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CAN COMMON-TYPE ATRIAL FLUTTER BE A SIGN OF AN ARRHYTHMOGENIC SUBSTRATE IN PAROXYSMAL ATRIAL FIBRILLATION? CLINICAL AND ABLATIVE CONSEQUENCES IN PATIENTS WITH COEXISTENT PAROXYSMAL ATRIAL FIBRILLATION/ATRIAL FLUTTER, by Moreira et al

Common atrial flutter due to right atrial reentry is often associated with atrial fibrillation, although the critical substrate for atrial fibrillation is more often left atrial. Moreira and coworkers performed sequential ambulatory monitoring and ablation studies in patients with paroxysmal atrial fibrillation to define the presence of atrial flutter and assess the impact of right atrial flutter ablation and segmental pulmonary vein isolation with cryoablation on spontaneous arrhythmias. As expected, right atrial ablation alone was effective for abolishing atrial flutter but failed to prevent atrial fibrillation. Interestingly, pulmonary vein isolation was substantially less effective in preventing recurrent atrial fibrillation in patients with prior atrial flutter than in those with no prior atrial flutter. These findings suggest that atrial flutter is a marker for more advanced atrial disease in patients with paroxysmal atrial fibrillation. Monitoring for atrial flutter may provide a noninvasive means of assessing the extent of arrhythmia substrate that might help predict outcomes and potentially facilitate more individualized ablation strategies. See p 2786 (editorial p 2774).

DOES COMORBIDITY ACCOUNT FOR THE EXCESS MORTALITY IN PATIENTS WITH MAJOR BLEEDING IN ACUTE MYOCARDIAL INFARCTION? by Spencer et al

Major bleeding after treatment for acute myocardial infarction is associated with a significantly worse overall outcome in randomized clinical trials. However the incidence in an unselected group of patients and the factors associated with an increased bleeding risk are not well described. 40 089 patients with acute myocardial infarction were enrolled in the Global Registry of Acute Coronary Events (GRACE) registry. The frequency of major bleeding in patients with acute myocardial infarction was 2.8%. In-hospital mortality was increased significantly (hazard ratio=1.9, 95% confidence interval 1.6–2.2) and accounted for 10% of all hospital deaths. Major bleeding was not a predictor of mortality after hospital discharge. Bleeding was associated with invasive procedures, age, and comorbidities, as well as premature discontinuation of antithrombotic therapy. This study suggests that bleeding is related to adverse outcomes and is often a marker for patients at higher risk for adverse outcomes. See p 2793 (editorial p 2776).

NF-κB IS A KEY MEDIATOR OF CEREBRAL ANEURYSM FORMATION, by Aoki et al

Cerebral aneurysms are an important source of morbidity and mortality, yet we know very little about the mechanisms of aneurysm formation. In this issue, Aoki and colleagues demonstrate that a key mediator of inflammation, known as NF-κB, is required for the development of cerebral aneurysms. Inhibition of this target prevented the formation of aneurysms in animal models. Most importantly, examination of human specimens revealed this NF-κB pathway to be activated in cerebral aneurysms. This study provides us with a potential new therapeutic target for patients in whom early aneurysm formation is noted. See p 2830.

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Images in Cardiovascular Medicine
Leiomyosarcoma Involving Main and Left Pulmonary Artery Treated Surgically With Homograft Replacement and Concomitant Left Pneumonectomy. See p e559.

Correspondence
See p e562.
Atrial Fibrillation–Atrial Flutter Interactions
Clinical Implications for Ablation

Albert L. Waldo, MD

An appreciation of the article by Moreira et al in the current issue of Circulation1 requires an understanding of the close interrelationship between atrial fibrillation (AF) and atrial flutter (AFL). These authors have understood this interrelationship and applied it to their data to advance the approach to both AF and AFL ablation. Key to this understanding is the recognition that cavitricuspid isthmus (CTI)–dependent AFL almost always develops from antecedent AF of variable duration.2–5 This is because in almost all instances, it is during the AF that a functional line of block (LoB) necessary for the development of AFL forms between the superior and inferior vena cavae. This LoB acts as a critical lateral boundary that prevents short-circuiting of the AFL reentrant circuit. Thus, in the vast majority of instances, without preceding AF, there can be no AFL. The most recent additional support of this concept comes from the report by Ellis et al,6 which found that of 363 patients who presented additional support of this concept comes from the report by Ellis et al,6 which found that of 363 patients who presented with only CTI-dependent AFL and who underwent CTI ablation, long-term follow-up (mean of 39 ± 11 months) demonstrated newly recognized AF in 82%. It also should be noted that, as Moreira et al1 recognize, in some patients, a LoB between the vena cavae may be fixed (ie, anatomic) rather than functional. In such patients, AF may not be required for AFL to develop.

As Moreira et al1 further recognize, their report does not answer all the questions about the interrelationships of AF and AFL as they relate to AF ablation, but they do raise and consider relevant questions. The most fundamental question is a logical extension of understanding the development of classic, CTI-dependent AFL: ie, “Can the successful elimination of AF prevent the development of AFL?” If eliminating AF by ablation means successfully isolating pulmonary vein triggers that precipitate AF, the pathophysiology of AFL would lead one to expect that AFL would no longer occur. But because triggers may be at places other than the pulmonary veins, successful isolation of only the pulmonary veins may not be enough. Then, too, isolation of all the pulmonary veins may not always be possible or even be attempted. In fact, in the study by Moreira et al,1 the mean number of pulmonary veins isolated was 3.06 in patients with AFL and AF (group I) and 2.89 in patients with AF only (group II). Even in the most experienced hands, after catheter ablation of AF, there is at least a 10% to 20% AF recurrence rate, and a still higher rate during the early postablation 2- to 3-month blanking period.7 Thus, if AFL was present before AF ablation, one should expect AFL recurrence after ablation, too, unless CTI ablation was performed. This is what occurred in the study by Moreira et al1 and was the experience in 108 patients with both AF and AFL reported by Wazni et al.8 Interestingly, after the early postablation period, the AFL recurrence rate was only 8% during long-term follow-up in patients treated only with AF ablation in the study by Wazni et al,8 reflecting the much lower incidence of AF recurrence during late follow-up. Then, too, in the occasional patient with a fixed intercaval LoB, even a premature atrial beat that results from ventriculoatrial conduction of a premature ventricular beat could be enough to precipitate AFL. Lastly, if our knowledge of AF mechanisms improves such that ablation of a targeted atrial substrate alone, eg, of a driver causing fibrillatory conduction, is all that may be needed for some, if not most, to prevent sustained AF, the triggers, which would still be present, may precipitate brief episodes of AF (eg, due to multiple reentrant wavelets). This might be sufficient to generate a LoB between the vena cavae and, thereby, result in the development of AFL.

But it is quite striking that the same ablation team (ie, Moreira et al),1 using the same techniques to ablate paroxysmal AF (PAF) in all their patients, found a remarkable difference in success rates between group I (AF/AFL patients) and group II (only-AF patients). Although the authors did not restudy all of the patients with AF recurrence, one would not expect that the enormous difference in success rates for preventing AF (33% in group I versus 89% in group II) would be explained simply by a greater failure rate of pulmonary vein isolation in group I. So, the authors asked another important, relevant question: “Is common-type AFL a sign of an arrhythmogenic substrate in [patients with] PAF?” They suggest the answer is “yes,” and they well may be right. But it should be noted that Wazni et al8 had a far greater success rate (86%) of AFL ablation in patients who also presented with AFL and in whom CTI ablation was performed in addition to pulmonary vein isolation.

In the context of considering the interrelationship of AF and AFL, one should ask, “Why don’t all patients who get AF also develop AFL?” It may be because they do not have that more extensive arrhythmogenic substrate. But, in the same context, why don’t patients who present with both AF and
AFL only get AFL? And why don’t all patients who initially present with only AFL subsequently develop sustained AF after CTI ablation? Or do they all develop AF if they are followed up long enough? Citing again the recent study by Ellis et al, perhaps they mostly do. Ah, questions, questions, questions. Clearly, there is much more to understand.

Another question relates to the need to do a “prophylactic” CTI ablation in patients with PAF who present without any history of AFL. Although 5 (8%) of such patients in group II later presented with a new onset of CTI-dependent AFL, Moreira et al concluded that “preventive CTI ablation should not be included in the strategy for treatment of PAF.” All 5 of those patients underwent successful CTI ablation, but then, not surprisingly, manifested problematic recurrence of PAF. So, this really gets us back to the first question, because if the AF had been prevented, it is fair to assert that the AFL would not have developed. It seems to make sense that if the expected efficacy rate of AF ablation is ≥80% for PAF patients without AFL, prophylactic CTI ablation would mostly be unnecessary. Thus, most such patients would not needlessly be exposed to the small, but not zero, risk of CTI ablation without much likelihood of benefit, and an already long procedure would not needlessly be prolonged further. In fact, the recently issued consensus statement from the Heart Rhythm Society recommends that prophylactic CTI ablation not be performed in this group of patients.

Finally, the present report also nicely serves to reemphasize the need to think mechanistically and to understand better the relationships of triggers and substrate in AF, and AFL as well. When we have understood these relationships, as with atrioventricular nodal reentrant tachycardia, atrioventricular reentrant tachycardia, and, per the latter, Wolff-Parkinson-White syndrome, we have been exquisite in applying ablation techniques to the vulnerable substrate.

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Bleeding Is Bad. . . . Isn’t It?

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Advances in antithrombin and antiplatelet therapy have traditionally been associated with reductions in myocardial infarction and other thrombotic events in patients who have experienced an acute coronary syndrome (ACS), are undergoing a percutaneous coronary intervention (PCI), or are receiving long-term therapy for secondary prevention of stable vascular disease. Although individual trials of anti-thrombotic therapy have rarely been able to demonstrate that the reduction in ischemic events leads to a reduction in mortality (the most important end point), meta-analyses have often suggested that this is the case. And because myocardial infarction is an independent correlate of mortality, a link between a reduction in ischemic events and reduced mortality makes intuitive sense.

Unfortunately, more potent anticoagulants and antiplatelet agents are also associated with an increased risk of bleeding, especially when used in combination with one another, as is usually the case. Furthermore, there is a well-established body of evidence that indicates an association between bleeding and ischemic events. It has been unclear, however, whether the link between bleeding and thrombosis is the result of bleeding after the initial development of a thrombotic complication (and its treatment) or if bleeding precedes the development of an ischemic complication and actually leads to (actually causes) thrombosis. In support of the former possibility is that patients who, because of the presence of thrombus, undergo a longer or more complicated procedure, receive a higher dose or longer duration of antithrombotic medication, or require an intra-aortic balloon pump are surely more prone to bleed. However, the possibility that hemorrhage actually leads to thrombosis rather than results from it is supported by the observation that hemorrhage is a potent stimulus for thrombosis; all patients would surely die if bleeding did not trigger at least a local thrombotic reaction. Furthermore, as the Global Registry of Acute Coronary Events (GRACE) investigators report in an analysis of 40,087 patients in this issue of Circulation, patients who experience a hemorrhagic complication often have their antithrombotic medications discontinued, which further increases the risk of thrombosis. Although it might seem easy to distinguish between these 2 very different explanations of the relationship between bleeding and death, most studies have not been able to do so. Also, historically, in patients with coronary disease and particularly those undergoing PCI, death usually resulted from thrombosis. Now, more frequently than ever before, it is recognized that death may result from hemorrhagic complications. In fact, fatal hemorrhage is probably a more common cause of death among patients undergoing PCI than is refractory coronary thrombosis that leads to fatal myocardial infarction.

Even though the relationship between bleeding and mortality has often been described as an independent one, many of the correlates of bleeding, as reported in the analysis of GRACE, are themselves associated with mortality. Older age, female sex, impaired renal function, and others are all associated with mortality, and no amount of statistical adjustment can provide certainty of a cause-and-effect relationship between correlates, particularly when a large number of covariates differ between the 2 groups of interest that are themselves associated with the outcome of interest (ie, death in patients who did and did not bleed). Perhaps as a result of the recognition of how difficult it is to separate whether bleeding leads to or results from thrombotic complications and of the clinical importance of bleeding, recent trials have begun to include bleeding as a component of their primary end point. Terminology such as “net clinical outcome” and “net adverse cardiac events (NACE)” has begun to enter the lexicon. However, composite end points are most appropriate when the individual components are of roughly equal importance, occur with approximately equal frequency, and correlate with one another. The “quadruple end point” of death, myocardial infarction, repeat revascularization, and bleeding satisfies few of these requirements—fewer, even, than the “triple end point” of death, myocardial infarction, and repeat revascularization. Because more potent anticoagulant and antiplatelet therapies typically reduce the frequency of thrombotic events but increase the frequency of hemorrhagic events, composite end points that include both thrombotic and hemorrhagic end points are problematic and have not been universally accepted.

The Organization for the Assessment of Strategies for Ischemic Syndromes (OASIS)-5 Trial, in which the Xa inhibitor fondaparinux was compared with enoxaparin in patients with non–ST-elevation ACS, is being touted as the strongest evidence that a reduction in bleeding may lead to a reduction in subsequent mortality. In OASIS-5, the frequencies of death, myocardial infarction, and refractory ischemia at 9 days (the prespecified primary end point) were virtually identical, but fondaparinux caused significantly less bleeding (an absolute 1.9% reduction) at 9 days. Remarkably, in the next 6 months, a difference in death (an absolute 0.7%) was detected between the enoxaparin and fondaparinux groups, in

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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favor of fondaparinux. However, one is reminded of a somewhat analogous situation in which the addition of a glycoprotein IIb/IIIa inhibitor to heparin reduced procedural infarctions by ≈40% and was associated with a reduction in mortality not when the drugs were administered, but in late mortality, long after their discontinuation. Additional analyses, however, revealed that >80% of the patients who died in the placebo arms of these trials had not experienced a procedural infarction. This finding was a blow to the theory linking procedural infarction to death and its corollary, that a reduction in early procedural infarction leads to greater long-term survival rates; many people now believe that the difference in late death was most likely the result of chance. The causes of death among patients assigned to enoxaparin in OASIS-5 have not yet been reported, and it has not yet been reported whether late deaths were related in any way, directly or indirectly, to the bleeding that occurred earlier. Preliminary analysis of the Harmonizing Outcomes With Revascularization and Stents in Acute Myocardial Infarction (HORIZONS) trial, in which bivalirudin reduced major bleeding in patients with ST-elevation myocardial infarction undergoing primary PCI and, to the surprise of many, was also associated with a significantly lower mortality at 30 days, may also support a link between bleeding and a reduction in mortality; further analysis and longer-term follow-up are needed.

If procedural infarction is associated with mortality, however, and bleeding is associated with mortality, why would it not be easy to demonstrate that drugs that reduce these events also reduce mortality? There are many possible explanations. One is that drugs that are potent inhibitors of platelet aggregation, such as glycoprotein IIb/IIIa inhibitors, reduce procedural infarction but also increase bleeding, so the beneficial effects from a reduction in infarction may be partially offset by the increase in bleeding. This was also just seen in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombosis in Myocardial Infarction 38 (TRITON-TIMI 38), in which the novel, more potent P2Y12 inhibitor prasugrel reduced infarction significantly more than clopidogrel but, as a result of its greater potency, was also associated with a significant increase in major and life-threatening bleeding. Similarly, drugs that reduce bleeding, such as the direct thrombin inhibitor bivalirudin, may be associated with a trend toward more frequent procedural infarction, particularly in higher-risk, troponin-positive patients; this, if true, might in part offset the expected improvement in clinical outcome that results from a reduction in bleeding.

One of the principle findings in the analysis of GRACE highlights an important concept to remember whenever registry outcomes are analyzed. Spencer and colleagues report that many of the correlates of bleeding in GRACE are themselves independently associated with mortality. No antithrombotic therapy can be expected to reduce the mortality that results from nonmodifiable risk factors associated with bleeding. Again, a parallel exists between the many PCI studies that have shown that older patients and those with diabetes, vein grafts, a greater number of lesions, and longer lesions, for example, have an increased frequency of procedural infarction. No antithrombotic agent can reduce the mortality that results from these nonmodifiable characteristics associated with procedural infarction.

There are many remaining issues, however, about which we have much to learn in order to better understand the relationship between bleeding and outcome. Many different definitions of bleeding have been used; not all are appropriate for both antiplatelet and anticoagulant agents, or for both chronic and acute clinical situations, or for both ACS and PCI. The definition of bleeding used in GRACE is yet another relatively unique definition; however, reported rates of bleeding with this definition are similar to those reported in other recent studies. Bleeding in GRACE was associated with in-hospital, although not longer-term, mortality. Other studies have suggested a relationship between bleeding and longer term (1-year) mortality. The GRACE analysis avoids a pitfall that many other analyses have not: A transfusion without a documented bleed was not sufficient for moderate or severe bleeding to be considered to have occurred. Many other analyses consider a transfusion to represent a moderately severe or major bleed, even without documented bleeding. However, we know that many ACS patients (several absolute percent) receive a transfusion because of anemia or procedural blood loss without a documented hemorrhagic complication. Indeed, baseline anemia is a powerful correlate, among the most powerful correlates, of transfusion. When long-term analyses indicate a great mortality in such patients, it may not necessarily be because they bled, even though they are "defined" as having bled. Does the mortality result from more complex coronary disease that leads to a more difficult and complicated procedure, with greater blood loss? Or underlying comorbid illnesses that led to the anemia? Or is transfusion itself deleterious? The answer to all of these questions is unknown but under active investigation. Regardless, the GRACE analysis provides further insight into the frequency, correlates, and clinical impact of bleeding on adverse outcomes in >40,000 patients with ST-elevation and non–ST-elevation myocardial infarction. It is now clearer than ever that mortality may result from either thrombosis or hemorrhage. Although antithrombotic medications that cause less bleeding may not reduce mortality as much as we would like or expect, bleeding is dangerous, uncomfortable to the patient, associated with much nonfatal morbidity, and expensive, and it is to be avoided whenever possible. The GRACE analysis reinforces just how much we have to learn about the most appropriate ways to characterize, prevent, and treat bleeding during the administration of antithrombotic therapy to optimize outcomes.

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Caloric restriction has emerged as an effective strategy for lengthening lifespan in a variety of species. In mammals, one mechanism for this phenomenon may be the prevention of detrimental age-related alterations in cellular function and presumably subsequent improvement in organ function. The effects of caloric restriction on the heart, at least in rats and mice, involve a number of changes in gene expression that are beneficial to the aged cardiomyocyte and/or protect the heart from ischemic injury. Although it is likely that all of the beneficial mediators of caloric restriction have not been identified, a number of proteins in the mammalian sirtuin family may play key roles in the regulation of health and longevity. In addition, recent evidence has suggested that alterations in whole-body energy metabolism contribute to the beneficial effects of caloric restriction. Indeed, caloric restriction in mammals leads to loss of adipose tissue and dramatically alters the action of this endocrine organ. As such, caloric restriction contributes to changes in adipose tissue–derived hormone (adipokine) secretion, which can govern whole-body metabolism. Furthermore, studies using isolated cardiac myocytes suggest that these adipokines may exert direct end-organ effects that are independent from alterations in whole-body metabolism. One adipokine that is significantly increased during caloric restriction is adiponectin. Previous work has shown that adiponectin exerts a host of protective effects on the cardiovascular system and as such may be an essential component mediating the effects of caloric restriction.

The focus on adiponectin in the cardiovascular system has been due largely to the fact that in humans, circulating adiponectin levels are negatively correlated with increased body mass index. Because increased body mass index is associated with a number of obesity-linked disorders, including cardiovascular disease, the reduction in serum adiponectin levels may contribute to disease development. On the basis of this rationale, adiponectin has been proposed to be beneficial in promoting cardiovascular health, and as such, its loss may contribute to the higher incidence of cardiovascular disease in obese individuals. Indeed, the beneficial effects of adiponectin on the cardiovascular system are quite extensive. In the vasculature, increased serum adiponectin levels assist in maintaining vascular tone via the stimulation of nitric oxide production in endothelial cells, promote angiogenesis after ischemic injury, protect against the formation of atherosclerotic lesions, and reduce smooth muscle cell growth and neointimal thickening in vascular lesions. In cardiac tissue, increased serum adiponectin lessens the development of concentric hypertrophy in a mouse model of aortic constriction and protects against ischemia/reperfusion injury in mice subjected to coronary artery ligation. The effects described in cardiac muscle are mediated, at least in part, by the activation of AMP-activated protein kinase (AMPK). Although AMPK activation by itself appears to inhibit hypertrophic growth in the cardiac myocyte, the protective effects of adiponectin during and after ischemia appear to be more complex and involve additional signaling pathways. Specifically, it has been shown previously that one of these auxiliary pathways may be the activation of cyclooxygenase-2, which also can protect the ischemic myocardium via a number of mechanisms. Although the evidence for the cardioprotective effects of adiponectin during ischemia is strong, it is still unclear whether AMPK is the central mediator of this effect.

In a study published in this issue of Circulation, Shinmura et al demonstrate the cardioprotective effects of short-term caloric restriction on isolated mouse hearts subjected to ischemia/reperfusion. This study provides evidence that short-term caloric restriction (10% reduction in normal caloric intake for 3 weeks followed by a 35% reduction for 2 weeks) significantly increases serum adiponectin levels before ischemia, which results in improved left ventricular function throughout reperfusion. Furthermore, the authors demonstrate that short-term caloric restriction reduces infarct size from 28% of the left ventricular in ad libitum–fed mice to 19% in calorie-restricted mice. Evidence suggesting that adiponectin is the key signaling molecule under these conditions is provided through the use of transgenic mice expressing an antisense adiponectin oligonucleotide. In these mice, serum adiponectin levels are close to baseline values of the ad libitum–fed wild-type mice and are not elevated during caloric restriction. Consistent with the author’s hypothesis, the cardioprotective effects of caloric restriction are completely lost, and left ventricular function and infarct size are virtually indistinguishable in the ad libitum–fed transgenic mice compared with the calorie-restricted transgenic mice. Additionally, when recombinant adiponectin is administered to the transgenic mice in vivo, the beneficial effects of caloric...
restriction are restored. Taken together, these findings strongly implicate adiponectin as being responsible for mediating the cardioprotective effects of short-term caloric restriction.

To explore the molecular mechanisms responsible for the beneficial effects of short-term caloric restriction and elevated serum adiponectin levels on the ischemic heart, the authors investigated AMPK phosphorylation status in hearts from calorie-restricted mice. In agreement with previous reports that adiponectin activates AMPK in the mouse heart, the 84% increase in serum adiponectin induced by short-term caloric restriction correlates with a significant increase in AMPK phosphorylation at its activation site. Subsequent administration of adenosine 9-D arabinofuranoside, a relatively nonspecific inhibitor of AMPK, to short-term calorie-restricted wild-type mice prevented both AMPK activation and the cardioprotective effects normally induced by short-term caloric restriction. On the basis of the evidence that the cardioprotective effect of short-term caloric restriction is mediated via the activation of AMPK, the authors propose that these effects form the basis of a novel form of preconditioning. That is, caloric restriction elevates serum adiponectin levels, which activates AMPK and subsequently preconditions the heart to better withstand a more severe ischemic insult. As such, Shinmura et al suggest that this mechanism may allow novel strategies aimed at stimulating the adiponectin-AMPK signaling axis to be developed for future use in ischemic heart disease and/or after acute myocardial infarction.

Although the study by Shinmura et al provides valuable insights into the effects of short-term caloric restriction on the heart, a number of questions are yet to be answered. For example, if adiponectin is the sole signaling molecule responsible for the cardioprotective effects of caloric restriction, it remains to be resolved why the cyclooxygenase-2 pathway are yet to be resolved, the data presented by Shinmura et al are consistent with previous reports indicating that AMPK activation is a necessary component of the signal transduction pathway responsible for the cardioprotective effects induced by elevated serum adiponectin. However, given the use of the nonspecific inhibitor of AMPK in this study (ie, adenosine 9-D arabinofuranoside), there may be alterations in a number of AMPK-dependent kinase signaling cascades, and it remains to be clarified whether the cardioprotective effects of adiponectin observed in this study can be attributed directly to AMPK activation or some other pathway. For example, although Shibata et al have shown that the addition of adiponectin during ischemia is cardioprotective, the fact that severe ischemia likely maximally activates AMPK, the addition of adiponectin during ischemia may exert its cardioprotective effects via alternative signaling pathways. Whether this also is possible in the short-term caloric restriction model described by Shinmura et al remains to be elucidated.

The above-mentioned comments notwithstanding, if AMPK is confirmed as the central mediator of the effects of adiponectin, the subsequent downstream effector pathways that protect the myocyte from ischemic damage remain to be elucidated. For example, it is unknown whether AMPK directly alters cell survival pathways to promote myocyte survival after ischemia or whether the protective effects of AMPK activation are purely metabolic in nature. To date, much of the research into the mechanisms by which AMPK activation protects the ischemic myocardium demonstrates reduced apoptosis, suggesting a direct link between AMPK and cellular survival pathways. However, it is also possible that vasodilatation induced by phosphorylation and activation of endothelial nitric oxide synthase by AMPK may lessen the severity of ischemia and enhance left ventricular function after ischemia. In addition, because AMPK is a pivotal regulator of energy homeostasis at both the cellular and whole-body levels, activation of AMPK by caloric restriction may profoundly alter myocardial energy metabolism. Although not fully explored, evidence presented by Shinmura et al suggests that cardiac energy metabolism may be involved. For instance, hearts from transgenic mice lacking the ability to increase serum adiponectin levels have a significant reduction in myocardial glycogen content when subjected to caloric restriction. Because glycogen becomes the primary source of myocardial ATP during no-flow ischemia, depleted glycogen stores before ischemia may be sufficient to adversely affect functional recovery after ischemia. This finding may partially explain the loss of protection induced by caloric restriction in these transgenic mice. Because myocardial glycogen levels and ATP levels immediately after ischemia were not reported, it is not known whether these hearts are more energetically compromised than hearts from calorie-restricted mice after ischemia. In addition, because glycogen levels were not determined in any other groups of hearts, it will be important to determine whether administration of recombinant adiponectin increases myocardial glycogen levels in the transgenic mice. This would provide insight into the role of glycogen in the cardioprotective effects of short-term caloric restriction and would help to determine whether alterations in cardiac energy metabolism play a role in this process.

An additional concept underlying the study of Shinmura et al is that the activation of cardiac AMPK before ischemia is cardioprotective. However, it has yet to be definitively proven whether acute ischemia-induced activation of AMPK is beneficial or detrimental to the heart during reperfusion. Although it is recognized that activation of myocardial AMPK either before or during ischemia may have a beneficial effect of increased energy supply to the heart by stimulating glucose uptake, glycogenolysis, and glycolysis, it also may be detrimental to the heart after reperfusion by promoting fatty acid oxidation and subsequently decreasing cardiac efficiency. Because the concentration of fatty acids in the perfusate of ex vivo perfused hearts may
profoundly alter functional recovery after ischemia,\textsuperscript{23} clinically relevant concentrations of fatty acids (ie, >1 mmol/L\textsuperscript{24}) must be present in the perfusate to adequately address whether AMPK activation is detrimental or beneficial to the ischemic heart on reperfusion. Therefore, on the basis of this limitation of the study by Shimamura et al, it is still unknown whether short-term caloric restriction and subsequent activation of AMPK are beneficial in models of ischemia/reperfusion injury that include clinically relevant concentrations of fatty acids. That being said, the present study and the work by Shibata et al\textsuperscript{9} do suggest that activation of AMPK at least before an ischemic insult is cardioprotective. Although more work is needed to definitively prove this suggestion, if it turns out to be true, caloric restriction--induced elevations of serum adiponectin and subsequent activation of AMPK may indeed represent a novel method of ischemic preconditioning.

In summary, the beneficial effects of long-term caloric restriction on longevity have become an exciting area of research\textsuperscript{1} and have the potential for significant clinical importance. Unfortunately, long-term caloric restriction in humans requires considerable patient discipline and is not likely sustainable despite potential health benefits. Therefore, short-term caloric restriction may be a more realistic approach. If short-term caloric restriction is achievable, the study by Shimamura et al highlights its potential cardiovascular benefits, specifically suggesting that this strategy may precondition the heart to better withstand a more severe ischemic insult. Interestingly, the studies performed by Shimamura et al involved nonobese mice, suggesting that adiponectin-mediated signaling is not maximally stimulated even when serum adiponectin levels are within the normal range. As such, stimulating the adiponectin-AMPK signaling axis either by caloric restriction or by pharmaceutical agents may be an effective therapeutic strategy applicable to a broad spectrum of patients, especially those with progressive ischemic heart disease at high risk for developing acute myocardial infarction, even in the absence of obesity.

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None.

References


Key Words: Editorials | adiponectin | AMP-activated protein kinase | diet | ischemia | metabolism
Inflammation Ushers in Calcification
A Cycle of Damage and Protection?

Catherine M. Shanahan, PhD

The layman’s term “hardening of the arteries” is synonymous with vascular disease and describes the ubiquitous calcification of the intima and media of the vessel wall that occurs in atherosclerosis and aging. Vascular calcification is a powerful predictor of myocardial infarction, occurring in atherosclerosis and aging. Vascular calcification of the intima and media of the vessel wall that act as mineralization inhibitors. However, in response to these insults, VSMCs may die by apoptosis and release apoptotic vesicle release associated with the generation of the first nidus of calcification and VSMC osteogenic change are, and whether calcification is a purely physiological mineralization processes. In the normal vessel wall, VSMCs are protected from calcification by local and circulating proteins such as matrix GlA protein and fetuin-A that act as mineralization inhibitors. However, in response to these insults, VSMCs may die by apoptosis and release apoptotic bodies or may be induced by signals and mechanisms that are still not well defined to release matrix vesicles. These small membrane-bound microparticles have the capacity to concentrate calcium and phosphate to allow crystal nucleation and thus act as the first nidus for mineralization. Concomitant with vesicle release, although the precise order and relationship between these 2 events remain unclear, VSMCs undergo an osteo/chondrocytic phenotypic transition and begin to express transcription factors normally associated with differentiated chondrocytes and osteoblasts such as Sox 9, Cbfa1/Runx2, and osterix that regulate the expression of a cascade of mineralization regulating proteins such as alkaline phosphatase, osteopontin, and osteocalcin. These proteins act to regulate mineralization by altering the dynamics of crystal growth or by altering the availability of calcium and phosphate ions in the local environment. Key unanswered questions remain about what these initial damage-inducing agents might be, what the time course and order/sequence of events leading to the first initiation of calcification and VSMC osteogenic change are, and whether calcification is a purely pathological response or represents a protective adaptive response in VSMCs and/or the vasculature.

The study of Aikawa et al4 in this issue of Circulation goes part way to answering some of these key questions. Using multiple state-of-the-art imaging modalities, the authors have been able to describe the earliest events in the relationship between inflammation and calcification and have elegantly shown that macrophage infiltration and inflammation precede both the osteogenic conversion of VSMCs and the vesicle release associated with the generation of the first nidus of microcalcifications, providing evidence for the first time in vivo that, at least in atherosclerosis, inflammation is a potent initiator of calcification. Using apolipoprotein E knockout mice fed a high-fat diet as a model, Aikawa et al analyzed atherosclerotic lesions at a number of early and late time points with both in vivo imaging and histological and electron microscopy techniques. They showed that osteogenesis associates with inflammation in very early stages of atherosclerosis by visualizing OsteoSense750, a previously described bisphosphonate-based imaging agent that binds calcium and hydroxyapatite (HA) and can be excited at 750 nm.5 Modern imaging techniques were imaged in the same near-infrared fluorescent range using nanoparticles excitable at 680 nm. Both of these activities were found to be highly correlated; they evolved in close proximity, overlapped at border regions, and increased with plaque progression, suggesting an intimate relationship between them. Indeed, inflammation volume and calcification volume increased over time, concomitantly further confirming an interrelationship. Moreover, OsteoSense750 signals correlated with the osteogenic conversion of VSMCs measured in histological sections via alkaline phosphatase activity (an early marker of osteogenic activity) and with expression of other mineralization-regulating proteins such as Cbfa1/Runx 2 and osteocalcin. Finally, fluorescent-activated cell sorter analysis was used to confirm that the cells expressing the inducible osteogenic marker osteopontin were not monocytic in origin and therefore likely to be VSMCs. Additionally, what was highly informative was the use of electron microscopy to identify preclinical microcalcifications well before histological stains such as von Kossa could detect their presence. Importantly, electron microscopy showed that microcalcifications and HA nanocrystals colo-
calized with cholesterol crystals and were present in membrane-bound vesicles in the size range of apoptotic bodies and matrix vesicles. They concluded that infiltrating macrophages drawn to sites of lipid accumulation induce VSMC death and VSMC phenotypic transition to an osteogenic phenotype via cytokine release. This scenario was strengthened by their observations that macrophage-conditioned media could increase expression of alkaline phosphatase in VSMCs and concurs with previous in vitro studies demonstrating that lipids and inflammatory cytokines released by macrophages accelerate osteogenic differentiation and calcification.

The idea that inflammation was likely to be the initial event very early on in plaque development leading to calcification is supported by further experiments using the same model with the addition of statin treatment. Statins, via their capacity to inhibit inflammation, reduced the increase in osteogenic and inflammatory activities with plaque progression, and this correlated with a reduction in calcification. Thus, early treatment of inflammation can ameliorate the calcification response in the vessel wall. In addition to the animal experiments, Aikawa et al applied the same methods and technologies to human carotid endarterectomy specimens ex vivo. This analysis showed the same coassociations of inflammation with OsteoSense throughout the lesions. Although these analyses on human lesions did not allow an accurate time course as did the mouse studies, they lend strong support to the notion that the events in the mouse lesions are mirrored in human atherosclerosis in vivo.

An additional aspect of the study by Aikawa et al was the analysis of inflammation and calcification in advanced plaques in the same apolipoprotein E knockout mouse model. Here, the results were very different. In contrast to the early plaque calcifications, in aged mice with more advanced plaques, calcification was spatially distinct from macrophage accumulation. The lack of association between these 2 factors in advanced plaques suggests perhaps that calcification is initiated by factors other than inflammation as plaques progress. Alternatively, it may reflect the possibility that once calcification is established, it has the propensity to progress by physicochemical processes, enhanced by the absence of mineralization inhibitors, the expression of which may be compromised by loss of or damage to the VSMCs that produce them. Alternatively, it may represent a continuation of active osteogenic processes by VSMCs. Indeed, Aikawa et al showed that areas of inflammation also were decreased in advanced calcified plaques, suggesting an interrelationship between inflammation and calcification, but this time a negative one. Thus, we are left with the intriguing possibility that calcification can dampen inflammation and is protective in a manner perhaps analogous to the calcific muffling of infections that occurs in other soft tissues. Thus, by ensuring their own survival and using calcification to seal off inflammation, VSMCs may be prolonging the lifetime integrity of the vessel wall. So, does any evidence exist that calcification is an adaptive/protective response? And is it possible that calcified deposits can act directly on inflammation?

The rapid initiation of osteogenic gene expression patterns in VSMCs in response to insults is highly suggestive of an adaptive response aimed at regulating mineralization. The proteins upregulated by osteogenic VSMCs include matrix Gla protein (MGP) and other “bone”-specific proteins that dampen and control the mineralization process. In addition, matrix vesicle release from VSMCs was first described as a protective mechanism against calcium overload, able to rescue VSMCs from apoptotic cell death. Moreover, vesicles released by VSMCs are loaded with calcification inhibitors, including matrix Gla protein and fetuin-A, that act to minimize their capacity to initiate calcification and facilitate their rapid phagocytosis. Potentially, elevated extracellular calcium at sites of apoptosis induces VSMC vesicle release, and in the absence of inhibitors or phagocytosis, microcalcifications result, with these events preceding the osteogenic differentiation of VSMCs. Indeed, this may be a universal initiation mechanism for both medial and intimal calcification, and it would be interesting to determine whether the technologies used in the Aikawa et al study could be adapted to investigate this idea in animal models of medial calcification.

However, other evidence argues against the notion of calcification as protective. Recent in vitro studies have shown that microcalcifications induce a proinflammatory response in macrophages, leading to a vicious cycle of macrophage infiltration, matrix breakdown, VSMC apoptosis, and plaque rupture. Interestingly, it was found that HA crystals of <2 μm induced the most proinflammatory response in macrophages, with larger particles being inert. However, these studies used naked HA, whereas in the vessel wall, HA is complexed with a number of protein components that may block inflammatory responses. Moreover, in the Aikawa et al study, calcification also colocalized with cathepsin K, an enzyme involved in bone reabsorption, suggesting that macrophages may respond differentially to HA in the vessel wall. Indeed, medial calcification occurs in the absence of macrophage infiltration, suggesting that in vivo not all calcification is proinflammatory and that this is an area that requires further investigation of the effects of calcified deposits on not only macrophages but also VSMCs.

Finally, clinical studies clearly show that not all calcifications in vivo are the same. Intravascular ultrasound has shown that spotty calcification within a plaque is more predictive of a cardiovascular event than plate-like heavy calcification. Using the knowledge obtained from the Aikawa et al study, it seems likely that these “spotty” areas represent inflammatory lesions and therefore are more likely to rupture. Plaques with advanced calcification are potentially more benign because they are no longer inflammatory. Because atherosclerosis is a progressive inflammatory disease, it is clear that as long as inflammatory stimuli are present, there will be continuous cycles of inflammation leading to macrophage infiltration and microcalcifications. If each inflammatory region is contained, potentially by macrocalcification, and the VSMC repair system is not overwhelmed, over a lifetime, this process will lead to calcified fibrous acellular plaques. If, however, inflammation is sustained, the plaque may become heavily calcified but will still be prone to rupture if an ongoing
inflammatory process is present with associated loss of VSMCs (the Figure). Although this hypothesis is controversial, these plaques also may rupture as a result of debonding around microcalcifications or may be susceptible to increased hemodynamic stresses and therefore prone to mechanical rupture.\textsuperscript{18,19} We should also remember that, within an individual, plaques are heterogeneous, exhibiting a continuum of inflammation and calcification, with any inflamed plaque, calcified or not, vulnerable to rupture.\textsuperscript{20}

In summary, the Aikawa et al study has provided important insights into the earliest cell biological mechanisms of atherosclerotic calcification in vivo and highlighted key issues that remain controversial in the field of atherosclerosis and calcification. If these technologies could be modified for use in the clinic, inflammation and microcalcification may eventually become the keys to identifying the vulnerable patient and the vulnerable plaque.

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**Disclosures**

None.

**References**


**Key Words:** Editorials ■ atherosclerosis ■ calcium ■ imaging ■ inflammation
Can Common-Type Atrial Flutter Be a Sign of an Arrhythmogenic Substrate in Paroxysmal Atrial Fibrillation?

Clinical and Ablative Consequences in Patients With Coexistent Paroxysmal Atrial Fibrillation/Atrial Flutter

Wendel Moreira, MD; Carl Timmermans, MD, PhD; Hein J.J. Wellens, MD, PhD; Yuka Mizusawa, MD; Suzanne Philippens, RN; David Perez, MD; Luz-Maria Rodriguez, MD, PhD

Background—The coexistence of atrial fibrillation (AF) and atrial flutter (AFL) is well recognized. AF precedes the onset of AFL in almost all instances. We evaluated the effect of 2 ablation strategies in patients with paroxysmal AF (PAF) and AFL.

Methods and Results—Ninety-eight patients with PAF/AFL were prospectively recruited to undergo pulmonary vein cryoisolation (PVI). Those with at least 1 episode of sustained common-type AFL were assigned to cavotricuspid isthmus cryoablation followed by a 6-week monitoring period and a subsequent PVI (n = 36; group I). Patients with PAF only underwent PVI (n = 62; group II). The study included 76 men with a mean age of 50 ± 10 years. Most patients (76 [78%]) had no structural heart disease. When the 2 groups were compared, residual AF after a blanking period of 3 months after PVI occurred in 24 patients (67%) in group I versus 7 (11%) in group II (P < 0.05).

Conclusions—In patients with PAF and no documented common-type AFL, PVI alone prevented the occurrence of AF in 82%, whereas in patients with AFL/PAF, cavotricuspid isthmus cryoablation and PVI were used successfully to treat sustained common-type AFL but appeared to be insufficient to prevent recurrences of AF. In this population, AFL can be a sign that non–pulmonary vein triggers are the culprit behind AF or that sufficient electrical remodeling has already occurred in both atria, and thus a strategy that includes substrate modification may be required. (Circulation. 2007;116:2786-2792.)

Key Words: atrial fibrillation ■ atrial flutter ■ electrophysiology ■ pulmonary veins ■ catheter ablation

It is known that for the macroreentry circuit of common-type atrial flutter (AFL) to occur, a line of block between the venae cavae needs to be present along with other fixed anatomic lateral boundaries (the crista terminalis and the tricuspid annulus).1–3 The former is almost always functional and usually develops during an initial period of transient atrial fibrillation (AF; Figure 1).4 Actually, Waldo suggests that in most instances, without preceding AF, there will be no classic AFL.5 Percutaneous ablative techniques for the treatment of AF can be performed with high success rates.5–10 Because of the likely role of AF as the initiator of AFL, we were interested in the best ablation approach for patients with paroxysmal atrial fibrillation (PAF) and AFL. Two cryoablation protocols in patients with PAF/AFL were evaluated prospectively in the present study with regard to the outcome of those arrhythmias.

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Methods

Ninety-eight patients with drug-refractory symptomatic PAF who were deemed to be candidates for pulmonary vein cryoisolation (PVI) were enrolled prospectively from July 2001 to July 2006. All subjects were given a transtelephonic monitoring (TTM) device and instructed to use it daily (preferably at the same time) and whenever they had symptoms. This monitoring began 30 days before PVI and continued to day 180 after PVI. From then on, a Holter monitor was used during clinic visits (1, 3, 6, 9, and 12 months) or when patients had symptoms.

On the basis of the data provided by the clinical history, cardiac status, Holter monitoring, and TTMs, patients were assigned to 2 ablation strategies. If at least 1 episode of sustained common-type AFL was documented, patients underwent cavotricuspid isthmus (CTI) cryoablation, followed by PVI after a 6-week monitoring
Each cryoapplication lasted 3 minutes with a constant target temperature of \(-90^\circ C\). After bidirectional isthmus conduction block was achieved, short-term success was defined if it persisted for 30 minutes after the last application without and with isoproterenol infusion (1 to 3 \(\mu g/min\)). Oral anticoagulation therapy was continued for all patients, and patients returned 6 weeks later for their PVI.

During this period, daily TTMs were provided to assess arrhythmia burden compared with the preablation TTM data.

The PVI cryoablation protocol has been described previously. Briefly, during the procedure (but after the transseptal punctures), intravenous heparin was given as a 100-IU/kg bolus dose, followed by boluses of 5000 IU every 1.5 hours if needed to maintain an activated clotting time \(\geq 300\) seconds. A decapolar catheter was positioned in the distal coronary sinus and a quadripolar catheter in the His bundle region via the femoral route. Double transseptal catheterization was performed under fluoroscopic and transesophageal guidance.

Left atrial angiography (to visualize the pulmonary vein [PV] ostia) was performed after adenosine administration. A deflectable, circumferential decapolar mapping catheter (LASSO, Biosense-Webster) was advanced into the left atrium and positioned at the ostium of each PV. A deflectable 10.5F cryoablation catheter (CryoCor Inc) with a 6.5-mm-tip electrode was inserted into the left atrium through a 12F, 65-cm-long sheath (DAIG, St Jude Medical Inc, St Paul, Minn, or Cook Inc, Bloomington, Ind), and a linear lesion was created by use of a point-by-point technique with gradual pullback of the cryocatheter in a ventricular atrial fashion. The first application was delivered at the ventricular insertion of the CTI. Each cryoapplication lasted 3 minutes with a constant target temperature of \(-90^\circ C\). After bidirectional isthmus conduction block was achieved, short-term success was defined if it persisted for 30 minutes after the last application without and with isoproterenol infusion (1 to 3 \(\mu g/min\)). Oral anticoagulation therapy was continued for all patients, and patients returned 6 weeks later for their PVI.

**Electrophysiological Study and Ablation**

All patients were studied in the fasting state without sedation. Those presenting in AF while in the catheterization room were converted to sinus rhythm by internal or external cardioversion.

Group I underwent CTI cryoablation first. The following protocol was used: intravenous heparin was given as a 100-IU/kg bolus dose after the venous sheaths were inserted; via the femoral route, a decapolar catheter was positioned in the distal coronary sinus (for evaluation of left atrial activation), a duodecapolar catheter (2-mm interelectrode spacing, Halo catheter, Biosense Webster, Baldwin Park, Calif) was placed to map the right atrial lateral wall, and a quadripolar catheter was inserted in the His bundle region. An additional deflectable 10.5F cryoablation catheter (CryoCor Inc, San Diego, Calif) with a 6.5-mm-tip electrode was inserted into the right atrium through a 12F, 65-cm-long sheath (DAIG, St Jude Medical Inc, St Paul, Minn, or Cook Inc, Bloomington, Ind), and a linear lesion was created by use of a point-by-point technique with gradual pullback of the cryocatheter in a ventricular atrial fashion. The first application was delivered at the ventricular insertion of the CTI. Each cryoapplication lasted 3 minutes with a constant target temperature of \(-90^\circ C\). After bidirectional isthmus conduction block was achieved, short-term success was defined if it persisted for 30 minutes after the last application without and with isoproterenol infusion (1 to 3 \(\mu g/min\)). Oral anticoagulation therapy was continued for all patients, and patients returned 6 weeks later for their PVI.

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Segmental isolation of PVs, guided by the recording of their potentials with the LASSO catheter, was performed with the CryoCor cryoablation system as described previously. Efforts were made to identify the arrhythmogenic PV (culprit PV) by use of adenosine (24 to 40 mg) or isoproterenol (1 to 5 \(\mu g\)). If the culprit PVs were not identified, all veins with potentials recorded at their ostium were targeted for ablation. Isolation of the PV was performed during sinus rhythm or coronary sinus pacing by the delivery of cryoablation at ostial sites that had the earliest bipolar potential.

At each effective target site, defined by the abolishment of a PV potential or a change in the PV potential activation sequence during cryothermal application, 3 minutes of cryoablation was delivered. If no changes in the electrogram were observed after 20 seconds despite a catheter tip temperature of \(-90^\circ C\), the application was stopped, and the catheter was repositioned. The early procedural end point was complete electrical isolation of PVs based on abolition of all ostial PV potentials or complete entrance conduction block into the PV.
Postablation Management

Every patient was monitored in the hospital for 24 hours, and oral anticoagulation therapy was started the day of the ablation. The same AADs were continued for at least 3 months after the procedure. After 3 months, the need for long-term anticoagulation was assessed by the number of recurrences of AFL/PAF and the presence of risk factors for thromboembolic events.

All patients had a Holter recording at hospital discharge and during each clinic visit (1, 3, 6, 9, and 12 months) or earlier if they had symptoms. They were also instructed to keep a diary of events associated with their TTMs. A blanking period of 3 months (starting after the PVI procedure) was used before recurrences were assessed.

We adopted the following definitions according to the latest American College of Cardiology/American Heart Association/European Society of Cardiology guidelines for the management of patients with AF15 and/or the guidelines for supraventricular arrhythmias16: (1) PAF was defined as self-terminating episodes of AF that lasted >30 seconds and up to 7 days (usually <24 hours); (2) common-type AFL was defined as organized atrial rhythm with a rate typically between 250 and 350 bpm in which the macroreentry circuit was dependent on the CTI in either a counterclockwise or clockwise fashion.

Statistical Analysis

Continuous variables are presented as mean±SD where appropriate. In cases of a nongaussian distribution, medians and quartiles are given. Categorical variables are expressed as numbers and percentages of patients.

Statistical analysis was performed with the Student t test for unpaired data. The χ² test was used to compare categorical variables. The McNemar test was applied to evaluate the differences in medication use from preablation to post-PVI. A Kaplan–Meier analysis was used to estimate the probability of freedom from medication use from preablation to post-PVI. A Kaplan–Meier analysis was used to estimate the probability of freedom from PAF recurrences and compared between them with the Wilcoxon and log-rank tests. All values were considered significant at P<0.05.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agreed to the manuscript as written.

Results

The study cohort consisted of 98 patients considered for segmental PVI after being appropriately screened by both the referring cardiologist and our group. Review of Holter recordings, TTMs, failure of AADs, functional capacity, type of AF, and imaging studies were the main criteria used to recruit patients. In patients with a prior history of coronary artery disease, myocardial perfusion studies were performed to exclude active ischemia.

The characteristics of the patients are shown in Table 1. The total number of patients was 98, with a mean age of 50±10 years (range 21 to 69 years). The majority of patients (n=76; 78%) had no structural heart disease. Of the 22 patients with structural heart disease, 6 had a history of coronary artery disease, and 16 had a history of arterial hypertension, all well-controlled at the time of ablation.

As previously mentioned, patients in group I first underwent CTI cryoablation and then, 6 weeks later, PVI. During this period, daily TTMs were compared with those before CTI cryoablation, and the average number of AF episodes per patient (1±1 registered daily AF episode) did not change. The long-term outcome of this group was as follows: most AFL recurrences occurred in the first 6 months, which confirmed our prior data,17 and 5 patients underwent a second successful CTI cryoablation. Two of those AFL patients continued to have symptomatic AF episodes; therefore, a second PVI was performed. On the other hand, 24 patients had recurrences of PAF. In the majority of these (17 patients), the arrhythmia was well-controlled with an AAD. Six patients underwent a second successful PVI, and the remaining patient opted to undergo the Maze operation (Figure 2).

