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Relation between particle metal content (with focus on iron) and biological responses

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Introduction

Recent epidemiologic studies have shown an association between exposure to ambient particulate matter (PM) less than 10 um in diameter (PM10) and less than 2.5 um in diameter (PM2.5) and increased mortality and morbidity. The PM components that may be responsible for these effects are not known. Transition metals may be involved in some of the effects because of their ability to catalyze redox reactions in lung cells leading to the production of reactive oxygen species and increased production of inflammatory mediators. The goal of this project was to test the hypotheses that 1) iron is mobilized (i.e. bioavailable) from PM, 2) mobilized iron, but not total iron content, is associated with biological responses, and 3) the size of the particles is related to the amount of mobilized iron. These hypotheses were tested using size-fractionated ash (CFA) from combustion of three different coals (Utah, Illinois, and North Dakota), particles from the exhaust of a diesel engine (DEP), and Standard Reference ambient particles. Iron was the metal specifically studied because it is the predominant transition metal in ambient particles as well as in these particles. The data reported in this paper are summarized in Table 1.

Methods

Mobilized (bioavailable) iron was measured in various ways: iron mobilized at acid pH (water at pH 2.5), iron mobilized at neutral pH in the presence of a metal chelator (1 mM sodium citrate, pH 7.4), and increases in the intracellular level of ferritin (an iron storage protein). To measure increased ferritin levels, cells from a human lung epithelial cell line were cultured and exposed to varying amounts of the test particles. (The data reported are for exposure concentration of 20 ug/cm^2.) The particles were removed from the collection substrates either by scraping (coarse particles) or by ultrasonic agitation in ethanol and resuspended in buffer at neutral pH prior to being delivered to the cells. The duration of the exposure was 24 hours. Particle effects were also evaluated by the production of interleukin-8 (IL-8), an inflammatory mediator.

Results

Size fractionated CFA were analyzed for their size distribution after resuspension by Scanning Electron Microscopy or ICP-MS. The results show that the particles in each size fraction are polydisperse suggesting that the resuspension process causes a certain extent of aggregation.

The CFA ash from the different coals contained different amounts of total iron. Iron could be mobilized at acidic pH and by citrate (at neutral pH) from all particles tested indicating that pH and the presence of a metal chelator are important when determining the availability of iron. However, simply resuspending metal-containing particles in water at acidic pH may not be reflective of what may occur in the lungs at neutral pH. There was no correlation between iron mobilized by citrate and total iron content. The form of iron that was mobilized (determined by Mössbauer Spectroscopy) was associated with the glassy aluminosilicate fraction. The other major form of iron present in these particles was insoluble iron oxide. All the particles tested increased the amount of intracellular ferritin in cultured human lung epithelial cells. The level of ferritin was proportional to the amount of iron mobilized by citrate and was dependent on the size of the particles, showing increased amount of iron mobilized with decreasing particle sizes.

CFA particles also increased the level of IL-8 in a dose-dependent fashion (not shown). The increase in IL-8 was inversely dependent on the size of the particles within a given CFA (with the exception of particles>10 um) and correlated with the amount of iron mobilized by citrate and with the level of ferritin.

Discussion

The results of this study suggest that iron mobilized by citrate (in the absence of cells) or intracellular ferritin level provide a reliable measure of iron bioavailable in the cells and may prove useful in determining whether particles have the potential to release iron in vivo. Bioavailable iron was also associated with increased level of the inflammatory mediator IL-8. These results point to the need of obtaining measures of PM characteristics that are biologically relevant.

This study has some limitations because it did not use fresh particles and used cells in culture exposed to high doses of particles. Moreover, resuspending particles from the collection substrate may cause changes in the particle's physical/chemical properties. Thus, these findings need to be validated in vivo. Substrates designed to better preserve those properties are needed for testing particles in biological systems.

