Module 2: From "Healthy" Obese to NASH – What Happens?

Maternal Insulin Resistance and NAFLD Development



Jacob E. (Jed) Friedman, PhD
Professor of Pediatrics, Biochemistry & Molecular Genetics
University of Colorado School of Medicine
Professor of Medicine-Endocrinology & Metabolism, and Reproductive Sciences
Director, NIH Molecular & Cellular Core Laboratory, Nutrition and Obesity Research Center

STOPNASH: Symposium on The Origins and Pathways of Nonalcoholic Steatohepatitis
NASPHGAN, October 7, 2015

University of Colorado Anschutz Medical Campus School of Medicine | University of Colorado Hospital | Children's Hospital Colorado

World-Wide Childhood Obesity Epidemic

Critical Early Life Factors affect Health Across the Lifespan



Genes | Gestational Exposure | Post-natal Environment

The Childhood Obesity Pipeline is Full and getting worse0po

Health and Wellness Center

Maternal obesity increases offspring risk for obesity and metabolic disease

Obese Mother
- ⇧ Cholesterol
- ⇧ Triglycerides
- ⇧ Leptin
- ⇧ Glucose
- ⇧ Insulin
- ⇧ Inflammation
- ⇧ Oxidative Stress

Offspring of Obese Mother
- ⇧ Cholesterol
- ⇧ Triglycerides
- ⇧ Leptin
- ⇧ Glucose
- ⇧ Insulin
- ⇧ Inflammation
- ⇧ Oxidative Stress
- ⇧ Fat Mass at Birth
- ⇧ Risk of Obesity & Metabolic Disease

Placenta of Obese Mother
- ⇧ Inflammation
- ⇧ Oxidative Stress
- ⇧ LPL Activity
- ⇧ Lipids

University of Colorado **Anschutz Medical Campus**

What We Don't Know!

- What are the consequences of exposure to maternal obesity during pregnancy and lactation on fetal metabolic systems and neonatal adiposity? liver, WAT, bone marrow, appetite control.
- What are the potential mediators of these effects?
  Maternal Lipids, inflammation, Breast milk? Microbiome?
- Is there a role for maternal nutrition in triggering epigenetic factors leading to NASH? DNA methylation/acetylation, in early infancy.
- What are the public health consequences of exposure to maternal obesity on the childhood obesity epidemic and the evolution of pediatric NASH?

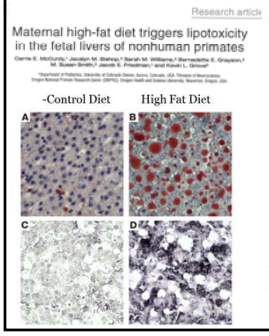
Collaborative Research
Oregon National Primate Research Center, University of Colorado

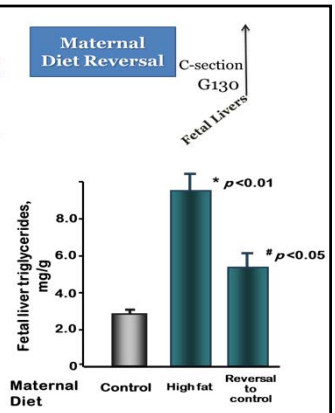
Kevin Grove, PhD
Oregon National Primate Research Center

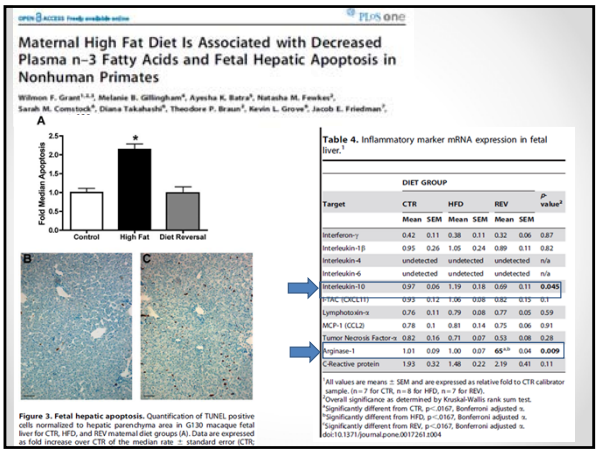
Long-Term Goal:

- **To develop a Non-Human Primate Model to study the effects of Maternal Diet, Obesity and GDM on the development of metabolic systems (liver, muscle, fat, heart, brain) in utero and the effects on infant behavior and post-natal disease pathways.**

Maternal Obesity is Non-Alcoholic Fatt







Maternal High Fat Diet Is Associated with Decreased Plasma n–3 Fatty Acids and Fetal Hepatic Apoptosis in Nonhuman Primates

Wilmon F. Grant[1,2,3], Melanie B. Gillingham[4], Ayesha K. Batra[5], Natasha M. Fewkes[2], Sarah M. Comstock[4], Diana Takahashi[6], Theodore P. Braun[5], Kevin L. Grove[6], Jacob E. Friedman[7]

A

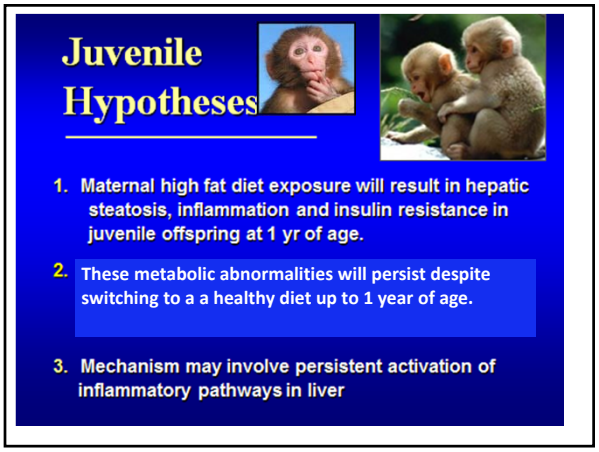
B C

Figure 3. Fetal hepatic apoptosis. Quantification of TUNEL positive cells normalized to hepatic parenchyma area in GI 30 macaque fetal liver for CTR, HFD, and REV maternal diet groups (A). Data are expressed as fold increase over CTR of the median rate ± standard error (CTR)

Table 4. Inflammatory marker mRNA expression in fetal liver.[1]

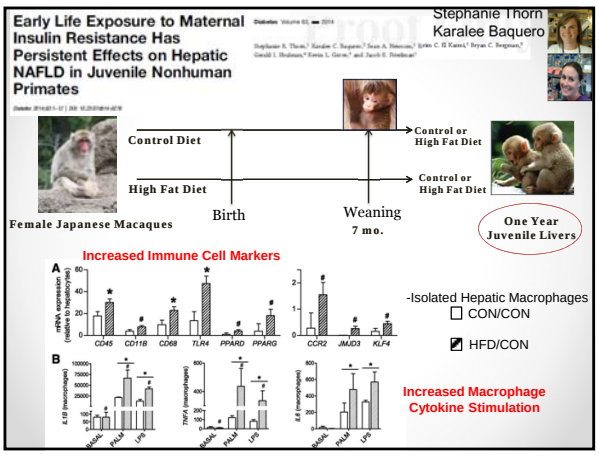
Target	DIET GROUP						P-value[2]
	CTR		HFD		REV		
	Mean	SEM	Mean	SEM	Mean	SEM	
Interferon-γ	0.42	0.11	0.38	0.11	0.32	0.06	0.87
Interleukin-1β	0.95	0.26	1.05	0.24	0.89	0.11	0.82
Interleukin-4	undetected		undetected		undetected		n/a
Interleukin-6	undetected		undetected		undetected		n/a
Interleukin-10	0.97	0.06	1.39	0.18	0.69	0.11	0.045
HTAC (CXCL11)	0.93	0.12	1.06	0.08	0.82	0.15	0.1
Lymphotoxin-α	0.76	0.11	0.79	0.08	0.77	0.05	0.59
MCP-1 (CCL2)	0.78	0.1	0.81	0.14	0.75	0.06	0.91
Tumor Necrosis Factor-α	0.82	0.16	0.71	0.07	0.53	0.08	0.28
Arginase-1	1.01	0.09	1.00	0.07	65[a,b]	0.04	0.009
C-Reactive protein	1.93	0.32	1.48	0.22	2.19	0.41	0.11