With regard to the long-term outcome of group II, 7 patients had PAF recurrences. All of these patients underwent a second PVI. Five patients were asymptomatic after the second procedure and did not require AADs. The remaining 2 patients were given an AAD. Interestingly, 5 of the 62 patients developed new-onset common-type AFL (along with sporadic PAF episodes) as the predominant arrhythmia, each of whom underwent a successful CTI ablation. Despite the CTI ablation procedure, all 5 patients continued to experience PAF. Their PAF was controlled with a repeat PVI (2 patients)

### Table 1. Characteristics of Patients With PAF Referred for PVI

<table>
<thead>
<tr>
<th></th>
<th>Group I: 36 Patients (37%) With CTI Ablation and PVI</th>
<th>Group II: 62 Patients (63%) With PVI Alone</th>
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<td>Age, mean±range, y</td>
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<td>51±10</td>
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<tr>
<td>Women</td>
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<td>16 (26)</td>
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<tr>
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<td>12 (19)</td>
<td>...</td>
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<td>4 (7)</td>
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<td>Recurrent AFL, n</td>
<td>5</td>
<td>...</td>
<td>NA</td>
</tr>
<tr>
<td>New AFL, n</td>
<td>...</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>Follow-up, mean±range, mo</td>
<td>23±20</td>
<td>27±16</td>
<td>NS</td>
</tr>
</tbody>
</table>

AHT indicates arterial hypertension; CAD, coronary artery disease; LA, left atrium; LVEF, left ventricular ejection fraction; NA, not applicable; NS, not significant; and SHD, structural heart disease.

Numbers represent number of patients (%) unless otherwise specified.
or an AAD (1 patient), and 2 patients had asymptomatic PAF and were not given an AAD (Figure 3). No differences existed in procedural characteristics with regard to PVI between group I (procedure time 337 minutes and fluoroscopy time 90 minutes) and group II (procedure time 331 minutes and fluoroscopy time 88 minutes; \( P = H11005 \) NS).

No significant differences were found when we compared the characteristics of the 2 groups (Table 1). The only 2 factors that reached statistical significance were residual PAF after PVI (24 [67%] versus 7 [11%] patients from groups I and II, respectively; \( P = H11021 \) 0.05) and the number of patients who required an AAD after the 3-month blanking period after PVI (17 patients [47%] from group I and 5 [8%] from group II, \( P = H11021 \) 0.05; Table 2).

The results of patients who underwent PVI taken as a whole are as follows: 67 patients (68%) were free of arrhythmia after the index procedure, and 13 (13%) were free of arrhythmia after a second PVI, which yielded an overall freedom from PAF (and AAD) of 82%. Seventeen patients (17%) had significant improvement with AAD and refused a second procedure.

The percentage of freedom from PAF in both groups is shown in Figure 4. After a mean follow-up of 26±17 months, no complications related to the procedure occurred, and no evidence existed of atypical (including left-sided) AFL.

**Discussion**

**Main Findings**

To the best of our knowledge, this is the first study analyzing a population with coexistent PAF/AFL, treated with 2 different strategies, to demonstrate that a previous history of AFL is a bad sign with regard to AF recurrences after catheter ablation. Additionally, the present study demonstrated that a prophylactic ablation of the CTI should not be included in the treatment of patients with PAF and no documented common-type AFL. Therefore, a careful screening of the index arrhythmia(s) (eg, PAF only or PAF/AFL) will enable the correct ablation strategy to be chosen and an optimal long-term outcome to be attained. The success rate in group II (PAF only) was 89%, whereas in group I (PAF/AFL), it was only 33%.

Those numbers were acquired by means of a follow-up in which daily TTMs and the common methods used to assess recurrences (patient’s symptoms with Holter and 12-lead ECG monitoring being used only during clinic visits) were utilized. Although TTMs represent only a fraction of the day, they still can be used to estimate the incidence of asymptomatic episodes; however, it is known that patients’ symptoms after AF ablation do not correspond to their actual arrhythmia burden (as demonstrated by Hindricks and colleagues\(^{18}\)), and continuous rhythm registration would be the ideal method for estimation of the success of the ablative procedure.

**Is Common-Type AFL a Sign of an Arrhythmogenic Substrate in PAF?**

Current advances in percutaneous ablative therapy for the treatment of AF have helped us understand more completely the importance of triggers and substrate in the pathophysiology of this arrhythmia. Segmental PV isolation, performed in a selected group of patients, has better success rates than use of AADs.\(^{19,20}\)

The association of AF and AFL is known, and for the macroreentry circuit of CTI-dependent AFL to occur, an intercaval line of block (in addition to the other anatomic barriers, the crista terminalis and the tricuspid annulus) is required.\(^{1-4}\) Waldo et al\(^{4}\) advocate that a functional line of block between the venae cavae during AF is critical for the pathogenesis of classic AFL. If this functional component of
the AFL circuit does not develop, AF will either persist or spontaneously convert back to sinus rhythm. In those patients in whom AF does not appear to precede the onset of AFL, a very high degree of block or even complete block between the venae cavae may already be present. If we extrapolate those findings to the present study population, one could say that patients from group I had a more critical substrate (intercaval functional line of block) for the development of sustained common-type AFL than did patients from group II.

Another important observation is that although ablation of the CTI permanently interrupted AFL in 86% of patients, residual AF was still present in 67%. That could be the result of non-PV sites acting as triggers (most of which were found in the right atrium) or conditions (eg, right atrial dilatation, electrical remodeling, and fibrosis) that may act as substrate together being responsible for the initiation and maintenance of AF in group I.

The ability to develop the intercaval functional line of block (essential in common-type AFL) could represent a stage in which electrical remodeling of atrial tissue (both right and left) is already important. In such a situation, elimination of the triggers in the PVs alone would not be enough to prevent AF. Any other premature beat could be sufficient to initiate AF, which thereafter perpetuates in the remodeled substrate. Electrical remodeling is known to occur before mechanical remodeling in AF. The latter could be present in those patients from the relatively healthy population in the present study (minimal or no structural heart disease, normal left atrial size, and PAF) who had the worst outcomes. AFL would then be a sign of right-sided triggers and/or advanced electrical remodeling (of both atria) in patients with PAF who also have a history of common-type sustained AFL. In this population, it is tempting to speculate that in substrate modification could potentially lead to better results.

The fact that patients from group II in the present study had only episodes of PAF indicates that no functional line of block was present (or if present, that it was a much shorter line of block). This could represent the absence of a substrate responsible for common-type AFL. Isolation of the PVs (triggers) provided freedom from AF in 89% of those patients during follow-up. These results might indicate that in those patients, prophylactic CTI is not necessary and therefore should not be included as an additional ablative step.

Can the Successful Elimination of AF Prevent the Development of AFL?

Wazni et al evaluated the effect of PVI/left atrial junction radiofrequency ablation in patients with AF/AFL. They showed that after a blanking period of 8 weeks, PVI/left atrial junction ablation alone (without concomitant CTI ablation) decreased the occurrence of not only AF but also AFL. Even though the present study was not designed to evaluate this specific issue, some of our findings are consistent with their results. During an observational period of >2 years, only 5 of 62 patients in the present study (all of whom had associated AF) developed new-onset common-type AFL. This reinforces the theory that AF is needed for the initiation of AFL and that elimination of the initiating arrhythmia (AF) prevents the appearance of common-type AFL. Further investigation of this concept is needed.

Class IC Drugs Versus Ablation for AF/AFL

Before the introduction of PV isolation as a treatment strategy for AF, class IC drugs were reported to be effective in...
converting AF to AFL, which could then be treated by CTI ablation. The proposed mechanism was the rate-dependent prolongation of the atrial refractory period by class IC agents, with an increase in the wavelength, thus facilitating the conversion of AF (multiple smaller reentry circuits) to AFL (a single macroreentrant loop). AADs could also have electrophysiological effects in the intercalar region, creating the needed line of block for common-type AFL. Also, in those studies, a great number of patients had improvement only in their symptoms (while still having bouts of AF), with a rate of conversion to sinus rhythm much lower than that achieved by present-day percutaneous ablation. The inefficiency of AADs in AF was also confirmed in the AFFIRM trial (Atrial Fibrillation Follow-up Investigation of Rhythm Management), with some suggestions of increased mortality.

Therefore, ablation of AF appears to be a better solution than the use of AADs to convert AF into AFL.

Study Limitations

The daily TTM recorded only a fraction of each day and did not represent a 24-hour period; episodes of asymptomatic arrhythmias could have been missed. In addition, the classification of AFL versus AF during follow-up event recordings and 24-hour Holter recordings was not done blindly, which could potentially be a source of bias. Even though the diagnosis of CTI-dependent flutter cannot be made with certainty from the event recorder or Holter tracings, we confirmed the diagnosis in all 10 patients with AFL after PVI by endocardial recordings during the subsequent electrophysiological study in which CTI ablation was performed.

Assessment of atrial anatomy was done with transthoracic echocardiography, which has known limitations for accurate evaluation of the right-sided structures. MRI could give a more precise estimation of right atrial morphology. Furthermore, some patients were taking AADs to control AF during the postablation period. We cannot rule out that AADs were the cause of the transformation of AF into AFL; however, the type of AADs and the percentage of patients taking them in the preablation period were comparable in both groups (Table 2). Thus, this possibility would be pertinent only to the AFLs observed in the postablation period (10 patients). Thus, this possibility would be pertinent only to the AFLs observed in the postablation period (10 patients).

Conclusions

We demonstrated that in patients with PAF and no documented common-type AFL, PVI alone prevents the recurrence of AF in 82%. Therefore, preventive CTI ablation should not be included in the strategy for treatment of PAF. Furthermore, in patients with coexisting PAF/AFL, CTI ablation and PVI were used successfully to treat sustained common-type AFL but appeared insufficient to prevent recurrences of AF. In this population, AFL can be a sign that non-PV triggers (including triggers in the right atrium) are the culprit behind AF or that sufficient electrical remodeling has occurred in both atria, and an ablative strategy that includes substrate modification is already required. Future work is needed to determine whether the combination of PAF/AFL could be a marker of early progression to persistent or permanent AF.

Disclosures

Drs Rodríguez and Timmermans have received research grants from CryoCor. Dr Wellens is a member of the Advisory Board of CryoCor. The remaining authors report no conflicts.

References

The relation between the common type of atrial flutter (AFL) and atrial fibrillation (AF) was described long ago. Patients with AFL have a tendency to develop AF. On the other hand, AF is often the event that triggers the macroreentry circuit of AFL. The present study prospectively evaluated the best catheter ablation strategy in patients having both AFL and AF. Further evaluation of these mechanisms and their proper treatment is required.

CLINICAL PERSPECTIVE

The relation between the common type of atrial flutter (AFL) and atrial fibrillation (AF) was described long ago. Patients with AFL have a tendency to develop AF. On the other hand, AF is often the event that triggers the macroreentry circuit of AFL. The present study prospectively evaluated the best catheter ablation strategy in patients having both paroxysmal AF and AFL or paroxysmal AF only with regard to outcome. If at least 1 episode of sustained AFL was documented, patients underwent cavotricuspid isthmus cryoablation, followed by pulmonary vein isolation after a 6-week monitoring period (group I). In patients in whom only paroxysmal AF was documented, only pulmonary vein isolation was performed (group II). During a follow-up of 26 ± 17 months, we found that pulmonary vein isolation only was highly successful in patients from group II (82% success rate), whereas in group I, the combination of cavotricuspid isthmus cryoablation and pulmonary vein isolation successfully treated AFL but was frequently insufficient to prevent recurrences of AF (recurrence rate of 67%). The latter finding suggests the following: (1) AFL can be a sign that non–pulmonary vein triggers are the culprit behind AF, and an electrophysiological evaluation of right atrial properties (eg, ectopic impulse formation and electrogram analysis) is required for an effective AF ablation, which would also involve the right atrium; and (2) sufficient electrical remodeling has already occurred in both atria, and thus, a strategy that includes bialtral substrate modification may be necessary for successful percutaneous ablative treatment of patients having both AF and AFL. Further evaluation of these mechanisms and their proper treatment is required.
Does Comorbidity Account for the Excess Mortality in Patients With Major Bleeding in Acute Myocardial Infarction?

Frederick A. Spencer, MD; Mauro Moscucci, MD; Christopher B. Granger, MD; Joel M. Gore, MD; Robert J. Goldberg, PhD; Philippe Gabriel Steg, MD; Shaun G. Goodman, MD; Andrzej Budaj, MD, PhD; Gordon FitzGerald, PhD; Keith A.A. Fox, MB, ChB, FRCP; for the GRACE Investigators

Background—Analyses from randomized controlled trials suggest that bleeding in patients with acute myocardial infarction is associated with poor outcomes. Because these data are not generalizable to all patients with acute myocardial infarction, we sought to better understand the scope of this problem in a “real-world” setting.

Methods and Results—We examined the frequency of major bleeding in 40,087 patients with acute myocardial infarction enrolled in the Global Registry of Acute Coronary Events. Regression analyses were used to examine the association between patient and treatment characteristics, bleeding, and hospital and postdischarge outcomes. Major bleeding occurred in 2.8% of patients. These patients were older, more severely ill, and more likely to undergo invasive procedures. Patients with bleeding were more likely to die during hospitalization (hazard ratio, 1.9; 95% confidence interval, 1.6 to 2.2) but not after discharge (hazard ratio, 0.8; 95% confidence interval, 0.6 to 1.0) than patients who did not bleed. Continuation of antithrombotic therapies after day 1 was lower in patients who experienced early bleeding. Moreover, in patients who bled, hospital mortality was increased in those who discontinued aspirin, thienopyridines, or low–molecular-weight heparins.

Conclusions—Major bleeding occurred in 1 in 35 patients with acute myocardial infarction; these patients accounted for ≈10% of all hospital deaths. Nevertheless, risk of hospital mortality associated with bleeding was much lower than reported in randomized controlled trials. These data suggest that although bleeding may be causally related to adverse outcomes in some patients in the real-world setting, it is often merely a marker for patients at higher risk for adverse outcomes. (Circulation. 2007;116:2793-2801.)

Key Words: death ▪ hemorrhage ▪ mortality ▪ myocardial infarction

Increased understanding of the essential, overlapping, and synergistic roles of platelet activation and thrombin generation/activity in patients with acute myocardial infarction (AMI) has led to the development of increasingly effective antiplatelet and thrombin-inhibition therapies. Percutaneous intervention, often performed in combination with 3, 4, or even 5 of these antithrombotic therapies, is being increasingly used in the management of patients with AMI. These advances have led to impressive improvements in our ability to achieve early and sustained coronary patency. However, the impact of these more aggressive treatment approaches on bleeding complications and hospital and long-term outcomes is not clear. Recent analyses from randomized controlled trials suggest that bleeding after treatment for AMI is associated with significantly worse overall outcomes.1,2 Because randomized controlled trial data are not necessarily generalizable to all patients with AMI,3 the objective of our large observational study was to better understand the magnitude and scope of this problem as it exists in the community setting.

Editorial p 2776
Clinical Perspective p 2801
Using data from a large multinational registry of patients with acute coronary syndromes (ACS), we report the frequency of major bleeding in patients with AMI, examine the relationship between major bleeding and antithrombotic therapy (with or without invasive procedures), and explore the
association between major bleeding and adverse patient outcomes. Finally, because patients who suffer major bleeding are likely to have antithrombotic therapies discontinued, we hypothesized that the cessation of these therapies may partially explain any observed association between major bleeding and excess mortality. We begin to explore this hypothesis using data from a subset of patients who suffered a major bleeding episode within the first 24 hours of hospital admission.

**Methods**

Full details of the Global Registry of Acute Coronary Events (GRACE) rationale and methodology have been published and are outlined below.4–6

**Site Selection**

GRACE is designed to reflect an unbiased and generalizable sample of patients with ACS. A total of 114 hospitals located in 14 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, France, Germany, Italy, New Zealand, Poland, Spain, United Kingdom, and United States) across 4 continents have contributed data to this observational study.

Patient enrollment at each study hospital is intended to reflect an unbiased sample of admissions for ACS that is independent of the annual volume of ACS patients seen at each of the participating hospitals. Individual hospital enrollment targets have been uniformly established as the first 10 qualifying cases of ACS discharged each month. Regular audits are performed at all participating hospitals.

**Patient Population**

Patients entered in the registry had to be ≥18 years of age, be admitted for ACS as a presumptive diagnosis, and have at least one of the following: ECG changes consistent with ACS, serial increases in biochemical markers of cardiac necrosis, and/or documentation of coronary artery disease.5–6 The qualifying ACS must not have been precipitated by trauma or surgery. For purposes of the present study, patients with unstable angina but no increase in cardiac markers or those who were subsequently found to have non-ACS primary diagnoses and patients transferred into or out of GRACE registry hospitals were excluded from further consideration. When required, study investigators received approval from their hospital ethics or institutional review board, and a signed consent form for follow-up contact was obtained.

**Data Collection**

Data were collected at each site by a trained coordinator using a standardized case report form. Demographic and clinical characteristics, treatment practices, and hospital outcome data, including information on occurrence and timing of major bleeding, were collected. Standardized definitions of all patient-related variables, clinical diagnoses, selected hospital complications, and outcomes were used. Medications and procedures received in hospital were categorized into those received during the “first 24 hours of admission” or those received “anytime during hospitalization.” Major in-hospital bleeding was defined as life-threatening bleeding requiring a transfusion of ≥2 U of packed red blood cells, resulting in a decrease in hematocrit of ≥10%, occurring intracerebrally, or resulting in stroke or death. Date and site of each episode of major bleeding were recorded. Bleeding that occurred after coronary artery bypass graft surgery was not included in our analyses.

**Data Analysis**

Differences in the demographic and clinical characteristics and treatment practices of study patients in relation to occurrence of major bleeding were analyzed with χ² tests for discrete variables. The Wilcoxon rank-sum test was used to analyze differences between respective comparison groups for continuous variables. Differences in clinical presentation (ST-segment elevation versus non–ST-segment elevation) and subsequent diagnosis (ST-segment elevation myocardial infarction [STEMI] versus non-STEMI) in relation to major bleeding were analyzed with χ² tests. Fisher’s exact test was used to compare mortality between patients with bleeding who stopped versus those who continued anticoagulant use. The Kaplan–Meier method was used to create survival curves for patients with and without in-hospital bleeding. Major bleeding was treated as a time-varying covariate (see below).

Cox proportional-hazards regression analyses were used to examine the association between patient demographic, clinical, and treatment characteristics and the occurrence of major bleeding. Because our initial case report form (1999) did not include information on the timing of glycoprotein IIb/IIIa inhibitors, we excluded these patients (n=5595) from our analyses. Of the remaining 34 492 patients, we had complete covariate data on 28 327 (81%), who comprised the final cohort for our bleeding models.

Candidate variables for inclusion in regression models included the demographic, clinical, and treatment characteristics listed in Table 1. Only procedures (eg, cardiac catheterization, percutaneous coronary intervention) occurring before major bleeding were considered for these analyses. These procedures and fibrinolytic therapy, for which timing was available, were modeled as time-varying covariates. Similarly, only medical therapies administered within the first 24 hours of hospitalization were included in these analyses. Candidate variables possibly associated with in-hospital bleeding (P<0.25 after univariate analysis) were included in the multivariable models. Variables with values of P>0.05 were eliminated in a backward fashion so that only variables with a statistically significant association with the outcome of interest were included in the final regression models. The assumption of proportional hazards was tested for each covariate by a covariate-by-time interaction. Recognizing that different demographic, clinical, and treatment factors may be associated with “early” bleeding (defined as occurring in days 0 to 1 or days 0 to 2) versus “late” bleeding (defined as occurring in days 2 to 30 or days 3 to 30), we reported early and late differential hazards if statistically significant (P<0.05) using SAS programming statements (SAS Institute, Cary, NC).7

Using similar methods, we also performed multiple Cox regression analyses to test whether hospital bleeding was related to hospital and postdischarge mortality. These analyses were performed in the cohort of patients with complete covariate data (n=32 240). In these analyses, bleeding was treated as a time-varying covariate, as was hospital stay, as follows: Let X(t) represent bleeding by day t (day t extends from admission day to 180 days after admission), where X(t)=0 if the patient never bled or bled after day t and X(t)=1 if the patient bled on or before day t. Let L(t) represent whether day t occurs in hospital or after discharge, where L(t)=0 if day t is after discharge (≥length of stay in hospital) and L(t)=1 if day t is before discharge. The bleeding hazard ratio for in-hospital mortality is then B1X(t)+B2X(t)×L(t) and B1X(t) for postdischarge mortality, where B1 is the estimate for bleeding by day t and B2 is the estimate for bleeding-by-hospital-stay interaction.

These analyses were conducted in the overall study sample and in patients who did or did not receive fibrinolytic therapy. Multiple Cox regression also was used to assess the adjusted relation between in-hospital bleeding and subsequent complications of recurrent myocardial infarction and stroke during hospitalization.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

The study population consisted of 40 087 men and women with AMI (53% STEMI, 47% NON-STEMI) enrolled in GRACE from April 1999 to March 2007. The median length of stay was 6 days (interquartile range, 3 to 10 days). Of these, 1140 patients (2.8%) experienced a major bleeding episode during hospitalization for AMI. The location of major bleeding was at the site of vascular access in 29% of patients,
Table 1. Admission Characteristics of Patients With and Without In-Hospital Bleeding

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>No Bleeding (n=30 947)</th>
<th>Bleeding in Hospital (0–30 d) (n=1140)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), y</td>
<td>66 (56–76)</td>
<td>74 (64–81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77 (67–88)</td>
<td>73 (62–84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27 (24–30)</td>
<td>26 (23–30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women, %</td>
<td>31</td>
<td>47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medical history, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>7.2</td>
<td>11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bleeding</td>
<td>1.1</td>
<td>3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>10</td>
<td>16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary artery bypass surgery</td>
<td>9.4</td>
<td>11</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>24</td>
<td>29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>44</td>
<td>42</td>
<td>0.29</td>
</tr>
<tr>
<td>Hypertension</td>
<td>58</td>
<td>65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Major surgery within previous 2 wk</td>
<td>4.2</td>
<td>4.2</td>
<td>0.88</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>25</td>
<td>28</td>
<td>0.06</td>
</tr>
<tr>
<td>Percutaneous coronary intervention</td>
<td>13</td>
<td>13</td>
<td>0.56</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>8.9</td>
<td>16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>7.7</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoker</td>
<td>59</td>
<td>53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TIA/stroke</td>
<td>8.4</td>
<td>10</td>
<td>0.02</td>
</tr>
<tr>
<td>Presentation characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, median (IQR), mm Hg</td>
<td>140 (120–160)</td>
<td>135 (111–160)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP, median (IQR), mm Hg</td>
<td>80 (70–90)</td>
<td>77 (63–90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse, bpm</td>
<td>79 (66–92)</td>
<td>83 (70–100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Killip class, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>81</td>
<td>69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II</td>
<td>13</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4.5</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1.2</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Cardiac arrest, %</td>
<td>2.3</td>
<td>4.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>91 (80–113)</td>
<td>100 (80–135)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFR (MDRD), mL/min</td>
<td>71 (55–86)</td>
<td>58 (41–76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Therapies, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-term aspirin</td>
<td>32</td>
<td>35</td>
<td>0.03</td>
</tr>
<tr>
<td>In hospital first 24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>90</td>
<td>83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>47</td>
<td>38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>73</td>
<td>62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium antagonist</td>
<td>12</td>
<td>14</td>
<td>0.11</td>
</tr>
<tr>
<td>Diuretic</td>
<td>26</td>
<td>41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GP IIb/IIIa inhibitor</td>
<td>24</td>
<td>34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IV inotropic agents</td>
<td>6.1</td>
<td>19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low–molecular-weight heparin</td>
<td>46</td>
<td>34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thienopyridine</td>
<td>40</td>
<td>43</td>
<td>0.08</td>
</tr>
<tr>
<td>Unfractionated heparin</td>
<td>45</td>
<td>51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Warfarin</td>
<td>2.2</td>
<td>2.1</td>
<td>0.84</td>
</tr>
<tr>
<td>Fibrinolytics</td>
<td>15</td>
<td>15</td>
<td>0.93</td>
</tr>
</tbody>
</table>

(Continued)
intracerebral in 6%, at another site in 53%, and not reported in 12%. Approximately 57% of patients with major bleeding received blood transfusions of ≥2 U, 4% underwent surgery to explore or repair the bleeding site, and 3% received transfusions and underwent surgery. Approximately half (49%) of the major bleeding events occurred the day of or the day after hospital admission. Patients with major bleeding in hospital were more likely to die in the next 6 months than patients without major bleeding (25.7% versus 9.3%; *P* <0.001). The 6-month survival curves of patients with and without major bleeding are shown in Figure 1.

### Demographic, Clinical, and Treatment Characteristics of Patients With or Without Major Bleeding

Patients experiencing major bleeding during hospitalization were older, more likely to be female, and more likely to have a history of a number of comorbidities than patients without major bleeding (Table 1). Patients with major bleeding were of lower body mass index; had worse renal function, lower blood pressure, and higher pulse rates on admission; and were less likely to present in Killip class I than patients without major bleeding. Patients with major bleeding were more likely to have a discharge diagnosis of STEMI.

Patients with major bleeding were more likely to be treated during hospitalization with glycoprotein IIb/IIIa inhibitors, unfractionated heparin, thienopyridines, diuretics, and intravenous vasopressor therapy but were less likely to be treated with aspirin, β-blockers, or low-molecular-weight heparins than those without major bleeding (Table 1). Overall use of fibrinolytic therapy did not differ significantly between patients with or without major bleeding. Patients with major bleeding also were more likely to have undergone percutaneous coronary intervention, intra-aortic balloon placement, or pulmonary artery catheter placement during hospitalization than patients without a major bleeding episode.

![Figure 1. Cumulative death rate stratified by the occurrence of major hospital bleeding as a time-varying covariate.](image-url)
After Cox proportional-hazards regression analysis, increasing age; female sex; history of prior bleeding, smoking, or peripheral arterial disease; presentation with glomerular filtration rate <30 mL/min or ST-segment deviation on initial ECG; treatment with intravenous vasopressor agent(s) or glycoprotein IIb/IIIa inhibitors in the first 24 hours; treatment with fibrinolytics; and use of cardiac catheterization, percutaneous coronary intervention, intra-aortic balloon pump, or pulmonary artery catheter were associated with bleeding in the first 30 days (Table 2). Treatment with aspirin or unfractionated heparin in the first 24 hours was inversely associated with major bleeding in the first 30 days. History of atrial fibrillation or presentation with increased heart rate was associated with late but not early bleeding. History of hypertension was inversely associated with early but not late bleeding, whereas presentation with ST elevation on initial ECG was inversely associated with late but not early bleeding.

**Major Bleeding and Hospital Outcomes**

Patients who experienced a major bleeding episode were more likely to die in hospital than those who did not bleed (20.9% versus 5.6%; \( P < 0.001 \)). One in 5 patients with a major bleed did not survive until hospital discharge; these patients accounted for \( \approx \)10% of all hospital deaths observed.

After the previously described demographic and clinical variables were controlled for, patients who had major bleeding during hospitalization were significantly more likely to die in hospital (hazard ratio [HR], 1.9; 95% confidence interval [CI], 1.6 to 2.2) than those who did not experience major bleeding. Major bleeding was not associated with subsequent recurrence of AMI (HR, 1.1; 95% CI, 0.9 to 1.4) or nonhemorrhagic stroke (HR, 1.8; 95% CI, 0.8 to 3.7) during hospitalization after controlling for potential confounders.

**Major Bleeding, Fibrinolytics, and Hospital Outcomes**

Of 5931 patients treated with fibrinolytic therapy, 3.1% suffered major bleeding. After adjustment for potential confounders, patients who received fibrinolytic therapy and bled were at significantly increased risk of in-hospital death (HR, 3.3; 95% CI, 2.3 to 4.7) compared with patients who had received fibrinolytic therapy and did not bleed. Of 26 043 patients who did not receive fibrinolytic therapy, 2.9% suffered major bleeding. After regression analysis, these patients also were at significantly increased risk of hospital death (HR, 1.7; 95% CI, 1.4 to 2.0) compared with their counterparts who did not bleed.

**Major Bleeding and Postdischarge Outcomes**

Patients who survived major bleeding in hospital were more likely to die during postdischarge follow-up than patients without major bleeding (7.9% versus 5.2%; \( P = 0.002 \)). However, after the previously described demographic and clinical variables were controlled for, hospital survivors of a major bleeding episode did not have an increased risk of 6-month mortality (HR, 0.8; 95% CI, 0.6 to 1.0). Similarly, major bleeding in hospital was not significantly associated with

| Table 2. Predictors of In-Hospital Bleeding Within 30 Days* |
|---------------------------------|---------------------------------|------------------|
| Variable                        | Total Cohort,† HR (95% CI)      |
| History of prior bleeding       | 2.70 (1.84–3.96)                |
| Glomerular filtration rate <30 mL/min‡ | 2.40 (1.76–3.29)               |
| Early bleeding (0–2 d)          | 1.83 (1.25–2.68)                |
| Late bleeding (3–30 d)          | 1.65 (1.18–2.29)                |
| Pulmonary artery catheter       | 2.04 (1.53–2.72)                |
| Early bleeding (0–1 d)          | 1.94 (1.57–2.39)                |
| Late bleeding (2–30 d)          | 1.94 (1.57–2.39)                |
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*For variables in which proportional-hazards assumption was violated, HR for both early and late bleeding are presented. Analyses were performed with early bleeding defined as 0 to 1 or 0 to 2 days and late bleeding as 2 to 30 or 3 to 30 days. Data are presented for whichever interval provided the largest difference in HR between early and late bleeding.

†Data for \( n = 28,327 \) patients with complete covariate information, 766 with major bleeding.

‡Reference category: glomerular filtration rate \( \geq 60 \) mL/min.
postdischarge mortality in patients who received fibrinolytic therapy (HR, 0.6; 95% CI, 0.2 to 1.6) or in those who did not receive fibrinolytic therapy (HR, 0.8; 95% CI, 0.6 to 1.1).

**Bleeding, Treatment, and Outcomes**

We identified 506 patients who suffered major bleeding on or the day after hospital admission for AMI and had received at least 1 antithrombotic therapy. Overall use of therapy after day 1 in patients who bled compared with those who never bled was as follows: aspirin, 69% versus 86%; thienopyridines, 51% versus 52%; unfractionated heparin, 29% versus 35%; low–molecular-weight heparin, 27% versus 50%; or glycoprotein IIb/IIIa inhibitors, 9% versus 14%.

Among patients who suffered major bleeding within the first hospital day, mortality rates were higher among those who discontinued aspirin, thienopyridines, or unfractionated heparin compared with those patients who bled but continued to be treated with these agents after the first day (52% versus 13%, P≤0.001; 58% versus 13%, P<0.001; 26% versus 16%, P=0.03, respectively) (Figure 2). The corresponding odds ratios for mortality associated with discontinuation of each agent were as follows: aspirin, 7.55 (95% CI, 4.43 to 12.88); thienopyridines, 8.91 (95% CI, 4.39 to 18.12); and unfractionated heparin, 1.91 (95% CI, 1.09 to 3.36).

**Discussion**

In this large observational registry, major bleeding occurred in 2.8% of all patients hospitalized with AMI. Although major bleeding occurred in only 1 in 35 patients, ~10% of deaths in our entire study population occurred in this high-risk patient subset. Our data provide insight into the clinical significance of major bleeding in patients with AMI in the real world, which patients are at risk, and the magnitude of associated morbidity and mortality.

**Risk Factors for Bleeding**

Similar to the results of an earlier analysis from GRACE, advancing age, female sex, and altered renal function were strongly associated with major bleeding in our study.\(^8\) Although not assessed in several other studies of ACS-associated bleeding,\(^1,2\) prior history of bleeding was a potent predictor of in-hospital bleeding in our study. These data suggest that information about history of bleeding should be collected routinely from every patient presenting with AMI and must be seriously weighed by physicians formulating a management plan. Other strong predictors of major bleeding were variables reflecting more severe illness (impaired renal function on presentation, use of intravenous vasopressor agents) or use of invasive procedures for severe illness (intra-aortic balloon pump, pulmonary artery catheter).

A recent randomized trial of antithrombotic therapy in ACS suggests that improvements in patient survival may be achieved by minimizing bleeding complications.\(^9\) Therefore, we were interested in exploring the possible associations between various antithrombotic and antiplatelet agents and bleeding in a nonrandomized trial setting. Although treatment with glycoprotein IIb/IIIa inhibitors in the first 24 hours of hospitalization was linked to the development of major bleeding, there was an inverse association between treatment with aspirin, unfractionated heparin, or low–molecular-weight heparins in the first 24 hours of hospitalization and subsequent major in-hospital bleeding events. This is in contrast to a recent analysis of the combined Organization to Assess Strategies for Ischemic Syndromes (OASIS-2) study and registry and Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) study in which unfractionated heparin, low–molecular-weight heparin, and hirudin were associated with bleeding.\(^1\) These data suggest that in the “real-world” setting, physicians do a reasonably good job of selecting patients at low risk for bleeding when deciding to administer these agents. It also suggests that at least in the community setting, type of anticoagulant therapy may have a smaller impact on bleeding than the characteristics of the patients in whom they are used (eg, comorbidities, severity of illness) and whether invasive procedures are performed.

Unfortunately, we were not able to examine the impact of dosing of antithrombotic therapies on the risk of bleeding. A previous analysis from Can Rapid Risk Stratification of Unstable Angina Patients Suppress Adverse Outcomes With Early Implementation of the ACC/AHA Guidelines (CRUSADE) suggests that patients with non-STEMI treated with excess doses of low–molecular-weight heparin or glycoprotein IIb/IIIa inhibitors were at increased risk for major bleeding.\(^10\) Similarly, in an analysis from the CURE study, bleeding risks increased with increasing aspirin dose, with or without clopidogrel, without any increase in efficacy.\(^11\)

**Bleeding and Hospital Outcomes**

One in every 5 patients who suffered major bleeding in our study did not survive to hospital discharge. This is even higher than the mortality rate observed in the aforementioned analysis of OASIS/CURE patients in which ~1 in every 8 patients who bled had died at 30 days after study enrollment.\(^1\) This increase in mortality likely reflects the older age and the higher prevalence of specific comorbidities (eg, diabetes mellitus, renal dysfunction, and congestive heart failure) in
It should be noted that after adjustment for demographic, clinical, and treatment characteristics, the HR for in-hospital mortality associated with bleeding in our study was much lower (1.9) compared with the HR for 30-day mortality in the OASIS/CURE study of 5.4. The HR for 30-day death rates associated with moderate or severe bleeding in the Rao et al analysis of the impact of bleeding in the pooled results of 4 randomized controlled trials (Global Use of Strategies To Open Occluded Coronary Arteries [GUSTO IIb], Platelet Glycoprotein IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy [PURSUIT], and Platelet IIb/ IIIa Antagonism for the Reduction of Acute coronary syndrome events in a Global Organization Network [PARAGON] A and B) also was higher than observed in our study (2.1 and 7.5, respectively). This difference is even more striking when one considers that neither of these studies included patients who received fibrinolytic therapy. The difference in adjusted HRs between our study and those deriving data from clinical trials reflects in part the higher unadjusted death rates observed in nonbleeding patients in our observational study. Indeed, the in-hospital mortality of patients who did not bleed in our study was 5.6% compared with 30-day mortality rates of only 2.5% and 2.9% in OASIS/CURE and the Rao et al analyses, respectively. We do not believe that having different definitions of major bleeding was a likely cause for the observed differences in adjusted bleeding risk; in each of the 3 studies, bleeding prompting blood transfusion was the lowest threshold for categorization of moderate or major bleeding. In addition, absolute mortality associated with major bleeding was higher in our study than observed in either of the other 2 studies.

Differences in the patient profile of our patients compared with those enrolled in clinical trials and use of a different mortality time point (in-hospital versus 30-day) may partially explain the differences in the impact of bleeding on death rates in our study compared with prior investigations. Nevertheless, our data suggest that in the community setting, bleeding, although still associated with worse outcomes, also is a marker for patients with more severe illness or comorbidities.

Bleeding and Postdischarge Outcomes

It has been suggested that the occurrence of major bleeding in hospital continues to affect mortality even after hospital discharge. In the Canadian Acute Coronary Syndrome Registry, major bleeding was a predictor of 1-year mortality. In the aforementioned report by Rao et al, mild, moderate, and severe bleeding remained significantly associated with 1-year mortality (HR, 1.4, 2.1, and 7.5, respectively). However, both of these studies were cumulative analyses including early in-hospital deaths. In the OASIS/CURE analysis, major bleeding remained associated with an increased hazard of death after 30 days after the exclusion of patients who died before 30 days. In contrast, although patients in our study who suffered major bleeding experienced higher postdischarge mortality (7.9% versus 5.2%), bleeding was no longer a predictor of late mortality after controlling for differences in clinical and treatment characteristics. These findings are concordant with that of a meta-analysis of 25 studies of ACS in which the impact of bleeding on mortality was confined to in-hospital or 30-day mortality.

Bleeding and Mortality: Potential Mechanisms

Although the GRACE registry was not specifically designed to explore the issue of bleeding and increased mortality in patients with AMI, available data allow us to make some observations and to generate hypotheses about possible mechanisms involved. First, as noted previously, patients who bled were older, sicker, and more likely to undergo invasive procedures. Although we attempted to control for these and other variables, it is still likely that in some patients bleeding was merely a marker for severe illness and did not contribute directly to their death.

That said, bleeding remained associated with in-hospital mortality after controlling for important confounders. Mechanisms by which bleeding may directly lead to death in patients with a myocardial infarction warrant further study. One potential mechanism includes the premature discontinuation of antiplatelet and antithrombotic therapies in patients who bled. Clearly, early cessation of these therapies in patients with ACS, particularly those who may have undergone percutaneous coronary intervention with stent placement, is problematic. Although we were able to explore this mechanism in only a limited fashion, our data suggest that this may be an important issue. Institution or continuation of all antiplatelet and/or antithrombotic therapies assessed after day 1 was lower in patients who experienced early bleeding (within 24 hours). Moreover, in patients who bled, hospital mortality was significantly increased in those who discontinued aspirin, thienopyridines, or low-molecular-weight heparins compared with those who continued these agents after the first day of hospitalization.

Given the proven efficacy of these agents for the prevention of recurrent ischemic events (particularly in patients undergoing stent implantation), the inability to use them would be expected to have adverse sequelae. Unfortunately, our sample size for this subanalysis was not large enough to allow a more detailed analysis. It must also be acknowledged that antithrombotic therapy may have been discontinued more often in sicker patients with more serious bleeds. We are unable to directly explore the relationship between bleeding, avoidance of antiplatelet and antithrombotic medications, and adverse outcomes in this observational study; future observational studies of bleeding in ACS should be designed to examine these relationships.

Other postulated mechanisms by which major bleeding may lead to recurrent coronary ischemia and/or increased mortality might include the adverse effects of resulting hypotension on end organs, platelet and coagulation activation associated with anemia, or even adverse effects of the resulting blood transfusions themselves. Finally, interventions performed to manage bleeding complications (eg, exploratory surgery) likely result in increased risk. Although we could not fully explore these issues, we believe that a better understanding of the pathophysiological relationship between bleeding and subsequent mortality is critical and deserves further study.
Study Strengths and Limitations

The strengths of the present investigation include its large sample size, the amount of data collected for each subject, and the use of a uniform predetermined definition of major bleeding throughout the study. Another strength is that our study population is more reflective of patients seen in physicians’ actual practice than patients enrolled in randomized controlled trials.

However, several limitations should be borne in mind when our study findings are interpreted. Most important, we acknowledge that in this large-scale observational study, we cannot exclude “underreporting” of bleeding events. That said, the observed rate of major bleeding in our study was slightly more than that observed in the aforementioned OASIS/CURE analysis. Obviously, no firm conclusions about a causal relationship between hospital bleeding and hospital outcomes can be made on the basis of this analysis. Because we did not stratify bleeding events by severity, we were unable to examine the relationship between increasing severity and subsequent outcomes. A number of prior analyses have demonstrated a consistent increase in the likelihood for dying with increasing bleed severity; the consistency of these findings lends strength to the validity of an association.\textsuperscript{1,2} Finally, we were unable to fully explore possible relationships between antithrombotic therapy (dosing, timing, and cessation), bleeding, and observed outcomes. Future observational studies specifically designed to address these associations are required.

Conclusions

This observational study of \(>40,000\) patients with AMI suggests that major bleeding events occur in \(\approx 1\) in every 35 patients. One in 5 of these patients will not survive until hospital discharge; these patients accounted for \(\approx 10\%\) of all hospital deaths observed. Our data suggest that in the community setting, bleeding tends to occur in patients who are older and have more comorbidities or more severe illness and is a marker for poor hospital outcome. A better understanding of the specific causes of AMI-associated bleeding and the relationship between major bleeding events and death is needed if we are to decrease morbidity and mortality in this high-risk patient subset.

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Disclosures

Dr Spencer has served on an advisory board and received a research grant from sanofi-aventis. Dr Mosucci has received grant support from Blue Cross Blue Shield of Michigan and has served as a consultant and on the speakers’ bureaus for Pfizer and the Medicine Company. Dr Granger has received research grants from Novartis, Proctor and Gamble, sanofi-aventis, The Medicines Company, Alexion, AstraZeneca, Boehringer Ingelheim, BMS, deCode Genetics, Genentech, and GSK. He has served as a consultant for Novartis, Proctor and Gamble, sanofi-aventis, The Medicines Company, Alexion, AstraZeneca, Genentech, GSK, INO Therapeutics, and Medicare. Dr Gore has received a research grant from sanofi-aventis. Dr Steg has received a research grant from sanofi-aventis; has served on the speakers’ bureaus for Boehringer Ingelheim, BMS, GSK, MSD, Novartis, Nycomed, sanofi-aventis, Sankyo, Servier, and ZLB-Behring; and has served on consulting or advisory boards for AstraZeneca, BMS, GSK, MSD, Pfizer, sanofi-aventis, Servier, and Takeda. Dr Goodman has received research grants or other research support from AstraZeneca, Bayer, Biovail, Boehringer Ingelheim, Bristol Myers Squibb, Eli Lilly, GlaxoSmithKline, Guidant, Hoffman La-Roche, Johnson & Johnson, Key Schering/Schering Plough, Merck Frosst, Pfizer, sanofi-aventis, and The Medicines Company; has received honoraria from AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Hoffman La-Roche, Key Schering/Schering Plough, Merck Frosst, Pfizer, sanofi-aventis, and The Medicines Company; and has served as a consultant or on the advisory boards for Bristol Myers Squibb, GlaxoSmithKline, Hoffman La-Roche, and sanofi-aventis. Dr Budaj has received research grants and honoraria from sanofi-aventis, GSK, AstraZeneca, Boehringer Ingelheim and has served as a consultant on or on the advisory boards for sanofi-aventis and GSK. Dr Fox has received research grants and honoraria and served as a consultant or on the advisory boards for sanofi-aventis, BMS, and GSK. The other authors report no conflicts.

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Analyses from randomized controlled trials suggest that bleeding in patients with acute myocardial infarction is strongly associated with poor outcomes. Because these data are not generalizable to all patients with acute myocardial infarction, we sought to understand better the scope of this problem in a “real-world” setting. Using data from >40,000 patients enrolled in the Global Registry of Acute Coronary Events, we found that 2.8% of patients with acute myocardial infarction experienced major bleeding during their hospital stay. These patients were older, more severely ill, and more likely to undergo invasive procedures. These patients accounted for ≈10% of all hospital deaths. Continuation of antithrombotic therapies after day 1 was lower in patients who experienced early bleeding. Moreover, in patients who bled, hospital mortality was increased in those who discontinued aspirin, thienopyridines, or low–molecular-weight heparins. Nevertheless, after adjustment for differences in age and comorbidities between patients with and without major bleeding, the risk of hospital mortality associated with bleeding was much lower than reported in randomized controlled trials. These data suggest that although bleeding may be causally related to adverse outcomes in some patients in the real-world setting, it is often merely a marker for patients at higher risk for adverse outcomes. The effect of antithrombotic therapy discontinuation after major bleeding in patients with acute myocardial infarction requires further study.
Influence of a Pressure Gradient Distal to Implanted Bare-Metal Stent on In-Stent Restenosis After Percutaneous Coronary Intervention

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Background—Fractional flow reserve predicts cardiac events after coronary stent implantation. The aim of the present study was to assess the 9-month angiographic in-stent restenosis rate in the setting of optimal stenting and a persisting gradient distal to the stent as assessed by a pressure wire pullback recording in the entire length of the artery.

Methods and Results—In 98 patients with angina pectoris, 1 de novo coronary lesion was treated with a bare-metal stent. After stent implantation, pressure wire measurements ($P_d$=mean hyperemic coronary pressure and $P_a$=mean aortic pressure) were performed in the target vessel: (1) $P_d/P_a$ as distal to the artery as possible (fractional flow reserve per definition); (2) $P_d/P_a$ just distal to the stent; (3) $P_d/P_a$ just proximal to the stent; and (4) $P_d/P_a$ at the ostium. Residual abnormal $P_d/P_a$ was defined as a pressure drop between $P_d/P_a$ measured at points 1 and 2. Fractional flow reserve distal to the artery after stenting was significantly lower (0.88 ± 0.21 versus 0.97 ± 0.05; $P \lt 0.001$), and angiographic in-stent binary restenosis rate was significantly higher (44.0% versus 8.1%; $P \lt 0.001$) in vessels with a residual abnormal $P_d/P_a$. Residual abnormal $P_d/P_a$ (odds ratio, 4.39; 95% confidence interval, 1.10 to 18.16; $P \lt 0.034$), reference vessel size (odds ratio, 0.17; 95% confidence interval, 0.04 to 0.69; $P \lt 0.013$), and stent length (odds ratio, 1.11; 95% confidence interval, 1.03 to 1.21; $P \lt 0.009$) were predictors of angiographic in-stent restenosis after 9 months.

Conclusions—A residual abnormal $P_d/P_a$ distal to a bare-metal stent was an independent predictor of in-stent restenosis after implantation of a coronary bare-metal stent. (Circulation. 2007;116:2802-2808.)

Key Words: atherosclerosis ■ collateral circulation ■ myocardial fractional flow reserve ■ restenosis ■ stents

Until recently, restenosis was a major problem in percutaneous coronary intervention (PCI). The introduction of coronary stents reduced the rate of restenosis, but in-stent restenosis still appears in a number of patients. Studies using intravascular ultrasound and pathological studies have shown that angiographic stenoses are associated with diffuse atherosclerosis in the distal part of the coronary tree, although this may not be identified by coronary arteriography.

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Coronary pressure measurement with determination of fractional flow reserve (FFR) has been proposed as a complementary technique for optimizing PCI results. In addition, FFR has been shown to have a predictive value after PCI. FFR measured after stenting with the pressure wire located distal to the stent indicates the effects on maximal flow of the stented segment and of the remaining part of the artery. A complete analysis of the coronary artery after PCI can be achieved by a pressure wire pullback during sustained hyperemia induced by intravenous adenosine. A pullback pressure recording in an epicardial coronary artery reflects the conductance of the entire artery as well as of the individual segments. In diffusely diseased coronary arteries, a marked pressure drop may occur between an implanted stent and the distal part of the artery.

The aim of the present study was to assess the in-stent restenosis rate in the setting of optimal stent implantation and a persisting pressure gradient in the vessel distal to the stent as assessed by a pressure wire pullback recording in the stented coronary artery.
Methods

Patient Population
From October 2002 to December 2004, 98 patients with a single lesion in a native coronary artery and planned PCI were enrolled at Odense University Hospital, Aarhus University Hospital, Skejby Sygehus, or Rigshospitalet, Copenhagen, Denmark. Patients with a total occluded artery or acute myocardial infarction were excluded. All patients were on aspirin (75 mg/d) and clopidogrel (loading dose 300 mg 24 hours before PCI, continued on 75 mg/d for 12 months). A total of 88 patients (90%) had a 9-month angiographic follow-up. The study population was divided into 2 groups: group I, patients with a pressure gradient in the nonstented part of the vessel distal to the stent; and group II, patients without a pressure gradient distal to the stent. Stent names are shown in the online-only Data Supplement. Patients with residual abnormal Pd/Pa had more left anterior descending artery lesions and fewer left circumflex artery lesions treated. The reference diameter was significantly lower in patients with residual abnormal Pd/Pa compared with patients without residual abnormal Pd/Pa (2.9±0.6 versus 3.3±0.5; P<0.001).

Intracoronary Pressure Measurements
Before pressure measurements and intervention, the patients had a 200-µg intracoronary nitroglycerin and a 5000- to 10 000-U intravenous heparin administration. A 0.014-inch pressure wire (PressureWire, Radi Medical Systems, Uppsala, Sweden, or WaveWire, JoMed, Helsingborg, Sweden) was passed through the target lesion and placed as distal to the coronary artery as possible. Maximal hyperemia was induced by intravenous adenosine (140 µg/kg per min), and the ratio Pd/Pa was calculated. Here, Pd represents mean hyperemic coronary pressure of the index vessel measured by the pressure wire, and Pa represents mean aortic pressure measured by the guiding catheter. After stenting, a slow manual pullback of the pressure sensor from the most distal position to the proximal part of the vessel was performed at maximal hyperemia and recorded on paper. Pressure measurements were performed in the whole length of the artery: Pd/Pa was measured as (1) distal in the artery as possible (per definition of FFR), (2) just distal to the stent, (3) just proximal to the stent, and (4) and at the ostium (Figure 1). A residual abnormal Pd/Po was defined as a pressure drop between hyperemic Pd/Po and Pa at the ostium.

Quantitative Coronary Angiography
Angiographic studies performed at baseline, after the procedure, and at follow-up were assessed at the Angiographic Core Laboratory (Catheterization Laboratory, Odense University Hospital, Odense, Denmark). The computer-based ACOM.PC V3.1 (Siemens Medical Systems, Inc) was used for quantitative coronary angiography. Quantitative analysis was performed offline by experienced personnel unaware of the pressure measurements. The same 2 projections were used at all time points. The following angiographic measurements were measured: reference diameter of the vessel, minimal luminal diameter, percent diameter stenosis (1−(minimal luminal diameter/reference segment diameter)×100), and late lumen loss (difference between minimal luminal diameter at the end of the procedure and at follow-up).

Study End Point and Definitions
The primary end point of the study was binary angiographic restenosis ≥50% after 9 months. Optimal stenting was based on visual estimates by the operator and defined as a residual stenosis of 0. The core laboratory qualitative comparative analysis could overrule the visual operator assessment.

