Elevated Plasma Fibrinopeptide A and Thromboxane B₂ Levels During Cardiopulmonary Bypass

GERALD C. DAVIES, M.B., B.S., MICHAEL SOBEL, M.D., AND EDWIN W. SALZMAN, M.D.

SUMMARY Patients who underwent operations in which cardiopulmonary bypass was used had elevations of plasma fibrinopeptide A which did not return to normal during bypass despite conventional heparin anticoagulation, suggesting inadequate heparin dosage and continued thrombin activity during the operation. Patients who underwent aortocoronary artery grafting had high plasma thromboxane B₂ levels and a rapid fall in platelet count at the onset of extracorporeal circulation. Thromboxane elevations were less marked in patients who underwent valve replacement. Platelet aggregation and coronary artery constriction secondary to thromboxane production may contribute to the morbidity of cardiopulmonary bypass.

CARDIOPULMONARY BYPASS is attended by risks of thromboembolism, hemorrhage and myocardial ischemia during surgery and in the immediate postoperative period, and many of its complications appear to result from interaction of blood components with the extracorporeal circuit.¹ We studied the effect of cardiopulmonary bypass on coagulation and platelet activation and consumption by measuring plasma levels of fibrinopeptide A (FpA) and thromboxane B₂ (TXB₂).

FpA is a 16-amino-acid peptide cleaved from the Aα chains of fibrinogen by thrombin.² Radioimmunoassay of FpA in plasma provides a sensitive index of in vivo thrombin activity.³ FpA is physiologically active, leading in experimental animals to changes in pulmonary blood flow, heart rate, stroke volume,⁴ and vascular permeability.⁵ Cardiopulmonary changes during respiratory distress syndrome, thromboembolism, septicemia, endotoxic shock and cardiopulmonary bypass⁶ have been attributed to FpA.

TXA₂, a product of platelet arachidonic acid metabolism, is the most potent known stimulant of platelet aggregation. In experimental animals TXA₂ causes profound coronary vasoconstriction, myocardial ischemia and infarction and death.⁶,⁷ TXA₂ production, with resulting microembolization and vasoospasm, could lead to myocardial ischemia during cardiopulmonary bypass. The substance is highly unstable, with a half-life of only 30 seconds,⁶ but it has a stable metabolite, thromboxane B₁ (TXB₁), for which a radioimmunoassay is available.⁹

Patients and Methods

Twenty healthy adult human volunteers who had received no medication for the previous 2 weeks served as normal controls. Blood samples were taken via a Minicath-19 infusion set (Deseret Pharmaceutical Co.) inserted into an antecubital vein with the aid of a tourniquet. Nine milliliters of blood were withdrawn into a polypropylene syringe and immediately transferred into a polypropylene tube containing 1.0 ml of an aqueous solution of 38 mg trisodium citrate, 0.5 mg soybean trypsin inhibitor, 1 mM e-aminocaproic acid, 3.6 mg anhydrs indomethacin and 10 μM Na₂EDTA. Blood for platelet counts was collected into a glass tube containing powdered Na₂EDTA.

Samples were centrifuged at 4°C for 20 minutes at 1000 g. Aliquots of 2 ml of plasma were assayed for FpA using the method described by Cronlund et al.,¹⁰ who provided the necessary reagents.

Another aliquot was centrifuged for 10 minutes at 4°C at 11,000 g to produce platelet-poor plasma (platelet count < 1000/mm³) for TXB₂ assays. Radioimmunoassay for TXB₂ was performed according to the method of Sors et al.,¹¹ after extraction of the plasma with 1:1 (v/v) cyclohexane/ethyl acetate and resuspension in 50 mM phosphate buffer (pH 7.4) containing 0.2% human γ-globulin. Antibody-to-TXB₂ was supplied by Dr. J. B. Smith and purified TXB₂ by Dr. J. Pike (Upjohn Co.). Platelet counts were performed using a Technicon Auto-Analyzer. Hematocrit determinations were by routine method.

Twelve patients undergoing various types of general surgery were also studied. Peripheral venous blood samples were taken before incision, 10 minutes after incision, and 1 hour after the start of the operation. TXB₂ levels were also measured at random intervals during the postoperative period.

