Arteriosclerosis in Normal and von Willebrand Pigs
Long-Term Prospective Study and Aortic Transplantation Study

Valentin Fuster, David N. Fass, Michael P. Kaye, Miguel Josa, Alan R. Zinsmeister, and E. J. Walter Bowie

With the technical assistance of James M. Byrne

From the Department of Medicine, Department of Surgery, and Department of Medical Statistics, Mayo Clinic and Mayo Foundation, Rochester, Minnesota

SUMMARY. In a long-term prospective study, five normal control pigs and five pigs with homozygous von Willebrand's disease received a nonfatty diet from age 3 months to 4 years; then the aortas were analyzed. The fibrous arteriosclerotic plaques in the distal abdominal aortic region involved an average of 28% of the surface area in control pigs and only 7% of the surface area in pigs with von Willebrand's disease (P < 0.01). In a subsequent study of 3-month-old pigs, the distal abdominal aortic segments from nine normal pigs were cross-transplanted with segments from nine other normal pigs (control study), and aortic segments from four normal pigs were transplanted into four host pigs with von Willebrand's disease (exchange study). All pigs received a 2% cholesterol diet for up to 6 months; then the transplanted aortic segments were analyzed. The donor normal aortic segments in the host normal pigs developed arteriosclerosis that involved an average of 20% of the surface; the endothelial fluorescent pattern of von Willebrand factor was identified. In contrast, the donor normal aortic segments in the host pigs with von Willebrand's disease had arteriosclerosis that involved an average of only 4% of the surface (P < 0.01); the endothelial cell von Willebrand factor was not identified. The long-term prospective study indicates that pigs with von Willebrand's disease are resistant to the development of spontaneous arteriosclerosis. The aortic transplantation data are compatible with the hypothesis that the absence of von Willebrand factor in pigs with von Willebrand's disease may cause impairment of platelet-arterial wall interaction and resistance to arteriosclerosis. (Circ Res 51: 587-593, 1982)

FOR several years, we have maintained a breeding colony of pigs with von Willebrand's disease (Bowie et al., 1973). These animals have the observed impairment of primary hemostasis and the other hemostatic abnormalities noted in the severe form of the disease in humans (Bowie et al., 1973; Mannucci et al., 1976): a serious autosomally transmitted bleeding tendency, a long bleeding time, reduced retention of platelets in a column filled with glass beads, reduced levels of factor VIII coagulant activity (VIII:C), very low levels (0.2% of normal) of factor VIII-related antigen (VIII R:Ag), and a lack of ristocetin cofactor (Willebrand factor VIII:RWF) in the plasma (Bowie et al., 1973; Fass et al., 1976). This ristocetin cofactor is closely allied with, or identical to, the factor VIII-related antigen.

There is increasing evidence that platelets, by adhering to a damaged vascular surface and subsequently releasing a platelet-derived growth factor (or factors), may play a role in the initiation of arteriosclerosis (Ross et al., 1974; Harker et al., 1976; Goldberg et al., 1980). In pigs with homozygous von Willebrand's disease, we have observed a resistance to high-cholesterol diet-induced aortic arteriosclerosis, and, as we have suggested, this might be related to the impairment of the platelet-hemostatic mechanism in these animals (Fuster et al., 1978).

This study had two purposes: (1) to compare the development of spontaneous aortic arteriosclerosis in normal and von Willebrand pigs given a controlled diet low in fat and cholesterol for up to 4 years—such an approach was essential because, in our previously reported prospective study on arteriosclerosis (Fuster et al., 1978), the pigs were under a high-fat and high-cholesterol diet and the study was short-term (for up to 6 months); and (2) to evaluate, by aortic transplantation, whether the donor normal pig aorta becomes resistant to the development of arteriosclerosis when it is transplanted into the host von Willebrand pig—such an approach might provide information on the possible contribution of the arterial wall or the circulating blood in the development of arteriosclerosis in the pig.

