Emerging Issue in Toxicology: The Metabolic Syndrome

Jack R. Harkema
July 25, 2012

Outline

• Obesity and Chronic Diseases
• The Metabolic Syndrome (MetS)
• Animal Models for MetS Research
• High Fructose Diets and MetS
• Intersection of Health and Environment: Metabolic Syndrome and Air Pollution

Increased Prevalence of Obesity

2000
2010

Increased Prevalence of Obesity
The Weight of the Nation

The Metabolic Syndrome

- Cluster of Risk Factors for CVD and Diabetes: Central Obesity; Dyslipidemia; Hyperglycemia; Hypertension; Insulin Resistance
- Dr. Gerald Reaves, Stanford University, 1988 Banting Lecture
- Syndrome X, Insulin Resistance Syndrome, CardioMetabolic Syndrome
- Increased risk of heart attack (2X), stroke (2X), and diabetes (5X)
- 20-25% of world population

Prevalence of the MetS Across Age Groups and Gender in Various Countries

Cornier M et al. Endocrine Reviews 2008;29:777-822
Pathophysiology of MetS

Underlying Factor: Insulin Resistance (IR)

Pathophysiology: Inflammatory Enhancement of IR

Insulin Resistance in Obesity as the Underlying Cause for the Metabolic Syndrome
Pathophysiology: Adipose Tissue Remodeling, Inflammation and Insulin Resistance


Pathophysiology: Obesity, Lipotoxicity and NAFLD

Cusi K. Gastroenterology 2012; 142: 711-725

Cusi K. Gastroenterology 2012; 142: 711-725
Proposed mechanisms that link insulin resistance/hyperinsulinemia to hypertension in fructose-fed rats


Animal Models for MetS Research

- Insects (e.g., dragonflies)
- Mice (genetic, diet-induced)
- Rats (genetic, diet-induced)
- Nonhuman Primates (diet-induced)
- Horses (diet-induced)
- Minipigs

Rodent Models for MetS Research

- Genetic Models of Obesity + T2DM
  - ob/ob (C57BL/6-ob/ob) Mice
  - db/db (C57BL/KsJ-db/db) Mice
  - Zucker Diabetic Fatty Rats (fa/fa)
  - Otsuka Long-Evans Tokushima Fatty Rats
  - Goto-Kakizaki Rats
- Genetic models develop obesity and non-insulin dependent diabetes, but MetS is a much broader constellation of pathophysiological changes

Leptin/Leptin Receptor Deficiency

• Very rare mutations in humans
• ob/ob Mice
  – Reduced BP
  – Do not develop dyslipidemia


Lipoprotein Profiles in Different Mouse Models of Metabolic Syndrome

Kennedy et al, Disease Models & Mechanisms (2010); 3:156-165

Diet-Induced Obesity and Health


Diet-Induced Metabolic Syndrome in Rodents

- High Fructose
- High Sucrose
- High Fat
- High Carbohydrate + High Fat
- Obesity-Resistant Rat Strain
- High-Fat + High-Carbohydrate Diet-Fed Spontaneously Hypertensive Rats (SHR)


High-Carbohydrate, High-Fat Diet-induced Metabolic Syndrome


Percentage of change in the various metabolic parameters in SDR-F and SHR-S rat models

Fructose-Fed Rhesus Monkeys: NHP Model of Insulin Resistance, Metabolic Syndrome, T2DM

- Stanhope and Havel, UCD/CNPC, Davis, CA
- Rhesus monkeys on HFrD for 12 months
- 30% fructose diet (Kool-Aid; 75g/day)
- IR, central obesity, dyslipidemia, and inflammation in 6 months (rapid onset)
- Subset developed T2 DM
- Efficient model for studying pathogenesis, prevention and treatment


Fructose and Glucose Metabolism


Fructose-Fed Rhesus Monkeys with MetS

Fructose-Fed Rhesus Monkeys with T2DM


Fructose-Fed Rhesus Monkeys: Adipokines


Fructose-Fed Rhesus Monkeys: Proinflammatory Markers

Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin.


