Causes of anemia in the uremic milieu

- Reduced **production** of erythropoietin
- **Shortened erythrocyte lifespan** caused by uremic toxins and oxidative stress
- **Impaired iron availability** due to inflammation-driven production of hepcidin, which inhibits intestinal iron absorption
- **Occult blood loss** due to uremic platelet dysfunction

Hemodialysis patients lose 7-15 ml (3-10 mg iron) of blood per day due to blood trapped in dialysis machine, sampling for lab tests, bleeding etc.
Ferric hydroxide core encased in a carbohydrate shell designed to act like ferritin and prevent rapid release of its iron content.

Crystal structure of ferritin (storage protein with antioxidative properties)

Carbohydrate shell
**Fenton reaction:**
\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \cdot\text{OH} + \text{OH}^- + \text{Fe}^{3+} \]

The hydroxyl radical is the most reactive and cytotoxic free radical known.

**Haber Weiss reaction:**
\[ \text{Fe}^{3+} + \text{O}_2^- \rightarrow \text{Fe}^{2+} + \text{O}_2 \]

These two reactions supply the fuel for continuous iron-catalyzed hydroxyl radical production and perpetuation of oxidative stress. *Does this occur in vivo?*
**Simple Visual Qualitative Assessment of Amount of Labile Iron in i.v. Iron Preparations**

**Reaction with tea (polyphenols):**

<table>
<thead>
<tr>
<th>SFG</th>
<th>IS</th>
<th>LMWID</th>
<th>FCM</th>
<th>FMX</th>
</tr>
</thead>
</table>

**Review**

*Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases*

Douglas B Kell*

**SFG**: Sodium ferric gluconate in sucrose; **IS**: Iron sucrose

**LMWID**: Low molecular weight iron dextran; **FCM**: Ferric carboxymaltose

**FMX**: Ferumoxytol; **IIM**: Iron isomaltoside 1000

Intravenous iron exerts some effects on markers of oxidative stress that are of unclear clinical significance.

<table>
<thead>
<tr>
<th>Table 3. Summary effect of intravenous iron therapy on anemia parameters and markers of oxidative stress and inflammation in RCT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome variables</strong></td>
</tr>
<tr>
<td>Anemia parameters</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
</tr>
<tr>
<td>Serum ferritin, mg/dl</td>
</tr>
<tr>
<td>Serum iron, μmol/l</td>
</tr>
<tr>
<td>TIBC, μg/dl</td>
</tr>
<tr>
<td>Transferrin, %</td>
</tr>
<tr>
<td>TSAT, %</td>
</tr>
<tr>
<td>Reticulocyte hemoglobin content, pg</td>
</tr>
<tr>
<td>Erythropoietin dose, units/week</td>
</tr>
<tr>
<td>Markers of oxidative stress</td>
</tr>
<tr>
<td>Plasma TBARS, μmol/l</td>
</tr>
<tr>
<td>Plasma MDA, μmol/l</td>
</tr>
<tr>
<td>Neutrophil respiratory burst, RLU</td>
</tr>
<tr>
<td>Markers of inflammation</td>
</tr>
<tr>
<td>β2-Microglobulin, mg/dl</td>
</tr>
</tbody>
</table>

Data in parentheses denote 95% confidence limits. RLU = Relative light units.

* By random-effects model meta-analysis. b An I² index ≥75% indicates medium-to-high heterogeneity.
In 20 healthy volunteers the effect of 100 mg ferric saccharate infusion was investigated.
Intravenous iron sucrose provokes oxidative damage to peripheral blood lymphocyte DNA in HD patients, especially among those with high ferritin levels.
Deleterious Effects of i.v. Iron on Mononuclear Cells

Iron Sucrose Impairs Phagocytic Function and Promotes Apoptosis in Polymorphonuclear Leukocytes

Hirohito Ichii Yuichi Masuda Tania Hassanzadeh Mateen Saffarian Sastry Gollapudi Nosratola D. Vaziri

Intravenous Iron Treatment Promotes Monocyte Cytokine Formation ex vivo

24 HD patients assigned to i.v. iron sucrose or saline

Intravenously administered iron is taken up by monocytes and activate the NF-κB pathway.
Multiple pathways stimulate NF-κB in the uremic milieu

- Reactive oxygen species
  - NOX
- Glutathione
  - SOD
- Nrf2
  - Keap1
  - Nrf2
- Upregulation of about 250 cytoprotective and antioxidant genes

Glomerular endothelium

Mesangial cells

Podocytes

Vascular endothelium

Proximal tubule cells

Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease

Stacey Ruiz1, Pablo E. Pergola2, Richard A. Zager3,4 and Nosratola D. Vaziri5

1 Reata Pharmaceuticals, Irving, Texas, USA; 2 Renal Associates, PA, San Antonio, Texas, USA; 3 Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; 4 Department of Medicine, University of Washington, Seattle, Washington, USA and 5 Department of Medicine, University of California, Irvine, Irvine, California, USA

Kidney Int 2013

Nrf2 activation is suppressed in animal models of CKD
Fe administration leads to transient liver oxidative stress development and transient Nrf2 activation.

