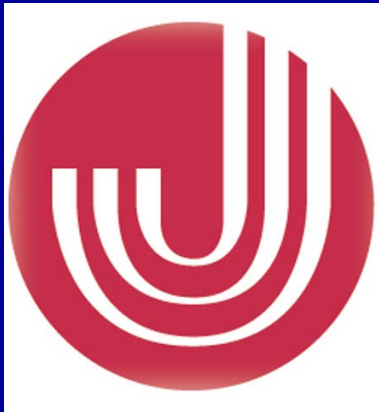


Early Life Origins of Risk for Obesity & Metabolic Disease



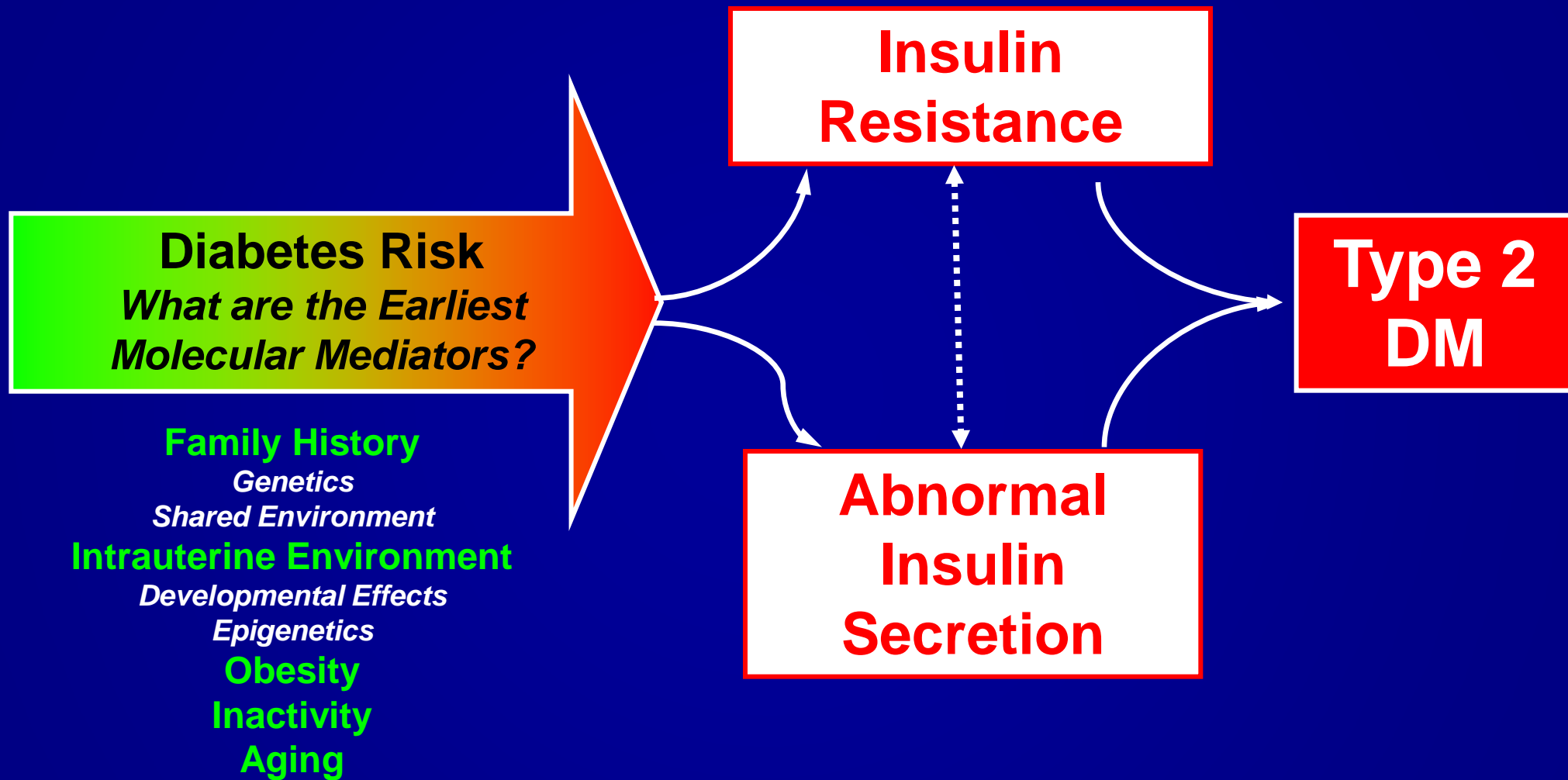
Mary-Elizabeth Patti MD
Endocrinologist and Investigator
Joslin Diabetes Center
Harvard Medical School

**Nutrition & metabolism are important at all stages of life
...*especially* pregnancy & early childhood.**

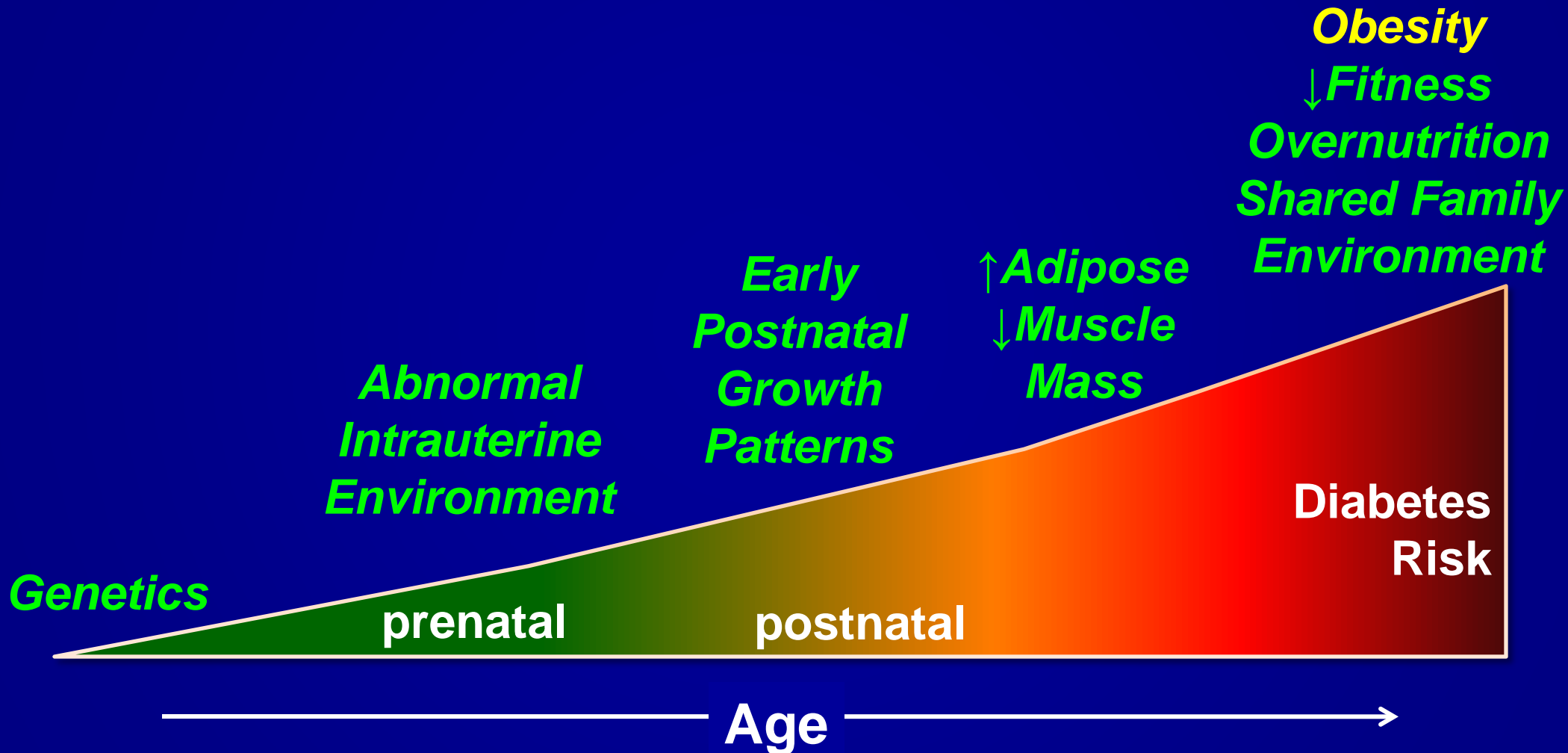
**Nutrition and metabolic status of *both* mother and father
are important for health of offspring.**



Progressive Pathogenesis of Type 2 DM



Diabetes Risk Increases Progressively Throughout the Lifespan



OCTOBER 4, 2010

Environment Special:
The oceans—why 70%
of our planet is in danger

The Facebook Movie:
The secret history of
social networking

TIME



**How the
first nine
months
shape
the rest
of your life**

The new science
of fetal origins

BY ANNIE MURPHY PAUL

www.time.com

October 2010

OVERVIEW

- **Maternal nutrition and metabolism and developmental programming as a risk factor for adult obesity and type 2 DM**
- **Mouse model of metabolic risk induced by maternal undernutrition**
- **Metabolic disease can be transmitted through *both the paternal & maternal lineage* to subsequent generations, even without further experimental nutritional modulation**
- **Potential epigenetic & metabolic mediators of these phenotypes**

The Original Fetal Origins Epidemiologist



**Ethel Margaret
Burnside**

Chief Health Visitor and Lady
Inspector of Midwives
Hertfordshire, England

Weight at Birth.	Weight 1st Year	Food	No. of Visits.	Condition, and Health Visits		
				W	V	T
8 1/2 lbs	24 1/2 lbs	B.	11	4		
Healthy & well developed.				Buckland School.		
7 lbs	18 1/2 lbs	B.	12	4		
born to Mary Jane A. Jackson.				Not much post		
8	20	B.C.	11	4	4	
Pl. born in first part but finally still per 27 yrs. old.						
8 1/2	22	B.B.	9	4	4	
Healthy & normal.				Buckland School.		

