Iron deposition in arterial wall macrophages is increased in atherosclerotic lesions,1,2 and increased iron stores have been suggested as a marker of cardiovascular risk.3 Several mechanisms have been proposed to explain the atherogenic role of iron through oxidative stress catalysis, including promotion of insulin resistance,3 decreased plasma antioxidant activity, and increased low-density lipoprotein (LDL) oxidation,5 direct endothelial toxicity,6 enhanced macrophage activation determining oxidized LDL uptake,7 and accelerated arterial thrombosis.8 Consistently with the previous findings, iron depletion has been shown to decrease atherogenesis in experimental models,3,9 blood donation was associated with decreased risk for myocardial infarction,10 and phlebotomy was suggested to decrease the progression of peripheral vascular disease.11 However, evidence of the atherogenic effect of iron is still controversial.12,13

Hepcidin has recently emerged as the key hormone regulating iron balance.14,15 Hepcidin, whose levels are increased by iron stores and inflammation, inhibits iron recycling from macrophages and absorption from enterocytes by binding the cellular iron exporter ferroportin, thus matching iron availability to body stores and providing a defense mechanism against extracellular pathogens during inflammation. Increased iron absorption resulting from decreased hepcidin release, related to common missense mutations of the hereditary hemochromatosis (HHC) gene (HFE) or other inherited defects, is the common pathogenic mechanism of HHC. Hepcidin may also be induced by subclinical inflammation and obesity,16 and can determine iron trapping into macrophages,17 increasing oxidative stress and transformation into foam cells in the presence of an atherogenic environment. Thus, it has been postulated that increased hepcidin may be responsible for iron-induced atherogenesis and may be involved in the mechanism linking inflammation to vascular disease.3 This theory would explain the controversial association between HFE mutations,18–21 or higher transferrin saturation,12,13 and susceptibility to vascular disease, which has so far been considered as a negative proof of the “iron hypothesis” of atherosclerosis.

Objective—Increased body iron stores and hepcidin have been hypothesized to promote atherosclerosis by inducing macrophage iron accumulation and release of cytokines, but direct demonstration in human cells is lacking. The aim of this study was to evaluate the effect of iron on cytokine release in monocytes ex vivo and the correlation with vascular damage and to evaluate the relationship among serum levels of hepcidin, cytokines, and vascular damage in patients with metabolic syndrome alterations.

Methods and Results—Manipulation of iron status with ferric ammonium citrate and hepcidin-25 induced monocyte chemoattractant protein (MCP)-1 and interleukin-6 in human differentiating monocytes of patients with hyperferritemia associated with the metabolic syndrome (n = 11), but not in subjects with hemochromatosis or HFE mutations impairing iron accumulation (n = 15), and the degree of induction correlated with the presence of carotid plaques, detected by echocolor-Doppler. In monocytes of healthy subjects (n = 7), iron and hepcidin increased the mRNA levels and release of MCP-1, but not of interleukin-6. In 130 patients with metabolic alterations, MCP-1 levels, as detected by ELISA, were correlated with hepcidin-25 measured by time-of-flight mass spectrometry (P = 0.005) and were an independent predictor of the presence of carotid plaques (P = 0.05).

Conclusion—Hepcidin and macrophage iron correlate with MCP-1 release and vascular damage in high-risk individuals with metabolic alterations. (Arterioscler Thromb Vasc Biol. 2011;31:683-690.)

