The shape of things to come
When FAT Attacks: How Fat Cells Are Waging War on Your Health
Why Dieting Is No Magic Bullet
Coincidence of Macrophage Infiltration into White Adipose Tissue and Onset of Hyperinsulinemia During Diet-Induced Obesity

(After: Xu et al, 2003 JCI vol.112)
F4/80+ Cells are the Predominant Source of TNF

*Weisberg JCI, 2003. Vol 112*
Summary

Macrophages infiltrate adipose tissue

Macrophage infiltration is associated with insulin resistance

Level of macrophage infiltration correlates with cell size

Increased activated macrophages in obese versus lean
Why does macrophage infiltration increase with obesity?

Is there a physiologic function for macrophages in adipose tissue?

Can histological studies assist us in our understanding?
The preponderance of MΦ in white adipose tissue of lean and obese mice are arranged in “crown-like structures” (CLS) surrounding what appear to be functional adipocytes.

The frequency of these macrophage aggregations increases ~30-fold in obese (db/db) vs lean mice.

Cinti et.al., J Lipid Res 2005. 16:2347-2355
These aggregated МΦ form syncitia that progress to multinucleate giant cells (MGC), a hallmark of chronic inflammation.
Do macrophages form CLS around adipocytes or another structure?

Electron microscopic studies revealed the histology of the CLS.
Electron microscopy reveals that these MΦ aggregations form exclusively around the ‘free’ lipid droplet (L) of dead adipocytes.

Cinti et al., J Lipid Res 2005. 16:2347-2355
Immunohistochemical localization of TNFα in EWAT of 8 week old db/db mouse

Higher magnification of ∇ crown like structure shown above left

Anti-TNFα

Anti-TNFα + competitive peptide con
Correlation between CLS Formation and Cytokine Production
High Fat Feeding Increases Body Weight in Mice
Progressive Adipocyte Death with High Fat Feeding in Intrabdominal Adipose Tissue Starting at 8 weeks
Pattern of Macrophage Markers with HFD
Increased CD11c

- **F4/80**
  - mRNA expression (ΔΔCt)
  - 4: , 8: , 12: , 16: 

- **CD11c**
  - mRNA expression (ΔΔCt)
  - 4: , 8: , 12: , 16: 

- **CD11b**
  - mRNA expression (ΔΔCt)
  - 4: , 8: , 12: , 16: 

*week of HF diet*
Increased Cytokine Production with HFD Parallels Adipocyte Death and Macrophage Infiltration

Week of HF diet
Adipocyte Death Correlates with Adipose Tissue Inflammation
Estrogen regulation of adiposity; *in vivo* and *in vitro* effects on adipose tissue and muscle metabolism
Estrogen

- Dominant form is $17\beta$-estradiol; also estrone and estriol
- Synthesized mainly in ovaries (in women), also some synthesis in other tissues in both men and women
- Enzyme *AROMATASE converts* testosterone to $17\beta$-estradiol

(Mendelson et al. Science 2005)
Women’s Health Initiative

- 15,641 post-menopausal women
- Randomized to estrogen, estrogen plus progesterone, or placebo
- 5+ year follow-up
Women randomized to HRT

- Lower BMI
- Lower waist : hip ratio
- Lower blood glucose
- Lower insulin
- Greater insulin sensitivity
Comparison of Sham vs Ovariectomized Mice
Operations performed in female C57Bl/6 mice (OVX / SHAM)

Model: Ovariectomized Mouse

Experimental Outcome Variables:

1. Whole body physiology
   - Food intake, body weight, insulin resistance
   - Energy expenditure, activity levels

2. Adipose tissue mass and cell size
   - Intra-abdominal (perigonadal)
   - Subcutaneous (inguinal)

3. QPCR, etc
   - Muscle (fat oxidation, fiber type)
   - Adipose tissue (inflammation)
   - Liver (lipogenesis/inflammation)

Subset of mice put in metabolic chambers
Weeks 9-11
Week 12
Tissues harvested
Physiological measurements of female sham-ovariectomized (SHAM) and ovariectomized (OVX) mice 12 weeks post-operation

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHAM</th>
<th>OVX</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>22.8 ± 0.1</td>
<td>27.5 ± 0.1*</td>
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<tr>
<td>Uterine weight (g)</td>
<td>0.081 ± 0.008</td>
<td>0.040 ± 0.008*</td>
</tr>
<tr>
<td>Plasma estradiol (pmol/l)</td>
<td>305.9 ± 176</td>
<td>12.3 ± 6*</td>
</tr>
<tr>
<td>Glucose¹ (mmol/l)</td>
<td>5.8 ± 0.26</td>
<td>7.4 ± 0.42*</td>
</tr>
<tr>
<td>Insulin¹ (pmol/l)</td>
<td>145.3 ± 23.4</td>
<td>207.8 ± 47.4</td>
</tr>
<tr>
<td>Triglycerides¹ (mmol/l)</td>
<td>0.34 ± 0.04</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>NEFA² (mmol/l)</td>
<td>0.66 ± 0.17</td>
<td>0.49 ± 0.10</td>
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¹Plasma measures determined after an 8 hour fast. N ≥ 7, *P ≤ 0.05.
²Non-esterified fatty acids
Time course of Body Weight and Food Intake
Energy Metabolism

Oxygen Consumption

SHAM  
OVX

Counts

Spontaneous Physical Activity

SHAM  
OVX

Counts
Adipose Depot Weights

Percent of total body weight

- **SHAM**
- **OVX**

**PGAT**

**SCAT**

Adipocyte Volume

- **SHAM**
- **OVX**

**PGAT**

**SCAT**
Role of Ovarian Hormones in Regulating Inflammation

Obesity is associated with increased inflammation in several tissues.