- Sample ledger with birth weight and weight at 1 yr, 1911-1948
- Data accessed by **David Barker**, who demonstrated link between low birth weight, accelerated early postnatal growth, and adult disease risk

The Dutch Hunger Winter

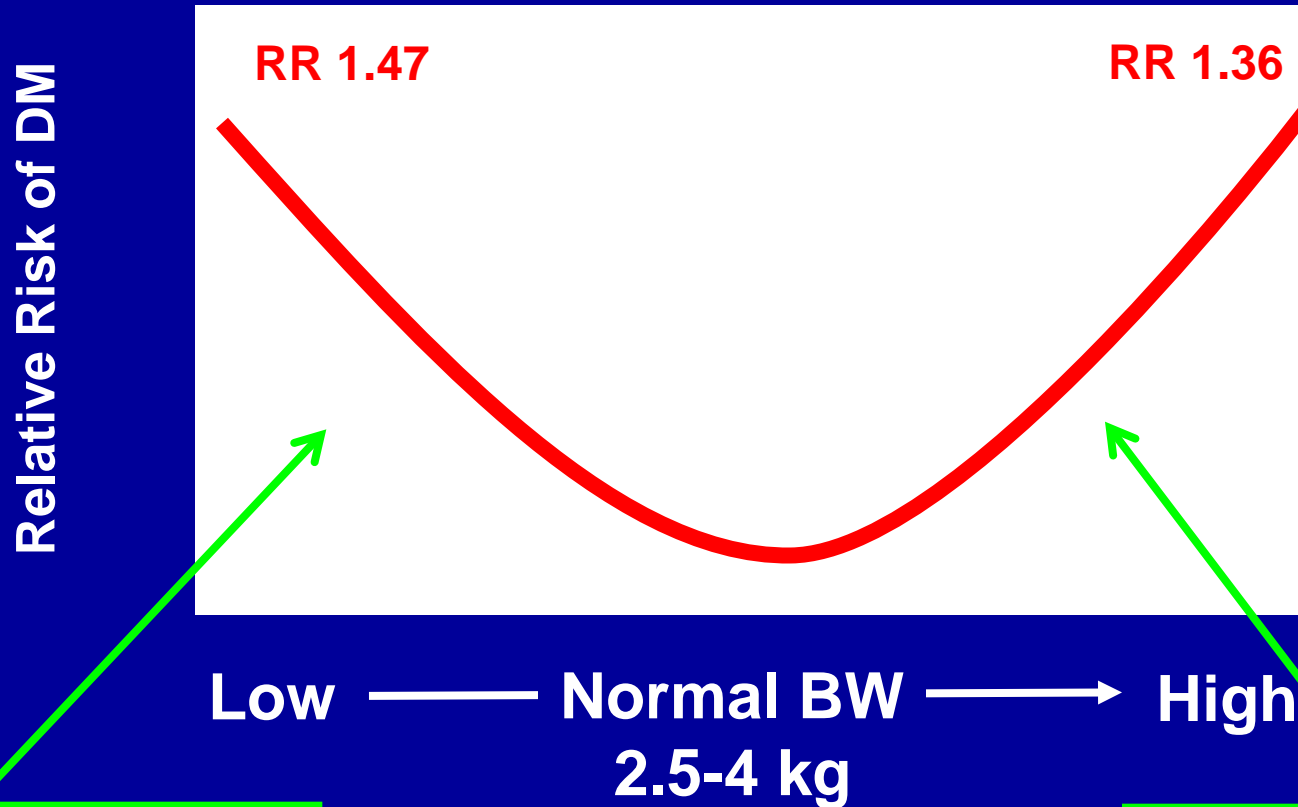
Rotterdam, 1945



- Western Netherlands affected by acute famine by the end of World War II
- Official rations: 400-800 calories/day
- Women exposed to famine during the 2nd and 3rd trimester of pregnancy delivered small babies.
- These low birth weight babies (exposed to famine *in utero*) had a higher prevalence of adult diseases:
 - diabetes
 - cardiovascular disease (heart attack, stroke)
 - hypertension
 - obesity

What Did These and Other Studies Teach Us?

Both Low and High Birth Weight Associated with Risk of Metabolic Disease in Humans



Higher Risk with LOW Birth Weight

Higher Risk with HIGH Birth Weight

Genes, Adverse Fetal Environment (UN or Obesity) → Poor Growth → LBW

Maternal Gestational Diabetes & Excess Weight Gain

Birth Weight is a *Biomarker of Development* Influenced by Nutrition, Environment, and Genetics



Placenta

Hypertension
Vascular anomalies
Hypoxia



Nutrition/Metabolism

Excess maternal weight gain & obesity
Undernutrition
Hyperglycemia
Iron deficiency

STRESS

Stressors

Infection
Glucocorticoids
Maternal smoking



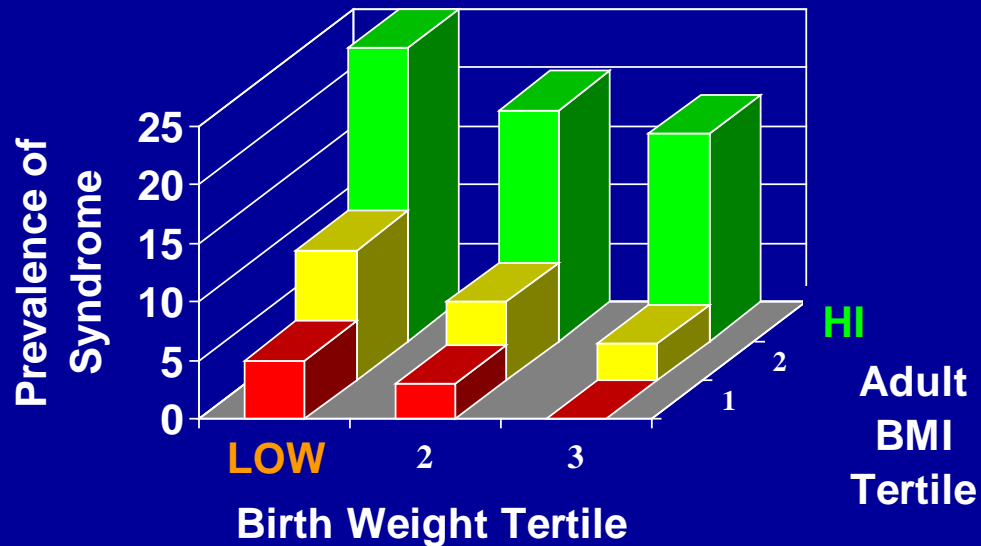
Genes

Ethnicity
Chromosomal anomalies
DNA polymorphisms
GCK, PPAR-γ, TCF7L2, GR, HNF4
Epigenetic regulation
Igf2/H19, Grb10



Postnatal Catch-Up Growth and Obesity During Adult Life Further Increase Risk

Metabolic Syndrome Risk



LBW Baby

Catch Up Growth

Thin Adult
at Higher Risk

Obese Adult
At Very High Risk

“DEVELOPMENTAL PROGRAMMING”

Alterations during critical periods of development influence risk of adult disease



Potential Mechanisms?

Patti Lab Models of Developmental Programming and DM Risk

Rodent models:

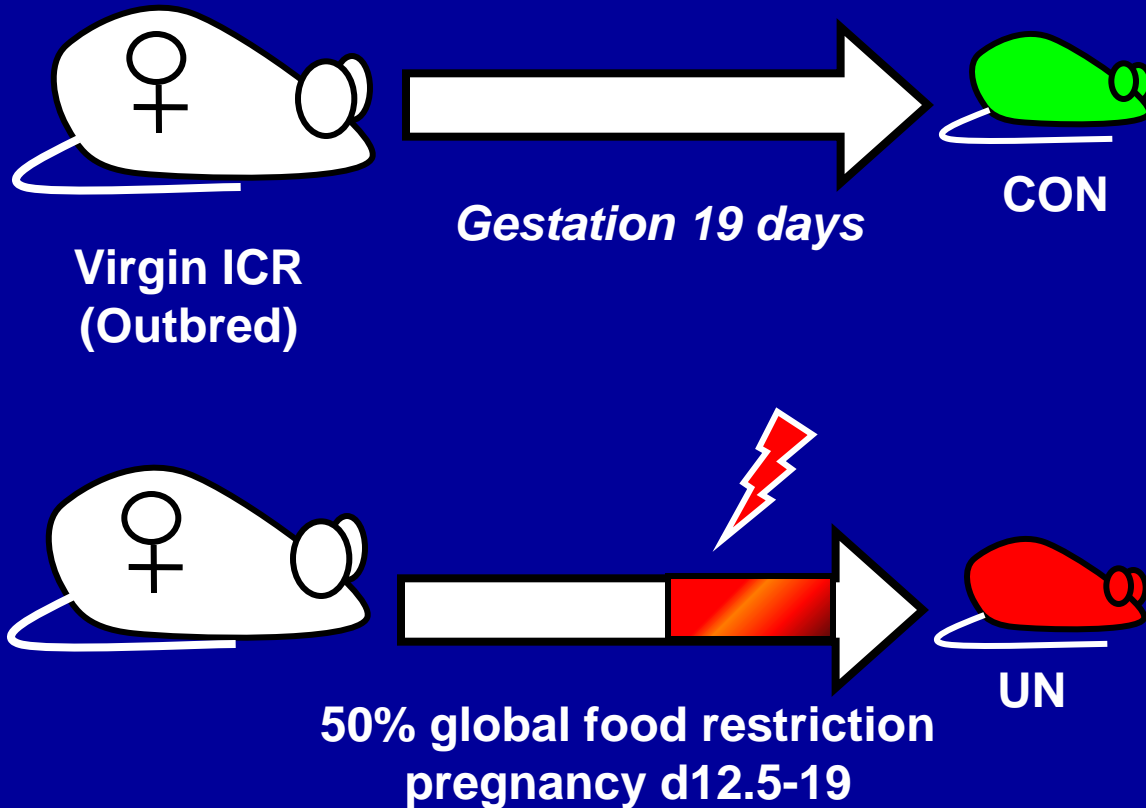
- **Exposure to maternal undernutrition**
- **Exposure to genetically-determined maternal insulin resistance – even without maternal diabetes or obesity**