Statistical Analysis
Statistical analysis was performed with the use of SPSS 14.0. Categorical data were presented as counts and percentages and compared by the Pearson χ² test or the Fisher exact test. Continuous data were expressed as mean±SD and compared by t test. Two-way ANOVA with 4 repeated measurements was used to test whether a significant change occurred in measurements at different wire positions. If this test was significant, a paired t test was used to compare the values between the relevant wire positions. Separate logistic regression analyses were performed to identify univariate predictors of binary angiographic restenosis, and a subsequent stepwise (forward conditional) regression analysis was performed with entry and removal criteria of 0.05 and 0.10, respectively. Logistic regression analyses were presented as odds ratio (OR) with 95% confidence intervals. All statistical tests were 2-tailed.

With anticipation of a mean binary angiographic restenosis (≥50%) rate of 25% after 9 months, the 166 patients enrolled provided 80% power and a 5% α level to detect a difference of 20% (event rate of 15% and 35%, respectively). A probability value <0.05 (2-sided) was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Baseline Characteristics and Procedural Results
The clinical features at baseline are shown in Table 1. Age and risk factors did not differ significantly between the 2 groups: group I with abnormal residual Pd/Pa (n=58) and group II without abnormal residual Pd/Pa (n=40).

Lesion Characteristics
The occurrence of 1- and 2-vessel coronary artery disease was similar in the 2 groups. Diameter stenosis and lesion type did not differ between the 2 groups. Lesions tended to be longer in patients with residual abnormal Pd/Pa (Table 2). Patients with residual abnormal Pd/Pa had more left anterior descending artery lesions and fewer left circumflex artery lesions treated. The reference diameter was significantly lower in patients with residual abnormal Pd/Pa compared with patients without residual abnormal Pd/Pa (2.9±0.6 versus 3.3±0.5; P<0.001).
**FFR and $P_d/P_a$ at Different Pressure Wire Positions**

Preinterventional FFR was significantly lower in vessels with residual abnormal $P_d/P_a$ compared with vessels without residual abnormal $P_d/P_a$ (Table 2). FFR before intervention correlated inversely with the angiographic diameter stenosis ($r = -0.562$, $P < 0.001$) (Figure 2). FFR increased significantly after PCI ($0.95 \pm 0.09$ versus $0.65 \pm 0.20$; $P < 0.001$).

No artery showed a sudden increase in distal coronary pressure by pressure wire pullback. This indicated that the pressure gradient, observed in the distal part of the artery, was due to a continuous loss of pressure in the artery distal to the stented segment and was not caused by an angiographically undetected focal narrowing. After the intervention, a significant within-subject change of $P_d/P_a$ occurred in the entire artery in patients with a residual abnormal $P_d/P_a$ (group I) ($P < 0.001$, 2-way ANOVA analysis; Figure 3), whereas the within-subject change of $P_d/P_a$ did not differ significantly along the entire length of the artery in patients without a residual abnormal $P_d/P_a$ (group II). In group I, FFR (pressure wire placed in the distal vessel) was significantly lower than hyperemic $P_d/P_a$ just distal to the stent ($0.88 \pm 0.12$ versus $0.94 \pm 0.11$; $P < 0.001$). In both groups, a small trans-stent gradient was present ($P_d/P_a$ just distal to the stent versus $P_d/P_a$ just proximal to the stent) (group I, $0.94 \pm 0.10$ versus $0.97 \pm 0.08$, $P < 0.001$; group II, $0.97 \pm 0.05$ versus $0.99 \pm 0.04$, $P = 0.001$). In group I, 29.3% (n = 17) of the lesions had no trans-stent pressure gradient, and in group II, 67.5% (n = 27) of the lesions had no trans-stent pressure gradient ($P = 0.001$).

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**Table 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Group I (Residual Abnormal $P_d/P_a$ Ratio)</th>
<th>Group II (No Residual Abnormal $P_d/P_a$ Ratio)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>58</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Men, %</td>
<td>84.5</td>
<td>84.6</td>
<td>NS</td>
</tr>
<tr>
<td>Age, mean±SD, y</td>
<td>61.9±10.2</td>
<td>62.4±8.1</td>
<td>NS</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>36.2</td>
<td>35.9</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, mean±SD, kg/m$^2$</td>
<td>26.3±4.0</td>
<td>27.9±4.0</td>
<td>NS</td>
</tr>
<tr>
<td>Prior myocardial infarction, %</td>
<td>43.1</td>
<td>35.9</td>
<td>NS</td>
</tr>
<tr>
<td>Prior PCI, %</td>
<td>6.9</td>
<td>7.7</td>
<td>NS</td>
</tr>
<tr>
<td>Stable angina pectoris, %</td>
<td>89.7</td>
<td>92.3</td>
<td>NS</td>
</tr>
<tr>
<td>1-vessel disease</td>
<td>67.8</td>
<td>69.0</td>
<td>NS</td>
</tr>
<tr>
<td>2-vessel disease</td>
<td>27.1</td>
<td>28.6</td>
<td>NS</td>
</tr>
<tr>
<td>3-vessel disease</td>
<td>5.1</td>
<td>2.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Medication

- Antianginal medication ≥2 drugs, %: 21.1 versus 33.3; NS
- Aspirin, %: 100 versus 100; NS
- Clopidogrel, %: 100 versus 100; NS
- Statin, %: 88.1 versus 89.3; NS

NS indicates not significant.

**Table 2. Lesion and Procedure Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Group I (Residual Abnormal $P_d/P_a$ Ratio)</th>
<th>Group II (No Residual Abnormal $P_d/P_a$ Ratio)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>58</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>LAD/CX/RCA, %</td>
<td>30/40/30</td>
<td>57/10/33</td>
<td>0.004</td>
</tr>
<tr>
<td>Reference diameter, mm</td>
<td>2.86±0.56</td>
<td>3.27±0.53</td>
<td>0.001</td>
</tr>
<tr>
<td>Diameter stenosis, %</td>
<td>66.4±13.9</td>
<td>69.5±14.3</td>
<td>NS</td>
</tr>
<tr>
<td>Lesion type (A/B/C), %</td>
<td>21/68/11</td>
<td>33/59/8</td>
<td>NS</td>
</tr>
<tr>
<td>Lesion length, mm</td>
<td>14.8±8.6</td>
<td>12.2±4.9</td>
<td>0.072</td>
</tr>
<tr>
<td>Stent length, mm</td>
<td>18.2±8.9</td>
<td>16.0±6.5</td>
<td>NS</td>
</tr>
<tr>
<td>Direct stenting, %</td>
<td>40.0</td>
<td>35.1</td>
<td>NS</td>
</tr>
<tr>
<td>FFR before intervention</td>
<td>0.62±0.21</td>
<td>0.71±0.19</td>
<td>0.033</td>
</tr>
<tr>
<td>FFR after intervention</td>
<td>0.88±0.12</td>
<td>0.97±0.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD unless otherwise indicated. LAD indicates left anterior descending artery; CX, circumflex artery; and RCA, right coronary artery.
FFR After PCI in the Individual Vessels With and Without a Residual Abnormal Pd/Pa Ratio Distal to the Stent

No significant differences in Pd/Pa were present just distal to the stent for the 3 major coronary arteries in group I (left anterior descending artery, 0.90±0.13; left circumflex artery, 0.98±0.08; right coronary artery, 0.94±0.07; P=NS) or group II (left anterior descending artery, 0.94±0.04; left circumflex artery, 0.98±0.06; right coronary artery, 0.97±0.04; P=NS). FFR after PCI was significantly lower in vessels with a residual abnormal Pd/Pa ratio compared with vessels without a residual abnormal Pd/Pa ratio (0.88±0.21 versus 0.97±0.05; P<0.001).

Angiographic Follow-Up and Event Rate

At 9-month follow-up, in-stent binary angiographic restenosis was demonstrated in 28.6% of patients with angiographic follow-up (Table 3) (25.7% of all patients). Target lesion revascularization was performed in 24.2% of patients with angiographic follow-up (21.8% of all patients). The in-stent binary angiographic restenosis rate was 21.1% (n=8) for right coronary artery lesions, 30.4% (n=7) for left anterior descending artery lesions, and 37.5% (n=9) for left circumflex artery lesions. In vessels with a residual abnormal Pd/Pa ratio, binary angiographic restenosis was seen in 44.0% compared with 8.1% in vessels without a residual abnormal Pd/Pa ratio (P<0.001). During the 9-month follow-up, no stent thrombosis was seen, and none of the patients died or suffered an acute myocardial infarction.

Predictors of In-Stent Binary Angiographic Restenosis

Logistic regression analysis was used to assess independent predictors of binary angiographic restenosis at 9 months. The parameters examined with the use of univariate logistic regression analysis are shown in Table 4. A residual abnormal Pd/Pa, reference vessel diameter, minimal luminal diameter after stent implantation, lesion length, and stent length were significantly associated with an increased rate of binary angiographic restenosis. To adjust for differences in lesion factors, we performed a multiple logistic regression analysis including the abnormal residual Pd/Pa ratio (as the variable of primary interest) and reference vessel diameter (as a well-known factor influencing binary angiographic restenosis) by forced entry and parameters with P<0.20 (from the univariate analysis) in a forward stepwise procedure. Included in the forward procedure were minimal luminal diameter after stent implantation, lesion length, stent length, FFR before PCI, and FFR after PCI. After these adjustments, a residual abnormal Pd/Pa ratio, reference vessel diameter, and stent length were found to be predictors of binary angiographic restenosis at 9 months (Table 5). Performing a backward stepwise procedure showed the same predictors of binary angiographic restenosis. The c statistic (area under the receiver operating characteristic curve) in the final model was 0.83.

Number of Patients Included

According to the power calculation, 166 patients were expected to be included. However, during the enrollment period

![Figure 2. FFR vs angiographic diameter stenosis. FFR correlates inversely to the angiographic diameter stenosis.](image)

![Figure 3. Mean values (and 95% confidence interval for the mean) of FFR distal in the vessel, hyperemic Pd/Pa just distal to the stent, hyperemic Pd/Pa just proximal to the stent, and hyperemic Pd/Pa at the ostium evaluated with a complete analysis of the stented coronary artery with a pullback pressure recording. Top, Patients with a residual abnormal Pd/Pa ratio (group I). Bottom, Patients without a residual abnormal Pd/Pa ratio (group II).](image)
the drug-eluting stents were implemented for clinical use, and the study was stopped before enrollment of the 166 patients in order not to have a selected cohort with bare-metal stents.

In regard to calculating the power with the observed difference of 44.0% / H11002 8.1% / H11005 35.9% in the event rate with binary angiographic restenosis, for a comparison of 2 independent binomial proportions with the use of the likelihood ratio statistic with a / H9273 2 approximation with a 2-sided significance level of 0.05, group sample sizes of 50 and 38 have an approximate power of 0.974 when the proportions are 0.44 and 0.081.

**Discussion**

The present study demonstrated that pressure reduction from an implanted stent to the distal part of the artery was a predictor of angiographic in-stent restenosis after 9 months. The pressure reduction was detected by a systematic analysis of the entire length of the stented coronary artery with a pullback pressure wire recording. A sudden increase in distal coronary pressure was not seen in any of the investigated arteries during the pullback procedure. Therefore, the pressure gradient observed in the distal part of the artery was due to a continuous loss of pressure in the artery distal to the stented segment and not to an angiographically undetected focal narrowing. A residual abnormal Pd/Pa ratio suggests diffuse disease in the remaining part of the artery, but diffuse disease was not documented by either angiography or intravascular ultrasound imaging.

In normal coronary arteries, no gradient exists between the ostium and the distal part of the artery despite induced hyperemia. In patients with angiographically mild disease, Gould et al13 demonstrated a base-to-apex myocardial perfu-

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**Table 3. Results of Quantitative Angiographic Analysis at Baseline and Follow-Up**

<table>
<thead>
<tr>
<th></th>
<th>Group I (Residual Abnormal Pd/Pa Ratio)</th>
<th>Group II (No Residual Abnormal Pd/Pa Ratio)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>58</td>
<td>40</td>
<td>...</td>
</tr>
<tr>
<td>No. of patients with angiographic follow-up</td>
<td>50</td>
<td>38</td>
<td>...</td>
</tr>
<tr>
<td><strong>Before intervention</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference diameter, mm</td>
<td>2.86±0.56</td>
<td>3.27±0.53</td>
<td>0.001</td>
</tr>
<tr>
<td>Minimal lumen diameter, mm</td>
<td>0.41±0.06</td>
<td>0.47±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Diameter stenosis, %</td>
<td>66.4±13.9</td>
<td>69.5±14.1</td>
<td>NS</td>
</tr>
<tr>
<td><strong>After PCI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference diameter, mm</td>
<td>2.59±0.51</td>
<td>2.94±0.56</td>
<td>0.003</td>
</tr>
<tr>
<td>Minimal lumen diameter, mm</td>
<td>8.1±11.0</td>
<td>7.6±11.8</td>
<td>NS</td>
</tr>
<tr>
<td>Follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients, n (%)</td>
<td>51 (84.7)</td>
<td>40 (90.5)</td>
<td>...</td>
</tr>
<tr>
<td>Reference diameter, mm</td>
<td>2.71±0.59</td>
<td>3.01±0.63</td>
<td>0.027</td>
</tr>
<tr>
<td>Minimal lumen diameter, mm</td>
<td>1.51±0.78</td>
<td>2.23±0.68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Late lumen loss, mm</td>
<td>1.09±0.72</td>
<td>0.71±0.59</td>
<td>0.011</td>
</tr>
<tr>
<td>Diameter stenosis, %</td>
<td>45.3±25.0</td>
<td>25.9±17.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Binary angiographic restenosis, %</td>
<td>44.0</td>
<td>8.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD unless otherwise indicated. NS indicates not significant.

---

**Table 4. Predictors for Binary Angiographic Restenosis (Univariate Analysis)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.03</td>
<td>0.97–1.09</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5.18</td>
<td>0.63–42.43</td>
<td>0.126</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.33</td>
<td>0.46–3.84</td>
<td>NS</td>
</tr>
<tr>
<td>Study vessel*</td>
<td>0.83</td>
<td>0.26–2.70</td>
<td>NS</td>
</tr>
<tr>
<td>FFR before PCI</td>
<td>0.08</td>
<td>0.01–0.92</td>
<td>0.043</td>
</tr>
<tr>
<td>FFR after PCI</td>
<td>0.01</td>
<td>0.00–1.16</td>
<td>0.058</td>
</tr>
<tr>
<td>Residual abnormal Pd/Pa ratio distal to the stent</td>
<td>9.16</td>
<td>2.49–33.79</td>
<td>0.001</td>
</tr>
<tr>
<td>Lesion length</td>
<td>1.09</td>
<td>1.02–1.17</td>
<td>0.011</td>
</tr>
<tr>
<td>Reference vessel diameter</td>
<td>0.21</td>
<td>0.07–0.62</td>
<td>0.005</td>
</tr>
<tr>
<td>Minimum lumen diameter after stent implantation</td>
<td>0.24</td>
<td>0.08–0.70</td>
<td>0.009</td>
</tr>
<tr>
<td>Stent length</td>
<td>1.08</td>
<td>1.01–1.14</td>
<td>0.017</td>
</tr>
<tr>
<td>Angiographic diameter stenosis, %</td>
<td>1.01</td>
<td>0.97–1.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; NS, not significant.

*Circumflex as reference.

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**Table 5. Predictors for Binary Angiographic Restenosis (Multivariable Analysis)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual abnormal Pd/Pa ratio distal to the stent</td>
<td>4.58</td>
<td>1.11–18.84</td>
<td>0.034</td>
</tr>
<tr>
<td>Reference vessel diameter</td>
<td>0.17</td>
<td>0.04–0.71</td>
<td>0.014</td>
</tr>
<tr>
<td>Stent length</td>
<td>1.11</td>
<td>1.03–1.21</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Predictors initially included in the model were as follows: residual abnormal Pd/Pa ratio distal to the stent, reference vessel diameter, minimum lumen diameter, lesion length, stent length, FFR before PCI, FFR after PCI distal to artery, and diabetes mellitus. CI indicates confidence interval.
sion gradient after dipyridamole administration by positron emission tomography without significant regional perfusion defects. It has been shown by coronary pressure measurements that a base-to-apex perfusion gradient can be due to abnormal resistance in atherosclerotic epicardial coronary arteries without segmental stenosis5 because diffuse coronary atherosclerosis with no focal stenoses results in a graded, continuous pressure drop in the artery. This is in accordance with the findings of the present study, in which optimal coronary stenting resulted in a minimum hyperemic pressure drop within the stented coronary artery segment, whereas the transtenotic hyperemic gradient was not eliminated fully after stenting when the entire artery was evaluated. The minimum hyperemic pressure drop across the stented segment might indicate a small resistance to blood flow, but the present study was not designed to obtain a total elimination of the transtenotic hyperemic gradient after stenting.

After treatment with bare-metal stents, several studies have demonstrated that FFR predicts major cardiac events.11,12 However, when FFR is reduced after stenting, it is important to distinguish between a persistent hyperemic gradient due to incomplete deployment and a gradient caused by diffuse disease proximal or distal to the treated lesion. Consequently, evaluation of stent deployment by FFR can be improved by calculations of Pd/Pa distal and proximal to the stent during maximal hyperemia to assess the conductance of the stented segment. In the present study, FFR distal to the artery tended to be related to development of in-stent restenosis.

Reference Vessel Size
The second independent predictor of angiographic in-stent restenosis after 9 months was reference vessel size. The ability to distinguish between large and small coronary arteries on the basis of quantitative coronary angiography is essential in PCI, and stent implantation in small arteries is a well-known independent risk factor of restenosis and major adverse cardiac events after PCI.14–16 The mechanisms behind the unfavorable outcome for small vessels are not well understood. In addition to a small postprocedural lumen diameter, a high plaque burden and pronounced diffuse disease may be important factors.17 Pathological and intravascular ultrasound studies have shown that an angiographically documented stenosis is associated with diffuse atherosclerotic changes in other parts of the coronary artery, although this may not be identified by coronary arteriography.18–20 These findings are in accordance with the present study, in which coronary arteries without diffuse disease had a larger reference vessel diameter.

Pressure Pullback Recordings With Intravenous Adenosine
In the present study, maximum hyperemia was achieved with continuous intravenous adenosine infusion because intracoronary adenosine was too short-acting for a pullback recording. A pullback pressure recording in the epicardial coronary artery reflects the conductance of the entire artery as well as of every individual segment. A pullback recording during adenosine infusion has the potential to differentiate between diffusely diseased coronary arteries and a focal problem, such as a coronary lesion or an underexpanded stent.

Residual Abnormal Pd/Pa and Restenosis
Several mechanisms might influence our findings of increased binary restenosis rate in lesions with a residual abnormal Pd/Pa distal to the stent. Lesions with a residual abnormal Pd/Pa might not have been covered completely by the stent, and our intention to stent from disease-free to disease-free vessel might not have been achieved. In addition, a positive remodeling may have made a diffusely diseased artery look angiographically normal, and a diffusely diseased vessel might appear smaller because of lack of a normal reference segment. This might result in underexpansion or undersizing of the stent. The same degree of in-stent neointimal hyperplasia would contribute to a larger relative lumen reduction in a smaller stent size compared with a larger stent.

Study Limitations
Several limitations related to the study design should be taken into account. First, a low FFR and a low Pd/Pa without a focal step-up during a manual pullback through the artery is a pathophysiological finding that hypothetically can be applied to “diffuse disease,” which is an anatomic description. However, we did not perform intravascular ultrasound imaging to confirm the presence of diffuse disease. Second, intravascular ultrasound imaging was not performed to confirm optimal stent expansion. Third, only disease distal to the stent was taken into account as a proximal and/or a distal “diffuse disease segment.” Multiple segments within a patient should have been taken into account in the statistical analysis, and this could raise the question of whether either the distal or the proximal segment would be the strongest predictor, but the study was not powered for this determination. Fourth, the regression analyses for restenosis are based on 88 patients having 25 events only, as evidenced by the very wide confidence limits. In addition, we performed statistical tests with no accounting for multiple testing.

All patients were treated with bare-metal stents, and the overall angiographic restenosis rate was 25.7%, which is comparable to results with the placebo group in drug-eluting stent trials.21,22 The use of drug-eluting stents has reduced restenosis rates dramatically, and our results cannot be extended to patients treated with drug-eluting stents.

Conclusion
A pullback recording during maximal hyperemia of a coronary artery treated with a stent is a rapid and simple method to analyze residual hyperemic gradients after coronary stenting. By this technique, it is possible to differentiate between a persistent gradient caused by incomplete stent deployment or by diffuse disease proximal or distal to the stent. Furthermore, a distal residual abnormal Pd/Pa seems to be a predictor of in-stent restenosis.

Disclosures
None.

References


CLINICAL PERSPECTIVE

Fractional flow reserve predicts cardiac events after coronary stent implantation. We assessed the 9-month angiographic in-stent restenosis rate in the setting of optimal stenting and a persisting gradient distal to the stent as assessed by a pressure wire pullback recording in the entire length of the artery. In 98 patients, 1 de novo coronary lesion was treated with a bare-metal stent. After stent implantation, pressure wire measurements (P_{\text{f}}=\text{mean hyperemic coronary pressure and P_{a}=\text{mean aortic pressure}) were performed in the target vessel: (1) P_{f}/P_{a} as distal to the artery as possible and (2) P_{f}/P_{a} just distal to the stent. Residual abnormal P_{f}/P_{a} was defined as a pressure drop between P_{f}/P_{a} measured at the 2 points. Fractional flow reserve distal to the artery after stenting was significantly lower (0.88±0.21 versus 0.97±0.05; P<0.001), and angiographic in-stent binary restenosis rate was significantly higher (44.0% versus 8.1%; P<0.001) in vessels with a residual abnormal P_{f}/P_{a}. Residual abnormal P_{f}/P_{a} (odds ratio, 4.39; 95% confidence interval, 1.10 to 18.16; P=0.034), reference vessel size (odds ratio, 0.17; 95% confidence interval, 0.04 to 0.69; P=0.013), and stent length (odds ratio, 1.11; 95% confidence interval, 1.03 to 1.21; P=0.009) were predictors of angiographic in-stent restenosis after 9 months. The present study demonstrated that pressure drop from an implanted stent to the distal part of the artery was a predictor of angiographic in-stent restenosis after 9 months. A pullback recording during maximal hyperemia of a coronary artery treated with a stent is a rapid and simple method to analyze residual hyperemic gradients after coronary stenting.
Cardioprotective Effects of Short-Term Caloric Restriction Are Mediated by Adiponectin via Activation of AMP-Activated Protein Kinase

Ken Shinmura, MD, PhD; Kayoko Tamaki, BS; Kiyomi Saito, PhD; Yasuko Nakano, PhD; Takashi Tobe, PhD; Roberto Bolli, MD

Background—Overeating and obesity are major health problems in developed countries. Caloric restriction (CR) can counteract the deleterious aspects of obesity-related diseases and prolong lifespan. We have demonstrated that short-term CR improves myocardial ischemic tolerance and increases adiponectin levels. Here, we investigated the specific role of adiponectin in CR-induced cardioprotection.

Methods and Results—Adiponectin antisense transgenic (Ad-AS) mice and wild-type (WT) mice were randomly assigned to a group fed ad libitum and a CR group (90% of caloric intake of ad libitum for 3 weeks, then 65% for 2 weeks). Isolated perfused mouse hearts were subjected to 25 minutes of ischemia, followed by 60 minutes of reperfusion. CR increased serum adiponectin levels by 84% in WT mice. Gel filtration analysis of the oligomeric complex distribution showed that CR produced a marked increase in the high–molecular-weight complex of adiponectin in WT mice; in contrast, CR did not change serum adiponectin levels or their oligomeric pattern in Ad-AS mice. CR improved the recovery of left ventricular function after ischemia/reperfusion and limited infarct size in WT mice; these effects were completely abrogated in Ad-AS mice. CR also increased the phosphorylated form of AMP-activated protein kinase and acetyl-CoA carboxylase in WT but not in Ad-AS mice. Recombinant adiponectin restored CR-induced cardioprotection in Ad-AS mice, and inhibition of AMP-activated protein kinase phosphorylation completely abrogated CR-induced cardioprotection in WT mice.

Conclusion—The cardioprotective effects of short-term CR are mediated by increased production of adiponectin and the associated activation of AMP-activated protein kinase. (Circulation. 2007;116:2809-2817.)

Key Words: ischemia ■ myocardial infarction ■ nutrition ■ reperfusion

More than 30% of American adults are obese (defined as a body mass index ≥30 kg/m²).1 The prevalence of obesity also is increasing in other developed countries. Obesity and overeating lead to the metabolic syndrome, resulting in increased cardiovascular disease.1–3 Novel nutritional approaches to control body weight and counteract the metabolic syndrome are becoming increasingly important.

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Caloric restriction (CR) has been widely investigated in experimental animals as a powerful intervention that can prevent and reverse aging-related changes.4–6 In general, the daily caloric intake in animals subjected to CR has been restricted to 50% to 70% of the average food intake in animals eating ad libitum (AL). Mounting evidence indicates that CR profoundly affects the physiological and pathophysiological alterations associated with aging and markedly increases lifespan in several species, including mammals.4–6 Although the ability of CR to prolong the lifespan in humans has not been demonstrated conclusively, it now seems plausible that CR may attenuate visceral fat accumulation and counteract the deleterious aspects of obesity.

Lifelong CR has been reported to restore the cardioprotective effects of ischemic preconditioning in middle-aged (10 months)7 and aged (24 months)8 rats, indicating that CR enhances the innate defense mechanisms against ischemic stress. In addition, we have recently demonstrated that short-term (4-week) CR confers cardioprotection in both young and aged hearts without causing consequences sometimes associated with CR such as increased mortality.

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The online-only Data Supplement, consisting of expanded Methods, can be found with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.107.725697/DC1.

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and malnutrition. Clearly, the use of short-term CR is easier to incorporate into clinical practice than lifelong CR; moreover, the development of CR mimetics that can replicate the cytoprotective effects of CR would be much easier to incorporate into clinical practice than a strict CR protocol.

The adipose tissue has been recognized as an endocrine organ that secretes many peptides collectively referred to as adipokines. An impressive amount of evidence indicates that adipokines play an important role in the regulation of the cardiovascular system. CR decreases perigonadal adipose tissue and alters serum levels of several adipokines. We have found that CR significantly elevates serum levels of adiponectin and lowers those of leptin in both young and old rats and increases myocardial levels of phosphorylated AMP-activated protein kinase (AMPK)-α at baseline without affecting the myocardial AMP-to-ATP ratio. On the basis of these findings, we hypothesized that the increase in circulating adiponectin levels effected by CR activates myocardial AMPK, resulting in protection against ischemia.

To test this hypothesis, we investigated in the present study the role of adiponectin in CR-induced cardioprotection using adiponectin antisense (Ad-AS) transgenic mice. We analyzed the oligomeric state of circulating adiponectin (which consists of 3 forms: the high–molecular-weight [HMW] form, the hexameric form, and the trimeric form) because, among the 3 oligomeric complexes, the HMW complex appears to be the most active and protective form, independent of total adiponectin levels. Our results demonstrate that increased production of adiponectin is essential for CR-induced cardioprotection against ischemia and suggest that the HMW form of adiponectin is likely to account for this effect by activating AMPK in the myocardium.

**Methods**

An expanded Methods section can be found in the online-only Data Supplement.

**Ad-AS Transgenic Mice**

Transgenic mice expressing an Ad-AS oligonucleotide were created as described previously. Briefly, an Ad-AS expression vector was constructed by inserting an inverted fragment of the mouse adiponectin cDNA into the unique EcoRI site between the cytomegalovirus immediate enhancer–chicken β-actin promoter and the 3′ flanking sequence of the rabbit β-globin gene of the pCAGGS expression vector. A Basic Local Alignment Search Tool (BLAST) analysis (National Center for Biotechnology Information, National Library of Medicine, Bethesda, Md) of the GenBank nucleotide database indicated that this antisense sequence showed no significant homology to any other mouse genes.

**CR Protocols**

CR was performed as described previously. Briefly, 8-week-old male Ad-AS and wild-type (WT) mice were housed in individual cages and fed AL for 3 weeks. After weaning, mice were randomly allocated into 2 groups. AL mice continued to be fed AL using control diet A for the subsequent 5 weeks. CR mice were fed 90% of the average caloric intake during the AL period for 3 weeks (10% restriction), followed by 65% of that for 2 weeks (35% restriction).

**Ischemia/Reperfusion Protocol and Measurement of Infarct Size**

Under anesthesia, the hearts were excised quickly and perfused with modified Krebs-Henseleit buffer according to the Langendorff procedure, as described previously.

All hearts were subjected to 25 minutes of global no-flow ischemia, followed by 60 minutes of reperfusion. Infarct size (percent of the left ventricle [LV]) was quantified as described previously. The perfusate was collected during reperfusion, and total creatine kinase (CK) activity released into the perfusate was measured with commercially available spectrophotometric assays (Sigma-Aldrich, St Louis, Mo).

**Measurement of Serum Adiponectin Levels**

Mice were fasted overnight, and blood samples were collected from the chest cavity when the hearts were excised. Serum levels of adiponectin were measured with a commercially available ELISA kit (R&D Systems, Minneapolis, Minn). Gel filtration analysis of the oligomeric complex distribution of adiponectin was performed with serum samples from each group (n=4) as described previously.

**Western Immunoblotting**

Standard SDS-PAGE Western immunoblotting was performed with 40 μg protein sample as described previously. DENMOMOIOO values (arbitrary density units) of the phosphorylated protein were normalized to the total amount of protein detected and expressed as a percentage of the corresponding values in AL WT mice. Polyclonal antibodies against AMPK, phosphorylated AMPK-α at the Thr172 residue, acetyl-CoA carboxylase (ACC), and phosphorylated ACC at the Ser27 residue were purchased from Cell Signaling (Beverly, Mass).

**Metabolites Analysis**

Myocardial glycogen content was determined from methanol precipitates of KOH-digested tissue using the amyloglucosidase method. ATP and creatine phosphate contents were determined spectrophotometrically from neutralized perchloric acid extracts of tissue samples as described previously.

**Rescue and AMPK Inhibition Experiments**

An Alzet micro-osmotic pump (model 1007D, DURECT, Cupertino, Calif) was implanted subcutaneously in the intrascapular region of Ad-AS CR mice 1 week before their death. The reservoir of each pump was preloaded with 96 μl sterile Tris-buffered saline or murine recombinant adiponectin (rAd; 1.55 μg/μl, BioVision, Mountain View, Calif). CR was continued for 1 additional week, and 5 hearts from each group were subjected to the same ischemia/reperfusion protocol as described above.

WT mice were treated with adenine 9-D arabinofuranoside (AraA, Sigma-Aldrich), an AMPK inhibitor. Thirty minutes before the mice were killed, 2 μg/g AraA or vehicle was injected intravenously. Four hearts from each group were used for Western immunoblotting. Six hearts from WT mice treated with AraA or vehicle were perfused with modified Krebs-Henseleit buffer containing 10 μM insulin, 0.4 mmol/L oleate, and 1% BSA to assess the physiological role of AMPK. Hearts were subjected to the ischemia/reperfusion protocol as described above.

**Statistical Methods**

Data are reported as mean±SEM. Serum adiponectin levels, cardiac parameters, infarct size, total CK activity, and densitometric measurements of Western immunoblots were analyzed by 2-way ANOVA (WT versus Ad-AS, AL versus CR), followed by Scheffé’s test for post hoc comparisons. One-way ANOVA was used when the effect of recombinant vehicle or adiponectin supplementation was compared among 3 groups. Differences were considered significant.
Table 1. Morphological and Cardiac Parameters at Baseline in Each Group

<table>
<thead>
<tr>
<th></th>
<th>WT Mice</th>
<th>Ad-AS Mice</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>AL</td>
<td>CR</td>
</tr>
<tr>
<td>Morphological</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW at baseline, g*</td>
<td>24.1±0.4</td>
<td>24.6±0.5</td>
</tr>
<tr>
<td>BW before death, g*</td>
<td>27.4±0.7</td>
<td>21.5±0.6$§$</td>
</tr>
<tr>
<td>Change in BW, %*</td>
<td>13.7±1.5</td>
<td>12.6±1.1$§$</td>
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<tr>
<td>LVW (mg)†</td>
<td>76.6±2.4</td>
<td>66.7±1.9$§$</td>
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<tr>
<td>LVW/BW (%)†</td>
<td>0.28±0.01</td>
<td>0.31±0.01$§$</td>
</tr>
<tr>
<td>Cardiac‡</td>
<td></td>
<td></td>
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<tr>
<td>HR, bpm</td>
<td>502±17</td>
<td>525±11</td>
</tr>
<tr>
<td>LVDP, mm Hg</td>
<td>91±4</td>
<td>85±4</td>
</tr>
<tr>
<td>dP/dt</td>
<td>3760±140</td>
<td>3780±160</td>
</tr>
<tr>
<td>−dP/dt</td>
<td>3380±110</td>
<td>3470±330</td>
</tr>
<tr>
<td>RPP$\times10^2$</td>
<td>507±20</td>
<td>499±20</td>
</tr>
</tbody>
</table>

BW indicates body weight; LVW, LV weight; HR, heart rate; and RPP, rate-pressure product.

$n=18$ each; $\dagger n=14$ each; $\ddagger n=6$ each.

$P<0.05$ vs AL.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Effect of CR on Body Weight and Serum Adiponectin Levels

No difference existed in body weight at baseline between WT and Ad-AS mice or between AL and CR mice (Table 1). Five weeks of CR decreased body weight to a similar extent in both strains. No mouse died during CR period in either strain.

Serum levels of adiponectin were lower in Ad-AS AL than in WT AL mice (Figure 1A). CR significantly increased serum adiponectin levels in WT mice. However, CR did not change these levels significantly in Ad-AS mice ($0.05<P<0.1$).

Gel filtration analysis of the oligomeric complex distribution of adiponectin showed an increase in all fractions with CR in WT mice; the increase was particularly pronounced for the HMW form (first peak) (Figure 1B). In contrast, the increase in the HMW form of adiponectin was not significant in Ad-AS CR mice (Figure 1C).

Effect of CR on Myocardial Ischemia/Reperfusion Injury

Although CR significantly reduced LV weight in both strains, no difference was observed in LV weight between WT AL and Ad-AS AL mice or between WT CR and Ad-AS CR mice (Table 1). In addition, no difference was present in LV function at baseline between WT and Ad-AS mice (Table 1). In WT mice, CR significantly improved the recovery of LV developed pressure (LVDP), peak positive dP/dt, and peak negative dP/dt throughout reperfusion compared with WT AL mice (Figure 2A, 2C, and 2D). In Ad-AS mice, CR improved percent recovery of dP/dt and −dP/dt at 30 and 40 minutes after reperfusion, but this effect was transient, and the difference between AL and CR was no longer statistically significant 50 minutes after reperfusion (Figure 2C and 2D).

In WT mice, CR significantly reduced infarct size as detected by 2,3,5-triphenyltetrazolium chloride (TTC) staining (Figure 3A and 3B). In contrast, infarct size in Ad-AS CR mice was equivalent to that in Ad-AS AL mice, indicating that the cardioprotective effect of CR is abrogated in Ad-AS mice.
mice. The results obtained with TTC staining were substantiated by the measurement of total CK activity released into the perfusate (Figure 3C). Attenuation of CK release during reperfusion was not observed in Ad-AS mice subjected to CR.

**Effect of CR on AMPK and ACC Phosphorylation**

Myocardial expression levels of total AMPK protein were similar between WT and Ad-AS mice and between AL and CR mice (Figure 3A). At baseline, myocardial levels of AMPK-\(\alpha\) phosphorylated at the Thr\(^{172}\) residue increased significantly with CR in WT mice (Figure 3A and 3B), suggesting that CR activated AMPK in WT hearts. In contrast, no increase occurred in myocardial levels of AMPK-\(\alpha\) phosphorylated at the Thr\(^{172}\) in Ad-AS CR compared with Ad-AS AL mice. As expected, myocardial levels of phosphorylated ACC at the Ser\(^{79}\) residue increased significantly with CR in WT mice, but no increase in phosphorylated ACC occurred in Ad-AS CR mice (Figure 4C and 4D). Prolonged ischemia caused a robust increase in the expression levels of phosphorylated AMPK-\(\alpha\) in both strains; no difference was present in phosphorylated AMPK-\(\alpha\) levels between AL and CR in either strain (Figure 4A and 4B).

**Effect of CR on Myocardial Metabolites**

Myocardial glycogen content at baseline was similar between AL and CR in WT mice (Table 2). In contrast, myocardial glycogen content in Ad-AS CR mice was less than that in Ad-AS AL mice, suggesting that activated AMPK plays a role in maintaining myocardial glycogen content under CR. No difference existed in myocardial ATP and creatine phosphate content between AL and CR in both strains.

**Restoration of CR-Induced Cardioprotection by Exogenous Adiponectin in Ad-AS Mice**

The delivery of rAd via implanted micro-osmotic pumps in Ad-AS CR mice resulted in circulating levels of adiponectin similar to those observed in WT mice treated with CR (Figure 5A). Consequently, the recovery of LV function after ischemia/reperfusion was significantly improved and the infarct size was reduced in Ad-AS CR mice implanted with micro-osmotic pumps delivering rAd compared with Ad-AS mice implanted with pumps delivering vehicle (Figure 5B and 5C). These results indicate that administration of exogenous adiponectin can restore the cardioprotective effect of CR in Ad-AS mice.
**Treatment With an AMPK Inhibitor Abrogates CR-Induced Cardioprotection**

Administration of AraA decreased the expression levels of phosphorylated AMPK in response to CR in WT mice (Figure 6A and 6B). The dose of AraA chosen in the present study did not affect LV function at baseline in either WT AL or WT CR mice (data not shown). The recovery of LVDP after ischemia/reperfusion was significantly better and the infarct size was smaller in WT mice treated with CR compared with WT AL mice, even though insulin and free fatty acid were added to the perfusate (Figure 6C and 6D). Inhibition of AMPK phosphorylation by AraA completely abrogated the cardioprotective effect of CR in WT mice, although administration of AraA in itself did not exacerbate the degree of ischemia/reperfusion injury (Figure 6C and 6D).

**Discussion**

This study provides 4 major findings: (1) short-term CR confers protection against myocardial ischemia/reperfusion injury in WT mice; (2) the increase in circulating adiponectin levels associated with CR is necessary for the cardioprotective effects of CR; (3) increased production of the HMW form of adiponectin during CR is associated with activation of AMPK (as indicated by the increase in phosphorylated AMPK-α); and (4) activation of AMPK plays an obligatory role in the cardioprotection afforded by short-term CR.

CR is currently the only known intervention that significantly prolongs the maximal lifespan in mammals.4–6 It is speculated to be of possible relevance in delaying the deleterious effects of aging in humans.4–6 However, the exact mechanisms by which CR prolongs lifespan and reverses senescent changes have not been clarified. Lifelong CR significantly attenuates tissue oxidative damage and decreases apoptosis.4–6 Recent reports suggest that CR provokes a mild stress response, resulting in enhanced cell defenses, probably coordinated by the endocrine system (called hormesis).5,6 This concept is analogous to the well-known phenomenon of preconditioning, in which a sublethal stress greatly enhances the tolerance of the organ to a subsequent more severe stress.26,27 Accordingly, we hypothesized that short-term CR preconditions organs and improves ischemic tolerance. As predicted by this hypothesis, short-term CR improved myocardial ischemic tolerance in rats9 and in mice. It is likely that multiple mechanisms account for the protective effect of CR against myocardial ischemia/reperfusion injury. However, on the basis of our previous work, we hypothesized that activation of AMPK, accompanied by changes in adipocyte-derived cytokines, is mainly responsible for CR-induced cardioprotection.9

CR markedly changes adipokine production9,12,13,28 specifically, circulating levels of adiponectin increase and those of leptin decrease during CR.9,13 Higami et al28 demonstrated that CR profoundly decreases mRNA levels of leptin in adipose tissue (∼1/10th) and increases those of adiponectin (1.5-fold) by CR. Thus, it is plausible that...
adipose tissue modulates the effects of CR by secreting humoral factors that promote health and prevent the aging process. However, direct evidence that adipose-derived factors are essential for the beneficial effect of CR is lacking.

To address this crucial issue, we investigated the role of adiponectin in CR-induced cardioprotection using mice expressing an Ad-AS gene. We chose heterozygous littermates because we sought to evaluate the specific role of adiponectin in CR using animals that were as healthy as possible. Although heterozygous Ad-AS mice showed a slight decrease in serum adiponectin levels, no differences were evident between WT and Ad-AS mice with respect to LV weight, baseline LV function, and the extent of myocardial ischemia/reperfusion injury (Table 1 and Figures 2 and 3). In contrast, the increase in adiponectin production by CR was completely suppressed in Ad-AS mice, suggesting that we successfully separated the role of adiponectin during CR from that under physiological conditions. Shibata et al29 reported that infarct size after in vivo ischemia/reperfusion was significantly increased in adiponectin knockout mice compared with WT mice, indicating that endogenous production of adiponectin has a protective effect against myocardial ischemia/reperfusion injury. In contrast, in the present study, infarct size in Ad-AS transgenic mice was similar to that in WT mice. The maintenance of normal circulating adiponectin levels at baseline in Ad-AS mice could explain the discrepancy between the results obtained with adiponectin knockout mice and results in Ad-AS mice. Our present findings that CR-induced cardioprotection is abrogated in Ad-AS mice and that exogenous administration of recombinant adiponectin via implanted micro-osmotic pumps (Figure 5) significantly improved percentage recovery of LVDP and reduced infarct size (Figure 6).

**Figure 5.** Serum adiponectin levels (A), percentage recovery of LVDP (B), and infarct size (C) in Ad-AS mice implanted with osmotic pumps. A, The delivery of recombinant adiponectin via implanted micro-osmotic pumps in Ad-AS CR (Ad-AS CR+rAd) achieved circulating levels of adiponectin similar to those observed in WT mice treated with CR. B and C, Consequently, percentage recovery of LVDP was significantly improved and infarct size was reduced in Ad-AS CR+rAd mice. Data are mean±SEM. *P<0.05 vs AL, +P<0.05 vs corresponding vehicle (v) group.

**Figure 6.** Western immunoblotting for AMPK (A and B), percentage recovery of LVDP (C), and infarct size (D) in WT CR mice treated with AraA. A, Representative Western immunoblots showing the expression of total and phosphorylated AMPK-α at the Thr172 residue. B, Densitometric analysis of phosphorylated AMPK-α (Thr172) signals. A and B, The increase in myocardial levels of phosphorylated AMPK-α in WT with CR was abrogated by pretreatment with AraA. C and D, The cardioprotection afforded by CR was completely blocked by the inhibition of AMPK activation. Data are mean±SEM. *P<0.05 vs AL, +P<0.05 vs corresponding vehicle group (AraA[−]).
Adiponectin in Ad-AS mice restores CR-induced cardioprotection, providing direct evidence that demonstrates, for the first time, a necessary role of increased adiponectin production in the beneficial effects of CR. Saito et al. reported a remarkable decrease in body weight and adipose tissue during 3 days of starvation in Ad-AS mice. In the present study, the decrease in body weight in Ad-AS mice was similar to that in WT mice, and no mice in either strain died during CR. However, myocardial glycogen content was less in Ad-AS mice treated with CR, associated with attenuated AMPK activation by CR (Table 2 and Figure 4B). These results further support the previous conclusion that adiponectin plays an important role in maintaining energy homeostasis under energy depletion in mammals.

The mechanisms by which adiponectin protects myocardium from ischemia/reperfusion injury have not been fully elucidated. Shibata et al. have demonstrated that adiponectin alleviates ischemia/reperfusion injury via AMPK- and cyclooxygenase-2–dependent mechanisms. We could not find any increase in cyclooxygenase-2 protein in CR hearts, and the expression levels of cyclooxygenase-2 remained at low levels (data not shown). Thus, it is unlikely that cyclooxygenase-2 mediates the cardioprotective effect of short-term CR. Recently, Tao et al. reported that disruption of adiponectin gene exacerbates myocardial ischemia/reperfusion injury as a result of increased oxidative/nitratative stress via enhanced induction of inducible nitric oxide synthase and gp91phox protein. CR reduces oxidative stress, but the involvement of the inducible nitric oxide synthase/gp91phox system in CR-induced cardioprotection remains to be clarified.

It is still controversial whether activation of AMPK is detrimental or protective to the ischemic heart. Most reports demonstrate that activation of AMPK improves myocardial ischemic tolerance, resulting in attenuated myocardial ischemia/reperfusion injury. However, AMPK-dependent acceleration of fatty acid oxidation during reperfusion has the potential to be detrimental in the setting of ischemia/reperfusion. In the present study, CR confers protection in isolated heart perfused with or without insulin and free fatty acids. Before the induction of ischemia, marked activation of AMPK was present in WT mice treated with CR. These results strongly suggest that activation of AMPK before ischemia is protective against myocardial ischemia/reperfusion injury. The loss of CR-induced cardioprotection by AraA treatment before ischemia supports this concept, although this compound is not specific for AMPK, and limitations of pharmacological inhibition should be taken into account when these results are interpreted. Interestingly, AMPK was activated in CR hearts, but myocardial glycogen content and high-energy phosphate content were similar to those in AL hearts. These results are consistent with previous reports on the energy metabolites in the CR heart and suggest that activated AMPK may compensate for the limited supply of energy during CR by increasing the uptake of substrates and glycolysis rather than by increasing glycogenolysis. The decreased myocardial glycogen content in Ad-AS mice with CR may contribute, at least in part, to greater damage after ischemia/reperfusion compared with WT mice treated with CR because glycogenolysis is protective against ischemia/reperfusion injury until the accumulation of deleterious metabolites (lactate, H+, NADH, and inorganic phosphate) outweighs the benefit of ATP production.

The increase in the cellular AMP-to-ATP ratio is a major regulator of AMPK activity, but recently, adipocyte-derived hormones also have been reported to activate AMPK. Most of the beneficial effects of adiponectin appear to be mediated by AMPK-associated signaling. In the present study, AMPK-α phosphorylated at the Thr172 residue, the activated form of AMPK, was increased by CR in WT mouse hearts, and the magnitude of cardiac AMPK phosphorylation during CR appears to correlate with the distribution of the HMW oligomers. Different oligomeric forms of adiponectin bind to the specific adiponectin receptors adip R1 and adip R2 in a distinct manner, activating different signaling pathways and exerting distinct functions on the target tissues. Tsao et al. reported that the globular domain of adiponectin, which can form only trimers, is more potent than other forms in activating AMPK in rat skeletal muscle. In contrast, Pajvani et al. showed that the HMW complex is the most active form of adiponectin in lowering blood glucose levels in mice. Furthermore, only the HMW form can protect endothelial cells from apoptosis. These considerations support the concept that increased production of the HMW complex by CR activates AMPK, resulting in cardioprotection against ischemia.

Further studies are necessary to determine whether the increase in the HMW form of adiponectin also contributes to the various effects of lifelong CR. Whether the metabolic adaptation to lifelong CR in the heart is related to changes in AMPK activity is controversial. It seems reasonable to assume that lifelong CR switches off ATP-consuming pathways and switches on ATP-saving pathways. Long-term CR experiments using Ad-AS mice might resolve the role of the adiponectin-AMPK signaling pathway in lifelong CR.

In summary, this study demonstrates a cause–effect relationship between increased adiponectin production and cardioprotection by short-term CR. In addition, the present data demonstrate that inhibition of AMPK activation, which occurs before ischemia in the CR heart, completely abrogates the cardioprotection afforded by CR. Therefore, we conclude that short-term CR preconditions the myocardium against lethal ischemic injury by enhancing adiponectin production and activating AMPK. In this connection, activation of AMPK has been shown to occur in the setting of ischemic preconditioning. The present results also suggest that activators of adiponectin-mediated signaling may be potentially useful as a novel class of cardioprotective agents (CR mimetics).

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Disclosures

None.

References


Clinical Perspective

Overeating and obesity are major health problems in advanced countries. They lead to the metabolic syndrome, resulting in the increased incidence of cardiovascular disease. Accumulation of visceral fat is proposed to be a fundamental pathology because adipokines secreted from visceral fat are closely related to the development of obesity-related diseases; among these adipokines, adiponectin is important because it has antiarteriosclerotic and cardioprotective properties. Caloric restriction (CR) has been widely investigated as a powerful intervention that can prevent and reverse aging-related changes. CR has recently attracted additional interest as a means of controlling body weight and counteracting the metabolic syndrome. Given the adverse effects of obesity, it is plausible that CR provides health benefits by decreasing fat mass. However, the exact mechanisms by which CR prolongs lifespan and reverses the deleterious aspects of obesity have not been clarified. Using the adiponectin antisense transgenic mouse, this study demonstrates that short-term CR confers cardioprotection and that the increase in circulating adiponectin levels, especially the increase in the high-molecular-weight form of adiponectin, associated with CR is necessary for CR-induced cardioprotection. Furthermore, downstream activation of AMP-activated protein kinase plays an obligatory role in this cardioprotection. These findings provide novel therapeutic approaches for managing obese patients with advanced arteriosclerosis. In addition to CR, activators of adiponectin-mediated signaling may be potentially useful as a novel class of cardioprotective agents (CR mimetics). The data also suggest that the increase in the high–molecular-weight form of adiponectin may be a marker of successful CR.
Age Decreases Endothelial Progenitor Cell Recruitment Through Decreases in Hypoxia-Inducible Factor 1α Stabilization During Ischemia

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Background—Advanced age is known to impair neovascularization. Because endothelial progenitor cells (EPCs) participate in this process, we examined the effects of aging on EPC recruitment and vascular incorporation.