Table 1

Particulate	Fe content nmol/mg PM	Fe mobilized in citrate nmol/mg PM	% Fe mobilized	Ferritin level ng/ug protein	IL-8 level pg/mL
Utah CFA >10 um	875	22	2.5	0.23	103
North Dakota >10 um	1268	22.3	1.8	0.14	106
Illinois >10 um	1964	24	1.2	0.36	120
Utah CFA 2.5-10 um	786	40.2	5.1	0.61	278
Noth Dakota CFA 2.5-10 um	1054	24.6	2.3	0.33	103
Illinois CFA 2.5-10 um	1964	27.9	1.4	0.52	216
SRM 1649 <10 um	536	52.9	9.9	1.51	ND[1]
SRM 1648 <10 um	696	24.9	3.6	1.15	ND
Utah CFA <2.5 um	892	56.7	6.4	0.95	402
North Dakota <2.5 um	554	44.1	8	0.38	127
Illinois CFA <2.5	2679	43.3	1.6	0.75	279
DEP <0.5 um	ND	17.1	ND	0.33	ND
Utah CFA <1 um	ND	ND	ND	1.29	ND
North Dakota <1 um	ND	ND	ND	0.51	ND
Illinois <1 um	ND	ND	ND	0.98	ND

[1] ND=Not Determined

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Study Team

- Ann Aust (PI), Utah State University
- John Veranth, University of Utah
- JoAnn Lightly, University of Utah
- Robert Waytulonis, University of Minnesota

Goals of the Study

Test the hypotheses that

- iron is mobilized (bioavailable) from PM
- mobilized iron, but not total iron, is associated with biological responses
- size of particles is related to iron mobilized