Methods

Swine

The original Poland-China pigs with von Willebrand's disease (Bowie, et al., 1973) were crossed with Yorkshire-Hampshire pigs to establish our present colony. Our control pigs were also a mixture of Poland-China and Yorkshire-Hampshire. All pigs were housed at the Mayo Institute Hills Farm, and all were studied concurrently.

Spontaneous Aortic Arteriosclerosis Study

Fifteen newborn pigs (five controls and 10 with homozygous von Willebrand's disease) were fed maternal milk
which was supplemented with cow's milk until 3 months of age, at which time they began to receive a diet low in fat and cholesterol (Table 1), 800 g/40 kg body weight, that was continued for up to 4 years. Five pigs with von Willebrand's disease that bled to death at 5, 8, 12, 12, and 12 months of age were excluded from the study. Thus, the study comprised five normal control pigs (three male and two female) and five pigs with von Willebrand's disease (four male and one female), and all pigs were under surveillance for more than 1 year. In each instance that a von Willebrand pig bled to death, the control animal closest in age was killed. The mean age of the control pigs was 37 (range, 20-47) months and of the von Willebrand pigs was 36 (range, 23-52) months.

The hemostatic data during life of the 10 study pigs are summarized in Table 2, and the methods used have been described previously (Mertz, 1942; Laurell, 1966; Bowie et al., 1971; Zimmerman et al., 1971; Weiss et al., 1973; Olson et al., 1975). The von Willebrand pigs have a form of disease similar to the human type I (Ruggeri and Zimmerman, 1980). In the colony from which these pigs were selected, serum cholesterol (Levine and Zack, 1964) averaged 101 mg/dl (±13 SD) in the control pigs and 88 mg/dl (±15 SD) in the pigs with von Willebrand's disease; serum triglycerides (Ellison and Caraway, 1976) averaged 53 mg/dl (±19 SD) in the control pigs and 47 mg/dl (±22 SD) in the pigs with von Willebrand's disease.

Postmortem Analysis

In all pigs, the entire aorta from the aortic valve to the abdominal trifurcation was removed and preserved in 10% formaldehyde solution. All of the aortas were analyzed blindly at the same time. Raised arteriosclerotic plaques were identified and quantitated by gross and microscopic examinations that conformed to methods previously described (Fuster et al., 1978). In brief, for gross examination, the aortas were opened longitudinally and were carefully inspected for lesions. Only one type of lesion was seen (Mitchell and Schwartz, 1965; Fritz et al., 1980; Griggs et al., 1981): a raised fibrous arteriosclerotic plaque consisting of a firm, gray, prominent elevation with predominantly collagen and elastic bundles, as detected microscopically. Quantification was done by recording the proportion of the aortic area affected. The area affected was measured by cutting out the involved portion from a drawing of the aorta and weighing the transparent acetate paper [the weight of the drawn aorta in the control pigs—average 23 g (range 175-265 g)—was not significantly different than in the von Willebrand pigs—average 219 g (range 165-251 g)]. Because the raised arteriosclerotic plaques involved primarily the distal abdominal aorta—between the origin of the renal arteries and the aortic trifurcation—the quantification of the arteriosclerotic plaques specifically related to the distal aortic region was of major interest [The weight of the drawn distal aorta in the control pigs—average 32 g (range 20-47 g)—was not significantly different from that of the von Willebrand pigs—average 30 g (range 19-25 g)].

After the entire aortas had been stained with Sudan IV (Holman et al., 1958), the raised fibrous arteriosclerotic plaques seen before staining became red, particularly at the periphery; this indicated some content of fat, which was also confirmed microscopically. In addition, in most pigs, a small degree of diffuse sudanophilia in the nonarteriosclerotic proximal aortic region was also observed.

For microscopic examination, a full-thickness sample, ≈ 5 mm² in surface area, was excised from each arteriosclerotic plaque. An apparently normal sample near each lesion but on the opposite side was excised to serve as a control. The tissues were cut as accurately as possible perpendicular to the surface. They were stained with hematoxylin and eosin, with Heidenhain's Weigert-Van Gieson stain, and with Sudan IV. Histological sections were photographed at a magnification of 160-400 X. From these pictures, the thickness of the intima from the surface down to the internal elastic membrane was measured. In each section, the thickness was measured at 10 equally separated levels and the average result was obtained.

