The Economist

DECEMBER 13TH–19TH 2003 www.economist.com

Gore anoints Dean
PAGES 12 AND 33

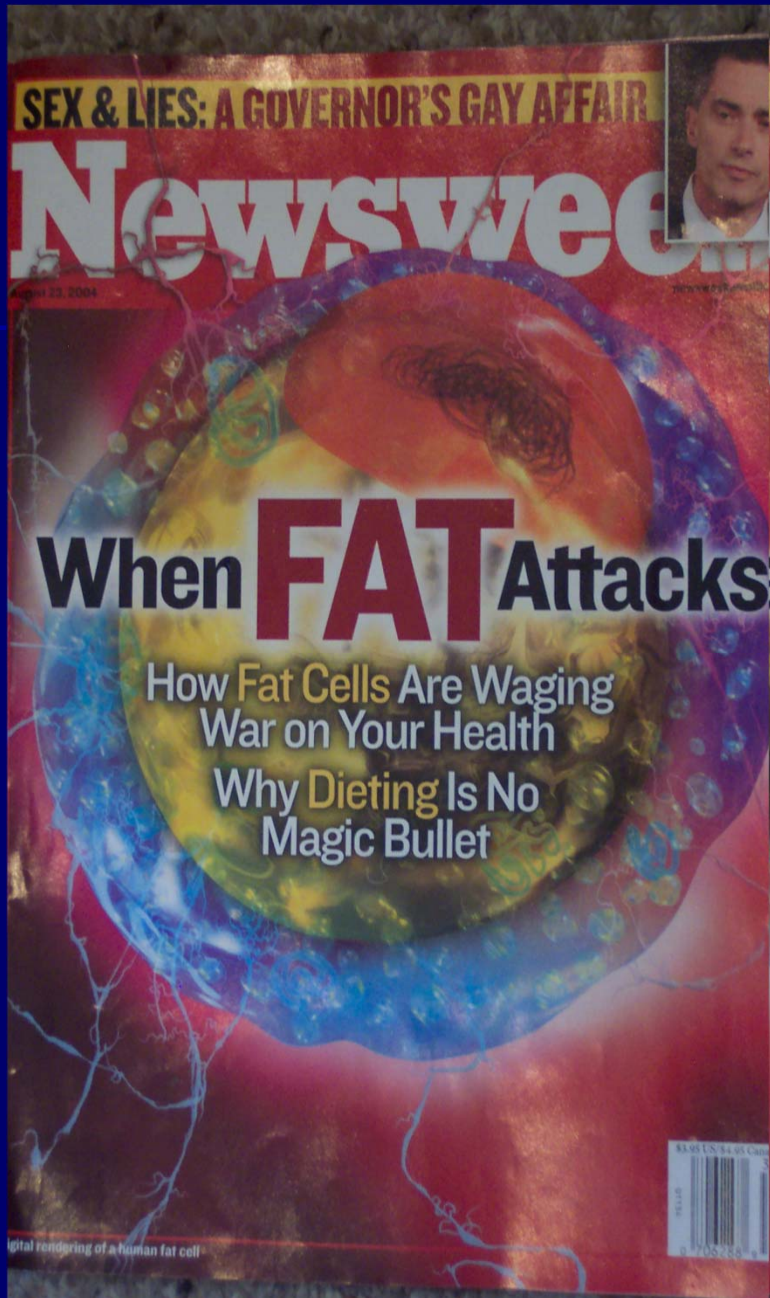
America's Taiwan test
PAGES 12 AND 29

The future of flight
PAGES 79–81

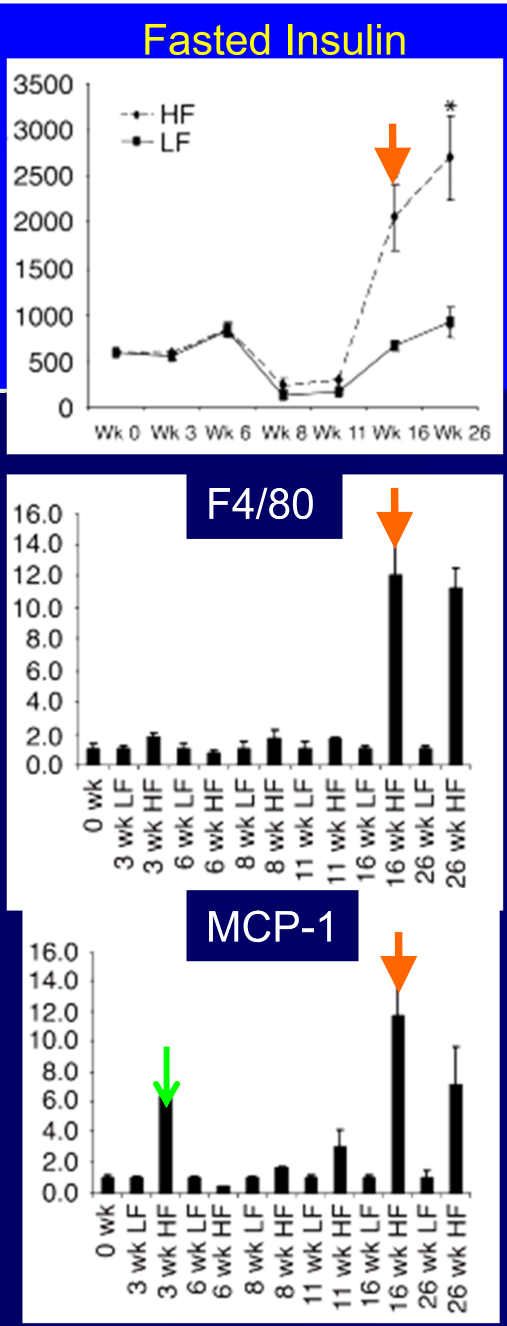
A SURVEY OF FOOD
AFTER PAGE 52

The shape of things to come

TUFTS HNRCA
Healthy Aging
THROUGH NUTRITION RESEARCH
JEAN MAYER USDA HUMAN NUTRITION RESEARCH CENTER ON AGING

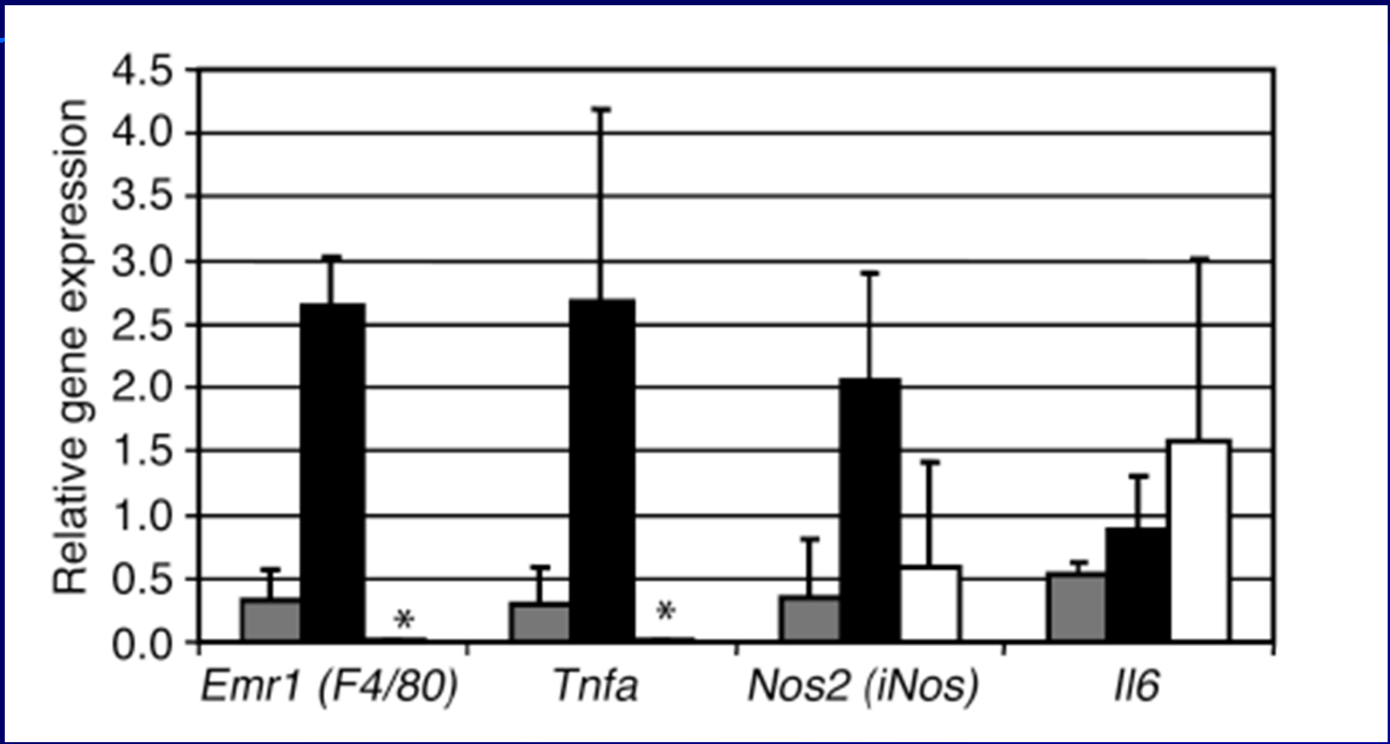

SEX & LIES: A GOVERNOR'S GAY AFFAIR
Newsweek
When FAT Attacks:
How Fat Cells Are Waging War on Your Health
Why Dieting Is No Magic Bullet

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



SVC:F4/80-
SVC: F4/80+
Adipocyte

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

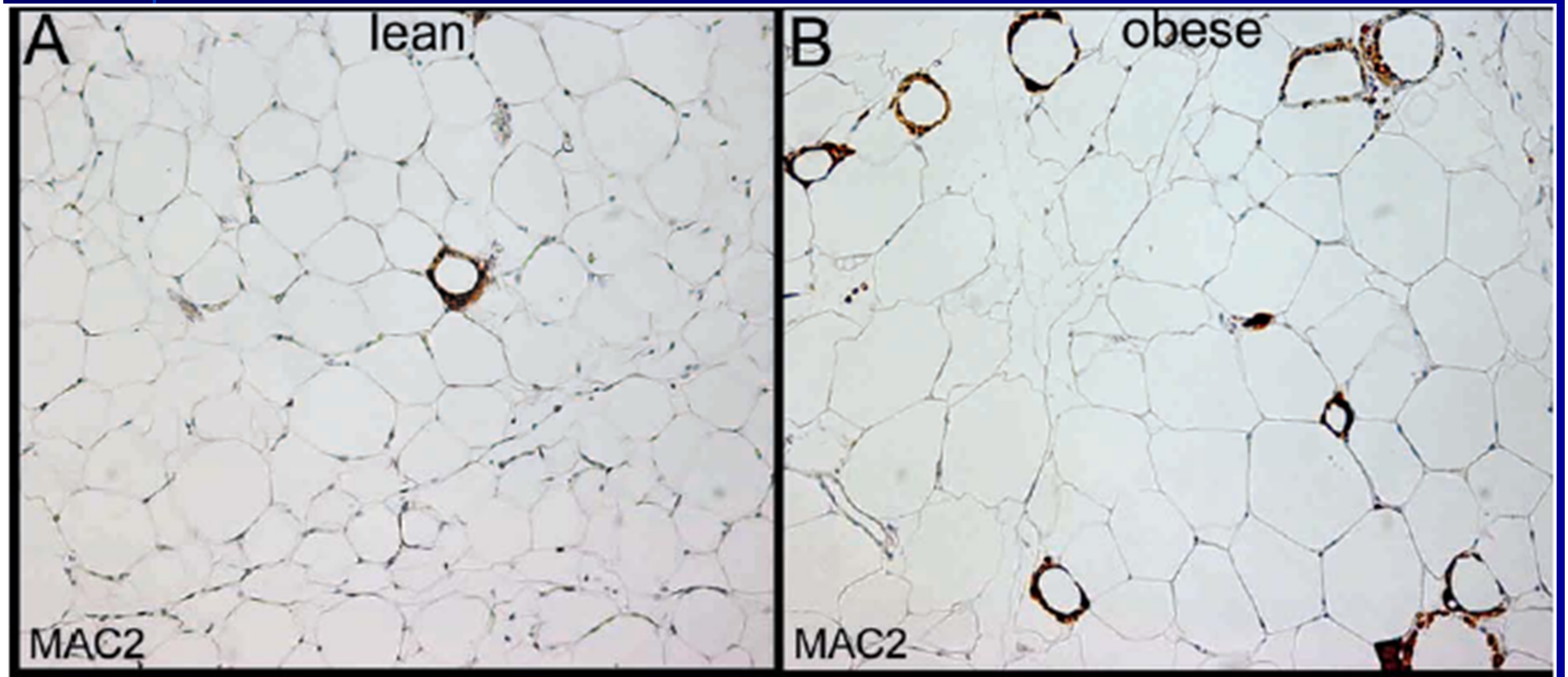
Why does macrophage infiltration increase with obesity?

