Deletion of p66\textsuperscript{shc} Gene Protects Against Age-Related Endothelial Dysfunction

Pietro Francia, MD; Chiara dell’Gatti, MD; Markus Bachschmid, PhD; Ines Martin-Padura, PhD; Carmine Savoia, MD; Enrica Migliaccio, MD; Pier Giuseppe Pelicci, MD; Marzia Schiavoni, MD; Thomas Felix Lüscher, MD; Massimo Volpe, MD; Francesco Cosentino, MD, PhD

Background—Enhanced production of reactive oxygen species (ROS) has been recognized as the major determinant of age-related endothelial dysfunction. The p66\textsuperscript{shc} protein controls cellular responses to oxidative stress. Mice lacking p66\textsuperscript{shc} (p66\textsuperscript{shc\textminus/w-}) have increased resistance to ROS and a 30% prolonged life span. The present study investigates age-dependent changes of endothelial function in this model.

Methods and Results—Aortic rings from young and old p66\textsuperscript{shc\textminus/w-} or wild-type (WT) mice were suspended for isometric tension recording. Nitric oxide (NO) release was measured by a porphyrinic microsensor. Expression of endothelial NO synthase (eNOS), inducible NOS (iNOS), superoxide dismutase, and nitrotyrosine-containing proteins was assessed by Western blotting. Nitrotyrosine residues were also identified by immunohistochemistry. Superoxide (O$_2^-$) production was determined by coelenterazine-enhanced chemiluminescence. Endothelium-dependent relaxation in response to acetylcholine was age-dependently impaired in WT mice but not in p66\textsuperscript{shc\textminus/w-} mice. Accordingly, an age-related decline of NO release was found in WT but not in p66\textsuperscript{shc\textminus/w-} mice. The expression of eNOS and manganese superoxide dismutase was not affected by aging either in WT or in p66\textsuperscript{shc\textminus/w-} mice, whereas iNOS was upregulated only in old WT mice. It is interesting that old WT mice displayed a significant increase of O$_2^-$ production as well as of nitrotyrosine expression compared with young animals. Such age-dependent changes were not found in p66\textsuperscript{shc\textminus/w-} mice.

Conclusions—We report that inactivation of the p66\textsuperscript{shc} gene protects against age-related, ROS-mediated endothelial dysfunction. These findings suggest that the p66\textsuperscript{shc} is part of a signal transduction pathway also relevant to endothelial integrity and may represent a novel target to prevent vascular aging. (Circulation. 2004;110:2889-2895.)

Key Words: aging ■ endothelium ■ free radicals ■ nitric oxide ■ genes

Shc proteins are adaptor proteins that exist in 3 different isoforms with relative molecular masses of 46, 52, and 66 kDa. P52\textsuperscript{shc}/p46\textsuperscript{shc} is involved in the transmission of mitogenic signals from tyrosine kinases to Ras.\textsuperscript{1} p66\textsuperscript{shc} has the same modular structure of p52\textsuperscript{shc}/p46\textsuperscript{shc} (SH2-CH1-PTB) and contains a unique N-terminal region (CH2); however, it is not involved in Ras regulation but rather functions in the intracellular pathway that converts oxidative signals into apoptosis. Indeed, embryo fibroblasts from mice carrying a targeted mutation of p66\textsuperscript{shc} (p66\textsuperscript{shc\textminus/m-}) are more resistant to oxidative stress–induced apoptosis.\textsuperscript{2} p66\textsuperscript{shc\textminus/w-} mice have an approximately 30% increase in life span and reduced early atherogenesis after long-term consumption of a high-fat diet,\textsuperscript{3} suggesting that p66\textsuperscript{shc} is implicated in aging and in the pathogenesis of aging-associated diseases in mammals. The biochemical function of p66\textsuperscript{shc} remains, however, unknown. Recent reports demonstrated that p66\textsuperscript{shc} acts as a downstream target of the tumor suppressor p53 and is indispensable to the ability of activated p53 to induce elevation of intracellular oxidants and apoptosis. Under basal conditions, p66\textsuperscript{shc\textminus/w-} cells have a reduced rate of intracellular oxidant formation and mitochondrial DNA alterations, which suggests that p66\textsuperscript{shc} acts through regulation of the intracellular redox state.\textsuperscript{4}

