Iron-Dependent Human Platelet Activation and Hydroxyl Radical Formation

Involvement of Protein Kinase C

Domenico Praticò, MD; Mehtap Pasin, MD; Orla P. Barry, PhD; Andrea Ghiselli, MD; Giuseppe Sabatino, MD; Luigi Iuliano, MD; Garret A. FitzGerald, MD; Francesco Violi, MD

Background—Iron is an important modulator of lipid peroxidation, and its levels have been associated with the progression of atherosclerosis. Little is known about the possibility that this metal, when released from tissue stores, may modulate the reactivity of blood cell components, in particular platelets. Therefore, we investigated a possible link between iron, oxygen free radical formation, and platelet function.

Methods and Results—Human whole blood was stimulated with collagen 2 μg/mL, and an irreversible aggregation with thromboxane (Tx)B₂ formation was observed (15±4 versus 130±10 ng/mL). Deferoxamine (DSF), a specific iron chelator, and catalase, an H₂O₂ scavenger, inhibited collagen-induced whole-blood aggregation. The aggregation was accompanied by an increase in hydroxyl radical (OH⁻) levels (30±8 versus 205±20 nmol/L dihydroxybenzoates), which were reduced by DSF and by 2 specific OH⁻ scavengers, mannitol and deoxyribose. Iron (Fe²⁺) dose-dependently induced platelet aggregation, TxB₂ formation (6±2 versus 135±8 ng/mL), and protein kinase C (PKC) translocation from the cytosol to the cell membrane when added to platelets that have been primed with a low concentration of collagen (0.2 μg/mL). In the same system, an increase in OH⁻ levels was observed (37±12 versus 230±20 nmol/L dihydroxybenzoates). Mannitol and deoxyribose, but not urea, were able to reduce OH⁻ formation, PKC activation, and platelet aggregation. Selective inhibition of PKC activity by GF 109203X prevented iron-dependent platelet aggregation without influencing OH⁻ production.

Conclusions—The present study shows that iron can directly interact with human platelets, resulting in their activation. Its action is mediated by OH⁻ formation and involves PKC activity. Our findings provide an additional contribution to the understanding of the mechanism(s) by which iron overload might promote atherosclerosis and coronary artery disease.

Key Words: platelets • free radicals • thromboxane • protein kinase C
Methods

Materials
Collagen was purchased from Biodata Corp. GF 109203X (bisindolylmaleimide-1, H-T-[5-(isoquinolinesulfonyl)-2-methylpiperazine HCl]) and H-89 (N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinoline sulfonamide) were from Calbiochem Corp. [γ-32P]ATP was from Amersham Corp. The protein kinase C (PKC) pseudosubstrate peptide (RFARKSLRQKNV) was from Gibco BRL. [3H]thromboxane (TxB) was from Cayman Chemical Co. All other reagents not specified otherwise were of analytical grade and were obtained from Sigma Chemical Co.

Whole-Blood Aggregation Studies
Blood, anticoagulated with 3.8% sodium citrate, was obtained from healthy volunteers (4 men, 4 women; age, 25 to 40 years; nonsmokers) who had not taken any drug or vitamin supplements for the 4 weeks before the study. Informed consent was obtained from all the subjects. Platelet aggregation (impedance method) was studied with a Chronolog 540 whole-blood aggregometer (Chrono-Log), as previously described. Briefly, the aggregation was evaluated under continuous stirring at 1000 rpm, and the system was calibrated so that a 5-Ω change in impedance caused a 25-mm deflection of the pen. Platelet aggregation was measured as the maximum aggregation intensity (Ω), which is a measure of the highest impedance level (mm). Collagen 2 μg/mL was used as the threshold concentration that gave an irreversible aggregatory response (>15 Ω). Platelet aggregation was also measured using a threshold concentration of thrombin (1 U/mL) or ADP (2 μmol/L) as agonist. Aspirin was incubated 3 minutes before the stimulus; all the other inhibitors used were always added 60 seconds before the stimulus.