Statistical Method

Data corresponding to the proportion of area in the aorta with arteriosclerotic plaques were first transformed using the arc sin square root transformation; then differences between control pigs and pigs with von Willebrand’s disease were assessed using 1-way analysis of covariance with age (in months) as the covariate.

Aortic Transplantation Study

Twenty-six newborn pigs (22 normal controls and four with homozygous von Willebrand’s disease) were fed maternal milk supplemented with cow’s milk until 3 months of age, at which time the pigs were divided in two groups for two studies: (1) a control aortic transplantation study, in which the aortic segments of nine normal pigs were cross-transplanted with the aortic segments of nine other normal pigs (since one of the pigs died at operation, a total of 17 pigs were hosts of a transplanted aortic segment); and (2) an exchange aortic transplantation study, in which the aortic segments from four normal pigs were transplanted into four host von Willebrand pigs.

The hemostatic data during life of the 25 study pigs is summarized in Table 2.

Operation

In all of the studies, a distal aortic segment proximal to the trifurcation was chosen for transplantation because this...
Willebrand factor in the donor aortic segment and in the postmortem the presence or absence of endothelial cell von Willebrand factor (Booyse et al., 1977).

Spontaneous Aortic Arteriosclerosis Study

In the control study, for each normal pig the difference between the extent of arteriosclerotic plaques in the donor aortic segment and in the host aortic region; this was performed in two of the host normal pigs of the control study and in two of the host von Willebrand disease pigs of the exchange study. Thus, from each of the four aortas, four full-thickness tissue portions of 0.5 cm² were excised proximal and distal to both suture lines of the transplanted segment. The tissue portions were frozen in isopentane, sectioned, and stained for immunofluorescence microscopy by means of rabbit anti-porcine von Willebrand factor (Booyse et al., 1977).

### Results

#### Spontaneous Aortic Arteriosclerosis Study

In all 10 pigs, raised arteriosclerotic plaques developed, almost exclusively in the distal abdominal aortic region (Table 3). In the five control pigs, the aortic arteriosclerotic plaques in the distal abdominal aortic region averaged 28% (±11 sd) of that surface area. When the entire surface of the aorta was considered, in the five control pigs the aortic arteriosclerotic plaques averaged 5% (±2 sd) of the surface. Intimal thickening over the plaques found in the control pigs averaged 0.8 mm (range, 0.5–1.1 mm).

In the five pigs with von Willebrand’s disease, the aortic arteriosclerotic plaques in the distal abdominal aortic region averaged 7% (±6 sd) of that surface area. When the entire surface of the aorta was considered, in the five pigs with von Willebrand’s disease the
TABLE 3
Spontaneous Development of Arteriosclerotic Plaques

<table>
<thead>
<tr>
<th>Pig no.</th>
<th>Control pigs</th>
<th>Sex</th>
<th>Age (mo)</th>
<th>Distal aorta (%)</th>
<th>Total aorta (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>44</td>
<td>41</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>37</td>
<td>40</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>47</td>
<td>26</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>37</td>
<td>24</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>20</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pigs with von Willebrand’s disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>52</td>
<td>15</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>24</td>
<td>9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>33</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>46</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>23</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

aortic arteriosclerotic plaques averaged 2% (±1 SD). The decreased extension of arteriosclerotic plaques in the pigs with von Willebrand’s disease when compared with the controls was highly significant \( P < 0.01 \). Maximal intimal thickening over the plaques found in the pigs with von Willebrand’s disease averaged 0.9 mm (range, 0.1-1.6 mm); this was not significantly different than in the controls.

Aortic Transplantation Study

In the control aortic transplantation study that involved 17 normal pigs, the extent of the arteriosclerotic plaques in the donor aortic segments averaged 20% of the surface (Table 4); this was only slightly larger than the extent of the arteriosclerotic plaques in the host aortic region (average, 17%) \( P = 0.06 \). Maximal intimal thickening over the plaques of the donor aortic segments averaged 2.6 mm (range, 0.7-5.0 mm).