[1]All values are means ± SEM and are expressed as relative fold to CTR calibrator sample. (n = 7 for CTR, n = 8 for HFD, n = 7 for REV).
[2]Overall significance as determined by Kruskal-Wallis rank sum test.
[a]Significantly different from CTR, p<.0167, Bonferroni adjusted α.
[b]Significantly different from HFD, p<.0167, Bonferroni adjusted α.
[c]Significantly different from REV, p<.0167, Bonferroni adjusted α.
doi:10.1371/journal.pone.0017261.t004



Juvenile Hypotheses

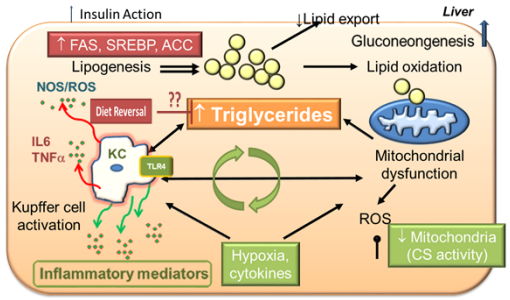
1. Maternal high fat diet exposure will result in hepatic steatosis, inflammation and insulin resistance in juvenile offspring at 1 yr of age.
2. These metabolic abnormalities will persist despite switching to a a healthy diet up to 1 year of age.
3. Mechanism may involve persistent activation of inflammatory pathways in liver



Early Life Exposure to Maternal Insulin Resistance Has Persistent Effects on Hepatic NAFLD in Juvenile Nonhuman Primates

Stephanie Thorn
Karalee Baquero

Programmed effects in juvenile



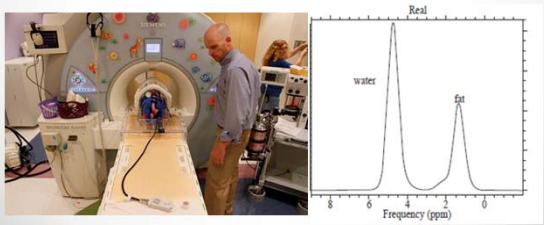
Programmed effects in Juvenile Liver (1 yr old) WSD/Control diets-Diabetes 2014.

Baur JA. *Nature*. 2008. 444:337-342
Poulsen MM. *Nutrition*. 2012. 32:701-708
Kendrick AA. *Biochem J*. 2011. 433:505-514
Giralt A. *Biochem J*. 2012. 444:1-10

Does hepatic steatosis occur in infants of obese-GDM Mothers? Infant "Papoose" for whole body MRI

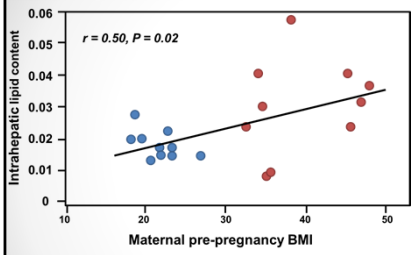
MRI for Neonatal Fat Measurement

- **Magnetic Resonance Spectroscopy (MRS)**
- **Cohort of 25 infants**
  - **13 born to normal weight controls**
  - **12 born to obese mothers with GDM**



Brumbaugh DE, et al. J Pediatr 2013;162:930-6.

• 68% Increase in Hepatic Fat in Neonates Born to Obese GDM mothers



Brumbaugh, 2013.

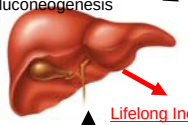
- *Can Excess Maternal Fat Delivery to the Fetal-Placental Interface Result in the Genesis of NAFLD?*

Visceral fat = less than 0.1% of total fat and independent of subcutaneous fat.