Accumulation of oxidative stress–damaged macromolecules with age has been documented consistently in tissues of different species and hypothesized to be the proximal causative mediator of age-associated diseases.\textsuperscript{5-7} Among different tissues, aging vessels are known to accumulate oxidative damage and undergo functional impairment.\textsuperscript{8-12}

The bioavailability of endothelium-derived nitric oxide (NO) represents a key marker of vascular health. The activity

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2889
of the L-arginine/NO pathway is a balance between synthesis and breakdown of NO by its reaction with superoxide anion (O$_2^-$). Under physiological conditions, the production of this molecule is not affected by O$_2^-$. Hence, the endothelium-derived NO may exert its well-known vascular protective effects. However, excessive generation of O$_2^-$ rapidly inactivates NO, leading to the formation of high concentrations of peroxynitrite (ONOO$^-$), a very powerful oxidant. Peroxynitrite easily penetrates across phospholipid membranes and produces substrate nitration, thereby inactivating regulatory receptors and enzymes such as free radical scavengers.

Decreased availability of NO plays a major role in the aging vessels. However, the cellular and molecular mechanisms underlying age-associated NO decline have not been fully elucidated and might involve (1) gradual loss of antioxidant defense mechanisms; (2) changes in expression or activity of endothelial NO synthase (eNOS) and (3) increased breakdown of NO because of enhanced O$_2^-$ production. Furthermore, the reported age-dependent upregulation of the inducible form of NOS (iNOS) might contribute to increased ONOO$^-$ formation and thus to the oxidative damage of vascular tissue.

Methods

Animals

Eighteen healthy young (6 to 7 months old) and 18 old (17 to 18 months old) p66$^{shc^{-/-}}$ and 18 young/18 old 129WT (wild-type) male mice were obtained from the Department of Experimental Oncology of the European Institute of Oncology (Milan, Italy). Control and knockout mice shared an identical genetic background because both mouse lines are of the 129Sv strain. The cohorts of WT mice and knockout mice were obtained from the Department of Experimental Oncology.

Animals

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Surgical Procedures

All of the experimental procedures were in accordance with the guidelines of our Institutions (Department of Experimental Medicine & Pathology, University of Rome “La Sapienza,” and Cardiovascular Research, Institute of Physiology, University of Zurich) and were approved by the local authorities for animal research. On the day of the experiment, mice were anesthetized through the intraperitoneal administration of 50 mg/kg sodium pentobarbital and then were euthanized. The chest and abdomen were opened with a medial sternotomy. The entire aorta from the heart to the iliac bifurcation was collected, rinsed in cold Krebs-Ringer bicarbonate solution (pH 7.4, 37°C, 95% O$_2$/5% CO$_2$) of the following composition (mmol/L): NaCl (118.6), KCl (4.7), CaCl$_2$ (2.5), KH$_2$PO$_4$ (1.2), MgSO$_4$ (1.2), NaHCO$_3$ (25.1), glucose (11.1), and calcium EDTA (0.026). The aorta was then cleaned of adhering tissues under a dissection microscope, frozen in liquid nitrogen, and stored at -80°C or was used immediately for organ chamber experiments and in situ measurement of NO production according to the study protocol.

Organ Chamber Experiments

Aortas were cut into rings (2 to 3 mm long). Each ring was connected to an isometric force transducer (SCAIMER, suspended in an organ chamber filled with 25 mL control solution (37°C, pH 7.4), and bubbled with 95% O$_2$/5% CO$_2$. Isometric tension was recorded continuously. After a 30-minute equilibration period, rings were gradually stretched to the optimal point of their length–tension curve (2±0.2 g) as determined by the contraction in response to norepinephrine (10$^{-6}$ mol/L). Concentration–response curves were obtained in a cumulative fashion. Several rings cut from the same artery were studied in parallel. Responses to acetylcholine (10$^{-7}$ to 10$^{-5}$ mol/L) and calcium ionophore A23187 (10$^{-7}$ to 10$^{-6}$ mol/L) were obtained during submaximal contraction to norepinephrine (10$^{-6}$ mol/L). The NO donor sodium nitroprusside (10$^{-10}$ to 10$^{-7}$ mol/L) was added to test endothelium-independent relaxation. Relaxations were expressed as a percentage of the precontracted tension.