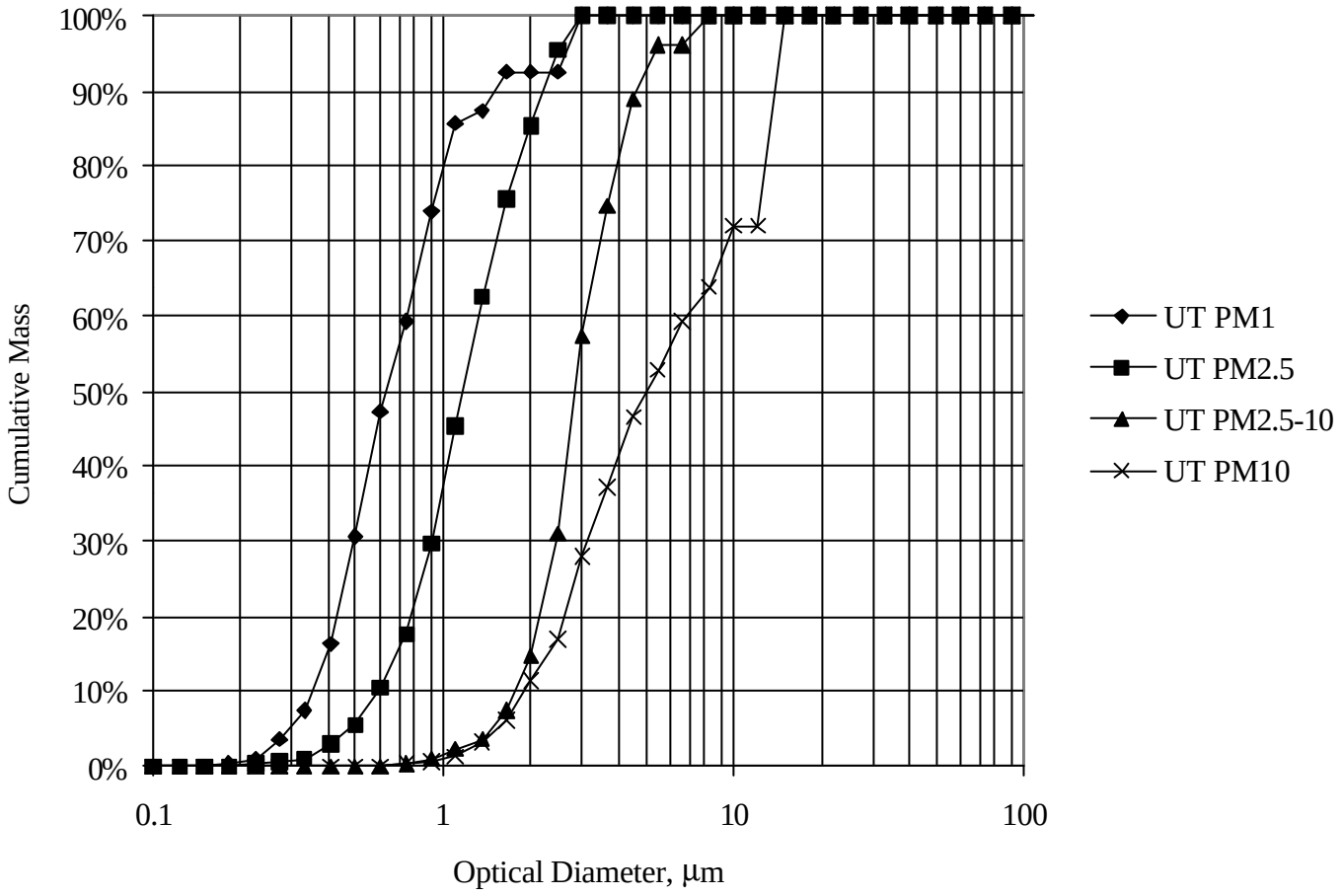
Methods

- Particles used
  - coal fly ash from 3 coals
  - DEP from 1994 diesel engine
  - Standard Reference Material of urban PM
- Collection methods
  - particles collected in different sizes using a cascade impactor

Methods (cont)

- Particles recovered from substrates by scraping (coarse PM) or ultrasonic agitation in ethanol (fine PM)
- Particle characterized
  - for size distribution by SEM
  - total iron by neutron absorption analysis
  - available iron
  - chemical form of iron by Mossbauer spectroscopy