Thirty-one patients undergoing cardiac surgery with cardiopulmonary bypass were studied. Bypass techniques involved the use of partial hemodilution, moderate hypothermia and a Harvey Bubble Oxygenator. The pump prime in each case was 1600–1700 ml Ringer’s lactate, 200 ml salt-poor albumin solution (50 g albumin), 3 g dextrose, and 3000 units porcine
mucosal heparin (Panheprin, Abbott). Systemic heparinization was obtained with an initial dose of 200 units heparin/kg, and further doses were given during the operation as dictated by the activated coagulation time (ACT), according to Bull et al.\textsuperscript{11, 12} We tried to keep the ACT longer than 400 seconds during bypass. No patient received platelet infusions during the period of sampling. Blood samples were drawn through indwelling venous or arterial cannulae or from ports in the different limbs of the pump oxygenator circuit. In all cases samples were taken as close to the patient as possible to minimize artifactual elevations of plasma levels. In some instances, samples were also taken directly from the aorta, from the pericardiotomy suction apparatus and from the left ventricle. All samples were assayed for TxB\textsubscript{2} and platelet counts were performed. Hematocrit determinations were made when a platelet count was performed. In selected patients, FpA levels were also assayed. The values given below are as measured and are not corrected for hemodilution of the patient by the pump prime.

**Results**

**Normal Subjects**

Plasma FpA in normal volunteers ranged from 0.6–2.3 ng/ml (mean 1.1 ± 0.51 ng/ml [SD]). TxB\textsubscript{2} in normal subjects was 0.08–0.32 ng/ml (mean 0.18 ± 0.07 ng/ml).

**General Surgical Patients**

General surgery, which included hernia repair, visceral carcinoma resections, mastectomy and prostatectomy, was associated with a transient rise in FpA levels in all patients. Preincision levels exceeded the normal range (mean elevation 30 ng/ml) and were further elevated 10 minutes after incision. The highest level recorded during operation was 100 ng/ml. Levels tended to return toward normal during the early postoperative period.

In general surgical patients, the TxB\textsubscript{2} level was never elevated outside the normal range before, during or after operation.

**Cardiopulmonary Bypass Patients**

Thirty-one patients undergoing cardiopulmonary bypass were studied. Twenty-one patients underwent aortocoronary artery saphenous vein grafts and 10 had valve replacement.

**Fibrinopeptide A**

Fifteen patients (nine with coronary graft and six with valve replacement) were studied to determine FpA variation during cardiopulmonary bypass. FpA levels were elevated in all samples taken from the radial artery or superior vena cava after incision but before heparinization. In 11 of the 15 patients the level was greater than 100 ng/ml. In all but two of these patients the initial FpA level fell after heparinization. The mean level just before bypass was 77.5 ± 40 ng/ml. It became progressively lower as bypass proceeded, but at no time during operation was a normal FpA level recorded. The mean level taken late in bypass was 37 ± 25 ng/ml.

In the 15 patients who had FpA determinations during bypass, a total of 73 ACT measurements were made. In 54 of these, the ACT was shorter than 400 seconds, a level below which fibrin monomer production has been shown to occur during cardiopulmonary bypass despite heparinization.\textsuperscript{13}

**Thromboxane B\textsubscript{2}**

Two groups were studied. In an initial group of 18 patients (13 with coronary grafts and five with valve replacement), blood samples were obtained during the period of extracorporeal circulation, usually early (first 30 minutes) and again later during bypass. Survey of these patients served as a pilot study and, as detailed below, led to examination of a second group of 13 patients who had frequent blood samples obtained serially, with particular attention to the time of collection during bypass.

**Initial Survey**

The results in the first 18 patients studied are shown in figure 1. The mean values before operation and after heparinization but before bypass were not significantly elevated above the normal range. Values during the first 30 minutes of bypass rose in most patients, and the mean value in early bypass was significantly higher than the prebypass mean. The mean rise in TxB\textsubscript{2} values above each patient's own prebypass level was 0.18 ± 0.30 ng/ml. Values obtained later during bypass tended to be lower, and the mean value in the late bypass period was not significantly different from the prebypass mean level.

