No Relationships to Disclose

Association of Iron, C-Reactive Protein, and Low-Density Lipoprotein Particle Size with Coronary Artery Calcification: Preliminary Results from The Muscatine Study

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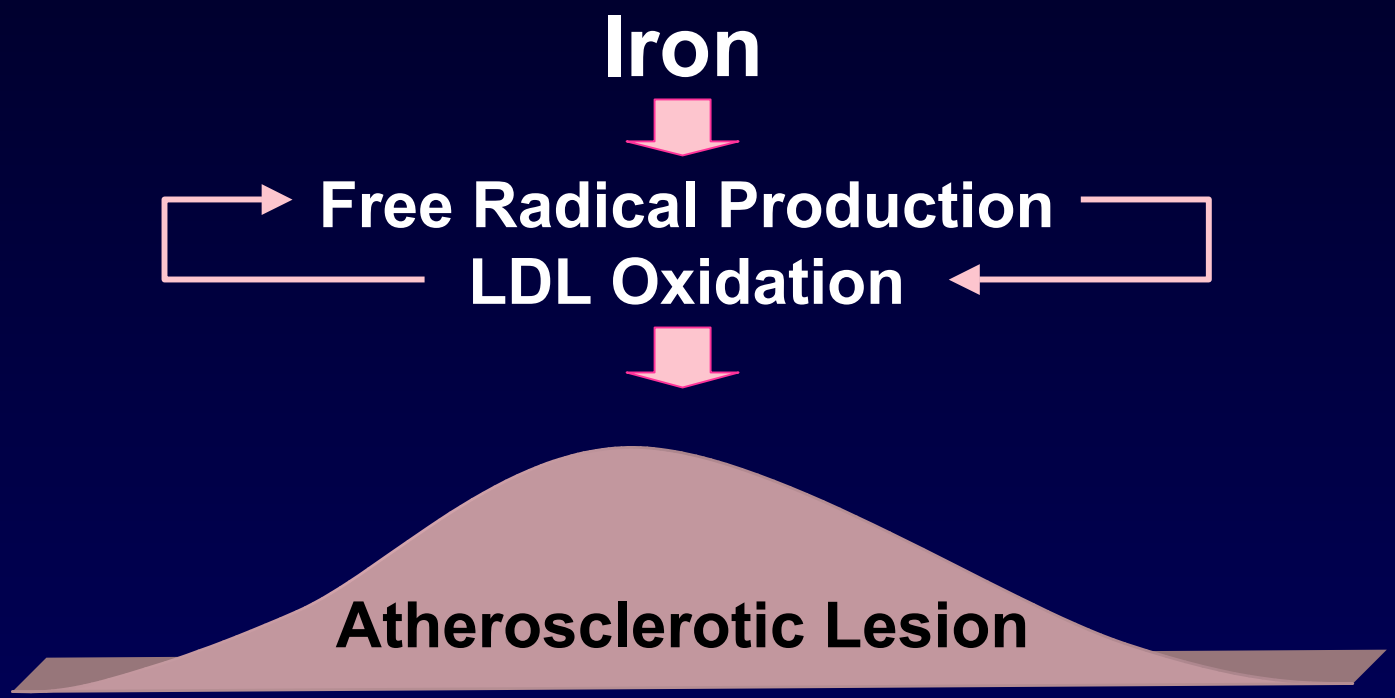
Why an interest in iron?



? Biomarkers of iron
? Biomarkers associated with preclinical atherosclerosis

Evidence for the Interaction of Iron and LDL in Atherosclerosis

- Atherosclerosis: a chronic inflammatory disease
- Interior of human lesions is a highly prooxidant environment (gruel samples catalyze the oxidation of LDL by macrophages & iron may be a catalyst)
- Acidic pH of the extracellular environment of lesions could favor the release of bound iron making it available for promotion of lipid peroxidation
- Ferritin gene expression ⇧ in human lesions

Assessment of Iron Status in Population Studies

- Functional
  - Serum iron
  - Total iron binding capacity (TIBC)
  - Transferrin receptor (TfR)
- Transport
  - Transferrin saturation
- Storage
  - Serum ferritin (C-reactive protein)