In the exchange aortic transplantation study, the donor aortic segments from the normal pigs in the four host von Willebrand pigs had arteriosclerotic plaques that involved an average of only 4% of the surface (Table 5); the extent of the arteriosclerotic plaques in the host aortic region also averaged 4%. Compared with the control aortic transplantation study, this decrease in extension of arteriosclerotic plaques in the donor aortic segments from the normal pigs when transplanted into the host von Willebrand pigs was significant \( P < 0.01 \). Maximal intimal thickening over the plaques of these donor aortic segments averaged 2.0 mm (range, 0.8-3.6 mm).

In the control study, the endothelial cell von Willebrand factor was identified in the transplanted aortic segments and in the host aortic regions of the host normal pigs. In the exchange study, the endothelial cell von Willebrand factor was identified neither in the transplanted aortic segment from the normal pigs, nor in the host aortic regions of the von Willebrand pigs. In four instances, aortic segments from von Willebrand pigs were transplanted into four normal host pigs; two of the normal pigs died during the procedure. In the remaining two normal pigs, the donor aortic segments from the von Willebrand pigs developed significant arteriosclerotic plaques (Table 5) and the endothelial cell von Willebrand factor was identified (Fuster et al., 1979). Unfortunately, the number of pigs in this last experiment was too small to include them in the statistical calculations of this paper.

Discussion

In a previous investigation, we observed that pigs with von Willebrand’s disease fed a high-fat and high-cholesterol diet for up to 6 months were less susceptible to the development of aortic arteriosclerosis than were normal control pigs (Fuster et al.,
such a finding has now been confirmed by Griggs et al., 1981). The present investigation shows the following: (1) When given a controlled low-fat and low-cholesterol diet for up to 4 years, pigs with von Willebrand’s disease have a pronounced resistance to the development of spontaneous aortic arteriosclerosis; and (2) in exchange aortic transplantation between normal and von Willebrand pigs, the donor normal pig aorta in the host von Willebrand pig becomes resistant to the development of arteriosclerosis.

Spontaneous Aortic Arteriosclerosis Study

In this long-term study of pigs fed a low-fat and low-cholesterol diet, the resistance to the development of aortic arteriosclerosis is more striking than in the previous short-term studies of dietary-induced arteriosclerosis (Fuster et al., 1978; Griggs et al., 1981). In addition, it is of interest that, in contrast to dietary-induced arteriosclerosis, in this study we observed only one type of arteriosclerotic lesion—the typical arteriosclerotic fibrous plaque noted in humans (Mitchell and Schwartz, 1965).

A possible explanation for the finding of resistance to spontaneous arteriosclerosis in pigs with von Willebrand’s disease may be in the impaired platelet-vascular interaction seen in this condition. In normal mammalian species, chronic endothelial injury may be caused by hemodynamic factors (Texon, 1960; Wesołowski et al., 1965; Frye, 1969; Sommer et al., 1972; Glagov, 1973; Cornhill and Roach, 1976; Nerem and Cornhill, 1980) and the platelet tends to adhere to the damaged vascular surface (Jorgensen et al., 1972; Lewis and Kottke, 1977). The von Willebrand factor in the plasma, the platelet, and the vascular wall favors such platelet-arterial wall interaction (Cornu et al., 1963; Bloom et al., 1973; Hoyer et al., 1973; Howard et al., 1974; Rand et al., 1980). Thus, it is reasonable to assume that the bleeding tendency in von Willebrand’s disease exists because of the reduction of von Willebrand factor in the plasma, platelet, and arterial wall (Baumgartner et al., 1977) and the consequent poor adhesion of platelets to the damaged vessel wall (Baumgartner et al., 1977; Legrand et al., 1978; Weiss et al., 1978; Kimura et al., 1979; Sakariassen et al., 1979; Bowie et al., 1980; Fuster et al., 1980; Nerem and Cornhill, 1980; Nyman, 1980).