Human studies:

- **Humans with history of low birth weight**

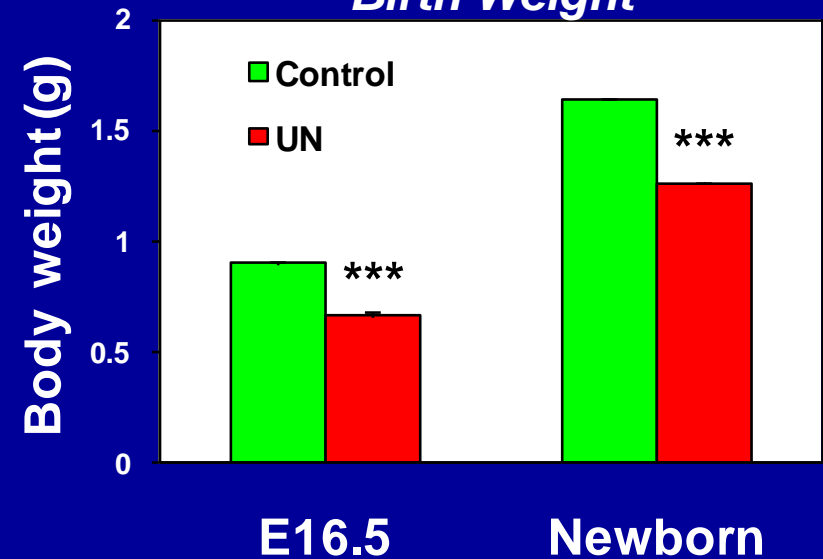
Mouse Model of Developmental Programming

Protocol: Exposure to Maternal Undernutrition



Effects in Offspring

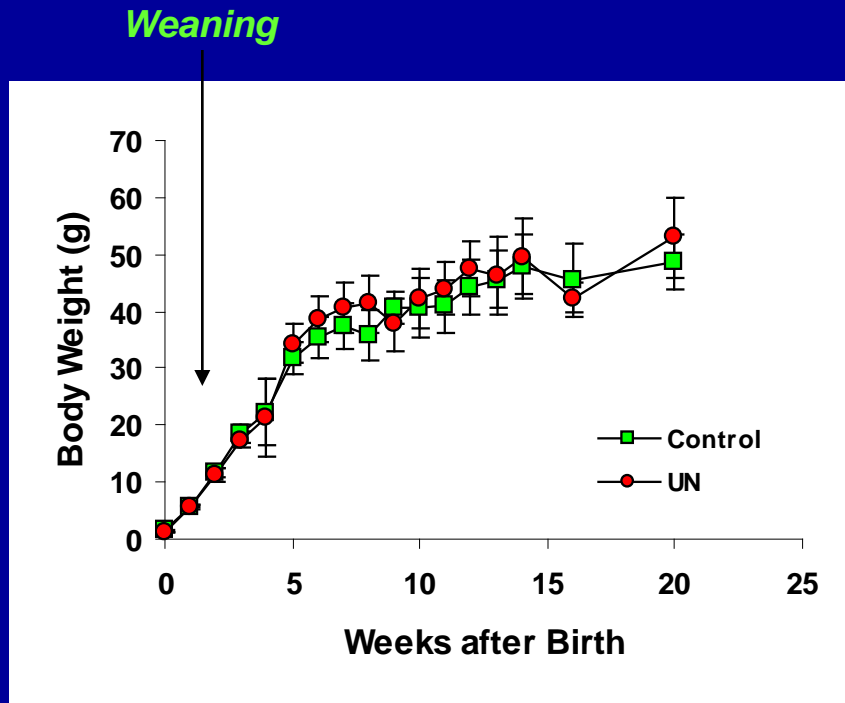
Reduced Fetal Growth & Birth Weight



Litter size equalized at birth
Effects similar with cross-fostering

Jimenez & Patti 2005, 2006, 2009.
Isganaitis & Patti 2009.
Woo & Patti 2011.

Undernutrition-Exposed Mice Have “Catch-Up” Growth & Maintain Normal Body Weight during Adult Life



Control Mouse

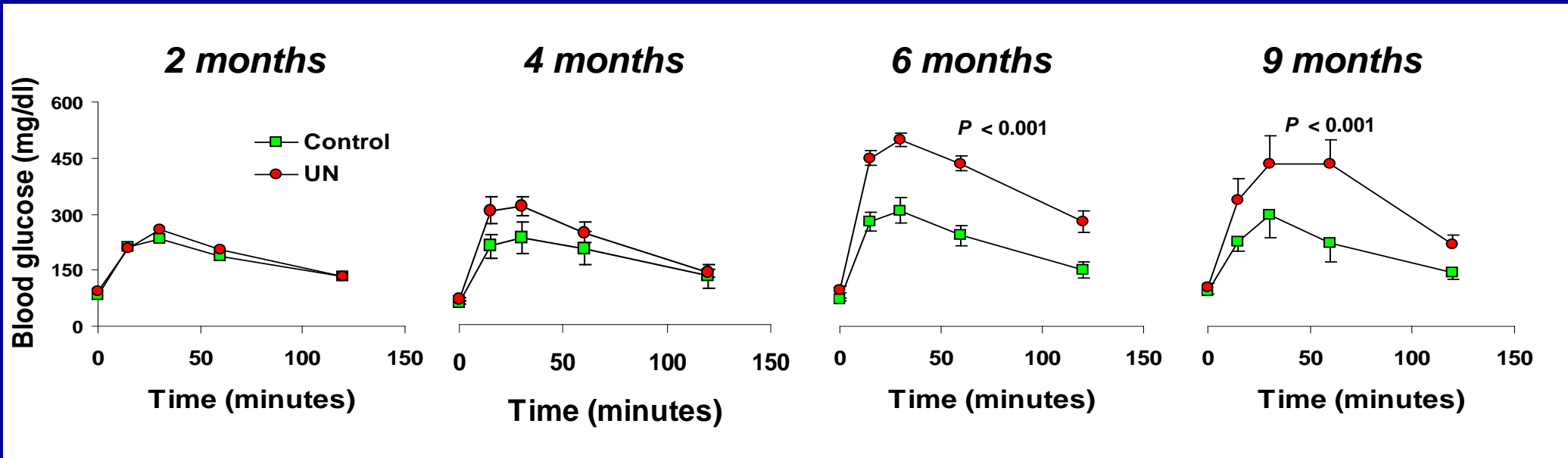


LBW Mouse



Purina 9F Chow at weaning

Exposure to Maternal Undernutrition Results in Impaired Glucose Tolerance and DM in Offspring Mice

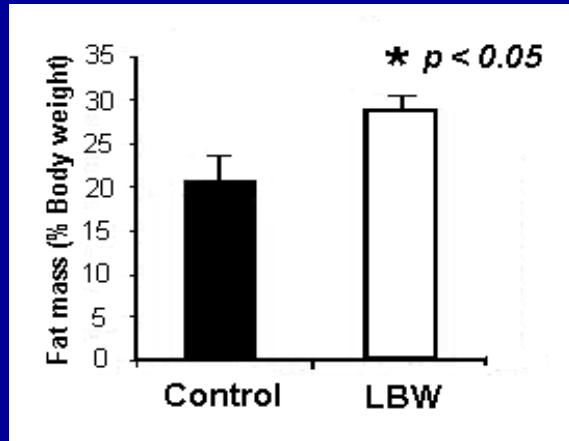


5X Increase in Risk of Diabetes in UN Mice

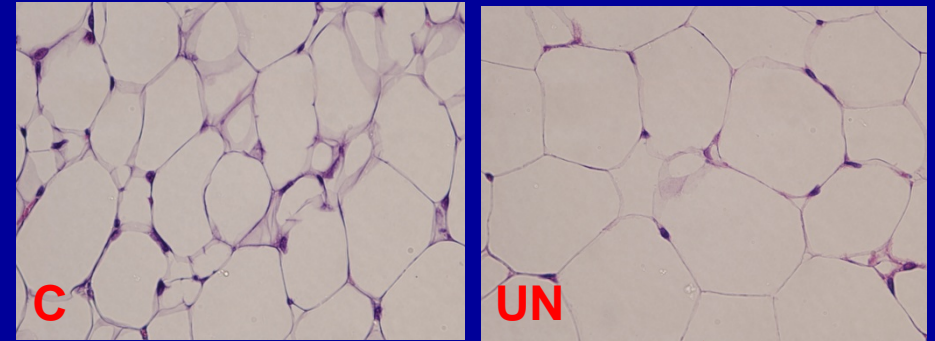
*Normal mice, outbred ICR; normal chow during postnatal life (Purina 9F)
Intraperitoneal GTT; 2g glucose/kg body weight*