Methods and Results—Murine neovascularization was examined by use of an ischemic flap model, which demonstrated aged mice (19 to 24 months) had decreased EPC mobilization (percent mobilized 1.4±0.2% versus 0.4±0.1%, P<0.005) that resulted in impaired gross tissue survival compared with young mice (2 to 6 months). This decrease correlated with diminished tissue perfusion (P<0.005) and decreased CD31vascular density (P<0.005). Gender-mismatched bone marrow transplantation demonstrated significantly fewer chimeric vessels in aged mice (P<0.05), which confirmed a deficit in bone marrow–mediated vasculogenesis. Age had no effect on total EPC number in mice or humans. Reciprocal bone marrow transplants confirmed that impaired neovascularization resulted from defects in the response of aged tissue to hypoxia and not from intrinsic defects in EPC function. We demonstrate that aging decreased hypoxia-inducible factor 1α stabilization in ischemic tissues because of increased prolyl hydroxylase-mediated hydroxylation (P<0.05) and proteasomal degradation. This resulted in a diminished hypoxia response, including decreased stromal cell–derived factor 1 (P<0.005) and vascular endothelial growth factor (P<0.0004). This effect can be reversed with the iron chelator deferoxamine, which results in hypoxia-inducible factor 1α stabilization and increased tissue survival.

Conclusions—Aging impairs EPC trafficking to sites of ischemia through a failure of aged tissues to normally activate the hypoxia-inducible factor 1α–mediated hypoxia response. (Circulation. 2007;116:2818-2829.)

Key Words: aging ■ vasculogenesis ■ hypoxia ■ ischemia

Advanced age has been associated with a decreased ability to form new blood vessels in response to ischemia, which results in higher rates of cardiovascular complications and diminished capacity for tissue regeneration. Whether this is due to an intrinsic decline in the regenerative capacity of putative vascular progenitors or a decline in a proregenerative niche remains unclear. Adult neovascularization occurs by 2 distinct processes: angiogenesis (the sprouting of new blood vessels from preexisting ones) and vasculogenesis (the recruitment, proliferation, and assembly of bone marrow–derived endothelial progenitor cells [EPCs] into new vessels). HIF-1α (hypoxia-inducible factor 1α) is the transcription factor known to regulate both of these processes through mediators such as vascular endothelial growth factor (VEGF) and stromal cell–derived factor-1 (SDF-1α). It has been postulated that aging results in a decline in progenitor cell function, also known as progenitor exhaustion. Current investigational stem cell therapies are based on replenishing this depleted supply of functional progenitor cells to areas of injury. Once delivered, it is hoped that progenitor cells will engraft at sites of injury and differentiate into native cell populations to regenerate tissue; however, clinical trials attempting to replenish progenitor cells after myocardial infarction have been disappointing and have often failed to maintain any lasting benefit. The inability to
retain progenitor cells in the injured myocardium suggests that administration of progenitor cells alone is insufficient for tissue repair.

Thus, we hypothesized that defects in neovascularization are not due to a lack of functional EPCs but to the body’s diminished ability to recruit EPCs to sites of injury. We have previously shown that SDF-1α plays an important role in the recruitment of EPCs to sites of ischemia. Given that hematopoietic, endothelial, neural, and skeletal/smooth muscle progenitor cells all respond to SDF-1α, we propose that deficits in SDF-1α expression may represent a significant mechanism for globally impaired tissue regeneration in the aged population.

In the present study, we investigated the inherent function of young and aged EPCs to respond to an ischemic insult. We studied the effects of SDF-1α stimulation of EPCs, as well as the host’s ability to upregulate SDF-1α signaling in vivo and in vitro. Furthermore, we investigated the role of HIF-1α and prolyl hydroxylases (PHD 1, 2, and 3) on SDF-1α expression. Finally, we used deferoxamine (DFO), a known HIF-1α stabilizer, to restore the “young” environment in aged mice and reverse age-related complications.

Methods

Mouse Ischemia Model

Young (4 to 6 months, Jackson Laboratories, Bar Harbor, Me) and aged (18 to 24 months, National Institute of Aging, Bethesda, Md) C57/BL6 mice underwent ischemic flap surgery in accordance with the guidelines of the New York University and Stanford University institutional animal care and use committees. Briefly, a peninsular skin flap (2.5×1.25 cm) was elevated dorsally and resutured after a silicone sheet was placed between the flap and wound bed. This created a reproducible ischemic gradient confirmed by color laser Doppler (Moor Instruments, Devon, United Kingdom) and oxygen-sensing probe measurements (Oxford Optronix, Oxford, United Kingdom). See the online-only Data Supplement for an expanded Methods section.

Mouse EPC Mobilization Assay

Peripheral blood was obtained from young (n=5) and aged (n=5) mice, and erythrocytes were lysed with ammonium chloride and separated into pellets. Cells were then washed with PBS/EDTA and separated into multichannel fluorescence-activated cell sorting for phycoerythrin-labeled Flk-1(VEGFR-2; BD Pharmingen, San Jose, Calif) and FITC-labeled CD11b (BD Pharmingen). Mouse EPCs were identified as Flk-1 (VEGFR-2; BD Pharmingen, San Jose, Calif) and FITC-labeled CD11b (BD Pharmingen). We used deferoxamine (DFO), a known HIF-1α stabilizer, to restore the “young” environment in aged mice and reverse age-related complications.

Mouse Neovascularization Assay

On postoperative day 14, mice (n=4 per group) were euthanized and flap sections harvested. Frozen sections were stained for CD31 (BD Pharmingen) as described previously.

Mouse Bone Marrow Transplantation Model

Gender-mismatched bone marrow transplantations were performed in 4 groups of mice (n=5 per group): young donor/young recipient, young donor/aged recipient, aged donor/young recipient, and aged donor/aged recipient. Bone marrow was harvested from femurs and tibia of male mice. The buffy coat was isolated with Histopaque 1083 (Sigma-Aldrich, St Louis, Mo) and washed with PBS/EDTA, and 1×10⁶ cells in 200 μL of DMEM (Gibco [Invitrogen], Carlsbad, Calif) were injected via the femoral vein into irradiated female mice (1.6 Gy for 1.5 minutes for 2 cycles). Animals were allowed 30 days to reconstitute.

Recruitment of Mouse EPCs to Ischemic Flap

Flap sections from bone marrow–transplanted animals were deparaffinized in Citrisolv (Fisher Scientific, Fairlawn, NJ). CD31-PE (BD Pharmingen) and Y-chromosome–FITC (Cambio, Cambridge, United Kingdom) containing was performed as described previously; sections were counterstained with DAPI and examined under a multichannel fluorescent microscope (Olympus, Center Valley, Pa). Cells positive for CD31, Y chromosome, and DAPI markers were designated EPCs.

Deferoxamine Rescue of the Ischemic Flap

Deferoxamine (DFO; 10 mg/kg; Calbiochem, San Diego, Calif) was injected intraperitoneally into aged mice (n=4) 1 day before surgery and every other day. On postoperative day 7, flaps were analyzed grossly and for CD31 staining and EPC mobilization as described above.

Enzyme-Linked Immunosorbent Assay

Quantikine human VEGF and murine SDF-1α ELISA kits (R&D Systems, Minneapolis, Minn) were used according to the manufacturer’s instructions.

EPC Chemotaxis

EPC migration was evaluated with the NeuroPore transwell assay (8-μm pore size) as described previously.

Human EPC Proliferation

EPCs (1×10⁶) were plated in 12-well fibronectin-coated plates (n=12) and serum starved for 24 hours with media containing 0.5% FBS. A [³H] thymidine incorporation assay was performed as described previously.

Determination of Human EPC Peripheral Blood Population

Peripheral blood (60 mL), isolated from younger (n=18, 18 to 35 years old) and older (n=21, 68 to 95 years old) patients in accordance with the guidelines of the New York University institutional review board, was separated with Histopaque 1077 (Sigma-Aldrich). The buffy coat was washed with PBS/10% FBS. Cells were labeled with AC133-PE and sorted by fluorescence-activated cell sorting as described previously.

Cell Culture

Fibroblasts and endothelial cells were harvested from healthy younger (n=9, 18 to 35 years old) and older (n=7, 68 to 95 years old) patients undergoing melanoma excision in accordance with guidelines of the New York University institutional review board. Young and aged fibroblasts were also obtained from the National Institute on Aging’s Coriell Institute cell repository in Camden, NJ. Tissue explants were digested with 0.07% Liberase III Blendzyme (Roche, Palo Alto, Calif), filtered through a 70-μm nylon mesh, washed with PBS/0.2% BSA, and purified with CD31-coated magnetic beads. Endothelial cells were cultured on gelatin-coated plates in EGM-2 (Cambrex, Exh Rutherford, NJ). Unbound fibroblasts were cultured in DMEM (Gibco) with 10% FBS/1% antibiotics. Cultures were exposed to hypoxia (0.5% O₂, 5% CO₂) or normoxia (21% O₂, 5% CO₂) for 12 hours before protein and RNA harvesting. Cells obtained from the National Institute on Aging were grown in αMEM/15% FBS.

Primary murine fibroblasts were harvested in a similar fashion and subjected to hypoxia or normoxia and 100 μmol/L DFO (Calbiochem) for 18 hours. For hydroxylated HIF-1α studies, 10 μmol/L of the proteasome inhibitor MG132 (Peptides International, Louisville, Ky) was added, and protein and RNA were harvested.

Quantitative Real-Time Polymerase Chain Reaction

RNA was harvested with the RNeasy Fibrous Tissue Mini-Kit (Qiagen, Valencia, Calif) and from cell culture with the RNeasy...
Mini-Kit (Qiagen) according to the manufacturer’s instructions. mRNA was reverse transcribed to cDNA with the RNA PCR Core kit (Applied Biosystems, Foster City, Calif). Quantitative gene expression was determined with the Roche LightCycler 1.2 instrument with the LightCycler FastStart DNA MasterPLUS SYBR Green I kit (Roche). Absolute gene transcription was normalized to β-actin.

Western Blot
A total of 50 to 80 μg of total protein extracted with RIPA buffer was separated on 7.5% SDS-PAGE, transferred to polyvinylidene fluoride membranes, and blocked with 5% milk/TBS with Tween. Separated on 7.5% SDS-PAGE, transferred to polyvinylidene difluoride membranes, and blocked with 5% milk/TBS with Tween. Western Blot

Luciferase Assay
A previously constructed murine SDF-1 luciferase reporter construct was created by cloning the 2-kilobase SDF-1 promoter into the pGL3-basic luciferase vector (Promega, Madison, Wis). The reporter plasmid was cotransfected with a constitutively expressed Renilla luciferase construct (pHRL-TK, Promega) into primary young and aged murine fibroblasts by use of the Lipofectamine Plus reagent (Invitrogen). Twenty-four hours after transfection, cells were incubated in hypoxia (1% O₂), normoxia, or normoxia with 100 μmol/L DFO for 18 hours. Luciferase activity was determined with the Roche LightCycler 1.2 instrument with the LightCycler FastStart DNA MasterPLUS SYBR Green I kit (Applied Biosystems, Foster City, Calif). Quantitative gene transcription was normalized to

Results

Progenitor-Mediated Vascular Remodeling Is Impaired With Age
We examined the impact of aging on ischemia-induced vascular regeneration using a well-described soft tissue ischemia model.4 Aged mice exhibited a marked impairment in tissue survival (Figure 1A and 1B) and tissue oxygenation 7 days postoperatively compared with young mice (perfusion ratio 0.88±0.04 versus 0.58±0.07, P<0.005; Figure 1C).

To assess the impact of aging on the bone marrow compartment, ischemic flaps from age-matched and gender-mismatched mice were analyzed for EPC-derived neovessels using the CD31/CD11b progenitor cells13,14 compared with young animals, which suggests a vasculogenesis-specific impairment with age (1.4±0.2% EPCs versus 0.4±0.1% EPCs, P<0.005; Figure 1D), which also correlated with decreased vascular density (number of vessels in section D per high-powered field: 1.96±0.24 versus 1.24±0.1, P<0.005; Figure 1E).

Statistical Analysis
All data are reported as mean±SEM, with the exception of fold induction, percentage increase, and ratios, which are reported as mean±SD. All in vitro cell experiments were repeated in triplicate, and all in vivo experiments (ischemic flap operations and bone marrow transplants) were performed with a minimum of 4 mice in each group. Statistical significance was evaluated with a paired Student t test or ANOVA, with P<0.05 considered significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Table. Primer Sequences

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FWD indicates forward; REV, reverse; and FIH, factor-inhibiting HIF.
versus 5.2 ± 1.2 vessels per high-powered field, *P* < 0.05; Figure 1F).

Lectin staining in situ also demonstrated patent vascular channels lined with EPCs (data not shown). These data suggest that aging impairs neovascularization in ischemia by reducing bone marrow–mediated vasculogenesis, which results in decreased vascular density and overall tissue survival.

**Figure 1.** Aging impairs progenitor-mediated vascular remodeling. A, Overall ischemic flap tissue survival showing poor flap survival in aged vs young mice. B, Representative ischemic flaps showing complete healing in the young mouse and necrosis in the aged mouse. C, Top, Color laser Doppler showing significantly decreased tissue perfusion in aged ischemic flaps; Bottom, oxygen tension and blood flow are also impaired in aged mice. D, Top, Fluorescence-activated cell sorting analysis demonstrating reduced EPC mobilization in aged mice; bottom, representative fluorescence-activated cell sorting histograms for young and aged mice. E, Decreased capillary density in aged flaps, defined as the ratio of CD31⁺ vessels in section B to that in section D. F, Higher percentage of chimeric blood vessels in young mice. Hpf indicates high-powered field.

**Intrinsic Function of Primitive Vascular Progenitors Is Relatively Preserved With Age**

It has been suggested that the observed impairments in vasculogenesis with age are due to “progenitor exhaustion.” However, examination of the mouse bone marrow compartment for primitive Sca-1⁺/c-kit⁺/lin⁻ (SKL) vascular progenitors, thought to be a more primitive precursor of...
EPCs, demonstrated no significant differences between young and aged animals (1470±475 versus 2220±272, \( P>0.05 \); Figure 2A).

Progenitor cell migration in vitro has been used to evaluate cell function and has been correlated with specific risk factors and severity of cardiovascular disease.\(^{20,21}\) Primitive bone marrow–derived SKL vascular progenitors purified from young and aged mice migrated in similar numbers toward an SDF-1\(\alpha\) gradient (596±84 versus 560±159, \( P>0.05 \); Figure 2B), which suggests that aged vascular progenitors functioned as well as their young counterparts.

To examine whether aged EPCs retained the ability to respond to an ischemic stimulus, systemic SDF-1\(\alpha\) was administered intraperitoneally, and the number of mobilized EPCs was analyzed. Strikingly, both young and aged animals mobilized comparable numbers of EPCs (% mobilized: 1.30±0.16% versus 1.35±0.25%, \( P>0.05 \); Figure 2C), which suggests not only that an adequate reservoir of EPCs exists in aged bone marrow but also that EPCs were capable of responding appropriately to a systemic stimulus. These findings further suggest that a defect in the signals necessary for EPC mobilization and recruitment may contribute in part or entirely to the decreased neovascularization seen with aging.

To confirm the human relevance of these findings, we examined the number and functional capacity of EPCs in healthy younger (\( n=18 \), mean age 25.7 years, range 18 to 35 years) and older (\( n=21 \), mean age 82.1 years, range 68 to 95 years) human subjects without evidence of cardiovascular disease,\(^{22}\) diabetes mellitus,\(^{20}\) or cholesterol-lowering statin therapy.\(^{23}\) No significant difference was present in baseline numbers of circulating EPCs between younger and older patients presented as percentage of baseline circulating mononuclear cells (% baseline, 0.184±0.09% versus 0.183±0.010%, \( P>0.05 \); Figure 2D). Likewise, no significant difference was present in EPC colony formation (19±8 versus 18±6 colonies, \( P>0.05 \); Figure 2E), migration toward SDF-1\(\alpha\) (604±103 versus 603±64 cells, \( P>0.05 \); Figure 2F), or hypoxia-induced proliferation (2524±400 versus 1973±79 cells, \( P>0.05 \); Figure 2G), which demonstrates that the intrinsic EPC function is preserved in humans with advancing age. Collectively, these data suggest that the decline in neovascularization that occurs with aging is not a consequence of decreased EPC number or function.

**Figure 2.** Intrinsic function of primitive vascular progenitors is relatively preserved with age. A, Relatively preserved numbers of Sca-1\(^+\)/c-kit\(^-\)/lin\(^-\) (SKL) cells in the murine bone marrow compartment between young and aged mice. B, Murine SKL cell migration to SDF-1\(\alpha\) stimulation demonstrates no significant difference. C, Mobilization of murine EPCs to intraperitoneal SDF-1\(\alpha\) administration is preserved. D, Baseline percentage of circulating EPCs (AC133\(^+\)) in human peripheral blood is unchanged with aging. E through G, Aged human EPC dynamics demonstrate no significant difference in (E) colony formation, (F) migration toward SDF-1\(\alpha\), or (G) hypoxia-induced proliferation compared with young controls. HPF indicates high-powered field.
Aging Neovascular Phenotype Corresponds With Altered Environmental Signals

To confirm the importance of progenitor depletion and peripheral tissue recruitment, we performed age- and gender-mismatched reciprocal bone marrow transplantations, which yielded 2 control groups (young donor/young recipient and aged donor/aged recipient) and 2 experimental groups (young donor/aged recipient and aged donor/young recipient). Interestingly, aged bone marrow transplanted into a young ischemic environment demonstrated mobilization of aged EPCs comparable to that in young controls (1.42 ± 0.34% versus 1.53 ± 0.46%, P > 0.05; Figure 3A). This resulted in similar increases in tissue perfusion ratio (0.81 ± 0.11 versus 0.84 ± 0.03, P > 0.05; Figure 3B) and vascular density (number of vessels in section B/number of vessels in section D: 1.42 ± 0.15 versus 1.56 ± 0.25, P > 0.05; Figure 3C). The number of CD31+/Y-chromosome+ chimeric neovessels (Figure 3E) was similar to that in young animal controls (23.4 ± 3.1 versus 28.8 ± 4.4, P > 0.05; Figure 3D), which demonstrates normal targeting and incorporation of aged EPCs into the host vasculature in response to ischemia.

Collectively, this adds further support to the theory that intrinsic EPC function remains intact even with aging given appropriate environmental signals.

However, young progenitors in an aged host exhibited marked deficiencies in mobilization from the bone marrow after an ischemic stimulus, similar to aged controls (0.41 ± 0.04 versus 0.42 ± 0.09, P > 0.05; Figure 3A). This resulted in markedly fewer CD31+/Y-chromosome+ chimeric neovessels (7.6 ± 2.8 versus 5.2 ± 1.2, P > 0.05; Figure 3E), reduced flap perfusion (0.57 ± 0.05 versus 0.50 ± 0.08, P > 0.05), and decreased vessel density (0.92 ± 0.08 versus 1.01 ± 0.10, P > 0.05; Figure 3B and 3C), similar to aged controls. Thus, these results also indicate the age-associated decline in vasculogenesis results from failure of peripheral tissues to generate a suitable signal for EPC recruitment rather than from primary EPC depletion or dysfunction.

Aging Impairs Systemic and Local Hypoxic Responses Required for Progenitor-Mediated Vascular Remodeling

We have previously demonstrated that SDF-1α is critical to EPC mobilization and trafficking to sites of neovasculariza-
tion. HIF-1α mediates ischemia-induced SDF-1α expression, and the ability to upregulate HIF-1α is thought to be impaired in aging. ELISA demonstrated a significant reduction in SDF-1α protein in ischemic flaps harvested from aged animals (347.4 ± 24.3% versus 52.1 ± 32.2% in section B, P<0.005; Figure 4B), which correlated with a marked decrease in HIF-1α protein stabilization (Figure 4A). Primary aged murine fibroblasts also demonstrated decreased HIF-1α expression compared with young cells (Figure 4C). This suggests that the observed deficiencies in SDF-1α within aged ischemic tissues may result from decreased HIF-1α stabilization.

These experiments were repeated in human microvascular endothelial cells and fibroblasts harvested from surgical specimens of healthy younger (n=9, age 18 to 35 years, mean 24.6 years) and older (n=7, age 68 to 95 years, mean 84.5 years) human subjects. Under hypoxia, aged human fibroblasts exhibited decreased HIF-1α stabilization (Figure 4D, bottom), which translated into a blunted ability to upregulate SDF-1α mRNA expression measured by real-time polymerase chain reaction (fold induction: endothelial cells 1.85 ± 0.21 versus 0.67 ± 0.35, P<0.005, and fibroblasts 1.90 ± 0.21 versus 0.90 ± 0.17, P<0.008; Figure 4D, top left). ELISA analysis of VEGF, another downstream target of HIF-1α, also demonstrated re-
duced baseline levels in aged cells (2.67±0.52 versus 124.93±14.98 pg/mL protein, aged versus young cells, \( P<0.0004 \)) and an inability to upregulate VEGF levels in response to hypoxia (25.30±5.30 versus 615.85±35.75 pg/mL protein, aged versus young, \( P<0.00001 \); Figure 4D, top right). Although the fold increase was higher in aged cells, the very low baseline levels of VEGF in cells from older subjects greatly magnified small changes in VEGF levels. Even with hypoxic stimulation, VEGF levels in aged cells were below baseline levels in young cells.

**Aging Increases HIF-1α Degradation Through Increased PHD Activity**

To elucidate the mechanism for decreased HIF-1α stability in aged cells, we investigated the contribution of PHDs, which hydroxylate proline residues (p402 and p564) in the presence of oxygen and iron,\(^{26}\) targeting HIF-1α for ubiquitin-mediated proteasome degradation.\(^{26}\) Additionally, HIF-1α function is mediated through the asparaginyl hydroxylase, factor-inhibiting HIF, which on hydroxylation of an asparagine group prevents the binding of the necessary transcriptional cofactor p300.\(^{26}\)

Primary aged murine fibroblasts demonstrated 170% to 250% higher baseline transcription of all hydroxylases compared with young controls (% increase aged/young: PHD1 171±10.7%, PHD2 253±41.6%, PHD3 215±39%; factor-inhibiting HIF 257±43.9%; Figure 5A), which correlated with decreased HIF-1α protein expression (data not shown). These data were further confirmed by examination of protein expression of PHD1, PHD2, and PHD3, which was also significantly higher in aged tissues (Figure 5B through 5D).

To establish the relevance of increased PHD levels with HIF-1α degradation in aging, we performed Western blots that specifically targeted the proline 564 residue. Because hydroxylated HIF-1α is degraded rapidly, the proteasome inhibitor MG132 was administered. Aged murine fibroblasts demonstrated increased levels of hydroxylated HIF-1α (Figure 5E). In addition, although hypoxic conditions decreased levels of hydroxylated HIF-1α in aged cells, these levels never returned to the baseline levels seen in young cells (Figure 5E). This demonstrates that aged cells exhibit increased baseline PHD1–3 function and increased HIF-1α degradation.

**DFO Promotes Ischemic Flap Survival Through Increased EPC Mobilization Via Increased HIF-1α and SDF-1α Levels**

To demonstrate the causal importance of decreased HIF-1α stabilization in vascular defects observed with aging, we performed a rescue experiment. DFO inhibits PHD hydroxylation of HIF-1α proline residues through sequestration of iron, a necessary cofactor in the reaction.\(^{16,26,27}\) Western blots on young and aged primary murine fibroblasts confirmed the ability of DFO to increase HIF-1α stabilization (Figure 6A) with downstream increases in SDF-1α and other HIF-responsive genes. Augmentation of HIF-1α stabilization with DFO in aged cells surpassed hypoxic HIF-1α stabilization in young cells (data not shown). Furthermore, although aged cells were unable to increase SDF-1α luciferase activity in hypoxia, DFO-treated aged cells demonstrated significant upregulation of luciferase activity that surpassed even that of young cells (fold induction 0.87±0.03 for aged, 1.67±0.09 for young, and 2.08±0.17 for aged+DFO; Figure 6B).

In vivo, mice were also treated with DFO. Fluorescence-activated cell sorting analysis demonstrated a marked increase in systemic Fk-1-1/CD11b+ EPCs in DFO-treated aged mice compared with aged controls (1.43±0.08% versus 0.56±0.12% EPCs, \( P<0.02 \)) and young controls (Figure 6C). This correlated with increased vascular density (1.96±0.24 versus 1.24±0.1 vessels in section B/vessels in section D per high-powered field, \( P<0.005 \); Figure 6D, left) and with significantly improved flap survival compared with their untreated aged counterparts, which approached the survival seen in young mice (Figure 6E and 6F). In contrast, we found no change in vascular density in normal skin (5.4±0.51 versus 4.8±0.38, \( P>0.05 \); Figure 6D, right) or serum SDF-1α and VEGF levels in DFO-treated animals (data not shown). These experiments confirm the well-established role of DFO to stabilize HIF-1α and reverse the age-related decline in HIF-1α and improve tissue survival.

**Discussion**

Progenitor-mediated regeneration occurs through differentiation of tissue-resident progenitor cells\(^{28}\) or trafficking of bone marrow–derived progenitor cells\(^{3,29}\) to sites of injury. Both require the local environment to generate appropriate chemotactic signals and progenitor cells to respond appropriately. Recently, there has been speculation about the hypothesis that aging may result from primary progenitor cell dysfunction or exhaustion.\(^{6,7}\) However, it has been demonstrated that senescent bone marrow cells retain the capacity to repopulate depleted bone marrow over multiple successive generations,\(^{30}\) which suggests that progenitor cells remain fully functional even with aging. In the present study, intrinsic EPC function and number remained intact both in humans and mouse model systems that used well-described assays of progenitor function.\(^{4,14}\) This led us to examine whether age-related defects in neovascularization could be attributed to a lack of hypoxia-induced signals necessary for EPC mobilization.

The repair and regeneration of the vascular system requires local vessel repair through angiogenesis and vasculogenesis. We have previously demonstrated that newly formed blood vessels in injured tissues can be composed entirely of EPCs.\(^{13}\) It has also been proposed that bone marrow cells recruited to areas of neovascularization are perivascular recruited bone marrow–derived circulating cells (RBCCs) that function as “helper” cells. These cells are postulated to augment neovascularization by secreting SDF-1α in response to upregulated local VEGF production.\(^{11}\) Regardless of the mechanism or cell type recruited, it is clear that the process of vasculogenesis requires an elaborate cascade of signaling events capable of mobilizing, homing, and retaining these cells. In the present study, using our bone marrow transplantation model, we demonstrate that vasculogenesis is markedly reduced with aging.

During development, gradients of oxygen tension regulate gene expression and determine spatial patterns of tissue organization via specific chemokines and growth factors that
guide primordial progenitor cells. In the adult, these oxygen gradients result from injury rather than tissue growth and can be demonstrated experimentally by reorientation of blood vessels to align with decreasing oxygen tensions and increasing chemokine concentrations. Central to the process of angiogenesis is the ability of local cells to sense these conditions of hypoxia and stimulate VEGF production through stabilization of intracellular HIF-1α, thus inducing migration of endothelial cells. We have demonstrated that vasculogenesis functions in a similar fashion in which local cells increase HIF-1α stabilization, which leads to augmented SDF-1α production and EPC recruitment.

The present investigation into HIF-1α stability as a common mediator for both angiogenesis and vasculogenesis indicates that aging prevents upregulation of HIF-1α because of constitutively active PHDs that increase HIF-1α degradation. This is clearly demonstrated by the inability to stabilize HIF-1α even under hypoxia, which leads to decreased levels of SDF-1α, impaired EPC mobilization, decreased vasculogenesis, and increased tissue necrosis. These data indicate an inability to mount a normal hypoxic response critical for the guidance and retention of reparative EPCs.

Interestingly, systemic administration of exogenous SDF-1α in aged animals promoted mobilization of EPCs but...
Figure 6. DFO enhances flap survival and increases EPC mobilization through increased HIF-1α and SDF-1α levels. A, HIF-1α Western blots of young and aged murine fibroblasts demonstrate the ability of DFO to stabilize HIF-1α levels in normoxia (N). B, SDF-1α luciferase activity of young, aged, and DFO-treated aged fibroblasts in response to hypoxia, expressed as fold induction to normoxia. DFO significantly increases SDF-1α reporter activity, to levels seen in young cells. C, DFO-treated aged mice mobilize EPCs to levels seen in young mice. D, Increased capillary density in DFO-treated flaps, defined as the ratio of CD31+ vessels in section B to that in section D, which is not observed in normal skin. E, Overall ischemic flap survival showing improved flap survival in aged mice treated with DFO similar to young controls. F, Near-complete healing in the DFO-treated group compared with flap necrosis in aged controls. Hpf indicates high-powered field.
failed to stimulate targeted EPC recruitment and increase tissue survival. Presumably, this results from limited tissue-specific SDF-1α expression to recruit and maintain EPCs. This suggests the stimulation of EPC-mediated repair is unlikely to be recaptured by chemokine monotherapy. However, global augmentation of HIF-1α with DFO promoted neovascularization only in areas of ischemia and did not produce neovascularization in uninjured skin. This selective effect has been confirmed by Duffy et al, who demonstrated no significant effect of DFO on human endothelial cells in the absence of ischemic coronary artery disease. The reasons for this are unclear, but recent work has examined the complex interplay between intracellular iron, PHD activity, and HIF stabilization and suggests that iron becomes limiting only in conditions of low oxygen availability. From this, one would predict that in vivo administration of DFO would not completely inhibit PHD function in normoxia but would linearly decrease PHD activity as oxygen availability decreases in the ischemic tissue, as we observed in the present study.

The findings of the present study have been confirmed by Rohrbach et al, who demonstrated an upregulation of PHD3 in aged cardiac tissue; however, unlike the present study, they found no difference in PHD1 or PHD2 in aged tissues. This difference may be due to the well-described tissue specific alterations in PHD expression to or to differences in the classification of the younger and older groups. In the study by Rohrbach et al, younger and older groups were 18 to 55 and 55 to 75 years old, respectively, whereas specimens in the present study were harvested from chronologically distinct groups at the extremes of age (18 to 35 years and 68 to 95 years, respectively). This may have provided a more robust determination of differences in PHD1 and PHD2. Nonetheless, their data corroborate the present findings that aging increases PHD-mediated HIF-1α degradation.

The precise mechanism for constitutively activated PHDs in aging remains unknown. Current theories of aging include cellular senescence, accumulation of somatic mutations that lead to cellular apoptosis, and free radical accumulation that causes direct cellular damage. One possible mechanism for the observed alteration in HIF-1α stability is through increased accumulation of reactive oxygen species with age. It has been shown the expression of PHDs, which are highly dependent on binding of oxygen to iron, is enhanced by reactive oxygen species. Therefore, it is possible that long-term exposure to reactive oxygen species may permanently upregulate PHD expression and function, thereby destabilizing HIF-1α. The present data demonstrating increased levels of hydroxylated HIF-1α with aging support the idea that reactive oxygen species may indeed play a role in increased HIF-1α degradation.

The potential of stem cell–based therapies has led to an explosion in the number of clinical trials that use stem cells. The present demonstration of the dysfunctional hypoxia response in aged tissue with subsequent impairments in downstream gene expression (SDF-1α and VEGF) offers a potential explanation for the disappointing outcomes of clinical trials to date. Therefore, therapeutic interventions designed to restore the “young” hypoxic response may be a powerful strategy to prevent age-associated disease and functional decline by recruiting and retaining native or delivered stem cells to areas of injury.

Sources of Funding

This study was supported in part by the National Institute on Aging (R01-AG025016-03 to Dr Gurtner), an American Heart Association postdoctoral fellowship (Dr Loh), and the National Institutes of Health Loan Repayment Program (Dr Edward I. Chang).

Disclosures

None.

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30. Chang et al Age Impairs Vasculogenesis and HIF Stabilization

CLINICAL PERSPECTIVE

Recent clinical trials of stem and progenitor cell treatments for ischemic disease have been disappointing. This has led to a reappraisal of the potential determinants of stem cell activity in vivo. Among investigators in the field, appreciation is growing that a receptive environment or “soil” is critical for stem or progenitor cells to exert a proregenerative effect. Advanced age is a well-established risk factor for increased morbidity and mortality after myocardial infarction and poor tissue regeneration after an ischemic insult. In the present report, we demonstrate a profound age-related decrease in the signals necessary for endothelial progenitor cell recruitment and vasculogenesis in both human and experimental systems.

Interestingly, we were unable to elucidate any differences in intrinsic progenitor cell function in younger and older humans, which suggests that the progenitor cells themselves are unaffected by aging. These “soil” abnormalities were subsequently traced to increased degradation of the transcription factor hypoxia-inducible factor 1α (HIF-1α), which resulted in reduced activation of the hypoxia response genes VEGF (vascular endothelial growth factor) and SDF-1α (stromal cell–derived factor 1). This ultimately results in impaired neoangiogenesis and increased tissue necrosis. Because VEGF is also an HIF-1α-dependent gene, these findings have broader implications for ischemia-induced angiogenesis as well. These age-related effects can be reversed by treatment with deferoxamine, which increases HIF-1α stabilization and subsequent VEGF and SDF-1α gene expression. The present report provides a mechanistic window into the well-established effects of aging on neoangiogenesis and suggests novel therapeutic strategies for increasing vascular growth in elderly patients.
NF-κB Is a Key Mediator of Cerebral Aneurysm Formation

Tomohiro Aoki, MD; Hiroharu Kataoka, MD, PhD; Munehisa Shimamura, MD, PhD; Hironori Nakagami, MD, PhD; Kouji Wakayama, MD; Takuya Moriwaki, MD, PhD; Ryota Ishibashi, MD; Kazuhiko Nozaki, MD, PhD; Ryuichi Morishita, MD, PhD; Nobuo Hashimoto, MD, PhD

Background—Subarachnoid hemorrhage caused by the rupture of cerebral aneurysm (CA) remains a life-threatening disease despite recent diagnostic and therapeutic advancements. Recent studies strongly suggest the active participation of macrophage-mediated chronic inflammatory response in the pathogenesis of CA. We examined the role of nuclear factor-κB (NF-κB) in the pathogenesis of CA formation in this study.

Methods and Results—In experimentally induced CAs in rats, NF-κB was activated in cerebral arterial walls in the early stage of aneurysm formation with upregulated expression of downstream genes. NF-κB p50 subunit–deficient mice showed a decreased incidence of CA formation with less macrophage infiltration into the arterial wall. NF-κB decoy oligodeoxynucleotide also prevented CA formation when it was administered at the early stage of aneurysm formation in rats. Macrophage infiltration and expression of downstream genes were dramatically inhibited by NF-κB decoy oligodeoxynucleotide. In human CA walls, NF-κB also was activated, especially in the intima.

Conclusions—Our data indicate that NF-κB plays a crucial role as a key regulator in the initiation of CA development by inducing some inflammatory genes related to macrophage recruitment and activation. NF-κB may represent a therapeutic target of a novel medical treatment for CA. (Circulation. 2007;116:2830-2840.)

Key Words: aneurysm ■ experimental animal models ■ endothelium ■ inflammation ■ NF-kappa B

Cerebral aneurysm (CA) is a common lesion, with a prevalence ranging from 1% to 5% in large autopsy studies.1 CA can cause a catastrophic subarachnoid hemorrhage, which has a 30-day mortality rate of 45%. An estimated 30% of survivors have moderate to severe disability.2 Despite its public importance, the mechanisms of the initiation, progression, and rupture of CAs remain to be elucidated.

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CA is characterized by the excessive degradation of extracellular matrix and accumulation of inflammatory cells in aneurysmal walls. Studies using human CA specimens revealed macrophage accumulation3 in aneurysmal walls. Macrophage accumulation also was prominent in experimentally induced CA in rats.4 By using the experimentally induced CA model, we have identified inducible nitric oxide synthase (iNOS),5 interleukin-1α (IL-1α),6 and matrix metalloproteinase (MMP) -2 and -97 as exacerbating factors of CA progression. These results suggest that the pathogenesis of CA may be linked to chronic inflammation mediated by macrophages in vascular walls. However, it is still unclear which inflammatory pathway is involved in CA formation and what triggers the inflammatory cascade.

Nuclear factor-κB (NF-κB) is a family of transcriptional factors regulating the expression of a variety of genes in response to inflammatory mediators,7 thus contributing to the initiation and progression of atherosclerosis8,9 and abdominal aortic aneurysm.10,11 NF-κB transactivates genes related to endothelial dysfunction, including vascular cell adhesion molecule-1 (VCAM-1)12,13 and monocyte chemotactic protein-1 (MCP-1).14,15 NF-κB also regulates the transcription of some proinflammatory genes such as iNOS16 and MMPs,17 which are of functional importance for the progression of CAs.

In the present study, we investigated the role of NF-κB in the initiation and progression of CAs by using an experimentally induced CA model in rats and mice. Here, we show for the first time that NF-κB participates in the initiation of CA formation by transactivating downstream...
genes related to macrophage recruitment and vascular inflammation.

Methods

Induction of Experimentally Induced CAs
In 7-week–old male Sprague-Dawley rats, CAs were induced as previously described by Nagata et al.18 We also induced CAs in NF-κB p50 subunit knockout mice, which have a background of C57/B6;129P2 (p50−/−; mice; The Jackson Laboratory, Bar Harbor, Me), and their littermates (p50+/−; mice), as previously described by Morimoto et al.19 Blood pressure was measured by tail-cuff method. At the indicated time point, animals were euthanized, and the anterior cerebral artery/olfactory artery (ACA/OA) bifurcation was stripped and observed under a light microscope after elastica van Gieson staining. Induced aneurysms were classified into 2 categories. Internal elastic lamina (IEL) disruption refers to a lesion with the discontinuity of IEL without apparent outward bulging of the arterial wall, which represents early change of CA formation. Aneurysm refers to an obvious outward bulging of the arterial wall with the fragmentation or disappearance of IEL. Three independent researchers assessed the three independent researchers assessed the histopathological changes. Aneurysm size was expressed as the maximum diameter of CAs. Animal care and experiments complied with Japanese community standards on the care and use of laboratory animals.

Immunohistochemistry and Cell Counting
After blocking, 5-μm frozen sections were incubated with primary antibodies for 1 hour at room temperature, followed by incubation with fluorescence-labeled secondary antibodies for 1 hour at room temperature. Then, the slides were observed under a fluorescence microscope system (BX51N-34-FL-1, Olympus, Tokyo, Japan). The primary antibodies used in the present study are listed in the online-only Data Supplement, expanded Methods section. To quantify macrophage accumulation, the number of CD68-positive cells was counted in a 100-μm² area in rats and in a 50-μm² area in mice.

Electrophoretic Mobility Shift Assay
Nuclear protein from the whole Willis ring was extracted with the Qproteome Nuclear Protein Kit (Qiagen, Hilden, Germany) according to the manufacturer’s directions. Electrophoretic mobility shift assay (EMSA) was performed by LightShift Chemiluminescent EMSA Kit (Pierce, Rockford, Ill). Nuclear extract (5 μg) was incubated with 20 fmol biotin 3′-end-labeled oligonucleotides containing the NF-κB sequence (5′-GGGATTCC-3′). After electrophoresis, transfer, and cross-linking, the signal was detected by a peroxidase/luminol system (Chemiluminescent Nucleic Acid Detection Module, Pierce). To confirm the specificity, a 200-fold excess of NF-κB oligonucleotides (cold probe) or mutated binding motif (5′-GGCCATTTC-3′) was added. Supershift assays using an anti-p50, anti-p52, or anti-p65 subunit antibody (Santa Cruz Biotechnology, Santa Cruz, Calif) were performed as previously described.20

RNA Isolation and Reverse Transcription
At the indicated time point, total RNA from the whole Willis ring was isolated with RNaseasy Fibrous Tissue Mini Kit (Qiagen). Total RNA was converted into cDNA through the use of Sensiscript reverse transcriptase (Qiagen). The conditions for the cDNA synthesis were as follows: 1 hour at 37°C, followed by heating at 93°C for 5 minutes.

Polymerase Chain Reaction
Polymerase chain reaction (PCR) was performed with HotStar Taq polymerase (Qiagen). β-Actin was used as an internal control. The primer sets and conditions for PCR are described in the online-only Data Supplement, expanded Methods section. PCR products were separated by the electrophoresis in 2% agarose gel. Densitometric analysis includes data of 6 samples per group.

NF-κB Decoy Oligodeoxynucleotide
NF-κB decoy oligodeoxynucleotide (ODN) was synthesized as previously described.10,11 The sequence of NB–κB decoy ODN was 5′-ctcggagaattccgccaa and 5′-ggagaattccgccaa. Scrambled decoy ODN (5′-tgctgagctagattc-3′ and 5′-tgctgagctagattc-3′) served as control. NF-κB (40 μg) or scrambled decoy ODN in 60 μL PBS was injected into the cisterna magna every 2 weeks under general anesthesia.

To confirm the specificity of NF-κB decoy ODN, EMSA for the NF-κB binding site was performed as described above with a 200-fold excess amount of cold NF-κB or scrambled decoy ODN. In addition, the specificity of NF-κB decoy ODN was examined by luciferase assay using bovine aortic endothelial cells transfected with the luciferase gene driven by the NF-κB binding site (BD Bioscience Clontech, Palo Alto, Calif), PRT-TK plasmids (Promega, Madison, Wis), and 50 nmol/L NF-κB or scrambled decoy ODN. Transfected cells were incubated for 12 hours with or without human recombinant 10 ng/mL tumor necrosis factor-α (TNF-α; PeproTec, London, UK). NF-κB activity was measured with the Dual-Luciferase Assay System (Promega) according to the manufacturer’s instructions.

Quantitative PCR
In NF-κB- or scrambled decoy ODN–treated rats, quantitative PCR was performed using QuantiTect SYBR Green PCR Kit (Qiagen) and LightCycler quick system 330 (Roche, Basel, Switzerland). Constructs were produced by the TOPO TA Cloning Kit (Invitrogen, Carlsbad, Calif) from cDNA according to the manufacturer’s directions. β-Actin was used as an internal control. The second derivative maximum method was used for crossing point determination using LightCycler Software 3.3 (Roche). In addition, 3 independent samples were examined in 1 experiment. The primer sets and conditions for PCR are described in the online-only Data Supplement, expanded Methods section. Sham-operated rats were used as control.

Immunohistochemistry for Human Samples
Human CA samples were obtained from 7 patients who underwent neck clipping for unruptured aneurysms with informed consent. The middle cerebral artery (n = 4) obtained at the superficial temporal artery–middle cerebral artery bypass surgery served as control. Paraffin sections (4 μm) were cut and mounted on slides. After deparaffinization and blocking of endogenous peroxidase activity with 0.3% H₂O₂, a mouse monoclonal antibody against the rat DNA-binding form of the NF-κB p65 subunit, which cross-reacts with human p65, was incubated for 12 hours at 4°C, followed by incubation with biotin-labeled secondary antibodies for 30 minutes at room temperature. Then, the slides were incubated with streptavidin-conjugated peroxidase. Finally, the signal was detected by 3,3′-diaminobenzidine system (Dako, Carpinteria, Calif).

Statistical Analysis
Data (mean±SD) were analyzed by use of the Mann–Whitney U test for a 2-group comparison and Kruskal-Wallis 1-way ANOVA on ranks, followed by the Turkey-Kramer test for multiple comparisons. The incidence of aneurysmal changes was analyzed by use of Fisher exact test. Differences were considered statistically significant at P<0.05.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

NF-κB Activation in an Experimentally Induced CA in Rats
Immunohistochemistry with an anti–NF-κB p65 subunit antibody that recognizes only the DNA-binding form

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revealed that NF-κB was highly activated in arterial walls at the ACA/OA bifurcation 2 weeks, 1 month, and 3 months after aneurysm induction whether they showed histological changes or not (Figure 1C through 1H). Although NF-κB also was activated at the contralateral ACA/OA bifurcation, which was not subjected to excessive hemodynamic stress as a result of CCA ligation, the extent of NF-κB activation was more abundant in the aneurysm side (Figure I of the online-only Data Supplement). In contrast, NF-κB activation occurred in only a few cells of the cerebral arterial walls before aneurysm induction (Figure 1A and 1B). The DNA-binding form of p65 was merged with endothelial NOS (Figure 2A) and CD68 (Figure 2B), showing that NF-κB was activated in both endothelial cells and macrophages. Activated NF-κB also was costained with MCP-1 (Figure 2C) and VCAM-1 (Figure 2D).

DNA Binding of NF-κB in an Experimentally Induced CA in Rats

In EMSA, 1 specific complex band could be detected in aneurysmal walls 1 and 3 months after aneurysm induction (0M; A) and at 2 weeks (0.5M; C), 1 month (1M; E), and 3 months (3M; G) after aneurysm induction. B, D, F, and H, Immunohistostaining of the DNA-binding form of the NF-κB p65 subunit in the same section as A (B), C (D), E (F), and G (H). Bar=50 μm.

Figure 1. Activation of NF-κB in an experimentally induced CA in rats. A, C, E, and G, Hematoxylin and eosin (HE) staining of the ACA/OA bifurcation of a rat before aneurysm induction (0M; A) and at 2 weeks (0.5M; C), 1 month (1M; E), and 3 months (3M; G) after aneurysm induction. B, D, F, and H, Immunohistostaining of the DNA-binding form of the NF-κB p65 subunit in the same section as A (B), C (D), E (F), and G (H). Bar=50 μm.

Figure 2. Double immunohistostaining of DNA binding form of the NF-κB p65 subunit (red) with endothelial NOS (eNOS) (A), CD68 (B), MCP-1 (C), and VCAM-1 (D) (green). E, Hematoxylin and eosin (HE) staining of the serial section. Bar=50 μm.

Figure 3. DNA binding of NF-κB in an experimentally induced CA in rats. A, EMSA for an NF-κB binding site in cerebral arteries of rats before aneurysm induction (0M) and at 1 month (1M) and 3 months (3M) after aneurysm induction. B, Competition with a 200-fold excess amount of cold κB oligonucleotide (cold) and mutated κB oligonucleotide (cold-mutant) in cerebral arteries of rats 1 month after aneurysm induction. C, Supershift assays using antibodies against p50, p65, and p52. Representative data of 3 independent experiments are shown.
against p50 and p65 but not by the antibody against p52 (Figure 3C).

**Aneurysm Formation in p50−/− Mice**

After 5 months of aneurysm induction, only 1 of 9 p50−/− mice (10%) presented IEL disruption, whereas 7 of 10 p50+/+ mice (70%) developed aneurysmal changes, including IEL disruption, which were aneurysms in 3 of the mice (Figure 4A). The incidence of aneurysmal changes was significantly lower in p50−/− mice than in p50+/+ mice (P=0.020; n=10 per group). Aneurysms induced in p50−/− mice (0 μm; n=10) were significantly smaller than in the p50+/+ mice (39.0±31.9 μm; n=10; P<0.01; Figure 4B). Blood pressure after aneurysm induction was not different between p50−/− and p50+/+ mice (p50−/−: 124.9±19.0 mm Hg, n=7; p50+/+: 125.1±18.1 mm Hg, n=10; Figure 4C). No anatomic difference was present in the Willis ring between p50−/− and p50+/+ mice (Figure 4D and 4E).

The expression levels of MCP-1, VCAM-1, MMP-2, MMP-9, IL-1β, and iNOS mRNA were elevated with aneurysm progression in p50+/+ mice (MCP-1: VCAM-1: MMP-2: MMP-9: IL-1β: P<0.01; iNOS: P=0.012), whereas mRNA expression of these molecules was not upregulated in p50−/− mice (P<0.01, p50+/+ versus p50−/− after 5 months; Figure 5A through 5G). Macrophage accumulation assessed by the number of CD68-positive cells in p50−/− mice (1.3±0.87 cells/50 μm²; n=10) was observed much less than in p50+/+ mice (3.7±1.6 cells/50 μm²; n=10; P<0.01; Figure 5H). Immunohistochemistry also demonstrated the upregulation of MCP-1, VCAM-1, MMP-2, MMP-9, IL-1β, and iNOS in aneurysmal walls of p50−/− mice. Although these genes were also expressed in an upregulated fashion in the contralateral ACA/OA bifurcation, expression levels were higher in the aneurysm side than in the contralateral side (Figure II of the online-only Data Supplement).

**Effect of NF-κB Decoy ODN on CA Formation**

Two weeks after the first injection (40 μg in 60 μL PBS), fluorescein isothiocyanate (FITC)–conjugated NF-κB decoy ODN (FITC-decoy) was incorporated into the intima and media of the cerebral artery (Figure 6A and 6B). Double immunohistochemistry with the anti–endothelial NOS antibody revealed that FITC-decoy was transferred into endothelial cells (Figure 6C and 6D). The specificity of NF-κB decoy ODN was confirmed by EMSA and luciferase assay. The NF-κB complex band in rat CASs 1 month after aneurysm induction disappeared by preincubation with a 200-fold excess amount of cold NF-κB decoy ODN but not with a 200-fold excess amount of cold
scrambled decoy ODN (Figure 6E). The TNF-α-induced increase in luciferase activity in bovine aortic endothelial cells was inhibited by NF-κB decoy ODN but not by scrambled decoy ODN (Figure 6F).

When injection of NF-κB decoy ODN started at the same time as aneurysm induction, the incidence of aneurysmal changes, including IEL disruption, was significantly lower in the NF-κB decoy group (40%, n=10) than in the scrambled decoy group (100%, n=10; P=0.011; Figure 6H). Aneurysms were significantly smaller in the NF-κB decoy group (5.0±6.7 μm; n=10) than in the scrambled decoy group (55.0±32.6 μm; n=10; P<0.01; Figure 6I). When injection of NF-κB decoy ODN was started 1 week after aneurysm induction, the incidence of aneurysmal changes was significantly lower in the NF-κB decoy group (P=0.011; n=10 per group; Figure 6J). Aneurysms also were significantly smaller in the NF-κB decoy group (6.5±10.5 μm; n=10) than in the scrambled decoy group (47.5±30.0 μm; n=10; P<0.01; Figure 6K). However, when the injection of NF-κB decoy ODN started 2 weeks after aneurysm induction, the incidence of CA formation did not differ significantly between the 2 groups (P=0.47; Figure 6L). No statistically significant difference in aneurysm size existed between the 2 groups (scrambled decoy: 55.3±36.0 μm, n=10; NF-κB decoy: 23.5±14.7 μm, n=10; P=0.17; Figure 6M). Systemic blood pressure was not different between the 2 groups in any time course experiment (Figure 6G).