The necessary bonding between platelets and damaged vessels to stanch bleeding may furnish an important clue to the origin of arteriosclerosis. In experimental arteriosclerosis induced by removal of the endothelium, platelets initially adhere to denuded subendothelial tissue and undergo intracytoplasmic degranulation (Stemerman et al., 1979); this sequence of events is followed by release of platelet material into the intima and by intimal migration and proliferation of smooth muscle cells (Stemerman et al., 1979; Goldberg et al., 1980). It has been suggested that one of the materials released by the platelets, the so-called platelet-derived growth factor (Ross et al., 1974; Ross and Vogel, 1978; Antoniades et al., 1979; Grotendorst et al., 1981), contributes to this migration and proliferation of the smooth muscle cells seen in the process of arteriosclerosis (Harker et al., 1976; Ross and Glomset, 1976).

Further insight into the importance of platelets in the development of arteriosclerosis has been gained by demonstrating that experimental animals with inhibited platelet function or with thrombocytopenia are resistant to the development of arteriosclerosis. Thus, Harker et al. (1976) observed that in experimental homocystinemia, platelet consumption correlated well with formation of intimal lesions and arteriosclerosis, and a platelet inhibitor, dipyridamole, reduced both. Friedman et al. (1977) prevented formation of balloon catheter-induced intimal lesions in rabbits by producing marked thrombocytopenia with administration of antiplatelet serum. Similarly, thrombocytopenia from administration of 32P was found to reduce arteriosclerosis in rabbits fed an egg yolk diet (Cohen and McCombs, 1968), whereas increased arteriosclerosis accompanied experimental thrombocytosis (Cohen and McCombs, 1967).

In the light of these findings, we considered the situation in pigs with von Willebrand’s disease. Because the platelets cannot effectively adhere to vascular surfaces, the animals are functionally “thrombocytopenic.” Furthermore, if such adherence is a necessary step in the generation of arteriosclerosis, the life-threatening hemostatic defect in these animals may be a preserving vascular phenomenon. Our results indeed suggest that pigs with von Willebrand’s disease are resistant to the development of spontaneous arteriosclerosis, and we postulate that the absence of von Willebrand factor may be responsible for the impairment of platelet-arterial wall interaction and the resistance to arteriosclerosis in these animals. To enhance our understanding of this possible relationship among the von Willebrand factor, the circulating platelet, and the reactivity of the arterial wall in the genesis of arteriosclerosis, we performed the aortic transplantation study.

Aortic Transplantation Study

The control aortic transplantation study between normal pigs demonstrated that the experimental design could be useful and gave valid information. First, gross aortic rejection was not observed—that is, during life, all pigs appeared healthy and free of a rejection type of illness and at postmortem examination the intima, media, and adventitial layers of the donor aortas were well-defined and preserved. Second, the donor-transplanted aortic segments from the normal pigs were as capable of developing extensive arteriosclerosis as the recipient host normal pig aortic regions, and the endothelial fluorescent pattern of von Willebrand factor was maintained. In the short period of 6 months, the development of arteriosclerosis in the donor and recipient host aortic regions was certainly in part related to the effects of the hyperlipidemic diet (Ross and Glomset, 1976; Fuster et al.,
1978); possible mechanical injury of the endothelium of the donor segment at the time of transplantation probably did not play a major role in the extent of the atherosclerotic process, since the extent of arteriosclerosis in the donor segment was not significantly larger than the extent of arteriosclerosis in the host aortic region.

In contrast, the exchange aortic transplantation study revealed that the donor aortic segments from the normal pigs in the four host von Willebrand pigs had a decreased capability of developing arteriosclerosis and the endothelial fluorescent pattern of von Willebrand factor could not be identified.

For complete interpretation of the data of these transplantation studies, we need to know whether the donor aortic segments are totally or partially replaced by the host aortas. We can say, however, that the development or resistance to arteriosclerosis correlated with the presence or absence of von Willebrand factor. Thus, the results obtained are compatible with our hypothesis: that is, in pigs with homozygous von Willebrand disease, the absence of von Willebrand factor may be responsible for the impairment of platelet-arterial wall interaction and the resistance to arteriosclerosis in these animals.