Metformin protects against the development of fructose-induced steatosis in mice


Air Pollution and Metabolic Syndrome

GLACIER
GREAT LAKES AIR CENTER
FOR INTEGRATED ENVIRONMENTAL RESEARCH
Our Center Objective

Explore and elucidate one of the most prevalent and important global health-environment interfaces:

*Air Pollution and the CardioMetabolic Syndrome*

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National Public Health Burden Associated with Exposure to Ambient PM2.5 and Ozone

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Increase in mortality per 10μg/m³ increase in PM2.5 or PM10

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Health Effects of Air Pollution

- Particle pollution associated with increasing the risk of new cases of chronic diseases (e.g., CVD; diabetes)
- Heavy highway traffic connected to higher risks for heart attack, allergies, and premature births.
- Growing evidence suggests breathing pollution from heavy traffic may cause new cases of asthma in children.

Long-term Exposure to Ambient Fine Particulate Pollution Induces IR in C57BL/6 Mice

PM2.5 Exposure: 6h/day; 5 days/wk; 10 months; mean concentration: 94.4 µg/m3
(7x ambient)

[Graph showing glucose levels over time]


Biological pathways linking fine particulate matter exposure with cardiovascular diseases

[Diagram showing pathways and interactions within the body]

[Graph showing biological pathways]
Our Current Research Questions

Q1. What multipollutant atmospheres in the Great Lakes Region adversely affect human health?

Q2. Does diabetes, obesity, or unhealthy diets make people more susceptible to the health effects of air pollution?

Proposed Pathogenesis

CARDIOVASCULAR EVENTS
- Diabetes
- Insulin resistance and Obesity

ATHEROSCLEROSIS
- Elevated Blood Pressure
- Vascular Dysfunction

ALTERATIONS OF LIPIDS IN THE BLOOD
- Impaired HDL function

CARDIOMETABOLIC SYNDROME
- Inflammation, Oxidative Stress, Autonomic Nervous System Imbalance

Air Pollution
- Ozone, PM

Lifestyle Factors
- High fat or sugar diets, Genetics

Center Projects

- Short-term studies
  - Air Pollution Mixtures
  - Cardiometabolic Responses

- Long-term studies
  - Obesity
  - Diet

- Animal studies
  - PROJECT 1: Dr. Breake
    - Aortic CF exposure: Human subjects, normoxic vs. hypoxic

- Human studies
  - PROJECT 2: Dr. Jame
    - Aortic CF exposure: Human subjects, normoxic vs. hypoxic

- Interspecies/diet interface
  - PROJECT 3: Dr. Javine
    - Aortic CF exposure: Interspecies interactions, susceptibility to CF

- Synergy with PM & Ozone
**Project 1: Short-Term Human Studies**

**Cardio-metabolic Effects of Exposure to Differing Mixtures and Concentrations of Coarse and Fine Concentrated Ambient Particles in Obese and Lean Adults**

Robert Brook, MD\(^1\), Elif Oral, MD\(^1\), Marianna Kaplan, MD\(^1\) and Jesus Araujo, MD\(^2\)

\(^1\)The University of Michigan, Ann Arbor, MI
\(^2\)University of California, Los Angeles, CA

**PROJECT 1: PRELIMINARY DATA**

Reduced insulin sensitivity in human subjects with 5-day ambient \( \text{PM}_{2.5} \) exposure

<table>
<thead>
<tr>
<th>OUTCOME</th>
<th>( \beta )</th>
<th>95% CI</th>
<th>( p )</th>
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</thead>
<tbody>
<tr>
<td>SDNN (msec) [HEART RATE VARIABILITY]</td>
<td>(-13.1)</td>
<td>(-25.3) to (-0.9)</td>
<td>(0.035)</td>
</tr>
<tr>
<td>(^1)HOMA-IR [METABOLIC INSULIN SENSITIVITY]</td>
<td>(0.7)</td>
<td>(0.1) to (1.3)</td>
<td>(0.023)</td>
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</tbody>
</table>

\(^1\) Homeostasis model assessment of insulin resistance: \( \{\text{glucose (mg/dL)} \times \text{insulin (µU•ml}^{-1}\} / 405 \) (Lower values denote better metabolic insulin sensitivity)