Measurement of NO

Direct in situ measurements of NO were carried out as described. Immediately before NO measurements, the active tip of the L-shaped porphyrinic NO microsensor was placed directly on the surface of the endothelial cell monolayer. For maximal stimulation of eNOS, the calcium ionophore A23187 was injected into the cell culture dish to yield a final concentration of 10$^{-7}$ mol/L.

Determination of eNOS, iNOS, Superoxide Dismutase, and Nitrotyrosine Expression by Western Blot

Aortas were isolated and immediately snap-frozen in liquid nitrogen. The frozen aortas were pulverized and solubilized in lysis buffer containing 2-mercaptoethanol. Proteins were separated on denaturing SDS–8% (eNOS and iNOS) and 12% (manganese superoxide dismutase [MnSOD]– and 3-nitrotyrosine–containing proteins)—polyacrylamide gels overnight. Equal amounts of proteins (30 µg/lane) were loaded. To verify the equal loading, the gel was stained with Coomassie, and the intensity of the protein bands was examined. Separated proteins were blotted onto an activated piece of nitrocellulose (Immobilon-P, Millipore). Membranes were blocked for 1 hour at room temperature with a buffer containing 5% milk powder. Blots were incubated with anti-NOS3 rabbit polyclonal antibody (1:1000 dilution; Santa Cruz Biotechnology, Inc), anti-NOS2 mouse monoclonal antibody (dilution 1:1000, Santa Cruz Biotechnology, Inc), anti-MnSOD rabbit polyclonal antibody (1:2000 dilution, Upstate USA, Inc), or anti-nitrotyrosine mouse monoclonal antibody (1:1000 dilution, Upstate USA, Inc) for 1 hour at room temperature. Membranes were then incubated with the secondary antibody (horseradish peroxidase–conjugated anti-mouse/rabbit immunoglobulin antibody; Amersham Pharmacia Biotech) at a dilution of 1:2000. Prestained markers (Bio-Rad Laboratories) were used for molecular mass determinations. To compare target protein expression with the expression of a control protein, we analyzed the expression of α-tubulin using an anti-α-tubulin mouse monoclonal antibody (dilution 1:5000, Sigma-Aldrich). All bands were detected by enhanced chemiluminescence (ECL+, Amersham International).

Measurement of Superoxide by Coelenterazine-Enhanced Chemiluminescence

O$_2^-$ concentration in aortic tissue was determined by using a coelenterazine-enhanced chemiluminescence method. Each tissue sample (5 mm in length) was placed into 2 mL modified Krebs-Ringer solution, pH 7.40, and prewarmed to 37°C for 1 hour under a supply of carbogen. Immediately before measurement, rings were transferred into scintillation tubes filled with 500 µL Krebs-Hepes solution, pH 7.40, at 37°C. Coelenterazine was added to give a final concentration of 5 µmol/L. O$_2^-$-generated chemiluminescence of
Immunohistochemical Detection of 3-Nitrotyrosine

Small blocks of thoracic aortas from young and old p66shc and WT mice were embedded in OCT and stored at −80°C. Slices of 5 μm were cut, blocked with PBS/1%BSA for 1 hour, incubated for 1 hour at room temperature with anti-nitrotyrosine rabbit polyclonal antibody (5 μg/mL dilution, Upstate USA, Inc), stained with diaminobenzidine, and counterstained with hematoxylin.Slides were viewed with an Olympus BX51 microscope.

Statistical Analysis

In all experiments, n equals the number of mice per experiment. Results are expressed as mean±SEM. Statistical evaluation of data was performed by using the Student t test or ANOVA followed by Bonferroni test, as appropriate. A value of P<0.05 was considered statistically significant.

**Results**

**Characteristics of Animals**

Systolic blood pressure, lipid profile, blood glucose levels, and peripheral blood cell count are shown in Table 1. p66shc and WT mice did not display any significant differences. Small and not statistically significant variations in the blood cell count occurred with aging in both WT and p66shc mice. Similar findings in WT mice already have been published.23 No age-dependent changes of systolic blood pressure, blood glucose, or lipid profile were found in either WT or mutant mice (Table 1).