**Effect of NF-κB Decoy ODN on the Expression of Downstream Genes in Aneurysmal Walls**

In the scrambled decoy group, the mRNA expression of MCP-1, VCAM-1, MMP-2, MMP-9, IL-1β, and iNOS was upregulated in aneurysmal walls compared with sham-
operated rats (MCP-1: *P* = 0.015; VCAM-1, MMP-2, MMP-9, IL-1β: *P* < 0.01, scrambled decoy group versus sham group; Figure 7A through 7F). In contrast, the mRNA expression level of these molecules was significantly lower in the NF-κB decoy group (*P* < 0.01, scrambled decoy group versus NF-κB decoy group; Figure 7A through 7F). In immunohistochemistry, MCP-1 and VCAM-1 were expressed mainly in the intima of aneurysmal walls 1 month after aneurysm induction in the scrambled decoy group (Figure 7G through 7I). However, only a few positive signals for MCP-1 and VCAM-1 could be detected in the NF-κB decoy group (Figure 7J through 7L). Macrophage infiltration into the arterial walls also was reduced in the NF-κB decoy group compared with the scrambled decoy group (NF-κB decoy group: 1.3 ± 1.1 cells/100 μm², *n* = 7; scrambled decoy group: 5.4 ± 1.5 cells/100 μm², *n* = 10; *P* < 0.01; Figure 7M).

**NF-κB Activation in Human CAs**

Immunohistochemistry using an antibody against the DNA-binding form of NF-κB p65 subunit revealed that NF-κB was highly activated in aneurysmal walls, especially in the intima (Figure 8B). In contrast, NF-κB activation occurred in only a few cells in the control cerebral artery (Figure 8A).

**Discussion**

In the present study, we provide the first evidence that NF-κB plays a crucial role in the formation of CAs by transcriptionally activating the expression of some proinflammatory genes, leading to macrophage recruitment and extracellular matrix degradation. In unstimulated cells, NF-κB is located in the cytoplasm through association with inhibitory IκB proteins that mask their nuclear localization signal. After activating signals, IκB is phosphorylated and proteolytically degraded, resulting in NF-κB translocation to the nucleus. Immunohistochemistry with an antibody directed against the nuclear localization signal of the p65 subunit of NF-κB demonstrated increased NF-κB activation in arterial walls, especially in the early stage of CA formation in rats (Figure 1). NF-κB activation also was observed in the intima of
human CA samples (Figure 8). Enhanced DNA binding of NF-κB in aneurysmal walls was confirmed by EMSA (Figure 3A). NF-κB consists of homodimers or heterodimers of 5 known subunits in mammalian cells: p50, p52, RelA (p65), RelB, and RelC. Experiments with specific antibody to various NF-κB subunits demonstrated that the NF-κB complex activated in aneurysmal walls consisted of p50/p65 heterodimers (Figure 3C).

NF-κB activation occurred mainly in the endothelial cells and macrophages (Figure 2A and 2B). Furthermore, activated NF-κB was costained with MCP-1 and VCAM-1 (Figure 2C and 2D), both of which are prerequisites for macrophage accumulation in vascular walls, suggesting that activated NF-κB may mediate macrophage recruitment into aneurysmal walls through upregulated expression of MCP-1 and VCAM-1.

To determine the functional importance of NF-κB activation in CA formation, we used mice deficient in the p50 subunit of NF-κB (p50−/− mice). The p50−/− mice are viable and demonstrate specific defects only in immune responses. The anatomy of the Willis ring and systemic blood pressure are not different between p50−/− and p50+/−
mice (Figure 4C through 4E). Nevertheless, the incidence of aneurysmal changes, including IEL disruption, and aneurysm size were significantly lower in p50/H11002/H11002 mice than in p50/H11001/H11001 mice (Figure 4A and 4B). As shown in Figure 5, mRNA expression of MCP-1 and VCAM-1 was upregulated in CAs of p50/H11001/H11001 mice. In contrast, upregulation of these 2 genes did not occur even after aneurysm induction in p50/H11002/H11002 mice. Macrophage accumulation in cerebral arterial walls also was reduced in p50−/− mice (Figure 5H). These findings indicate that the NF-κB p50 subunit plays an important role in the initiation of CA formation through the transactivation of downstream genes causing macrophage accumulation.

We next used NF-κB decoy ODN to determine when NF-κB plays a role in the pathogenesis of CA. NF-κB decoy ODN was shown to bind NF-κB and to inhibit the binding of

![Figure 7. Effect of NF-κB decoy ODN on the expression of downstream genes and macrophage infiltration in aneurysmal walls. A through F, Quantitative reverse-transcription PCR analysis of MCP-1 (A), VCAM-1 (B), MMP-2 (C), MMP-9 (D), IL-1β (E), and iNOS (F) 1 month after aneurysm induction. Data were analyzed by the Kruskal-Wallis 1-way ANOVA on ranks, followed by the Turkey-Kramer test (n=3 per group). G through L, Immunohistochemistry of MCP-1 (H and K) and VCAM-1 (I and L) at the ACA/OA bifurcation 1 month after aneurysm induction in NF-κB decoy ODN-treated (K and L) or scrambled decoy ODN-treated (H and I) rats. Hematoxylin and eosin staining of the same section as H (G) and K (J). Bar=50 μm. M, Number of macrophages infiltrated into aneurysmal walls in NF-κB decoy ODN– or scrambled decoy ODN–treated rats. Data were analyzed by the Mann–Whitney U test (n=10 per group).]
NF-κB to DNA.\textsuperscript{11} NF-κB decoy ODN was injected into the cisterna magna every 2 weeks in rats. The dose of NF-κB decoy ODN was determined by a pilot study with FITC-decoy, and successful transfer to the cerebral arteries was confirmed (Figure 6A through 6D). The specificity of NF-κB decoy ODN was demonstrated in previous studies.\textsuperscript{23,24} We also confirmed its specificity in our model by EMSA, in which the addition of cold NF-κB decoy ODN inhibited the binding of NF-κB to DNA in CAs (Figure 6E). Luciferase assay revealed the inhibitory effect of NF-κB decoy ODN to TNF-α–induced NF-κB activation in vitro (Figure 6F). These data reinforce our hypothesis that NF-κB decoy ODN specifically inhibits DNA binding of NF-κB.

Incidence of aneurysmal changes, including IEL disruption, and aneurysm size were significantly reduced when injection of NF-κB decoy ODN was started within 1 week after aneurysm induction (Figure 6H through 6M), strongly suggesting that NF-κB may be involved in the early stage of CA formation. The expression of MCP-1 and VCAM-1 was inhibited by NF-κB decoy ODN (Figure 7A, 7B, and 7G through 7L), consequently reducing the number of macrophages accumulating in arterial walls (Figure 7M). These results indicate that macrophage recruitment into aneurysmal walls depends on the NF-κB pathway.

As we previously demonstrated,\textsuperscript{4–6} MMP-2, MMP-9, IL-1β, and iNOS were expressed in an upregulated fashion in aneurysmal walls with CA progression. In p50\textsuperscript{−/−} mice, mRNA expression of these molecules was not upregulated (Figure 5D through 5G). The upregulated expression of these genes also was inhibited by NF-κB decoy ODN (Figure 7C through 7F). MMP-2 and MMP-9 cause the loss of crucial extracellular matrix components, including collagen and

Figure 8. NF-κB activation in human CAs. A and B, Immunohisto- staining for the DNA-binding form of the NF-κB p65 subunit in a human control middle cerebral artery (MCA) (A) and aneurysmal wall (B). Bar = 30 μm. C and D, Hematoxylin and eosin (HE) staining of serial sections of A (C) and B (D).
elastin.\(^4\) IL-1\(\beta\) and iNOS\(^5\) induce apoptosis in medial
smooth muscle cells (SMCs). Extracellular matrix degradation and apoptosis of SMCs result in thinning of the
media, leading to progression and rupture of CAs. Our
findings suggest that degenerative changes in aneurysmal walls also may be regulated by NF-\(\kappa B\). Because these
genes are expressed mainly in macrophages and SMCs in
aneurysmal walls,\(^6\) decreased expression of these genes in
aneurysmal walls by the treatment with NF-\(\kappa B\) decoy
ODN or p50 deficiency may be derived in part from the
decreased macrophage infiltration.

CAs tend to be formed at the arterial bifurcation suffering
from high shear stress.\(^{25,26}\) Fluid shear stress promotes the
translocation into the nucleus of NF-\(\kappa B\) in cultured endothelial
cells by activating I\(\kappa B\) kinase.\(^{27}\) Therefore,
NF-\(\kappa B\) activation caused by excessive hemodynamic stress
resulting from anatomic architecture and hypertension is
considered the first step in CA formation. In fact, NF-\(\kappa B\)
was activated at the ACA/OA bifurcation before histological
changes occurred (Figure 1C and 1D). Even in the
contralateral side, which is influenced only by hemodynamic
stress resulting from hypertension, NF-\(\kappa B\) activation
and upregulated expression of downstream genes were
observed (Figures I and II of the online-only Data Supple-
ment). However, the activation or upregulation was more
prominent in the aneurysm side than in the contralateral
side, suggesting that hemodynamic stress resulting from
anatomic architecture may enhance the inflammatory re-
action in an NF-\(\kappa B\)–dependent manner. One major direct
downstream pathway of NF-\(\kappa B\) activation is upregulation of
MCP-1 and VCAM-1 gene expression, resulting in
macrophage recruitment into the aneurysmal walls. Mac-
rophages secrete MMP-2 and MMP-9, causing extracellu-
lar matrix degradation in aneurysmal walls, and release
nitric oxide via iNOS upregulation, which mediates
apoptosis in SMCs. We previously found extensive apoptotic
cell death of SMCs in aneurysmal walls of experimentally
induced CA in rats.\(^{28}\) Although it is still controversial
whether NF-\(\kappa B\) has a proapoptotic or an antiapoptotic
effect,\(^{29,30}\) another possible potential role of NF-\(\kappa B\) in the
pathogenesis of CA is NF-\(\kappa B\)–mediated regulation of
apoptosis in SMCs.

In the present study, we have identified for the first time
that NF-\(\kappa B\) is a key regulator of CA formation. Our results
shed light on the pathogenesis of CA and support the
notion that CA is a chronic inflammatory disease mediated
mainly by macrophages. NF-\(\kappa B\)–targeted therapy may
provide preventive therapeutic options for CA, the major
cause of a catastrophic subarachnoid hemorrhage.

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Disclosures

None.

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Subarachnoid hemorrhage caused by the rupture of cerebral aneurysm (CA) is one of the most severe forms of stroke. Many people die of aneurysmal subarachnoid hemorrhage despite recent diagnostic and therapeutic advancements. At present, patients with unruptured CAs must undergo microsurgical clipping or endovascular coiling to prevent CA rupture because no effective medical treatment for it exists. In the present study, we have demonstrated for the first time that nuclear factor-κB (NF-κB) is a key mediator of CA formation by using an experimentally induced CA model in mice and rats. NF-κB was activated in cerebral arterial walls in the early stage of aneurysm formation with upregulated expression of downstream genes. NF-κB p50 subunit–deficient mice showed decreased incidence of CA formation with less macrophage infiltration into the arterial wall. NF-κB decoy oligodeoxynucleotide also prevented CA formation when it was administered at the early stage of aneurysm formation in rats. Macrophage infiltration and expression of downstream genes were dramatically inhibited by NF-κB decoy oligodeoxynucleotide. In human CA walls, NF-κB also was activated, especially in the intima. These data indicate that inflammation elicited by NF-κB activation plays a crucial role in the initiation and progression of CA formation. Anti-inflammatory agents, especially those with an inhibitory effect on NF-κB, may be a promising medical treatment for CA in the future.
Osteogenesis Associates With Inflammation in Early-Stage Atherosclerosis Evaluated by Molecular Imaging In Vivo

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Background—Arterial calcification is associated with cardiovascular events; however, mechanisms of calcification in atherosclerosis remain obscure.

Methods and Results—We tested the hypothesis that inflammation promotes osteogenesis in atherosclerotic plaques using in vivo molecular imaging in apolipoprotein E/H11002/H11002/H11002 mice (20 to 30 weeks old, n=35). A bisphosphonate-derivatized near-infrared fluorescent imaging agent (excitation 750 nm) visualized osteogenic activity that was otherwise undetectable by x-ray computed tomography. Flow cytometry validated the target specifically in osteoblast-like cells. A spectrally distinct near-infrared fluorescent nanoparticle (excitation 680 nm) was coinjected to simultaneously image macrophages. Fluorescence reflectance mapping demonstrated an association between osteogenic activity and macrophages in aortas of apolipoprotein E/H11002/H11002/H11002 mice (R2=0.93). Intravital dual-channel fluorescence microscopy was used to further monitor osteogenic changes in inflamed carotid arteries at 20 and 30 weeks of age and revealed that macrophage burden and osteogenesis concomitantly increased during plaque progression (P<0.01 and P<0.001, respectively) and decreased after statin treatment (P<0.0001 and P<0.05, respectively). Fluorescence microscopy on cryosections colocalized near-infrared fluorescent osteogenic signals with alkaline phosphatase activity, bone-regulating protein expression, and hydroxyapatite nanocrystals as detected by electron microscopy, whereas von Kossa and alizarin red stains showed no evidence of calcification. Real-time reverse-transcription polymerase chain reaction revealed that macrophage-conditioned media increased alkaline phosphatase mRNA expression in vascular smooth muscle cells.

Conclusions—This serial in vivo study demonstrates the real-time association of macrophage burden with osteogenic activity in early-stage atherosclerosis and offers a cellular-resolution tool to identify preclinical microcalcifications. (Circulation. 2007;116:2841-2850.)

Key Words: atherosclerosis ■ calcification ■ inflammation ■ imaging

Calcification is a characteristic feature of atherosclerosis and is predictive of cardiovascular events. Clinico pathological studies suggest that atherosclerotic plaques are prone to rupture at interface areas between high- and low-density tissue, particularly in the superficial nodules of calcium deposition. In addition, microcalcification in the thin fibrous cap may cause microfractures that lead to plaque rupture and acute cardiovascular events. Furthermore, calcification impacts clinical outcome not only by complicating atherosclerosis but also by impairing the movement of aortic valve leaflets, increasing arterial stiffness, which in turn affects cardiac function, or by causing plaque fracture during angioplasty.

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Cardiovascular calcification has been viewed conventionally as a passive degenerative process; however, recent evidence suggests that calcification is a tightly regulated process of mineralization akin to bone formation. We reported previously that myofibroblast-like cells, due to their plasticity, respond to various stimuli by undergoing activation
and sequential phenotypic differentiation. Moreover, accumulating data suggest that proatherogenic stimuli promote phenotypic conversion of vascular and valvar myofibroblasts into osteoblastic cells, promote expression of bone-regulating proteins (eg, alkaline phosphatase, osteopontin, osteocalcin, osteonectin, and collagen types I and II) and transcription factors (eg, Runx2/Cbfa1 and Osterix), and eventually promote calcification. However, the precise cellular and molecular mechanisms that lead to ectopic calcification remain incompletely understood.

The inability to spatially and temporally resolve and quantify dynamic pro-osteogenic molecular mechanisms in vivo also accounts for the limited knowledge in the field. In the present study, we used emerging molecular imaging tools to visualize and quantify osteogenic activity in early-stage atherosclerosis that was otherwise undetectable by conventional imaging modalities or routine histological methods. Although previous in vitro studies suggested the potential role of inflammation in calcification, in vivo evidence remains scant. Novel imaging technologies allow detection in vivo of the expression and activity of proinflammatory and pro-osteogenic molecules. We therefore tested the specific hypothesis that atherosclerotic plaque inflammation, determined as macrophage infiltration, triggers osteogenic activity. Serial imaging studies in apolipoprotein (apo) E-deficient (apoE−/−) mice provide evidence that atherosclerotic inflammation precedes osteogenic activity and promotes calcification, which possibly explains the epidemiological link between inflammation, hypercholesterolemia, and calcification. The results of the present study provide new insights into the biology of inflammation-triggered osteoblastic activity in early stages of atherosclerosis and aid the exploration of novel, more refined therapeutic strategies to combat calcific cardiovascular disease.

**Methods**

An expanded Methods section is available online as a Data Supplement.

**Animal Protocol**

We studied osteogenic changes in carotid arteries of apoE−/− mice (20 to 30 weeks old [n = 35] and 72 weeks old [n = 5]) that consumed an atherogenic diet (Teklad TD 88137; 42% milk fat, 0.2% total cholesterol, Harlan, Indianapolis, Ind) from 10 weeks of age. Mice were randomized either to continue with the atherogenic high-cholesterol diet (n = 15, sequential imaging group; n = 5, cathepsin K group; n = 5, histological control) or to consume the high-cholesterol diet admixed with a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (n = 10, statin group, 0.01% wt/wt atorvastatin, Pfizer, Groton, Conn). Age-matched wild-type C57BL6 mice (n = 5, Jackson Laboratory, Bar Harbor, Me) and apoE−/− mice lacking probe injection (n = 5) served as controls. The Subcommitte on Research Animal Care at Massachusetts General Hospital approved all procedures.

**Intravital Laser Scanning Fluorescence Imaging and 3D Reconstruction**

Mice received imaging agents or saline via intravenous injection 24 hours before imaging. We performed multichannel fluorescence imaging using an intravital laser scanning fluorescence microscope specifically developed for imaging small experimental animals. Excitation at 633 and 748 nm and image collection of the different channels was done serially to avoid cross talk between channels. Image stacks were processed and analyzed with ImageJ software (version 1.38a, Bethesda, Md). For 3-dimensional (3D) reconstruction, image stacks were split into individual channels and imported into Amira software (version 3.1, Mercury Computer Systems, Chelmsford, Mass).

**Macroscopic Fluorescence Reflectance Imaging Ex Vivo**

After mice were euthanized, aortas were perfused with saline, dissected, and imaged to map the macroscopic near-infrared fluorescent (NIRF) signals elaborated from each imaging agent by use of a fluorescence reflectance imaging system equipped with multichannel filter sets (Omega Optical, Brattleboro, Vt).

**Molecular Imaging Agents**

**Macrophage-Targeted Fluorescent Nanoparticles**

We used a cross-linked iron oxide fluorescent nanoparticle, an agent that elaborates fluorescence detectable through the NIRF window (excitation/emission 673/694 nm), for in vivo detection of macrophage accumulation.

**Calcification**

We used bisphosphonate-conjugated imaging agent (OsteoSense750; VisEn Medical Inc, Woburn, Mass), which elaborates fluorescence detectable through the NIRF window (excitation/emission 750/780 nm), to detect osteogenic activity.

**Cathepsin K Activity**

Protease-activatable imaging agent detects the activity of cathepsin K in atherosclerotic lesions. The cathepsin K probe consists of the backbone of a cathepsin K–cleavable peptide substrate that contains a fluorochrome. After enzymatic cleavage, the fluorochromes separate, which results in amplification of the signal. This agent elaborates fluorescence detectable through the NIRF window (excitation/emission 673/694 nm).

**Statistical Analysis**

An expanded statistical analysis section is available in the online-only Data Supplement. Statistical analyses for comparison of multiple groups used 1-way ANOVA followed by the Tukey post hoc test, performed with GraphPad Prism software (version 4.0, GraphPad Software, San Diego, Calif). The ΔΔCt (change in cycle threshold) of real-time reverse-transcription polymerase chain reaction data for alkaline phosphatase mRNA was used in the Mann–Whitney U test. Data are presented as mean±SEM. Probability values <0.05 were considered significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agreed to the manuscript as written.

**Results**

**Topographic Association Between Macrophage Burden and Osteogenic Activity in the Aorta of ApoE−/− Mice**

Fluorescence reflectance imaging was used to map osteoblastic activity and macrophage burden in aortas of apoE−/− mice (n = 15). We administered a bisphosphonate-derivatized NIRF imaging agent (OsteoSense, excitation 750 nm; plaque target-to-background ratio 5.4±0.5 versus 1.7±0.2 in un.injected controls; P <0.01) via tail-vein injection 24 hours before imaging to monitor osteogenesis. To visualize macrophages, we coinjected mice with a spectrally distinct NIRF nanoparticle (excitation 680 nm; plaque target-to-background ratio 6.8±0.9 versus 1.4±0.4 in uninjectected controls; P <0.01). Fluorescence reflectance imaging in apoE−/− mice yielded strong macrophage-
targeted, fluorescent nanoparticle–derived signals at the level of aortic root, aortic valve, arch, and abdominal aorta that correlated with osteogenic activities in the same regions ($R^2=0.93$; Figure 1A and 1B). These results suggest that calcification associates with sites of macrophage accumulation.

**Osteoblast-Like Cells but Not Cells of Myeloid Origin Are the Source of the Osteogenic Signal in Atherosclerotic Aortas**

The cellular source of OsteoSense signal was examined with flow cytometry. We identified OsteoSense-positive cells in aorta of apoE$^{-/-}$ mice but not in wild-type control mice (Figure 1C). Spleens of injected apoE$^{-/-}$ mice and wild-type controls were OsteoSense negative. The present data show that OsteoSense colocalized preferentially with osteopontin-positive cells in lesion-rich aortas but not with monocytes, macrophages, or dendritic cells in control aortas or spleen tissues. E, Electron microscopic analysis revealed early stages of calcium deposition in aortic lesions. Star (*) indicates cholesterol crystals; double arrowhead indicates smooth muscle cell. Bar=3 μm. Apoptotic bodies (arrowhead, bar=300 nm) and matrix vesicles (arrow, bar=100 nm) contained electron-dense (dark) needlelike structures compatible with nanocrystals of hydroxyapatite.

**Early Atherosclerotic Lesions Contain Hydroxyapatite Nanocrystals**

Electron microscopic analysis revealed early stages of calcium deposition in aortic lesions of 20- to 30-week-old apoE$^{-/-}$ mice (Figure 1E). Both apoptotic bodies and matrix vesicles contained electron-dense needlelike structures compatible with nanocrystals of hydroxyapatite that were associated with the initial process of mineralization. The cholesterol cleaves appeared as large, long, colorless crystals.

**Active Processes of Early Osteogenesis Occur in Inflamed Atherosclerotic Plaques Before Development of Advanced Calcification**

Macrophage-rich atherosclerotic plaques (Mac3-positive area $23.4\pm4.8\%$) of 20- to 30 week-old mice had increased osteogenic activity detected as alkaline phosphatase activity (alkaline phosphatase–positive area $17.1\pm3.1\%$), whereas von Kossa staining showed negligible calcification (von Kossa–positive area $0.7\pm0.5\%$; Figure 2A). In contrast, calcified lesions (von Kossa–positive area $21.8\pm2.5\%$) of
aged mice had decreased macrophage accumulation (0.8±0.2% and alkaline phosphatase activity (12.2±1.9%; Figure 2B). Notably, early plaques showed no evidence of microscopic calcification, whereas fluorescence microscopy detected an OsteoSense-positive area (von Kossa-positive area 0.4±0.2% versus OsteoSense-positive area 3.2±0.6%; Figure 2C and 2D), which suggests that OsteoSense enhanced the areas of osteogenesis that were not detected by von Kossa staining. Cross sections through advanced plaques demonstrated prominent calcification as detected by von Kossa staining (left) that correlated with OsteoSense positive signal (right). Bar=200 μm. C, Cross sections of early plaques showed no evidence of microscopic calcification (von Kossa, left) while yielding NIRF signals for osteogenic activity (right). C, Cross sections through advanced lesion demonstrated prominent calcification as detected by von Kossa staining (left) that correlated with OsteoSense positive signal (right). Bar=50 μm. D and F, Quantitative analysis of von Kossa and OsteoSense (% positive area). OS indicates OsteoSense; Mφ, macrophage; and pos, positive.

Changes in Osteogenic Activity During Plaque Progression and Antiinflammatory Treatment
To monitor the dynamic changes in inflammation and osteogenesis in atherosclerotic plaques, we used intravital multi-channel, high-resolution laser scanning fluorescence microscopy. At 20 weeks, mice were randomized either to continue consumption of an atherogenic high-cholesterol diet or to consume a high-cholesterol diet admixed with statin (Figure 3A). We performed 50 sequential intravital microscopy sessions on carotid arteries of untreated (n=15) and statin-treated (n=10) mice at 20 and 30 weeks of age. At 20 weeks, macrophages correlated with little if any osteogenic activity as shown by imaging and histology (Figure 3B); however, by 30 weeks, macrophage accumulation increased in association with advanced osteogenic signal (Figure 3C). Statin treatment prevented progression of macrophage burden and osteogenesis (Figure 3D). Quantitative analyses further demonstrated that macrophage-derived (680 nm) and osteoblast-derived (750 nm) NIRF signal intensities increased over time concomitantly (P<0.01 and P<0.001, respectively) and decreased after antiinflammatory statin treatment (P<0.0001 and P<0.05, respectively; Figure 3E). Areas of inflammation and calcification also increased during plaque progression in parallel (P<0.001) and decreased with statin treatment (P<0.001 and P<0.05, respectively; Figure 3F), which supports our hypothesis that inflammation promotes osteogenic activity.

3D Evaluation of Calcium and Macrophage Burden in Atherosclerotic Plaques
Using the same model of in vivo calcification in atherosclerotic carotid arteries, we evaluated further the temporal and spatial associations of cellular inflammation and calcification (Figure 4A). Moreover, defining colocalization as the degree of overlap between 2 different fluorescent labels (green signifying inflammation and red, calcification) within the same image, we demonstrated that microcalcifications and inflammation evolve within close proximity of one another and overlap at the border regions (colocalized pixels appear white; Figure 4B). Overlap (r=0.62 at 20 weeks versus r=0.81 at 30 weeks) and Pearson’s coefficients (r=0.14 at 20 weeks versus r=0.46 at 30 weeks) increased over time. To assess whether the degree of inflammation and calcification can be monitored over time, 3D reconstructed images were derived from Z-stack data sets at 20 and 30 weeks of age. In addition, we displayed color-coded sequential images of the carotid artery at 0°-90°-180° at 20 and 30 weeks and thereby visualized the progression of calcification and inflammation in living mice (Figure 4C). Image acquisition through a portion of carotid plaque in 3-μm steps allowed assessment of the 3D distribution of calcium and macrophage burden (online-only Data Supplement Movie; Figure 2). We observed an overall increase in volume of inflammation of 160% (green=0.13 mm³ at 20 weeks versus yellow=0.34 mm³ at 30 weeks) and in volume of calcification of 25% (red=0.15 mm³ at 20 weeks versus blue=0.19 mm³ at 30 weeks) in the 10-week-interval between 2 imaging sessions.

Aged ApoE⁻/⁻ Mice Exhibit Increased Osteogenic Signal Spatially Distinct From Macrophages
We next analyzed atherosclerotic plaques in aged (72 weeks old, n=5) apoE⁻/⁻ mice. Gross morphology, intravital microscopy (Figure 5A), and fluorescence microscopy (Figure 5B) demonstrated that calcification in more advanced plaques in aged mice appeared spatially distinct from macrophage accumulation. Osteogenic NIRF signal intensities increased with age (P<0.05), whereas macrophage-derived inflamma-
tion did not change significantly (Figure 3E). Areas of inflammation in advanced plaques decreased at 72 weeks ($P<0.01$), but calcification increased significantly compared with 30 weeks ($P<0.001$; Figure 3F).

**Intravital Fluorescence Microscopy Visualizes Microcalcifications Undetectable by Computed Tomography**

Computed Tomography (CT) was performed on 30-week-old (n=5) apoE$^{-/-}$ mice 24 hours before intravital microscopy. In addition, we assessed calcification of mature atherosclerotic plaques in the aortic arch of aged apoE$^{-/-}$ mice (n=5). Although intravital microscopy detected a strong osteogenic signal in early plaques that resided in the carotid bifurcation (Figure 6A) as confirmed by gross anatomy (Figure 6B), CT imaging in the same area showed no evident signs of calcification (Figure 6C). Notably, CT readily identified prominent calcification in advanced plaques of aged mice in the lesser curvature of the aortic arch (Figure 6D). Calcium score, calculated as calcified plaque area in square millimeters multiplied by signal intensity, in the arch of old mice was 833±149, whereas young mice displayed a calcification score of 0 in their carotid arteries.

**Cathepsin K Activity Colocalizes With Calcified Areas in Early Atherosclerosis**

We further investigated the relationship between cathepsin K activity and calcified areas in early atherosclerotic lesions. Intravital microscopy of carotid atherosclerotic plaques was performed 24 hours after injection of the cathepsin K (excitation 680 nm) and OsteoSense750 agents (n=5). A significantly enhanced NIRF signal was observed in mice injected with the cathepsin K activatable agent (plaque target-to-background ratio 3.2±0.7 versus 1.1±0.4 in un.injected controls, $P<0.05$). Cathepsin K activity preceded osteogenic activity at 20 weeks and colocalized with areas of calcification in 30-week-old apoE$^{-/-}$ mice (Figure 7).

**Organic Culture of Human Carotid Plaques Shows Spatial Distribution of Macrophages and Osteoblast-Like Cells**

To determine the relationship between inflammation and calcification in human atherosclerotic plaques, we used serial fluorescence reflectance imaging of surgically obtained carotid atheroma specimens (n=6) incubated with macrophage-targeted fluorescent nanoparticle and OsteoSense750. NIRF signal derived from both agents evolved over 24 hours (Figure 8A). Correlative histological analysis of cryosections...
demonstrated colocalization of fluorescent nanoparticle–associated signal with macrophages (CD68) and of OsteoSense-derived NIRF signals with immunoreactive osteopontin and osteocalcin (not shown) and calcium deposits detected by hematoxylin-and-eosin and von Kossa staining (not shown; Figure 8B).

**Discussion**

The present study provides in vivo evidence that macrophage infiltration precedes osteogenic activity in the atherosclerotic microenvironment, thus extending the paradigm that arterial calcification is an inflammatory disease. Key findings documented here (1) demonstrate that arterial calcification is associated with macrophage burden, as detected by simultaneous mapping of spectrally distinct NIRF signals amplified by calcification- and inflammation-targeted imaging agents; (2) unravel in vivo processes of mineralization, which were undetectable by conventional histological and imaging approaches; (3) show the progression of plaque calcification in apoE-deficient mice from 20 to 72 weeks of age; (4) demonstrate that conditioned media from macrophages induce the osteogenic potential of vascular smooth muscle cells; (5) show that early introduction of statin therapy retards calcification; and (6) quantify the dynamic pro-osteogenic molecular processes at the initial stages of atherosclerosis. These results concur with our hypothesis on inflammation-triggered osteogenic activities and therefore suggest that antiinflammatory treatment can prevent the progression of arterial calcification. Because atherosclerosis and aortic valve stenosis share similar mechanisms and epidemiological risk factors, the present findings also apply to calcific aortic valve disease and suggest that cellular-resolution molecular imaging can identify microcalcifications and subclinical valvular lesions and potentially predict risk for devastating clinical complications in patients.

Pioneering work by Demer and colleagues demonstrated in vitro that macrophage-derived cytokines such as

**Macrophage-Conditioned Media Increase Alkaline Phosphatase mRNA Expression in Human Vascular Smooth Muscle Cells**

To address further whether macrophages promote an osteogenic phenotype in vascular cells, we examined alkaline phosphatase expression by human primary smooth muscle cells treated with culture media from human primary macrophages. Real-time reverse-transcription polymerase chain reaction showed that macrophage-conditioned media produced a statistically significant increase in alkaline phosphatase mRNA expression by smooth muscle cells in 6 hours compared with the control cell-free media (mean 3.1±0.7-fold increase, *P*<0.05; n=4; Figure 8C).
interleukin-1β, interleukin-6, interleukin-8, tumor necrosis factor-α, insulin-like growth factor-1, and transforming growth factor-β induce osteogenic differentiation and mineralization of vascular cells, which suggests that these proinflammatory molecules promote atherosclerosis-associated calcification by regulating the differentiation of calcifying vascular cells. The present study expands this proinflammatory paradigm of arterial calcification and shows the in vivo real-time association of inflammation and early calcification by mapping osteogenesis to sites of inflammation in the aorta. Notably, macrophage burden and osteogenic activity colocalized predominantly in regions of high mechanical stress, including the lesser curvature of the aortic arch, the aortic root, the innominate artery, the carotid bifurcation, and the aortic valve. These results suggest that detection of osteogenic activity at a specific anatomic site serves as a surrogate marker for calcification in other atherosclerosis-prone regions. We also demonstrate that macrophages promote an osteogenic phenotype of vascular wall cells in vivo, which concurs with previous reports by others and supports our in vivo data in the present study. Although the relative contribution between various macrophage products to osteogenesis remains elusive, several lines of evidence, including ours, suggest the important role of macrophages in calcification in the proinflammatory microenvironment. The in vivo and in vitro data documented here provide comprehensive understanding and mechanistic insights into the initiation of calcification in atherosclerosis and further suggest that calcification is a complex yet predictable and therefore preventable process that responds to mechanical signals, as well as local and systemic factors that regulate vascular cell differentiation and function.

Previous histopathological studies have shown that calcification occurs reproducibly in advanced atherosclerotic lesions of aged apoE−/− mice. Such studies have provided a model of advanced arterial calcification that assessed the presence of osteogenic cells and elucidated the factors that participate in differentiation of osteoblastic/chondroblastic cells within the setting of fibrosclerotic lesions. The present study explored further the pathogenesis of atherosclerotic plaques and identified the initial stages of arterial calcification in early atherosclerosis. In addition, we investigated the progression of plaque development and aging at cellular resolution in vivo. Intravital imaging showed that in early-stage atherosclerosis, inflammation precedes calcification, which suggests that macrophages promote the proinflammatory milieu and send specific signals to vascular wall cells to initiate osteogenic differentiation. Then, both processes developed in parallel and within close proximity. This stage of “microcalcification” may cause plaque rupture and microfractures that may result in the acute clinical events predicted by the theoretical model of Vengrenyuk and colleagues. Once equilibrium in the arterial wall shifts toward calcification, deposition of hydroxyapatite could progress quickly, as shown in the present study. Microparticles of calcium phosphate may elicit proinflammatory responses from macrophages, which suggests a positive-feedback amplification loop of calcification and inflammation that drives disease progression. In addition, macrophages and smooth muscle
cells may undergo apoptotic changes, providing new foci for calcium deposition. The eventual appearance of acellular fibroplastic plaques with prominent mineralization is associated with the classic view of calcification as a degenerative and geriatric disease.

The balance of osteoblastic and osteoclastic activities regulates osteogenesis. The cathepsins are among the most potent elastases characterized to date. Elastolysis induced by inflammation in the atherosclerotic plaques may alter smooth muscle cell phenotype and promote their osteogenic differentiation. Moreover, cathepsin K is a major protease expressed during bone resorption. Cathepsin K deficiency results in accumulation of bone matrix and development of ectopic calcification. The present study demonstrated that spectrally distinct NIRF signal derived from enzymatically active cathepsin K preceded osteoblastic activity in early atherosclerosis and colocalized later with prominent calcification, which suggests that osteoblastic and osteoclastic activities evolve in parallel.

Previous studies used histological methods to analyze static conditions in advanced atherosclerotic lesions at the time of death, when calcification had already become prominent and irreversible. Not surprisingly, limited resolution of imaging technologies failed to recognize initial osteogenesis or cellular-level microcalcifications. The present study used imaging techniques for which the resolution exceeds that of conventional imaging modalities (eg, MRI, intravascular ultrasound, and optical coherence tomography). Furthermore, the NIRF imaging agent binds to nanocrystals of hydroxyapatite elaborated by vascular smooth muscle cells that undergo vesicle-mediated calcification or differentiation into osteoblast-like cells, thereby producing images undetectable by earlier approaches. In the present study, for example, molecular agent–based intravital fluorescent microscopy but not x-ray CT detected early osteogenesis and microcalcifications.

The increased risk of mortality and morbidity associated with cardiovascular calcification has driven the development of new therapeutic strategies to prevent and even reverse this process. Although recent clinical trials failed to show the reduction of calcific aortic stenosis, a growing body of research indicates that statins may have therapeutic advantages in cardiovascular calcification. Previous studies on hypercholesterolemic rabbits indicated that in addition to their effect on inflammation, statins may inhibit osteogenic pathways in myofibroblasts. The present study demonstrates that statin treatment in early-stage atherosclerosis reduces inflammation and osteogenic activity concomitantly, hence supporting the concept that early pharmacological modification of proinflammatory processes retards the progression of arterial calcification. Therefore, clinical molecular imaging of microcalcifications and osteogenic activity (“precalcification”) may identify high-risk atherosclerotic plaques and aortic valve lesions while the disease is still silent and may enable the monitoring of osteogenic activity during therapeutic interventions, thereby providing a powerful tool for personalized preventive cardiovascular medicine.

Figure 8. Relationship of inflammation and calcification in human atherosclerotic plaques. A, Organoid culture of human carotid endarterectomy specimens detected slower uptake of OsteoSense than macrophage-targeted fluorescent nanoparticle. Arrow depicts lumen. D indicates distal; P, proximal. Bar=1 cm. B, Correlative histological analysis on cross sections demonstrated colocalization of fluorescent nanoparticle with macrophages (CD68; arrows) and of osteogenic signal with immunoreactive osteopontin (*). Bar=200 μm. HE indicates hematoxylin and eosin. C, Real-time reverse-transcription polymerase chain reaction showed that the conditioned media of human primary macrophages (Mφ; 4 donors) increased ALP mRNA expression by human primary smooth muscle cells (SMC; 4 donors) compared with control (Ctrl) cell-free media (mean 3.1±0.7-fold, **P<0.05, n=4), GAPDH-normalized threshold cycles (ΔΔCt) were compared with Mann–Whitney U-test. Relative fold changes were calculated by comparative Ct method, 2−ΔΔCt.
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Disclosures
Dr Weissleder is a shareholder of VisEn Medical in Woburn, Mass. The remaining authors report no conflicts.

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Dr Weissleder is a shareholder of VisEn Medical in Woburn, Mass. The remaining authors report no conflicts.

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CLINICAL PERSPECTIVE
Arterial calcification may trigger acute coronary events. Although conventional structural imaging modalities can identify prominent late-stage calcification, they cannot detect and quantify the dynamic pro-osteogenic processes in vivo at the earlier stages of subclinical disease. Our innovative functional imaging strategies not only monitor plaque initiation and progression but also provide robust evidence for coregistration of osteogenic processes with inflammation, thus extending the paradigm that cardiovascular calcification is an inflammatory disease. The present serial in vivo study demonstrates further that statin treatment concomitantly inhibits arterial inflammation and osteogenic activity in early-stage atherosclerosis, which suggests that early pharmacological modification of proinflammatory pathways can retard the progression of cardiovascular calcification. Because atherosclerosis and aortic valve stenosis share similar mechanisms and epidemiological risk factors, our findings also apply to calcific aortic valve disease that currently has no therapeutic options other than surgical valve replacement. Development of catheter-based fluorescence sensors and tomographic fluorescence imaging would enable simultaneous imaging of different biological processes such as inflammation and osteogenesis in patients before severe complications occur. Therefore, clinical molecular imaging of microcalcifications and osteogenic activity (“precalcification”) may identify high-risk atherosclerotic plaques and aortic valve lesions while the disease is still silent and may enable the monitoring of osteogenic activity during therapeutic interventions. In the near future, the combined use of molecular imaging, novel biomarkers, and genetics will identify subclinical lesions, providing a powerful tool for personalized preventive cardiovascular medicine.
Background—Tetrahydrobiopterin (BH₄) is a key regulator of endothelial nitric oxide synthase (eNOS) activity and coupling. However, the extent to which vascular and/or systemic BH₄ levels are altered in human atherosclerosis and the importance of BH₄ bioavailability in determining endothelial function and oxidative stress remain unclear. We sought to define the relationships between plasma and vascular biopterin levels in patients with coronary artery disease and to determine how BH₄ levels affect endothelial function, eNOS coupling, and vascular superoxide production.

Methods and Results—Samples of saphenous veins and internal mammary arteries were collected from 219 patients with coronary artery disease undergoing coronary artery bypass grafting. We determined plasma and vascular levels of biopterins, vasomotor responses to acetylcholine, and vascular superoxide production in the presence and absence of the eNOS inhibitor N⁵-nitro-L-arginine methyl ester. High vascular BH₄ was associated with greater vasorelaxations to acetylcholine (P<0.05), whereas high plasma BH₄ was associated with lower vasorelaxations in response to acetylcholine (P<0.05). Furthermore, an inverse association was observed between plasma and vascular biopterins (P<0.05 for both saphenous veins and internal mammary arteries). High vascular (but not plasma) BH₄ was associated with reduced total and N⁵-nitro-L-arginine methyl ester–inhibitable superoxide, suggesting improved eNOS coupling. Finally, plasma but not vascular biopterin levels were correlated with plasma C-reactive protein levels (P<0.001).

Conclusions—An inverse association exists between plasma and vascular biopterin levels in patients with coronary artery disease. Vascular but not plasma BH₄ is an important determinant of eNOS coupling, endothelium-dependent vasodilation, and superoxide production in human vessels, whereas plasma biopterins are a marker of systemic inflammation. (Circulation. 2007;116:2851-2859.)

Key Words: atherosclerosis ■ endothelium ■ free radicals ■ nitric oxide ■ nitric oxide synthase

Tetrahydrobiopterin (BH₄) is an essential cofactor for endothelial nitric oxide synthase (eNOS) and is required for nitric oxide (NO) synthesis in the vascular endothelium.¹ Reduced BH₄ availability leads to eNOS uncoupling and the production of superoxide radicals instead of NO.² ³ Recent evidence suggests that BH₄-dependent eNOS uncoupling may be an important mechanism that mediates endothelial dysfunction and increased superoxide generation in vascular disease states.⁴ ⁵

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Several studies have found that BH₄ levels in either endothelial cells or vascular tissues are reduced in vascular disease models. However, BH₄ biosynthesis can be increased by cytokine stimulation in inflammatory cells⁶ and in cultured endothelial cells⁷ ⁸ through induction of the rate-limiting enzyme GTP cyclohydrolase I (GTPCH).⁹ ¹⁰ Because both local and systemic inflammation are features of atherosclero-
sis, it is unclear how vascular disease states could be associated with reduced biopterin biosynthesis as the sole cause of BH4 deficiency. Indeed, plasma levels of neopterin, a metabolic by-product of biopterin biosynthesis, are elevated in patients with inflammatory conditions, including coronary artery disease (CAD). Other studies have suggested that reduced vascular BH4 is due to oxidation of BH4 by reactive oxygen species such as peroxynitrite to form dihydrobiopterin (BH2) and finally biopterin. Loss of BH4 through oxidation adds additional mechanistic complexity because selective changes in BH4 could be masked by little or no change in the overall level of total biopterins, comprising the sum of BH4, BH2, and biopterin. Furthermore, no studies have examined whether vascular biopterins are regulated in parallel with systemic biopterin levels. Indeed, the extent to which vascular and/or systemic biopterin levels are altered in human atherosclerosis and the importance of BH4 levels in determining endothelial function remain unclear.

Accordingly, we sought to systematically define the relationships between plasma and vascular biopterin levels in patients with CAD and to determine how BH4 levels influence eNOS coupling, endothelial function, and inflammation in human atherosclerosis.

**Methods**

**Study Subjects**

For the present study we initially screened 303 patients with CAD undergoing elective coronary artery bypass grafting (CABG) at the John Radcliffe Hospital, Oxford, UK. Of these subjects, 219 fulfilled the inclusion criteria and agreed to participate. Exclusion criteria were any inflammatory, infective, liver, or renal disease, overt clinical heart failure, malignancy, or acute coronary event during the last 2 months. Patients receiving nonsteroidal anti-inflammatory drugs, dietary supplements of folic acid, or antioxidant vitamins were also excluded. Individual characteristics of the patients are presented in Table 1. The study was approved by the local Research Ethics Committee, and each patient gave written informed consent.

**Tissue and Plasma Samples**

Samples of saphenous vein (SV) (n=101) and internal mammary artery (IMA) (n=101) were obtained at the time of CABG, as we have described previously. Paired vessel segments were snap frozen and stored at –80°C for measurement of biopterin content or were transferred to the laboratory for functional studies within 30 minutes in ice-cold Krebs-Henseleit buffer. For endothelial denudation experiments, endothelium was removed by gently pulling the vessel ring over a 2-mm-diameter wooden stick to produce mild abrasion of the luminal surface of the vessel, without undue stretching, followed by a flushing of the lumen with PBS to remove any residual endothelial debris, as described previously. Because the number of assays performed on each vessel was limited by the small quantity of tissue, a subset of the total population was studied in each individual experiment. Blood samples were obtained immediately before surgery, after overnight fasting. Samples were centrifuged at 2500 rpm for 10 minutes, and serum/plasma was stored at –80°C.

**Oxidative Fluorescent Microphotography**

In situ superoxide production was determined in vessel cryosections with the oxidative fluorescent dye dihydroethidium. Cryosections (30 μm) were incubated with dihydroethidium (2 μmol/L) in Krebs-HEPES buffer, with or without Nω-nitro-L-arginine methyl ester (L-NAME) (100 μmol/L).

**Table 1. Individual Characteristics of Patients**

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>Men/women</th>
<th>Age, y</th>
<th>SV, n</th>
<th>IMA, n</th>
<th>Risk factors, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>219</td>
<td>188/31</td>
<td>65.91 ± 8.9</td>
<td>101</td>
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<tr>
<td>Men/women</td>
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<tr>
<td>Age, y</td>
<td>65.91 ± 8.9</td>
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<tr>
<td>IMA, n</td>
<td>101</td>
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<td>Risk factors, n (%)</td>
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<tr>
<td>Hypertension</td>
<td>144 (65)</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>160 (73)</td>
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<td>Smokers/ex-smokers</td>
<td>29/114 (13/52)</td>
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<tr>
<td>Diabetes mellitus</td>
<td>61 (28)</td>
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<tr>
<td>Family history</td>
<td>115 (53)</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>27.7 ± 7.5</td>
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<td>Triglycerides, mmol/L</td>
<td>1.60 ± 0.80</td>
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<tr>
<td>Cholesterol, mmol/L</td>
<td>3.98 ± 0.88</td>
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<td>High-density lipoprotein, mmol/L</td>
<td>1.09 ± 0.26</td>
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<tr>
<td>Ejection fraction of left ventricle, %</td>
<td>52.0 ± 11.2</td>
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<tr>
<td>Plasma biopterin levels</td>
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<tr>
<td>Plasma BH₄, nmol/L</td>
<td>21.3 (11.5–42.1)</td>
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<tr>
<td>Plasma BH₂, nmol/L</td>
<td>15.1 (11.6–19.2)</td>
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<tr>
<td>Plasma biopterin, nmol/L</td>
<td>3.89 (2.57–6.58)</td>
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<tr>
<td>Plasma total biopterin, nmol/L</td>
<td>46.9 (30.9–70.3)</td>
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<tr>
<td>BH₄/total biopterin ratio</td>
<td>0.51 ± 0.24</td>
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Angiographic extent of CAD, n (%)

1-vessel disease 6 (3)
2-vessel disease 59 (27)
3-vessel disease 154 (70)

**Medication, n (%)**

Statins 200 (91)
Angiotensin-converting enzyme inhibitors 137 (63)
Calcium channel blockers 68 (31)
Angiotensin receptor blockers 16 (7)
β-Blocker 181 (83)
Nitrate 99 (45)
Aspirin 186 (85)
Clopixol 64 (29)
Diuretics 49 (22)

Values are expressed as mean ± SD or as median (25th to 75th percentile), unless otherwise indicated.

Fluorescent images of the endothelium (×40, Zeiss LSM 510 META laser scanning confocal microscope, Carl Zeiss, Inc, Oberkochen, Germany) were obtained from each vessel quadrant. In each case, segments of vessel rings (with and without L-NAME) were analyzed in parallel with identical imaging parameters. Dihydroethidium fluorescence was quantified by automated image analysis with Image-Pro Plus software (Media Cybernetics, Bethesda, MD); all analyses were performed in a blinded fashion by 2 independent observers.

**Vasomotor Studies**

Endothelium-dependent and endothelium-independent dilatations were assessed in SV obtained at the time of CABG with the use of isometric tension studies. Four rings from each vessel were precontracted with phenylephrine (3×10⁻⁶ mol/L), then endotheli-
um-dependent relaxations were quantified with acetylcholine (10⁻⁹ to 10⁻⁵ mol/L). Finally, relaxations to the endothelium-independent NO donor sodium nitroprusside (SNP) (10⁻⁶ to 10⁻⁴ mol/L) were evaluated in the presence of the NO inhibitor L-NAME (100 μmol/L), as we have described previously.¹⁶

**Determination of Vascular Superoxide Production**

Vascular superoxide production was measured in paired segments of SV and IMA with the use of lucigenin-enhanced chemiluminescence, as described previously.¹⁶,¹⁸ Vessels were opened longitudinally to expose the endothelial surface and equilibrated for 20 minutes in oxygenated (95% O₂/5% CO₂) Krebs-HEPES buffer (pH 7.4) at 37°C. Lucigenin-enhanced chemiluminescence was measured with the use of low-concentration lucigenin (5 μmol/L),¹⁷ NOS-derived superoxide production was determined by the difference in superoxide production after incubation with the NO inhibitor L-NAME (100 μmol/L).

**Determination of Plasma and Vascular Biopterin Levels**

BH₄, BH₃, and biopterin levels in plasma or vessel tissue lysates were each determined separately by high-performance liquid chromatography followed by electrochemical (for BH₄) and fluorescent (for BH₃ and biopterin) detection, as described previously,¹⁹ with some modifications. Biopterin levels were expressed as pmol/g of tissue for vessels and nmol/L for plasma (for details, see the online-only Data Supplement).

**Western Blotting**

Protein was extracted from frozen segments of SV with lysis buffer (50 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 0.1% sodium dodecyl sulfate, 0.5% deoxycholate, 1% Nonidet P-40) containing protease inhibitors (Complete; Roche) and 1 mmol/L phenylmethylsulfonyl fluoride. Protein lysates (5 to 15 μg) were separated by electrophoresis on 4% to 12% NuPAGE Bis-Tris gels (Invitrogen, Carlsbad, Calif) and transferred to nitrocellulose. Western blotting for eNOS and GAPDH were performed by goat polyclonal antibodies (BD Biosciences, San Jose, Calif, and Chemicon International, Temecula, Calif, respectively) and Tie-2 with the use of a goat polyclonal antibody (R&D Systems, Minneapolis, Minn). eNOS/Tie-2 levels were normalized to GAPDH for quantification.