Key Words: atherosclerosis ● cytokines ● free radicals/free-radical scavengers ● macrophages ● risk factors ● iron

Serum Hepcidin and Macrophage Iron Correlate With MCP-1 Release and Vascular Damage in Patients With Metabolic Syndrome Alterations

Luca Valenti, Paola Dongiovanni, Benedetta Maria Motta, Dorine W. Swinkels, Paola Bonara, Raffaella Rametta, Larry Burdick, Cecelia Frugoni, Anna Ludovica Fracanzani, Silvia Fargion
We recently showed that serum ferritin levels, reflecting iron stores, were an independent predictor of vascular damage in a population of patients with metabolic alterations defining the metabolic syndrome (MetS), but only in those negative for HFE genotypes determining decreased release of hepcidin.22,23 In addition, recent data confirmed that individuals carrying the major C282Y HFE mutation decreasing hepcidin may be at decreased risk for atherosclerosis, whereas vascular damage correlated with iron stores in patients predisposed to iron accumulation.24 Moreover, we reported an association between hepcidin and carotid vascular damage independently of other risk factors.22

However, although oxidative stress leading to increased cytokines release, and especially macrophage chemoattractant protein (MCP)-1, has been suggested to play a role,25 the mechanism linking increased macrophage iron content with accelerated atherogenesis are far from clear, and there is still no evidence of a modifying effect of HFE genotype on the atherogenic effect of iron in macrophages.

The aim of this study was to evaluate the following: (1) the effect of increased intracellular iron content induced by treatment with iron salts and/or hepcidin on the transcription and release of atherogenic cytokines in human differentiating macrophages derived from patients with hyperferritinemia; (2) the modifying effect of HFE genotype on cellular iron handling and cytokine release; (3) the correlation between iron induced cytokine release in vitro and vascular damage, and finally; and (4) the correlation between serum levels of hepcidin, proinflammatory mediators, and vascular damage in patients with metabolic alterations with and without hyperferritinemia.

Methods

Patients

Experiments in differentiating monocytes were performed on cells isolated from patients on maintenance phlebotomies for nonalcoholic fatty liver disease (NAFLD)/metabolic alterations associated with iron overload,26 related to the presence or not of HFE genotypes predisposing to iron overload, as defined below (n = 9 and 11, respectively), and from subjects with HHC (n = 6). All patients were iron-depleted and on maintenance phlebotomies at the time of evaluation (Table 1). Additional experiments were conducted in monocytes of male control subjects with HFE wild-type (wt)/wt, normal iron parameters, and absence of MetS (n = 7).

Vascular damage, iron status, HFE genotype, hepcidin-25, and MCP-1 levels were evaluated in 130 consecutive patients with NAFLD with or without hyperferritinemia attending the outpatient service of the Metabolic Liver Disease Center in Milan, for whom serum and DNA samples were available (Table 2).27

Table 1. Clinical Features of Twenty Patients With Nonalcoholic Fatty Liver and MetS Alterations and of Six With C282Y+/+ HHC, From Whom Peripheral Blood Monocytes Have Been Isolated

<table>
<thead>
<tr>
<th>HFE Status</th>
<th>Normal</th>
<th>Iron Overload</th>
<th>HHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>11</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Age, yr</td>
<td>50±9</td>
<td>48±12</td>
<td>49±11</td>
</tr>
<tr>
<td>Sex, F</td>
<td>2 (18)</td>
<td>2 (22)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Ferritin, ng/mL*</td>
<td>808±414 (median, 588)</td>
<td>529±310 (median, 546)</td>
<td>708±281 (median, 580)</td>
</tr>
<tr>
<td>Transferrin saturation, %*</td>
<td>37±11</td>
<td>36±16</td>
<td>87±10</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>9 (82)</td>
<td>7 (78)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Carotid plaques</td>
<td>7 (64)</td>
<td>2 (22)</td>
<td>1 (17)</td>
</tr>
</tbody>
</table>

*Parameters evaluated at diagnosis; at the time of monocyte collection, all patients were iron depleted and on maintenance venesections. Except where noted otherwise, values in parentheses are percentages.