It was apparent that the early bypass samples varied widely from patient to patient. Very high values were...
found in two patients who could not be obviously distinguished clinically from the patients with lower levels. Even if the two patients with high values are excluded from analysis, the mean rise in early bypass remains significant ($p < 0.01$). It seemed possible that differences in time of collection of samples might have contributed to the apparent variation among patients, especially because the values early in bypass were clearly higher, on the average, than those collected later. We therefore decided to study a series of patients with frequent blood sampling during bypass, paying particular attention to the time of collection after onset of bypass.

**Subsequent Group**

Thirteen patients were studied by serially sampling the blood during bypass. Samples were drawn every 30-60 seconds during the first few minutes of bypass and at least every 5 minutes thereafter. Except when noted below, samples were drawn from the venous outflow line. The study group included five patients who underwent valve replacement and eight who underwent aortocoronary grafts with the saphenous vein.

The pattern of TxB₂ elevation observed in each of the valve replacement patients (fig. 2 and table 1) was a slow rise, reaching a peak 5-30 minutes after the start of bypass. The maximum level recorded was 0.46 ng/ml in two patients.

In contrast, each patient who underwent aortocoronary artery grafting (fig. 3 and table 2) showed a large, rapid rise in the TxB₂ concentration within the first 10 minutes of bypass. The highest level recorded was 2.4 ng/ml, and the other seven patients showed peak early bypass levels of 0.62 ng/ml or higher. The difference in the mean peak values in the two groups is statistically significant ($p < 0.01$). Typical results of serially sampling in each group are shown in figures 4 and 5.

No difference was detected between mixed venous TxB₂ levels and those in blood taken directly from the left ventricle. TxB₂ levels rose earlier in the preoxygenator limb of the bypass circuit than in the postoxygenator limb (fig. 4) in five of the six patients in whom this was examined. There was no consistent difference in TxB₂ in blood from the superior or inferior vena cava.

**Hematocrit (tables 1 and 2)**

The hematocrit was reduced by dilution by the pump prime and by intraoperative fluid therapy. In

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Maximum thromboxane B₂ (TxB₂) levels reached and time of peak value in patients undergoing valve replacement.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Maximum thromboxane B₂ (TxB₂) levels reached and time of peak value in patients undergoing aortocoronary bypass grafting.

---

**Table 1. Valve Replacement Patients**

<table>
<thead>
<tr>
<th>Thromboxane B₂ (ng/ml)</th>
<th>Platelet count/μl</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preincision</td>
<td>Max. level</td>
<td>early bypass</td>
</tr>
<tr>
<td>0.21</td>
<td>0.25</td>
<td>210,000</td>
</tr>
<tr>
<td>0.16</td>
<td>0.28</td>
<td>220,000</td>
</tr>
<tr>
<td>0.30</td>
<td>0.46</td>
<td>196,850</td>
</tr>
<tr>
<td>0.22</td>
<td>0.46</td>
<td>150,000</td>
</tr>
<tr>
<td>0.20</td>
<td>0.21</td>
<td>200,000</td>
</tr>
</tbody>
</table>

n | Mean | SD | Mean | SD | Mean | SD |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.218</td>
<td>0.051</td>
<td>5</td>
<td>0.332</td>
<td>0.119</td>
</tr>
</tbody>
</table>

$0.05 < p < 0.1$
addition, in some patients, venesection was used with volume replacement by crystalloid solution and subsequent return of red cells to the circulation.

The mean initial hematocrit of the patients (taken just after the induction of anesthesia) was 40.7 ± 4.9 and the mean fall during bypass was 39.3 ± 8.1% of the initial value. The two groups of patients did not differ in the initial hematocrit values or the falls during bypass.

**Platelet Counts (Tables 1 and 2)**

Preoperative platelet counts ranged from 135,000 to 323,000/μl. Platelet counts frequently rose somewhat after the induction of anesthesia. The highest platelet count recorded after the induction of anesthesia but before commencement of bypass was termed the initial platelet count. The mean initial platelet count for all patients was 206,780 ± 59,840/μl. There was no difference in the mean initial platelet count between valve replacement and aortocoronary artery graft patients.

