

# Aspirin Treatment Reduces Platelet Resistance to Deformation

Steven M. Burris, Clark M. Smith II, Gundu H. R. Rao, and James G. White

**The present investigation has evaluated the influence of aspirin, its constituents, and other nonsteroidal anti-inflammatory agents on the resistance of human platelets to aspiration into micropipettes. Aspirin increased the length of platelet extensions into the micropipette over the entire negative tension range of 0.04 to 0.40 dynes/cm after exposure to the drug in vitro or after ingestion of the agent. Other cyclooxygenase inhibitors, ibuprofen and indomethacin, did not increase platelet deformability. The influence of aspirin was mimicked to some degree by high concentrations of salicylic acid, but acetylation of platelets with acetic anhydride had little influence on platelet deformability. Incubation of platelets with both salicylic acid and acetic anhydride had no more effect than salicylic acid alone. Benzoic acid, chemically similar to salicylic acid, had a minimal effect. The studies demonstrate that aspirin makes platelets more deformable, while components of the drug or other nonsteroidal anti-inflammatory agents and cyclooxygenase inhibitors do not have the same influence on resistance to deformation. (Arteriosclerosis 7:385-388, July/August 1987)**

Recent studies in our laboratory have used the technique of micropipette elastimetry to evaluate the resistance of platelets to deformation.<sup>1,2</sup> Investigations into the effects of chilling and of antimetabolic agents demonstrated that intact microtubule coils are critical for platelet resistance to aspiration into micropipettes.<sup>1,2</sup> Exposure of platelets to cytochalasin B before aspiration showed that actin microfilament assembly was also important in platelet stability.<sup>1</sup>

Exposure of platelets to aggregating agents dramatically altered platelet deformability. Thrombin, ADP, and the ionophore, A23187, stimulated platelet shape change and internal transformation associated with constriction of circumferential microtubule coils.<sup>3,4</sup> Agonist-activated platelets were significantly softer than resting platelets on aspiration.<sup>5</sup>

Aspirin inhibits platelet cyclooxygenase, blocks thromboxane generation and prevents platelet secretion caused by potent agonists but does not affect shape change.<sup>6-13</sup> It seemed reasonable, therefore, to determine whether or not aspirin would also block the influence of aggregating agents on deformability. We found that aspirin itself caused significant softening of resting platelets. Further studies revealed that other cyclooxygenase inhibitors did

not affect platelet deformability, and the effect of aspirin was not fully reproduced by components of the drug.

## Methods

### Materials

Acetylsalicylic acid (ASA), salicylic acid, benzoic acid, and indomethacin were obtained from Sigma Chemical Company (St. Louis, Missouri). Acetic anhydride was obtained from Upjohn Company (Kalamazoo, Michigan). All the agents were suspended in  $\text{Ca}^{++}/\text{Mg}^{++}$ -free Hanks' Balanced Salt Solution (HBSS) to a final stock concentration of 10 mmol/L. All of the drugs were added to platelet suspensions at a final concentration of 100  $\mu\text{M}/\text{L}$  and were incubated for 30 minutes at room temperature before the deformability studies were done. Salicylic acid was also incubated with platelets at a concentration of 200  $\mu\text{M}/\text{L}$ .

### Platelet Preparation

Blood was collected by venipuncture from healthy adult human donors after informed consent was obtained in accordance with the committee on human subjects at the University of Minnesota. Blood samples were obtained from volunteers who had taken no medications for 2 weeks prior to venipuncture or had ingested 650 mg of aspirin 2 hours earlier. The blood samples were immediately mixed with 3.8% trisodium citrate or citrate-citric acid, pH 6.5 (93 mmol/L sodium citrate, 70 mmol/L citric acid, and 140 mmol/L dextrose) in a ratio of nine parts blood to one part anticoagulant.<sup>14,15</sup> Platelet-rich plasma (PRP) was separated from whole blood by centrifugation at 100 g for 15 minutes. Samples of PRP were mixed with an equal volume of the citrate-citric acid anticoagulant; then one volume of PRP mixture was added to nine volumes of  $\text{Ca}^{++}/\text{Mg}^{++}$ -free HBSS containing adenosine 5 mmol/L, theophylline 3 mmol/L, and human serum albumin 0.1%. The diluted platelets were incubated in this mixture for 15