Adiposity is Increased in Mice Exposed to Maternal UN

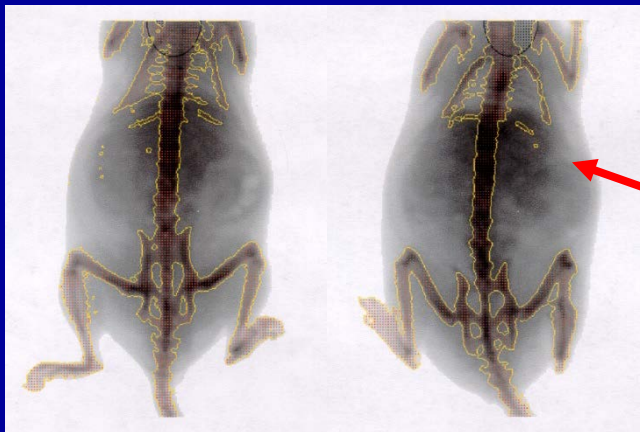
DEXA Scan, Age 12 Months
Chow Diet



Gonadal Adipose Tissue Histology, 3 wks



Larger Adipocytes
↑ Lipogenic Gene
Expression



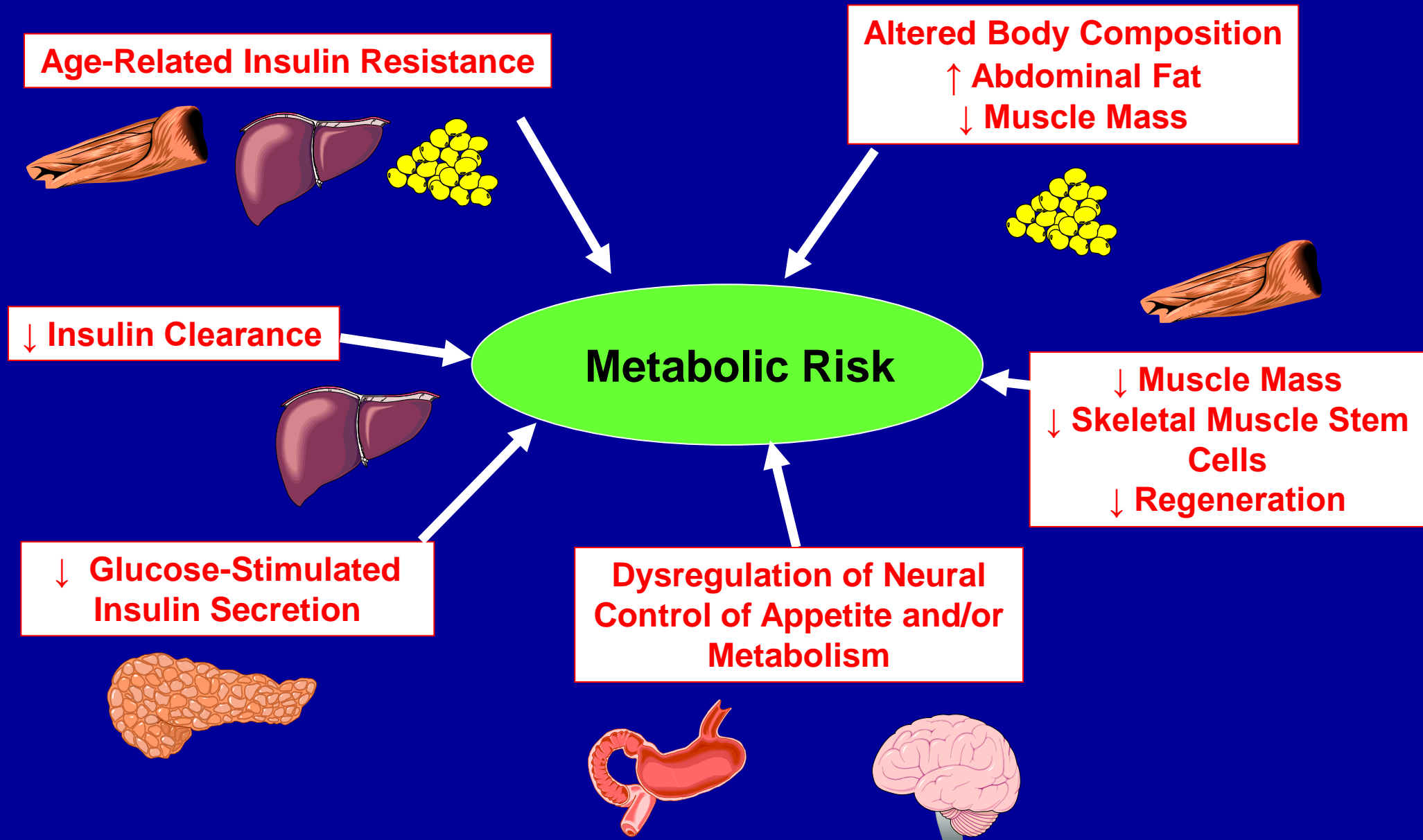
More abdominal fat

Aris
Lytras



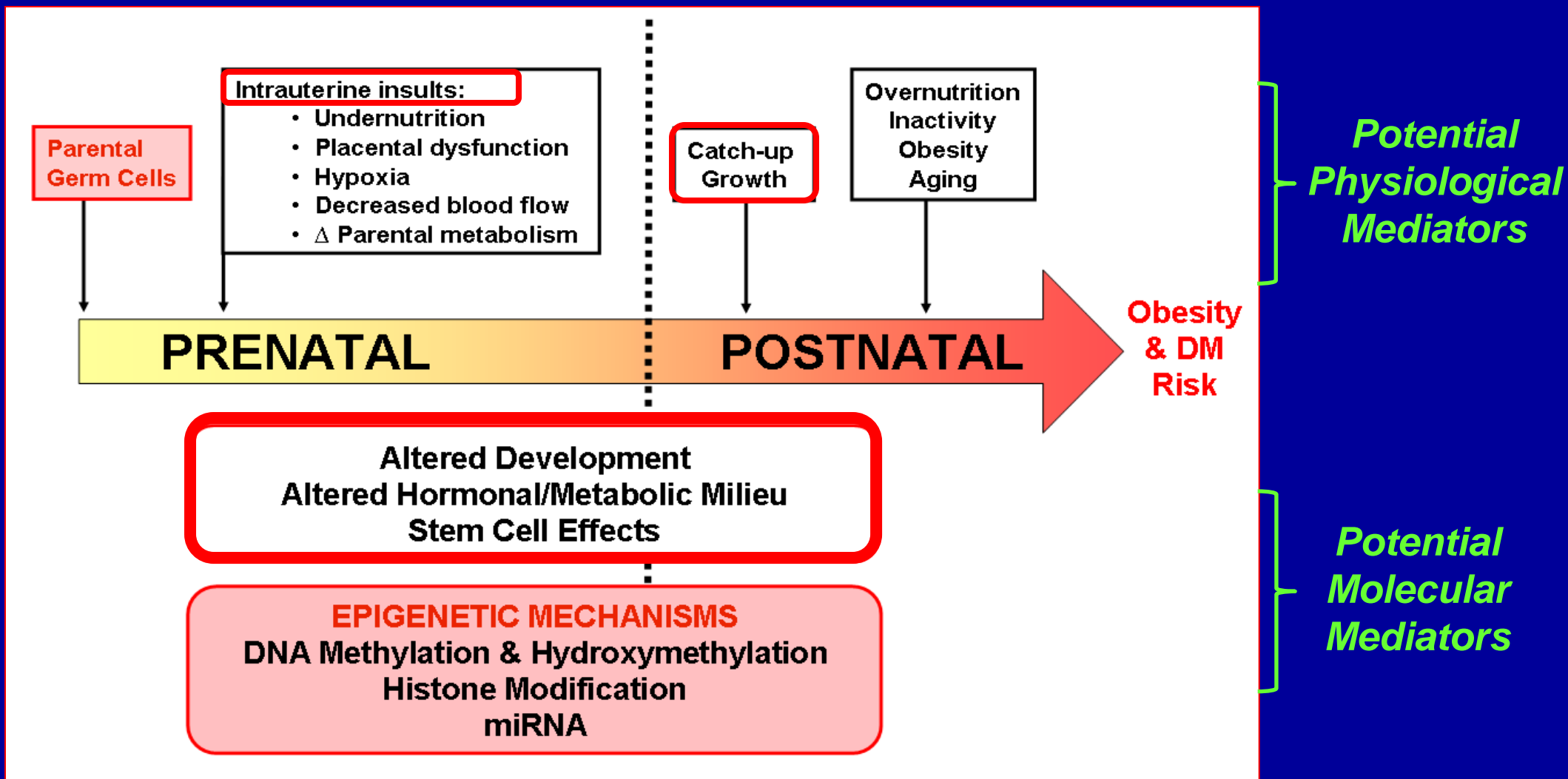
No change in food intake

Multiple Tissues Are Affected by Exposure to Maternal UN

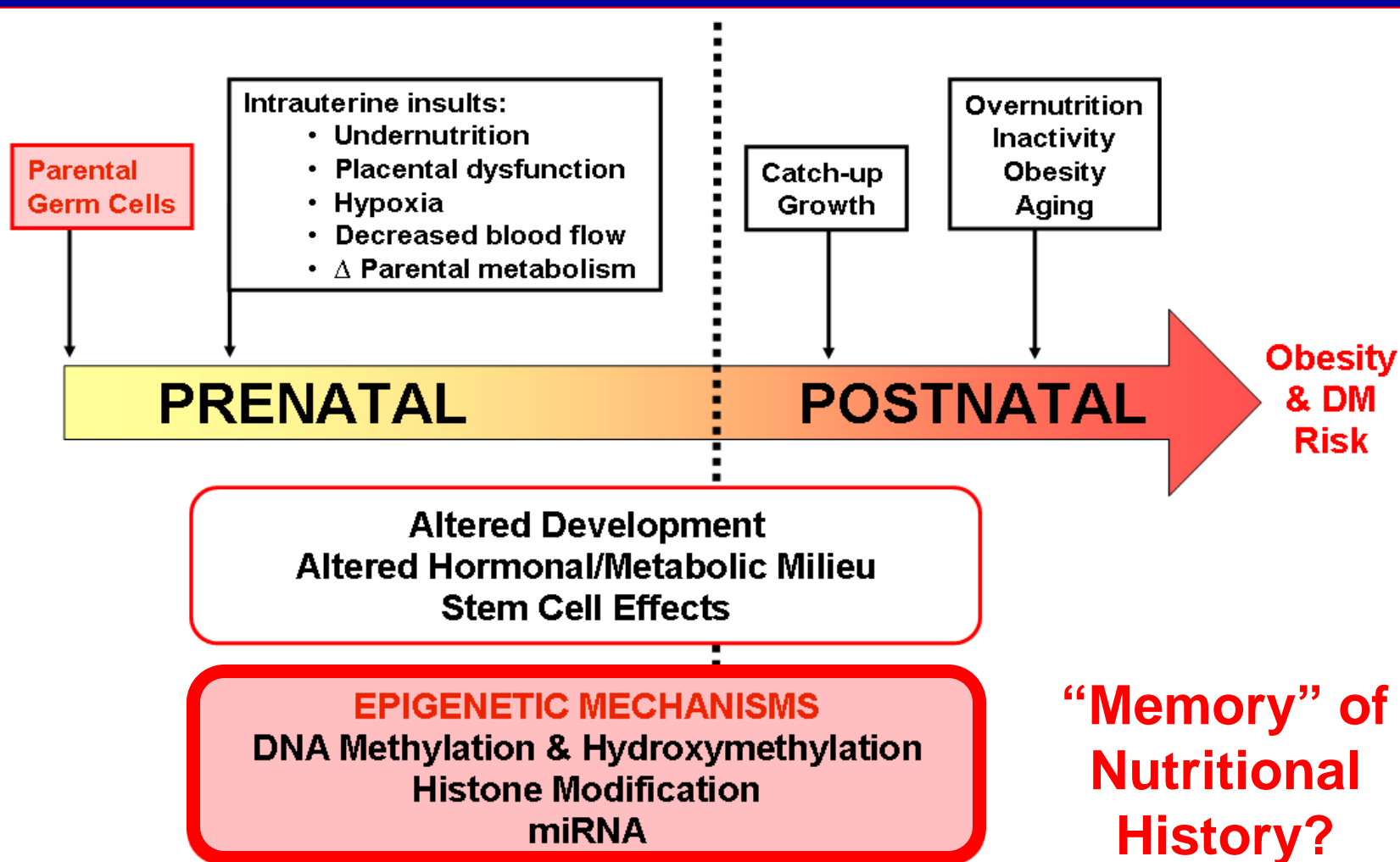


When & How Are these Developmentally-Mediated Phenotypes “Programmed”?

When & How Are these Developmentally-Mediated Phenotypes “Programmed”?



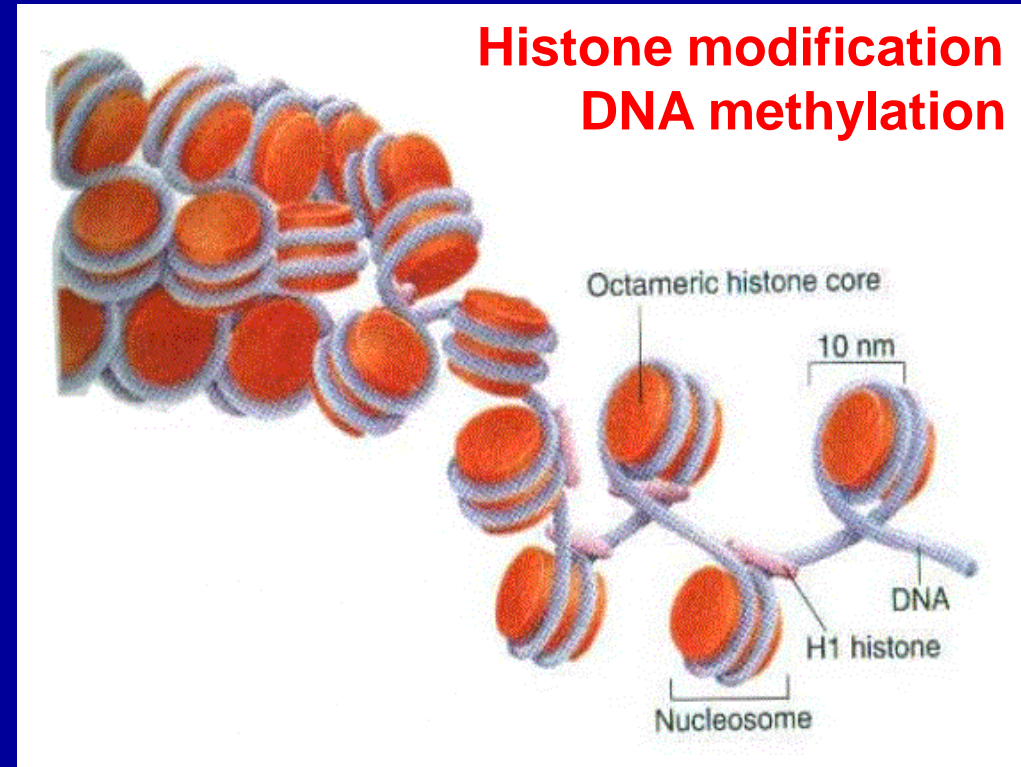
When & How Are these Developmentally-Mediated Phenotypes “Programmed”?



Hypothesis: Epigenetic Alterations Contribute to Metabolic Disease Risk

Epi-genetics:

- Changes in gene expression **NOT** due to changes in DNA sequence
- Can be influenced by nutritional or other environmental stimuli
- May be “permanent” and affect cellular function long after the causal nutritional or other stressor is gone
- Can be inherited



DNA CLOSED
= INACTIVE



DNA OPEN
= ACTIVE