Size Distribution of Size-Fractionated Resuspended CFA

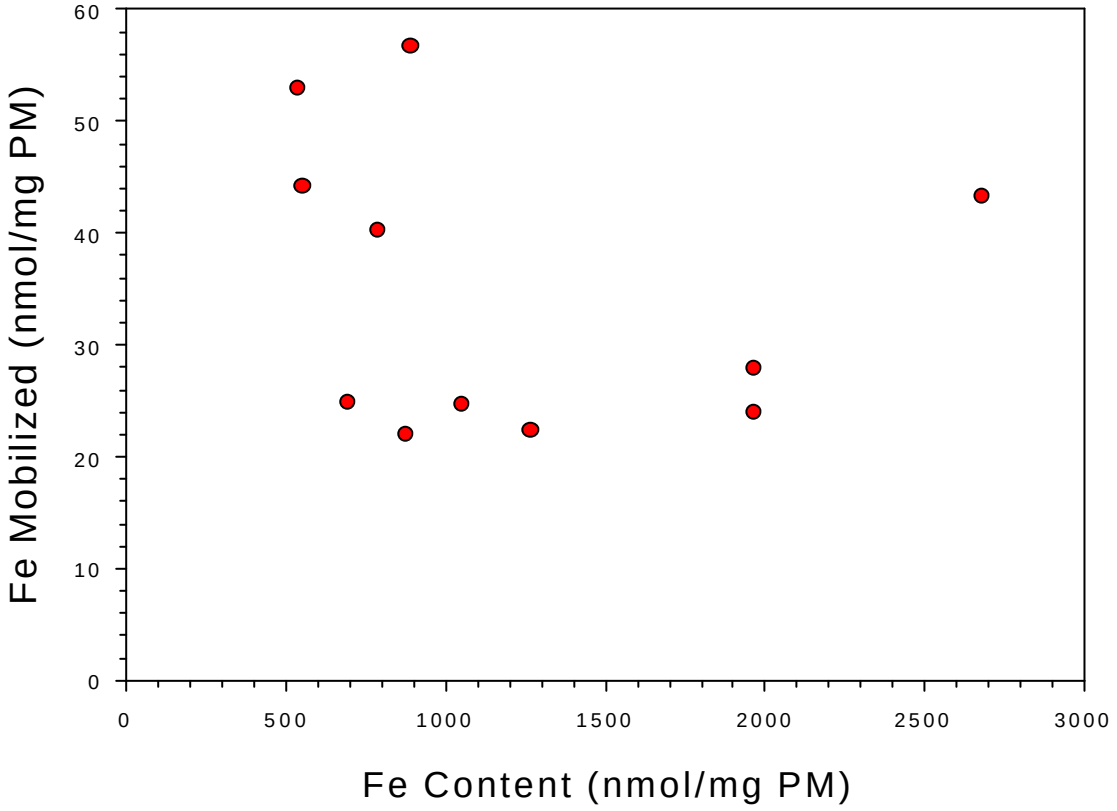


Measure of Fe Mobilization

- Fe released in acidic solution
- Fe released in neutral solution with citrate (a metal chelator)
- Ferritin level in the cells (ferritin is a Fe-binding protein)