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.

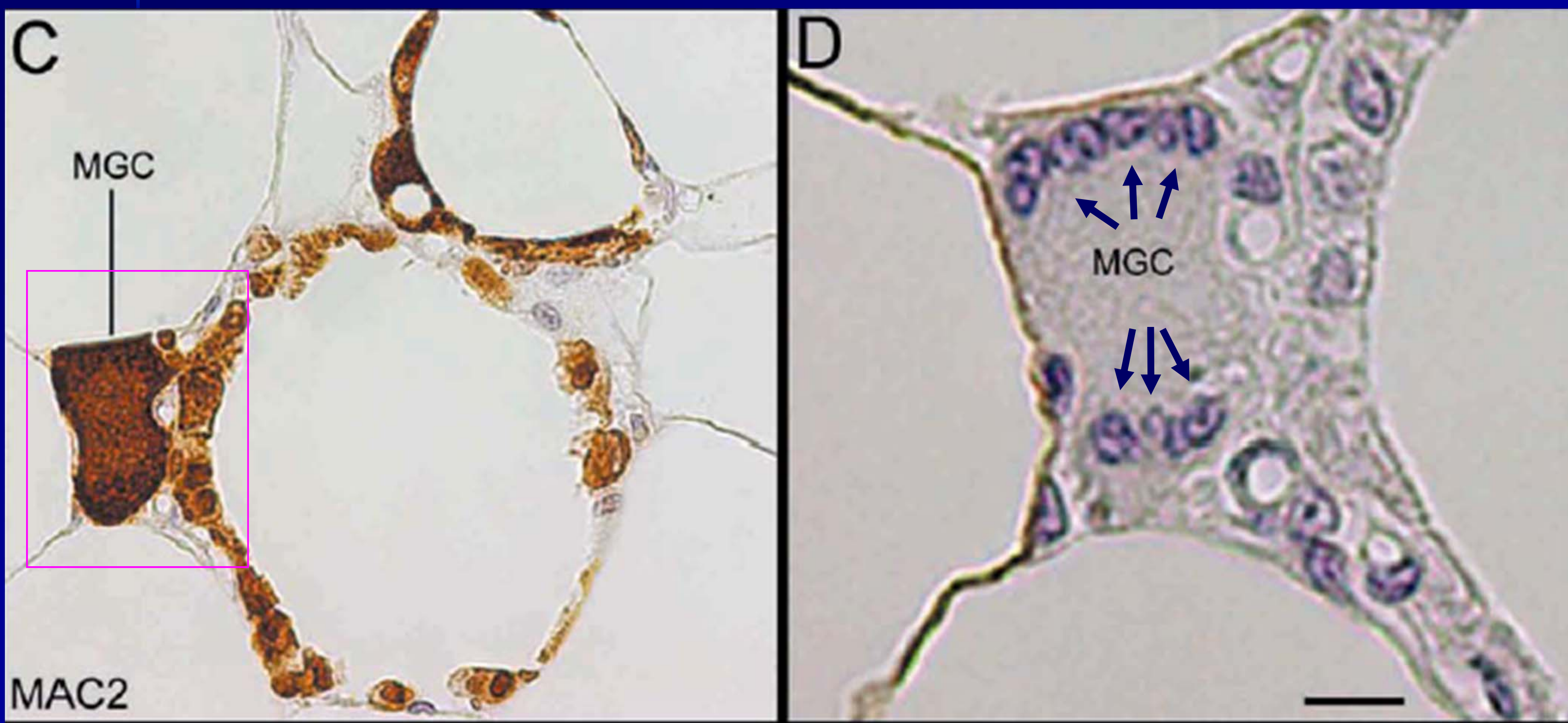


Cinti et.al., J Lipid Res 2005. 16:2347-2355

These aggregated MΦ form syncitia that progress to multinucleate giant cells (MGC), a hallmark of chronic inflammation.

MAC2 Immunostaining

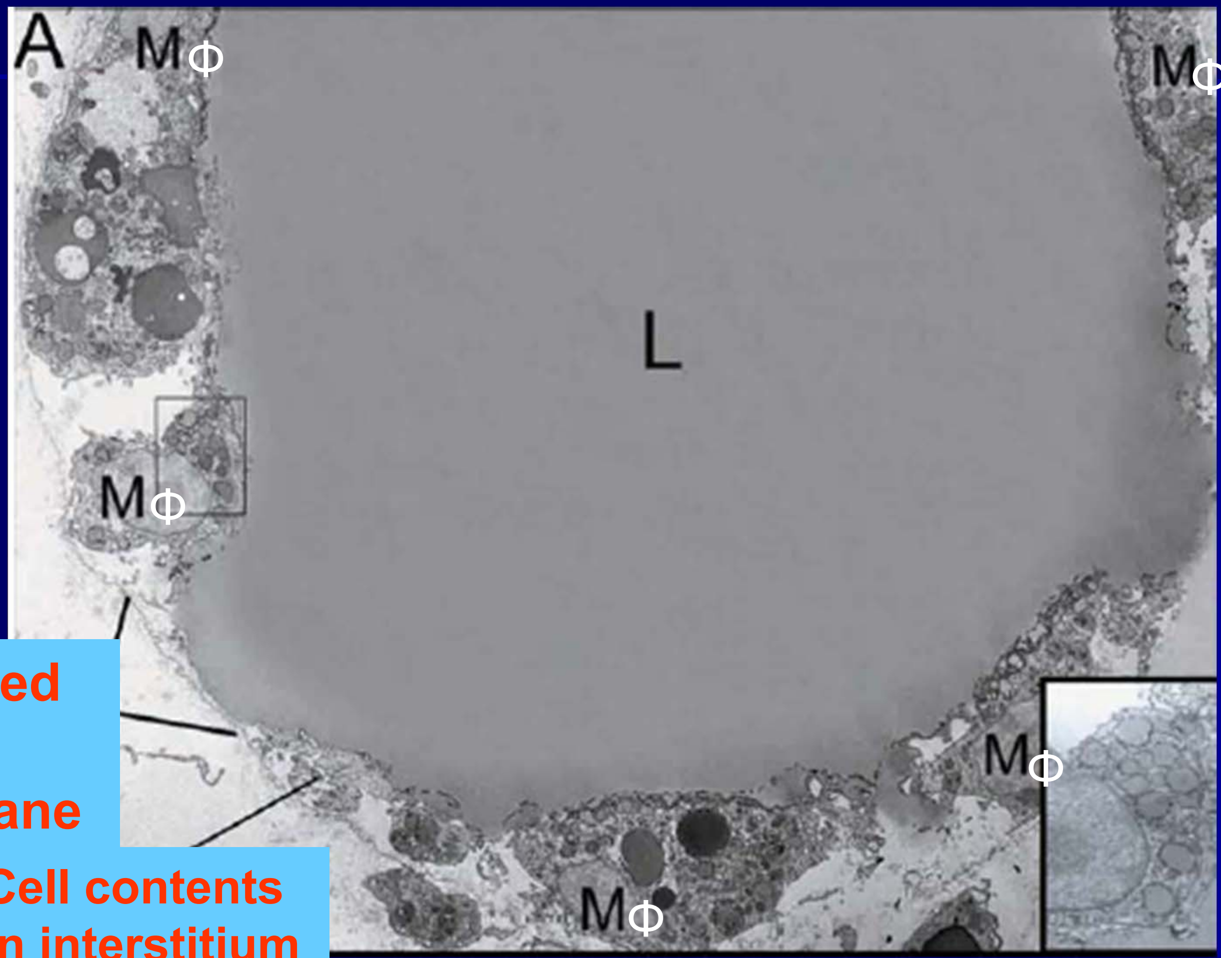
H&E



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.