Pattern of Iron Indices

Iron Overload

- ⇧ Serum iron
- ⇧ Trans saturation
- ⇧ Ferritin
- Normal TfR
- ⇩/N TIBC

Inflammation/Infection

- ⇩/N Serum iron
- ⇩/N Trans saturation
- N/⇧ Ferritin
- Normal TfR
- ⇩/N TIBC

Transferrin Receptor/ Ferritin Ratio

- Serves as a single laboratory index to assess body iron status
- < 100 adequate iron stores; 500 at point of storage iron depletion; > 2000 functional depletion
- Reciprocal relationship between TfR and ferritin justifies the use of the ratio
- Iron regulatory protein (IRP), regulates mRNA translation of both TfR and ferritin

Hypothesis 1:

Serum indices of iron status are associated with coronary artery calcification (CAC)

Specific Aims:

- To assess whether transferrin receptor, ferritin, and/or transferrin receptor/ferritin ratio are associated with CAC
- To examine whether serum iron, percent transferrin saturation, and/or total iron binding capacity are associated with CAC

Hypothesis 2:

LDL characteristics are associated with coronary artery calcification

Specific Aims:

To determine whether:

- LDL particle size, and/or
- LDL subclass patterns

are associated with CAC.

Methods: Subject Selection

- 184 men; exclusions: 2 no EBCT scans, 3 non-white
- Cross-sectional evaluation of 179 men from the Second CAC study of the Muscatine, Iowa, longitudinal cohort
- To be eligible for the Second CAC Study:

  (1) Biennial school surveys (mean age 15 years)

  (2) Young Adult Survey (mean age 27 years)

  (3) First CAC Study (mean age 33 years)

Methods:
Low-Density Lipoprotein Particles

- LDL particle size determines LDL phenotype. The classification is based on a calibrated scan from the major and minor LDL subclass peaks (GGE).
- Phenotype A: large LDL particles (> 263 Å)
- Phenotype B: small LDL particles (< 258 Å)
- Phenotype I (or AB): intermediate (258 to 263 Å)

Methods:
Main Outcome Variable

CAC as detected by Electron Beam CT

- 40 slices per scan
- Two scans per subject
- Calcified lesion present: area ≥ 1.03 mm^2 (3 or more contiguous pixels) with a CT density ≥ 130 Hounsfield Units on one or both scans
- Quantification of calcium: by one technician and one radiologist at a central reading station

Descriptive Statistics for 179 Men (Mean ± Standard Deviation)

Age	37.3 ± 3.0 years
Serum iron	99.1 ± 37.1 µg/dl
TIBC	295.4 ± 40.7 µg/dl
Trans Saturation	34.2 ± 13.4%
TfR	5.0 ± 1.6 µg/ml
Ferritin	142.4 ± 105.6 ng/ml
TfR/Ferritin Ratio	84.0 ± 152.1
Total Cholesterol	185.8 ± 34.4 mg/dl
LDL Cholesterol	113.3 ± 29.7 mg/dl

Results according to CAC Status

- Prevalence of CAC: 31.8%
- With CAC (n = 57) vs. without CAC (n = 122):
  - Larger body size:
    BMI, $p < 0.0005$
    waist, $p < 0.005$
    waist/hip ratio, $p < 0.01$
  - Higher systolic blood pressure, $p < 0.005$