Table 2. Clinical Features of One Hundred Thirty Patients With Nonalcoholic Fatty Liver Disease and MetS Abnormalities With Complete Characterization of Vascular Damage, Iron Status, Serum Hepcidin-25, and MCP-1 Levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>54.4±12</td>
<td>28–77</td>
</tr>
<tr>
<td>Sex, F</td>
<td>23 (18)</td>
<td>...</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.7±3.5</td>
<td>19.2–39</td>
</tr>
<tr>
<td>Smoke, current or previous</td>
<td>28 (21)</td>
<td>...</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.17±0.9</td>
<td>3.46–10.9</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>4.2±3.3</td>
<td>3.8–32.7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>32 (24.2)</td>
<td>...</td>
</tr>
<tr>
<td>Treated hypertension</td>
<td>53 (41)</td>
<td>...</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>3.21±0.8</td>
<td>0.72–5.33</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.31±0.4</td>
<td>0.54–2.46</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.44±0.7</td>
<td>0.46–5.1</td>
</tr>
<tr>
<td>ALT, IU/mL</td>
<td>38.1±24</td>
<td>10–163</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>34.0±11</td>
<td>7–61</td>
</tr>
<tr>
<td>Ferritin, ng/mL</td>
<td>399 [175–640]</td>
<td>10–1366</td>
</tr>
<tr>
<td>Hyperferritinemia, &gt;320 mol/L/240 F</td>
<td>188 (37.1)</td>
<td>...</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages; values in brackets are interquartile range.
basis of a previous study in this series of patients.\textsuperscript{20,30} Patients homozygous for the C282Y \textit{HFE} mutation, ie, affected by classic HHC, were used as positive controls of altered macrophage iron handling.\textsuperscript{31}

Hepcidin quantification was performed by time-of-flight mass spectrometry as described recently\textsuperscript{32} and in serum samples stored at $-80^\circ$C before evaluation.\textsuperscript{22} MCP-1 quantification was performed in serum samples and cell supernatants by the CCL2/MCP-1 ELISA kit (R&D Systems, Minneapolis, MN).

\section*{Isolation of Monocytes and Gene Expression Analysis}

Mononuclear cells were separated using the Lymphoprep method from peripheral blood\textsuperscript{33} and treated as described previously.\textsuperscript{34} Twenty-four hours after plating, the supernatant was removed leaving differentiating macrophages attached, and new supplemented medium was added in the presence or not of 150 \(\mu\)mol/L ferric ammonium citrate (FAC), hepcidin-25 0.5 or 3 \(\mu\)mol/L (H-5926; Bachem, Weil am Rhein, Germany), \(\mathrm{H}_2\mathrm{O}_2\) 10 \(\mu\)mol/L, unless otherwise specified. Cell purity, as confirmed by flow cytometry (with anti-CD14 antibodies), was $>95\%$ in each case. After further 24 hours cells were harvested and total RNA extracted by the phenol-chloroform method. First-strand cDNA was synthesized with equal amounts (1 \(\mu\)g) of total RNA by the Superscript VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA) with random hexamers. Quantitative real-time PCR analysis of transferrin receptor (TfR)-1, whose expression is decreased with increasing intracellular iron availability,\textsuperscript{35} heme oxygenase-1, induced by oxidative stress and involved in neutralizing iron induced oxidative stress,\textsuperscript{36} MCP-1, interleukin (IL)-6, tumor necrosis factor (TNF)\(\alpha\), tissue factor, and CD36 mRNA levels, was performed by using the SYBR green chemistry (Fluocycle, Euroclone, Siziano, Italy). Custom-designed primers and reaction protocols are available on request. All the reactions were performed in triplicate by the Opticon 2 analyzer (Bio-Rad, Hercules, CA) in a 25-\(\mu\)L final volume. Results were normalized for 18S and \(\beta\)-actin mRNA levels.