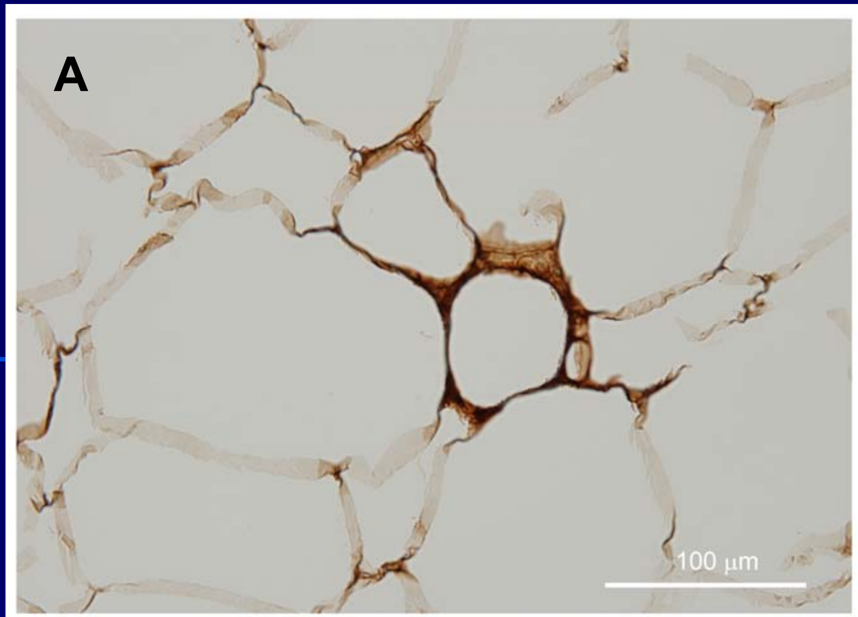


Disrupted plasma membrane

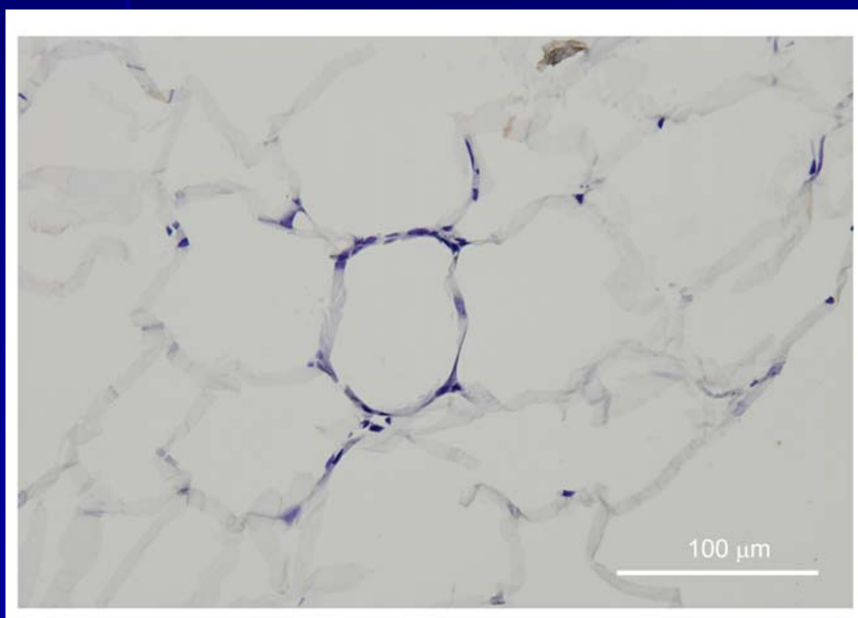
Cell contents in interstitium

Cinti et.al., J Lipid Res 2005. 16:2347-2355

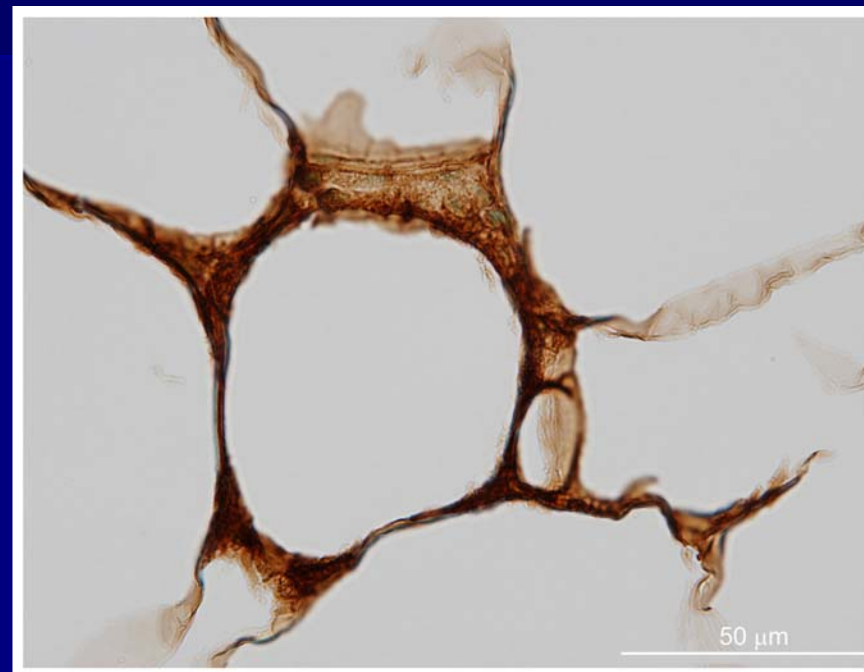
Immunohistochemical localization of TNFα in EWAT of 8 week old db/db mouse



Anti-TNFα



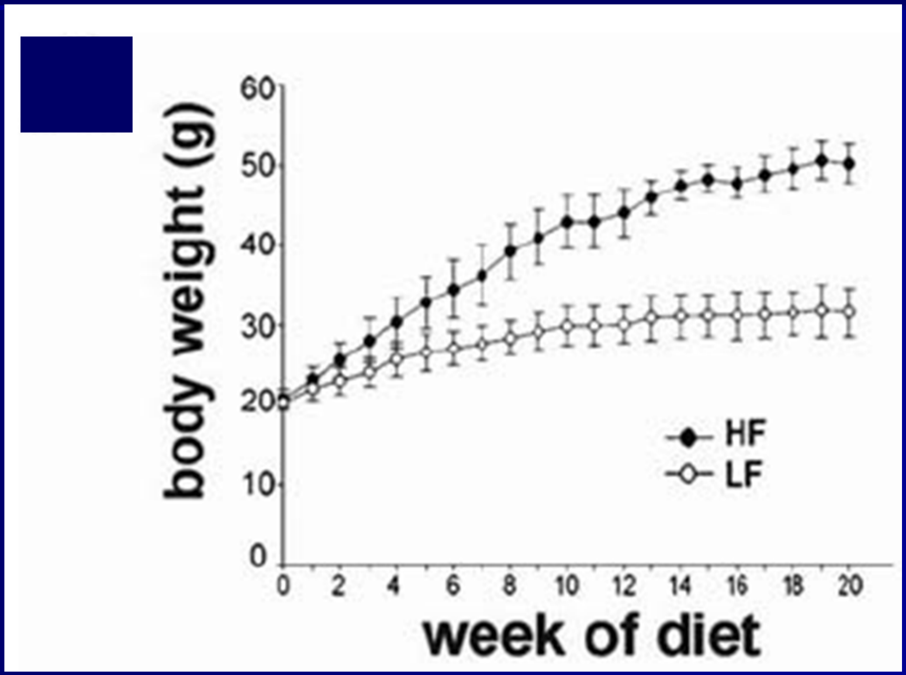
Anti-TNFα + competitive peptide con



Higher magnification of ŅCrown Like StructureÓshown above left

Correlation between CLS Formation and Cytokine Production

High Fat Feeding Increases Body Weight in Mice

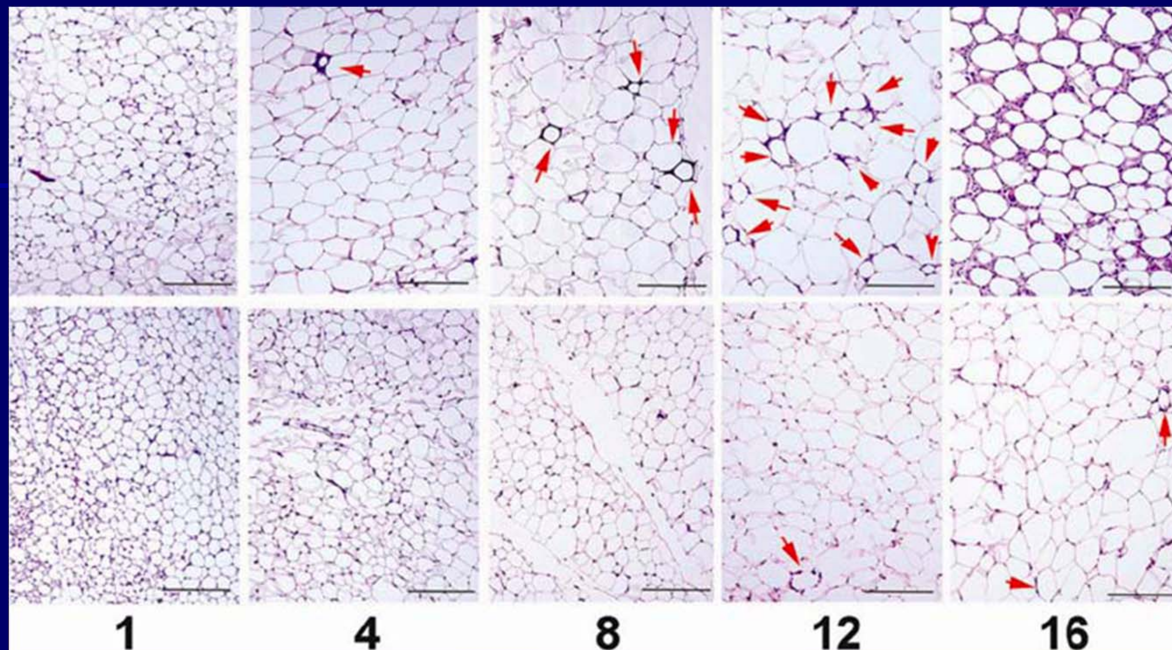


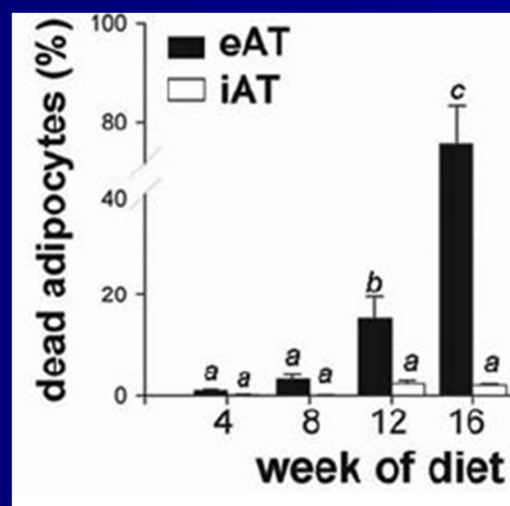
Progressive Adipocyte Death with High Fat Feeding in Intrabdominal Adipose Tissue Starting at 8 weeks

eWAT

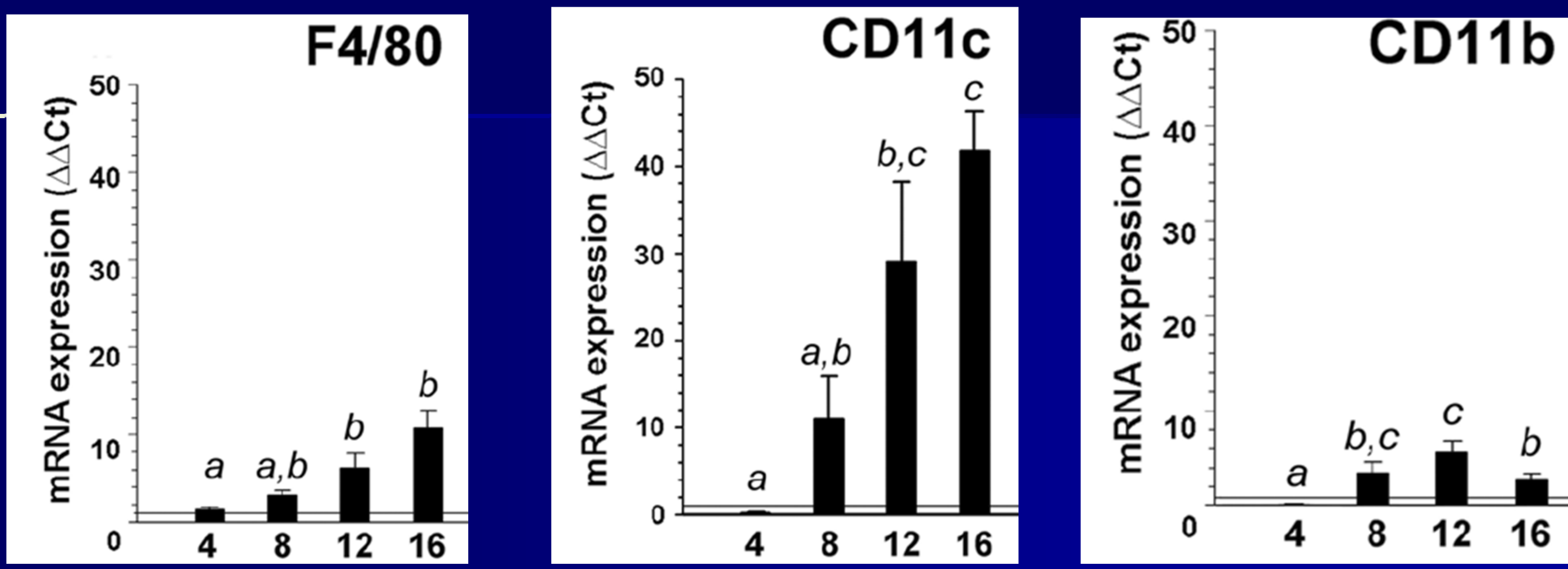
iWAT

Weeks HFD



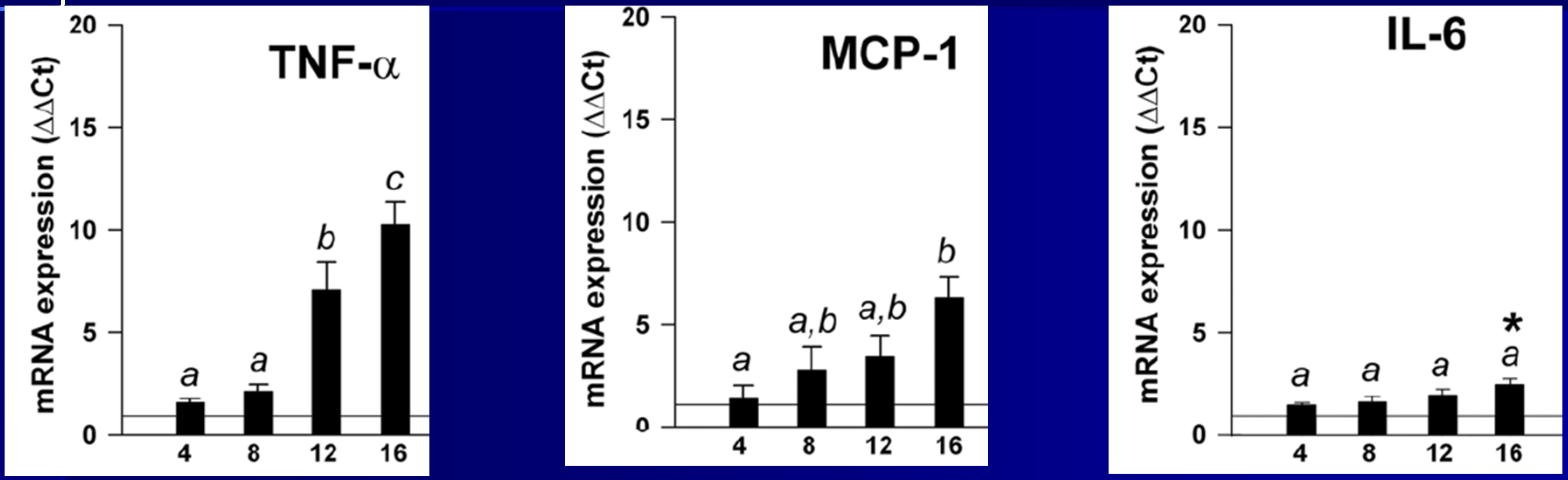


Pattern of Macrophage Markers with HFD Increased CD11c



week of HF diet

Increased Cytokine Production with HFD Parallels Adipocyte Death and Macrophage Infiltration