The maximum fall in platelet count during the first 30 minutes of bypass was expressed as a percentage of the initial level. For all patients the mean fall was 32.7 ± 16.2%. In coronary artery graft patients in whom platelet counts were studied in detail, the mean fall was 41.3 ± 14.7% of the initial platelet count, significantly greater than the fall of 20.7 ± 9.5% in five valve replacement patients (p < 0.05). The

**Table 2. Aortocoronary Graft Patients**

<table>
<thead>
<tr>
<th>Thromboxane B2 (ng/ml)</th>
<th>Platelet count/μl</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preincision</td>
<td>Initial count</td>
</tr>
<tr>
<td>0.15</td>
<td>0.44</td>
<td>165,000</td>
</tr>
<tr>
<td>0.17</td>
<td>1.09</td>
<td>360,000</td>
</tr>
<tr>
<td>0.24</td>
<td>0.89</td>
<td>—</td>
</tr>
<tr>
<td>0.3</td>
<td>1.4</td>
<td>245,000</td>
</tr>
<tr>
<td>0.34</td>
<td>1.3</td>
<td>230,000</td>
</tr>
<tr>
<td>0.4</td>
<td>2.4</td>
<td>145,000</td>
</tr>
<tr>
<td>0.22</td>
<td>1.6</td>
<td>220,000</td>
</tr>
<tr>
<td>0.18</td>
<td>0.62</td>
<td>135,000</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.25</td>
<td>214,290</td>
</tr>
<tr>
<td>sd</td>
<td>0.089</td>
<td>77,430</td>
</tr>
</tbody>
</table>

p < 0.005
platelet counts were not corrected for hemodilution, but because the mean fall in hematocrit was the same in the two groups, the difference in fall in platelet count between coronary graft and valve replacement patients cannot be attributed to differences in hemodilution.

**Discussion**

In patients undergoing general surgery, platelet survival is shortened by operation,

and there is an increased urinary output of 5-hydroxy-indole acetic acid, presumably as a result of release of platelet serotonin. Platelet factor 4 in plasma has recently been reported to be increased in the postoperative period. We have not been able to detect any changes in plasma TxB2 levels in such patients because of injudicious time of sampling, as in our early studies in cardiopulmonary bypass, because of insensitivity of the radioimmunoassay, or because TxB2 is not generated in response to operation, although the last possibility seems unlikely.

General surgical patients showed transient FpA elevations, indicating thrombin activity during operation and in the early postoperative period. Since this increased propensity to form fibrin may be balanced by changes in the fibrinolytic mechanism known to occur during and after surgical procedures, its clinical significance is not certain.

The elevated FpA levels found during cardiopulmonary bypass even before the commencement of extracorporeal circulation probably reflect the prior insertion of arterial and venous cannulae as well as a response to sternotomy and other operations such as saphenous vein mobilization. Decreased fibrinogen survival and increased turnover of fibrinogen have been demonstrated in severely burned patients, in whom it was shown that fibrinogen consumption occurs in the wound itself. In patients with thromboembolism, raised levels of FpA rapidly return to normal when heparin is administered in adequate doses.

Persistent elevation of FpA levels in such patients indicates continued thrombin activity despite supposedly adequate heparin therapy. In the burned patients reported by Simon et al., failure of heparin therapy to prevent fibrinogen consumption may have reflected insufficient heparin dosage. Heparinization of our patients did not return the elevated FpA levels to normal, which implies continued thrombin activity despite heparin therapy. This view is supported by the fact that the ACT in these patients was often found to be shorter than 400 seconds, the level that Young et al. reported was necessary to prevent generation of fibrin monomer during cardiopulmonary bypass in monkeys. The persistent release of physiologically active peptide from fibrinogen and thrombin-induced platelet activity could account for some of the cardiovascular and pulmonary problems during bypass.

Collection artifact can be a major problem in the estimation of plasma FpA. Generation of FpA occurs during the passage of blood through indwelling catheters but is abolished by adequate systemic heparinization. Thus, the finding of elevated FpA levels throughout bypass, irrespective of their source, implies inadequate heparinization.