**Question:** What happens when iron is injected into the inflamed, pro-oxidative and Nrf2 exhausted uremic milieu?
Does iron-mediated oxidative stress translate into increased risk for atherosclerotic lesions?

- **Iron sucrose** administration was associated with higher maximum serum non-transferrin bound iron concentrations compared to iron dextrane.
- Both compounds produced similar ROS generation and cytokine activation that was more pronounced among ESRD patients.

*Fig. 3* Intracellular ROS generation after IV Iron in ESRD patients
Iron in arterial plaque: A modifiable risk factor for atherosclerosis

Jerome L. Sullivan

Burnett School of Biomedical Sciences, University of Central Florida College of Medicine, Orlando, Florida, USA

- Ferrous iron leads to the generation of the hydroxyl radical, known to damage membrane lipids, oxidize low-density lipoprotein, and promote atherogenesis.

- It is believed that most intravenous iron formulations release bioactive iron, especially if given rapidly enough to oversaturate receptors.

- Under the influence of the increased concentrations of hepcidin, iron is primarily sequestered in macrophages and that iron-laden macrophages within the plaque promote atherosclerosis.
Hypothesis: Atherosclerosis may be increased by the usually recommended doses of intravenous iron.
The detrimental effects of i.v. iron were partly due to increased oxidative stress and induction of mononuclear adhesion to endothelial cells.
“The inconsistent effects of iron in atherosclerotic mouse models do not support the hypothesis that iron is an important aggravating factor in the pathogenesis of atherosclerosis.”
A retrospective cohort was created from the clinical database of a large dialysis provider merged with data from the USRDS. 117,050 patients contributed 776,203 unique iron exposure periods. There were no consistent associations of either high or bolus dose vs. low or maintenance respectively among pre-specified subgroups.

Strategies favoring large doses of intravenous iron were not associated with increased short-term cardiovascular morbidity and mortality. Investigation of the long-term safety of the various intravenous iron supplementation strategies may still be warranted.
Emerging Links Between Iron and Vascular Calcification and Bone Mineral Metabolism

The most common adverse event associated with ferric carboxymaltose was asymptomatic hypophosphatemia.

### TABLE 3. Serum phosphate, calcium, and potassium at baseline, at the lowest value observed (nadir), and at the final study examination in patients after treatment with IV ferric carboxymaltose or oral ferrous sulfate*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline mean</th>
<th>Change from baseline</th>
<th>p Value†</th>
<th>p Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>To nadir</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV ferric carboxymaltose</td>
<td>3.7 ± 0.5</td>
<td>−1.9 ± 0.6</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oral ferrous sulfate</td>
<td>3.7 ± 0.5</td>
<td>−0.3 ± 0.5</td>
<td>&lt;0.001</td>
<td>0.1 ± 0.6</td>
</tr>
<tr>
<td>p Value‡</td>
<td>0.795</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mEq/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV ferric carboxymaltose</td>
<td>9.2 ± 0.4</td>
<td>−0.5 ± 0.4</td>
<td>&lt;0.001</td>
<td>0.0 ± 0.4</td>
</tr>
<tr>
<td>Oral ferrous sulfate</td>
<td>9.2 ± 0.4</td>
<td>−0.2 ± 0.3</td>
<td>&lt;0.001</td>
<td>0.1 ± 0.4</td>
</tr>
<tr>
<td>p Value‡</td>
<td>0.448</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV ferric carboxymaltose</td>
<td>4.2 ± 0.4</td>
<td>−0.4 ± 0.3</td>
<td>&lt;0.001</td>
<td>−0.2 ± 0.4</td>
</tr>
<tr>
<td>Oral ferrous sulfate</td>
<td>4.2 ± 0.3</td>
<td>−0.3 ± 0.3</td>
<td>&lt;0.001</td>
<td>−0.1 ± 0.4†</td>
</tr>
<tr>
<td>p Value‡</td>
<td>0.949</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Results are given as mean ± SD for the safety population.
† Within-group comparison.
‡ Between-group comparison.
Iron deficiency stimulates FGF23 transcription in osteocytes