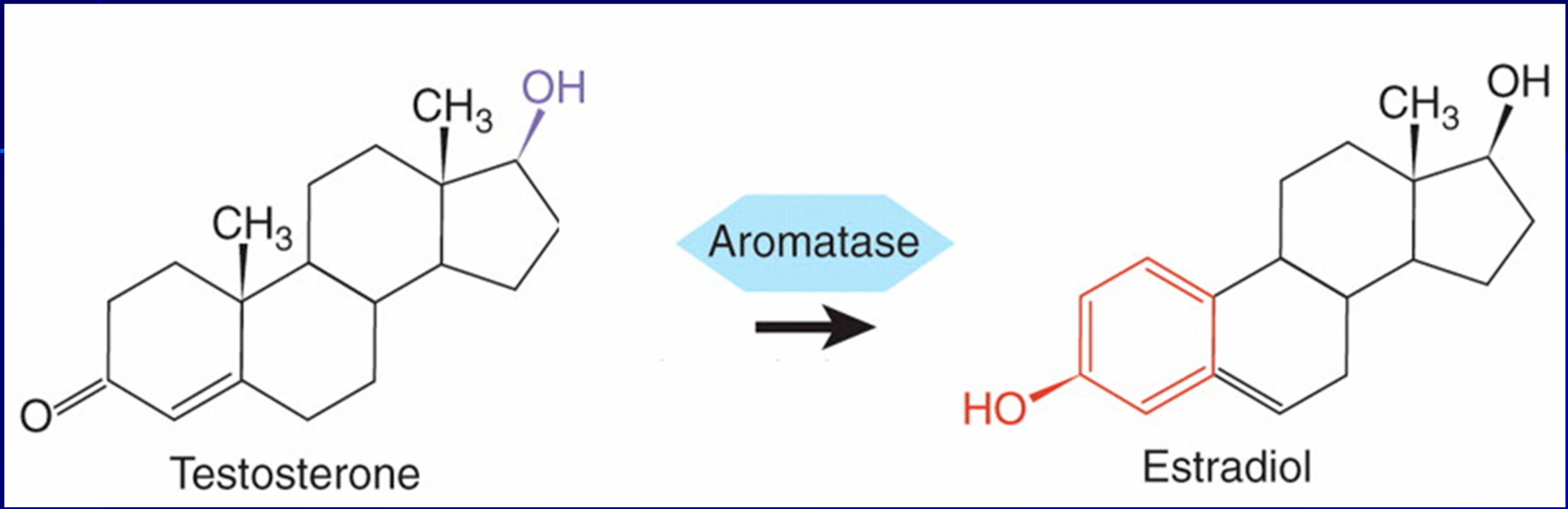


week of HF diet

Adipocyte Death Correlates with Adipose Tissue Inflammation

Estrogen regulation of adiposity; *in vivo* and *in vitro* effects on adipose tissue and muscle metabolism

Estrogen



(Mendelson et al. Science 2005)

- Dominant form is 17β-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 17β-estradiol

Women's Health Initiative

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

Effect of oestrogen plus progestin on the incidence of diabetes in postmenopausal women: results from the Women's Health Initiative Hormone Trial

K. L. Margolis[1,9] · D. E. Bonds[2] · R. J. Rodabough[3] · L. Tinker[3] · L. S. Phillips[4] · C. Allen[5] · T. Bassford[6] · G. Burke[2] · J. Torrens[7] · B. V. Howard[8] · for the Women's Health Initiative Investigators

(Margolis et al Diabetologia 2004)

- **Women randomized to HRT**
  - – Lower BMI
  - – Lower waist : hip ratio
  - – Lower blood glucose
  - – Lower insulin
  - – Greater insulin sensitivity

Comparison of Sham vs Ovariectomized Mice

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)**

Operations performed in female C57Bl/6 mice (OVX / SHAM)

Subset of mice put in metabolic chambers

Tissues harvested

day 0

Weeks 9-11

Week 12

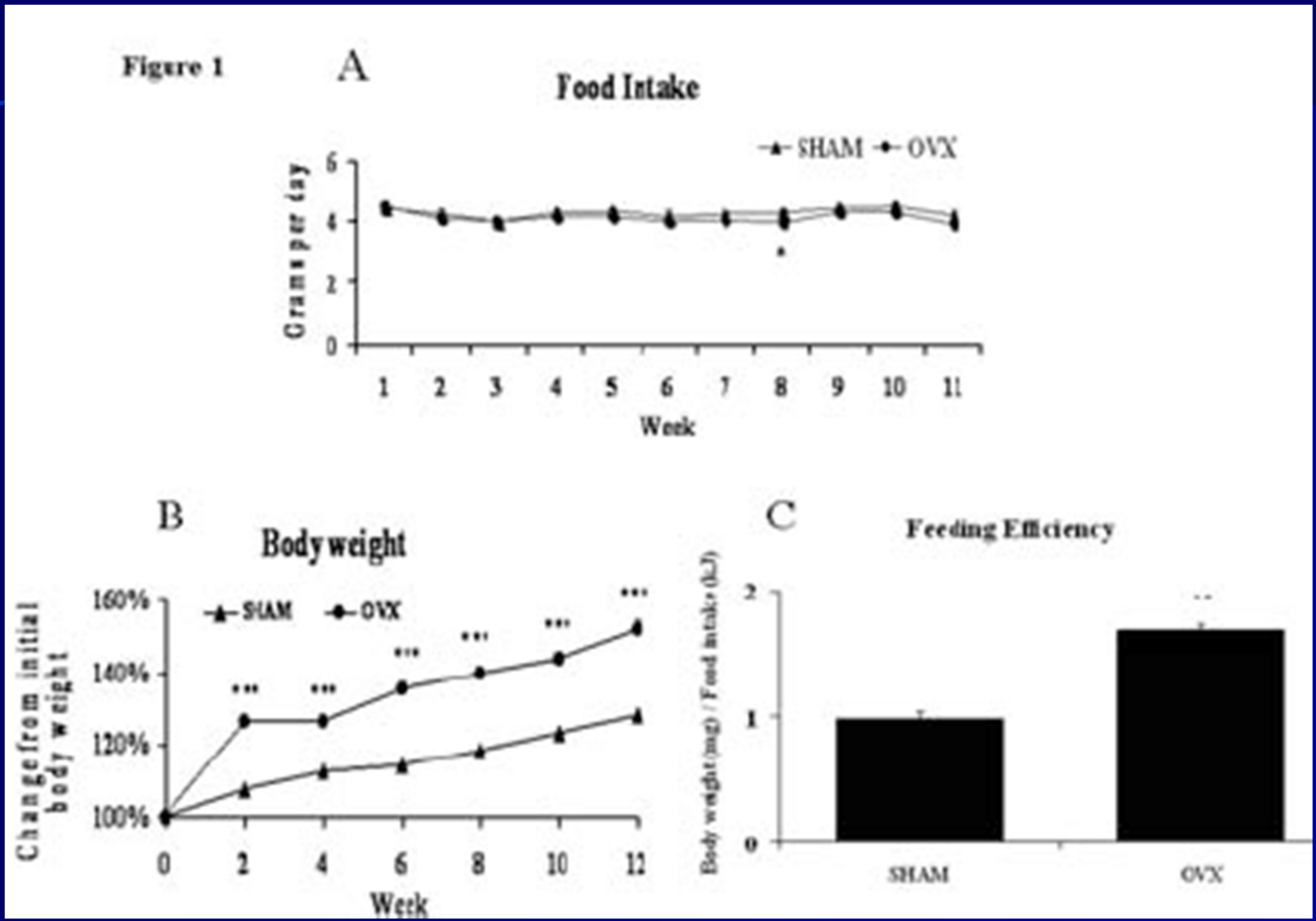
Physiological measurements of female sham-ovariectomized (SHAM) and ovariectomized (OVX) mice 12 weeks post-operation

Variable	SHAM	OVX
Body weight (g)	22.8 ± 0.1	27.5 ± 0.1*
Uterine weight (g)	0.081 ± 0.008	0.040 ± 0.008*
Plasma estradiol (pmol/l)	305.9 ± 176	12.3 ± 6*
Glucose[1] (mmol/l)	5.8 ± 0.26	7.4 ± 0.42*
Insulin[1] (pmol/l)	145.3 ± 23.4	207.8 ± 47.4
Triglycerides[1] (mmol/l)	0.34 ± 0.04	0.34 ± 0.03
NEFA[2] (mmol/l)	0.66 ± 0.17	0.49 ± 0.10

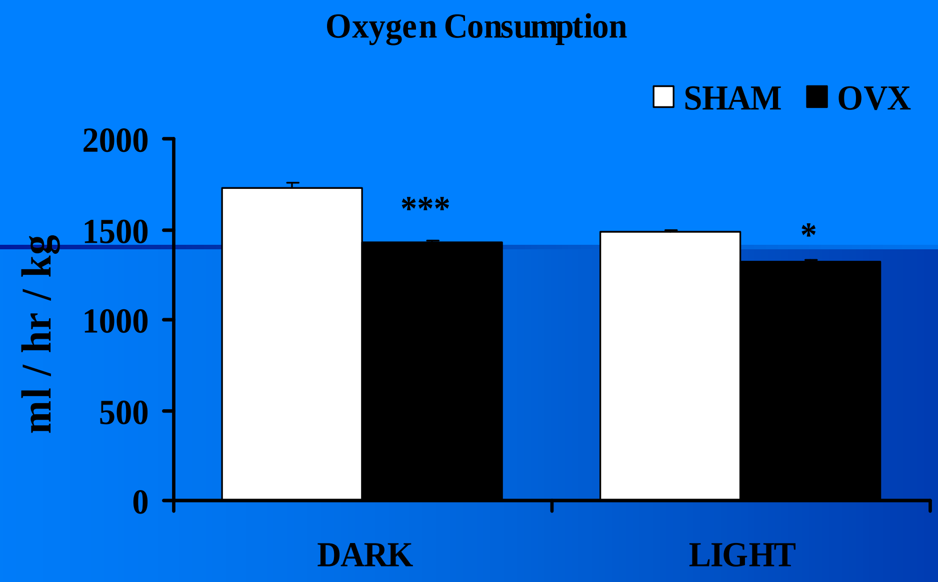
[1]Plasma measures determined after an 8 hour fast. N $\geq$ 7, *$P \leq 0.05$.