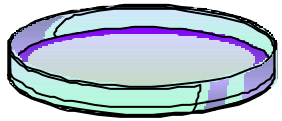
- 107 nmol/mg
- 27 nmol/mg

Iron Mobilized in Citrate versus Iron Content

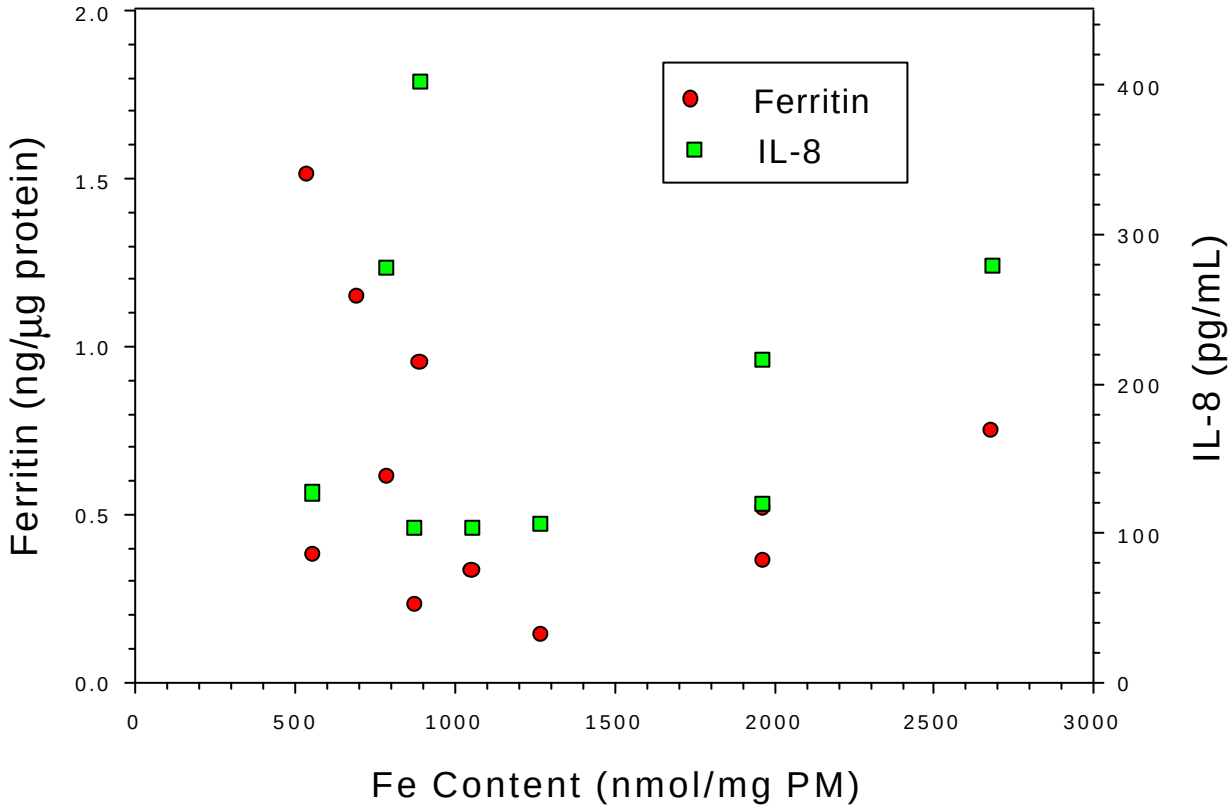


Biological Measures

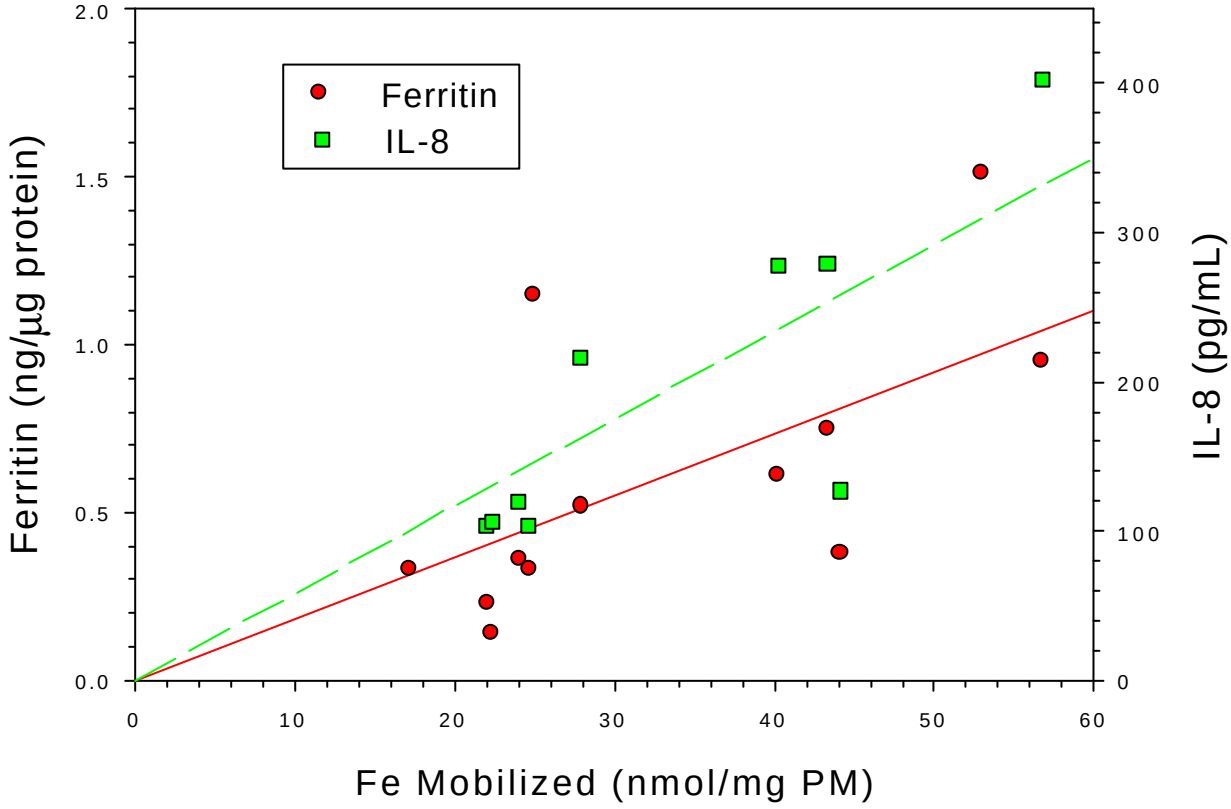
- Human epithelial cell line exposed to resuspended particles (20 ug/cm^2)
- Change in the level of the inflammatory mediator interleukin-8 (IL-8)