Sequence can be read

Evidence for Epigenetic Contribution to Metabolic Disease

Fetal programming – birth weight and adult disease effects in humans and mice

Human obesity:

- Prader-Willi syndrome – disruption at imprinted locus causes obesity and diabetes
- therapy with valproate (HDAC inhibitor) increases obesity

Altered maternal nutrition at conception associated with reduced methylation at IGF2 locus (Dutch hunger winter population)

Disruption in H3K9 demethylase JHDM2a causes obesity

Intergenerational transmission

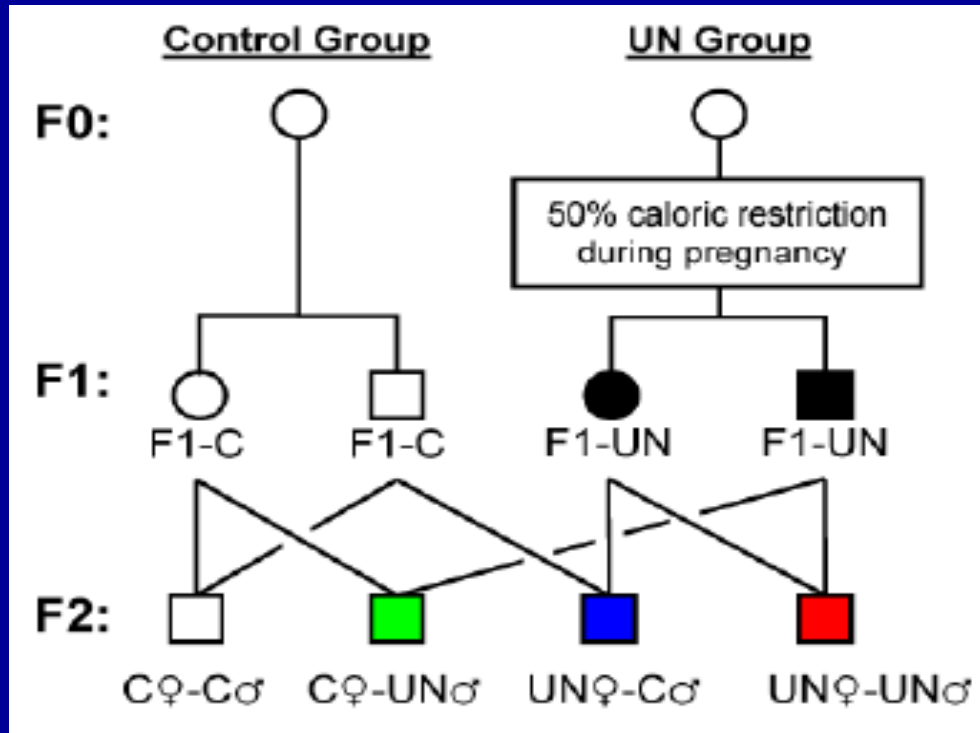
Multigenerational Effects

- Developmental & nutrition-associated phenotypes can progress to subsequent generations, even in the setting of normal nutrition during pregnancy.
 - *Paternal GF food supply associated with premature mortality in grandsons*
 - *Paternal GM food supply associated with mortality of granddaughter*
- Insulin resistance can be transmitted to offspring of IUGR rats despite embryo transfer and cross-fostering
- Can we observe multigenerational effects in our model?
- Can we use these data to dissect epigenetic vs. other molecular mechanisms?