---

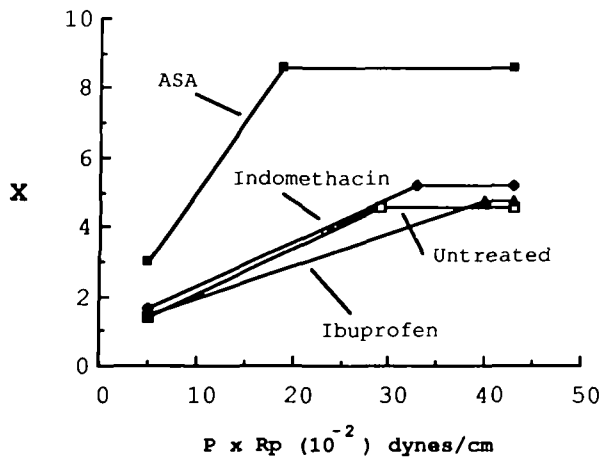
From the Departments of Laboratory Medicine and Pathology, Pediatrics, and Biomedical Engineering, University of Minnesota Health Sciences Center, Minneapolis, Minnesota.

This work was supported in part by USPHS Grants HL-27355, HL-11880, CA-21737, GM-22167, and HL-30217; March of Dimes Grant MOD1-886; and Minnesota Medical Foundation Grant HDRI-3685.

Address for reprints: James G. White, M.D., UMHC Box 490, University of Minnesota Health Sciences Center, Harvard at East River Road, Minneapolis, Minnesota 55455.

Received November 20, 1987; revision accepted March 19, 1987.

Laurence A. Harker, M.D., kindly acted as Guest Editor for this manuscript.



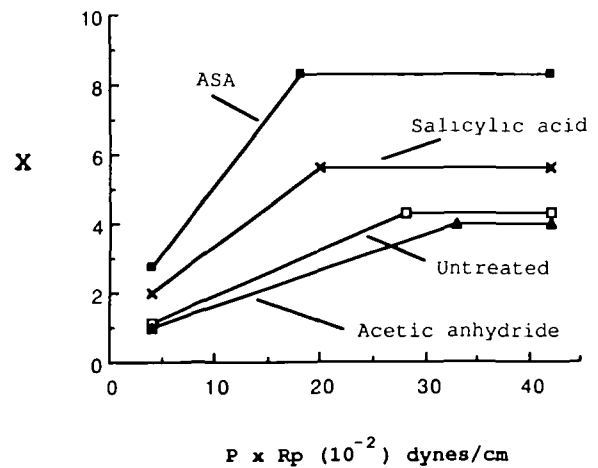
**Figure 1.** Effects of aspirin, ibuprofen, and indomethacin on the stress response of normal platelets. The lines represent the computerized linear regression of the tension-extension data with correlation coefficients ( $R^2$ )  $\geq 0.90$ .

minutes at room temperature to inhibit activation prior to and during micropipette aspiration.<sup>1, 2, 5</sup>

### Micropipette Aspiration of Platelets

Micropipette aspiration of platelets was performed in the same manner as recently reported.<sup>2, 5</sup> Briefly, boron silicate glass capillaries were pulled to form micropipettes with internal diameters ranging from 0.7 to 0.8  $\mu\text{m}$  which remained constant in the initial 4  $\mu\text{m}$  of the pipette. The internal diameters of the pipettes were measured on the video monitor with a calibrated 10- $\mu\text{m}$  grid as previously described.<sup>1, 2, 5</sup>

The pipette was placed in a micromanipulator and pressure was regulated by adjusting the height of a reservoir of water. The reservoir heights were calibrated with a  $\text{H}_2\text{O}$ -filled manometer and monitored with a pressure transducer and recorder. The micropipette cell aspirations were visualized and recorded with a videomicroscope apparatus for analysis at a later date.<sup>1, 2, 5</sup> The tip of the pipette was advanced through the platelet suspension under direct vision while a constant negative pressure of 1.0 cm  $\text{H}_2\text{O}$  (0.04 dynes/cm) was maintained. A portion of the cell was aspirated into the pipette upon contact with the platelet; aspiration pressure was sequentially increased by 1-cm increments to a maximum of 10 cm  $\text{H}_2\text{O}$  (0.42 dynes/cm) resulting in progressively larger extension lengths into the pipette until a maximum length was achieved. Control cells were studied with the same pipette