[2]Non-esterified fatty acids

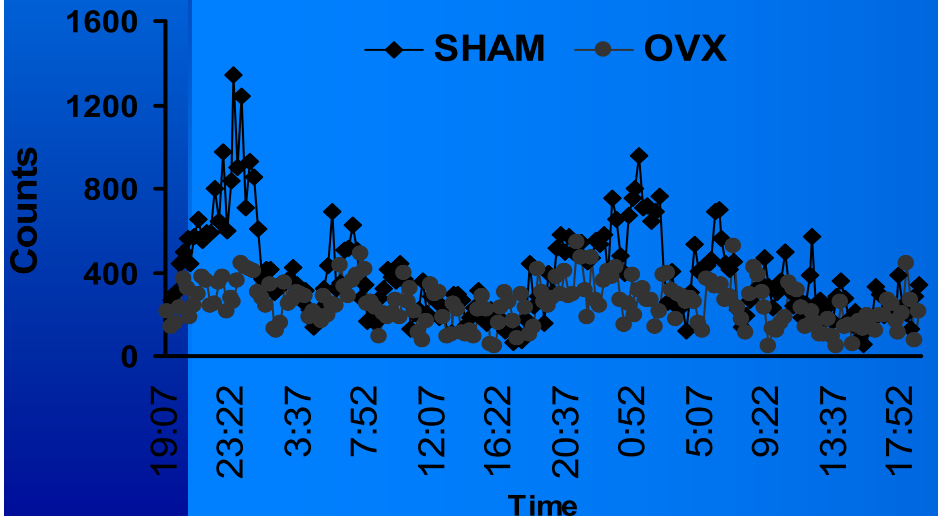
Time course of Body Weight and Food Intake

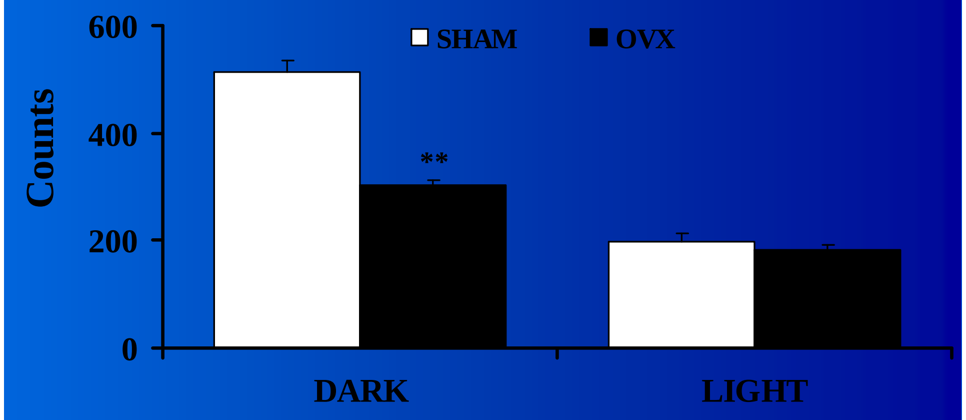


Energy Metabolism

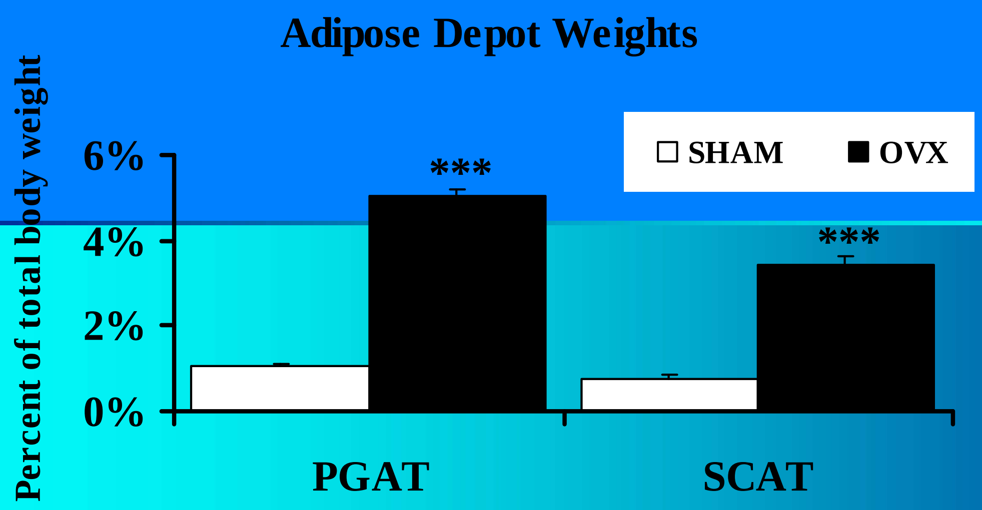


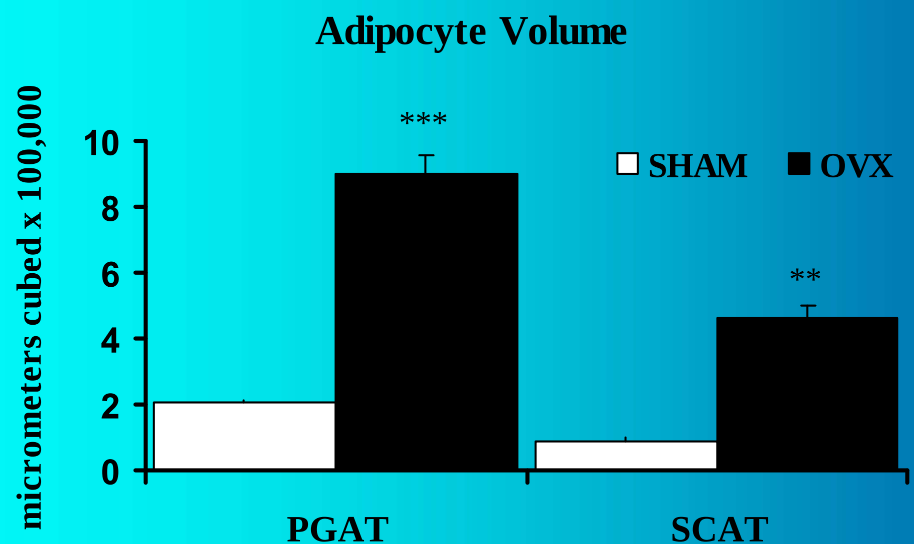
Spontaneous Physical Activity





Adipose Depot Weights and Cell Size



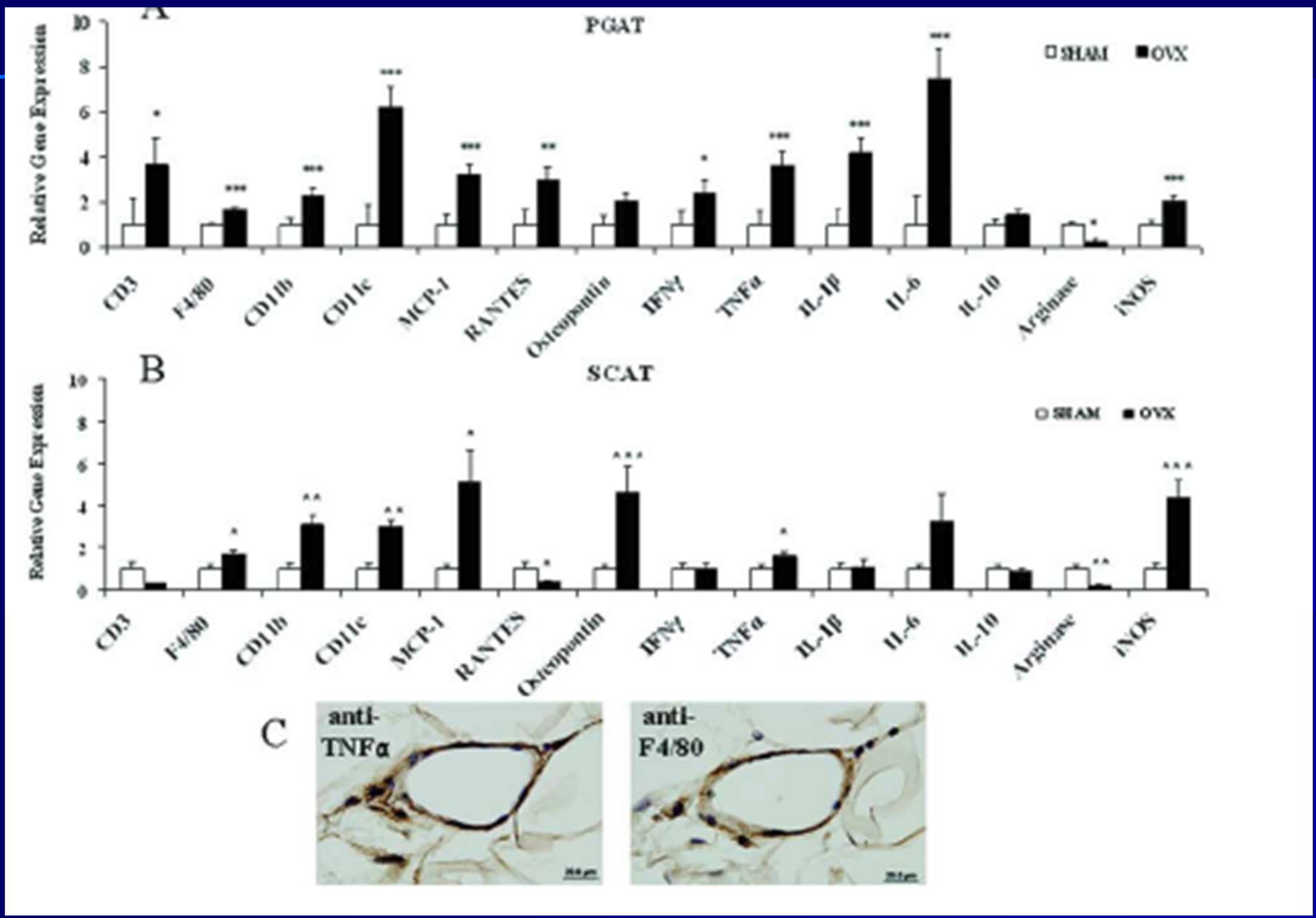


Role of Ovarian Hormones in Regulating Inflammation

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

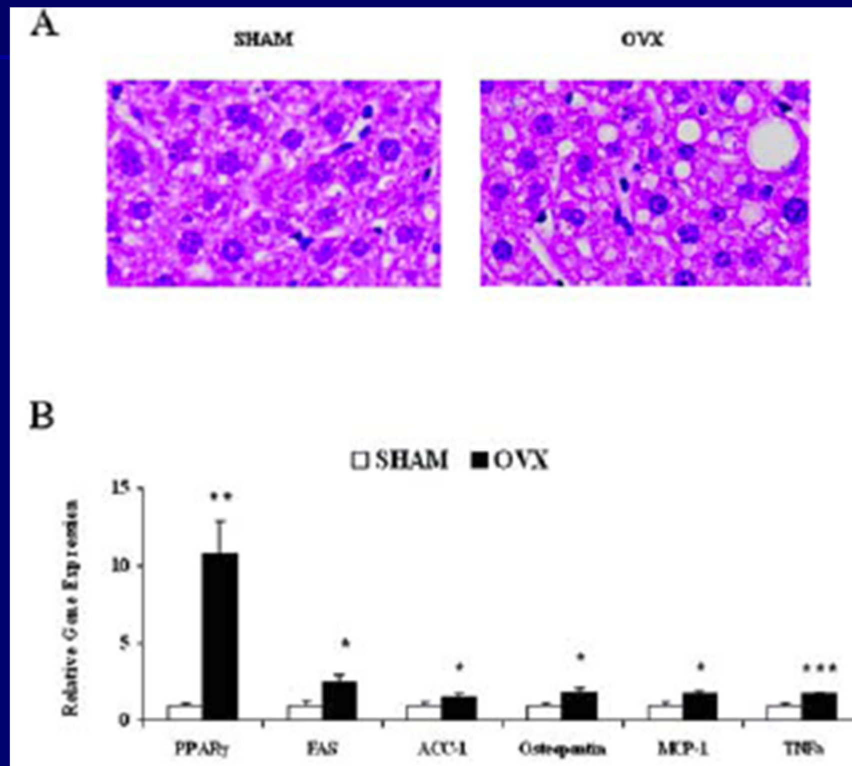
Both intradominal and subcutaneous depots are inflammed