\section*{Evaluation of Basal Activation Status of Monocytes}

Basal activation status of monocytes was estimated based on the percentage of CD69 positivity of CD14\textsuperscript{+} cells at cytofluorimetric analysis.\textsuperscript{37} The percentage of monocytes positive for CD69 or CD14 was quantified within 2 hours on a FACSscan flow cytometer (Becton Dickinson, San Jose, CA). Reactions were performed in triplicate by the Opticon 2 analyzer (Bio-Rad, Hercules, CA) in a 25-\(\mu\)L final volume. Results were normalized for 18S and \(\beta\)-actin mRNA levels.

\section*{Evaluation of Vascular Damage}

Vascular damage was determined by measurement of the common carotid arteries intima media thickness (CC-IMT) and plaque detection by echocolor–Doppler ultrasonography, as described previously.\textsuperscript{27}

\section*{Statistical Analysis}

Data are shown as means±SD for normally distributed variables, mean and interquartile range for nonnormally distributed variables (log-transformed before analysis), and frequencies for categorical variables. Data were compared by \(\chi^2\) and Wilcoxon tests, as required, and variables were correlated by Spearman’s rho test. Gene expression levels were compared by Wilcoxon signed-rank test for paired samples and by Wilcoxon test for unpaired samples. Logistic regression analysis was performed to adjust the association between MCP-1 and the presence of plaques for confounding variables. Values of \(P<0.05\) were considered statistically significant. All analyses were performed with the JMP 6.0 statistical analysis software (SAS institute, Cary, NJ).

\section*{Results}

\subsection*{Risk-Associated HFE Genotypes Influence Iron Handling, But Not Oxidative Stress, in Differentiating Human Monocytes of Patients With Hyperferriтинemia}

We first confirmed that treatment with iron salts increased intracellular iron status in monocytes of patients with “normal” \textit{HFE} genotype and evaluated whether \textit{HFE} genotypes predisposing to iron overload were associated with altered iron metabolism in macrophages, by comparing the expression of TIR-1 mRNA, a sensitive marker of cellular iron status, in differentiating monocytes isolated from patients with hyperferriтинemia related or not to the presence of \textit{HFE} genotypes associated with risk for disease. TIR-1 mRNA transcripts are stabilized during iron deficiency by direct binding of iron regulated proteins to the 3’ untranslated regions.\textsuperscript{35} Results are shown in Figure 1A. Treatment with FAC determined a significant increase in intracellular iron availability, as detected by a significantly higher decrease in TIR-1 mRNA levels, in monocytes of patients with normal \textit{HFE} genotypes than in those of patients carrying \textit{HFE} genotypes that are associated with risk for iron overload \((P=0.01)\), whereas TIR-1 levels were not significantly downregulated in monocytes of patients with HHC. In contrast, FAC induced a similar upregulation of heme oxygenase-1 mRNA levels irrespective of the \textit{HFE} genotype (Figure 1B). These data suggest that monocytes of patients with \textit{HFE} genotypes associated with risk for iron overload had a partial defect of iron retention reminiscent of that observed in classic HHC,\textsuperscript{38} but not reduced oxidative stress.

\subsection*{Effect of Iron Treatment on the Expression and Release of Atherogenic Factors in Human Differentiating Monocytes is Influenced by HFE Genotype}

We next evaluated whether manipulation of iron status modified the release of atherogenic mediators in monocytes of patients with hyperferri틴emia subdivided according to the \textit{HFE} genotype (Table 1). Treatment with FAC did not significantly affect either TNF\(\alpha\), tissue factor, or CD36 mRNA levels in monocytes of patients with normal \textit{HFE} genotypes than in those of patients carrying \textit{HFE} genotypes that are associated with risk for iron overload \((P=0.01)\), whereas TIR-1 levels were not significantly downregulated in monocytes of patients with HHC. In contrast, FAC induced a similar upregulation of heme oxygenase-1 mRNA levels irrespective of the \textit{HFE} genotype (Figure 1B). These data suggest that monocytes of patients with \textit{HFE} genotypes associated with risk for iron overload had a partial defect of iron retention reminiscent of that observed in classic HHC,\textsuperscript{38} but not reduced oxidative stress.
does not influence basal activation status but is associated with defective iron retention and a lack of proinflammatory response to iron challenge in human monocytes.