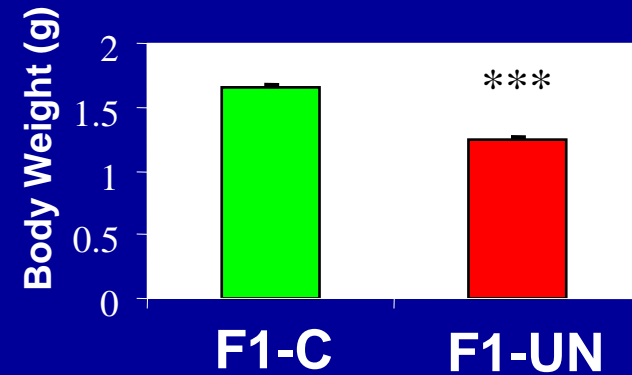
Food abundance for the grandfather associated with lifespan effects in grandchildren
Overkalix, Sweden



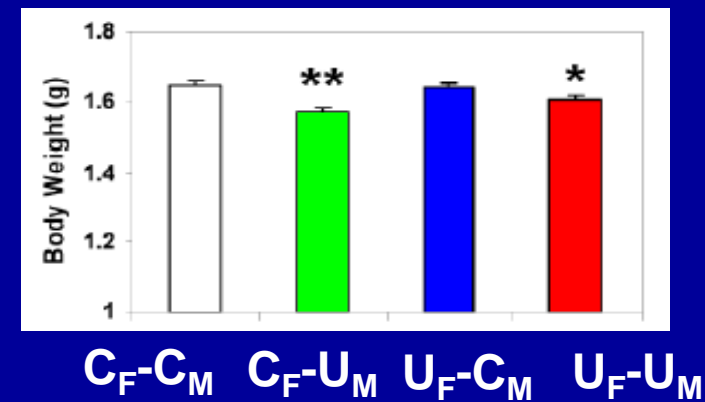
Multigenerational Effects of Nutritional History



Birth Weight – F1 Generation



Birth Weight – F2 Generation



***Normal Nutrition During 2nd Generation Pregnancy**

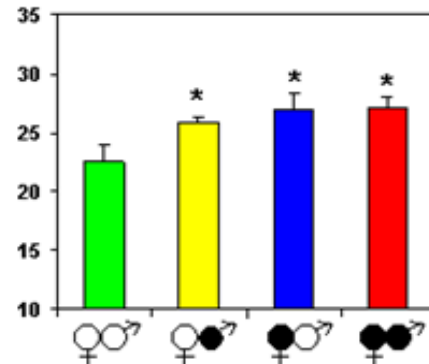
Paternal Effects Dominant

Multigenerational Metabolic Disease in Our Model

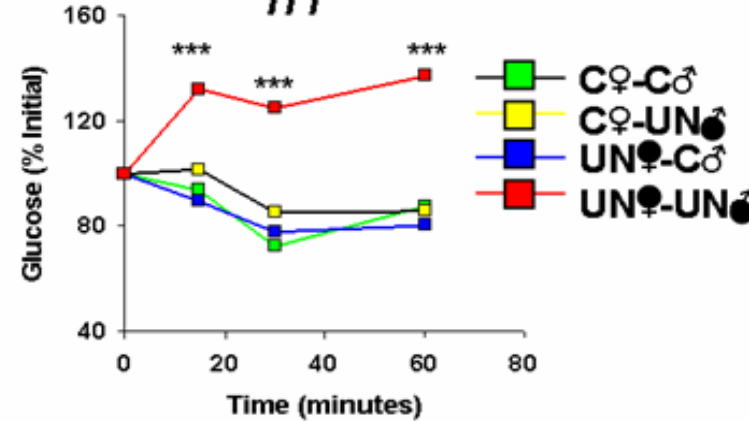
Obesity

Altered Gene Expression
 ↓ adipose *Dlk1* in both paternal and maternal

A. Fat Mass (DEXA)

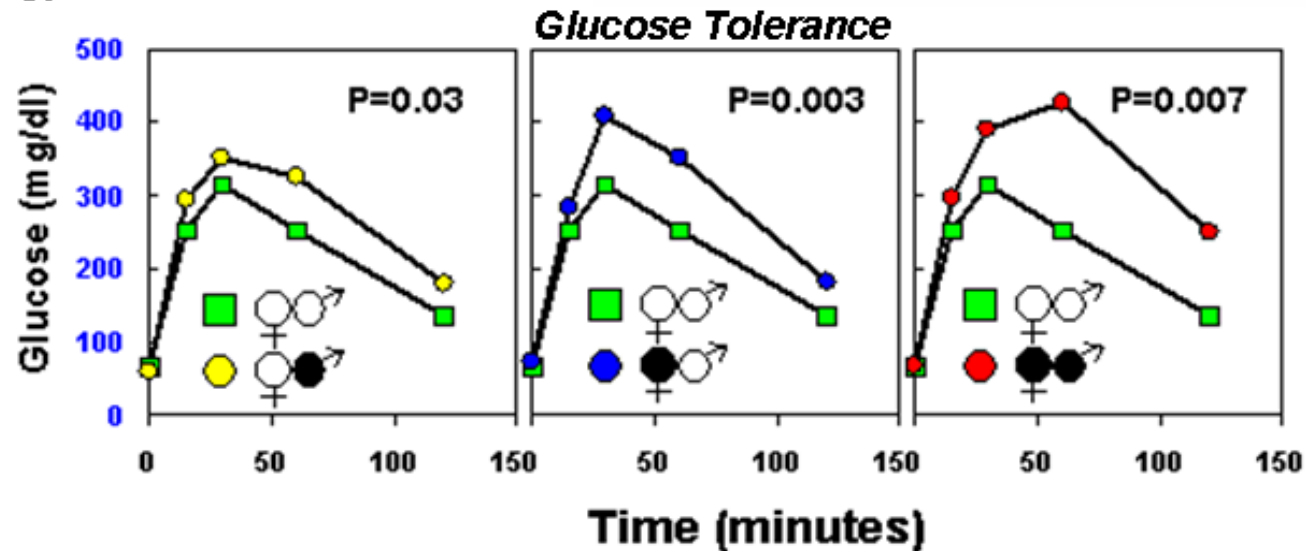


B. ITT



Insulin Resistance

C. Glucose Tolerance



Impaired Glucose Tolerance
 ↓ GSIS

Potential Mechanisms Mediating Multigenerational Metabolic Disease?

	Maternal Lineage Only
Nuclear DNA inheritance	✓
Mitochondrial DNA inheritance	✓
Epigenetic modification in germ cells and other tissues	✓
Maternal environment during pregnancy <ul style="list-style-type: none">•Hormones•Nutrients•Uterine environment	✓

Potential Mechanisms Mediating Multigenerational Metabolic Disease?

	Maternal Lineage Only	Paternal Lineage Only
Nuclear DNA inheritance	✓	✓
Mitochondrial DNA inheritance	✓	
Epigenetic modification in germ cells and other tissues	✓	✓
Maternal environment during pregnancy <ul style="list-style-type: none">•Hormones•Nutrients•Uterine environment	✓	

Potential Mechanisms Mediating Multigenerational Metabolic Disease?

	Maternal Lineage Only	Paternal Lineage Only	Both Maternal and Paternal Lineage
Nuclear DNA inheritance	✓	✓	✓
Mitochondrial DNA inheritance	✓		✓
Epigenetic modification in germ cells and other tissues	✓	✓	✓
Maternal environment during pregnancy <ul style="list-style-type: none"> •Hormones •Nutrients •Uterine environment 	✓		✓

Paternally-Mediated Multigenerational Effects: Test the Hypothesis that Epigenetic Mechanisms Contribute to Offspring Phenotypes

	Maternal Lineage Only	Paternal Lineage Only	Both Maternal and Paternal Lineage
Nuclear DNA inheritance	✓	✓	✓
Mitochondrial DNA inheritance	✓		✓
Epigenetic modification in germ cells and other tissues	✓	✓	✓
Maternal environment during pregnancy <ul style="list-style-type: none"> •Hormones •Nutrients •Uterine environment 	✓		✓

Isolated Paternal Effects:

- ↓ Birth Weight
- ↓ Glucose Tolerance
- ↑ Obesity

“You are What Your Father Ate”

**History of UN Exposure
in Fathers**

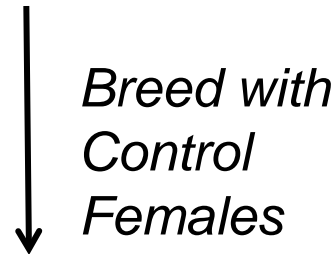


**Obesity & Glucose
Intolerance in
Offspring**

*Exposure during
Development*

*Jimenez & Patti,
Diabetes 2009*

**HFD-Induced Obesity
in Fathers**

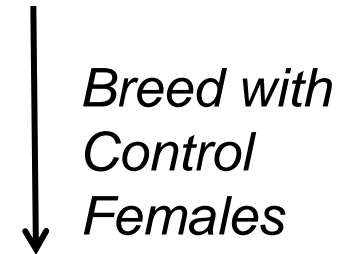


**Mild Glucose
Intolerance in
Daughters**

Exposure during Adult Life

*Ng & Morris,
Nature 2010*

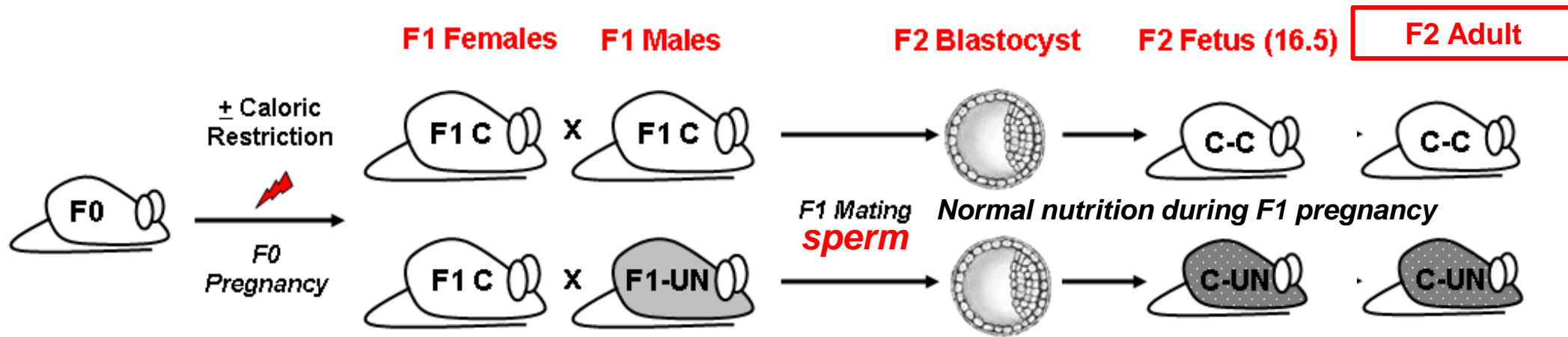
**Low Protein/High
Sucrose Diet in Fathers**