Washed-Platelet Aggregation Studies
Blood was centrifuged at 160g for 15 minutes to obtain platelet-rich plasma (PRP). Washed platelets (WPs) were harvested from PRP after centrifugation and resuspended in HEPES buffer, pH 7.4, as previously described. Platelet aggregation was measured photometrically at 37°C under continuous stirring.

Measurement of OH• Radical Formation
The production of OH• was determined by incubating samples with 5 mmol/L salicylic acid and measuring its hydroxylated byproducts 2,3- and 2,5-dihydroxybenzoate, as previously described. Briefly, at the end of the reaction, samples (500 mL) were placed into precylindrical 10-mL glass tubes containing 10 μL of 10 mmol/L 3,4-dihydroxybenzoate (internal standard) and 25 μL of 1.0N HCl. Samples were extracted twice into 5.0 mL of high-performance liquid chromatography (HPLC)–grade diethyl ether, and the organic layers were collected and evaporated to dryness under nitrogen. Samples were reconstituted with 1.0 mL of mobile phase just before HPLC analysis. Reverse-phase HPLC analysis was carried out on a Supelco LC-18, 5-mm (250×4.6-mm) analytical column (Supelco) with a Perkin-Elmer series 410 LC pump equipped with a Perkin-Elmer SEC-4 solvent environmental control.

Evaluation of PKC Activity
PKC activity was measured as previously described. Briefly, 3 minutes after the agonists had been added, platelets were lysed in ice-cold buffer containing 20 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA, 5 μg/mL soybean trypsin inhibitor, 1 μg/mL leupeptin, and 10 μg/mL aprotinin. Cytosolic and particulate fractions were separated by ultracentrifugation as described by Szallasi et al. The protein content was determined with a microbicinchoninic acid assay (Pierce) with BSA as the standard. Total PKC activity was measured in each fraction with a mixture of 50 mmol/L Tris-HCl (pH 7.4), 2 mmol/L EGTA, 50 μmol/L ATP, [γ-32P]ATP 0.15 μCi/tube, 15 mmol/L magnesium acetate, 10 μmol/L α-pseudosubstrate peptide (RFARKGSLRQKNV), 100 μg/mL phosphatidyl-l-serine, and 10 mmol/L PMA.

Biochemical Analyses
TxA2 production was measured as its stable metabolite TxB2 in the supernatant at the end of platelet aggregation curves by a gas chromatography/mass spectrometry assay as previously described. Free hemoglobin concentration was determined spectrophotometrically as previously described.

Statistical Analysis
All results are reported as mean±SD. Data were analyzed by ANOVA followed by Tukey’s test. Statistical significance was assumed for a value of P<0.05.