**Determination of Serum C-Reactive Protein Levels**

Serum levels of C-reactive protein (CRP) were measured by immunonephelometry with a high-sensitivity method (Dade Behring Marburg GmbH, Marburg, Germany).

**Statistical Analysis**

All variables were tested for normal distribution with the use of the Kolmogorov-Smirnov test. Non-normally distributed variables were log-transformed for analysis and are presented as median (25th to 75th percentiles) and range. Comparisons of categorical variables between groups were performed by χ² test. Comparisons of continuous variables between 3 groups (ie, between the 3 tertiles of a splitting variable) were performed by 1-way ANOVA followed by Bonferroni post hoc analysis for further comparisons between individual groups. The comparison of biopterin levels in vessels before and after endothelial denudation was performed by paired t test. Comparisons of continuous values between 2 independent groups (ie, for the L-NAME–induced change in superoxide production between the 2 groups used for the dihydroethidium experiment) were performed by unpaired t test. The dose–response curves for the vasomotor responses to acetylcholine and SNP were compared between groups by ANOVA for repeated measurements with the use of a dose (factor 1) by group (factor 2) interaction. Correlations between continuous variables were assessed by determining the Pearson correlation coefficient.

In linear multiple regression, we used the following as dependent variables in 4 respective models: (1) total vascular superoxide production in SV rings; (2) L-NAME–inhibitable vascular superoxide production in SV rings; (3) plasma BH₄; and (4) plasma total biopterin levels (for details, see the online-only Data Supplement). All probability values were 2-tailed, and P<0.05 was considered statistically significant. All statistical analyses were performed with the use of SPSS 12.0.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agreed to the manuscript as written.

**Results**

**Associations Between Vascular and Plasma Biopterins in Patients With CAD**

We first compared levels of total biopterins in plasma and in vascular tissue (IMA [n = 67] and SV [n = 77]) collected from patients undergoing CABG. We divided our population into tertiles according to plasma total biopterin levels, and we observed that plasma and vascular levels of total biopterins varied widely between individual patients (Table 2). However, we found an inverse relationship between plasma and vascular total biopterins in both IMA (r = −0.251, P = 0.051) and SV (r = −0.317, P = 0.007). Indeed, the 3-fold increase in plasma total biopterins between the highest and lowest tertiles was associated with a 2-fold reduction in vascular total biopterins (Figure 1). In addition, no significant association was present between plasma and vascular absolute BH₄ levels, suggesting that they behave as 2 independent compartments (Figure 1). Measurement of vascular biopterins in paired vascular segments with or without endothelial denudation revealed that 88.8±3.6% of vascular BH₄ and 75.0±4.5% of vascular total biopterins were localized to the endothelium (Figure 2). Denudation of the vascular endothelium in these vessels was confirmed by Western blotting of endothelium-specific proteins eNOS and Tie-2 in vascular segments from 4 patients (Figure 2). These findings suggest that vascular biopterin levels are determined principally by endothelial biopterin content and that vascular and plasma biopterin levels are regulated differentially in patients with CAD.

**Vascular and Plasma BH₄ Levels and Endothelial Function**

We next examined whether vascular biopterins were associated with relaxation responses of SV (n = 74) to acetylcholine as a measure of NO-mediated endothelial function. To address this question, we divided our population into tertiles according to vascular total biopterins or vascular BH₄ levels, and we compared the vasomotor responses of SV to acetylcholine between the 3 tertiles. No significant relationship existed between vascular total biopterins and acetylcholine relaxations (P = 0.1). However, analysis of acetylcholine dose–response curves according to tertiles of vascular BH₄ revealed that patients with vascular BH₄ in the highest and middle tertiles had significantly greater relaxations to acetylcholine compared with those in the lowest tertile (Figure 3A). In contrast, no differences were present between vascular BH₄ tertiles in relaxation responses to the direct NO donor SNP (Figure 3B). Surprisingly, the relationship between plasma BH₄ and relaxation to acetylcholine was the inverse of the association observed between vascular BH₄ and acetylcholine relaxations. Patients with plasma BH₄ in the highest tertile had significantly reduced NO-mediated endothelial function compared with patients in the lowest tertile (Figure 3C),
whereas no significant association existed between plasma total biopterins and vasomotor responses to acetylcholine \((P=0.647)\). No significant association existed between the absolute values of vascular BH4 or biopterin and the vasomotor responses to acetylcholine \((P=NS\) for all). Relaxation responses to SNP were also not significantly different between plasma BH4 tertiles (Figure 3D).

**Vascular and Plasma BH4 Levels and Superoxide Production in Human SV and IMA**

To further examine the biological significance of plasma and vascular biopterins in human atherosclerosis, we examined the relationship between vascular BH4 and vascular superoxide production. We divided our population into tertiles according to vascular BH4 levels, and we observed that patients in the highest tertile of vascular BH4 had significantly lower superoxide production in both SV \((n=90)\) and IMA \((n=82)\) than those in the lowest vascular BH4 tertile (Figure 4A and 4B). Because BH4 regulates eNOS coupling, we then examined whether vascular BH4 levels may affect eNOS-dependent superoxide production by measuring the change in vascular superoxide induced by eNOS inhibition. Indeed, we observed that the L-NAME–induced decrease in vascular superoxide was significantly greater in SV and IMA from patients with low vascular BH4 compared with those with high vascular BH4, reflecting greater eNOS coupling in the presence of high vascular BH4 (Figure 4C and 4D). In addition, a significant association was present between vascular BH4 and the ratio of BH4/(BH4+biopterin) in both vessel types (Figure 4E and 4F), suggesting that higher absolute levels of vascular BH4 are associated with less BH4 oxidation. On the other hand, no association existed between vascular BH4 (or biopterin) and vascular superoxide generation (data not shown). In multivariate analysis, we found that vascular BH4 was the only independent predictor of both vascular total superoxide \((\beta [SE]=-2.487 [0.572]\); \(P=0.0001)\) and L-NAME–induced change in vascular superoxide \((\beta [SE]=2.895 [0.643]; P=0.0001)\) in SV. To visualize the relationship between vascular BH4 and superoxide production, we performed dihydroethidium staining in SV from 5 patients at the highest and 5 patients at the lowest tertile of vascular BH4. We observed that endothelium-derived superoxide was decreased by L-NAME in vessels with low BH4 (by \(-4.7 \pm 2.0\ U/mm of endothelium)\), suggesting eNOS uncoupling, whereas the opposite was observed in vessels with high BH4 levels (by \(+3.7 \pm 2.0\ U/mm of endothelium\); \(P<0.05\) versus low BH4) (Figure 5). Taken together, these findings suggest that vascular BH4 may be a key regulator of eNOS coupling in the vasculature of patients with CAD.

We next examined possible relationships between plasma BH4 and vascular superoxide production. We observed that plasma BH4 was not associated with either total vascular superoxide or the L-NAME–induced change in vascular superoxide in either SV or IMA (Figure 6A through 6D). Correspondingly, no association existed between plasma BH4 and the ratio BH4/(BH4+biopterin) in plasma (Figure 6E and 6F). These findings suggest that vascular rather than plasma BH4 has a greater impact on eNOS coupling and vascular superoxide production. To further evaluate the biological importance of eNOS coupling, as determined by L-NAME–inhibitable vascular superoxide production, we examined whether this measure was related to NO-mediated endothelial function. Indeed, L-NAME–induced change of vascular su-
Biopterins are associated with inflammation and impaired endothelial function. These findings suggest a distinct and contrasting biological importance for systemic versus plasma biopterins in patients with CAD. Vascular biopterins are associated with maintained nitric oxide-mediated endothelial function in both arteries and veins, independently of the patient’s individual characteristics. Finally, plasma BH4 is positively correlated with CRP levels and inversely associated with endothelial function. These findings suggest a distinct and contrasting biological importance for systemic versus plasma biopterins in patients with CAD. Vascular biopterins are associated with maintained nitric oxide coupling and endothelial function, whereas plasma biopterins are associated with inflammation and impaired endothelial function.

The proposed importance of BH4 in vascular homeostasis relates to its role as an essential cofactor for eNOS. Recent studies have shown that BH4 availability mediates coupling of oxygen reduction to heme-catalyzed L-arginine oxidation to form NO and L-citrulline. Therefore, BH4 deficiency is believed to lead to eNOS uncoupling, resulting in impaired endothelium-dependent vasodilation and the production of superoxide radicals from the uncoupled enzymatic form. 

In experimental models, risk factors for atherosclerosis are accompanied by endothelial dysfunction, while plasma biopterins are associated with inflammation and impaired endothelial function.
BH4 is associated with the ratio of BH4/(BH2+biopeterin) [B] was determined as an indicator of BH4 oxidation. Values shown are mean±SEM according to tertiles of vascular BH4. The highest vascular BH4 tertiles in both SV and IMA were associated with low vascular superoxide (A and B), less L-NAME–inhibitable superoxide production (C and D), and an increased BH4/(BH2+B) ratio in both vessel types (E and F). *P<0.05 vs lowest tertile; **P<0.01 vs lowest tertile; †P<0.05 vs medium tertile; ‡P<0.01 vs medium tertile.

In contrast to the positive associations between vascular biopeterin levels, reduced vascular superoxide, and improved endothelial function, we found no similar associations with plasma BH4. Rather, plasma biopeterin levels were associated with CRP, a marker of systemic inflammation. This observation is in agreement with the fact that plasma neopterin, a by-product of BH4 biosynthesis, is well established as a marker of systemic inflammation. Indeed, neopterin was raised in parallel with BH4 after cytokine stimulation of endothelial cells. Of particular importance for our findings, several previous studies have shown that patients with CAD have elevated plasma neopterin levels. Elevated plasma neopterin is associated with accelerated CAD progression and increased clinical events, which are also characterized by elevated CRP. Our study now clarifies the implication of these observations because we show that increased plasma biopeterin levels are associated with plasma CRP in a manner similar to plasma neopterin but are also associated with...
reduced vascular biopterin levels and reduced NO-mediated endothelial function, which would be expected to increase CAD progression and risk. Despite the fact that endothelial BH₄ is an important determinant of eNOS activity, little is known about the pathophysiological control of endothelial BH₄. In mammalian cells, the biosynthesis of BH₄ begins with GTPCH, which catalyzes the rearrangement of GTP to dihydromeopterin triphosphate, which is subsequently converted to BH₄ by the sequential action of 6-pyruvoyl-tetrahydrobiopterin synthase and sepiapterin reductase. In contrast to the latter 2 enzymes, GTPCH activity is limiting in most tissues, and it is the major regulator of BH₄ synthesis. The GCH1 gene, encoding GTPCH, is expressed in several cell types such as macrophages, hepatocytes, and endothelial cells. Several in vitro studies suggested that GCH1 expression in endothelial cells or macrophages is induced by cytokines. However, GCH1 upregulation in human endothelial cells requires simultaneous exposure to high concentrations of multiple cytokines (such as interleukin-1β, interleukin-6, tumor necrosis factor-α, and interferon-γ) and lipopolysaccharide, which may not be clinically relevant. Indeed, our data suggest that in patients with CAD, systemic inflammatory stimuli that are sufficient to increase plasma biopterins (in

![Figure 5](image5.png)

**Figure 5.** Vascular BH₄ and endothelium-derived superoxide production. Endothelium-derived superoxide production was measured by dihydroethidium staining in IMA from 5 patients at the highest and 5 patients at the lowest tertile of vascular BH₄. L-NAME induced a decrease in endothelium-derived dihydroethidium fluorescence in vessels with low BH₄ levels, whereas it increased dihydroethidium fluorescence in vessels with high BH₄ levels. The arrowheads show dihydroethidium staining representing superoxide production by endothelial cell nuclei.

![Figure 6](image6.png)

**Figure 6.** Plasma BH₄ levels and superoxide production. Superoxide production was measured with the use of lucigenin chemiluminescence in SV (n=84; A, C, and E) and IMA (n=85; B, D, and F). The L-NAME–induced difference in vascular superoxide was determined as an index of eNOS coupling, and the ratio of BH₄/([BH₄] + biopterin [B]) was determined as an indicator of BH₄ oxidation. Values shown are mean±SEM according to tertiles of plasma BH₄. No significant differences existed between plasma BH₄ tertiles.

![Figure 7](image7.png)

**Figure 7.** Relationship between eNOS coupling and NO-mediated endothelial function. Relaxations to acetylcholine (ACh) (A) or SNP (B) were determined in SV rings from patients undergoing CABG. Dose–response relaxation curves (mean±SEM) are shown according to tertiles of the L-NAME–induced difference in vascular superoxide production as an index of eNOS coupling. Acetylcholine relaxations in the highest tertile of L-NAME–induced difference in vascular superoxide production were significantly greater compared with the lowest tertile, whereas SNP relaxations were identical between the tertiles (n=72 patients; *P<0.01 vs lowest tertile).
BH4, BH2, and biopterin) as an index of the overall biopterin present study we used the total biopterins levels (the sum of regulate endothelial BH4 levels in vivo.

Biopterins are not passively diffused from plasma to endothelium. In addition, we observed that inflammatory alterations in intracellular BH4 bioavailability in vascular eNOS uncoupling, and endothelial dysfunction. It is also likely that BH4 oxidation contributes to loss of vascular BH4 on the basis of previous observations in experimental models and our current finding that increased vascular superoxide was associated not only with reduced vascular BH4 but with a reduced BH4/(BH4 + biopterin) ratio. More work is required to delineate the relative importance of mechanisms that regulate endothelial BH4 levels in vivo.

Because BH4 levels are dependent on both its biosynthesis and its oxidative conversion to BH2 and biopterin, in the present study we used the total biopterins levels (the sum of BH4, BH2, and biopterin) as an index of the overall biopterin biosynthesis, representing an indirect index of GTPCH activity. We observed an inverse relationship between plasma and vascular biopterins, with plasma (but not vascular) biopterins being positively associated with circulating CRP levels. However, we observed no significant association between absolute levels of plasma and vascular BH4. In contrast to total biopterins, BH4 bioavailability is dependent on both biosynthesis and oxidative degradation and appears to be differentially regulated in the plasma and vascular compartments. It seems likely that the proinflammatory stimuli in human atherosclerosis in vivo are able to increase the biosynthesis of biopterins in cell types with major contribution to the circulating biopterin pool (such as inflammatory cells or the liver) but not in human endothelium. Under these conditions, BH4 is increased in the circulation because of its increased synthesis, but this effect is accompanied by an impairment of endothelial function, possibly as a result of the effect of the coexisting inflammation rather than due to alterations in intracellular BH4 bioavailability in vascular endothelium. In addition, we observed that the inflammatory component (as evidenced by CRP levels) was the only independent predictor of plasma biopterin levels but not of vascular biopterin levels. The observed discordance between plasma and vascular biopterins suggests that the circulating biopterins are not passively diffused from plasma to endothelial cells (or vice versa) but that this transfer is mediated by a more complex mechanism.

A limitation of the present study is the absence of any information about biopterin levels in vessels from healthy individuals. By definition, these measurements require access to surgical material obtained during CABG, and no measurement in healthy human vessels is possible. In addition, this study presents an association between BH4 and vascular superoxide production/endothelium-dependent vasodilation, which does not prove a cause-and-effect relationship. Finally, it would be interesting to determine whether vascular BH4 is correlated with the eNOS protein levels in human vessels. However, the high variability and technical limitations of Western blotting make quantitative comparisons in these small vessels difficult.

In conclusion, we demonstrate that vascular BH4 levels are a key determinant of eNOS coupling, vascular superoxide production, and endothelium-dependent vasodilation in vessels from patients with atherosclerosis. In contrast, plasma biopterin levels are associated with plasma CRP levels and inversely correlated with vascular biopterins and with vascular endothelial function. Discordant regulation of vascular and plasma biopterins in human atherosclerosis provides important mechanistic insights into the relationships between inflammation, endothelial dysfunction, and vascular oxidative stress, with direct implications for therapeutic strategies aimed at improving endothelial function.

Sources of Funding
The study was supported by the Marie Curie Intra-European Fellowship, within the 6th European Community Framework Program (Dr Antoniades). This work was also supported by grants from the British Heart Foundation (RG/02/006 to Professor Channon, FS/03/105/16340 to Dr Shirodaria) and the Margarete Waitz-Stiftung Foundation (Dr Rinze).

Disclosures
None.

References

Figure 8. Correlation of biopterin levels with CRP. Plasma total biopterin levels (left; n=183) and vascular total biopterin levels in both SV (n=82) and IMA (n=70; right) are shown according to serum CRP levels. Values of CRP and total biopterins were log-transformed for analysis and are shown on log-scale axes. HSV indicates human SV.


**CLINICAL PERSPECTIVE**

Endothelial nitric oxide synthase, which is the main source of nitric oxide in vascular endothelium, may be uncoupled in the absence of its cofactor tetrahydrobiopterin (BH4). However, the extent to which vascular and/or systemic BH4 levels are altered in human atherosclerosis and the importance of BH4 bioavailability in determining endothelial function and oxidative stress remain unclear. In the present study we define the relationships between plasma and vascular biopterin levels in patients with coronary artery disease, and we determine how BH4 levels affect endothelial function, endothelial nitric oxide synthase coupling, and vascular superoxide production in saphenous veins and internal mammary arteries obtained during coronary artery bypass grafting. We demonstrate an inverse association between plasma and vascular biopterins in patients with coronary artery disease. We also support that vascular but not plasma BH4 is an important determinant of endothelial nitric oxide synthase coupling, endothelium-dependent vasodilation, and superoxide production in human vessels. On the other hand, plasma biopterins are a marker of systemic inflammation, being positively correlated with C-reactive protein levels in human circulation. These findings suggest a distinct and contrasting biological importance for systemic versus plasma biopterins in patients with coronary artery disease. Vascular biopterins are associated with maintained endothelial nitric oxide synthase coupling and endothelial function, whereas plasma biopterins are associated with inflammation and impaired endothelial function.
Carcinoid tumors are relatively rare neuroendocrine malignancies most commonly originating from enterochromaffin cells in the gastrointestinal tract. The incidence is ~1 in 100,000 of the general population. They usually grow slowly over years, commonly causing no symptoms at all until they become large or have metastasized. Carcinoid tumors of midgut origin may secrete large amounts of vasoactive substances, including 5-hydroxytryptamine (5-HT), tachykinins, and prostaglandins. These are largely inactivated by the liver. Carcinoid syndrome occurs when tumor cells metastasize to the liver as the vasoactive substances produced are able to reach the systemic circulation via the hepatic vein. Clinically, this is characterized by flushing, diarrhea, and bronchospasm.

Over the past decade, several new therapies for carcinoid tumors have emerged to reduce symptoms and cause tumor regression. Most notably, the development of somatostatin analogs, which inhibit the release of various biogenic amines and peptides, including serotonin, has resulted in a marked improvement in symptoms. These may also have contributed to increased survival, although this has not been proved. Rarely, surgical resection is curative for nonmetastatic disease. Otherwise, reduction of symptoms, improvement in quality of life, and improvement in survival by inhibition of tumor hormones or reduction of tumor load are the main goals. Metastatic disease of the liver may be debulked surgically or by hepatic artery embolization in selected patients. Interferon therapy and targeted radionuclide therapy may stabilize or reduce the tumor. Chemotherapy is rarely an option except for pancreatic, bronchial, and high-grade neuroendocrine tumors.

Carcinoid heart disease (CHD) was first reported in 1954. Several series have reported CHD in up to 70% of cases of carcinoid syndrome. Development is thought to relate to the vasoactive substances secreted by the metastatic tumor cells in the liver, reaching the right heart. This is associated with deposition of fibrous tissue on the endocardial surfaces of the heart. More recent reports have suggested that this number has reduced, perhaps as a result of the introduction of somatostatin analogs and other antitumor therapies designed to reduce the tumor load and the production of tumor secretory products. Exceptionally, CHD may present in carcinoid tumors without liver metastases or in primary ovarian carcinoid tumors in which 5-HT is thought to reach the systemic circulation directly, bypassing portal circulation and the liver.

Presentations

Up to 20% of patients with carcinoid syndrome present with CHD at diagnosis. CHD is remarkably well tolerated initially. Patients may be in functional New York Heart Association class I despite severe right-sided valve lesions. Eventually, the signs and symptoms of right heart failure, including shortness of breath on exertion, ankle edema, and fatigue, develop as CHD progresses. Case reports have demonstrated presentations resulting from pericardial effusions, restrictive cardiomyopathy,10 constrictive pericarditis,11 and patent foramen ovale presenting with cyanosis and hypoxia secondary to a combination of right heart disease and interatrial shunts.12

Clinical Examination

Initially, clinical examination reveals prominent CV waves of tricuspid regurgitation; a right ventricular heave can be palpated; and auscultation reveals the pansystolic murmur of tricuspid regurgitation, early diastolic murmur of pulmonary regurgitation, and systolic murmur of pulmonary stenosis at the left sternal edge. Murmurs may be difficult to detect because velocities in the right heart are low. Peripheral edema, ascites, and pulsatile hepatomegaly develop as the disease progresses.

Biochemical Markers and Pathogenesis of CHD

The pathogenesis of CHD and the development of carcinoid plaques remain incompletely understood, although a growing body of evidence points toward serotonin (5-HT) playing a key role.

Evidence for 5-HT–induced valvulopathy has arisen from a variety of sources. The appetite suppressants fenfluramine and phentermine have been withdrawn from the market because of the development of valve pathology with changes similar to those seen in carcinoid patients.13 These drugs display a serotoninergic action on human tissue.14

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Carcinoid heart valves demonstrate accumulation of tissue growth factor-β latency–associated peptide and latent binding protein.\(^{15}\) 5-HT has been shown to increase synthesis and upregulate tissue growth factor-β, as well as stimulating collagen synthesis by heart valve interstitial cells.\(^{16}\) These findings may contribute to the pathophysiology of carcinoid heart valve involvement because 5-HT receptors are present in human heart valves.

In animal models, both long-term 5-HT administration and the deficiency of 5-hydroxyindoleacetic acid (5-HIAA) transporter gene can induce morphological and echocardiographic changes consistent with cardiac fibrosis and valvulopathy similar to those seen in human CHD.\(^{17,18}\)

5-HT is metabolized to urinary 5-HIAA by monoamine oxidases in the liver. Mean 5-HIAA level has a high sensitivity (100%) but a very low specificity for the development of CHD. Therefore, it has been postulated that although 5-HT is important, other factors combined with serotonin must be required for the development of CHD.\(^{19}\) The tachykinins neuropeptide K and substance P have been shown to be elevated in CHD and may be an important part of the pathogenesis of CHD.\(^{6}\) Peak 5-HIAA is a significant predictor of the progression of CHD.\(^{20}\)

N-terminal brain natriuretic peptides are released by the atria and ventricles of the heart in response to wall stress.\(^{21}\) Brain natriuretic peptide is released in a variety of valvular lesions and ventricular dysfunction. Significantly greater median levels of N-terminal brain natriuretic peptides are found in patients with CHD than in those without CHD. A high sensitivity may allow accurate differentiation between those with and without CHD and its use as a possible screening test for CHD.\(^{7}\)

**Morphological and Histological Features of CHD**

The carcinoid plaque, composed of smooth muscle cells, myofibroblasts, and elastic tissue, forms a white fibrous layer lining the endocardial surface of cardiac valves superficial to normal valve tissue. Native, underlying valve morphology is unharmed.\(^{22}\) Plaques develop on the endocardium of the right ventricle and atrium, the valve leaflets, and the subvalvular apparatus, including chordae and papillary muscle. Deposition of plaques has been found in the vena cava, pulmonary artery, coronary sinus, and coronary arteries.\(^{23}\) The tricuspid valve plaques have a preponderance to develop on the ventricular side of the leaflets, causing adherence to mural endocardium and creating a substrate for regurgitation of blood volume. Fibrous tissue at the valve annulus causes constriction at the ring, resulting in a degree of valvular stenosis. For the pulmonary valve, the predominant lesion is stenosis because plaques develop at the pulmonic root, causing constriction of the root and diminishing an already small orifice.\(^{24}\)

**Investigations**

**ECG and Chest X-Ray**
The ECG and chest x-ray may provide clues to the diagnosis of CHD. The cardiothoracic ratio may be enlarged. The ECG in patients with CHD has a higher frequency of low-voltage QRS complexes in CHD patients than those without; however, they are not sensitive.\(^{5,24}\)

**Cardiac Imaging in CHD**

**Echocardiography**
The echocardiographic features of CHD are well described.\(^{3,25}\) Appearance are pathognomic in the absence of exposure to the appetite suppressants fenfluramine and phentermine, ergot-derived dopamine agonists, and ergot alkaloid agents such as methysergide and ergotamine.\(^{13,26}\)

Multiple views of each valve should be obtained for optimal evaluation of right-sided heart valves. The tricuspid valve is visualized in the parasternal long-axis view of the right ventricular inflow tract, parasternal short-axis view, apical 4-chamber view, and subcostal long-axis view. The pulmonary valve is visualized in the parasternal long-axis view of the right ventricular outflow tract, parasternal short-axis view, and subcostal short-axis view.\(^{27}\)

Classically, both tricuspid and pulmonary valve leaflets and their corresponding subvalvular apparatus are thickened. Excursion of the leaflets is reduced. Eventually, valve leaflets become retracted, fixed, and noncoapting, leading to the valve remaining in a semiopen position. Functionally, a combination of valvular regurgitation and stenosis occurs (Figures 1 and 2). A “dagger-shaped” continuous-wave Doppler profile, resulting from severe tricuspid regurgitation that causes early peak pressure and rapid decline and representing equalization of right atrial and ventricular pressures, is seen in severe disease. The tricuspid valve, with or without pulmonary valve involvement, is involved in most cases of CHD. Indeed, it is the combination of these that creates the most hemodynamic disturbance. Pulmonary stenosis is thought to worsen the severity of tricuspid regurgitation; conversely, the severity of pulmonary stenosis may be underestimated because of low cardiac output and severe tricuspid regurgitation.

The right atrium and ventricle are typically enlarged. As the ventricle becomes volume overloaded, paradoxical motion of the interventricular septum occurs. Right ventricular function seemingly remains intact until quite late in the disease course. The increasing elevation in right ventricular pressure and increasing size of the right atrium may lead to reopening of patent foramen ovale in severe CHD.\(^{28}\)

Left-sided lesions occur in up to 15% of all cases.\(^{5,29}\) Involvement is characterized by diffuse thickening of valve leaflets and is usually less severe than right-sided valvular lesions (Figure 3). Serotonin is thought to be inactivated as it passes through lung parenchyma.\(^{30}\) Involvement of left-sided valves is thought to be due to the presence of a patent foramen ovale with a right-to-left shunt, bronchial carcinoid, or high levels of circulating vasoactive substances. Small pericardial effusions are present in up to 10% of cases. Myocardial metastases are rare.\(^{31}\) When transthoracic echocardiography cannot adequately visualize structures, transesophageal echocardiography should be undertaken.\(^{32}\)

**Cardiac Magnetic Resonance Imaging/64-Slice Computed Tomography**

Cardiac magnetic resonance imaging has been shown to provide clear anatomic and functional information on both
the pulmonary and tricuspid valve in CHD. This can be of use, particularly in evaluating the pulmonary valve when it is difficult to visualize by echocardiography and when limited ultrasound acoustic windows provide sparse echocardiographic data or in providing accurate data of right ventricular function.33,34 Recently, 64-slice coronary angiography has demonstrated similar anatomic information.34

Management
Without intervention, CHD patients may develop progressively worsening symptomatic right heart failure. Life expectancy is significantly reduced. The Mayo Clinic showed a mean life expectancy of 1.6 years for those with cardiac disease compared with 4.6 years for those without cardiac disease in patients with metastatic midgut carcinoid tumors.5 Recent improvements in medical and surgical therapy over the past decade may have improved the prognosis.

Medical
Treatment of carcinoid disease rarely achieves cure. However, with modern antitumor therapy, its progression can be substantially slowed. Many patients survive for many years


Figure 2. Carcinoid involvement of tricuspid valve (TV). A, Right ventricular inflow view. Fixed, retracted, and thickening of tricuspid valve leaflets and associated chordae. B, Continuous-wave Doppler showing dagger-shaped profile of tricuspid regurgitation (TR). C, Apical 4-chamber view showing dilated right ventricle with tricuspid valve leaflets failing to coapt resulting in constant semiopen position. D, Color Doppler demonstrating severe tricuspid regurgitation into a dilated right atrium.
after resection of a primary carcinoid tumor or palliative treatment of metastatic disease. Therefore, cardiac intervention should be considered in CHD to offer symptomatic palliation.

Medical management consists of relieving symptoms of right heart failure with a combination of loop and thiazide diuretic therapy. The use of digoxin may play a role, but no convincing data for the right ventricle are available. Intuitively, optimizing somatostatin analog therapy should reduce circulating vasoactive substances and carcinoid syndrome and therefore may stabilize CHD.

In patients not suitable for cardiac valve surgery, the use of balloon valvuloplasty has been reported. Identification of suitable patients, with predominantly stenotic valvular lesions, will be problematic in that most patients with CHD also have significant valvular regurgitation. Success of the procedure has been very limited. Although a couple reports have shown some functional and hemodynamic benefit, others have noted either a lack of symptomatic benefit or a rapid relapse of symptoms and valvular stenosis when initial benefit did occur.

Surgical
Cardiac surgery offers definitive therapy for symptoms. Marked symptomatic improvement, of >1 New York Heart Association class, occurs after valve replacement. There also may be survival benefit with cardiac surgery, although this is difficult to prove, given the other morbidities of this patient group. Median survival of 6 years with the greatest at 11 years after cardiac valve replacement compares very favorably with medically treated patients. Several series report high perioperative mortality, although the operative risk has declined from >20% in the 1980s to <10% more recently. The main perioperative complications are bleeding and right ventricular failure. Despite some patients having relatively mild pulmonary valve disease, pulmonary valve replacement in addition to tricuspid valve replacement has been shown to reduce right ventricular size after surgery compared with patients with isolated tricuspid valve replacement. Right ventricular dysfunction may not recover postoperatively. The optimal timing of surgery in relation to the severity of valve dysfunction and symptoms has not been identified. However, on the basis of these data, cardiac surgery at the onset of either symptoms or right ventricular dysfunction with pulmonary valve replacement in addition to replacement of the tricuspid valve may be considered prudent.

More controversial is the choice of valve prosthesis. No large series have compared the choice of valve prosthesis. Initial reports favored the use of mechanical prosthesis on the basis of the assumption of damage to a bioprosthetic valve with vasoactive substances. There have been several case reports of bioprosthetic valve degeneration. Carcinoid plaques have caused pulmonary valve allograft failure as early as 3 months after implantation and tricuspid biological graft dysfunction after as little as 4 years. However, the advent of somatostatin analogs and other antitumor therapies may theoretically protect the valve from deposition of further carcinoid plaques. Tissue valves have the advantage of not requiring anticoagulation and consequently lower the risk of bleeding in patients with hepatic dysfunction, reduce the risk of valve thrombosis (mechanical valve thrombosis is 4% per year), and allow further procedures such as hepatic dearterIALIZATION to proceed at a later date. Therefore, choice of prosthesis should be tailored to individual patient risk of bleeding, life expectancy, and future interventions.

There have been several reports of patients presenting with dyspnea, hypoxia, and cyanosis. Interatrial shunts via patent foramen ovale associated with valvular disease were described. Surgical closure of patent foramen ovale and percutaneous transcatheter closure devices in patients at high surgical risk have produced dramatic relief of symptoms. Elevated right atrial pressure secondary to valvular disease may have contributed to stretching of the foramen ovale and development of a right-to-left shunt.

Perioperative Anesthetic Management
Carcinoid crises characterized by hypotension, bronchospasm, and flushing can be precipitated by surgery and by drugs that release catecholamine and histamines. During the perioperative period, it can be difficult to differentiate between carcinoid crisis and hypotension secondary to myocardial dysfunction. Perioperative octreotide, aimed at reducing serotonin release, is the most efficacious treatment for preventing crises during surgery and is the mainstay treatment of carcinoid crisis. Intravenous octreotide (50 to 100 μg/h) should be started at least 2 hours before surgery. The infusion should continue for 48 hours after surgery. Patients may then require subcutaneous octreotide, depending on previous somatostatin analog requirements and current control of carcinoid syndrome. Avoiding or minimizing the use of drugs

known to precipitate mediator release such as opioids, the neuromuscular relaxant atracurium, and catecholamine producers like dopamine and epinephrine may reduce the risk of carcinoid crisis.48,49

Conclusions

Although carcinoid tumors are rare malignancies, cardiac involvement is relatively common. Despite severe disease, patients may possess relatively few signs or symptoms in the early stages. Echocardiography is the investigation of choice, revealing a unique valvular appearance. Gradual decline in right ventricular function and increasing severity of valvular disease lead to right heart failure and a poor outlook if treated medically. In view of the increasing longevity of patients with carcinoid tumors as a result of better control of carcinoid symptoms and treatment of metastatic disease, patients should be considered for surgical therapy to relieve cardiac symptoms. A multidisciplinary team experienced in dealing with these complex patients is required to provide informed decisions on optimal patient management.

Disclosures

None.

References

Prospects for Percutaneous Valve Therapies

Ted Feldman, MD; Martin B. Leon, MD

Developmental efforts to achieve percutaneous catheter-based therapies for cardiac valve repair and replacement have advanced rapidly over the past several years. A variety of methods to treat mitral regurgitation (MR) and to replace aortic and pulmonic valves have already been successfully employed in patients. These innovative clinical transcatheter valve therapies were anticipated more than a decade ago by creative experimentalists who helped develop predicate techniques in animal models. For example, in 1992, a catheter-delivered ball-in-cage prosthetic aortic valve was implanted in a canine model by Pavcnik and a stent-mounted bioprosthetic valve was placed by Andersen, who used a retrograde transarterial approach in a swine model. Clearly, the catheter-based technologies used in clinical studies today in patients with aortic stenosis were derived from the fusion of known successful aortic valve replacement (AVR) surgical devices and adaptive interventional modalities, first studied in experimental animal models. Similarly, approaches for transcatheter treatment of MR have also borrowed heavily from preexisting and accepted surgical techniques, such as the edge-to-edge leaflet coaptation technique and reduction ring mitral annuloplasty. Importantly, recognition that the coronary sinus parallels the mitral annulus has spurred unique catheter-based transvenous approaches to treat MR by indirectly reducing mitral annular dimensions. Because many of the new percutaneous approaches to valve therapy have been developed by surgeons, a collaboration has emerged between thoughtful surgeons and interventionalists, combining skill sets and experiences to accelerate the developmental pathways of less-invasive transcatheter valve therapies.

Unmet Clinical Needs

Growing recognition exists that percutaneous alternatives to surgical therapies are required in some patient subgroups with valvular heart disease. Among patients with either mitral and/or aortic valve disease, an expanding population of elderly patients with significant comorbidities may benefit from traditional surgical methods, but these methods are associated with unacceptable perioperative mortality or prolonged postoperative recoveries. In the EuroHeart Survey on Valvular Heart Disease, which monitored treatment patterns in >5000 patients from 25 countries, almost one third of symptomatic patients with mitral or aortic valve disease who met accepted guidelines for valve replacement or repair were never referred for surgery. Even more disturbing was an echocardiography laboratory report of 740 consecutive patients with severe aortic stenosis. Surprisingly, 62% of the patients with severe aortic stenosis were treated conservatively without surgical AVR, and these medically managed patients had a dismal prognosis (1-, 5-, and 10-year survival was 60%, 32%, and 18%, respectively). The clinical efficacy of surgical therapy for MR in patients with congestive heart failure and for those with ischemic MR has remained uncertain, and perioperative risks are clearly increased. Therefore, given the poorly characterized risk–benefit profile in these patients, surgical mitral annuloplasty is rarely employed as a stand-alone therapy and is used most commonly in conjunction with coronary artery bypass graft procedures. An increasing patient cohort also exists with congenital heart disease, which is treated with surgical right heart conduit procedures, in whom degeneration of bioprosthetic pulmonic valves is common and presents formidable long-term clinical challenges. These patients often undergo repetitive operative procedures with progressively increasing risk, or bare stent placement, or both. In these compelling circumstances, the addition of percutaneous pulmonic valve replacement to the treatment armamentarium affords a highly attractive alternative. It should be clearly articulated that the principle intention of the new transcatheter valve therapies is initially to expand the pool of patients with valvular heart disease who may become treatment candidates and not to replace current successful low-risk surgical therapies.

Preclinical Assessments

Preclinical development and testing of catheter-based devices for valvular heart disease has been hampered by difficulty in developing appropriate animal models. The best attempts to simulate human valvular heart disease have been the creation of functional or ischemic MR lesions in dogs, pigs, and sheep. Pacing tachycardia models (at heart rates of 200 to 220 bpm for several weeks), which induce a dilated congestive cardiomyopathy with associated MR, have been used to assess coronary sinus indirect annuloplasty procedures. In these models, reduction in mitral annular dimensions has been demonstrated with improved MR, but extrapolation to human clinical scenarios using the same devices has been problematic. Similarly, coronary artery ligation techniques have been used in sheep models to produce ischemic MR, but the
reproducibility of the model can be difficult and making the transition to the more complex human anatomy has resulted in less consistent efficacy. Even more frustrating has been the inability to approximate the pathoanatomy of mitral valve prolapse (degenerative MR) and calcific aortic stenosis outside of the human clinical setting. Therefore, animal models have been used primarily to examine early and late healing responses of implanted devices, to test catheter delivery systems, and to refine operator implantation techniques. All transcatheter valve replacement therapies are subjected to careful finite element analysis and long-term durability testing on conventional ex vivo pulse duplicators (200 to 600 million cycles) to characterize device failure modes.

Clinical Trial Methodologies

The clinical trial pathways for emerging catheter-based valve therapies are still in a state of rapid evolution. Often a delicate balance must be struck between the requirements to satisfy the rigorous US Food and Drug Administration standards for a permanent transcatheter valve therapy and the economic realities of small start-up device companies. Initial first-in-humans experiences in 10 to 30 patients are necessary to establish safety and general operator use principles and to determine proof-of-concept feasibility. Next, larger, more rigorous multicenter registries (30 to 100 patients) are recommended to encourage device design iteration, to improve and standardize operator technique, to uncover device failure modes, and to compare clinical safety and efficacy outcomes with natural history data and surgical series. Finally, pivotal randomized controlled trials are performed in several hundred patients with a frozen device design versus an appropriate surgical or medical therapy control group. At every stage problems abound, including agreement on the target patient population and control group therapy, definitions of end points, duration of follow-up, and physician training requirements. Finally, one can legitimately raise the question of whether percutaneous catheter-based valve therapies in a morbid elderly patient cohort should be held to the same high standards as surgical therapies performed in younger and healthier patients.

Most assuredly, to propose transcatheter valve alternatives will require the demonstration of incremental clinical benefits in well-characterized patient cohorts studied with the use of rigorous clinical trial methodologies. Thus, these new therapy approaches must have substantive clinical value and cannot simply be the fashionable extrapolation of previous catheter-based treatments for vascular disease.

Percutaneous Pulmonary Valve Replacement

The first successful human percutaneous implantation of a catheter-based stent valve was accomplished in the pulmonic position by Bonhoeffer in 2000. A bovine jugular vein valve was sutured onto a platinum stent and the stent-valve device was compressed on an 18-mm balloon catheter and enclosed within an 18F sheath. The stent valve was delivered percutaneously via the femoral vein in a 12-year-old boy with a severely stenosed pulmonary valve in a right ventricle-to-pulmonary artery conduit. After sheath retraction, the stent valve was deployed by balloon inflation at the point of greatest obstruction within the degenerated Carpentier-Edwards prosthetic valve and immediate hemodynamic improvement was observed. This created a functional pulmonic valve in a case where a bioprosthetic valve had failed, which otherwise would have required a repeat high-risk cardiac operation. The trileaflet bovine jugular vein valve used in this landmark case was well suited to the lower-pressure right heart circulation and proved ideal as a valve material for this first application in humans. Subsequently, this device in a variety of similar formulations has been employed in ~200 patients worldwide with congenital heart disease. Seventeen of these have been stent-within-stent procedures. Infrequent procedural complications (~6% of cases) have included homograft rupture, device dislodgement, coronary compression, and jailing of the right pulmonary artery. One- and 5-year freedom from subsequent surgical procedures to treat device failures has been 90% and 80%, respectively (Philipp Bonhoeffer, MD, personal communication, 2007). In addition to sparing these patients the rigors of an additional or repeat high-risk surgical cardiac procedure, pulmonic valve replacement in this population has been shown to have a profound impact on the physiology of right ventricular outflow tract obstruction occurring late after repair for other congenital heart defects, with improvements in symptoms, aerobic and anaerobic exercise capacity, right ventricular volumes, and systolic and diastolic function.

Surgical Aortic Valve Replacement—The “Gold Standard,” but Not for Everyone

Few therapies in cardiovascular medicine are as accepted and standardized as the surgical treatment of symptomatic patients with severe valvular aortic stenosis. The recent American College of Cardiology/American Heart Association Guidelines for management of valvular heart disease list four class I recommendations for AVR in patients with severe aortic stenosis. Surgical AVR has had an illustrious history for >40 years, with low mortality, continued improvement in operative and perioperative patient management techniques, and increased valve durability. Unlike many current therapies, we can state unequivocally that surgical AVR in symptomatic patients with severe aortic stenosis both relieves symptoms and prolongs life.

Despite clear benefits of AVR in patients with aortic stenosis, morbidity and mortality of valve replacement surgery remains significant in several subgroups. Average perioperative mortality reported from the recent Society of Thoracic Surgeons database is 5.5% to 6.8% for AVR combined with coronary bypass surgery. Surgical mortality increases by 33% in low-volume centers and increases to >10% in octogenarians. Other traditional operative risk factors include female gender, pulmonary hypertension, chronic pulmonary disease, prior cardiac surgical procedures, chronic renal insufficiency, reduced ejection fraction (especially an ejection fraction <30% with prior myocardial infarction), and New York Heart Association class III and IV congestive heart failure. In addition, other imponderables contribute to surgical mortality, such as heavy calcification of the thoracic aorta (so-called porcelain aorta), chest-wall radiation exposure, and chest-wall deformities. These higher
It is essential that no component of the stent valve (the catheter-based aortic valves should be similar to surgical to function immediately. The hemodynamic performance of diseased native valve to allow the new tissue valve to begin must remain securely implanted with displacement of the by either balloon expansion or after withdrawal of a sheath profiles of some devices to 18F. The stent valve is deployed aortic stent valves were large, stiff devices (24F to 26F in expanding or self-expanding stent (or cage) and mounted on trileaflet valve is sewn or affixed to a circular balloon-pericardium has been the valve material of choice. The favor the use of biological trileaflet tissue valves, and aortic valve implant by Cribier. Design engineers currently over the past 4 years, since the first successful percutaneous based aortic valve system are formidable and have evolved The current design requirements for a permanent catheter-based aortic valve surgery is either not considered or results in disproportionate perioperative mortality and morbidity. This so-called high-risk group has been the focus for new transcatheter AVR therapies.

Transcatheter Aortic Valve Replacement General Considerations
The current design requirements for a permanent catheter-based aortic valve system are formidable and have evolved over the past 4 years, since the first successful percutaneous aortic valve implant by Cribier. Design engineers currently favor the use of biological trileaflet tissue valves, and pericardium has been the valve material of choice. The trileaflet valve is sewn or affixed to a circular balloon-expanding or self-expanding stent (or cage) and mounted on a catheter system for deployment. The earliest percutaneous aortic stent valves were large, stiff devices (24F to 26F in diameter), but iterative designs have reduced the system profiles of some devices to 18F. The stent valve is deployed by either balloon expansion or after withdrawal of a sheath that releases a self-expanding stent. The deployed stent valve must remain securely implanted with displacement of the diseased native valve to allow the new tissue valve to begin to function immediately. The hemodynamic performance of the catheter-based aortic valves should be similar to surgical counterparts with minimal transvalvular gradients (<10 mm Hg) and measured aortic valve areas of 1.5 to 2.0 cm². It is essential that no component of the stent valve obstruct the native coronary arteries or interfere with function of the mitral valve apparatus. Also, there must be circumferential apposition of the stent valve to the aortic annulus to prevent the occurrence of paravalvular aortic regurgitation. Successful application of catheter-based aortic valve technology depends greatly on careful attention to operator technique and procedural methodology. All current catheter-based aortic stent valve procedures begin with conventional balloon aortic valvuloplasty to provide an enlarged passage-way for the insertion of the larger stent-valve device. Careful patient screening for vascular access and aortic annulus dimensions for correct valve sizing are critical. Because of increased periprocedural complications associated with the earlier transvenous antegrade approach, the transarterial retrograde approach is now the preferred technique. This requires femoral artery access (and usually a surgical arterial repair for closure); negotiation of the femoral, iliac, and aortic vasculature; and retrograde crossing of the calcified aortic valve. In general, the implanted catheter-based aortic valves are oversized relative to the annulus to ensure stability and immobility of the implanted stent and to minimize paravalvular regurgitation. During valve deployment, to ensure hemodynamic stability and to minimize movement effects of pulsatile aortic flow heart on stent-valve positioning, additional hemodynamic support devices or temporary rapid right ventricular pacing (to heart rates ≥200 beats per minute) may be necessary. After implantation of the stent valve, postdilation with slightly larger balloon catheter may be needed to reduce paravalvular regurgitation.

The Cribier-Edwards Valve
The Cribier-Edwards Aortic Bioprosthesis (Edwards Lifesciences Inc., Orange, Calif) has been in development for 8 years. The leaflet material is currently thin, durable equine pericardium. The 3 valve leaflets are hand-sewn to a stainless steel, tubular, slotted balloon expandable stent (Figure 1, left). Currently stent valves are available that are 23 mm and 26 mm in diameter and that accommodate aortic annulus sizes between 18 mm and 24 mm. At the time of implantation, the sterile stent valve is carefully mounted and crimped (with a specialized crimping tool) onto conventional balloon dilatation catheters. At present, during retrograde procedures, the balloon catheter and stent valve assembly are placed within a tip-deflecting catheter, which is then inserted into a 22F to 24F flexible arterial sheath. Active flexion of the tip-deflecting catheter navigates aortic tortuosity and assists with native valve crossing. After successful balloon valvuloplasty and retrograde positioning of the stent valve, rapid ventricular pacing is initiated and balloon inflation deploys the stent valve in the subannular position.
An alternative to the percutaneous access approach for the Cribier-Edwards bioprosthesis involves the use of a transaortic, transapical device access strategy. A left anterolateral intercostal incision is used to expose the left ventricular apex. Using direct needle puncture, a 33F sheath is inserted into the left ventricle. The stent valve, which is identical to the percutaneous transfemoral version, is mounted in the antegrade direction on a shorter catheter and is positioned in either a catheterization laboratory or an operating room with the use of fluoroscopy, aortography, and echocardiography. This technique encourages a close collaboration between surgeons and interventionalists and avoids the sometimes forbidding iliofemoral arterial access anatomy that may preclude the percutaneous retrograde approach.

The first successful percutaneous aortic stent valve implantation was performed by Cribier on April 16, 2002, in a patient with critical aortic stenosis and cardiogenic shock, multiple comorbidities, and no therapy options because he was refused surgical AVR. The antegrade approach was used to implant a 23-mm bovine pericardial stent valve. The valve performed flawlessly, and the patient experienced dramatic hemodynamic improvement, but he died 17 weeks later after a lower limb amputation for peripheral vascular disease. After 3 additional “compassionate use” cases performed by Cribier’s team in Rouen, France, a European registry was initiated for patients without surgical options (Initial Registry of EndoVascular Implantation of Valves in Europe (I-REVIVE)). This was followed by another single-center registry in Rouen that used the antegrade approach in high-risk patients who had been refused surgical AVR (Registry of Endovascular Critical Aortic Stenosis Treatment (RECAST)). Cribier recently reported the procedural results, clinical outcomes, and impressions from the 36 patients treated in his center who enrolled in these registries. In 3 patients, because of procedural complications, valve implantation was never attempted. Overall, successful stent-valve placement was achieved in 27 patients (75%); 23 patients were treated using the antegrade approach and 4 were treated using the retrograde approach. Reasons for failure to successfully implant the stent valve included hemodynamic instability, stent-valve embolization into the aorta after deployment, and failure to cross the native valve from the retrograde direction. Hemodynamic results after successful stent-valve implantation were uniformly excellent; echocardiography mean aortic valve gradients decreased from 37 to 9 mm Hg (P<0.0001) and mean aortic valve areas increased from 0.6 to 1.7 cm² (P<0.0001). Ejection fraction increased from 45% pretreatment to 53% at 1 week (P=0.02). Postprocedural aortic regurgitation (moderate or severe) was present in 17 patients (63%) and was always paravalvular in origin. Aortic insufficiency was more common when the only available device size was 23 mm in diameter. Aortic insufficiency has been diminished with the availability of the larger, 26-mm diameter device and the use of additional balloon dilatation after initial prosthesis deployment when needed. Procedure-related in-hospital complications were disturbing, including 6 deaths and 1 stroke (26%). An additional 10 patients died in the ensuing 6 months. In all cases, out-of-hospital mortality was noncardiac and caused by comorbid conditions. Importantly, extended follow-up in the surviving patients shows continued excellent valve function and maintained symptom relief. The longest survivor from this series received the stent valve >3 years ago.