**↑ Expression of Lipid
Synthesis Genes in
Offspring Liver**

*Carone & Rando,
Cell 2010*

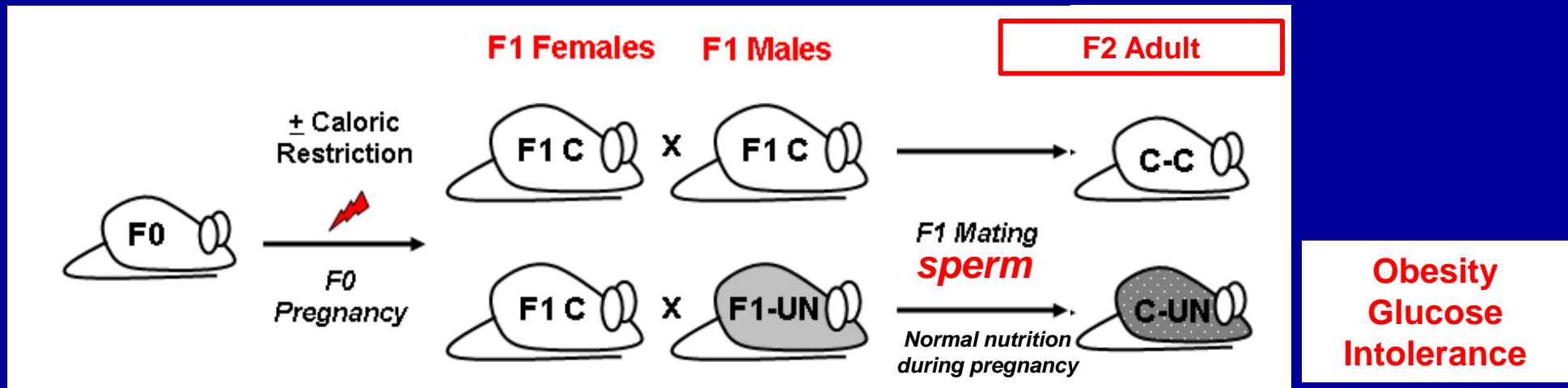
Multigenerational Phenotypes via Paternal Lineage Implicates Epigenetic Modification of Sperm



**Compare Sperm
Epigenome:
C vs. UN Father
Does DNA
Methylation
Differ?**

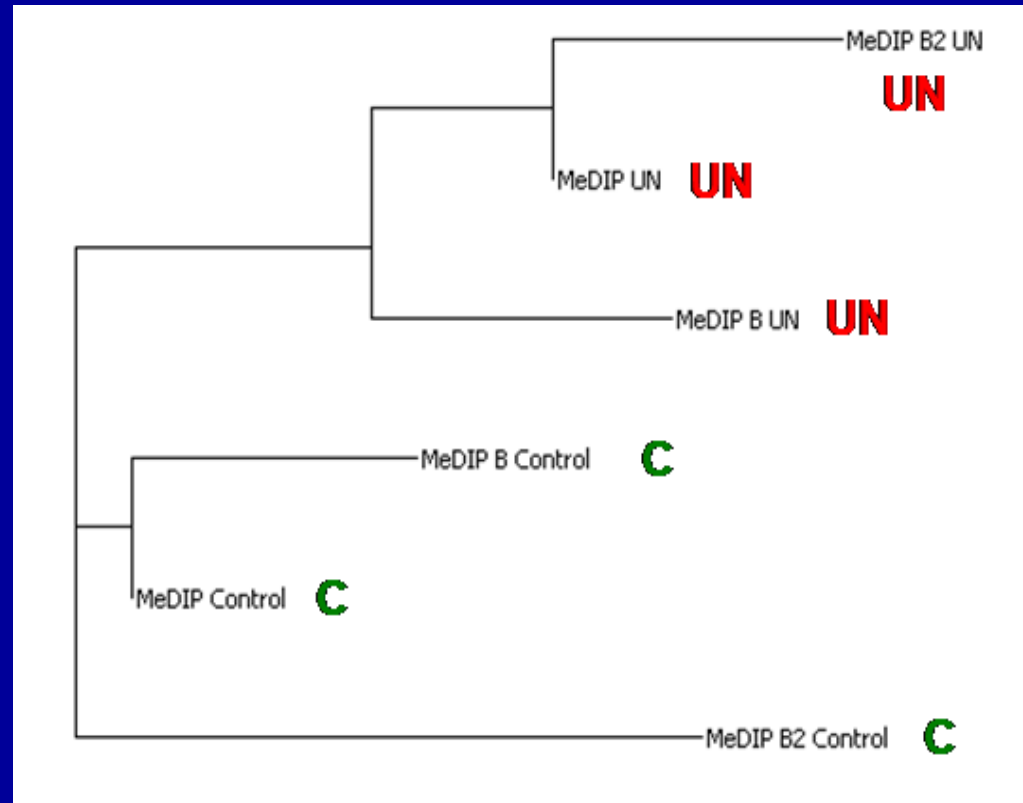
**Obesity
Glucose
Intolerance**

Multigenerational Phenotypes via Paternal Lineage Implicates Epigenetic Modification of Sperm



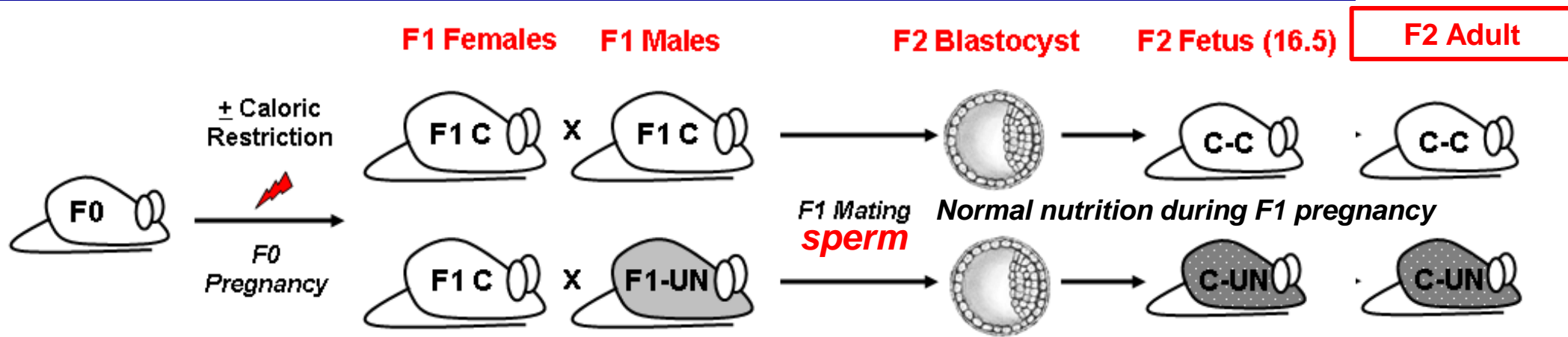
**Compare Sperm
Epigenome:
C vs. UN Father
Does DNA
Methylation
Differ?**

History of Nutritional Exposure During Development Alters DNA Methylation in Sperm



Unsupervised clustering of methylation patterns

Multigenerational Transmission via Paternal Lineage Implicates Epigenetic Modification of Sperm

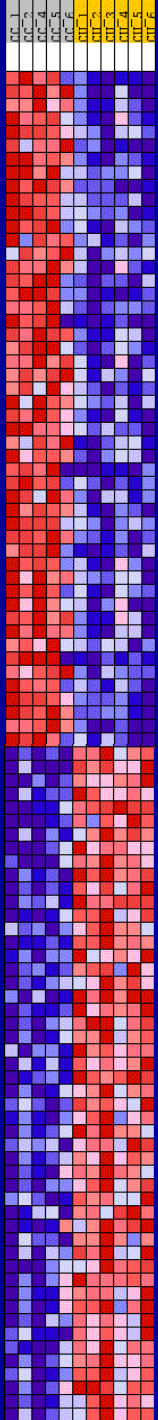


SPERM:
Altered Methylation?
Other Modifications?
miRNA?
Altered maintenance of
histones at key
developmental loci?

Are there effects on fetal
development and
patterns of gene
expression?
ED 16.5 LIVER &
PLACENTA

Obesity
Glucose
Intolerance

Fetal Liver Gene Expression is Altered in F2 Offspring of Males Exposed to Maternal UN



Downregulated
in offspring of
males with
history of
prenatal UN
exposure

Upregulated in
offspring of
males with
history of
prenatal UN
exposure

DAVID Ontology (FDR <0.05):

Downregulated in C x U:

Cell Cycle
Cytoskeleton

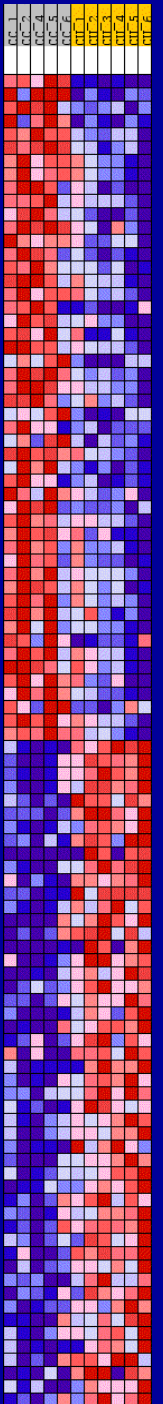
Upregulated in C x U:

Lipid β -Oxidation
Microsome

n=4949 genes with $q < 0.05$

N=5 pools per group, each with 2-3 litters

Placental Gene Expression is Altered in F2 Offspring of Males Exposed to Maternal UN



Downregulated in F2 offspring of males with history of prenatal UN exposure

Upregulated in offspring of males with history of prenatal UN exposure

DAVID Ontology (FDR <0.05):