**Figure 2.** Effects of aspirin, salicylic acid, and acetic anhydride on the stress response of normal platelets. The lines represent the computerized linear regression of the tension-extension length data with correlation coefficients ( $R^2$ )  $\geq 0.90$ .

that was used to evaluate treated cells. Minor differences in pipette internal diameter were also accounted for in the data analysis as described below.

The changes in extension lengths were measured with a Hi-pad Digitizer (Bausch & Lomb, Houston, Texas) interfaced with a Terak 8510 Graphic Computer (Terak Corporation, Scottsdale, Arizona). A dimensionless extension parameter ( $X$ ) was obtained by dividing the extension length in micrometers by the radius of the pipette ( $R_p$ ) also in micrometers. A tension stress parameter ( $P \times R_p$ ) was defined by multiplying the aspiration pressure ( $P$ ) by the pipette radius. The stress response of the platelet was characterized by four quantifiable parameters. The cell extension length aspirated at the lowest tension was termed  $X_i$ . The maximum extension length aspirated from the cell was referred to as  $X_m$  and the tension at which  $X_m$  was reached was designated  $T_m$ . The slope of the linear portion of the stress response was also determined by linear regression analysis.

### Data Analysis

Linear regression analysis of the data was done with a multiple regression interactive computer program developed by Weisberg.<sup>16</sup> By plotting the residuals of the linear regression, it was possible to assess linearity throughout the range of tensions. The tension at which the stress response deviated from a linear function was also detected in the analysis. The statistical significance of the differ-

**Table 1. Initial Cell Extension, Slope, Maximum Extension Length, and Tension at Maximum Extension Length of Untreated and Treated Platelets**

| Platelet treatment | $X_i$             | Slope                   | $X_m$             | $T_m$              |
|--------------------|-------------------|-------------------------|-------------------|--------------------|
| Untreated          | $1.15 \pm 0.13$   | $12.71 \pm 0.65$        | $4.30 \pm 0.30$   | $0.280 \pm 0.03$   |
| Aspirin            | $2.72 \pm 0.25^*$ | $29.27 \pm 1.88^*$      | $8.32 \pm 0.61^*$ | $0.180 \pm 0.02^*$ |
| Ibuprofen          | $1.18 \pm 0.15$   | $9.97 \pm 0.58^\dagger$ | $4.51 \pm 0.32$   | $0.390 \pm 0.04^*$ |
| Indomethacin       | $1.42 \pm 0.24$   | $13.64 \pm 0.64$        | $4.98 \pm 0.64$   | $0.317 \pm 0.03$   |

The results within each experimental category were based on 40 to 50 cells. Values are mean  $\pm$  SD.

\*Statistically different from untreated,  $p < 0.001$ .

†Statistically different from untreated,  $p < 0.01$ .

$X_i$  = initial cell extension;  $X_m$  = maximum extension length;  $T_m$  = tension at maximum extension length.

**Table 2. Deformability Parameters of Untreated and Treated Platelets**

| Platelet treatment                  | $X_i$        | Slope         | $X_m$        | $T_m$         |
|-------------------------------------|--------------|---------------|--------------|---------------|
| Untreated                           | 1.15 ± 0.13  | 12.71 ± 0.65  | 4.30 ± 0.30  | 0.280 ± 0.03  |
| Aspirin                             | 2.72 ± 0.25* | 29.27 ± 1.88* | 8.32 ± 0.61* | 0.180 ± 0.02* |
| Salicylic acid                      | 2.00 ± 0.24* | 20.78 ± 1.35* | 5.63 ± 0.81* | 0.205 ± 0.02* |
| Acetic anhydride                    | 0.98 ± 0.25  | 11.20 ± 1.02  | 4.00 ± 0.19  | 0.334 ± 0.02* |
| Salicylic acid and acetic anhydride | 1.89 ± 0.46† | 20.08 ± 1.47* | 5.85 ± 0.62† | 0.245 ± 0.03  |
| Acetic anhydride and salicylic acid | 2.15 ± 0.36* | 22.51 ± 1.77* | 5.43 ± 0.70† | 0.302 ± 0.02  |

Results within each experimental category were based on 40 to 50 cells. Values are mean ± SD.