Results
Whole-Blood Platelet Aggregation and Fenton-Type Reaction
The threshold concentration of collagen (2 μg/mL) was used to achieve a full, irreversible aggregatory response (impedance, >15 Ω). Collagen-induced whole-blood aggregation was inhibited by the iron chelator deferoxamine (DSF) in a dose-dependent fashion (IC50, 4.5±1.1 mmol/L) (Figure 1A). Conversely, DSF did not affect either thrombin- or ADP-induced whole-blood platelet aggregation (data not shown). Concentrations of DSF up to 20 mmol/L were ineffective in preventing collagen-induced platelet aggregation in PRP or in...
WPs (data not shown), ruling out a possible direct inhibitory effect of this compound on platelets. A significant increase in free hemoglobin was observed in the supernatant of collagen-induced whole-blood aggregation (0.2 ± 0.03 versus 1.2 ± 0.04 μmol/L). Because DSF is a potent and specific iron chelator, it is likely that this metal plays an important role in this setting by catalyzing, in the presence of H₂O₂, a Fenton-type reaction (see equation). To investigate the role of H₂O₂, we used catalase, a specific H₂O₂ scavenger. Collagen-induced whole-blood aggregation was dose-dependently inhibited by catalase (IC₅₀, 1.7 ± 1.5 mg/mL) (Figure 1B). Furthermore, we used deoxyribose and mannitol, specific OH⁻ scavengers, and urea, which reacts poorly with this radical, as a negative control. We found a dose-dependent inhibition of the aggregatory response by both OH⁻ scavengers (deoxyribose, IC₅₀, 1.5 ± 0.1 mmol/L; mannitol, IC₅₀, 1.3 ± 0.12 mmol/L) (Figure 2A and 2B), whereas urea was without effect (data not shown). The requirement of both OH⁻ and H₂O₂ was further confirmed when DSF and OH⁻ scavengers were combined. In this setting, we observed a significant reduction in their IC₅₀ (40%), suggesting a synergistic effect of these inhibitors (data not shown). Because ADP has been involved in the amplification of platelet reactivity by erythrocytes, we investigated whether the addition of an enzyme system known to scavenge ADP could prevent collagen-induced whole-blood aggregation. Creatine phosphate/creatine phosphokinase (CP/CPK) at 20 mmol/L/50 U/mL, which is able to totally prevent in vitro platelet aggregation induced by up to 40 μmol/L ADP, reduced collagen-induced aggregation response only by 20±4% compared with control (P>0.05). Similar results were observed when apyrase (2 U/mL) was used (data not shown). Aspirin (100 μmol/L) significantly inhibited collagen-induced whole-blood aggregation (25±5 versus 4±2 W, P<0.005) and TxB₂ formation (130±10 versus 14±6 ng/mL, P<0.005). Because aspirin, a cyclooxygenase inhibitor, was able to prevent collagen-induced whole-blood aggregation, we decided to study the involvement of this metabolic pathway further. DSF as well as catalase dose-dependently inhibited platelet TxA₂ formation (IC₅₀, 7.4±1.1 mmol/L and 12.4±1.2 mg/mL, respectively) (Figure 3A and 3B). The OH⁻ scavengers mannitol and deoxyribose were also able to inhibit platelet TxA₂ production (data not shown). CP/CPK (20 mmol/L/50 U/mL) and apyrase (2 U/mL) reduced TxA₂ production by 18±5% and 20±3%, respectively.

To investigate whether collagen-induced whole-blood aggregation was actually associated with OH⁻ production, we
assessed its formation by measuring salicylate hydroxylation.

We observed that collagen-induced whole-blood aggregation had significantly higher values of OH$, expressed as the sum of the 2 main hydroxylated salicylate byproducts (2,3- and 2,5-dihydroxybenzoates). A 5- to 6-fold increase in OH$ levels compared with controls was measured (Table 1). A negligible amount of OH$ production was detected with thrombin or ADP used as agonist (data not shown). DSF dose-dependently inhibited the production of OH$ (IC$_{50}$, 4.4±1 mmol/L). Similar results were obtained when mannitol or deoxyribose, but not urea, was used (Table 1).

**Iron-Dependent Platelet Activation and OH$ Formation**

Next, we decided to study a system in which iron was added externally to human platelets. Iron (Fe$^{2+}$), in the form of ferrous chloride, alone did not evoke any platelet response measured as platelet aggregation and TxB$_{2}$ formation at concentrations up to 100 µmol/L (data not shown). When WPs were primed with a subthreshold concentration (STC) of collagen (0.2 µg/mL), which gave an aggregatory curve of <10%, and increasing concentrations of Fe$^{2+}$ were added, a dose-dependent increase in platelet aggregation (EC$_{50}$, 2.1±0.5 µmol/L) and TxB$_{2}$ formation was observed (Figure 4A). Fe$^{3+}$, in the form of ferric ammonium sulfate, was without effect either alone or when added to primed platelets (data not shown). As for whole blood, DSF, mannitol, deoxyribose, and aspirin were all able to significantly reduce Fe$^{2+}$-induced platelet activation (Table 2). Although iron at concentrations up to 100 µmol/L or STC collagen alone did