Consequences of Maternal Overfeeding on Fetal Liver

Fetal Hepatic Fat Accumulation
Oxidative Stress
Inflammation
Gluconeogenesis

Placental Inflammation
Placental Nutrient Transfer



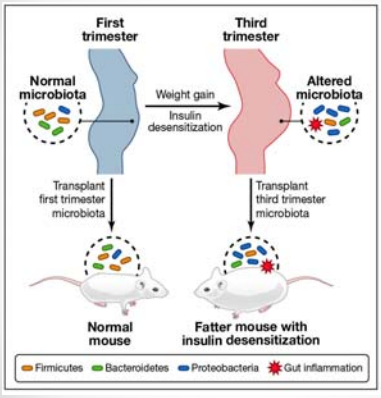
Lifelong Increased Risk of a Proinflammatory Response to Overnutrition

Recruitment and Activation of Bone Marrow WBC Precursors

US adults: 20-30%
Obese adults: 60%
US kids 9-19*: 17%
Obese kids: 55%

Excess FFA/TG Delivery

Host remodeling of the gut microbiome and metabolic changes during pregnancy.



Koren et al Cell. 50(3):470-80, 2012.

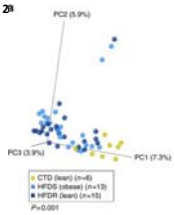
ARTICLE

Received 2 Jan 2014 | Accepted 14 Apr 2014 | Published 20 May 2014

High-fat maternal diet during pregnancy persistently alters the offspring microbiome in a primate model

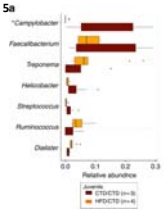
Jun Ma[1,2,*], Amanda L. Prince[3,*], David Bader[1,3,*], Min Hu[1], Radhika Ganu[1], Karalee Baquero[4], Peter Blundell[4], R. Alan Harris[1,2], Antonio E. Frias[4], Kevin L. Grove[4] & Kjersti M. Aagaard[1,2,3]

Figure 2a



Maternal gut microbiome clusters according to diet

Figure 5a



Offspring gut microbiome clusters according to maternal diet

Microbiome

- Best studied is gut microbiome
  - 100 trillion microorganisms (10x more than the total of all other cells in the whole body)
  - Using 16S rRNA sequences, composition of microbiomes can be determined
  - >90% from 2 phyla: Firmicutes and Bacteriodetes
  - Human gut microbiome established by 1 year of age and depends on genetics, mode of delivery (vaginal vs C-section), and form of infant feeding
  - Later influenced by demographics, diet, and lifestyle
  - Evidence supports a role in inflammation, intestinal cell health and hormone production, efficiency of energy harvest from food, and appetite.

Gut microflora may stimulate hepatic fat deposition and promote NASH through several mechanisms:

1. **It promotes obesity by improving energy yield from food.**
2. **It regulates gut permeability, low-grade inflammation and immune balance.**
3. **It modulates metabolism/genes directly in the liver.**
4. **It regulates bile acid metabolism.**
5. **It increases ethanol production by bacteria—ROS and mitochondrial function.**

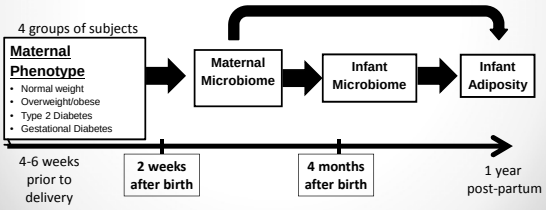
ADA/Glaxo-SmithKline Sponsored study:

University of Colorado Denver

Objectives:

To determine how maternal obesity and diabetes act to colonize the microbiome of the mother-infant pairs.

To establish how maternal characteristics during a critical window of development impacts the infant microbiome community, and whether it plays a role in infant adiposity?