- A negative relationship between iron administration and serum iFGF23 level in a dialysis population.
- If high levels of FGF23 are harmful, iron therapy may have a beneficial effect on bone metabolism by reducing FGF23 levels in a dialysis population.
Effect of ferric carboxymaltose on serum phosphate and C-terminal FGF23 levels in non-dialysis chronic kidney disease patients: post-hoc analysis of a prospective study

- Ferric carboxymaltose induces reduction in serum phosphate levels that persists for three months.
- Ferric carboxymaltose causes a significant decrease in C-terminal FGF23 levels

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>28.9(10)</th>
<th>25.6(12.7)</th>
<th>&lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium, mg/dl</td>
<td>67.8(61.7)</td>
<td>502.5(263.3)</td>
<td>230(144.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phosphate, mg/dL</td>
<td>9.3(0.4)</td>
<td>9.3(0.5)</td>
<td>9.3(0.5)</td>
<td>ns</td>
</tr>
<tr>
<td>PTH, pg/ml</td>
<td>138.1(2.5-600)</td>
<td>124(2.5-736)</td>
<td>106.5(2.5-613)</td>
<td>ns</td>
</tr>
<tr>
<td>1,25(OH)_2 D, pg/mL</td>
<td>9.7(4.4)</td>
<td>10(3.7)</td>
<td>10.4(5.4)</td>
<td>ns</td>
</tr>
<tr>
<td>FGF23, RU/mL</td>
<td>442(44.9-4079.2)</td>
<td>340(68.5-2603.3)</td>
<td>191.6(51.3-2465.9)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

TAST: transferrin saturation.
Iron deficiency associated with normal intact FGF23 but markedly higher c-terminal FGF23. I.v. iron lowers c-terminal FGF23 in humans by reducing fgf23 transcription.
fgf23 transcription in osteocytes is up-regulated by iron deficiency, but a counter-balancing increase in post-translational FGF23 cleavage maintains normal net production of intact protein.

Correction of iron deficiency with iron dextran restores normal fgf23 transcription, thereby decreasing production of FGF23 fragments while maintaining normal production of intact protein.

Correction of iron deficiency with ferric carboxymaltose restores normal fgf23 transcription, but production of intact FGF23 protein increases, perhaps because of a greater magnitude of concomitant inhibition of FGF23 cleavage by carboxymaltose.

**Conclusion:** We need to learn more about the interactions between iron deficiency and effects of different iron solutions on mineral metabolism and vascular calcification.
The association between higher serum ferritin level and lower bone mineral density is prominent in women ≥45 years of age (KNHANES 2008–2010)

Ferritin Ferroxidase Activity: A Potent Inhibitor of Osteogenesis

Abolfazl Zarjou, Viktória Jeney, Paolo Arosio, Maura Poli, Erzsébet Zavaczkí, György Balla, and József Balla

Novel data indicate suppressive effect of iron overloading on vascular calcification in uremic rats.

Iron dextran reduced Runx2 and the phosphate transporter Pit-1.

Ferritin prevents calcification and osteoblastic differentiation of vascular smooth muscle cells.

Induction of heme oxygenase/ferritin system prevented phosphate-mediated calcification.

On the other hand...

Serum ferritin levels are associated with vascular damage in patients with nonalcoholic fatty liver disease.

Iron compartmentalization into macrophages.
Increased hepcidin may increase iron deposition in macrophages which enhance oxidative stress in atherosclerotic plaques.

Hepcidin inhibits iron efflux from ferroportin-expressing cells, such as duodenal enterocytes, reticuloendothelial macrophages and hepatocytes.

Inflammation (IL-6) + STAT3

Liver

Hypoxia

HIF-2

Hepcidin

FPN

Spleen

Bone marrow

Red blood cells

Plasma iron

EPO

Hepcidin

Hypoxia

Increased hepcidin may increase iron deposition in macrophages which enhance oxidative stress in atherosclerotic plaques.

Is Increased Hepcidin Pro-atherogenic?

Serum Hepcidin Is Associated With Presence of Plaque in Postmenopausal Women of a General Population

Tessel E. Galesloot, Suzanne Holewijn, Lambertus A.L.M. Kiemeney, Jacqueline de Graaf, Sita H. Vermeulen, * Dorine W. Swinkels*

Arterioscler Thromb Vasc Biol 2014

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, Raffaela Rametta, Larry Burdick, Cecelia Frugoni, Anna Ludovica Fracanzani, Silvia Fargion

Arterioscler Thromb Vasc Biol. 2011

Pharmacological Suppression of Hepcidin Increases Macrophage Cholesterol Efflux and Reduces Foam Cell Formation and Atherosclerosis


Arterioscler Thromb Vasc Biol 2012
Hepcidin-25 is related to cardiovascular events in chronic haemodialysis patients