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Salicylate Hydroxylation, nmol/L Dihydroxybenzoates</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>30±8</td>
</tr>
<tr>
<td>Collagen, 2 µg/mL</td>
<td>205±20</td>
</tr>
<tr>
<td>Collagen+DSF, 1 mmol/L</td>
<td>148±11*</td>
</tr>
<tr>
<td>Collagen+DSF, 5 mmol/L</td>
<td>85±12†</td>
</tr>
<tr>
<td>Collagen+DSF, 10 mmol/L</td>
<td>43±8†</td>
</tr>
<tr>
<td>Collagen+mannitol, 5 mmol/L</td>
<td>53±8†</td>
</tr>
<tr>
<td>Collagen+deoxyribose, 5 mmol/L</td>
<td>46±10†</td>
</tr>
<tr>
<td>Collagen+urea, 5 mmol/L</td>
<td>200±16</td>
</tr>
</tbody>
</table>

DSF, mannitol, deoxyribose, and urea were incubated 60 seconds before adding the agonist. Results are expressed as mean±SD and represent the mean of 5 separate experiments.

*P<0.05; †P<0.001.

Figure 4. A, Aggregation tracings representing dose-dependent effects of iron (Fe$^{2+}$) on WPs primed with low concentration of collagen (CL) (0.2 µg/mL). B, Effects of PKC inhibitor GF 109203X 5 µmol/L (GF) and protein kinase A inhibitor H-89 5 µmol/L on platelet aggregation induced by Fe$^{2+}$ (10 µmol/L) added to platelets primed with collagen (0.2 µg/mL). Both inhibitors were incubated 60 seconds before stimulus was added. Traces are representative of 5 similar experiments.
not induce any \( \text{OH}^- \) formation (data not shown), a significant increase in \( \text{OH}^- \) level was detected when iron was added to platelets primed with an STC of collagen (Table 3). Mannitol, deoxyribose, and catalase caused a significant decrease in \( \text{OH}^- \) formation, confirming its role as second messenger in iron-induced platelet activation. In this setting, urea was without effect (Table 3).

**Iron-Dependent Platelet Activation and PKC Activation**

Because previous reports suggested that oxygen free radicals can activate PKC in different cell types,\textsuperscript{26,27} WPs were incubated with a specific inhibitor of this enzyme, GF 109203X, before STCs of collagen and Fe\textsuperscript{2+} were added. We observed a significant inhibition of platelet aggregation (Figure 4B), which suggested an involvement of PKC activity in \( \text{OH}^- \)-mediated platelet activation. Inhibition of PKC activity by GF 109203X (5 \( \mu \)mol/L) was also accompanied by a significant reduction in TxB\textsubscript{2} levels (data not shown). Similar results were obtained when H-7, another PKC inhibitor, was used (data not shown). Both compounds at concentrations up to 20 \( \mu \)mol/L were without effect on a full, irreversible platelet aggregation induced by collagen (data not shown). H-89, a specific inhibitor of protein kinase A, was without effect in the same system, which ruled out a role for this kinase in iron-induced platelet aggregation (Figure 4B). Interestingly, in the presence of GF 109203X, although platelet activation was significantly reduced, only a weak reduction in OH\textsuperscript{•} formation was observed (Table 3), which suggested that its formation is independent of PKC activity. To directly investigate the involvement of PKC in iron-dependent platelet aggregation, the activity of platelet total PKC was evaluated in vitro. When platelets were primed with STC collagen or iron alone, no change of PKC activity in the soluble (cytosol) or particulate fractions was observed. However, when iron was added to primed platelets, translocation of total PKC activity was observed, with a 50% loss in the soluble (cytosol) fraction and a concomitant increase in the particulate fraction (Figure 5), which supported a role for PKC in this setting. Mannitol prevented this phenomenon, confirming the role played by the \( \text{OH}^- \) in such activation. Urea, as negative control, was without effect (data not shown).