Specimen Processing

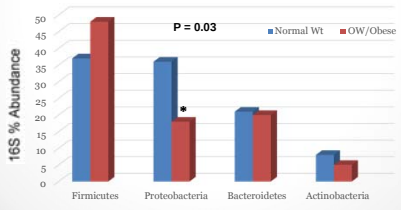
- **Bacterial DNA extraction**
- **16s rRNA (V1V2) amplification and sequence analysis (Illumina Miseq) of all samples**
- **Sequence sorting by OTU (phyla, etc) and relative abundance calculated (Explicet)**
- **2 week infant stool: Shotgun sequencing with sequence classification/analysis using MG-RAST.**
- **SCFA analysis (Acetate, Butyrate, Proprionate by MS-MS.**

Gammaproteobacteria is suppressed infants born to obese mothers

Supplementary Table 2B. Infant gut microbiome according to maternal pre-pregnant BMI (class)

OTU Name	ALL (%)	Normal Weight (%)	Overweight/Obese (%)	p-valu
Firmicutes/Bacilli	17.8 ± 23.9	13.3 ± 19.1	24.5 ± 29.3	0.25
Firmicutes/Clostridia	14.1 ± 17.2	14.0 ± 13.1	14.4 ± 22.7	0.33
Firmicutes/Negativicutes	7.2 ± 11.8	6.2 ± 11.2	8.7 ± 12.9	0.92
Proteobacteria/Gammaproteobacteria	28.0 ± 22.8	34.8 ± 22.7	17.7 ± 19.6	0.03
Bacteroidetes/Bacteroidia	20.5 ± 27.0	20.9 ± 26.6	20.1 ± 28.6	0.55
Actinobacteria/Actinobacteria	7.2 ± 13.9	3.9 ± 6.1	12.2 ± 20.2	0.11

Results presented as Mean ± SD unless otherwise noted.



Importance of ɣ-Proteobacteria

- **"Pioneering Bacteria", marker of transition to mature MB. Low abundance found in infant stool in Premature neonates. Increases on HFD.**
- **May have disproportionate impact on intestinal microenvironment (LPS, oxygen tension, intestinal mucous production).**
- **Microenvironment changes may create conditions for critical establishing TLR4 based immunity, that persists into childhood?**

Question:

Are these changes meaningful biologically?

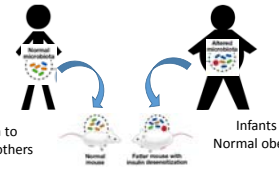
From Bedside (or toilet) to Bench: Germ Free Mice

Hypothesis: *the reduction in the early pioneering γ-proteobacteria in obese offspring may allow a more pro-inflammatory gut to develop and SCFA may influence body fat in GF mice*

Kristine Kuhn, MD, PhD

Lyndsey Babcock

Taylor Soderborg, M.D./PhD. Candidate

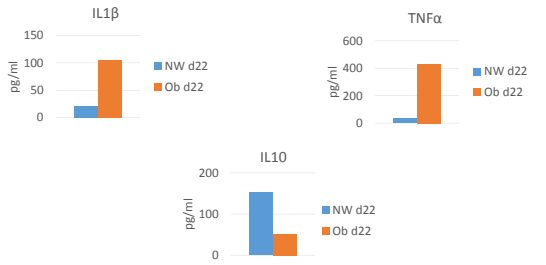


Infants born to Normal wt mothers

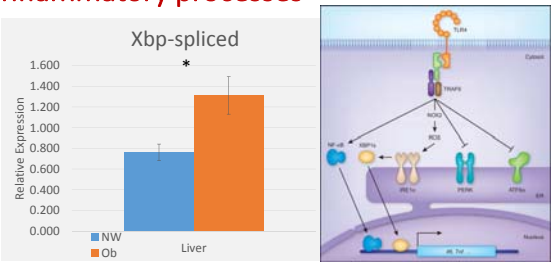
Infants born to Normal obese mothers

Erin Severs, BA, BS, CVT, RLATg

Increased Pro-inflammatory shift in plasma cytokines –hepatic portal vein.