An important advance in transcatheter Cribier-Edwards AVR was led by Webb and colleagues, who refined the retrograde implantation approach with the use of improvements in equipment and procedural techniques. The availability of a 26-mm valve prosthesis has reduced the likelihood of valve embolization and the severity of paravalvular regurgitation. The use of a supportive tip-deflecting loading catheter assists with catheter navigation and native valve crossing. Webb et al reported the initial 18 cases performed with the retrograde technique. Fourteen successful stent valve implants occurred, with 2 procedure-related deaths (1 death as a result of complications from an iliac artery rupture and the other related to inadvertent obstruction of the left main coronary artery by the prosthesis). In the expanded patient series from Webb et al in Vancouver of >40 retrograde cases (John Webb, MD, unpublished observations, 2007), procedure-related 30-day mortality has been <10%. In the United States, a 3-center phase I safety and feasibility study, Percutaneous Endovascular Implantation of Valves (RE-Val), is ongoing in patients with severe aortic stenosis and high–surgical risk characteristics as determined by a logistic EuroSCORE of >20% and independent surgical assessments of risk profile. Thus far, in the first 55 patients, in whom the retrograde approach was used, there have been 47 successful valve implants (87%) and 4 (7.3%) procedure-related deaths at 30 days (1 death was caused by procedural valve embolization, 1 death was caused by refractory heart failure 2 weeks after failed valve implantation, and the other 2 deaths were caused by complications of acute thoracic aorta dissection during failed valve crossing). Other periprocedural complications have included stroke (5 patients) and iliac artery rupture (5 patients). The major limitation of the current Cribier-Edwards aortic bioprosthesis resides in the large device profiles rendering transfemoral access treacherous in patients with diseased arterial vasculature. As in prior series, short-term hemodynamic results have been excellent and the frequency of moderate or severe paravalvular regurgitation has been markedly reduced (to <10%).

The major limitation of the current Cribier-Edwards aortic bioprosthesis is the large device profile. Nevertheless, the clinical trial process using this device has evolved significantly over the past 4 years and current clinical outcomes are sufficiently encouraging to justify a randomized clinical trial versus an appropriate control group in critical aortic stenosis patients who are at high risk for standard surgical AVR.

The CoreValve System

The ReValVing System (CoreValve, Paris, France) consists of 3 components: a self-expanding frame with a trileaflet porcine pericardial valve, a delivery catheter that has been reduced in size from 25F to most recently 18F, and a loading system. The self-expanding nitinol support frame (total length 45 mm) has a diamond-cell configuration and incorporates 3 different areas of radial force (Figure 1, right). The upper-frame portion anchors in the aorta above the coronary...
sinuses with low radial force, the middle portion contains the valve leaflets, and the lower portion has high radial force and implants in the subannular region. Despite crossing the coronary ostia, the frame geometry is narrowed and unopposed to the aortic wall in its middle portion, thus allowing normal flow and free access to the coronary ostia through the stent struts. The single-layer porcine pericardial tissue valve is sewn to the frame with polytetrafluoroethylene (PTFE) sutures. The present system can be used in patients with distal aortas <45 mm in diameter and with aortic annulus sizes <23 mm in diameter. After balloon aortic valvuloplasty, the CoreValve ReValving system is positioned in a transaortic valve location and the overriding sheath is withdrawn over several minutes to expose and anchor the frame-valve unit. During the critical several minutes of valve deployment, percutaneous external hemodynamic support is used to maintain patient stability. Because of the self-expanding nature of the nitinol frame, continuous further expansion of the system occurs over 30 to 60 minutes, which provides apposition at the nitinol frame, continuous further expansion of the system occurs over 30 to 60 minutes, which provides apposition at the proximal and distal implantation zones, minimizing paravalvular regurgitation.

Since the initial report of the first successful implantation of the CoreValve stent valve prosthesis in 2005, several cases have been made in device design, operator technique, and clinical outcomes. The largest reported clinical series of CoreValve cases includes 25 cases; the initial 10 cases used and clinical outcomes. The largest reported clinical series of CoreValve cases includes 25 cases; the initial 10 cases used the first-generation 25F system (bovine pericardial valve material), and the subsequent 15 patients were treated with the second-generation 21F system (porcine pericardial valve material). The average baseline peak and mean transvalvular aortic pressure gradients were 69.3 mm Hg and 44.2 mm Hg, respectively, and after successful CoreValve implantation, gradients were reduced to 21.3 mm Hg and 12.4 mm Hg, respectively (both \( P < 0.0001 \)). Postprocedure aortic regurgitation grade was unchanged (0.9 preprocedure and 0.7 postprocedure), and no cases of moderate or severe paravalvular regurgitation occurred. Major in-hospital cardiovascular and cerebral events occurred in 8 (32%) patients, including death in 5 patients (20%). Among the 8 patients with device success who survived to discharge, no adverse events occurred within 30 days after discharge.

An informal report of the complete CoreValve clinical experience includes the aforementioned Siegburg series and the international ongoing multicenter trial (unpublished CoreValve company records, 2007). A total of >100 patients have been treated; 14 were treated with the 25F first-generation device and 63 were treated with the 21F second-generation device, and the remainder with the most recent 18F device. Patients were at high risk for surgical AVR (either compassionate use or logistic EuroSCORE \( > 20 \% \)), and significant improvement occurred between first- and second-generation patient cohorts with device success increasing from 86% to 93% and 30-day death rate decreasing from 43% to 15%. The third-generation 18F device is now in use and promises to expand the treatment potential of this prosthesis. A limitation of the CoreValve approach has been the requirement for extracorporeal cardiopulmonary support during deployment. An alternative has been the use of a percutaneous left ventricular assist device for ventricular unloading, which obviates the need for ventilatory support and extracorporeal oxygenation. The lower-profile 18F device may not require as much dependence on temporary cardiopulmonary support, thus simplifying the procedure.

**Other New Technologies**

Several other new percutaneous AVR technologies are being developed. These new devices offer creative design features and attempt to address some of the deficiencies of the first generation technologies. Common characteristics include retrograde valve crossing, reduced profiles, pericardial tissue valve leaflets, and the ability to retrieve and reposition the device before final deployment and release. Some of the most promising of these new aortic valve technologies are described below.

The Direct Flow percutaneous aortic valve (Santa Rosa, Calif) is a stentless, inflatable, fabric-cuff, equine pericardial tissue valve. After advancement across the diseased native aortic valve, the distal ring is positioned and inflated with contrast to secure fixation. Tethers are used to align the valve correctly and the proximal ring is then deployed. Once valve position, function, and sealing are confirmed, a permanent polymer is infused to replace the contrast, followed by detachment of the control tethers (Figure 2, left).

The Lotus percutaneous aortic valve by Sadra Medical (Saratoga, Calif) comprises a nitinol continuous braid frame with a bovine pericardial trileaflet tissue valve (Figure 2, middle). The 19F outer diameter catheter is delivered across the aortic valve and unsheathed. The self-expanding nitinol prosthesis passively shortens and self-centers with low radial force, which allows the valve to begin functioning. When it is optimally located, the nitinol frame is actively shortened and locked to its final height (19 mm), which increases the radial force and secures the bioprosthesis. The frame-valve assembly is attached to the catheter deployment system by 15 arms and the device can be re-elongated, retrieved, and repositioned at any time before final release from the catheter.

Finally, the AorTx percutaneous aortic valve (Palo Alto, Calif) consists of a low-profile, folded, metallic frame that incorporates a pericardial tissue valve (Figure 2, right). The frame is positioned, springs open to unfold the trileaflet valve, and with high radial force is securely implanted. As with the previously mentioned designs, the system is fully retrievable and can be repositioned before final detachment from the catheter delivery system.

**Transcatheter Aortic Valve Replacement Clinical Trial Considerations**

Given the success of surgical AVR for normal-risk patients, the focus for new transcatheter AVR technologies has been on high–surgical risk patients. Optimal characterization of patient risk requires a combination of clinical judgment and application of standard quantitative risk assessment algorithms. The 2 most commonly applied risk assessment tools are the EuroSCORE and the Society of Thoracic Surgeons risk scoring systems. In general, high surgical risk for AVR is defined as the upper 10% risk decile or, alternatively, as a 30-day procedure death rate in excess of 15%. Less tangible risks, such as patient frailty, chest-wall pathology, and...
thoracic aorta calcification are often omitted from these risk assessment algorithms and must be considered separately when overall patient risk is determined.

Undoubtedly, successful validation of transcatheter AVR as a meaningful clinical procedure will require appropriately designed randomized clinical trials. A template clinical trial pathway might involve randomization of high surgical risk patients to either transcatheter versus surgical AVR by use of a noninferiority methodology and a primary end point of all-cause death at 1 year. In critical aortic stenosis patients deemed inoperable by current standards, a different randomized clinical trial design would be appropriate, using best medical therapy and/or balloon aortic valvuloplasty as the control arm (versus transcatheter AVR). Nevertheless, secondary quality-of-life measures and other death end points would be important in such clinical trials, and long-term follow-up should be required to assess valve durability of these new devices. In the future, if transcatheter AVR is proven to be safe and effective in the high surgical risk patients, additional clinical trials comparing transcatheter AVR to analogous surgical procedures in lower risk aortic stenosis patients, patients with aortic stenosis and coronary disease, and patients with aortic regurgitation may be considered.

Surgical Mitral Valve Repair
Surgical treatment of MR remains controversial and includes mitral valve replacement and various combinations of mitral leaflet repair procedures and reduction ring annuloplasty. The choice of operation depends on both the etiology of MR and the experience of the operating surgeon. Because MR can have various causes, no single treatment approach can be applied broadly, and a complete understanding of the pathophysiology of MR is essential for selection of the optimal surgical therapy. Patients with degenerative MR, caused by mitral valve prolapse, are usually treated with direct leaflet repair therapies, such as leaflet resection plus sliding repair or direct edge-to-edge repair, almost always combined with an annuloplasty ring. Patients with functional MR caused by dilated cardiomyopathy and resulting in incomplete leaflet coaptation are typically treated with annuloplasty approaches. Ischemic MR, a subcategory of functional MR, is often caused by varying amounts of incomplete leaflet coaptation and leaflet tethering, and it is surgically managed by a combination of coronary revascularization and annuloplasty procedures. Importantly, the beneficial impact of MR surgery in patients with reduced left ventricular function and congestive heart failure has not been well established. In particular, long-term surgical outcomes after annuloplasty procedures are discouraging in patients with ischemic MR. Nevertheless, given these caveats, a multitude of percutaneous technologies have been developed to mimic the various aforementioned surgical procedures for treatment of MR in defined patient populations.

Transcatheter Mitral Valve Therapies
Leaflet Repair
Direct leaflet repair has been accomplished using a surgical approach pioneered by Alfieri in the early 1990s. Suturing of the free leaflet edges of the mid-part of the line of coaptation results in a double-orifice mitral valve. This edge-to-edge, or “bow tie,” repair can be successful as an isolated surgical approach in patients with regurgitation localized to the middle segments of the anterior or posterior leaflets in the absence of a grossly dilated annulus. The edge-to-edge repair, often combined with an annuloplasty ring, obliterates the gap in coaptation caused by the redundant leaflets.

Surgical edge-to-edge repair has had mixed clinical results. It has been used as a bail-out procedure in cases of both functional and degenerative MR when more conventional surgical approaches had suboptimal outcomes. Isolated edge-to-edge surgical repair in a patient cohort with optimal leaflet morphology had 90% freedom from reoperation and recurrent MR >2+ at 5 years, and almost 80% freedom from reoperation and recurrent MR >2+ after 12 years. This...
latter report clearly demonstrates that isolated surgical edge-to-edge repair can be durable in selected patients.

Edge-to-edge repair has been duplicated using percutaneous clip- and suture-based devices. After transseptal puncture, MitraClip (Evalve, San Francisco, Calif) is delivered to the left atrium via a 24F guide catheter and positioned in the mid–left atrial cavity above the mitral orifice (Figure 3). The clip must be aligned in the center of the valve orifice, with the clip arms perpendicular to the line of coaptation. The process of steering the MitraClip guide catheter into optimal position is accomplished using steering knobs on the guide catheter and clip-delivery catheter, using both fluoroscopic and transesophageal echocardiographic guidance. When the clip is centered above the origin of the regurgitant jet along the line of leaflet coaptation, the clip is opened. The opened clip arms are passed through the mitral orifice; the open arms minimize the chance for chordal entanglement. After the clip is passed into the left ventricle below the mitral leaflets, it is pulled back, the leaflets are grasped, and the clip arms are closed to create a double orifice. The device can be repositioned if control of the MR is not adequate and removed if it appears to be unsuccessful. A second clip can also be placed if a first clip appears inadequate in decreasing the magnitude of MR.

This device approach has been successfully used in a phase I clinical trial in the United States, with results at 6 and 12 months reported recently. Surgical candidates with moderately severe or severe MR and cardiac symptoms or no symptoms with signs of left ventricular dysfunction were included in the clinical Endovascular Valve Edge-to-Edge Repair Study (EVEREST-I). Patients fulfilled the American Heart Association/American College of Cardiology guidelines criteria for surgical treatment of MR, and echocardiograms were evaluated using the American Society for Echocardiography methods for assessment of MR severity. Mitral leaflet morphology and MR jet origin must be suited to this approach. The regurgitant jet must arise from the central two thirds of the line of coaptation. Leaflet coaptation length and depth must be ≥2 mm and ≤11 mm, respectively. When flail segments are present, the flail gap must be <10 mm and the flail width <15 mm. These rigorous clinical and morphological criteria effectively exclude patients with severe annular dilatation. Less than 20% of echocardiograms evaluated by the core laboratory are considered appropriate for treatment with the MitraClip device.

A total of 70 patients were enrolled in this phase I trial and in the run-in portion of the subsequent EVEREST-II trial (see below) with >6 months follow-up in 27 patients. Compared with results from the most recent Society of Thoracic Surgeons database, patients referred for this percutaneous procedure were significantly older; median age was 71 years for the clip procedure compared with 59 years for surgical repairs. In these 70 patients, clips were successfully implanted in 90% and no intraprocedural major complications occurred. Acute procedure success, defined as successful clip placement with reduction in MR severity to ≤2+, was 73%. Major adverse events within 30 days included partial clip detachment without embolization in 7% of patients, all of whom underwent successful elective valve surgery, and a postprocedure stroke in 1 patient, which resolved in 1 month. Average length of hospital stay was <2 days. Even when a clip was placed and the results were suboptimal and required surgery afterward, mitral leaflet repair using standard surgical techniques has been possible as late as 18 months after the index interventional procedure. Actuarial 2-year freedom from death, mitral valve surgery, or recurrent MR >2+ has been 80% among patients discharged with successful clip therapy. The mitral clip procedure may be difficult to generalize to most interventional operators, as a significant operator learning curve is required to reduce procedure time and achieve consistent results, in addition to a familiarity with transesophageal echocardiographic techniques for therapy guidance.

The encouraging success of the Evalve clip procedure, particularly the favorable procedural safety results, has led to a randomized trial comparing this device with mitral valve surgery in the United States. EVEREST II is currently randomizing patients to percutaneous repair versus a standard surgical approach (2:1 allocation), with clinical and echocardiographic safety and efficacy end points. Importantly, in the
surgical literature there has never been a prospective, echocardiography core laboratory–evaluated, intention-to-treat trial of mitral valve repair therapy. The EVEREST II trial will be important not only in the assessment of a new percutaneous mitral valve therapy but also in defining the contemporary results of surgery for mitral valve disease.

The edge-to-edge mitral valve repair can also be accomplished using the Mobius percutaneous suture device (Edwards Lifesciences Inc., Orange, Calif). A 16F guide catheter is used to deliver a 10F therapy catheter. The therapy catheter uses suction to capture and immobilize a portion of the mitral leaflet, and then deliver a suture. The device is rotated and the procedure is repeated on the second leaflet. A nitinol fastener is delivered over the suture and the suture is clipped. Early first-in-man experiences that use this suture-based edge-to-edge approach at multiple investigator sites are accumulating. Percutaneous suture-based edge-to-edge mitral valve repair has been successful in reducing MR and creating a double-orifice mitral inlet, but additional device enhancements and refinement in patient selection and operator technique are required to achieve consistent procedure results.

**Coronary Sinus Annuloplasty**

The mainstay of surgical therapy for MR has been ring reduction annuloplasty, either as a stand-alone treatment for MR or in conjunction with mitral leaflet repair. A simplified interventional approach to simulate surgical annuloplasty has been to work from within the coronary sinus to geometrically deform the anteroposterior dimension of the mitral annulus. This procedure is dependent on the anatomic juxtaposition of the mitral annulus and the circumnavigating coronary sinus. Anchors or stents can be placed in both the distal coronary sinus or great cardiac vein and in the coronary sinus ostium, with a bridging connector, which either immediately or over time constrains the coronary sinus and reduces the cross-sectional area of the mitral annulus, thereby improving MR.

The Monarc device (Edwards Lifesciences Inc.) has been implanted in >80 patients outside the United States. The coronary sinus and anterior interventricular vein are cannulated via the right internal jugular vein with deployment of distal and proximal self-expanding stent anchors that are separated by a connecting bridge element. The connecting bridge is a coiled spring, held in an open position by biodegradable material in the spring interstices. Thus, tension on the coronary sinus (and mitral annulus) develops over 3 to 6 weeks as the spring shortens as a result of biodegradation of the embedded material. This device is in early phase I clinical trials, with published reports on only the first few patients treated. In the earliest experience, bridge fractures between the 2 anchors occurred in 3 of the 4 implanted patients (detected at days 22, 28, and 81 after device implantation) but were not associated with clinical sequelae other than worsening MR. After a redesign of the bridge element, >80 additional procedures have been performed without fractures or other device failures. Importantly, because tension on the mitral annulus is slow and gradual (coincident with bridge shortening), the efficacy in reducing MR can only be determined during follow-up assessments.

The Carillon device, delivered via a transjugular puncture and requiring a 9F guiding catheter, is progressively shortened before final deployment of the proximal anchor on the basis of the reduction in MR measured using echocardiography. This device has been inserted successfully in a small number of patients both outside and inside the United States and has already undergone a device redesign to improve coronary sinus securement and fixation. The first treated patient had ischemic MR, with sustained reduction of MR after 2 years. A continuation of the first-in-humans clinical trial is underway.

The percutaneous transvenous mitral annuloplasty system (Viacor, Wilmington, Mass) consists of a nitinol wire shaping ribbon between proximal and distal anchors that are placed in the coronary sinus. Tension applied to the shaping ribbon between the 2 anchors deforms the coronary sinus geometry, thereby reducing the mitral annulus dimensions (Figure 4). The Carillon device, delivered via a transjugular puncture and requiring a 9F guiding catheter, is progressively shortened before final deployment of the proximal anchor on the basis of the reduction in MR measured using echocardiography. This device has been inserted successfully in a small number of patients both outside and inside the United States and has already undergone a device redesign to improve coronary sinus securement and fixation. The first treated patient had ischemic MR, with sustained reduction of MR after 2 years. A continuation of the first-in-humans clinical trial is underway.

The percutaneous transvenous mitral annuloplasty system (Viacor, Wilmington, Mass) is the third in this genre of coronary sinus shape deforming devices. Percutaneous transvenous mitral annuloplasty was invented by cardiac surgeons and consists of a 7F multilumen polytetrafluoroethylene catheter, within which variable stiffness rods are inserted. The rods deform the shape of the midportion of the coronary sinus, which diminishes the septal to lateral dimension of the mitral annulus and reduces the severity of MR in animal models. After the optimal number and stiffness of rods have been inserted in a temporary diagnostic catheter, a permanent version of the device is implanted. Importantly, the system shape and stiffness can be adjusted over time by addition or substitution of rods, depending on the patient response and changes in the severity of MR. A small series of temporary implants were inserted in the operating room to establish proof of concept. Presently, a 30-patient clinical trial is ongoing at 3 centers outside the United States.
Another similar coronary sinus approach is the so-called percutaneous septal-sinus shortening procedure. An anchor is placed in the coronary sinus and a cord traversing the left atrium is attached to a septal occluder in the fossa ovalis and tensioned to modify mitral annulus geometry.61 This system is in the process of initiating first-in-humans clinical trials.

Important limitations may be associated with coronary sinus annuloplasty as a methodology to reduce MR. The coronary sinus does not directly parallel the mitral annulus in many patients, but rather is positioned about 1 cm cranial and somewhat tangential to the annulus plane.62 Thus, tension created within the coronary sinus is transmitted imprecisely and indirectly to the annulus and might dissipate into the surrounding tissues over time, reducing the efficacy of MR reduction. Moreover, the coronary sinus crosses over branches of the circumflex coronary artery in approximately half of the patients. It remains unclear whether any of the aforementioned devices will induce important circumflex artery compression and ischemia. The coronary sinus has become a frequently utilized anatomic space over the past several years and it would be important to maintain access for lead placements during procedures such as resynchronization therapy. Finally, issues of erosion and thrombosis of the coronary sinus with these devices can only be ascertained after increased clinical experiences. Despite these potential disadvantages, if a safe and predictable device reduces MR by use of a simple transvenous coronary sinus implant, this would become a worthwhile therapy for many patients.

Direct Mitral Annuloplasty
To overcome some of the potential limitations of indirect annuloplasty via the coronary sinus, direct approaches to the mitral annulus are being developed. The Mitralign device (Mitralign, Tewksbury, Mass) uses anchor pledgets that are placed directly into the mitral annulus, and a drawstring to cinch the annulus in a manner analogous to surgical plication annuloplasty (Figure 5). A relatively small (20%) reduction of the posterior annulus can normalize the septal–lateral dimension and eliminate ischemic MR.63 The surgical version of this approach was initially described in 1977,64 and acceptable clinical surgical results have been reported recently.65,66 The Mitralign annuloplasty system places anchors directly into the mitral annulus from the left ventricular side and tethers them with a plication suture. Transventricular access to the periannular space on the left ventricular side of the posterior leaflet is achieved with retrograde left ventricular catheterization by use of standard guiding catheter shapes. Clinical studies with the Mitralign device will commence in the next several months.

Transpericardial Annulus and Left Ventricular Remodeling
The Coapsys surgical system (Myocor, Maple Grove, Minn) uses pads placed on either side of the ventricle with a cord passing through the left ventricular cavity to apply tension to both the mitral annulus and the basal left ventricular cavity (Figure 6).67 Uniquely, this off-pump surgical procedure is a direct approach to achieve both left ventricular remodeling and an associated mitral annuloplasty. Initial results of the Coapsys surgical system implanted in patients with ischemic MR during coronary revascularization, have shown sustained reductions in MR and improved ventricular chamber dimensions for as long as 1 year after the procedure. A percutaneous transpericardial method to simulate this surgical procedure (iCoapsys) is under development in preclinical models.68

Percutaneous Mitral Repair Trial Considerations
Patient selection and trial design for the development of these novel technologies remains challenging.69 There have been
no prior randomized trials and little organized follow-up to evaluate surgical approaches to treat MR. Thus, no established methods exist to compare percutaneous and surgical valve therapy approaches. Percutaneous therapy is inherently different, and the standards for measuring clinical outcomes after percutaneous therapy may not be comparable to surgery. Clinical trials designed to demonstrate noninferiority for efficacy end points and superiority for safety end points may be reasonable goals for catheter-based versus surgical mitral valve procedures.

It has been demonstrated that asymptomatic patients with severe MR have a poor prognosis compared with those with less severe MR. A clinical trial is urgently needed to demonstrate that early mitral valve therapy might benefit these patients. The lower morbidity of percutaneous therapies may be well suited for such an asymptomatic, lower-risk population. Alternatively, evidence exists to indicate favorable outcomes with watchful waiting in this patient group; thus, a randomized clinical trial versus either medical or surgical therapy would be critical to evaluate the utility of any therapy in this patient population. Indirect or direct annuloplasty approaches are better suited to patients with functional MR as a result of heart failure or coronary ischemia. This population is often not treated surgically, and comparisons with medical therapy may be more appropriate.

A paucity of data on the results of mitral repair surgery complicates comparisons with catheter-based technologies. There have been no prospective intention-to-treat trials to define the rate of prosthetic replacement when valve repair is intended, and this conversion rate will impact the relative merits of surgical and percutaneous methods. The usual end point in long-term surgical reports is freedom from reoperation rather than the combination of freedom from death, reoperation, and recurrent MR. Patient selection and surgical outcomes have never been reported with the use of rigorous intention-to-treat principles, core laboratory evaluation of baseline echocardiograms, use of American Society for Echocardiography criteria for grading severity of MR, or prospective echocardiographic follow-up.

Combinations of mitral annuloplasty and leaflet repair approaches may ultimately be necessary for optimal percutaneous therapy in some patients with MR. The use of device combinations will be extremely difficult to evaluate from a clinical trial perspective. The potential for novel nondevice therapies is also just being recognized. Recently, autologous clinical trial perspective. The potential for novel nondevice neous therapy in some patients with MR. The use of device approaches may ultimately be necessary for optimal percutaneous use in animal models. The tether places tension on the septal-lateral dimension of the mitral valve and also remodels the basal left ventricle with reorientation of the papillary muscles.

Conclusions

The field of percutaneous valve replacement and repair is clearly developing rapidly. Transcatheter aortic and pulmonic valve replacement and a variety of mitral valve therapy approaches have been successfully performed in hundreds of patients. A variety of operator technique and device-related problems have been encountered and solved. Significant challenges in patient selection and clinical trial design have yet to be resolved. The patient populations who may ultimately benefit most from treatment using these new technologies will be better defined during the course of the clinical trial process. In aggregate, these creative new transcatheter approaches may change the face of valve therapy and promise to extend treatment to a larger proportion of the valve disease population.

Disclosures

Dr Feldman is on the scientific advisory board of Myocor and has received grant support from Edwards Lifesciences, and Myocor. He is principal investigator for the Evalve EVEREST trial. Dr Leon is on the scientific advisory boards of Edwards Lifesciences, Guided Delivery Systems, Mitralign, and Sadra Medical. He has received grant support from Edwards Lifesciences and owns equity in Guided Delivery Systems, Mitralign, and Sadra Medical. He is principal investigator for the Edwards Lifesciences PARTNER trial.

References


Key Words: aortic stenosis mitral regurgitation mitral valve aortic valve
AHA Scientific Statement

Safety of Magnetic Resonance Imaging in Patients With Cardiovascular Devices

An American Heart Association Scientific Statement From the Committee on Diagnostic and Interventional Cardiac Catheterization, Council on Clinical Cardiology, and the Council on Cardiovascular Radiology and Intervention

Endorsed by the American College of Cardiology Foundation, the North American Society for Cardiac Imaging, and the Society for Cardiovascular Magnetic Resonance

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Abstract—Advances in magnetic resonance (MR) imaging over the past 2 decades have led to MR becoming an increasingly attractive imaging modality. With the growing number of patients treated with permanent implanted or temporary cardiovascular devices, it is becoming ever more important to clarify safety issues in regard to the performance of MR examinations in patients with these devices. Extensive, although not complete, ex vivo, animal, and clinical data are available from which to generate recommendations regarding the safe performance of MR examination in patients with cardiovascular devices, as well as to ascertain caveats and contraindications regarding MR examination for such patients. Safe MR imaging involves a careful initial patient screening, accurate determination of the permanent implanted or temporary cardiovascular device and its properties, a thoughtful analysis of the risks and benefits of performing the examination at that time, and, when indicated, appropriate physician management and supervision. This scientific statement is intended to summarize and clarify issues regarding the safety of MR imaging in patients with cardiovascular devices. (Circulation. 2007;116:2878-2891.)

Key Words: AHA Scientific Statement ■ magnetic resonance imaging ■ cardiovascular devices

Advances in magnetic resonance (MR) imaging and MR angiography over the last 2 decades have led to MR becoming an increasingly attractive imaging modality. MR imaging provides excellent spatial resolution and multiplanar 3-dimensional analysis, while not exposing patients to ionizing radiation, the risks of invasive procedures, or potentially nephrotoxic iodinated contrast agents. MR imaging has thus developed into a broadly applied diagnostic tool for patients with cardiovascular and other disease states, and the number of patients undergoing scanning each year is increasing. At the same time, an increasing number of patients are being treated with permanently or temporarily implanted cardiovascular devices.

There remains confusion and controversy regarding which patients with cardiovascular devices can safely undergo MR examination. This has led to the unsafe examination of patients with certain devices and to the misinformed and inappropriate refusal to refer or scan patients with other devices, thus depriving the patient and treating physician of clinically useful information. Furthermore, many of the reported cases of MR-related injuries and most of the few fatalities that have occurred have been the result of failure to...
follow established safety guidelines or the use of outdated information related to the safety aspects of biomedical implants and devices. Accordingly, this scientific statement is intended to summarize and clarify issues regarding the safety of MR imaging in patients with cardiovascular devices.

It is beyond the scope of this document to provide guidelines for every cardiovascular device. Furthermore, most devices have been tested under very specific circumstances (eg, magnetic field strength, radiofrequency [RF] energy levels, and type of RF transmission coils). Additionally, devices may undergo manufacturing modification, particularly with regard to metallic composition, while retaining the same basic name, and new devices will be introduced into the market constantly. Therefore, for specific guidelines for specific devices, particularly when there is doubt as to the safety of scanning a patient with a given device, the reader is encouraged to refer to a more detailed source of safety information, such as dedicated Web sites,1,2 reference manuals,3 or, when available, the manufacturer’s product information. Broader information on MR examinations is available at several well-recognized expert Web sites4–7 and in published and online documents.8–17

General Safety Considerations
Risks associated with MR imaging generally arise from 3 distinct mechanisms related to MR imaging: (1) the static main magnetic field; (2) RF energy; and (3) gradient magnetic fields. There are several potential risks associated with MR scanning of specific cardiovascular devices that result from these processes.5,9–12,14,16–21 Most of these risks can be understood by consideration of the areas discussed below.

Static Magnetic Fields
Most currently used clinical MR scanners are 1.5 to 3 tesla (T), which corresponds to ~30,000 to 60,000 times the strength of the Earth’s magnetic field. The greatest risk from the main magnetic field is attraction of a ferromagnetic object into the scanner. For the purposes of this statement, the term “ferromagnetic” is used to denote a substance that experiences an attractive force in the presence of a magnetic field. As a result of ferromagnetic interactions, a device may be moved, rotated, dislodged, or accelerated toward the magnet. Thus, a ferromagnetic object might be accelerated toward the magnet at dangerously high velocities and/or with dangerously high forces, creating a “projectile effect” that could lead to significant patient injury or damage to the MR system. Device function may also be altered or negated as a result of interactions with the strong static magnetic fields. Most, but importantly not all, currently implanted cardiovascular devices are either nonferromagnetic or weakly ferromagnetic. The higher the static magnetic field of the MR system, the greater the resultant ferromagnetic forces on weakly or overtly ferromagnetic materials. Thus, findings from ex vivo studies at fields of 1.5 T or lower may not necessarily apply to imaging of devices at higher field strengths (eg, 3 T and higher), which produce significantly greater forces. For devices in which the ferromagnetism of the device is a significant safety concern, consideration should be given to performing the study at the lowest field strength available to reduce whatever ferromagnetic risk might be present. Finally, all healthcare professionals are reminded that currently used MR scanners are typically superconducting and thus are always “on.”

RF Energy
During MR imaging, RF energy is “pulsed” into the body to generate the MR image. The body will absorb some of the RF power and therefore will heat up (usually less than 1°C) directly owing to ohmic heating. The dosimetric term used to characterize RF energy is the specific absorption rate (SAR, measured in watts per kilogram). SAR increases with the square of the field strength.22 Certain metallic devices (such as leads) can act as an “antenna” and concentrate this RF energy, which leads to excessive local heating, especially at the tip of these devices. An example of such an interaction is the heating (and subsequent melting at the skin entry site) of a Swan-Ganz (pulmonary artery) thermodilution catheter.23

These concerns are most relevant for electrically conductive implants such as wires or leads, particularly when such wires or leads form large loops. Fractured leads may pose a particularly high risk of thermal injury. Concentration of RF energy is frequency dependent and therefore changes for a given device in a different field strength. The multiparametric nature of this risk results in the seemingly paradoxical situation of being able to identify implants/leads that test as being safe at a given field strength/frequency yet are unsafe at a higher or lower one. RF energies used in the MR imaging process can also induce electrical currents in wires and leads, which could possibly induce arrhythmias.

Gradient Magnetic Fields
Time-varying magnetic fields called gradients (dB/dt, measured in teslas per second) are used to encode for various aspects of the image acquisition. Although the gradients are much weaker than the main magnetic field, the gradients are repeatedly and rapidly turned on and off. The rapidly changing magnetic fields from the gradients can induce electrical currents in electrically conductive devices and may directly excite peripheral nerves. Although current-generation scanners operate at levels that will not directly excite cardiomyocytes, the gradients can induce currents within electrically conductive wires and leads that could cause arrhythmias.

In addition to the above considerations, several other issues merit mention. The location of the device relative to the anatomy to be studied is also an important consideration in assessing the risk-benefit ratio of the study. An understanding of the risks involved in such study requires an expert understanding of the physics involved in MR scanning. For example, some MR imaging studies of the brain may theoretically produce maximal dB/dt values over a cardiac pulse generator and leads implanted in the upper thorax. Therefore, particularly in cases in which there is a relative contraindication to device examination and the examination location is distinct from the device location, consultation with a person with expertise in MR physics and MR safety is recommended.

The very flow of electrically conductive blood in the presence of powerful static magnetic fields produces very small voltages that may produce electrocardiographic aberrations, including elevation of the ST segment, T-wave abnor-
thorough and effective screening procedures for patients who are to undergo MR examinations are essential. Indeed, most MR examination adverse events are believed to be due to deficiencies in screening methods. Therefore, all patients should undergo a thorough screening procedure for cardiovascular and other implants and devices, including an interview with a healthcare worker specifically trained in MR safety and completion of a standardized screening form, which should then be thoroughly reviewed by the MR technologist or physician. MR screening forms are available for download at several Web sites. Whenever possible and practical, particularly if there is doubt regarding patient reliability, any implanted devices should be identified via wallet-sized cards the patient may have been given and/or procedure notes. If the specific identity of a device cannot be confirmed, but it is believed for clinical reasons that the scan should be performed at that time, consideration should be given to performing the study at the lowest field strength available to reduce whatever ferromagnetic risk might be present. Inpatients should be examined for the presence of temporary devices (eg, pulmonary artery catheters or temporary pacing leads). If, during scanning, a metallic object is identified that the patient has not reported having implanted, the study should be stopped and the patient further questioned until the metallic object is identified. MR technologists should be well trained on MR safety issues, because they may often represent the “last line of defense.”

### Patient Screening

Given the risks associated with MR imaging of certain cardiovascular (as well as other) implants and devices, thorough and effective screening procedures for patients who are to undergo MR examinations are essential. Indeed, most MR examination adverse events are believed to be due to deficiencies in screening methods. Therefore, all patients should undergo a thorough screening procedure for cardiovascular and other implants and devices, including an interview with a healthcare worker specifically trained in MR safety and completion of a standardized screening form, which should then be thoroughly reviewed by the MR technologist or physician. MR screening forms are available for download at several Web sites. Whenever possible and practical, particularly if there is doubt regarding patient reliability, any implanted devices should be identified via wallet-sized cards the patient may have been given and/or procedure notes. If the specific identity of a device cannot be confirmed, but it is believed for clinical reasons that the scan should be performed at that time, consideration should be given to performing the study at the lowest field strength available to reduce whatever ferromagnetic risk might be present. Inpatients should be examined for the presence of temporary devices (eg, pulmonary artery catheters or temporary pacing leads). If, during scanning, a metallic object is identified that the patient has not reported having implanted, the study should be stopped and the patient further questioned until the metallic object is identified. MR technologists should be well trained on MR safety issues, because they may often represent the “last line of defense.”

### Safety Terminology for Implants and Devices

Terminology applied to implants and devices relative to the MR environment has evolved over the years. In 1997, the Food and Drug Administration (FDA), Center for Devices and Radiological Health, proposed definitions for the terms “MR safe” and “MR compatible” (Table 1). With this terminology, MR testing of an implant or object for MR safety involved assessments of magnetic field interactions, heating, and, in some cases, induced electrical currents, whereas MR compatibility testing required all of these plus characterization of artifacts. In addition, it may have been necessary to evaluate the functional or operational aspects of an implant or device relative to specific MR imaging conditions. Over time, however, it became apparent that these terms were often applied incorrectly or used interchangeably. Therefore, to clarify the terminology and, more importantly, because the misuse of these terms could result in serious accidents for patients and others, the American Society for Testing and Materials International developed a new set of terms: “MR safe,” “MR conditional,” and “MR unsafe” (Table 1). Notably, the US FDA is not mandating retesting (and relabeling) of implants and devices that have already received approved labeling with the older terminology. Therefore, the reader should be aware that there may be confusion with regard to the labeling of certain biomedical implants.

The labeling approved by the FDA using the latest American Society for Testing and Materials International designation is given for each device type discussed that has been labeled with this newer terminology. In addition, a more general discussion of safety issues is also provided that uses the expertise of the writing group to synthesize the FDA labeling using the American Society for Testing and Materi-
als terminology with the latest experimental and clinical data, as well as expert consensus opinion, to give guidance to as broad a target audience as possible for issues regarding MR safety and cardiovascular devices.

**MR Imaging After Device Implantation**

In general, if a device is a nonferromagnetic “passive” implant (ie, there is no electronically or magnetically activated component) made from a nonferromagnetic material (eg, titanium, titanium alloy, or nitinol), and if there are no concerns associated with MR-related heating, the patient with the device may undergo MR imaging immediately after implantation. The issue of when patients who have been treated with weakly ferromagnetic devices may undergo MR examination has not been established definitively for every device and thus remains controversial. For weakly ferromagnetic devices, it is theoretically possible that the forces present during an MR examination could move or dislodge such a device. On the other hand, some devices, such as many intravascular coils and stents that are firmly implanted into the vessel wall or adjacent tissues during the implantation process, may be further passively or actively anchored to the vessel wall or adjacent tissues and are subject to constant hemodynamically generated forces from the beating of the heart and resultant blood flow that are often much greater than the forces associated with the MR examination. However, it is generally believed that the tissue healing process that occurs over the weeks after implantation may in some cases provide an additional degree of device anchoring, and thus, it has been advocated by some to wait ≈6 weeks before MR imaging of certain devices.

For some weakly ferromagnetic devices, there are currently sufficient data and consensus that it can be recommended that patients with such devices can undergo MR examination any time after scanning. For weakly ferromagnetic devices for which there are not currently enough data and consensus to make the recommendation that scanning can be performed safely any time after implantation, the writing group recommends that the physician weigh the risks and benefits of scanning patients with such devices on a case-by-case basis and adopt the following approach: For cases that occur in the days to weeks after device implantation in which there is a clear potential clinical benefit of scanning the patient at that time (eg, acute back pain and lower-extremity weakness after trauma), the benefits of the MR examination will likely outweigh any risks of the examination, and MR examination should generally be performed. For patients in whom it makes little difference whether the scan is performed at a given time or weeks later (eg, those with chronic back pain), it may be prudent to defer MR examination until ≈6 weeks after such device implantation.

**Coronary Artery and Peripheral Vascular Stents**

**Background Data**

Most coronary and peripheral vascular stents are composed of either 316L stainless steel or nitinol. Less commonly, stents may be composed of or contain variable amounts of platinum, cobalt alloy, gold, tantalum, MP35N, or other materials. Most coronary and peripheral vascular stents exhibit nonferromagnetic or weakly ferromagnetic characteristics. Most of the stents currently used for carotid procedures are made of nitinol and are nonferromagnetic or only weakly ferromagnetic. Implantation of the stent against the vessel wall provides for immediate anchoring of the stent. It is generally believed that additional anchoring of the stent into the vessel wall occurs over ≈6 to 8 weeks primarily due to tissue ingrowth. Although this latter phenomenon may have led to recommendations that MR scanning be deferred for 6 to 8 weeks in patients treated with nonferromagnetic coronary stents, there are no good clinical data or rationale to support this recommended delay.

In 1 study, ex vivo testing at 1.5 T on 19 different coronary stents revealed 2 to be nonferromagnetic and the remaining 17 to be at worst “minimally” ferromagnetic. Other ex vivo studies of various coronary stents also led to the conclusions that MR examination with those stents tested would be safe. Studies of peripherally implanted stents yielded generally similar results, with the exception of a stainless steel Zenith/Cook iliac stent (Cook), which at 3 T was found to have ferromagnetic properties. Studies conducted thus far have not suggested any increased risk of stent subacute or late thrombosis after MR examination.

More recently, ex vivo study has been conducted on several of the more commonly used coronary drug-eluting stents, including 2005 to 2006 versions of the Cypher (Johnson & Johnson/Cordis), Taxus Express (Boston Scientific), Taxus Liberte (Boston Scientific), and Endeavor (Medtronic) stents. These ex vivo studies demonstrated a lack of ferromagnetic interactions at 3 T that would pose a risk for stent migration. Therefore, for those drug-eluting stents tested, it is believed that MR examination may be performed immediately after implantation. In those studies that evaluated stent heating, only minimal to modest heating (<1°C for a single stent and <2°C for 2 long, overlapping stents) was evident. The effect of the MR examination on heating of the drug or polymer coating used in drug-eluting stents is unknown, although heating of the stent (and possible resultant effects on the drug/polymer coating) might be somewhat mitigated by flowing blood. A recent retrospective review of patients with myocardial infarction who underwent MR examination within 2 weeks (median 3 days) of stent implantation detected no increased incidence of clinical adverse events at 30-day and 6-month follow-up compared with those who had undergone stent implantation at more distant time points. Thirty-nine percent of the stents implanted in the study group were drug-eluting stents, and no adverse cardiovascular events occurred in patients treated with drug-eluting stents.

**Labeling/Recommendations**

Most coronary and peripheral vascular stents that have been tested have been labeled as “MR safe”; the remainder have been labeled as “MR conditional.” Tested coronary artery stents (including tested drug-eluting coronary stents) that are nonferromagnetic (all currently used coronary stents) can be safely scanned at 3 T or less any time after implantation. MR
examination at \( \leq 3 \) T in patients with peripheral stents that are nonferromagnetic can be performed immediately after implantation. The timing of MR examination at \( \leq 3 \) T in patients with peripheral stents that are weakly ferromagnetic should be determined on a case-by-case basis. For cases in which there is a clear potential clinical benefit of scanning in the days to weeks after implantation, the benefits of the MR examination will likely outweigh the risks of the examination, and MR examination should generally be performed. In patients with chronic conditions in which it makes little difference whether the scan is performed at a given time or weeks later, it may be prudent to defer MR examination until \( \approx 6 \) weeks after device implantation.

The reader should be aware that local artifact remains an issue for many stents. The degree of in-stent stenosis cannot be assessed reliably in the case of coronary stents or peripheral stents, although patency of the peripheral stent can usually be inferred from a complete assessment of the MR examination.

### Aortic Stent Grafts

#### Background Data

The majority of endovascular aortic stent grafts, but not all, are made from nonferromagnetic or weakly ferromagnetic materials. An ex vivo study of stent grafts at 3.0 T found that most exhibited nonferromagnetic or weakly ferromagnetic properties, with the exception of several EndoFit stent grafts and extenders (Endomed Inc). Thus far, there have been several published studies of MR examinations in patients with aortic stent grafts that have not noted any adverse clinical events related to the MR examinations. The MR characteristics of the Zenith AAA endovascular graft (Cook) have been evaluated through bench testing in MR systems with static fields of \( \leq 1.5 \) T, and this stent graft was found to exhibit significant deflection and torque of the stainless steel metallic component of the endovascular graft and therefore did not meet standard “MR safe” bench test criteria.

A practical consideration in MR examinations of endovascular stents relates to the potential magnetic susceptibility effects (artifacts) induced by the metallic components of the stent grafts. Most stent grafts create minimal artifacts, which allows for diagnostic visualization of the endostent lumen and for evidence of endostent leak. However, 3 stent grafts (Zenith AAA endovascular graft [Cook], Endologix AAA stent [Endologix], and Lifepath AAA stent [Edwards Life-sciences Corp]) have been reported to show severe susceptibility artifact that makes evaluation of the endostent lumen or surrounding tissues problematic.

#### Labeling/Recommendations

Most aortic stent grafts that have been tested have been labeled as “MR safe”; the Zenith AAA endovascular graft stent has been labeled as “MR unsafe.” Patients with stent grafts made from nonferromagnetic materials may be scanned immediately after implantation at 3 T or less. The timing of MR examination at 3 T or less in patients with aortic stent grafts that are weakly ferromagnetic should be weighed on a case-by-case basis. For cases in which there is a clear potential clinical benefit of scanning in the days to weeks after implantation, the benefits of the MR examination will likely outweigh the risks of the examination, and MR examination should generally be performed. In patients with chronic conditions in which it makes little difference whether the scan is performed at a given time or weeks later, it may be prudent to defer MR examination until \( \approx 6 \) weeks after device implantation.

The approved manufacturer’s labeling for the Zenith AAA endovascular graft states in part, “Adverse events have not been reported clinically in patients who have undergone MRI. However, sufficient data are not available to demonstrate MRI safety and there may be potential risks (eg, device migration, vessel damage) that could be associated with force applied to the metallic components of the Zenith AAA Endovascular Graft. Therefore, a careful assessment of these potential risks and the potential benefits to the patient should be completed before use of MR imaging.” The writing group agrees with this approach.

Although patients with the Endologix AAA or Lifepath AAA stents may undergo MR imaging, because of the artifacts created by these stents, MR examination is not recommended as the modality of choice for examinations specifically targeted toward evaluation of the stent grafts.

### Prosthetic Heart Valves, Annuloplasty Rings, and Sternal Suture Wires

#### Background Data

Prosthetic heart valves and annuloplasty rings are made from a variety of materials. Bioprosthetic heart valves are composed primarily of nonmetallic materials (usually porcine tissue or bovine pericardium) but may contain small amounts of metal (used for scaffolding rings), depending on whether or not they are “stentless” or have other design features. Mechanical heart valves are composed of a variety of metals, including titanium alloy, MP35N, pyrolytic carbon, Eligloy, chromium cobalt alloy, nitinol, 316L stainless steel, and 316LVM stainless steel. Some annuloplasty rings contain no metal, whereas others may be composed in part of titanium, chromium cobalt, and other metallic materials. Sternal wires are most commonly composed of stainless steel or similar alloys.

Many heart valve prostheses and annuloplasty rings have been evaluated to determine whether they are acceptable for patients undergoing MR examinations with scanners operating at 1.5 T or less. Of these, several displayed measurable yet relatively minor magnetic field interactions in relation to the static magnetic fields of the MR systems used for testing. The forces exerted on these valves and rings are less than the forces exerted by gravity and considerably less than those exerted by the beating heart and resultant pulsatile blood flow. A recent study using tissue samples excised during heart valve replacement surgery demonstrated that the forces required to pull a suture through a valve annulus tissue were significantly greater than magnetically induced forces at \(< 4.7 \) T. Accordingly, patients with degenerative valvaral diseases are unlikely to be at risk for valve dehiscence (loosening or unseating of the valve from its sewed-in position in the heart) during exposure to static magnetic fields up to 4.7 T.
MR-related heating of prosthetic heart valves and annuloplasty rings has been assessed with ex vivo techniques. These studies indicated that temperature increases are relatively minor, with studies reporting heating ranging from 0°C to 0.8°C. With vascular stents, any heating is likely to be somewhat dissipated by flowing blood. Although there is a theoretical possibility of an electromagnetic interaction with a heart valve that contains metal in the disk or leaflet that could inhibit opening and closing of the mechanical heart valve prosthesis (referred to as the Lenz effect), this has never been demonstrated experimentally or reported clinically. Those valves and rings that have undergone testing thus far at 3 T have not demonstrated clinically significant magnetic field interaction or MR-related heating and thus have been found to be safe for clinical MR examinations.1,3

Numerous clinical studies have demonstrated the safety of performing MR examinations in patients with prosthetic heart valves. Of note, 28 patients recently underwent apparently uneventful cardiac MR imaging after percutaneous pulmonary valve implantation. As of this writing, we are unaware of any case of a patient incident or injury related to the presence of a heart valve prosthesis or annuloplasty ring in association with an MR examination.

Labeling/Recommendations

The majority of prosthetic heart valves and annuloplasty rings that have been tested have been labeled as “MR safe”; the remainder of heart valves and rings that have been tested have been labeled as “MR conditional.” On the basis of the above studies and findings, the presence of a prosthetic heart valve or annuloplasty ring that has been formally evaluated for MR safety should not be considered a contraindication to an MR examination at 3 T or less (and possibly even 4.7 T in some cases) any time after implantation. MR examination of patients with sternal wires is generally considered to be safe.

Cardiac Closure and Occluder Devices

Background Data

Cardiac closure and left atrial appendage occluder devices are typically made from metals that include nitinol, titanium, titanium alloy, MP35N, 316L stainless steel, and 304V stainless steel. In addition, nonmetallic fabrics and other materials are often used for these devices. In tests for magnetic field interactions conducted at 1.5 T, devices made from 304V stainless steel displayed weakly ferromagnetic qualities, whereas those made from nitinol, titanium, titanium alloy, and MP35N were nonferromagnetic. Several closure devices have been evaluated at 3 T. For those tested, studies demonstrated acceptable deflection angles, torque, and MR-related heating with regard to the intended in vivo uses of these specific devices. Two to date, at least 1 left atrial appendage occlusion device, the Watchman left atrial appendage device (Atritech, Inc), has been tested at 3 T. Findings indicated that patients with this device can be safely scanned at 3 T (Frank Shellock, unpublished data, 2006).

Labeling/Recommendations

The majority of cardiac closure and occluder devices that have been tested have been labeled as “MR safe”; several that have been tested are labeled as “MR conditional.” Patients with nonferromagnetic cardiac closure and occluder devices may undergo MR procedures at any time after implantation. The timing of MR examination at 3 T or less in patients with cardiac closure or occluder devices that are weakly ferromagnetic should be weighed on a case-by-case basis. For cases for which there is a clear potential clinical benefit of scanning in the days to weeks after implantation, the benefits of the MR examination will likely outweigh the risks of the examination, and MR examination should generally be performed. In patients with chronic conditions in which it makes little difference whether the scan is performed at a given time or weeks later, it may be prudent to defer MR examination until approximately 6 weeks after device implantation.