**Age-Associated Changes of Vascular Function**

Endothelium-dependent relaxation to acetylcholine was markedly reduced in old versus young WT mice. Surprisingly, p66shc mice did not show significant age-dependent impairment of endothelial function (Table 2, Figure 1a). Similar responses were obtained with the receptor-independent agonist calcium ionophore A23187 (data not shown). Endothelium-independent relaxation to sodium nitroprusside did not differ in mutant and WT mice (Table 2). Furthermore, the contractions in response to norepinephrine (10−6 mol/L) did not differ between WT and mutant mice (Table 2).

**Age-Dependent Changes of NO Release**

We assessed NO release from aortic rings after stimulation with the calcium ionophore A23187 (10−6 μmol/L). In the WT mice, maximal NO levels decreased significantly in old animals (Table 2, Figure 1b). In the p66shc mice, instead, similar levels of NO release were found in young and old animals (Table 2, Figure 1b), which indicates that aging does not significantly affect NO availability in the absence of p66shc.

**Table 1. Characteristics of the Animals**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>p66shc−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>Age, mo</td>
<td>6.5±0.3</td>
<td>17.5±0.9*</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>113±11</td>
<td>116±7</td>
</tr>
<tr>
<td>Blood glucose, mg/dL</td>
<td>64±6</td>
<td>81±7</td>
</tr>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>153±7</td>
<td>178±9</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>106±10</td>
<td>138±10</td>
</tr>
<tr>
<td>Blood cell count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red blood cells, 10^3/μL</td>
<td>9.15±1.23</td>
<td>8.64±1.40</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>14.4±1.4</td>
<td>12.3±1.6</td>
</tr>
<tr>
<td>White blood cells, 10^3/μL</td>
<td>9.39±1.01</td>
<td>8.76±1.39</td>
</tr>
<tr>
<td>Platelets, 10^9/μL</td>
<td>776±98</td>
<td>1039±73</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

**Table 2. Comparison of Different Parameters in Young and Old WT and p66shc−/−**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>p66shc−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>Maximal contraction to NE 10^{-6} mol/L, g</td>
<td>1.3±0.2</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Relaxations, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>56±8</td>
<td>19±3*</td>
</tr>
<tr>
<td>SNP</td>
<td>101±4</td>
<td>102±3</td>
</tr>
<tr>
<td>Maximal NO release, nmol/L</td>
<td>462±95</td>
<td>224±34†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SNP indicates sodium nitroprusside; NE, norepinephrine.

*P<0.05 vs young WT; †P<0.05 vs young WT.
Because NO availability is determined by the levels of NOS enzymes, endothelial and inducible NOS expression was assessed. Young WT and p66shc/H11002/H11002/H11002 mice did not show significant changes in the expression of eNOS (Figure 2a). Furthermore, we did not observe age-related changes of eNOS expression in either WT or mutant mice (Figure 2a). Conversely, old WT mice displayed an almost doubled expression of iNOS versus the matched young individuals, whereas no age-dependent changes of iNOS expression were found in p66shc/H11002/H11002/H11002 mice (Figure 2b).

**SOD Expression**

To determine whether an upregulation of antioxidant defense mechanisms might explain the increased NO bioavailability in p66shc−/− animals, we assessed the expression of the pivotal free radical scavenger SOD. Western blot analysis did not reveal any age-dependent difference in MnSOD expression (Figure 3). Cu/Zn SOD expression levels were comparable as well (data not shown).

**Vascular Superoxide Production**

Aortic O$_2^-$ production was assessed by using a coelenterazine-enhanced chemiluminescence method. A significant increase of O$_2^-$ production was observed in the aortas of old WT mice compared with the young animals, whereas no significant age-dependent changes were found in p66shc−/− mice (Figure 4).

**3-Nitrotyrosine Content**

Western blot analysis for total 3-nitrotyrosine–containing proteins revealed an increased prevalence of nitrated tyrosine residues in the aortas of old WT mice (Figure 5a). By contrast, nitrotyrosine immunoreactivity detected in young p66shc−/− mice remained unchanged in old animals (Figure 5a).