\*Statistically different from untreated,  $p < 0.001$ .

†Statistically different from untreated,  $p < 0.01$ .

$X_i$  = cell extension;  $X_m$  = maximum extension length;  $T_m$  = tension at maximum extension length.

ences between individual linear regressions was determined by fitting the data to four separate discriminant models. These were intercept and slope different, different intercept but same slope, same intercept but different slope, and same intercept and slope. The resulting sum of squares, residuals, F ratio, and coefficient of determination ( $R^2$ ) were used to assess the validity of the models, and  $p$  values were determined from the ratio.

## Results

Treatment of platelets with ASA in vitro increased the deformability of the cells upon micropipette aspiration as seen in Figure 1 and Table 1. ASA increased the length of cell extensions into the micropipette with a doubling of initial ( $X_i$ ) and maximum ( $X_m$ ) extension lengths, and also significantly reduced the tension at which maximum extension was achieved. ASA treatment was not associated with any alteration of cell shape that could be visualized through the videomicroscope that was used during the micropipette aspirations. The effect of ASA on the mechanical properties of platelets was also observed after oral ingestion of the drug by healthy human volunteers. Platelets withdrawn by venipuncture 2 hours after ingestion of a 650 mg tablet of ASA manifested increased deformability to essentially the same degree as platelets treated in vitro (data not shown).

Unlike ASA, other inhibitors of platelet cyclooxygenase, ibuprofen and indomethacin,<sup>17</sup> did not increase platelet deformability. As seen in Figure 1 and Table 1, treatment of platelets with ibuprofen in vitro tended to increase resistance to aspiration. Although ibuprofen did not increase  $X_i$  or  $X_m$ , more force was required to reach maximum platelet extension after exposure to ibuprofen. Indomethacin, an irreversible inhibitor of cyclooxygenase, also had little effect on platelet micropipette aspiration.<sup>17</sup> Neither ibuprofen nor indomethacin was associated with any obvious change in platelet shape.

Since aspirin hydrolyzes to acetate and salicylic acid in plasma and in tissues, platelets were treated with equimolar concentrations of acetic anhydride or salicylic acid to determine which metabolite of ASA was responsible for the mechanical effect.<sup>18, 19</sup> As described in Figure 2 and Table 2, salicylic acid affected platelet deformability like ASA but the effect was not as pronounced. Salicylic acid significantly altered all of the deformability parameters in

the same manner as ASA, but the magnitude of the influence of salicylic acid on platelet deformability was less than the effect of ASA ( $p < 0.05$ ). Doubling the concentration of salicylic acid to 200  $\mu\text{M/L}$  did not alter platelet deformability more than the lower concentration of the drug. Contrary to ASA and salicylic acid, acetic anhydride tended to decrease platelet deformability as exemplified by a modest increase in  $T_m$ . Acetic anhydride did not significantly change  $X_i$ ,  $X_m$ , or slope. Incubation of platelets with benzoic acid, the basic ring structure of ASA, had little effect on platelet deformability (data not shown).

The result of treatment with salicylic acid for 30 minutes followed by exposure to acetic anhydride for another 30 minutes was similar to treatment with acetic anhydride followed by salicylic acid. These regimens altered platelet deformability more like treatment with salicylic acid rather than reproducing the effect of exposure to ASA (see Table 2). Unlike isolated salicylic acid or ASA treatment, the  $T_m$  of the dually exposed platelets was not decreased.  $T_m$  was unchanged if the platelets were treated with acetic anhydride first, but was somewhat increased after initial treatment with salicylic acid followed by acetic anhydride.