It is important to recognize that there may be other explanations for the differences in the susceptibility to arteriosclerosis between normal and von Willebrand pigs. However, up until present, we have not been able to identify any other factor that may explain such difference, including the various lipoprotein fractions, blood sugar, platelet count, and blood hematocrit, which are all independently similar in both groups of pigs (Fuster et al., 1978; Griggs et al., 1981).

Finally, we should consider how these studies may relate to the incidence of arteriosclerosis in humans with von Willebrand's disease, although little information is available on the subject. Kernoff et al. (1981) detected significant atherosclerosis in the abdominal aorta of an elderly man (73 years old) with severe von Willebrand's disease. However, this man had been over many years under an intensive program of fresh frozen plasma and cryoprecipitate infusions which tend to correct the platelet defect; in addition, it is remarkable that at his age the major coronary, cerebral, and medium size arteries were free from atherosclerosis. Silwer et al. (1966) reported that there was no protection against arteriosclerosis in humans with this disorder. At the time the study was performed, among other problems previously discussed (Fuster et al., 1978), nothing was known about the measurement of von Willebrand factor. The findings of Silwer et al. (1966) are perhaps not unexpected because the occurrence of the severe type of von Willebrand's disease that we find in our pigs is rare in humans (Mannucci et al., 1976). Most humans with von Willebrand's disease have detectable levels of von Willebrand factor in their plasma. We have found that our carrier pigs, which have about 40% of von Willebrand factor in their plasma, are not resistant to the development of arteriosclerosis (Fuster and Bowie, 1979). We are now planning to perform a study on pigs which have an abnormal form of von Willebrand factor molecule in their plasma. We feel that this study may give evidence which is more directly related to the human disease.

We greatly appreciate the valuable help of Dr. Paul E. Zollman (Director of Veterinary Medicine at Mayo Institute Hills Farm). We would also like to express our thanks for the expert editorial assistance of Nancy D. Nelson, the technical assistance of O. Arian Hildestad, and the excellent secretarial assistance of Darlene K. Albrect.

Dr. Fuster, Fass, and Bowie are affiliated with the Department of Medicine, Drs. Kaye and Fosa, with the Department of Surgery, and Dr. Zinsmeister, with the Department of Medical Statistics.

Supported in part by National Institutes of Health grants HL-19001 and HL-17430.

Address for reprints: Valentin Fuster, M.D., Department of Medicine, Mayo Clinic, Rochester, Minnesota 55905.

Received March 16, 1982; accepted for publication August 12, 1982.

References


Booyse FM, Quarfoot AJ, Bell S, Fass DN, Lewis JC, Mann KG, Bowie EJW (1979) Cultured aortic endothelial cells from pigs with von Willebrand's disease: In vitro model for studying the molecular defect(s) of the disease. Proc Natl Acad Sci USA 74: 5702-5706


Cohen P, McCombs HL (1968) Platelets and atherogenesis. II. Amelioration of cholesterol atherogenesis in rabbits with reduced platelet counts as the result of IP administration. J Atheroscler Res 8: 389-396


on experimental atherosclerotic lesion formation in rabbits. J Clin Invest 60: 1191-1201


Wesolowski SA, Fries CC, Sabini AM, Sawyer PN (1965) The significance of turbulence in hemic systems and in the distribution of the atherosclerotic lesions. Surgery 57: 155-161

Zimmerman TS, Ratnoff OD, Powell AE (1971) Immunologic differentiation of classic hemophilia (factor VIII deficiency) and von Willebrand's disease. With observations on combined deficiencies of antihemophilic factor and procoagulant (factor V) and on acquired circulating anticoagulant against antihemophilic factor. J Clin Invest 50: 244-254

INDEX TERMS: Arteriosclerosis • von Willebrand's disease • Aortic transplant • Platelets and arteriosclerosis
V Fuster, D N Fass, M P Kaye, M Josa, A R Zinsmeister and E J Bowie

Circ Res. 1982;51:587-593
doi: 10.1161/01.RES.51.5.587

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/51/5/587