Nitrotyrosine residues were also measured in situ by immunohistochemistry with a polyclonal antibody against 3-nitrotyrosine. Aortas from old WT mice exhibited a markedly enhanced immunostaining both in the endothelium and smooth muscle cells compared with age-matched p66shc−/− (Figure 5b).
Discussion

The main finding of our study is that long-living p66shc−/− mice are protected against age-related endothelial dysfunction. Investigation of the underlying mechanisms revealed that deletion of the p66shc gene lowers aortic $\text{O}_2^-$ production, thereby reducing NO breakdown and increasing its bioavailability. Several lines of evidence support this conclusion. As expected, endothelium-dependent relaxation to acetylcholine was markedly reduced in old versus young WT mice. Interestingly enough, p66shc−/− mice did not show significant age-dependent impairment of endothelial function. Preservation of vascular function was not due to a selective protection from muscarinic receptor impairment or a difference in guanylate cyclase activity, because we observed a similar extent of vasorelaxation in WT and mutant mice when we used the receptor-independent agonist calcium ionophore A23187 or the endothelium-independent agent sodium nitroprusside.

To investigate whether the preserved endothelial function in the old p66shc−/− mice was associated with increased bioavailability of NO, we assessed NO release from aortic rings after stimulation with the calcium ionophore A23187. In the WT, maximal NO levels age-dependently decreased, whereas they remained unchanged in p66shc−/− mice.

The hypothesis that in p66shc−/− mice such preserved NO availability might be due to an upregulation of the main free radical scavengers has been ruled out because the expression of MnSOD and Cu/Zn SOD was comparable in old and young animals.

As far as eNOS expression is concerned, conflicting data have been reported on the regulation of eNOS during aging, possibly because of vascular bed and species-dependent differences.8–12 It was reported that eNOS is upregulated with age as a compensatory mechanism to counterbalance oxidative stress.10,11 On the contrary, there is evidence that age-induced decline of NO release is coupled with eNOS mRNA and protein downregulation.12 In the present study, WT and p66shc−/− mice did not show significant changes in the expression of eNOS. Therefore, it appears from our data that changes of eNOS expression are not responsible either for the age-related decline of NO release in WT mice or for its enhanced availability in p66shc−/− mice. By contrast, analysis of iNOS expression revealed marked differences among WT and mutant mice. Expression of iNOS increased significantly in the old WT mice, whereas no age-dependent changes were found in the p66shc−/− mice. This finding might contribute to explaining the preserved NO availability and vasorelaxant responses observed in old p66shc−/− mice. Indeed, age-dependent upregulation of iNOS is involved in ONOO− formation and, in turn, increased oxidative damage of aging vascular tissue.10,12,21

Because $\text{O}_2^-$ is the main inactivator of NO, we next tested the hypothesis that in p66shc−/− mice a decreased vascular production of $\text{O}_2^-$ contributes to increased NO availability. In this regard, an enhanced $\text{O}_2^-$ production was observed in the aortas of old versus young WT mice, whereas no significant age-dependent changes were found in p66shc−/− mice.

In aged vessels, the reaction of NO and $\text{O}_2^-$ leads to ONOO− formation and, in turn, increased protein 3-nitrotyrosine content.11,12,24,25 Accordingly, nitrotyrosine immunoreactivity detected in young p66shc−/− mice remained unchanged in old animals. Nitrotyrosine residues were also measured in situ, by immunohistochemistry with
a polyclonal antibody against 3-nitrotyrosine. As shown, nitrotyrosine immunoreactivity was detected in both endothelium and smooth muscle cells of aged animals. However, aortas from old WT mice exhibited a markedly enhanced immunostaining compared with age-matched p66\textsuperscript{shc} mice. The age-dependent tyrosine nitration process is responsible for inactivation of several enzymes. It was recently shown by our group that in aged animals nitration of MnSOD occurs. In the present study, we did not selectively assess MnSOD activity and its level of nitration. However, because our results show lower O2\textsuperscript{−} production and reduced protein nitration in aortas from old p66\textsuperscript{shc} mice, it is likely that in these animals MnSOD might be preserved from nitration and, hence, from inactivation.

In conclusion, we report for the first time that inactivation of the p66\textsuperscript{shc} gene protects against age-dependent, free radical–mediated endothelial dysfunction. Such prevention of endothelial impairment might contribute to the extended life span of p66\textsuperscript{shc} mice. Although other unknown p66\textsuperscript{shc}–related processes might be involved in the observed effects on endothelial function, a different modulation of intracellular redox state is the most likely explanation. However, important questions remain. Can the preserved endothelial function and longer life spans of the p66\textsuperscript{shc} mice be extended to humans? Why do mammals have a p66\textsuperscript{shc} at all, if mice that lack it live longer? Indeed, phenotypical and histopathologic analysis revealed no obvious abnormalities in the p66\textsuperscript{shc} mice. Accordingly, systolic blood pressure, lipid profile, blood glucose levels, and peripheral blood cell count did not significantly differ between WT and mutant mice.