**Autonomic imbalance may be the mechanism linking PM to insulin resistance**

SDNN was inversely associated with HOMA-IR (\( \beta = -1.3 \) pmol/day, \( p = 0.030 \))

*BMI and/or age-adjusted (per 10 \( \mu g/m^3 \) of 5-day \( \text{PM}_{2.5} \) average)
Project 2: Short-Term Animal Studies

Cardiometabolic, Autonomic, and Airway Toxicity of Acute Exposures to PM$_{2.5}$ from Multipollutant Atmospheres in the Great Lakes Region

Jack Harkema, DVM, PhD$^1$, Greg Fink, PhD$^1$
James Wagner, PhD$^1$, Masako Morishita$^2$, Tim Dvonch$^2$, Cathie Spino$^2$, and Bhramar Mukherjee$^2$

$^1$Michigan State University, East Lansing, MI
$^2$The University of Michigan, Ann Arbor, MI

Project 2: Animal Toxicology Studies

High Fructose Diet

- Fructose has the same chemical formula as glucose (C$_6$H$_{12}$O$_6$), but with different stereochemistry.
- Metabolism of fructose differs from glucose, and is insulin independent.
- In 8 wks, rats develop hyperglycemia, insulin resistance, dyslipidemia, high blood pressure, and hepatic steatosis.
Study 1: Dearborn Study Design

Diet | Air | CAPs (PM2.5) | O3 (0.5 ppm) | O3 & CAPs Mixture
--- | --- | --- | --- | ---
Normal (ND) | 8 rats | 8 | 8 | 8
High Fructose (HFrD) | 8 | 8 | 8 | 8

* Male rats on diet for 8 wks prior to and during exposure
  * Daily 8h-exposures for two weeks (5 & 4 days/wk; 9 total exposure days)
  * ~400 µg/m³ Concentrated Ambient Fine Particles (CAPs)
  * Animal necropsies one day after last exposure

Urban/Industrial Exposure Site

*AirCARE 2 in Dearborn, MI

Salina Elementary School
Steel Manufacturing Plant

Michigan Department of Environmental Quality Monitoring Site

Cardiovascular Telemetry (BP, ECG)

* 8 rats with implanted telemeters in each exposure chamber
  * 30-second recordings every 5 minutes during daily 8-hour exposures
**Chamber Concentrations**

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<tr>
<th>Air Pollutant</th>
<th>Average Daily Concentrations (Mean ± Standard Deviation)</th>
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<tr>
<td>Ozone (O3)</td>
<td>0.50 ± 0.03 ppm</td>
</tr>
<tr>
<td>CAPs (PM2.5)</td>
<td>444 ± 196 µg/m³</td>
</tr>
<tr>
<td>O3 &amp; CAPs Mixture</td>
<td>O3: 0.49 ± 0.04 ppm&lt;br&gt;CAPs: 356 ± 261 µg/m³</td>
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**Summary of Current Findings**

- High fructose diet produced facets of the CMS in rats (e.g., hypertension, insulin resistance, hyperglycemia, and hepatic steatosis).
- Acute exposures to O3, CAPs, or CAPs+O3 caused reductions in BP and HR that were markedly enhanced in HFrD-fed rats.
- Enhanced BP and HR responses in HFrD-fed rats suggest a diet-induced dysfunction in the autonomic nervous system.
- CAPs+O3 caused a greater decrease in BP and HR in the first few days of exposure, but a quicker adaptive response with repeated exposures.
- Preliminary data suggests that TRP ion channel(s) may mediate ozone-induced drop in BP.

**Effect of 8h Ozone on HR in Rats fed Normal or HFr Diets (Compared to Filtered Air Exposure)**

![Graph showing the effect of 8h ozone on HR in rats fed normal or HFr diets compared to filtered air exposure. The graph includes data points and error bars indicating statistical significance.]
Daily Effect of Ozone Exposure on Blood Pressure
In Rats Fed Normal or High Fructose Diets

Days of Exposure

Change in Mean Arterial Pressure (mm Hg)

Normal Diet
High Fructose Diet

Week 1 Week 2

Effect of 8h Ozone on BP in Rats fed Normal or HFr Diets
(Compared to Filtered Air Exposure)

Effect of 8h CAP on BP in Rats fed Normal or HFr Diets
(Compared to Filtered Air Exposure)

Effect of 8h CAP on HR in Rats fed Normal or HFr Diets
(Compared to Filtered Air Exposure)
How do air pollutants cause a drop in HR and BP?

