012-2017
doi: 10.1161/hc4201.097835

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 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/104/17/2012