Is there increased inflammation with loss of ovarian hormones?
Increased inflammation in both intrabdominal and subcutaneous adipose depots.
**Summary**

Increased inflammation with ovariectomy

Both intradominal and subcutaneous depots are inflammed

Increased infiltration of T Cells and increased expression of interferon gamma

In liver increased inflammation along with increased Lipogenic genes

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<tr>
<th></th>
<th>F4/80, CD11b</th>
<th>CD11c</th>
<th>CD3</th>
<th>MCP1, osteopontin</th>
<th>RANTES</th>
<th>TNFα</th>
<th>IL-6, IL-1β</th>
<th>IFNγ</th>
<th>IL-10</th>
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<tr>
<td><strong>Perigonadal</strong></td>
<td>↑</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑↑</td>
<td>↑,↑</td>
<td>↑</td>
<td>↔</td>
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<tr>
<td><strong>Subcutaneous</strong></td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
<td>↑↑</td>
<td>↓</td>
<td>↑</td>
<td>↑, ↔</td>
<td>↔</td>
<td>↔</td>
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Increased Fat Accumulation and Inflammation in Liver
Loss of Ovarian Hormones Promotes Both Obesity and Inflammation
Acknowledgements

**Martin Obin**

- Tara D’Eon
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  - Sandra Souza
  - James Perfield
  - Katherine Strissel
  - Hideaki Miyoshi

- Michael Mendelsohn
- Richard Karas MD PhD
- Mark Aronovitz

- Susan Fried
Numerous studies demonstrate ovariectomy increases body fat which can be prevented by estrogen treatment.

Little is known about the mechanism.

**HOWEVER, STUDIES ARE CONFOUNDED**

There’s approximately a 20% difference in food intake between ovariectomized (OVX) and OVX-estrogen treated mice.
Estrogen regulation of adiposity in ovariectomized pairfed mice

**AIMS**

- To determine if chronic estrogen regulates adiposity in mice when food intake is carefully controlled.
- To elucidate metabolic and molecular mechanism by which E2 regulates adiposity.

**Study Design**

- C57 Bl/6 mice were ovariectomized
- Allowed 7 days to recover
- Randomized to placebo (OVX-C) or estrogen (OVX-E2) subcutaneous implant pellets (n=4-5/group)
- Pairfed daily for 40 days
- Fasted overnight
OVX-E2 mice had lower body fat

- Adiposity Index
- Weight (g)

- Periovarian
- Perirenal
- M/O
- Subcutaneous

* Significant difference
** Highly significant difference
Adipose tissue gene expression cont.
Estrogen downregulates lipogenic gene expression in Cultured Human Adipocytes

- C
- 10nM E2
- ICI 182 780

**SREBP-1c**
- a
- b
- c

**FAS**
- a
- b
- c
Estrogen decreases expression of genes involved in lipogenesis in liver.
Estrogen decreases expression of genes involved in lipogenesis in muscle

Relative gene expression

- LXR
- SREBP-1c
- FAS
- ACC-1
- ACC-2

OVX-C
OVX-E2

Muscle
Estrogen increases expression of PPAR-δ and downstream genes involved in oxidative metabolism.

Muscle

OVX-C ■ OVX-E2

** *

***
E2

PPAR-δ

↑ LPL expression

PPAR-δregulated genes upregulated by E2

- PDHK (20-fold increase)
  - increases fat oxidation by reducing glucose derived Acetyl CoA

- ACOX (10-fold increase)
  - Involved in β-oxidation

- UCP2, UCP 3 (3-fold increase)
  - Increase energy consumption
OVX mice have decreased expression of type I oxidative fiber markers in skeletal muscle (quadriceps).

Muscle

- PPARδ
- FoxO1

↓

Type I oxidative fibers

MLC = myosin light chain, normalized to cyclophilin B, **p<0.01, ***p<0.001

SHAM
YOUR LOST WEIGHT

"Ready to head back?"
Summary

- Estrogen reduces adiposity in pairfed mice
- Estrogen treatment is related to smaller adipocytes and better regulation of lipolysis (lower basal and higher stimulated lipolysis)
- E2-induced genomic changes potentially contributing to reduced adiposity include:
  - Decreased expression of lipogenic-related genes (SREBP-1c pathway)
  - Increased expression in PPAR-δ and downstream targets involved in oxidative metabolism
# Acknowledgements

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Funding:
- NIDDK
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- USDA
- Atkins Foundation
Fiber Type and Gender

- Female muscle
  - Greater relative type I oxidative fiber area
  - Consistent with protection against insulin resistance and diabetes

- Male muscle
  - Greater relative type II glycolytic fiber area

Despite these gender differences, effects of ovarian hormones on fiber type, and any relationship this may have to menopause-associated disease risk, is unknown.
Estrogen

Adipose Tissue

↓ lipogenesis (ACC-1, FAS)
↓ FA uptake (LPL)
↑ lipolysis

Muscle

↑ FA uptake (LPL)
↑ Fat oxidation (AMPK, ACOX, PDK4)
↑ Energy expenditure (UCP2, UCP3)

Liver

↓ lipogenesis (ACC-1, FAS)

Decreased Adiposity

Estrogen

Insulin sensitivity?