Increased infiltration of T Cells and increased expression of interferon gamma

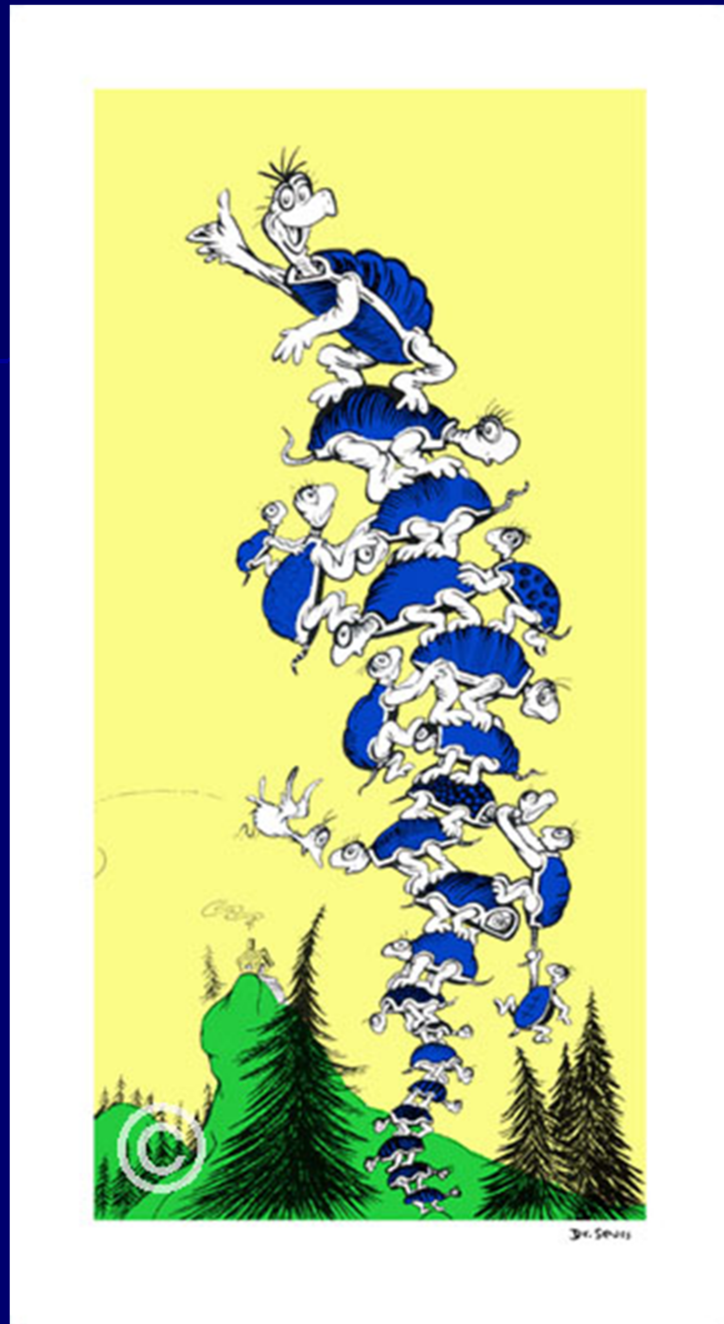
In liver increased inflammation along with increased Lipogenic genes

	F4/80, CD11b	CD11c	CD3	MCP1, osteopontin	RANTES	TNFα	IL-6, IL-1β	IFNγ	IL-10
Perigonadal	↑	↑↑	↑	↑	↑	↑↑	↑,↑	↑	↔
Subcutaneous	↑	↑	↔	↑↑	↓	↑	↑, ↔	↔	↔

Increased Fat Accumulation and Inflammation in Liver



Loss of Ovarian Hormones Promotes Both Obesity and Inflammation



Acknowledgements

Martin Obin

Tara D'Eon

Nicole Rogers

- **Sandra Souza**
- **James Perfield**
- **Katherine Strissel**
- **Hideaki Miyoshi**

- **Michael Mendelsohn**
- **Richard Karas MD PhD**
- **Mark Aronovitz**

- **Susan Fried**

Estrogen decreases adiposity in rodents

- Numerous studies demonstrate ovariectomy increases body fat which can be prevented by estrogen treatment.
- Little is know 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

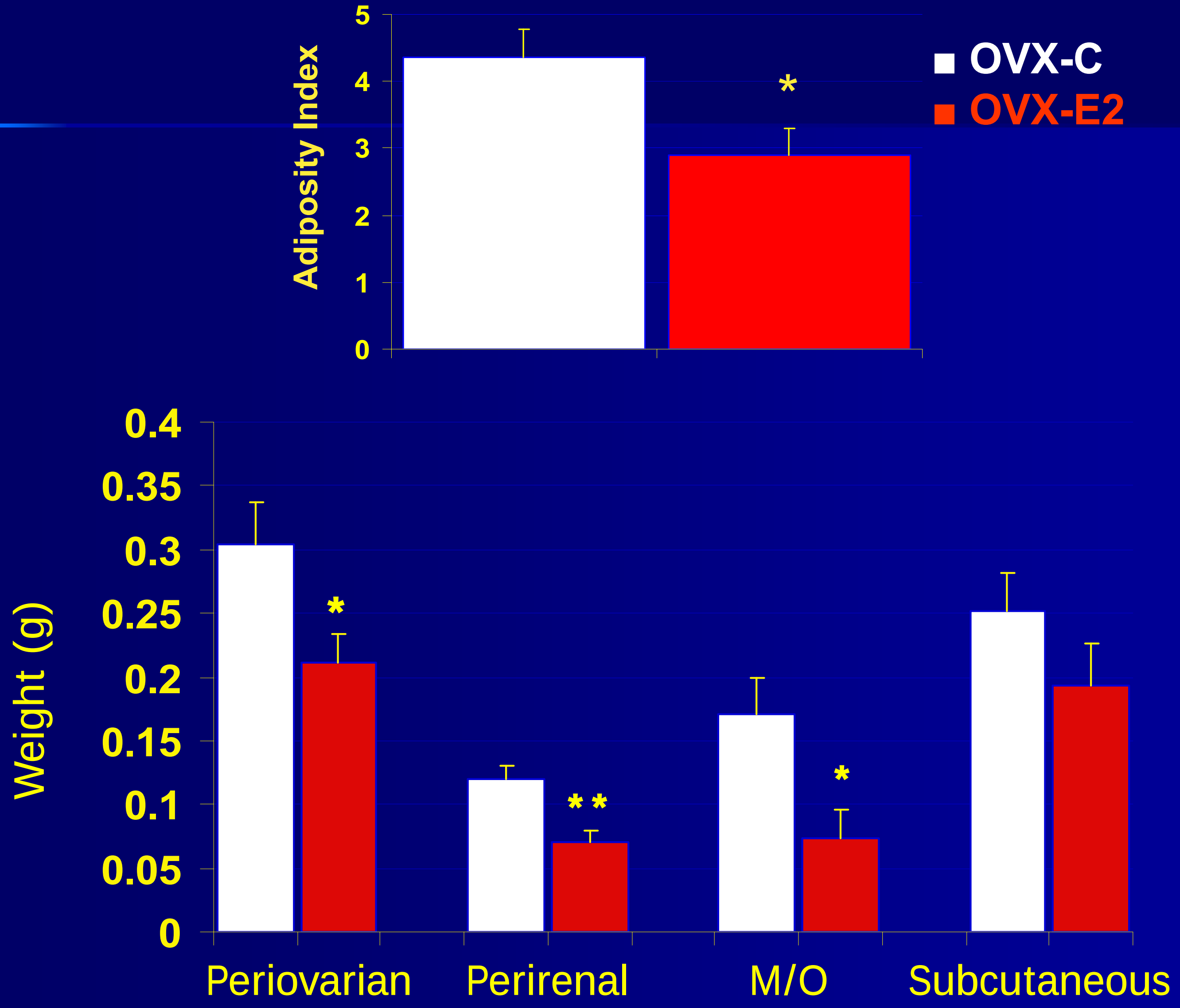
- To determine if chronic estrogen regulates adiposity in mice when food intake is carefully controlled
- To elucidate metabolic and molecular mechanism by which E2 regulates adiposity

D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS: Estrogen regulation of adiposity and fuel partitioning: Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem*, 2005

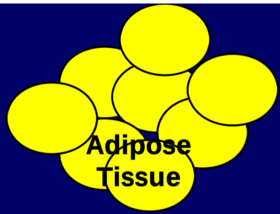
Study Design

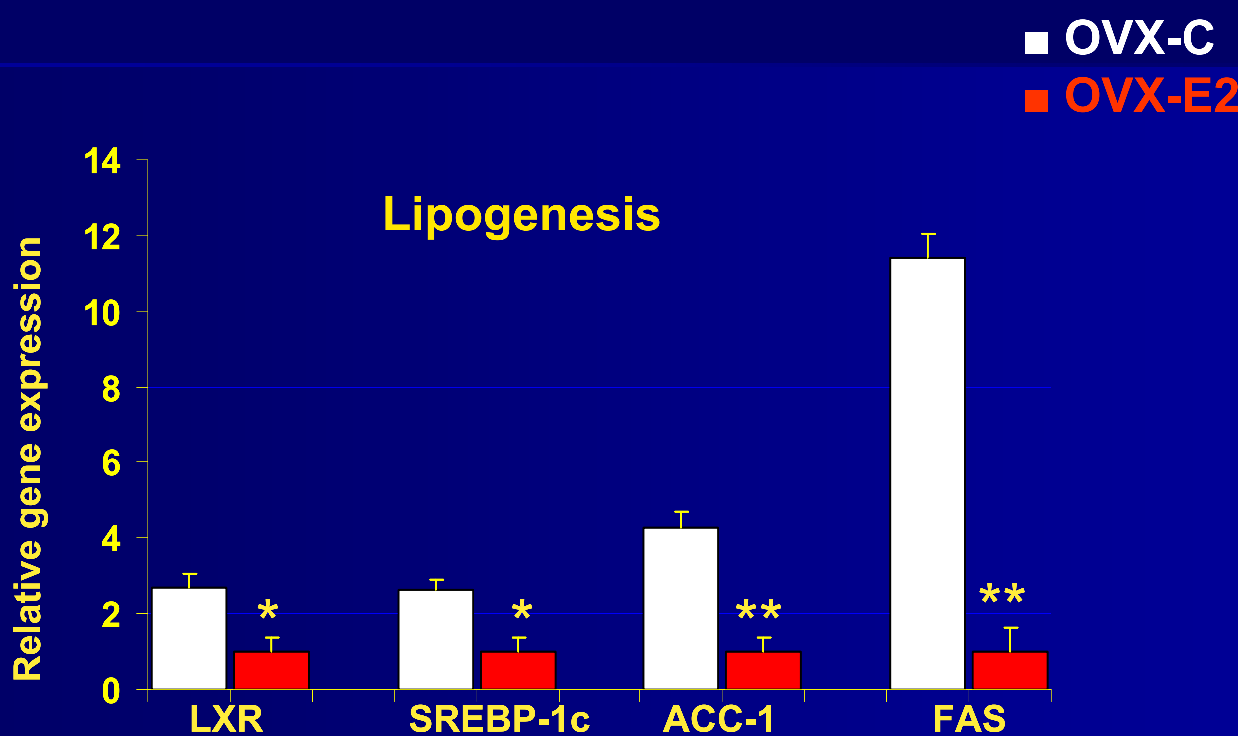
- C57 Bl/6 mice were ovariectomized
- Allowed 7 days to recover
- Randomized to placebo (OVX-C) or estrogen (OVX-E2) subcutaneous implant pellets (n=4-5/group)
- Pairfed daily for 40 days
- Fasted overnight