**Inflammatory Response to Iron Challenge in Monocytes Correlates With Vascular Damage in Patients With Hyperferritinemia**

To evaluate the relationship between iron-dependent induction of cytokines and vascular damage, we analyzed whether inducibility of MCP-1 and IL-6 in monocytes of patients with hyperferritinemia (Table 1) was associated with the presence of carotid plaques, an index of advanced vascular disease. Results are shown in Figure 1D. Despite basal expression of cytokines not being significantly different in untreated monocytes (data not shown), both MCP-1 and IL-6 mRNA levels were significantly more upregulated after iron challenge in patients with carotid plaques than in those without carotid plaques.

**Effect of Iron and Hepcidin-25 Treatment on Cytokines in Monocytes Derived From Healthy Subjects With Normal Iron Metabolism**

To assess whether MCP-1 and IL-6 induction after iron challenge represented a physiological response to iron overload related to increased iron availability and hepcidin upregulation in monocytes, we next evaluated the dose–response relationship between FAC and hepcidin-25 treatment and MCP-1 and IL-6 mRNA levels in monocytes of 7 healthy male subjects with normal iron metabolism and absence of metabolic alterations. Data are presented in Figure 2A. Surprisingly, neither FAC nor hepcidin-25 induced a significant upregulation of IL-6 mRNA levels in monocytes of most of the healthy subjects (n=6/7, 86%), whereas increasing concentrations of both FAC and hepcidin-25 induced a progressive increase in MCP-1 mRNA levels. However, we observed a very strong upregulation of IL-6 in 1 healthy control. The absence of MCP-1 upregulation after H2O2 treatment indicates that the effect of iron is not attributable to an unspecific induction by oxidative stress but is likely a specific response to intracellular iron overload.

Next, we confirmed that increased MCP-1 transcription induced by iron and/or hepcidin-25 treatment translated into increased release of this chemokine in differentiating monocytes, by measuring MCP-1 protein levels in cell supernatants by ELISA (Figure 2B). Data indicate that the maximum MCP-1 induction by FAC was already observed at 24 hours.

**Serum Hepcidin-25, MCP-1, and Vascular Damage in Patients With Metabolic Alterations**

We finally evaluated the relationship among iron status, hepcidin-25, and MCP-1 serum levels and vascular damage in 130 patients with NAFLD and MetS alterations. Heparin-25 levels were not correlated with available demographic and anthropometric features, the presence of diabetes, dyslipidemia, hypertension, central obesity, the metabolic syndrome, and with indices of inflammation or of altered

---

**Figure 1.** Effect of ferric ammonium citrate (FAC 150 μmol/L) treatment on TIR-1 mRNA levels (A), heme oxygenase-1 (HO-1) mRNA levels (B), and mRNA levels (C) of MCP-1 and IL-6 in monocytes of subjects with iron overload subdivided according to HFE genotype: “normal,” “hepatic iron overload,” and hereditary hemochromatosis (HHC). *P<0.05 vs untreated cells; °P<0.05 vs normal HFE. D, Association between the induction of MCP-1 and IL-6 mRNA after iron challenge in monocytes of subjects with iron overload and the presence of carotid plaques. *P<0.05 vs absence of plaques.
metabolism, but, as previously reported,22 with serum ferritin, an index of body iron stores (Rho = 0.43; P < 0.0001), and with serum MCP-1 levels (Rho = 0.24; P = 0.005; Figure 3A).