## Discussion

ASA caused platelets to become soft after exposure to the drug in vitro and after oral ingestion. The agent increased the length of cell extensions into the micropipette over the entire range of negative tensions, and lowered the tension required for maximum cell deformation. Previous platelet aspiration studies emphasized the importance of an unencumbered surface for extension into the pipette. The state of assembly of the circumferential microtubule and its location in the resting, compared to activated, platelets were found to be major factors determining the availability of platelet surface for deformation. ASA had no effect on cell shape or location of the circumferential microtubule and yet increased the length of cell extensions drawn into the pipette at all tensions.

ASA reduced the minimum tension required to reach maximum cell deformation. Previous manipulations that increased platelet extensibility and maximum cell deformation had not shown this effect. Neither exposure to cold nor treatment with agonists decreased the tension at which maximum cell deformation was first achieved.<sup>2, 5</sup> Although the previous manipulations may have increased platelet

extensibility primarily by increasing the availability of the surface for deformation, ASA profoundly affected platelet deformability through a decrease in cell elasticity.

Aspirin, a well-known inhibitor of platelet cyclooxygenase, blocks the conversion of arachidonic acid to thromboxane A<sub>2</sub>.<sup>6,9</sup> Hence, agonist-induced secretion of granule contents and irreversible aggregation are prevented without affecting platelet shape change.<sup>10,13</sup> Aspirin hydrolyzes to acetate and salicylic acid in plasma and tissues so that platelet cyclooxygenase is irreversibly inhibited by ASA-induced acetylation.<sup>18,19</sup> On the other hand, salicylic acid does not suppress cyclooxygenase activity.<sup>20,21</sup>

The increase in platelet deformability caused by ASA was not due to blockade of prostaglandin metabolism. Unlike ASA, other inhibitors of cyclooxygenase, such as ibuprofen and indomethacin, had little effect on platelet deformability. Ibuprofen tended to increase resistance to deformation as exemplified by an increase in the tension needed for maximum deformation. Indomethacin had no discernible effect.

The influence of ASA on platelet deformability was reproduced to some extent by the salicylic acid moiety of the compound. Salicylic acid significantly altered all of the deformability parameters in the same manner as ASA, although to a lesser degree. Doubling the concentration of salicylic acid did not increase platelet deformability more than the lower concentration of the drug. Acetylation of platelets with acetic anhydride had no influence on the initial or maximum cell extensions, and, contrary to ASA or salicylic acid, tended to increase the tension needed for maximum cell deformation. Benzoic acid, having a ring structure similar to that found in ASA and salicylic acid, had little influence on platelet deformability.

The effect of ASA did not appear to be due to a synergistic effect of acetylation and salicylic acid. Sequential incubation of platelets with acetic anhydride and salicylic acid did not increase platelet deformability more than exposure to salicylic acid alone. The potency of ASA in altering platelet deformability infers some specificity to the effect not shared by individual or combined treatment with more simple congeners of the drug.

The reduced resistance to deformation accompanying ASA treatment may counteract inhibitory actions of the drug on platelet reactivity. Increased deformability may mechanically augment platelet interaction with surfaces by facilitating the ease with which multiple point contacts are formed for firm attachment. Our group and others have reported augmented surface contact and spreading of ASA-treated platelets to vascular subendothelium under flow conditions compatible with the altered mechanical properties of the cell.<sup>22,23,24</sup> The softening effect of ASA on platelets may be another factor contributing to the variable efficacy of the drug in preventing thrombosis in clinical trials.<sup>25</sup>