Because oxygen free radical production is a distinct trait of the biology of aging, we propose that the p66\textsuperscript{shc} is part of a signal transduction pathway also relevant to endothelial integrity. These findings shed some light on new putative interventions to prevent vascular aging.

Acknowledgments
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References


Deletion of p66

Gene Protects Against Age-Related Endothelial Dysfunction

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/content/111/3/379.3.full.pdf

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In the article by Huynh et al, “Aspirin, Warfarin, or the Combination for Secondary Prevention of Coronary Events in Patients With Acute Coronary Syndromes and Prior Coronary Artery Bypass Surgery,” which published in the June 26, 2001, issue (Circulation. 2001;103:3069–3074), the authors now realize errors appeared in Tables 3 and 4. The percentages of events and complications were presented on the basis of the number of patients’ visits rather than on the total number of patients.

Overall, the corrected results did not change the implication of the study. There was no benefit of warfarin alone or combined with aspirin in the secondary prevention of ischemic events in this study of patients with previous coronary artery bypass surgery and an acute coronary syndrome; there was a significant excess in minor bleeding compared with the aspirin-alone group.

Corrected versions of Tables 3 and 4 appear below.

### TABLE 3. End-Point Events According to Treatment

<table>
<thead>
<tr>
<th>Events</th>
<th>Warfarin + Placebo (n=45)</th>
<th>Aspirin + Placebo (n=46)</th>
<th>Warfarin + Aspirin (n=44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary end point, n (%)</td>
<td>18 (40.0)</td>
<td>13 (28.3)</td>
<td>11 (25.0)</td>
<td>0.27</td>
</tr>
<tr>
<td>Death, n (%)</td>
<td>1 (2.2)</td>
<td>0 (0.0)</td>
<td>2 (4.5)</td>
<td>0.34</td>
</tr>
<tr>
<td>MI, n (%)</td>
<td>4 (8.9)</td>
<td>1 (2.2)</td>
<td>2 (4.5)</td>
<td>0.34</td>
</tr>
<tr>
<td>UA, n (%)</td>
<td>16 (35.6)</td>
<td>13 (28.3)</td>
<td>10 (22.7)</td>
<td>0.41</td>
</tr>
<tr>
<td>PCI, n (%)</td>
<td>6 (13.3)</td>
<td>1 (2.2)</td>
<td>3 (6.8)</td>
<td>0.12</td>
</tr>
<tr>
<td>Repeat CABG, n (%)</td>
<td>2 (4.4)</td>
<td>2 (4.3)</td>
<td>2 (4.5)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

UA indicates unstable angina requiring rehospitalization; PCI, percutaneous coronary intervention; and MI, myocardial infarction. Primary end point is any-cause mortality, MI, or UA requiring hospitalization.

### TABLE 4. Complications and Adherence to Protocol by Patients

<table>
<thead>
<tr>
<th>Complications</th>
<th>Warfarin + Placebo (n=45)</th>
<th>Aspirin + Placebo (n=46)</th>
<th>Warfarin + Aspirin (n=44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor bleeding, n (%)</td>
<td>10 (22.2)</td>
<td>2 (4.3)</td>
<td>9 (20.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Major bleeding, n (%)</td>
<td>1 (2.2)</td>
<td>0 (0.0)</td>
<td>2 (4.5)</td>
<td>0.34</td>
</tr>
<tr>
<td>Blood transfusions, n (%)</td>
<td>2 (4.4)</td>
<td>0 (0.0)</td>
<td>2 (4.5)</td>
<td>0.34</td>
</tr>
<tr>
<td>Compliance, %*</td>
<td>90.1</td>
<td>86.7</td>
<td>86.1</td>
<td>0.66</td>
</tr>
<tr>
<td>Protocol completion, %*</td>
<td>77.6</td>
<td>78.5</td>
<td>69.9</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Compliance and protocol completion were calculated per visit.