- Trigeminocardiac reflex (TCR) causes bradycardia (increased parasympathetic activity) and is the most powerful autonomic reflex in the body.
- Irritants induce stimulation of airway sensory nerves and transient receptor potential channels (TRPs; e.g., TRPA1)
- Does O₃ and/or CAPs cause a drop in HR and BP through TCR and/or TRPs?
- What are the mechanism(s) underlying HFxD enhancement of the exposure-induced bradycardia and hypotension?

Exposure-induced nasal airway Inflammation and epithelial remodeling

Project 3: Long-term Animal Studies

Long-term Metabolic Consequences of Exposures to Multipollutant Atmospheres in the Great Lakes Region

Sanjay Rajagopalan, MD and Qinghua Sun, MD

The Ohio State University, Columbus, OH
Project 3: Aim 1

Hypothesis: Near-roadway CAP exposure promotes development of obesity and insulin resistance.

Design: C57Bl/6 model fed normal chow or high-fat chow and exposed to FA/CAP for 12 or 18 weeks. KKAY mice exposed to CAP over 8-10 weeks

• To assess effects of multi-pollutant CAP (regional vs. near-roadway) on glucose and insulin homeostasis, inflammation and insulin signaling pathways.
• To identify inflammatory chemokine mediators.
• To investigate temporal response of multi-pollutant CAP and CMS effects.

KKAY Mice = Heterozygous for the yellow spontaneous mutation (Ay). Progressively develop insulin resistance, obesity over 6-12 weeks.

Effect of Regional CAP Exposure in a Model of Genetic Type II DM (KKAY Mouse Model)

EXPOSURE CONCENTRATIONS (Columbus Regional: 12/28/2011 - 02/28/2012)

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<tr>
<th>Exposure</th>
<th>Ambient FA</th>
<th>CAPS</th>
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<tr>
<td>FA</td>
<td>8.27 µg/m³</td>
<td>1.91 µg/m³</td>
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Enrichment Factor = 12.22

4-5 wk-old KKAY mice exposed to FA or CAP (normal diet)

Regional CAP Exposure and Insulin Resistance Measures in Genetic Type II DM (KKAY Model)
Effects of Regional CAP Exposure on Metabolic Indices

Short term CAP exposure has profound effects on metabolism and reduces oxygen consumption, VCO2 production and thermogenesis.

Regional CAP exposure † F4/80+ CD11c+ Adipose Tissue Macrophages (ATMs) in Type II Diabetic Mice

5-weeks of CAP exposure sufficient to induce VAT infiltration by ATMs. Analysis is on stromal vascular fraction (SVF) of epididymal fat.

Recent studies have suggest that high-fat diet (HFD) induces hypothalamic inflammation (arcuate nucleus) rapidly (24 h)
— Hypothalamic inflammation and ER stress regulates peripheral inflammation.

Regional CAP Exposure Increases Inflammation in Hypothalamus within 5 weeks

Prior studies have demonstrated that hypothalamic inflammation occurs within 1 week in response to HFD feeding in the arcuate nucleus (Thaler et al. 2012)

Working hypothesis

Diverse Stimuli in (Air Pollution, High Fat Feeding)

↓

Rapid Hypothalamic Inflammation

↓

Autonomic Imbalance/ Vagal Anti-Inflammatory Reflux

↓

Peripheral Inflammation

↓

Type II Diabetes

Summary & Conclusions

- Metabolic syndrome is a growing global problem and currently affecting 25% of our population.
- A variety of animal models are used in MetS research.
- Recognition of the strengths and limitations of the individual animal models is needed for proper selection and interpretation of results.
- Environmental air pollution may enhance the magnitude and onset of facets of the metabolic syndrome and associated chronic diseases.
- More studies are needed to understand how toxicants (or pharmaceuticals) may adversely affect those suffering from metabolic syndrome and associated chronic diseases (e.g., CVD, diabetes).
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Questions?