Results according to LDL Phenotype

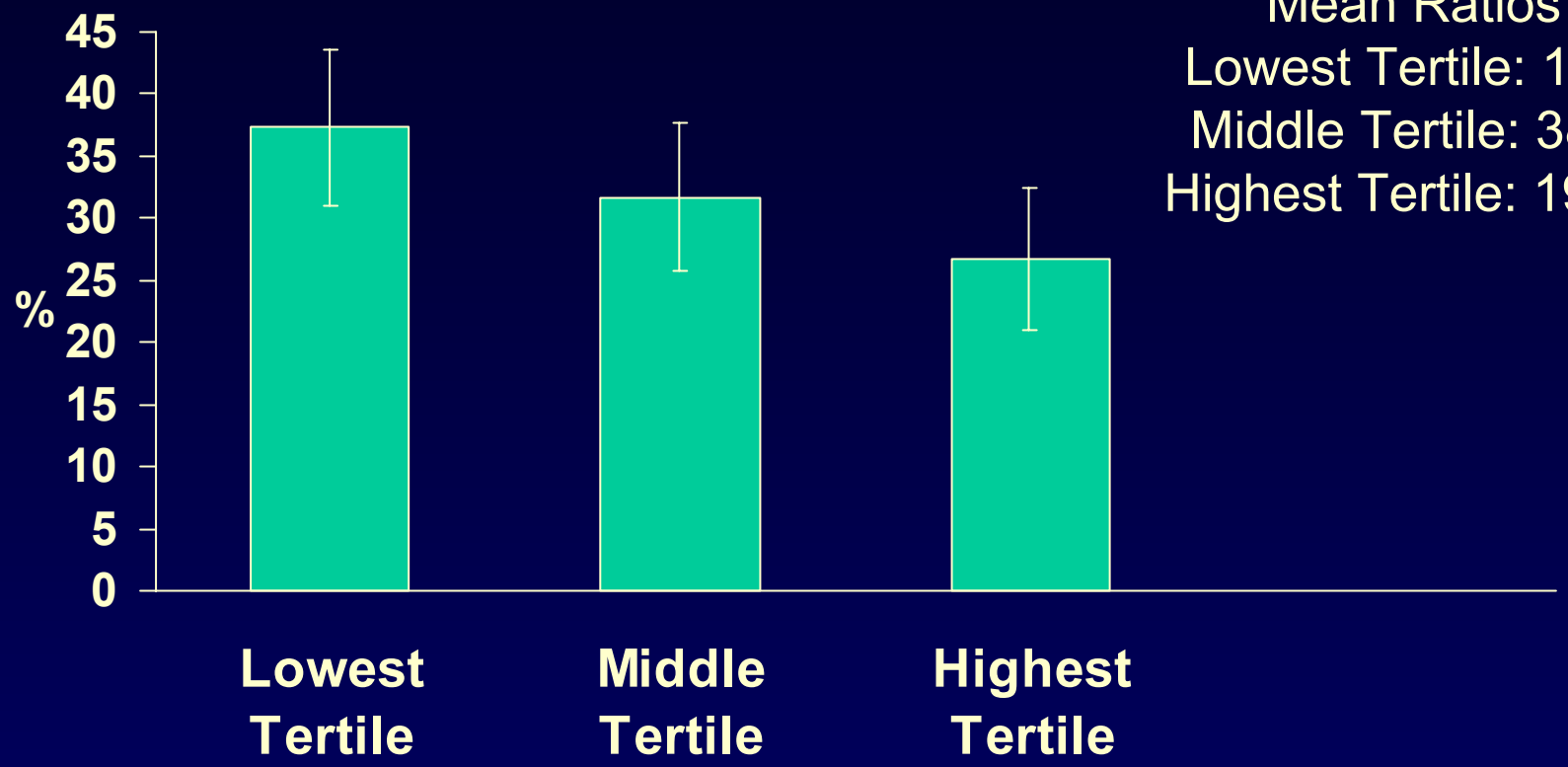
Phenotype B (n= 68) vs. phenotype A (n = 103)

Phenotype B profile:

- Larger body size, $p < 0.005$
- Higher diastolic blood pressure, $p < 0.025$
- Higher insulin levels, $p < 0.005$
- Less favorable lipid levels, $p < 0.005$

  ⇧ triglycerides, ⇧ total chol, ⇩ HDL, ⇧ total chol/HDL ratio, ⇧ LDL/HDL ratio, ⇧ apo B, ⇩ LDL/apo B ratio

Prevalence of CAC according to Tertiles of TfR/Ferritin



Odds Ratios* for the Presence of Coronary Artery Calcification for Selected Iron Indices, LDL Phenotype, and CRP

TfR (≤ 3.2 vs. > 3.2 μg/ml)

Ferritin (≥ 122 vs. < 122 ng/ml)

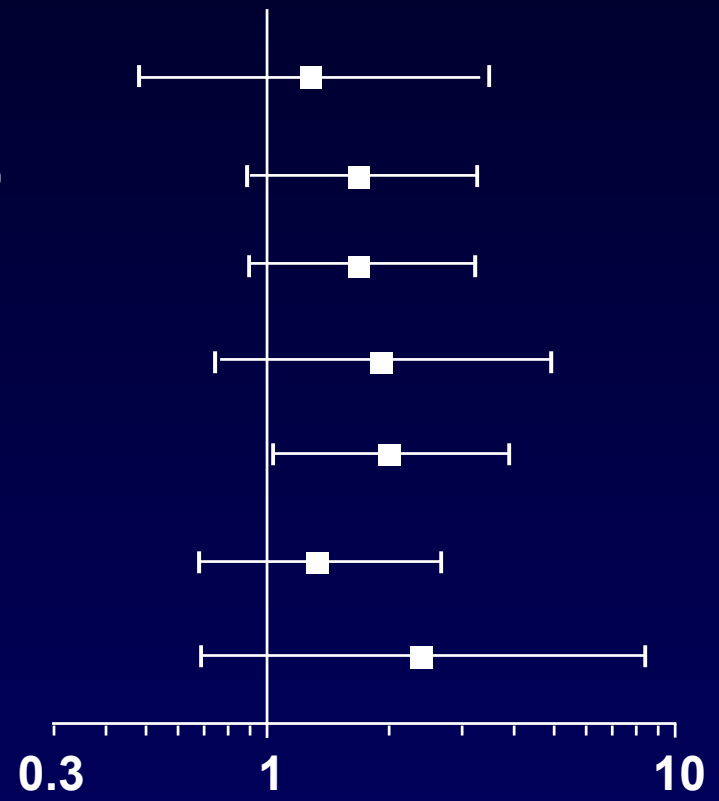
TfR/ferritin (≤ 36.5 vs. > 36.5)