DOI: 10.1161/01.CIR.0000155489.11621.70
In the article by Haïssaguerre et al, “Mapping and Ablation of Ventricular Fibrillation Associated With Long-QT and Brugada Syndromes,” which appeared in the August 26, 2003, issue (Circulation. 2003;108:925–928), the authors would like to note the following errors:

1. In the byline, Jerónimo Farré’s name incorrectly appeared as “Gerónimo Farre.”
2. José Angel Cabrera and Jerónimo Farré work at Fundación Jiménez Díaz in Madrid, Spain.
3. The work of Drs Cabrera and Farré was supported by Redes Temáticas de Cooperación, Red Cardiovascular C01/03.

DOI: 10.1161/01.CIR.0000155483.25082.D4

In the article by McRae and Ginsberg, “Initial Treatment of Venous Thromboembolism,” which appeared in the August 31, 2004, supplement sponsored by the Society for Vascular Medicine and Biology (Circulation. 2004;110[suppl I]:I-3–I-9), an error appeared in Table 2. The footnote of the table erroneously states that “For enoxaparin, 100 anti-Xa U/kg corresponds to a dose of 100 mg/kg.” The legend should have read, “For enoxaparin, 100 anti-Xa U/kg corresponds to a dose of 1 mg/kg.”

DOI: 10.1161/01.CIR.0000155484.25082.1A

In the article by Bauer et al, “Acute Improvement in Global and Regional Left Ventricular Systolic Function After Percutaneous Heart Valve Implantation in Patients With Symptomatic Aortic Stenosis,” which appeared in the September 14, 2004, issue (Circulation. 2004;110:1473–1476), two errors of note appeared in the table on page 1474. Under “Endocardiographic data,” the rows for “LV end-systolic volume, mm Hg” and “LV end-diastolic volume, mm Hg” should have appeared as the following:

<table>
<thead>
<tr>
<th>LV end-diastolic volume, mL</th>
<th>102±36 (baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end-systolic volume, mL</td>
<td>49±25 (baseline)</td>
</tr>
</tbody>
</table>

DOI: 10.1161/01.CIR.0000155485.32706/1C

Because of a typesetting error, several mathematical symbols appeared incorrectly in the article by Solomon et al, “Effect of Candesartan on Cause-Specific Mortality in Heart Failure Patients: The Candesartan in Heart failure Assessment of Reduction in Mortality and morbidity (CHARM) Program,” which appeared in the October 12, 2004, issue (Circulation. 2004;110:2180–2183). On page 2180, in the abstract and in the text of the article, there were several instances in which “LVEF=40%” should have appeared as “LVEF≤40%.” In addition, in the last sentence of the first paragraph of the article, please note that “9% borderline risk” should read “9% borderline significant risk.” The corrected version is available online at http://circ.ahajournals.org/cgi/content/full/110/15/2180. (The previous version can be accessed by selecting the “Previous Version of This Article” link.) We regret these errors.

DOI: 10.1161/01.CIR.0000155486.26868.C9

In the AHA Scientific Statement by Drew et al, “Practice Standards for Electrocardiographic Monitoring in Hospital Settings: An American Heart Association Scientific Statement From the Councils on Cardiovascular Nursing, Clinical Cardiology, and Cardiovascular Disease in the Young,” which appeared in the October 26, 2004, issue (Circulation. 2004;110:2721–2746), Figure 4 contained an error. The text in the figure refers to the “Angle of Lewis.” The correct name is “Angle of Louis.” The Association regrets this error.

DOI: 10.1161/01.CIR.00001155490.19245.B0
In the article by Noujaim et al, “From Mouse to Whale: A Universal Scaling Relation for the PR Interval of the Electrocardiogram of Mammals,” which appeared in the November 2, 2004, issue (Circulation. 2004;110:2802–2808), the name of Ary L. Goldberger, MD, was misspelled as “Goldberg” in reference 12. The authors regret this error.

DOI: 10.1161/01.CIR.0000155482.89456.78

In the article by Spargias et al, “Ascorbic Acid Prevents Contrast-Mediated Nephropathy in Patients With Renal Dysfunction Undergoing Coronary Angiography or Intervention,” which appeared in the November 2, 2004, issue (Circulation. 2004;110:2837–2842), the name of author Panagiotis Iokovis was spelled incorrectly as “Panagiotis Iocovis.” The authors regret this error.

DOI: 10.1161/01.CIR.0000155487.34492.0D


DOI: 10.1161/01.CIR.0000155488.34492.E9