**Discussion**

Erythrocytes have long been known to play an important role in hemostasis and thrombosis.\textsuperscript{1–4} In vivo, it has been suggested that the risk of developing thrombosis may be increased in patients with erythrocytosis, and it was proposed that shear-induced release of ADP from RBCs could be important in promoting thrombus formation.\textsuperscript{9,28,29} Furthermore, a clinical study showed an increase in urinary excretion of 11-dehydro-TxB\textsubscript{2}, a marker of in vivo platelet activation, in patients with essential thrombocythemia.\textsuperscript{30} More recently, Weiss et al\textsuperscript{11} suggested that other RBC properties apart from ADP release may play a role in this cell-cell interaction. RBCs are carriers of hemoglobin, which contains iron; thus, it is possible that these compounds, when released, modulate the reactivity of surrounding blood cell components, in particular platelets. Interestingly, a lot of attention has been focused recently on iron body levels and the risk of cardiovascular disease.\textsuperscript{31} It has been hypothesized that such association reflects an interaction between iron, oxygen free radicals, and fatty acids that ultimately leads to lipid peroxidation and atherosclerosis.\textsuperscript{31,32} However, some studies have suggested an inverse correlation.\textsuperscript{17,18} Iron could also play a role by activating platelets,\textsuperscript{20,34} which are implicated in the development of such a disease.\textsuperscript{35} To explore this issue, we used an in vitro system that could reproduce a physiological environment in which RBCs and platelets are both present, i.e., whole blood. In the present study, we demonstrated that RBCs can modulate platelet reactivity in response to a common agonist, such as collagen, and that their action is mediated through the release of free iron, because DSF, a specific iron chelator, was able to prevent it. We showed that once released, this metal can catalyze a Fenton-like reaction, with a significant formation of \( \text{OH}^- \). This radical acted as a second messenger, because specific \( \text{OH}^- \) scavengers simul-

**TABLE 2. Platelet Activation Measured as Aggregation (\% Light Transmission) and TxB\textsubscript{2} Formation in WPs Primed With STC Collagen and Iron (Fe\textsuperscript{2+})**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Light Transmission, %</th>
<th>TxB\textsubscript{2}, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP + collagen, 0.2 ( \mu )g/mL</td>
<td>10±4</td>
<td>6±2</td>
</tr>
<tr>
<td>WP + Fe\textsuperscript{2+}, 10 ( \mu )mol/L</td>
<td>6±2</td>
<td>4±2</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+}</td>
<td>78±6</td>
<td>135±8</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + mannitol, 5 mmol/L</td>
<td>20±4*</td>
<td>15±3*</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + deoxyribose, 5 mmol/L</td>
<td>18±2*</td>
<td>13±2*</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + aspirin, 100 ( \mu )mol/L</td>
<td>15±3*</td>
<td>12±2*</td>
</tr>
</tbody>
</table>

Mannitol and deoxyribose were incubated 60 seconds before the agonist was added. Aspirin was incubated for 3 minutes before collagen was added. Results are expressed as mean±SD and represent the mean of 6 separate experiments.

\*P<0.001.

**TABLE 3. \text{OH}^- \) Formation in WPs Primed With STC Collagen Added With Iron (Fe\textsuperscript{2+}) (10 \( \mu \)mol/L), Measured as the Sum of 2,3- and 2,5-Dihydroxybenzoate Formed and Expressed as Salicylate Hydroxylation**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Salicylate Hydroxylation, nmol/L Dihydroxybenzoates</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP + collagen, 0.2 ( \mu )g/mL</td>
<td>60±10</td>
</tr>
<tr>
<td>WP + Fe\textsuperscript{2+}, 10 ( \mu )mol/L</td>
<td>37±12</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+}</td>
<td>230±20</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + DSF, 10 mmol/L</td>
<td>70±12*</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + mannitol, 5 mmol/L</td>
<td>97±10*</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + deoxyribose, 5 mmol/L</td>
<td>102±10*</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + urea, 5 mmol/L</td>
<td>210±22</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + catalase, 20 mg/mL</td>
<td>70±8*</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + GF, 5 ( \mu )mol/L</td>
<td>208±14</td>
</tr>
<tr>
<td>WP + collagen + Fe\textsuperscript{2+} + H-89, 5 ( \mu )mol/L</td>
<td>218±16</td>
</tr>
</tbody>
</table>

DSF, mannitol, deoxyribose, urea, catalase, GF 109203X (GF), and H-89 were always added 60 seconds before the agonists were added. Results are expressed as mean±SD and represent the mean of 5 separate experiments.