Inferior Vena Cava Filters

Background Data

Many inferior vena cava (IVC) filters are made of nonferromagnetic materials, whereas some others are composed of weakly ferromagnetic materials. Devices such as IVC filters are attached with hooks. As is typical for healing processes throughout the body, it is generally believed that IVC filters become incorporated securely into the vessel wall, primarily due to tissue ingrowth, within approximately 4 to 6 weeks after implantation. Therefore, it is unlikely that such implants would become moved or dislodged as a result of exposure to static magnetic fields of MR systems operating at up to 1.5 T.

Studies of MR examination of both animals and humans with implanted IVC filters have thus far not reported complications or symptomatic filter displacement. Several animal studies have even used “real-time” MR for the placement of IVC filters.

Labeling/Recommendations

Most IVC filters that have been tested have been labeled as “MR safe”; the remainder of IVC filters that have been tested are classified as “MR conditional.” Patients who have been treated with nonferromagnetic IVC filters can undergo MR examination any time after filter implantation. In patients who have been treated with a weakly ferromagnetic IVC filter (Gianturco bird nest IVC filter [Cook], stainless steel Greenfield vena cava filter [Boston Scientific]), it is advised that the patient wait at least 6 weeks before undergoing an MR examination (because these older devices initially may not be anchored as firmly in place as other devices discussed in the present report), unless there is a strong clinical indication to perform the MR examination sooner after implantation, and as long as there is no reason to suspect that the device is not positioned properly or that it is not firmly in place. Most studies of IVC filters have generally been conducted at 1.5 T or less, although many IVC filters have now been evaluated at 3 T and deemed acceptable for MR examination.

Embolization Coils

Background Data

The earliest embolization coils were stainless steel; more recently developed coils are often made from platinum or...
other alloys. Commonly used embolization coils are either nonferromagnetic or weakly ferromagnetic.

Because of the shape of certain coils, the theoretical potential of coil heating during an MR examination exists. An ex vivo study of the Guglielmi detachable coil (Boston Scientific) found that there were no magnetic field interactions and that the temperature increase was minimal during extreme MR imaging conditions.71 Subsequently, >100 patients with Guglielmi detachable coils have reportedly undergone MR imaging without incident.72 Other embolization coils made from nitinol, platinum, or platinum and iridium with similar configurations have been evaluated and found to be safe for patients undergoing MR procedures performed in studies at 3 T or less.3,18,72–75 To date, there have been no reports of adverse events associated with MR examinations conducted on patients with platinum coils implanted in the neurovasculature.

Coils composed of stainless steel may create local artifact, which limits the usefulness of the MR examination if the coil is in the region of interest. Platinum coils, in contrast, create less local artifact, and some (but not necessarily all) do not significantly affect the quality of diagnostic information.3,18,72–75

Labeling/Recommendations

Most embolization coils that have been tested have been labeled as “MR safe”; the remainder that have been tested have been labeled as “MR conditional.”71 Patients who have been treated with nonferromagnetic embolization coils can undergo MR examination any time after coil implantation. The timing of MR examination at 3 T or less in patients with embolization coils that are weakly ferromagnetic should be weighed on a case-by-case basis. For cases in which there is a clear potential clinical benefit of scanning in the days to weeks after implantation, the benefits of the MR examination will likely outweigh the risks of the examination, and MR examination should generally be performed. In patients with chronic conditions in which it makes little difference whether the scan is performed at a given time or weeks later, it may be prudent to defer MR examination until ≈6 weeks after device implantation. Patients with tested coils1–3 may undergo MR examination at up to 3 T, according to the conditions under which they were tested.

Loop Recorder (Event Monitor)

Background Data

The 9526 Reveal Plus insertable loop recorder (ILR; Medtronic) is a single-use, subcutaneously implanted programmable device that contains 2 surface electrodes used to continuously record the patient’s electrocardiogram. The Reveal Plus ILR contains no lead wires; however, the electromagnetic fields produced during MR imaging may adversely affect the data stored by the Reveal Plus ILR.

Ex vivo evaluation of the Reveal Plus ILR did not suggest significant risk of device movement or dislodgment.76 Clinical MR study of 10 patients with these loop recorders demonstrated no subjective symptoms experienced by patients, no adverse clinical events, and no damage to the devices, although rhythm monitoring was not performed during these examinations. Of note, interrogation of the devices after MR revealed tachyarrhythmias and bradyarrhythmias recorded during the examinations that were believed to be artifacts.77

Labeling/Recommendations

The Reveal Plus ILR has been labeled as “MR conditional.”71 Patients with a Reveal Plus ILR can undergo MR examination any time after implantation, provided there is no reason to believe the device is not well implanted. Because of the theoretical risk of electromagnetic fields adversely affecting data stored by the device, all stored data should be downloaded before scanning. Because this device contains ferromagnetic components, the strong magnetic fields associated with the MR system can create sufficient magnetic field interactions for the Reveal Plus ILR such that the patient may feel slight movement of this device.78 Although this does not represent a safety hazard, the patient should be informed of this possibility to avoid undue concern.

Hemodynamic Monitoring and Temporary Pacing Devices

Background Data

Cardiovascular catheters, such as pulmonary artery hemodynamic monitoring/thermodilution catheters (including the Swan-Ganz catheter [Edwards Lifesciences]), and temporary transvenous cardiac pacing devices generally contain nonferromagnetic components but may incorporate nonferromagnetic, electrically conductive materials.3,78,79 The MR examination may induce sufficient voltages and currents in electrically conductive material so as to result in thermal injuries and burns to adjacent tissue (including myocardial tissue).80,81 Although the theoretical risk exists that MR examination in patients with retained temporary epicardial leads, which consist of electrically conductive material, could lead to cardiac excitation or thermal injury, such retained leads are typically relatively short in length, usually do not form large loops, and are generally not believed to pose a significant risk during MR scanning.

Hartnell et al79 reported on 51 patients with retained temporary epicardial pacing wires who underwent clinical MR examinations. Of those patients examined with electrocardiographic monitoring, no arrhythmias were noted, and for all patients, no symptoms suggestive of arrhythmia or other cardiac dysfunction were noted (although the anatomic region examined and the energies used in the examinations were not specifically described).79 To date, there is no report of complications related to the MR scanning of a patient with retained epicardial leads.

There is 1 report in the literature of a Swan-Ganz thermodilution catheter that “melted” at the skin entry site in a patient undergoing MR examination.23 It was postulated that the RF fields transmitted by the MR system caused heating of the copper wires within the catheter.

One ex vivo study of temporary transvenous pacing leads reported temperature increases of up to 63.1°C.82 Preliminary results of a recent study confirmed that even unconnected temporary transvenous pacing (as well as permanent pacing) leads can undergo high temperature increases at 1.5 T.83 In a
chronic-pacemaker animal model undergoing MR examination at 1.5 T, temperature increases of up to 20°C were measured, although pathologial and histological examination did not demonstrate heat-induced damage of the myocardium. The MR imaging conditions that generated such elevated lead temperatures included use of the body RF coil to transmit RF energy over the area of the lead (eg, an MR examination of the chest/thorax).

To the best of our knowledge, there are no studies assessing the safety of temporary pacemakers (lead and external pulse generator). Unlike permanent devices, temporary pacemakers use unfixed leads that are more prone to movement, longer leads that may be more prone to induction of lead currents, and a less sophisticated pulse generator, which makes them likely more susceptible to electromagnetic interference.

Labeling/Recommendations

Those few catheters that contain conducting wires and those few temporary transvenous pacing wires that have been tested have been labeled as “MR unsafe.” Patients with pulmonary artery hemodynamic monitoring/thermodilution catheters (such as the Swan-Ganz catheter) and similar catheters that have conductive wires or similar components should not undergo MR examinations because of the possible associated risks, unless in vivo testing provides labeling information or instructions for use that permit examinations to be performed safely. Patients with nonferromagnetic pulmonary artery catheters that contain no electrically conductive pathways in the catheter may undergo MR examination; however, it must be emphasized that such conditions must be verified before such patients undergo MR examination. Patients with retained temporary epicardial pacing wires are believed to be able to safely undergo MR procedures, and patients do not need to be routinely screened for the presence of such wires before scanning. Because of the possible risks involved with temporary-pacemaker external pulse generators, such generators should not be introduced into the MR environment. Although temporary transvenous lead heating might be minimized or avoided by scanning anatomic regions above (eg, head/brain) or below (eg, lower extremities) cardiac pacing leads, scanning of patients with temporary transvenous pacing leads (without the generator) is not recommended. Furthermore, because the harsh electromagnetic environment associated with the MR system can alter the operation of an external pulse generator or damage it, it may not be possible to reliably pace the patient during the MR examination, which makes the issue of scanning a patient with a temporary transvenous lead irrelevant in most cases.

Permanent Cardiac Pacemakers and Implantable Cardioverter Defibrillators

Background Data

Due to the wide prevalence of cardiovascular diseases, a significant proportion of patients who would ideally be referred for MR examinations will have permanent cardiac pacemakers or implantable cardioverter defibrillators (ICDs). It has been estimated that a patient with a pacemaker or implanted defibrillator has a 50% to 75% likelihood of having a clinical indication for MR imaging over the lifetime of their device. These devices contain metal with variable ferromagnetic qualities, as well as complex electrical systems, and additionally consist of 1 or several leads implanted into the myocardium. The potential for movement of the device, programming changes, asynchronous pacing, activation of tachyarrhythmia therapies, inhibition of pacing output, and induced lead currents that could lead to heating and cardiac stimulation has led to concerns regarding the performance of MR examinations in patients with permanent pacemakers and ICDs. These factors might lead to clinical sequelae that include changes in pacing/defibrillation thresholds, pacemaker ICD dysfunction or damage (including battery depletion), arrhythmia, or death. Deaths associated with MR examination of patients with pacemakers/ICDs have been reported. As best as can be determined, all of these deaths occurred in the setting of MR examinations that were not supervised or monitored by a physician. Because of these factors, it was not possible to determine the precise mechanism of death as it relates to the MR examination and the presence of a pacemaker/ICD in most cases, although in 1 recent report, ventricular fibrillation was believed to have been the cause of death in at least 3 patients.

There have been small to modestly sized prospective human trials in recent years at 0.5- to 2.0-T field strength that have reported on the relative safety of MR examination in the setting of pacemakers. Only 1 study has placed no anatomic limitations on MR procedures used for the patients studied. Martin and colleagues reported on a series of 54 patients who underwent a total of 62 MR examinations using a 1.5-T MR system. Pacemakers were examined before and after MR imaging. Pacemaker-dependent patients were excluded from the study, and heart rhythm was monitored during the examination. Pacing threshold changes were noted in 40 of 107 leads, of which 10 were judged to be significant, 2 of which required a change in programmed output. No episodes of pacing above the upper rate limit or arrhythmias were noted. A small series of patients with ICDs who were undergoing neurological MR examination found that none of the 8 patients scanned experienced significant adverse clinical events; in 1 patient, a change in programming was noted. One study involving ex vivo device testing and in vivo animal testing found that ICDs manufactured after 2000 may be more resistant to changes in function during MR examination. Several other small series have reported on the results of MR scanning in patients with pacemakers or ICDs and it is believed that at least several hundred patients with these devices have undergone examination. Recent studies of patients with pacemakers or ICDs have confirmed the findings of these earlier studies, and these study investigators, among others, have proposed strategies and protocols for safe pacemaker/ICD scanning. No deaths have been reported in studies in which patients were deliberately scanned and properly monitored, although cases of changes in pacing threshold, programming changes, need for device reprogramming, and possibly battery depletion have been noted. In addition, incidents in which pacemaker or ICD dysfunction has occurred in patients who have undergone MR examination at some time are listed on the
FDA Web site, although possible causative associations usually cannot be established with confidence.92

Writing on behalf of the FDA, Faris and Shein90 have both acknowledged and pointed out the shortcomings of research thus far on studies of MR imaging of patients with pacemakers and ICDs. They go on to state that “while FDA recognizes that there are pacemaker and ICD patients for whom, on a case-by-case basis, the diagnostic benefit from MR imaging outweighs the presumed risks, we believe that those risks have not yet been characterized and mitigated sufficiently to justify the routine use of MR examination in those populations.” Faris and Shein recently reiterated their position in an updated editorial.108

Labeling/Recommendations

The present writing group believes that despite the above discussion of patients with pacemakers or ICDs who have been scanned safely, the following must be noted: (1) these studies were conducted at institutions with expertise in MR imaging and electrophysiology; (2) the number of patients who experienced adverse events that have gone unreported is unknown; (3) considerable controversy exists over safety issues regarding MR scanning of patients with pacemakers and ICDs; and (4) the presence of a pacemaker or ICD should still be considered a strong relative contraindication to routine MR examination, which is therefore discouraged. Patients who have a pacemaker or ICD should not undergo an MR study if an alternative diagnostic test is available, and MR imaging should only be considered in cases in which the potential benefit to the patient clearly outweighs the risks to the patient. Risks to the patient are likely increased in centers without highly experienced personnel in both function and programming of the device and operations/pulse sequences of the MR scanner. Thus, scanning should only be performed at extremely experienced centers with expertise in MR imaging and electrophysiology. If such scanning is performed, the risks of MR scanning should be discussed specifically and clearly with the patient, and the written informed consent should specifically list risks, including (1) pacemaker/ICD dysfunction, (2) pacemaker/ICD damage, (3) arrhythmia, and (4) death. Any institution at which MR scanning of pacemakers/ICDs is performed should have some formal program of quality control to track adverse events. The patient’s heart rhythm and vital signs should be monitored throughout the MR examination. A physician with pacemaker/ICD expertise should be in attendance during scanning, and a “crash cart,” including defibrillator, must be available throughout the procedure to address any adverse events. A person with expertise in MR physics and safety should be involved with the scan to optimally plan the scan to minimize risk. The pacemaker/ICD should be interrogated before and after the procedure.

Specific comments regarding such scanning of non–pacemaker-dependent patients, pacemaker-dependent patients, and patients with ICDs are given below and in Table 2, based in part on previous recommendations1,3,9,93,96,109 and on the general consensus of the present writing group. Recommendations regarding the scanning of patients with permanent pacemakers and ICDs can be expected to evolve over time as more studies become available.

Those pacemakers that have been tested have been labeled as “MR unsafe.”1 At present, MR examination of non–pacemaker-dependent patients is discouraged and should only be considered in cases in which there is a strong clinical indication, in which the benefits clearly outweigh the risks, and then according to the criteria listed in the text and Table

Table 2. Recommendations for the Performance of MR Examinations in Patients With Pacemakers or ICDs

<table>
<thead>
<tr>
<th>General recommendations:</th>
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<tr>
<td>MR examination of non–pacemaker-dependent patients is discouraged and should only be considered in cases in which there is a strong clinical indication and in which the benefits clearly outweigh the risks</td>
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<tr>
<td>MR examination of pacemaker-dependent patients should not be performed unless there are highly compelling circumstances and when the benefits clearly outweigh the risks</td>
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<tr>
<td>MR examination of patients with ICDs should not be performed unless there are highly compelling circumstances and when the benefits clearly outweigh the risks</td>
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Scanning should only be performed at extremely experienced centers with expertise in MR imaging and electrophysiology.

Establish and document the risk-benefit ratio for the patient.

Obtain written and verbal informed consent. Written informed consent should specifically list risks, including (1) pacemaker/ICD dysfunction, (2) pacemaker/ICD damage, (3) arrhythmia, and (4) death.

A physician with ACLS and pacemaker/ICD expertise should decide whether it is necessary to reprogram the pacemaker/ICD before the MR examination and should be in attendance for the entire study.

A person with expertise in MR physics and safety should be involved with the scan to optimally plan the scan to minimize risk, and consideration should be given to using scanning parameters (eg, lowest RF power levels, weakest/slowest necessary gradient magnetic fields) that are believed to minimize study risk.

Prescanning steps outside the MR environment:

For non–pacemaker-dependent patients, pretest pacemaker functions

For pacemaker-dependent patients, pretest pacemaker functions and reprogram to asynchronous mode

For patients with ICDs, pretest ICD functions and disable therapy and detection for tachycardia/bradycardia modes

The patient’s heart rhythm and vital signs should be monitored throughout the MR procedure.

Appropriate personnel and a “crash cart,” including defibrillator, must be available throughout the procedure to address an adverse event.

Maintain visual and voice contact with the patient throughout the procedure.

Instruct the patient to alert the MR system operator to any unusual sensations or problems.

After the examination:

For non–pacemaker-dependent patients, a physician with electrophysiological expertise should interrogate the pacemaker and reprogram as needed

For pacemaker-dependent patients, a physician with electrophysiological expertise should interrogate the pacemaker function and reprogram the pacemaker

For patients with ICDs, a physician with electrophysiological expertise should perform postscan device reprogramming and defibrillation threshold testing

ACLS indicates advanced cardiovascular life support.
2. There are few current data on the performance of MR examination of pacemaker-dependent patients, and MR examination of pacemaker-dependent patients should not be performed unless there are highly compelling circumstances in which the benefits clearly outweigh the risks and then according to the criteria listed in the text and Table 2. MR examination of patients with ICDs should not be performed unless there are highly compelling circumstances in which the benefits clearly outweigh the risks and then according to the criteria listed in the text and Table 2. Although 1 study discussed above found that ICDs manufactured after 2000 may be more resistant to changes in function during MR examination, this finding should not be taken as a "green light" to routinely scan patients with such ICDs. Fractured leads may pose a particularly high risk of thermal injury, and MR examination should not be performed in patients with pacemakers or ICDs with known lead fractures. The writing committee emphasizes that efforts by industry to manufacture pacemakers and ICDs that are specifically designed to be acceptable for patients undergoing MR procedures should be intensified, an approach preferable to the current "ad hoc" methods described above.

Retained Transvenous Pacemaker and Defibrillator Leads

Background Data
Retained transvenous pacemaker and defibrillator leads (leads left in the body after explantation of the permanent pacemaker or ICD generator) pose significant theoretical risks, including heating and cardiac excitation. Retained fractured leads may pose a particularly high risk of thermal injury.

Labeling/Recommendations
To the best of our knowledge, no clinical studies have specifically addressed the risks of retained transvenous pacemaker or ICD leads. It is the consensus of the writing group that patients with retained transvenous pacemakers or ICD leads be approached similarly to those with pacemakers or ICDs, as outlined above. MR examination of patients with retained transvenous leads is discouraged, and MR examination should only be considered in centers with expertise in MR and electrophysiology, and only in cases in which there is a strong clinical indication. MR examination should not be performed in patients with known retained transvenous leads that have fractures.

Hemodynamic Support Devices

Background Data and Labeling/Recommendations
Hemodynamic support devices, including intra-aortic balloon pumps, right ventricular assist devices, and left ventricular assist devices, are complex devices with variable degrees of ferromagnetic materials, moving parts, and electrical components. Although formal evaluation of these devices in regard to MR safety has not been conducted, it is believed that these devices should be considered absolute contraindications to MR examination, particularly given that most hemodynamic support systems involve equipment likely to be affected by the electromagnetic fields used during MR imaging.

Summary and Conclusions
Advances in MR imaging over the last 2 decades have led to MR becoming an increasingly attractive imaging modality, one that provides excellent spatial resolution and multiplanar 3-dimensional analysis while not exposing patients to the risks associated with computerized tomography and invasive procedures. MR will increasingly be used in the population as a whole and in many cases may be the best imaging modality available for the increasing number of patients with permanently implanted and temporary cardiovascular devices. Extensive, although not complete, ex vivo, animal, and clinical data are available from which to generate recommendations regarding the safe performance of MR examination in patients with cardiovascular devices, as well as to ascertain caveats and contraindications regarding MR examination for patients with certain cardiovascular devices. Safe MR imaging involves a careful initial patient screening, accurate determination of the cardiovascular (and other) device and its properties, a thoughtful analysis of the risks and benefits of performing the examination at that time, and, when indicated, appropriate physician supervision.

The recommendations in the present statement are meant to serve as a guide for physicians, MR technologists, nurses, and other healthcare professionals. The reader is reminded that discussions of device safety are based on research through mid-2006 and are based only on devices that are commercially available as of this writing; recommendations in this statement will not necessarily apply to devices developed in the future. When doubt remains as to the safety of performing an MR examination, the reader is urged to consult a more detailed source of information, such as dedicated Web sites, reference manuals, or, especially, the manufacturer's product information when available. Because of the increasing use of MR examinations, as well as the increasing number of cardiovascular devices implanted in patients, efforts by industry, working in collaboration with academia, to manufacture devices, including pacemakers and ICDs, that are specifically designed to be safe for MR examination should be continued and intensified.
### Writing Group Disclosures

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<th>Writing Group Member</th>
<th>Employment</th>
<th>Research Grant</th>
<th>Other Research Support</th>
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*Modest.
†Significant.

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*Significant.


Leiomyosarcoma Involving Main and Left Pulmonary Artery Treated Surgically With Homograft Replacement and Concomitant Left Pneumonectomy

Sak Lee, MD; In-Kyu Park, MD; Sang-Ho Cho, MD; Do-Kyun Kim, MD

A 47-year-old woman presented with progressive dyspnea on exertion and generalized weakness for 6 months. On physical examination, a grade-III ejection systolic murmur was heard on her upper sternal border. Her chest x-ray showed mild cardiomegaly with multiple, variable-sized nodular opacities in the right lung (Figure 1). Echocardiography revealed severe supravalvular pulmonary stenosis (peak and mean pressure gradient 76 mm Hg and 45.6 mm Hg, respectively) due to diffuse tubular supravalvular stenosis associated with increased right ventricular pressure (93.9 mm Hg) and left pulmonary artery narrowing. Multislice computed tomogram showed an intravascular mass lesion at the suprapulmonic valvular area, narrowing the lumen and extending into the left pulmonary artery and upper and lower lobar arteries, with near total occlusion of the left pulmonary artery. Left lung volume loss and hypovascularity with left pleural effusion were also noted (Figure 2). A lung perfusion scan showed no perfusion in the left lung and uniform distribution of the radiotracers in the right lung (Figure 3). A positron emission computed tomographic scan was performed to evaluate the character and extent of the mass, which revealed a malignant mass of intense FDG (18F-2-fluoro-2-deoxyglucose) uptake along the main pulmonary artery and nodules and consolidations in the lungs (Figure 4). The positron emission computed tomographic scan revealed no uptake in any other organ suggesting metastasis.

The patient underwent surgical resection of the main pulmonary artery and left pulmonary artery along with concomitant left pneumonectomy under total circulatory arrest using conventional cardiopulmonary bypass technique. The main pulmonary artery, from the pulmonic valve to the proximal right pulmonary artery, was reconstructed with a homograft valved conduit, and pleural biopsy was performed on the nodules suspected of pleural seeding. The pathological diagnosis confirmed leiomyosarcoma confined to the excised pulmonary artery, and left lung without pleural metastasis. The patient suffered from bronchopleural fistula in the postoperative period, which was eventually occluded with the application of glue. Afterward, she made an uneventful recovery and was referred for chemotherapy. A postoperative chest computed tomographic scan taken 2 months after the operation shows patent pulmonary homograft with no evidence of tumor recurrence (Figure 5).

Disclosures

None.
Figure 2. Preoperative multislice chest computed tomogram shows intravascular mass lesion at suprapulmonic valvular area, narrowing the lumen (arrow) and extending into left pulmonary artery and upper and lower lobar arteries with near total occlusion of the left pulmonary artery. H indicates head; R, right; L, left; and F, feet.

Figure 3. Preoperative lung perfusion scan shows no perfusion in the left lung and uniform distribution of the radiotracers in the right lung. ANT indicates anterior; POST, posterior; RAO, right anterior oblique; LAO, left anterior oblique; RPO, right posterior oblique; LPO, left posterior oblique; LT LAT, left lateral; and RT LAT, right lateral.
Figure 4. Preoperative positron emission computed tomogram reveals a malignant mass of intense FDG uptake along the main pulmonary artery, and nodules and consolidations in the lungs.

Figure 5. Postoperative multislice chest computed tomogram taken 2 months after the operation shows patent pulmonary homograft with no evidence of tumor recurrence.
Letter by Tartière et al Regarding Article, “Cardiac Structure and Ventricular–Vascular Function in Persons With Heart Failure and Preserved Ejection Fraction From Olmsted County, Minnesota”

To the Editor:

We read with great interest the article by Lam et al comparing cardiac structure and ventricular–vascular function in subjects with heart failure and normal ejection fraction, hypertensive subjects, and controls. The main result of this noninvasive study is that end-systolic elastance, effective arterial elastance, and total arterial compliance do not differ between heart failure subjects and hypertensive subjects, but that heart failure subjects show lower end-diastolic volume, cardiac index, and relaxation capacity and greater diastolic stiffness than do hypertensive subjects. This study highlights major issues in the comprehension of heart failure. Nevertheless, we think that the noninvasive study contains some limitations that could modify the interpretation of the results.

First, 2 of the major determinants of effective arterial elastance and total arterial compliance used by Lam et al are brachial systolic pressure and pulse pressure, respectively. Their estimation of the end-systolic pressure is based on the product of brachial systolic blood pressure/1100.9, whatever the group, and to assess arterial compliance, brachial pulse pressure is used as an estimate of aortic pulse pressure. These estimations assume that pulse amplification is equivalent among groups and that pulse pressure and systolic blood pressure are the same from the aorta to the brachial artery, which is probably inaccurate, according to previously published results that showed pulse pressure amplification ranges from 1.09 to 1.67.

Second, end-systolic elastance is estimated using the noninvasive single-beat method. Two of the determinants of this parameter are brachial diastolic and end-systolic pressure. If brachial diastolic blood pressure is an acceptable surrogate for central diastolic pressure, end-systolic pressure is probably overestimated in both non–heart failure groups because of a different pulse amplification.

Third, Lam et al do not discuss pressure reflection as a source of increased vascular load. This part of the load has been related to cardiovascular damage and prognosis and is not assessed by effective arterial elastance and total arterial compliance.

Because effective arterial elastance, total arterial compliance, and end-systolic elastance were probably overestimated differently among groups in this study, we think that this study cannot support the absence of a stiffer ventricular–vascular phenotype in heart failure with normal ejection fraction.

In summary, the authors are to be congratulated for having made such a comprehensive study in such a large population; given these methodologic limitations, however, we think that their conclusions are to be taken with caution, and it is likely, as they suggest, that other studies using the carotid pressure or the estimated aortic pressure, or made during exercise are warranted to confirm these results.

Disclosures

None.

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Response to Letter Regarding Article, “Cardiac Structure and Ventricular–Vascular Function in Persons With Heart Failure and Preserved Ejection Fraction From Olmsted County, Minnesota”

We appreciate the comments of Tartière et al on our study1 and agree that these are valid concerns about the noninvasive estimation of systolic vascular and ventricular stiffness. As acknowledged in our paper, “Although total vascular load and indirect measures of vascular stiffening and other assessments of arterial impedance and its impact such as characteristic impedance, wave reflections, and pulse-wave velocity,” whereas effective arterial elastance, derived from the ratio of end-systolic pressure to stroke volume, is a useful index of total vascular load which, importantly, can be linked to measures of ventricular elastance in the assessment of vascular–ventricular coupling, there remains concern that arterial systems with very marked systolic wave reflections and wave transmission effects may be inadequately represented by this parameter. In the human validation study by Kelly et al,2 however, this noninvasive parameter demonstrated near-equivalency to the invasive parameter derived from arterial pressure–flow data over a wide range of systolic pressures and resistances, in normotensive and hypertensive subjects, young and old. The noninvasive determination of end-systolic elastance was similarly validated by Chen et al3 against the invasive gold standard in a spectrum of patients and disease conditions that ranged from noncardiac disease to heart failure and transplantation. On the basis of these previous data in wide spectra of patients, the noninvasive estimates were assumed to be similarly accurate in all 3 of our study groups. Any systematic deviation from true measurements based on central pressure was also equal among groups, because the measurements were made in a uniform manner regardless of group. Hence, the between-group comparisons are meaningful. Further, restricting the analysis to older patients, in whom one would expect to find the stiffest arterial systems, yielded consistent results (Table 3). Importantly, our data do in fact demonstrate systolic vascular–ventricular stiffening in patients with heart failure and normal ejection fraction which compared with healthy controls. “Superimposed” worsening diastolic dysfunction was observed in patients with heart failure and normal ejection fraction, compared with hypertensive controls without heart failure, but that does not imply that the “underlying” systolic vascular–ventricular stiffening does not contribute to the pathophysiology of this syndrome.

In conclusion, we reiterate that our data do not exclude a role for increased vascular and ventricular systolic stiffening in patients with heart failure and normal ejection fraction. Recent4 and future work will help to define this role, particularly during exercise or other stressors in which systolic vascular–ventricular stiffening may lead to exaggerated hypertensive responses and further load-dependent diastolic dysfunction.

Disclosures

None.

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Letter by Herring and Paterson Regarding Article, “Common NOS1AP Variants Are Associated With a Prolonged QTc Interval in the Rotterdam Study”

To the Editor:

We read with interest the recent study by Aarnoudse et al., which provides convincing evidence for an association between 2 common single-nucleotide polymorphisms in the neuronal nitric oxide synthase (nNOS/NOS-1) activator protein gene and prolongation of the heart rate–adjusted QT interval on ECGs from subjects in the Rotterdam study. This study builds on the findings of a recent genome-wide association study showing a similar link.2 These results are both surprising and clinically relevant, as it is not immediately obvious how NOS-I may influence the QT interval and Long-QT syndrome, which is strongly associated with sudden cardiac death. Is it possible that the myocyte alone is not the defective site for NOS-1 and Long-QT syndrome interactions?

In the same issue of Circulation, the review by Samuels3 points out that many cases of sudden cardiac death are precipitated by the autonomic nervous system. Increased sympathetic drive to the heart increases myocardial oxygen demand, reduces coronary perfusion time, and can cause calcium overload in ventricular myocytes, which can be proarrhythmic. Patients with certain forms of Long-QT syndrome and Brugada syndrome are also particularly susceptible to adrenergic-induced arrhythmia. It is interesting, therefore, to note a body of physiological evidence that has convincingly demonstrated a role for nNOS/NOS-1, both in the autonomic control of cardiac function and in cardiac contraction and calcium handling. Defective nNOS-cGMP activity in the sympathetic innervation of the heart leads to increased norepinephrine release and tachycardia, while the ability of the vagus nerve to release acetylcholine and counteract these effects is impaired.3 Postsynaptically, defective myocyte nNOS activity leads to increased contractility, prolonged calcium transients, and reduced L-type calcium current inactivation, which may be reflected in prolongation of the action potential duration. It is not, perhaps, as surprising as it may first seem, therefore, that defective nNOS/NOS-1 activity may increase corrected QT interval, an ECG surrogate for action potential duration, and also predispose to cardiac neurotransmission to amplify the dysfunctional electrical phenotype. It remains to be demonstrated experimentally whether the single nucleotide polymorphisms in NOS-1-AP described by Aarnoudse et al can directly alter cardiac norepinephrine release and ventricular action potential duration.

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Dr Herring is a clinical lecturer in cardiovascular medicine at Oxford University and specialist registrar at the Oxford Radcliffe Hospitals NHS Trust. Dr Paterson is the Professor of Cardiovascular Physiology at Oxford University.

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Disclosures

None.

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Response to Letter Regarding Article, “Common NOS1AP Variants Are Associated With a Prolonged QTc Interval in the Rotterdam Study”

We thank Drs. Herring and Paterson for their thoughtful comments on our article.1 As they have pointed out, no clear mechanism for the role of the NOS1AP gene in the heart in general, or of common genetic variants specifically, is yet understood. They suggest that apart from the effect of less active NOS-I in the cardiomyocyte, it might also affect the sympathetic innervation of the heart, thus contributing to catecholamine-induced sudden cardiac death.

Although we cannot exclude this mechanism, our data cannot provide any direct support for this hypothesis, because of the limited physiological resolution of a simple genetic association. If NOS1AP variants, via altered activation of NOS-I, indeed modulate norepinephrine release from sympathetic innervation, NOS1AP alleles could be associated with different resting heart rates. However, we find no association of NOS1AP alleles with resting heart rate (P=0.54 for rs10494366, P=0.93 for rs10918594). Nor did we find a statistically significant difference in the hazard of sudden cardiac death by NOS1AP genotype, but larger samples will be required to exclude such a possibility. The relationship of NOS1AP variation and Long-QT syndrome, if any, remains to be defined. Resequencing of affected individuals to identify rare variants and genotyping of known common variants are needed to identify causation or modification of Long-QT syndrome.

In conclusion, the finding that common genetic variation in NOS1AP is convincingly associated with QT interval duration points to the exciting potential of genome-wide association studies to identify genes and pathways not previously recognized to contribute to myocardial repolarization. The role of autonomic function in myocardial repolarization and arrhythmia triggering is well established, but our data cannot provide direct support for a role of NOS1AP in modulation of autonomic function. As Drs Herring and Paterson conclude, experimental work needs to be done to unravel the underlying mechanism by which NOS1AP variants modulate QT interval duration; a direct effect on autonomic modulation of cardiac electrogensis is one exciting possibility.

Disclosures

None.

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Music and the Heart

Luciano Bernardi, MD, a Cardiologist From Italy, Believes That Music Can Have a Beneficial Effect on the Cardiovascular System

Dr Luciano Bernardi, associate professor at the Department of Internal Medicine, University of Pavia, Italy, feels convinced that music could serve as a useful tool in the management of cardiovascular disease. He discusses the evidence with Emma Baines, BSc.

Listening to music, whether a Mozart concerto or the latest album from the top of the popular music charts, may not only help you to unwind at the end of a stressful day. It could also lower your blood pressure and improve your heart rate variability, according to Dr Luciano Bernardi, associate professor of internal medicine at the University of Pavia, Italy.

The idea that music has an effect on heart rate and blood pressure has existed for some time. In 1918, Hyde and Scalapino reported that minor tones increased pulse rate and lowered blood pressure, whereas “stirring” music increased both blood pressure and heart rate. Dr Bernardi became interested in the effects of music on the heart as a result of his research on the ways that he could influence the rhythms of the autonomic nervous system to improve respiratory and cardiovascular function.

He explains, “We are interested in the effects of the autonomic nervous system on circulation and the heart. There are rhythms in the cardiovascular system and the autonomic nervous system that can send information to the blood vessels and the heart and affects these rhythms. But talking about rhythms involves the idea that external rhythms can influence internal ones.”

Initially, Dr Bernardi investigated the effects of research on how controlled breathing techniques such as those used in yoga, meditation, and prayer could help regulate internal rhythms. “If, instead of breathing naturally, you superimpose a slow, steady rate of respiration on the body, you modulate the whole cardiovascular system,” he says.

In one study, he found that reciting either the rosary prayer or a yoga mantra enhanced heart rate variability and baroreflex sensitivity by slowing the breathing rate down to 6 breaths per minute. In a more recent study, he has shown that breathing at this slow rate reduced blood pressure and enhanced baroreflex sensitivity in hypertensive patients.

After several studies had shown that one could modulate internal rhythms through controlled breathing, Dr Bernardi became interested in whether other ways existed to modulate the rhythms of the autonomic nervous system. He says, “We wondered if perhaps other external stimuli could have an effect on the rhythms of the autonomic nervous system, and we decided to test the effects of different types of music.”

Dr Bernardi worked with Peter Sleight, MD, from the department of cardiovascular medicine, John Radcliffe Hospital, Oxford, United Kingdom, to test which aspects of music could produce changes in cardiovascular and respiratory variables. They designed a study in which 24 healthy volunteers, half of whom had extensive musical training.

On other pages...

Spotlight: Evelyn Regar, MD, PhD
Dr Evelyn Regar is an interventional cardiologist at the Erasmus Medical Center, Rotterdam, the Netherlands. She talks about her career and her thoughts on training to become a cardiologist as a woman in the Netherlands. Page f141

Long-Term Follow-up in Cardiac Surgery
Ron van Domburg, BSc, PhD, is responsible for collating the data on some of European cardiology’s longest follow-up studies and has spent nearly 30 years working as an epidemiologist in the Netherlands. Page f143
listened through headphones to 6 short tracks of music chosen for factors such as rhythm, syncopation, and speed while the researchers monitored their blood pressure, heart rate, breathing rate, cerebral artery flow velocity, and baroreflex.

The musical samples used in the study came from pieces by Beethoven, Vivaldi, the Red Hot Chili Peppers (a currently popular band), sitar music by Deborah Caudhuri, a dodecaphonic orchestral work, and techno music. These 6 samples of music played for 2 minutes each in a random order without pauses, and then repeated in a different random order for 4 minutes each. In addition, the sequence included a 2-minute period of silence.

Unlike earlier studies, this study found no effect of musical style or preference on any cardiovascular parameters. It showed that only 1 factor mediated the physiological effect of listening to music: tempo. Fast music, whether classical or techno, caused increases in blood pressure, heart rate, and breathing rate, and reduced baroreflex sensitivity. Slow music, on the other hand, whether classical music or reggae-style sitar music, caused a significant fall in heart rate and breathing frequency compared with the baseline.

Dr Bernardi says, “We discovered that controls and musicians all behaved the same way when listening to music. The faster the tempo, the faster the respiration, heart rate, blood pressure, and so on. When the music was slower, it had a slowing effect.”

They also observed an order effect. Slow-tempo music seemed to lower heart rate more when it followed a faster piece of music than if it came first. “Quicker music, whether it is Vivaldi or techno, has an arousing effect on the system which concentrates the attention. If you follow this with a slower track, you reach a more profound level of relaxation,” Dr Bernardi says.

The most surprising observation had to do with the effect of the 2-minute silence in the middle of the music sequence. It had a greater impact in reducing heart rate and blood pressure than did the slowest-tempo music. “The silence had a totally different effect on heart rate and other parameters when it came after music than it did at baseline,” Dr Bernardi recalls. “Silence between music had the most profound relaxing effect. In fact, it acted as though it were music with a zero frequency.”

He explained this effect as similar to the relaxed state produced during transcendental meditation: “First, you have to concentrate hard, giving your attention to something. Then, when you release the attention, you become very relaxed,” he said. “Music may be able to achieve the same effect.” He said that this finding suggests that listening to music that alternates a quick tempo with slower passages or pauses could help induce relaxation as an alternative to meditation, and it could have a potential use in managing patients with cardiovascular disease.

“Our job at the moment is to find out the possibilities for using music to modulate the heart,” he says. “Really, more research needs to be done. But, if our finding is supported, then if you have high blood pressure I wouldn’t suggest listening to too much techno! Or, at least, you should follow it with more relaxing music.”

Dr Bernardi has himself has always benefited from playing and listening to different kinds of music. He played rock music on the guitar and saxophone as a young man. More recently, he became interested in Renaissance and medieval music and has taken up playing the lute and other ancient instruments.

Emma Baines is a freelance medical writer.

References
Spotlight: Evelyn Regar, MD, PhD

Racing Driver, Pilot, Engineer, or Doctor? In the End, Evelyn Regar, MD, PhD, Chose Medicine

Dr Evelyn Regar practises as an interventional cardiologist at the Thoraxcenter, Erasmus Medical Center, Rotterdam, the Netherlands. She talks to Mark Nicholls about her mentors, her career, her hopes for the future, and her thoughts on training to become a cardiologist as a woman in the Netherlands.

A natural curiosity about the mechanics of the heart would eventually persuade Evelyn Regar, MD, PhD, to pursue a career in cardiology. Born in Munich, she has several relatives who have an interest and aptitude for mechanical engineering. “I looked at the heart as the main organ and began to think it was pretty versatile with rhythm and a lot of mechanics involved on valvular, myocardial, and coronary artery levels. I found it an interesting organ,” she explains.

Dr Regar’s initial interest in medicine stems from her teenage years. She says, “I was intrigued by the complexity of the human being, physically and physiologically, though in between I did want to become a racing driver and then a pilot! But I turned back to medicine.” She did her preclinical studies at the University of Regensburg, Bavaria, Germany, for 2 years, before training at the Technical University of Munich, Germany, from 1990 to 1994. From there, she trained in internal medicine and cardiology at the University Hospital, Munich, where she remained until 1999.

Her choice of a subject for her thesis would shape the direction of her career. “When I was looking for a thesis for my doctorate degree, I had the opportunity to do research with a group involved in coronary intervention,” says Dr Regar. “I became very enthusiastic about the possibilities of that, particularly being in the early 1990s, a time when stents and more treatments such as atherectomy and rotablator were becoming available and there was a great deal of innovation. It was an exciting time.”

For the past 8 years, Dr Regar, now 38 years of age, has worked at the Erasmus Medical Center in Rotterdam, where she now practises as an interventional cardiologist and has the post of clinical head of the catheter laboratory at the Thoraxcenter (Figure 1).

During her training and early career, she says she has benefited from the knowledge and expertise of a number of renowned clinicians and tutors, but she points to 3 in particular who inspired her.

With gratitude, she mentions Rudolph Blasini, MD, a professor from the Technical University of Munich, as the person who gave her the opportunity to do her doctorate thesis on intracoronary ultrasound. She also points to Harald Mudra, MD, a professor from the Ludwig Maximilians University, Munich, for introducing her to interventional cardiology. And, in more recent years, she has found a mentor and inspiration in Patrick Serruys, MD, PhD, professor of interventional cardiology at the Erasmus Medical Center (Figure 2). “He is a very inspiring person in terms of the huge knowledge that he has, his persistence and dedication, and his amazing capability to motivate people,” she says.

Areas of particular interest for Dr Regar include coronary imaging, the prevention of acute myocardial infarction, and treatment of coronary artery disease. She adds, “In interventional cardiology, I am also really intrigued by the potential of light-based technologies for coronary diagnosis, and I am looking forward to exciting developments in the next couple of years.” Dr Regar recently gave a presentation at the European Society of Cardiology meeting in September 2007 in Vienna, Austria, on bioabsorbable drug-eluting stents, and she has also edited a book on optical coherence tomography.

Dr Regar considers her natural curiosity a motivating force in her career. She explains, “I think that, as in all women, my main motivation is curiosity. I am curious, I want to understand the cause of disease, I want to improve outcomes. Also, working with patients, you realise that relatively simple procedures such as percutaneous coronary
intervention can improve the quality of life or even save lives. That makes it a very rewarding job.”

She has found combining her career as a cardiologist with her role as mother to her 1½-year-old daughter Vivienne manageable, yet challenging. When asked how she has found being a woman in cardiology, Dr Regar says, “We should not forget that men also have to work hard for their careers. But, as far as equality is concerned, that is something that differs very much within various parts of Europe.”

She continues: “The proportion of women in leading positions varies between 2% in Italy to 10% in Germany and 18% in Norway, despite an equal graduation rate of the sexes in their early careers. In Munich, I got the opportunity to gain a lot of knowledge. The Thoraxcenter in the Netherlands offers ideal conditions to follow up a career as a cardiologist and scientist. It was Dr Serruys who accepted me as the first woman for training in interventional cardiology in Rotterdam.”

Before that time, an impressively long list of national and international fellows had trained in interventional cardiology at the Thoraxcenter—all men. Dr Regar says, “I enjoy the privilege of working with a highly skilled, intelligent, and dedicated group of people at an institute like the Thoraxcenter.”

She advises young people seeking to pursue a career in cardiology to hone their focus. “Cardiology offers so many possibilities; people find out what they really like and what they find satisfying and then pursue it,” she says.

On a personal level, Dr Regar aims to continue her research, very much focussed on trying to understand why apparently healthy people die from sudden myocardial infarction. “I also enjoy training young people,” she says. “For me, that is a way of giving back all the energy and patience that has been invested in me to somebody else. It is really satisfying to see how people develop and grow.”

As for the future in cardiology, she believes the field should focus on offering better treatment for chronic heart failure and for the cardiovascular complications of diabetes mellitus. She also mentions electrophysiology as a rather young subspeciality holding the promise of novel treatment strategies for arrhythmias in the future. In interventional cardiology, Dr Regar believes that unravelling late stent thrombosis is an important short-term task. “We were able to do so in the past with brachytherapy, and our knowledge on drug-eluting stents is increasing by the day. Physicians and patients need to understand the risk and benefits of drug-eluting stent therapy—fear is not a good advisor.”

Dr Regar continues, “In the longer run, our tools to treat coronary artery disease will improve further, we will have dedicated devices to treat chronic total occlusions, and with magnetic navigation we have already today a technology in our hands that has the potential to fundamentally change our ways of working in the cath lab.”

Dr Regar explains further: “Noncoronary interventions such as percutaneous valve replacement will increase. It will be interesting to see how noninvasive imaging and development in drug therapy will change the entry of patients into catheter laboratories. However, invasive treatment will continue to be rather demanding on human, logistical, and financial resources. The increasing cost of health care that confronts most industrialised countries should drive our effort for innovative, cost-effective therapies.”

Mark Nicholls is a freelance medical writer.
Long-Term Follow-up in Cardiac Surgery

Ron van Domburg, BSc, PhD, is the Man Responsible for Collating the Data on Some of European Cardiology’s Longest Follow-Up Studies

Dr Ron van Domburg has spent nearly 30 years working as an epidemiologist at the Erasmus Medical Center in Rotterdam, the Netherlands. He talks to James Butcher, PhD, about his involvement in some of the longest follow-up studies ever done in cardiology, including a 30-year analysis of survival outcomes after coronary artery bypass grafts.

In today’s global research arena, it seems unusual for a successful academic to have worked at only 1 institution throughout a 27-year career. But that is exactly what Dr Ron van Domburg, an epidemiologist who has participated in one of the longest follow-up cardiology studies ever undertaken, has done. “The climate here is very good—we have the freedom to do what we want,” explains Dr van Domburg, who joined the Erasmus Medical Center in Rotterdam in 1980 and has worked there ever since. “In the Netherlands, we don’t have a culture that we go abroad. Only very few people from medicine go abroad to do research. It is more or less the other way around. Because of Patrick Serruys, MD, PhD, professor of interventional cardiology, people are attracted to come here and work with him.”

Dr van Domburg’s primary role involves providing statistical and epidemiological advice to cardiovascular clinicians, especially on coronary artery bypass grafts (CABG) and percutaneous coronary interventions.

“My specialism is follow-up after CABG and percutaneous coronary interventions,” he says. “I was involved in the first percutaneous coronary interventions and the first CABG surgical procedures, and since then I have been involved in all the follow-up studies every 5 or 6 years.”

Indeed, Dr van Domburg presented data at the European Society of Cardiology’s annual meeting in Vienna, Austria, in September 2007, on the outcome of 1041 patients who underwent a first isolated venous CABG procedure between 1971 and 1980.

Remarkably, he and his colleagues have managed to collect data on 98% of the patients, with a mean follow-up of 30 years, by reviewing hospital records for cardiac events and by contacting general practitioners. Because patients had a mean age at baseline of 53 years, most of them died during the 30 years that followed, providing accurate data on the life expectancy of patients treated in the hospital with CABG. “The main finding is that although mortality is higher in people after CABG than in the normal population in the first 15 years after the index operation, survival rates decreased more slowly in the CABG group thereafter and eventually converged to the normal population,” explains Dr van Domburg.

Dr van Domburg graduated in mathematical statistics from Delft University, Delft, the Netherlands, in 1980, at the age of 29. For his first job, he worked as a statistician/scientific system manager at the departments of epidemiology and clinical and experimental information processing at the Erasmus Medical Center.

“When I started, no databases existed on which we could perform data analyses, so during the first years I developed computer programs in order to collect the data,” he recalls. “I developed a software program to generate patient discharge letters at the coronary care unit, the medium care unit, the intensive care unit, and the medical intensive care unit.”

Figure 1. Coronary artery bypass graft: Dr van Domburg has been involved in the follow-up of these operations almost since the first procedures were carried out in Rotterdam.
catheterisation laboratory, cardiothoracic surgery departments, and outpatient clinic,” he says. He also designed a dedicated database to collect the baseline characteristics and other clinical relevant data.

Between 1984 and 1987, he developed a database management system—a software program called CLINT—that preceded programs such as Dbase and Access. “The program included tools such as generating a dictionary, data entry, edit queries, clinical trial tools, and data analysis—including extensive syntax rules,” says Dr van Domburg. “The software is still used and is working throughout the hospital network.”

Since then, his work has moved from information and communications technology towards clinical epidemiology; he defended his PhD thesis, entitled Long-Term Survival and Predictors of Mortality in Coronary Heart Disease, in 1998. Since then, he has served as an author on approximately 300 publications, including a 2007 paper in The Lancet entitled “Early and Late Coronary Stent Thrombosis Of Sirolimus-Eluting and Paclitaxel-Eluting Stents in Routine Clinical Practice: Data From a Large Two-Institutional Cohort Study.”

“We have a lot of interest at the moment in drug-eluting stents, and I am involved in the longest and greatest study that used drug-eluting stents in all patients,” Dr van Domburg says. “We have a consecutive cohort since 2002 that includes about 8000 patients with drug-eluting stents in which we did angioplasty. It is very exciting because we are doing the follow-up of them and writing a lot of papers.” In total, Dr van Domburg estimates that the research group of Dr Patrick Serruys, to which he belongs, has published around 80 papers on drug-eluting stents in high-impact journals.

But research represents only 1 aspect of Dr van Domburg’s busy academic life, which also includes a heavy teaching load. “I always have about 10 students and 10 to 20 fellows around, and most of the time I am helping them. It might be with the design of studies or the analysis of studies, advising them on what kind of statistical analysis should be performed.”

Dr van Domburg explains that he especially enjoys working with the research fellows who come from all over the world to work at the Erasmus Medical Center. “Mostly, they have a deadline because they are working here for only 2 years, and then they have to be finished for their thesis, so there is a drive to work hard, and mostly they go home only to sleep, and they work 14 or 15 hours a day. It is a pleasure to work with those kinds of guys,” he says.

Dr van Domburg also supervises the medical students who have to do a 6-month scientific study in which they collect data and then write up a thesis. “Mostly, I tell them to collect data for their successors, and they can write about data collected by their predecessors. In this way, they can write up a study during the 6 months. I guide them to do that properly, and that takes a lot of time,” he says.

“By the end, they know if they like clinical research and if they are able to do it. About half of them tend to like clinical research by the end of the 6 months, and the other half say ‘Okay, it was nice, but no more, thanks,’” he concludes wistfully.

James Butcher is a freelance medical journalist.

Reference