Downregulated in C x U:

Symporter Activity
Transmembrane Transporters (e.g. GLUT3)

Upregulated in C x U:

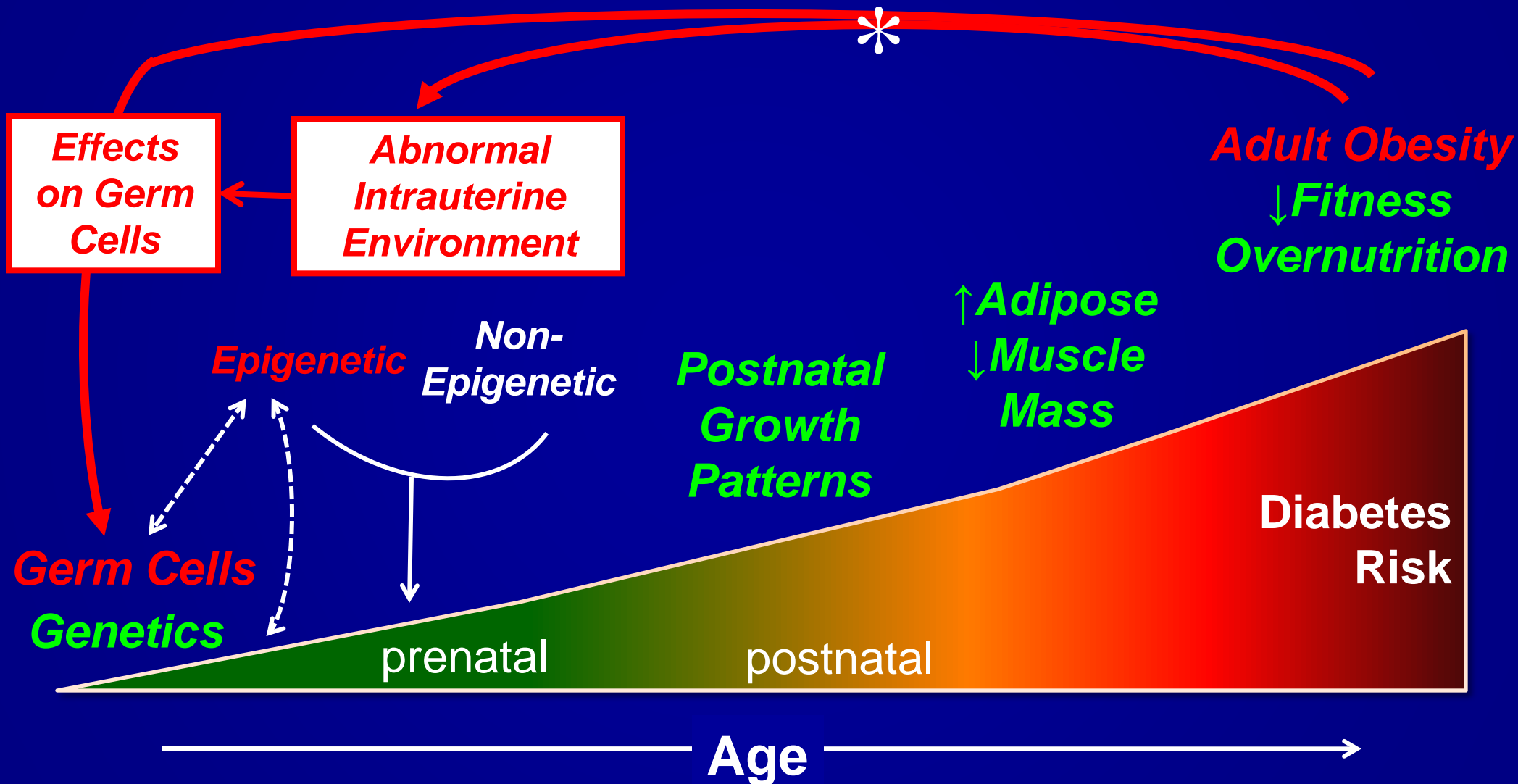
Inflammatory Genes



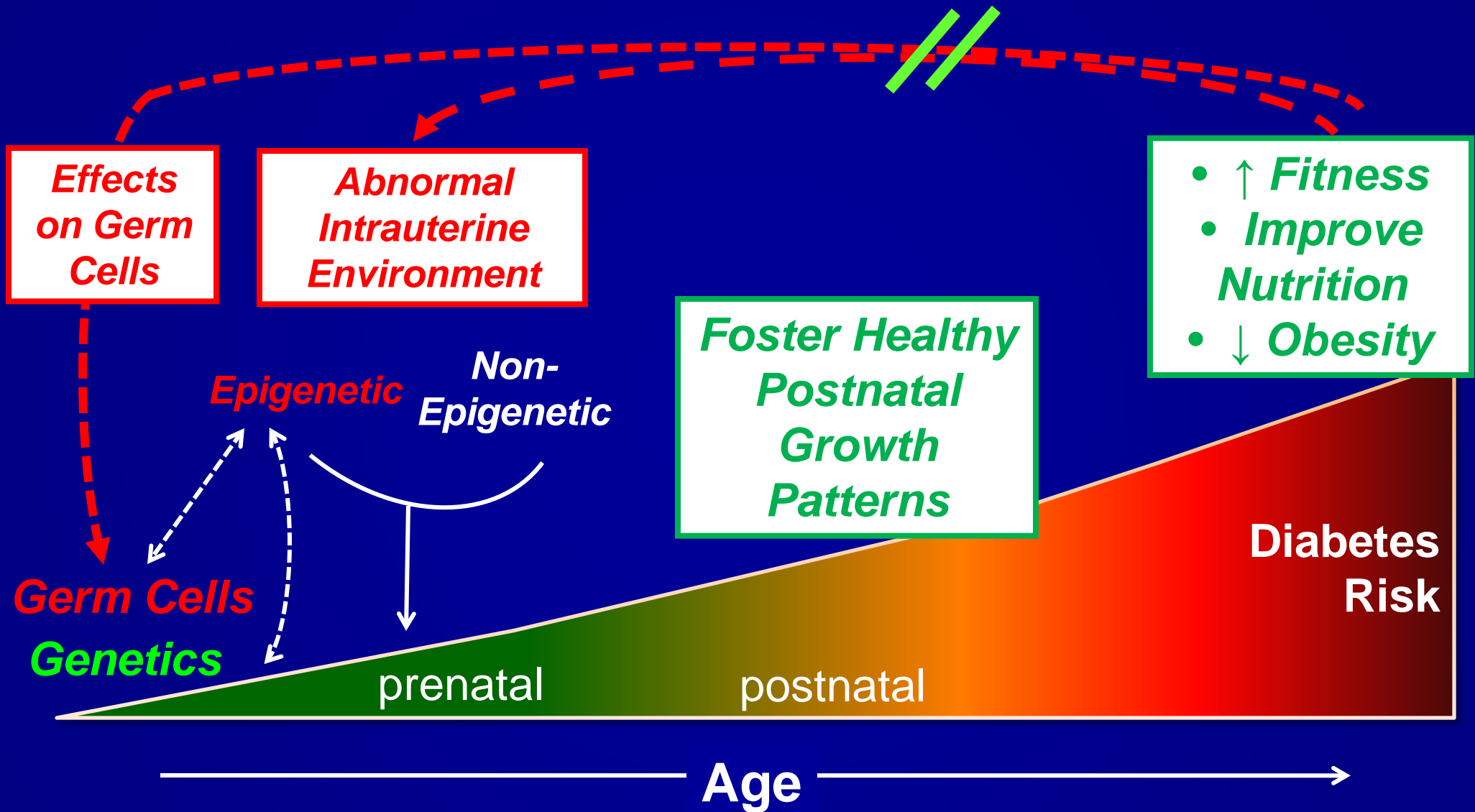
n=479 genes with $q < 0.05$

N=5 pools per group, each with 2-3 litters

A Vicious Cycle of Risk?

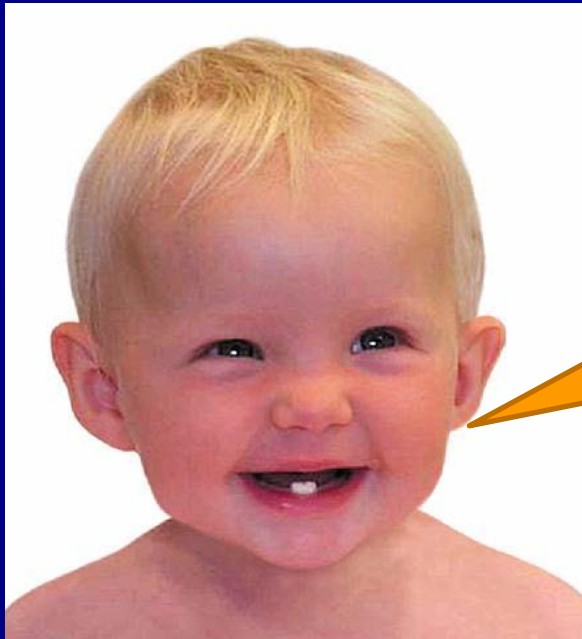


Let's Be Optimistic: Opportunities to Reduce Risk!



**Nutrition & metabolism are important at all stages of life
...especially pregnancy & early childhood.**

**Nutrition and metabolic status of both mother and
father are important for health of offspring.**



**I'm happy because now
I can blame my father
AND my mother AND
my grandparents for
EVERYTHING!**

Aris Lytras **Josh Schroeder** **Elvira Isganaitis**

Alison Burkart
Aparna Sharma



Mike Chen
Wen Kong

Amy Wagers
Max Cerletti

Josep Jimenez
Melissa Woo

Anne Ferguson-Smith
Lizzie Radford

Steven Bauer
Allan Vaag
Evan Rosen

ADA
Lilly Foundation