In contrast, MCP-1 levels were not significantly correlated with the other available demographic and anthropometric features, and with indices of dysmetabolism and inflammation, including CRP levels (rho = 0.03, P = 0.78). Interestingly, hepcidin levels were significantly correlated with MCP-1 in patients without (n = 98, rho = 0.12, P = 0.05), but not in those with diabetes (n = 32, rho = 0.12, P = 0.05), suggesting that in the presence of diabetes, hyperglycemia and its metabolic consequences plays the major role in determining serum MCP-1 levels. However, serum MCP-1 levels were significantly higher in patients with (n = 44) than in those without (n = 86) carotid plaques (121 pg/mL [interquartile range, 78 to 193 pg/mL] versus 85 pg/mL [interquartile range, 60 to 148 pg/mL]; P = 0.03; Figure 3B).

Patients with CRP levels above median value (≥0.1 mg/dL) had higher ferritin (542 ± 250 ng/mL versus 366 ± 260; P = 0.02) and a trend for higher hepcidin-25 (3.5 ± 3.2 versus 2.5 ± 1.7 nmol/L; P = 0.08) and IMT levels (0.92 ± 0.2 versus 0.80 ± 0.2; P = 0.08) but not higher MCP-1 levels (117 ± 80 versus 113 ± 60 pg/mL; P = 0.7) or prevalence of plaques (59 versus 63%; P = 0.7). These data suggest that in this series of patients the association between iron indices and MCP-1 levels, and the association between MCP-1 and vascular damage, is independent of subclinical inflammation.

At multivariate analysis (Table 3), presence of carotid plaques was significantly associated with ferritin (P = 0.013) and MCP-1 (P = 0.05) serum levels, independently of classic risk factors for atherosclerosis and of the presence of HFE mutations.

**Discussion**

In this study, we evaluated the effect of the manipulation of intracellular iron status by treatment with iron salts or the hormone hepcidin on the release of atherogenic cytokines in human differentiating monocytes of patients with mild iron overload at high risk for cardiovascular disease.22,27 The study was prompted by accumulating evidence indicating that
iron overload may represent a risk factor for cardiovascular diseases and that the mechanism may be mediated by iron accumulation in arterial plaques macrophages, with consequent induction of inflammation, release of proinflammatory mediators, and transformation into foam cells. Indeed, infiltration of the arterial wall by activated macrophages that phagocytose oxidative stress–modified lipoproteins and secretion of proinflammatory, fibrogenic, and extracellular matrix remodeling mediators play a pivotal role in the pathogenesis of atherosclerotic lesions.41

Our results indicate that treatment with iron salts determining increased intracellular iron increased the release of MCP-1 and IL-6 independently of oxidative stress. Furthermore, the iron-dependent induction of MCP-1 and IL-6 was associated with the severity of vascular damage in these patients, suggesting that macrophage activation by iron may be involved in the pathogenesis of vascular damage progression in these patients also in vivo.

IL-6 is a proinflammatory cytokine involved in the acute phase response, whose serum levels correlate with cardiovascular risk and have been linked to the inflammatory status within atherosclerotic plaques.42 MCP-1 (also known as CCL2), a chemokine involved in macrophage recruitment at inflammation sites released by macrophages, but also by smooth muscle cells and endothelial cells, plays a crucial role in both the initiation and progression of atherosclerosis, and MCP-1 serum levels reflect the atherosclerotic plaque burden. Higher MCP-1 has also been reported to represent a negative prognostic factor in acute coronary syndromes.43–45

A dose-dependent induction of MCP-1 transcription and release in the supernatant induced by iron treatment was confirmed in monocytes of healthy subjects with normal iron parameters, suggesting that this represents a physiological response to increased intracellular iron availability. These data are in line with previous experimental evidence, suggesting that iron may induce MCP-1 release by oxidative stress in macrophages. Indeed, intravenous iron treatment induces MCP-1 release by monocytes in mice and patients with end-stage renal disease,46,47 whereas the iron chelator deferoxamine has recently been shown to decrease nuclear factor κB activation, MCP-1 release, macrophage infiltration in vascular tissue, and atherosclerosis progression in ApoE−/− mice.25 In contrast, possibly because of different cellular iron handling caused by the absence of additional acquired and/or genetic factors, we could not confirm an induction of IL-6 by iron treatment in monocytes of most of the healthy subjects.