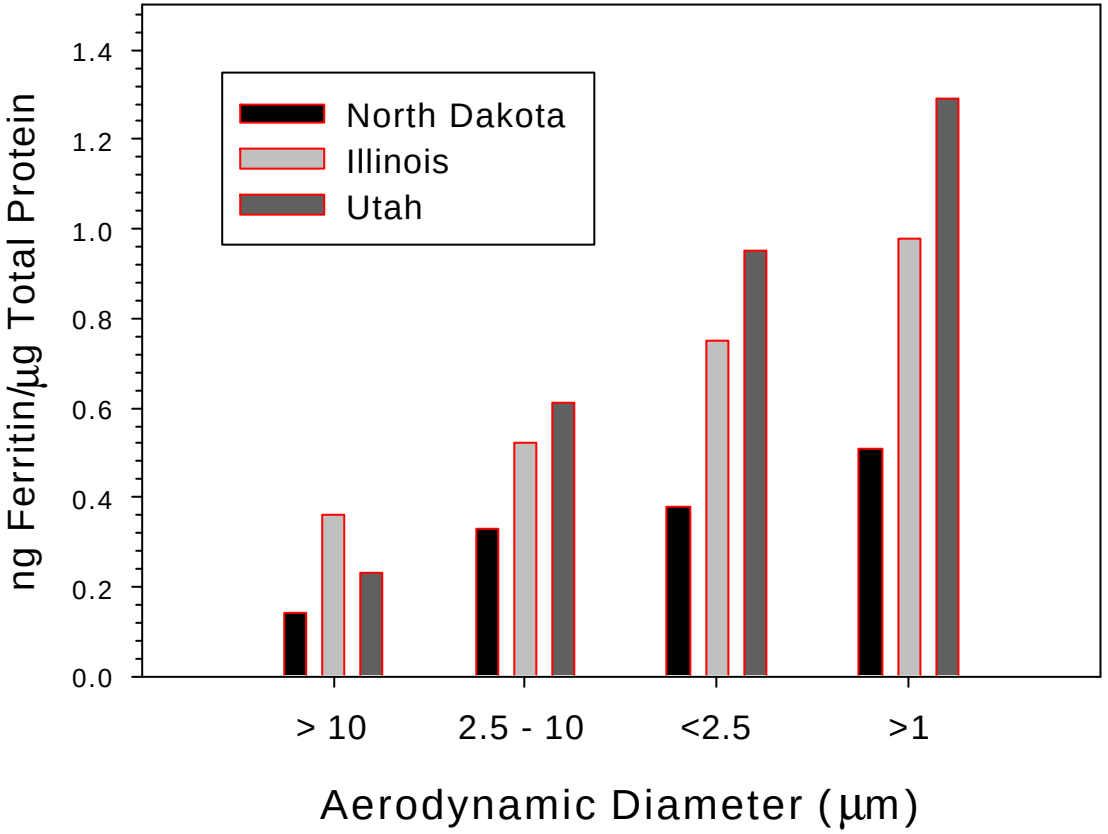
Ferritin and IL-8 Levels as a Function of Iron Content