\*P<0.001.
taneously prevented its formation and the increase in platelet activation. We further confirmed these observations when iron was added to WPs. We postulated that Fe$^{2+}$ in the presence of H$_2$O$_2$ formed during the priming of platelets with collagen$^{36}$ would favor the formation of OH$^-$, which subsequently would activate platelets. We are aware that the availability of iron to stimulate OH$^-$ generation in vivo might be limited. Interestingly, the concentrations of bleomycin-detectable iron in normal human samples are generally $\approx$2 to 3 $\mu$mol/L, which is close to the amount of iron we found to activate primed platelets in our in vitro system.$^{37}$ Another important issue is the source of H$_2$O$_2$ in collagen-primed platelets. A possible origin for H$_2$O$_2$ is the dismutation of the superoxide anion, which could be formed during the priming process. If this is correct, the presence of superoxide dismutase would increase OH$^-$ generation. We have previously reported that primed platelets in the presence of superoxide dismutase produced discrete amounts of H$_2$O$_2$ that catalyzed platelet aggregation.$^{38}$ Furthermore, we identified the NADPH membrane enzyme system as a source of superoxide anion during the priming of platelets, because a specific inhibitor of this enzyme suppressed this phenomenon.$^{29}$

In the present study, we have accumulated evidence that further supports and extends this hypothesis. First, although iron by itself was unable to induce any platelet activation, when it was added to primed platelets, a full platelet aggregation occurred. Catalase, an H$_2$O$_2$ scavenger, and DSF, an iron chelator, both prevented such activation, confirming the pivotal role of H$_2$O$_2$ and metal in this activation. The requirement for both H$_2$O$_2$ and iron was confirmed by our observation of a synergistic inhibitory effect when DSF and the OH$^-$ scavengers were coincubated. Second, coincubation of primed platelets with Fe$^{2+}$ provokes an increase in OH$^-$ levels. Its functional role as second messenger in this cellular activation was demonstrated by use of specific scavengers for this radical. Mannitol and deoxyribose, but not urea, reduced OH$^-$ levels along with a reduction in iron-dependent platelet aggregation. Finally, we showed that OH$^-$ acts via a PKC-dependent mechanism, which as a final step leads to the activation of arachidonic acid metabolism. Two specific inhibitors of PKC, but not a protein kinase A inhibitor, significantly reduced platelet activation induced by OH$^-$.

We further confirmed the involvement of PKC by demonstrating translocation of total kinase activity from the platelet cytosol to the platelet membrane fraction, which was prevented by OH$^-$ scavengers. Taken together, these results indicate that in this setting, iron, promoting the formation of OH$^-$, specifically activates PKC. Our finding is in agreement with other reports that showed that oxygen free radicals are able to activate PKC in different cell lines.$^{26,27}$ Iron may be an important modulator of lipid peroxidation, and increased iron concentrations in plasma have been associated with progression of atherosclerosis.$^{15,31}$ a multifactorial process in which platelets and other cellular blood components have been involved.$^{34}$ In this study, we demonstrated that iron may also play a role in such a disease via a new mechanism: it modulates platelet function via a PKC mechanism. Although under normal conditions virtually all of the circulating iron is bound to proteins such as transferrin and ferritin, iron release from stores can occur from injured cells in conditions such as inflammation. It is known that the low pH or oxidant stress in a microenvironment of activated phagocyte cells can itself provide the iron necessary for Fenton chemistry by metabolizing iron from ferritin or by degrading heme proteins to release iron.$^{37}$ In this setting, iron may stimulate lipid peroxidation and platelet activation, 2 phenomena strictly interrelated in the pathogenesis of cardiovascular disease.$^{37,39}$ For instance, high levels of iron stores could increase the risk of myocardial infarction by favoring platelet activation in a setting of plaque fissuring or rupture. In conclusion, we suggest that whereas an increase in lipid peroxidation mediated by iron stores may accelerate the progression of atherosclerosis, a direct interaction between iron and platelets may be involved in the thrombotic complication of such a disease.

Acknowledgments
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References


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