When platelets are maximally stimulated in vitro by addition of exogenous thrombin to platelet-rich plasma (average platelet count 400,000/μl), they will produce 10–15 ng/ml of TxB2 (unpublished data). It can be calculated that maximal stimulation of approximately 1–2% of the circulating platelet mass would raise TxB2 levels by 0.1 ng/ml in vivo.

TxB2 levels in plasma may be of platelet origin or produced in other tissues, such as lung, spleen and white cells. Generation of TxB2 during collection of samples is minimized by rapid withdrawal of blood and transfer into indomethacin-containing anticoagulant solution. Omission of indomethacin causes elevation of TxB2 levels due to generation in the test tube.

Our finding that TxB2 tends to rise earliest in the preoxygenator limb of the bypass circuit suggests that platelet damage may occur in the extracorporeal circuit but that damaged platelets are chiefly sequestered in the patient rather than retained in the pump-oxygenator. The principal site of generation of TxA4 would then be in the patient. The absence of preferential TxB2 elevation in left ventricular samples makes it unlikely that the lung contributes much TxB2 to the circulation and suggests that the platelet is a more likely source.

Within minutes of commencing cardiopulmonary bypass there is a profound fall in the platelet count, and further decrease then occurs at a slower rate throughout the period of bypass. In animals, after termination of bypass, some of the platelets may be returned to the circulation from sites of sequestration in spleen and liver. In man there is no clear evidence of this, although autotransfusion of platelets during bypass is known to occur, probably from an extrascular splenic storage pool. Thrombocytopenia following bypass in man usually persists for several days.

During extracorporeal circulation, there may be relative thrombocytopenia due to hemodilution or actual loss from the circulation as platelets are retained in the extracorporeal circuit or sequestered in the liver and spleen or the lungs. Hepatic sequestration appears to be the most important method of clearing damaged platelets from the circulation, at least in experimental animals.

Hemodilution accounts for much of the abrupt fall in platelet count during the first few minutes of bypass, but actual platelet loss probably explains the small, persistent drop during the procedure. Even when small prime volumes are used or when the prime is fresh whole blood, there is a reduction in platelet number due to actual disappearance of platelets from the circulation.

Evidence of functional damage may be detected in those platelets that remain in the circulation. Disordered function may be manifest as a reduction in platelet adhesiveness, decreased sensitivity to aggregating agents, or a decline in the amount of releasable and total ADP and ATP in the platelet.

"Downloaded from http://circ.ahajournals.org/ by guest on November 12, 2017"
Some of these changes are reversible and may return to normal after neutralization of heparin by the administration of protamine. However, in some patients, return of platelet function toward normal does not coincide with heparin reversal, and the defect may persist for several days after the operation.

Beurling-Harbury and Galvan suggested that the defect in platelet function induced by cardiopulmonary bypass results from the platelets undergoing a partial release reaction. In addition to secretion of ADP and ATP, mentioned above, there would also be release of serotonin, calcium, platelet factor 4, and β-thromboglobulin, and production of prostaglandin endoperoxides and TXA.

The high levels of TXB₂ in our patients, especially in those who underwent aortocoronary grafts, suggest that sufficient TXA₂ release has occurred to cause platelet aggregation and coronary arterial constriction. The peak production of TXA₂, as evidenced by elevated TXB₂ levels, may have been even higher than quoted, since the time of sampling in patients studied early in the series may have been inappropriate for detecting peak TXB₂ release.

The differences between patients with valvular disease and those with coronary revascularization are of interest. Beurling-Harbury and Galvan recently showed a preoperative decrease in the total and releasable ADP and ATP in patients with valvular heart disease, suggesting an element of preoperative platelet damage. No details of preoperative platelet function in coronary artery disease patients were included in their report, although these patients were found to have a lower blood loss during the operation and to require less transfusions than valve replacement patients.

It is possible that platelets of patients with valvular disease have already undergone release, to some extent, and their capacity to produce TXA₂ upon stimulation may be reduced compared with platelets from patients with coronary artery disease, perhaps because of depletion of membrane phospholipid. It is also possible that patients with chronic valvular disease who preoperatively have had accelerated platelet turnover develop a larger extravascular platelet pool and thus may be able to replace early platelet loss more efficiently than can patients undergoing coronary artery surgery.