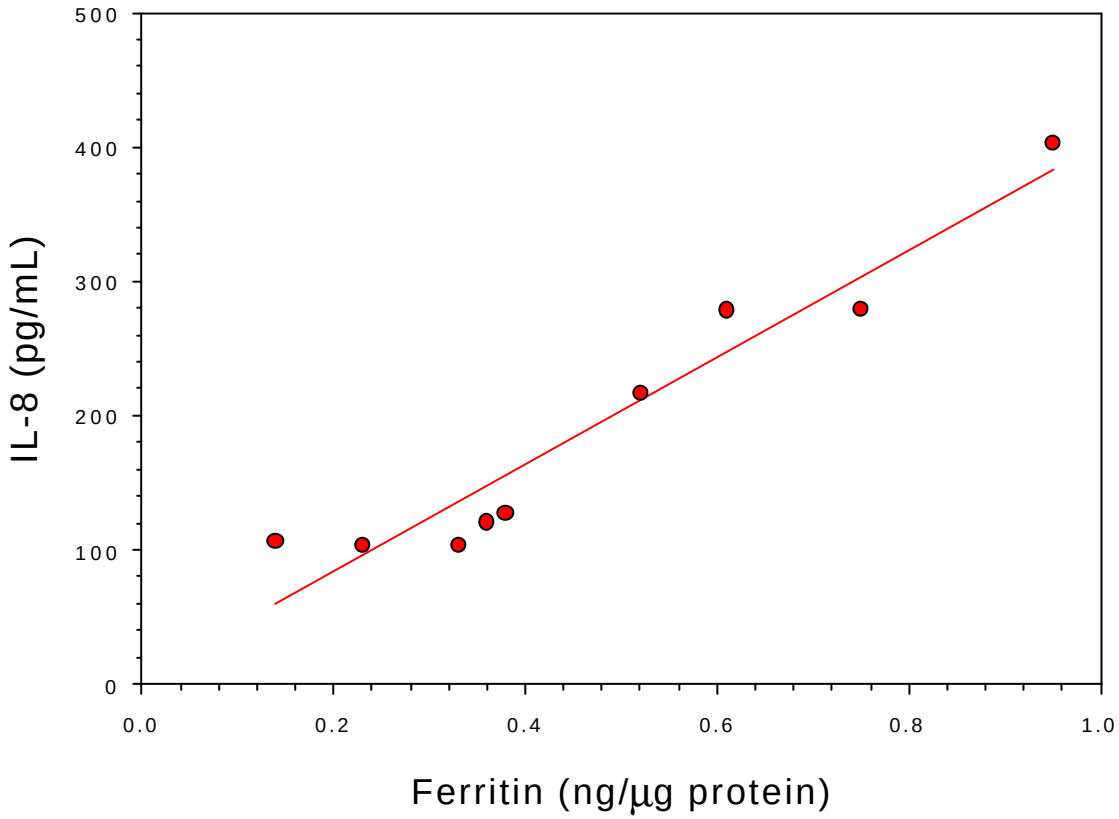
Ferritin and IL-8 Levels as a Function of Mobilized Iron



Relationship between Ferritin and Particle Size



IL-8 Level as a Function of Ferritin Level



Conclusions

- There is no correlation between total Fe content and bioavailable Fe content
- There is a correlation between Fe mobilized in citrate and ferritin (*i.e.* intracellular Fe levels), Both measures are correlated with IL-8 level
- Ferritin may be a measure of bioavailble Fe

Conclusions (Cont.)

- The amount of bioavailable Fe depends on the chemical form of Fe and the size of the particle
  - Fe in the form of ferric iron in aluminosilicates was more bioavailable that Fe in the form of mixed oxides
  - Fine PM induced a greater increase in ferritin level than coarse particles

Caveats and Comments

- Removing particles from their substrate is a problem. More appropriate substrates that preserve the characteristics of the particles are needed
- In vitro exposure do not reflect in vivo exposure and usually require high concentrations of particles

Caveats and Comments

- This study suggests the need to measure PM parameters that may more toxicologically relevant
- This study also suggests that metal (i.e Fe) may play a role in the toxicity of particles