OVX-E2 mice had lower body fat

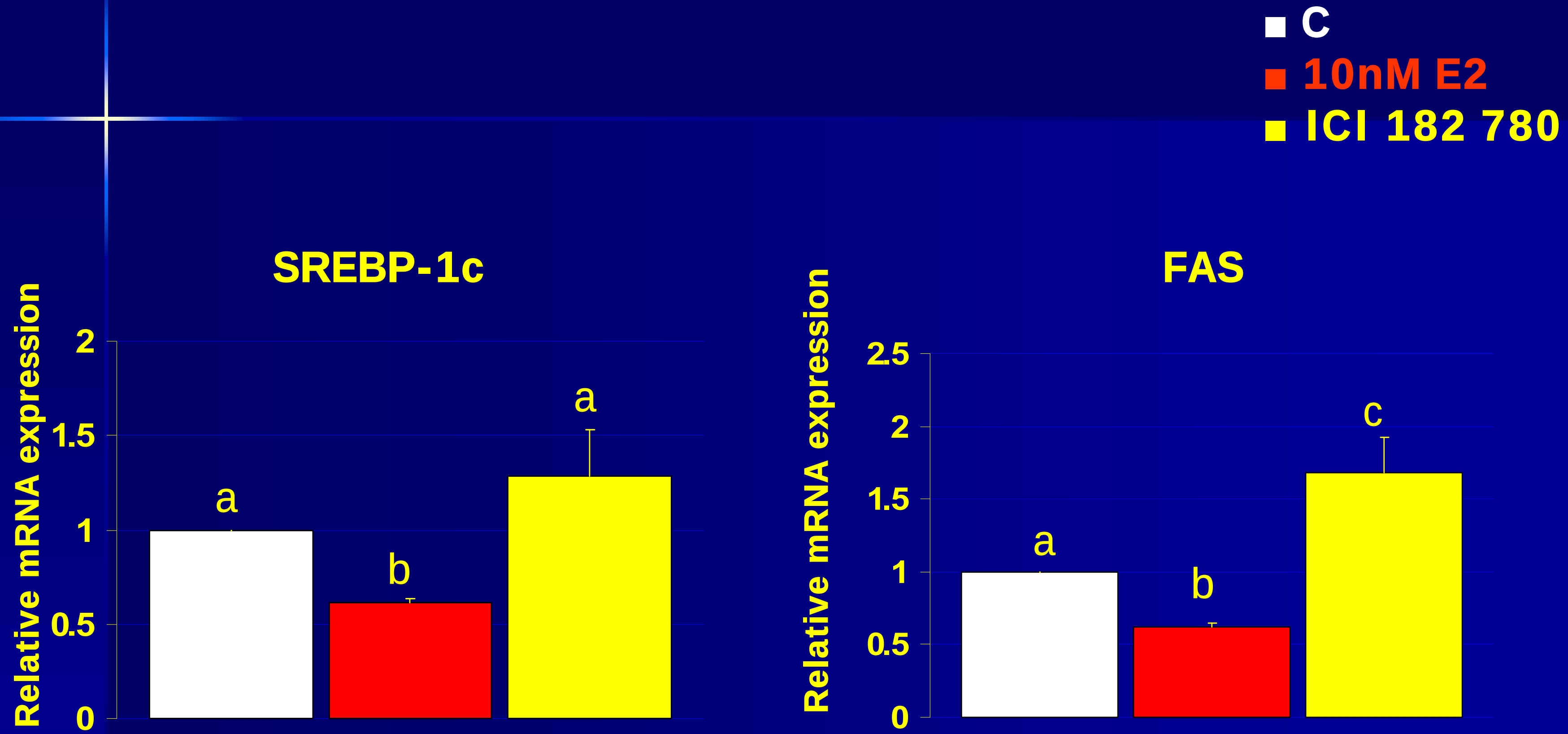


Adipose tissue gene expression cont.

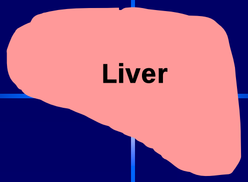


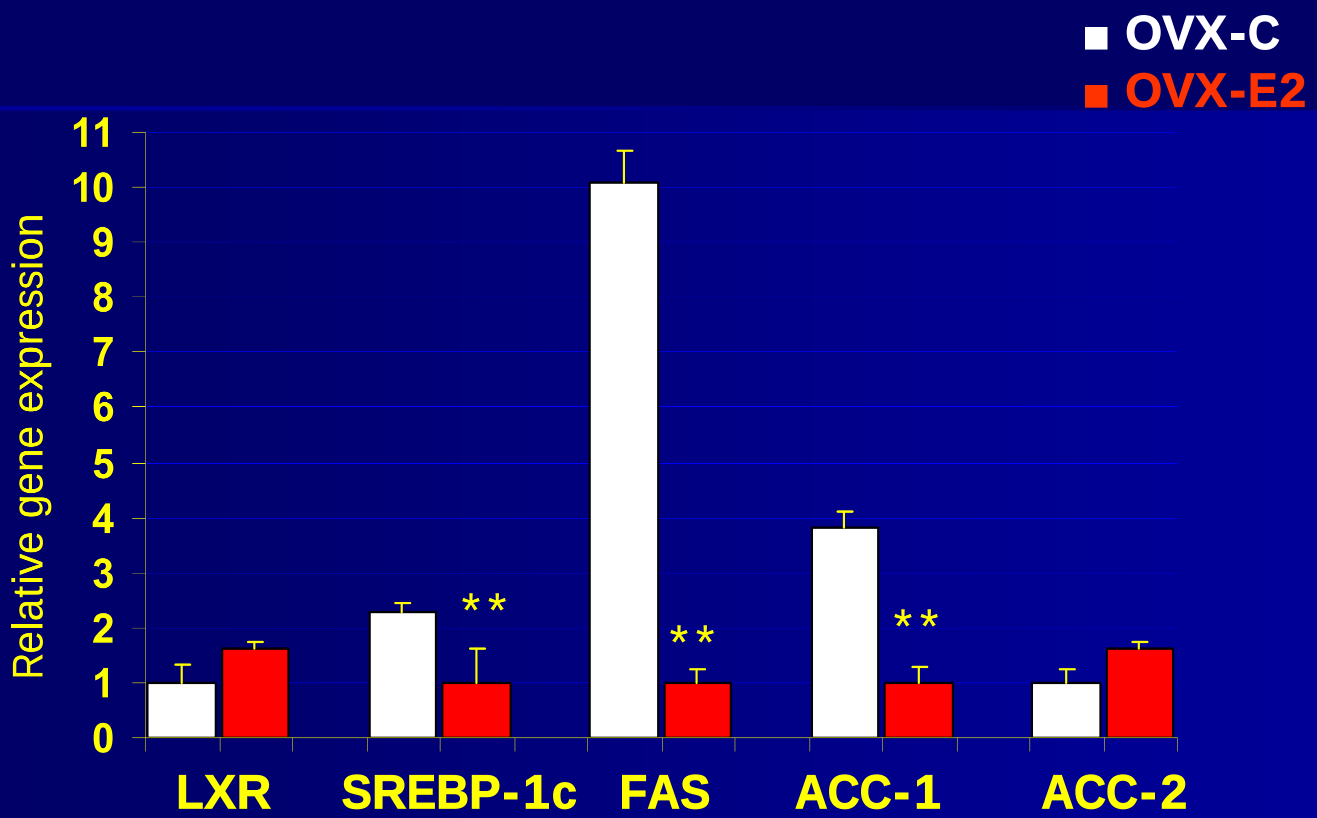


Estrogen downregulates lipogenic gene expression in Cultured Human Adipocytes



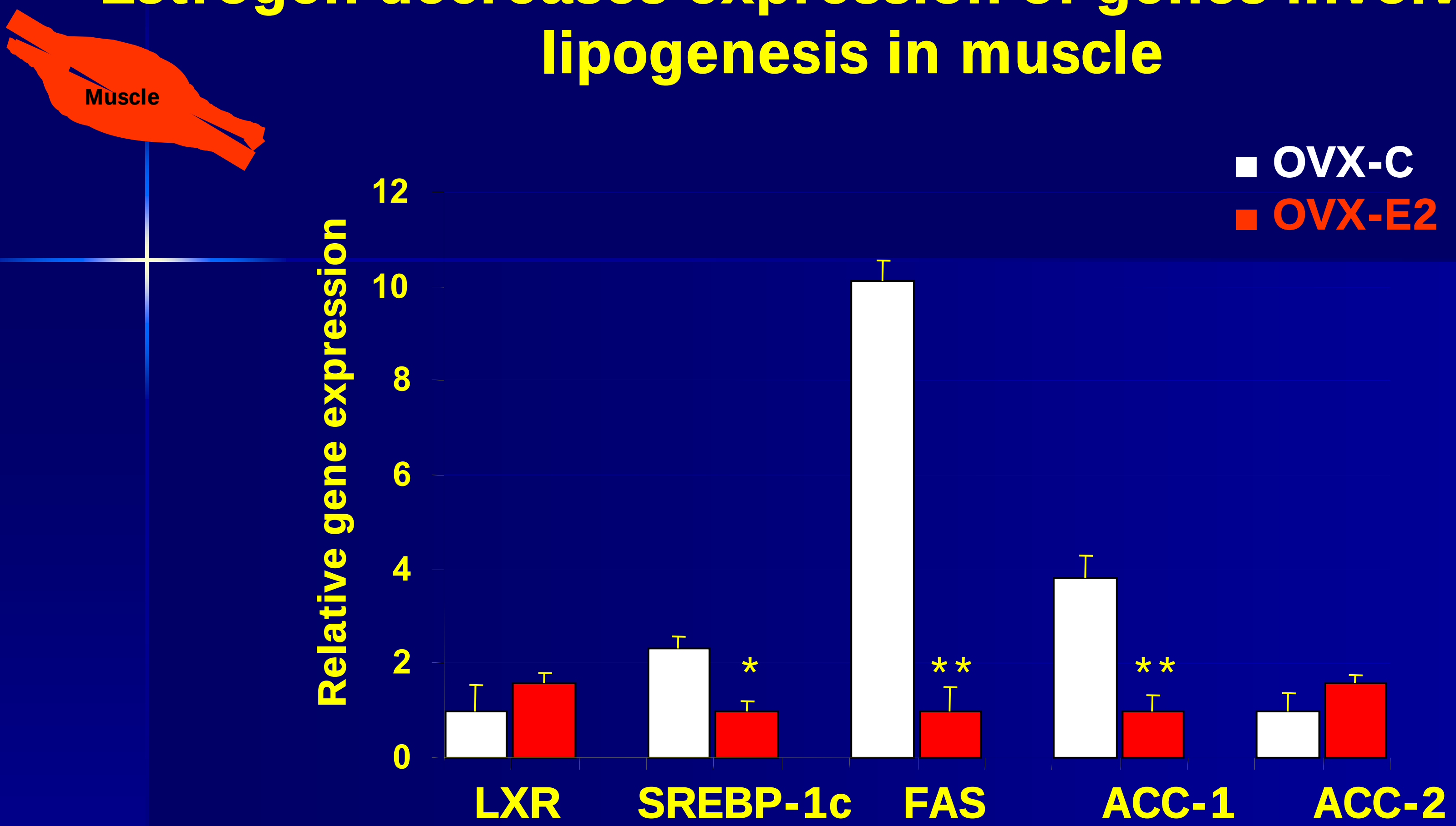
Estrogen decreases expression of genes involved in lipogenesis in liver