Acknowledgment

We thank Drs. Malcolm Cronlund and J. Bryan Smith for the contribution of valuable reagents and Janet Ploetz for technical assistance.

References

26. Ashmore PG, Svitick V, Ambrose P: The incidence and effects of particulate aggregation and microembolism in pump-
Evaluation of Aortic Valve Replacement in Patients with Valvular Aortic Stenosis

WALTER L. HENRY, M.D., ROBERT O. BONOW, M.D., JEFFREY S. BORER, M.D., KENNETH M. KENT, M.D., JAMES H. WARE, PH.D., DAVID R. REDWOOD, M.D., SAMUEL B. ITSCOITZ, M.D., CHARLES L. MCINTOSH, M.D., ANDREW G. MORROW, M.D., AND STEPHEN E. EPSTEIN, M.D.

SUMMARY  Echocardiographic and hemodynamic studies were obtained in 42 consecutive patients undergoing aortic valve replacement for isolated aortic stenosis. Concentric left ventricular (LV) wall thickening, the most common preoperative abnormality, occurred in 95% of patients. LV dilation with reduced fractional shortening was noted in approximately 25% of patients but was severe in only one patient. Six months after operation, LV wall thickness had decreased on average but had not returned to normal and fractional shortening was unchanged. Repeat measurements in 13 patients an average of 37 months after operation were unchanged compared with measurements made 6 months after operation. When patients were subdivided into those with LV dilatation and those without, we found that patients with dilated ventricles preoperatively had a greater decrease in LV internal dimension and mass than those without preoperative dilatation.

The patient data also were examined for possible association with mortality. One operative (2%) and five late cardiac (13%) deaths occurred. No preoperative or 6-month postoperative echocardiographic or hemodynamic measurement was strongly associated with these deaths, nor were any late deaths due to congestive heart failure.

Compared with preoperative measurements in symptomatic patients who were operated for isolated aortic regurgitation, patients with aortic stenosis had smaller left ventricles with less depression of systolic function, as well as less aortic root and left atrial dilatation. Our data do not support the concept that the aortic valve should be replaced before the onset of symptoms to prevent irreversible LV damage in patients with isolated aortic stenosis.

ALTHOUGH MOST PATIENTS who undergo aortic valve replacement for aortic valve disease survive operation and are symptomatically improved, some die despite an apparently successful operation. In patients with aortic regurgitation, late death often is related to irreversible left ventricular dysfunction that had developed before operation.1-11 To determine whether preoperative left ventricular dysfunction could also account for some of the late postoperative deaths in patients with aortic stenosis, we prospectively studied patients undergoing operation for this condition.

One goal of the study was to identify preoperative measurements associated with either operative or late mortality. Patients were also examined to determine the alterations in cardiac structure and function that develop in response to a severe left ventricular pressure load and to assess the changes that occur after the pressure load is relieved by operation. Finally, measurements obtained in patients with aortic stenosis were compared with those obtained in patients with aortic regurgitation.

Methods

Patients

The patient population consisted of all patients undergoing aortic valve replacement for long-standing valvular aortic stenosis who were operated on between January 1972 and June 1976. Patients included in the study population had an aortic valve index of less than 0.80 cm²/m² and less than 1+/4+ aortic regurgitation visualized by aortic root cineangiography (34 patients) or no murmur of aortic regurgitation on physical ex-

From the Cardiology and Surgery Branches, NHLBI, NIH, Bethesda, Maryland 20205.
Address for correspondence: Walter L. Henry, M.D., Division of Cardiology, University of California, Irvine, Medical Center, 101 City Drive South, Building 53, Orange, California 92668.
Received December 27, 1978; revision accepted October 9, 1979.
Circulation 61, No. 4, 1980.
Elevated plasma fibrinopeptide A and thromboxane B2 levels during cardiopulmonary bypass.
G C Davies, M Sobel and E W Salzman

Circulation. 1980;61:808-814
doi: 10.1161/01.CIR.61.4.808
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1980 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on
the World Wide Web at:
http://circ.ahajournals.org/content/61/4/808