## References

1. **White JG, Burris SM, Tukey DT, Smith CM, Clawson CC.** Micropipette aspiration of human platelets: Influence of microtubules and actin microtubules on deformability. *Blood* 1984;64:210-214
2. **Burris SM, Smith CM II, Tukey D, Clawson CC, White JG.** Micropipette aspiration of human platelets: Influence of rewarming on deformability of chilled cells. *J Lab Clin Med* 1986;107:238-243
3. **White JG.** Fine structural alterations induced in platelets by adenosine diphosphate. *Blood* 1968;31:604-622
4. **White JG.** Platelet morphology. In: Johnson SA, ed. *The circulating platelet*. New York: Academic Press, 1971:45-121
5. **Burris SM, Smith CM II, Tukey DT, Clawson CC, White JG.** Micropipette aspiration of human platelets after exposure to aggregating agents. *Arteriosclerosis* 1986;6:321-325
6. **Vane JR.** Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature* 1971;231:232-235
7. **Smith JB, Willis AL.** Aspirin selectively inhibits prostaglandin production in human platelets. *Nature* 1971;231:235-237
8. **Roth GJ, Stanford N, Majerus PW.** Acetylation of prostaglandin synthase by aspirin. *Proc Natl Acad Sci USA* 1975;72:3073-3076
9. **Burch JW, Stanford N, Majerus PW.** Inhibition of platelet prostaglandin synthetase by oral aspirin. *J Clin Invest* 1978;61:314-319
10. **Zucker MB, Peterson J.** Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet function by acetylsalicylic acid ingestion. *Proc Soc Exp Biol Med* 1968;127:547-551
11. **Weiss JH, Aldeort LM, Kochwa S.** The effect of salicylates on the hemostatic properties of platelets in man. *J Clin Invest* 1968;47:2169-2180
12. **O'Brien JR.** Effects of salicylates on human platelets. *Lancet* 1968;1:779
13. **Evans G, Packham MA, Nishizawa EE, Mustard JF, Murphy EA.** The effect of acetylsalicylic acid on platelet function. *J Exp Med* 1968;128:877-882
14. **White JG.** Fine structural alterations induced in platelets by adenosine diphosphate. *Blood* 1968;31:604-622
15. **Rao GHR, White JG.** Role of arachidonic acid metabolism in human platelet activation and irreversible aggregation. *Am J Hematol* 1985;19:339-347
16. **Weisberg S.** *Applied linear regression*. New York: John Wiley and Sons, 1980;150-162
17. **All M, McDonald JWD.** Reversible and irreversible inhibition of platelet cyclooxygenase and serotonin release by non-steroidal anti-inflammatory drugs. *Thromb Res* 1978;13:1057-1065
18. **Kelly CA.** Determination of the decompensation of aspirin. *J Pharm Sci* 1970;59:1053-1056
19. **Levy G.** Pharmacokinetics of salicylate in man. *Drug metabolism rev* 1979;9(1):3-19
20. **Vargaffig BB.** Salicylic acid fails to inhibit generation of thromboxane A<sub>2</sub> activity in platelets after in vivo administration to the rat. *J Pharm Pharmacol* 1978;30:101-109
21. **Rao GHR, Reddy KR, White JG.** Effect of acetaminophen and salicylate on aspirin-induced inhibition of human platelet cyclooxygenase. *Prostaglandins Leukotrienes Med* 1982;9:109-115
22. **Rao GHR, Escolar G, White JG.** Epinephrine reverses the inhibitory influence of aspirin on platelet-vessel wall interactions. *Thromb Res* 1986;44:65-74
23. **Weiss HJ, Tschopp TB, Baumgartner HR.** Impaired interaction (adhesion-aggregation) of platelets with the subendothelium in storage pool disease and after aspirin ingestion. A comparison with von Willebrand's disease. *N Engl J Med* 1975;293:619-623
24. **Escolar G, Bastida E, Ordinas A, Castillo R.** Interaction of platelets with subendothelium in humans treated with aspirin and dipyridamole alone or in combination. *Thromb Res* 1985;40:419-424
25. **Kelton JG.** Antiplatelet agents. Rationale and results. *Clin Haematol* 1983;12(1):311-354

Index Terms: platelet • deformability • aspirin



# Arteriosclerosis, Thrombosis, and Vascular Biology



JOURNAL OF THE AMERICAN HEART ASSOCIATION

## Aspirin treatment reduces platelet resistance to deformation.

S M Burris, C M Smith, 2nd, G H Rao and J G White

*Arterioscler Thromb Vasc Biol.* 1987;7:385-388

doi: 10.1161/01.ATV.7.4.385

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 1987 American Heart Association, Inc. All rights reserved.

Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://atvb.ahajournals.org/content/7/4/385>

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

**Reprints:** Information about reprints can be found online at:  
<http://www.lww.com/reprints>

**Subscriptions:** Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:  
<http://atvb.ahajournals.org/subscriptions/>