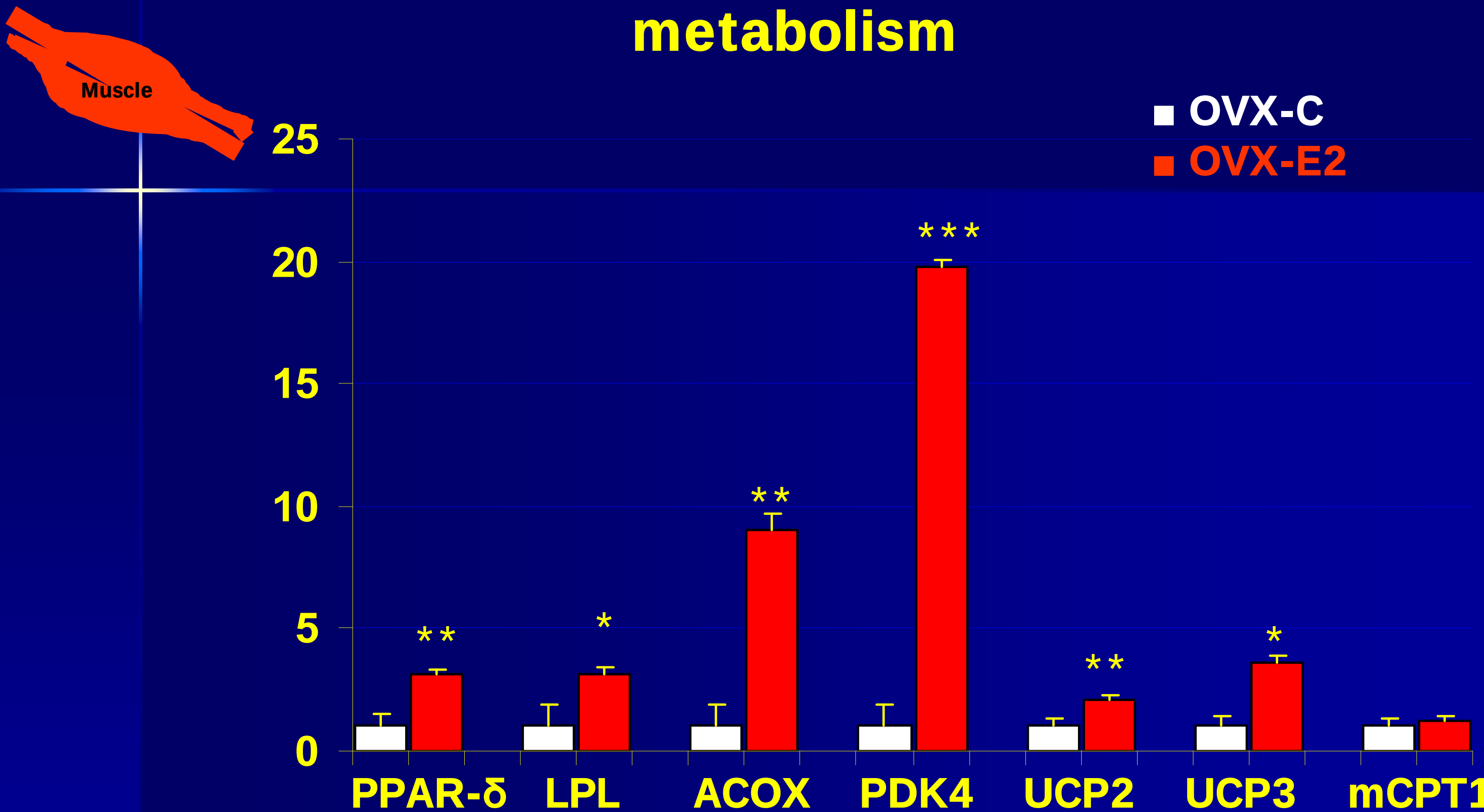


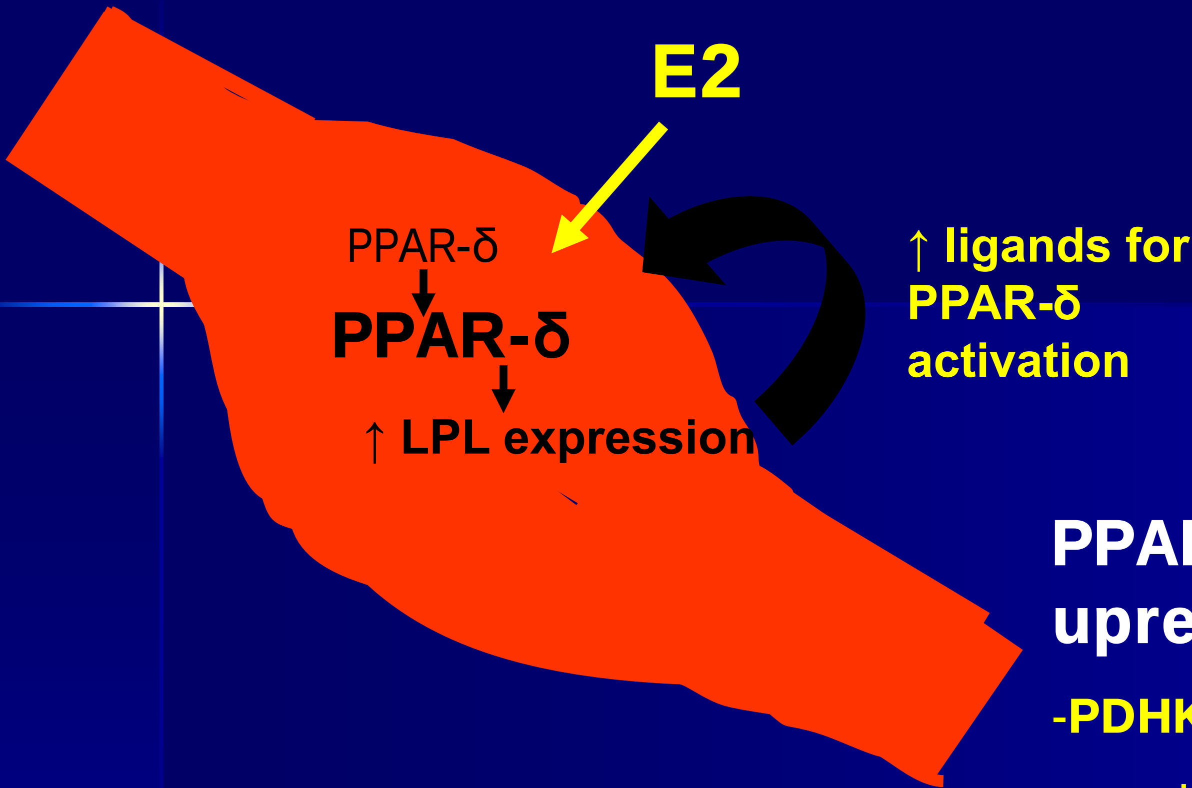
-

Estrogen decreases expression of genes involved in lipogenesis in muscle



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





PPAR-δ regulated 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

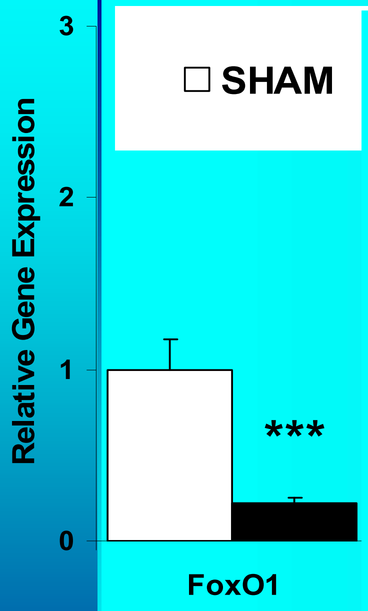
-**UCP2, UCP 3 (3-fold increase)**

- 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<0.01 ***p<0.001

"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

Tara D'Eon
Nicole Rogers
Martin Obin

- Sandra Souza
- James Perfield
- Katherine Strissel
- Hideaki Miyoshi
- Brooke Hussain
- Victoria Viera

Michael Mendelsohn

- Richard Karas
- Mark Aronovitz

Atkins Research Coalition

- Sam Klein
- Sharon Wardlaw
- Jay Horton
- Mike Goran
- Charles Burant

Laurie Goodyear
Carole Wiltzck
Michael Hirschmann

- Susan Fried
- Paul Pilch
- Neal Ruderman
- James Kirkland
- Tamara Pirtskhalava

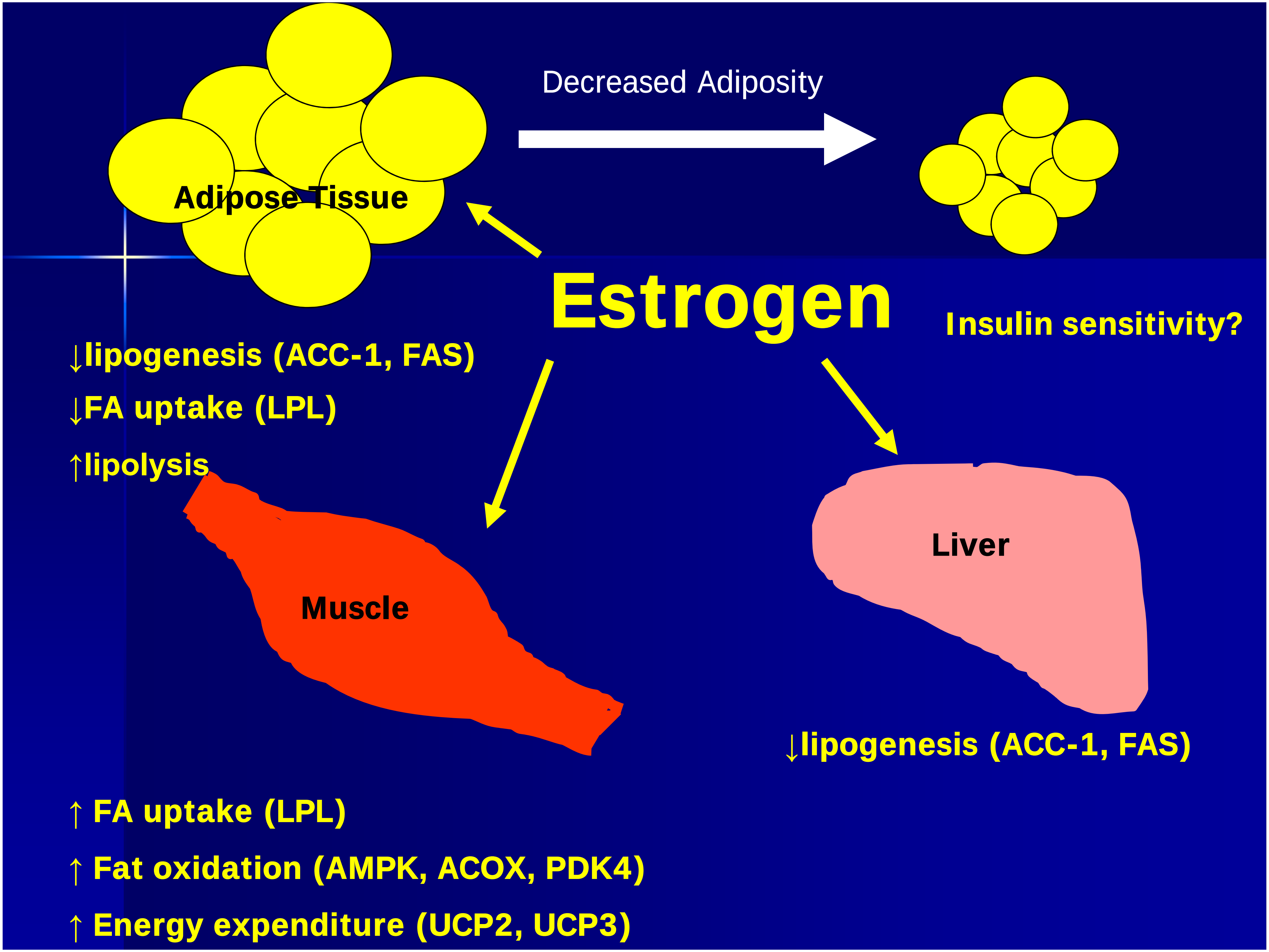
- Steve Smith
- Deb Clegg

- Funding:
- NIDDK
- ADA
- 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.


Decreased Adiposity
Adipose Tissue
Estrogen
Insulin sensitivity?
↓lipogenesis (ACC-1, FAS)
↓FA uptake (LPL)
↑lipolysis
Muscle
Liver
↓lipogenesis (ACC-1, FAS)
↑ FA uptake (LPL)
↑ Fat oxidation (AMPK, ACOX, PDK4)
↑ Energy expenditure (UCP2, UCP3)