Trans Sat (≥ 50% vs. < 50%)

TIBC (≤ 292 vs. >292 μg/dl)

LDL Phenotype: B vs. A

CRP (≥ 0.5 vs. < 0.5 mg/dl)



*Adjusted for age, total cholesterol, HDL, BP, diabetes, smoking as assessed by the Framingham score.

Odds Ratios* for the Presence of Coronary Artery Calcification for LDL Phenotype/Iron Indices or LDL Phenotype/CRP

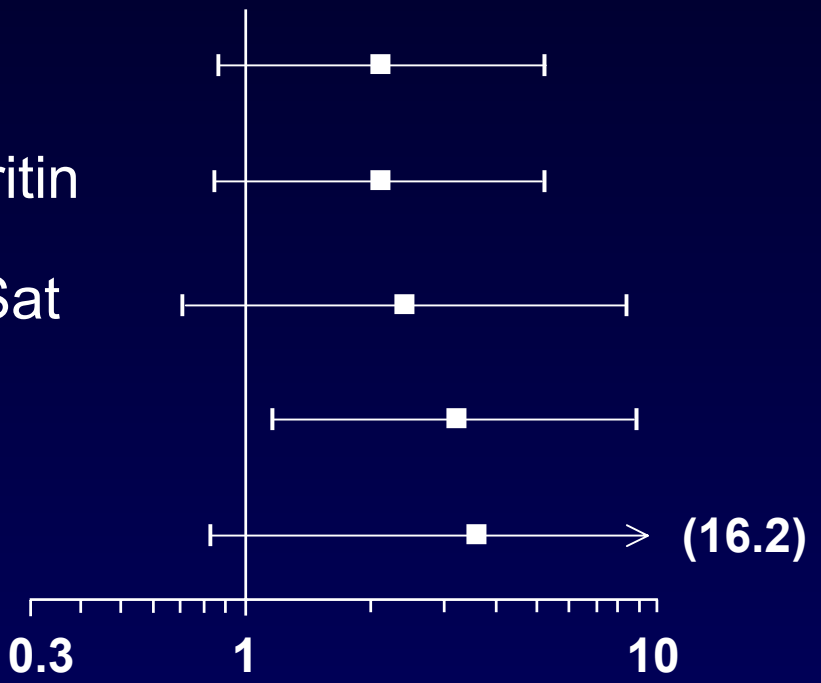
B, ↑ Ferritin vs. A, ↓ Ferritin

B, ↓ TfR/ferritin vs. A, ↑ TfR/ferritin

B, ↑ Trans Sat vs. A, ↓ Trans Sat

B, ↓ TIBC vs. A, ↑ TIBC

B, ↑ CRP vs. A, normal CRP



*Adjusted for age, total cholesterol, HDL, BP, diabetes, smoking as assessed by the Framingham Score.

Preliminary Conclusions

After adjustment for traditional CVD risk factors as assessed by the Framingham score:

- ↑ iron status, or ↑ C-Reactive protein, or LDL phenotype B have possible associations with CAC
- LDL phenotype B + ↑ iron status have higher odds of CAC vs. LDL phenotype A + ↓ iron status
- LDL phenotype B + ↑ CRP have higher odds of CAC vs. LDL phenotype A + normal CRP

Study Limitations

- Cross-sectional study -- cannot assess time sequence
- Small sample size at this time -- results are preliminary
- Other indices of oxidation not measured, e.g., serum antioxidants, copper, and selenium
- Blood donation status unknown

Study Strengths

- Includes several iron measurements → can assess pattern of iron indices
- Younger adults minimize iron changes associated with age and chronic diseases
- The Second CAC Study provided data on other CVD risk factors
- High participation rate

The End

Thank you