Importantly, we also showed that treatment with hepcidin, which acts by blocking cellular iron export,35 mimicked the effect of iron salts on MCP-1 release, as also reported in preliminary form by others.48 In addition, we obtained evidence that,38 monocytes of patients with HFE genotypes predisposing to mild iron overload show reduced ability to accumulate intracellular iron. Iron-induced upregulation of MCP-1 and IL-6 was hampered in monocytes of patients carrying these disease risk HFE genotypes, thus suggesting that the lack of upregulation of proinflammatory mediators may contribute to explain the lack of association between iron overload and accelerated atherosclerosis in HHC patients and explain the contradictory data on the effect of HFE mutations on vascular damage.3,22 Consistently, despite the fact that iron overload has been associated with increased serum MCP-1 levels, patients homozygous for the C282Y HFE mutation had lower MCP-1 levels than those whose iron overload was explained by other genetic/acquired factors and healthy controls.49 Thus, iron-dependent release of MCP-1 by macrophages may be a mechanism involved in the pathogenesis of accelerated atherosclerosis associated with iron overload and
is consistent with the apparently paradoxical protective effect of HFE mutations.

To assess whether this mechanism may be relevant in vivo, we measured serum MCP-1 levels in a series of patients at high risk for vascular disease because of MetS and fatty liver, who typically display a high prevalence of altered iron metabolism, with complete characterization of carotid atherosclerosis and iron metabolism, including serum hepcidin-25 evaluation, and HFE genotype determination. In line with a possible induction of MCP-1 release by increased iron in monocytes/macrophages, serum MCP-1 levels, whose primary source is represented by activated macrophages, were significantly correlated with hepcidin-25, and MCP-1 levels were an independent predictor of the presence of carotid plaques, indicating an advanced atherosclerotic process.

Thus, the emerging details of the physiology of hepcidin suggest a resolution of the apparent paradox of an important role of iron in atherogenesis in the absence of increased plaque burden in HHC. Hepcidin, induced by iron and inflammation, acts to block iron recycling from macrophages by binding and causing internalization and degradation of ferroportin, the sole cellular iron exporter. Low hepcidin levels are observed in iron deficiency anemia and HHC, both characterized by reduced macrophage iron stores. The failure of vascular wall macrophages to retain iron in HHC may therefore prevent the progression of atherosclerotic plaques.

In conclusion: (1) increased intracellular iron in human differentiating monocytes, induced either by treatment with iron salts or hepclin-25, induces MCP-1 transcription and release; (2) the ability of iron treatment to induce MCP-1 is correlated with the severity of vascular damage; and (3) serum MCP-1 is associated with hepclin-25 and vascular damage in high-risk individuals with metabolic syndrome alterations. These data support the hypothesis that MCP-1 release by intracellular monocytes/macrophages, which can be iron-loaded because of intraplaque hemorrhage, systemic iron overload, and local inflammation, is involved in the pathogenesis of iron-induced atherosclerosis.

Acknowledgments
We thank Dario Tavazzi, and Erwin Wiegierink for technical assistance; Harold Tjalma for coordinating the hepcidin measurements; and Cristina Bertelli and Daniela Bignamini for clinical assistance.

Sources of Funding
This work was supported by FIRST/PUR University of Milano 2006–2008; Ricerca Corrente 2006, 2007; and Progetto a Concorso 2009–2010 Fondazione Ospedale Policlinico MaRe IRCCS.

Disclosures
D.W.S. is the medical director of the Hepcidinanalysis team (http://hepcidinanalysis.com).

References


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Arterioscler Thromb Vasc Biol. 2011;31:683-690; originally published online December 23, 2010;

doi: 10.1161/ATVBAHA.110.214858

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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