405 pts in CONTRAST study

Neelke C. van der Weerd¹,², Muriel P.C. Grooteman¹,³, Michiel L. Bots⁴

¹Department of Nephrology, VU Medical Centre, Amsterdam, The Netherlands,
²Department of Nephrology, University Medical Centre Utrecht, Utrecht, The Netherlands,

**Table 3. Relationship between hepcidin-25 and cardiovascular events**

<table>
<thead>
<tr>
<th>Model</th>
<th>Hazard ratio¹</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: crude analysis</td>
<td>1.11</td>
<td>0.99–1.24</td>
<td>0.053</td>
</tr>
<tr>
<td>Model 2: adjustment for demographic and clinical parameters related to all-cause mortality²</td>
<td>1.16</td>
<td>1.04–1.29</td>
<td>0.011</td>
</tr>
<tr>
<td>Model 3: additional adjustment for demographic and clinical parameters related to hepcidin-25³</td>
<td>1.15</td>
<td>1.02–1.29</td>
<td>0.026</td>
</tr>
<tr>
<td>Model 4a: additional adjustment for biochemical parameters⁴</td>
<td>1.24</td>
<td>1.09–1.42</td>
<td>0.002</td>
</tr>
<tr>
<td>Model 4b: additional adjustment for ferritin</td>
<td>1.32</td>
<td>1.13–1.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 4c: additional adjustment for hsCRP</td>
<td>1.24</td>
<td>1.05–1.46</td>
<td>0.013</td>
</tr>
</tbody>
</table>

- Hepcidin-25 was associated with fatal and non-fatal CV events
- Inflammation appears to be a significant confounder in the relation between hepcidin and mortality
Prevalence and clinical implications of testosterone deficiency in men with end-stage renal disease

Juan Jesús Carrero¹,²,³,* Abdur Rashid Qureshi¹,* Ayumu Nakashima¹, Stefan Arver⁴, Paolo Parini⁵, Bengt Lindholm¹, Peter Bárány¹, Olof Heimbürger¹ and Peter Stenvinkel¹

Testosterone insufficiency

33%

Testosterone deficiency

44%

Normal Testosterone

23%

Serum testosterone, nmol/L
Testosterone deficiency is a cause of anaemia and reduced responsiveness to erythropoiesis-stimulating agents in men with chronic kidney disease

<table>
<thead>
<tr>
<th>Model</th>
<th>Prediction of ESA dose (U/kg/week)</th>
<th>$\beta$ (SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total testosterone (in nmol/L)</td>
<td>$-0.28 (2.3)$</td>
<td>0.007</td>
</tr>
<tr>
<td>2</td>
<td>1 + Age (in years) and SHBG (in nmol/L)</td>
<td>$-0.28 (2.5)$</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>2 + Davies comorbidity score</td>
<td>$-0.27 (2.4)$</td>
<td>0.006</td>
</tr>
<tr>
<td>4</td>
<td>3 + CRP (in mg/L) and albumin (in g/L)</td>
<td>$-0.26 (2.6)$</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Probability to poor ESA responsiveness (ESA wk/Kg<121) in ESA treated hemodialysis men.

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone deficiency &lt;10 nmol/L</td>
<td><strong>2.68</strong></td>
<td>1.17-6.14</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Adjusted for age, SHBG, co-morbidities, albumin, CRP
Testosterone Suppresses Hepcidin in Men: A Potential Mechanism for Testosterone-Induced Erythrophagocytosis

Eric Bachman, Rui Feng,* Thomas Travison,* Michelle Li, Gordana Olbina, Vaughn Ostland, Jagadish Ulloor, Anqi Zhang, Shehzad Basaria, Tomas Ganz, Mark Westerman, and Shalender Bhasin

FIG. 1. A, Study design, showing simultaneous suppression of endogenous testosterone secretion (GnRH = GnRH agonist, leuprolide) and weekly injection of testosterone enanthate for 20 wk. B, Testosterone suppresses hepcidin dose-dependently in men. Log-transformed serum hepcidin levels (ng/ml) are shown at various sampling points in the 20-wk testosterone treatment study.

Early changes in hepcidin levels were predictive of subsequent changes in hemoglobin and hematocrit.

Testosterone administration inhibits hepcidin transcription and is associated with increased iron incorporation into red blood cells.
Inhibition of Hepcidin

Inhibition of inflammation

S-mulation of CD34+ erytroid progenitors

Hypoxia

HIF-2

Iron

Plasma

Liver

Duodenum

FPN

Hepcidin

Hepcidin

Hepcidin

Hepcidin

HIF-2

Spleen

Bone marrow

Stimulation of CD34+ erytroid progenitors