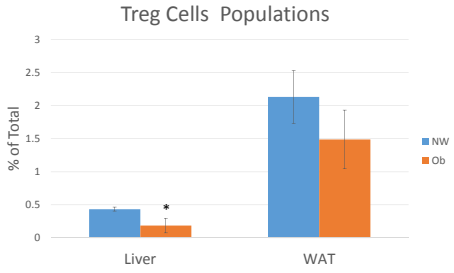


ER stress and heightened susceptibility for inflammatory processes

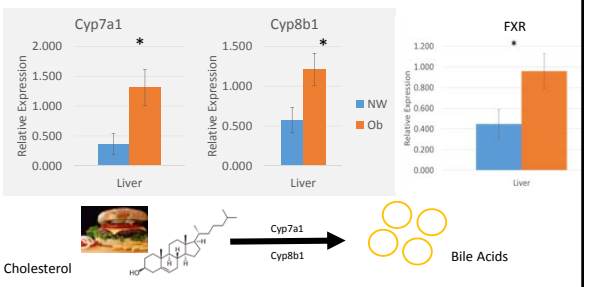


Engel AI., Barton, GM., 2010

Treg cells reduced in Liver in mice with Ob infant microbiome



Surprise: Bile acid enzymes and BA receptor FXR are induced in mice with MB from Ob Infants



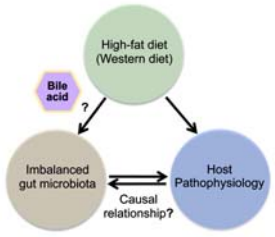
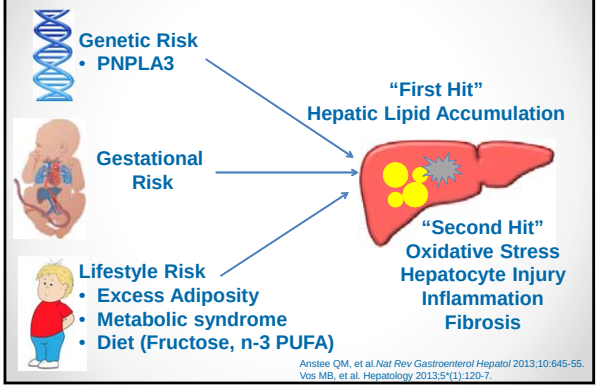
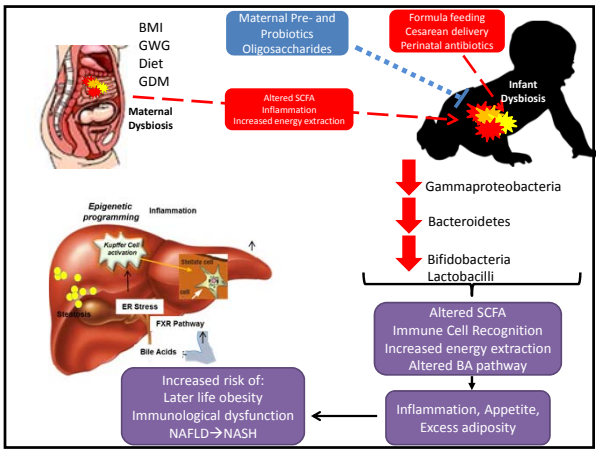


Figure 1. Relationship between a high-fat diet, imbalanced gut microbiota, and host pathophysiology.

Future Risks for NAFLD



Anstee QM, et al. *Nat Rev Gastroenterol Hepatol* 2013;10:645-55.
Vos MB, et al. Hepatology 2013;5*(1):120-7.


BMI
GWG
Diet
GDM
Maternal Dysbiosis
Maternal Pre- and Probiotics Oligosaccharides
Formula feeding
Cesarean delivery
Perinatal antibiotics
Altered SCFA
Inflammation
Increased energy extraction
Infant Dysbiosis
Gammaproteobacteria
Bacteroidetes
Bifidobacteria
Lactobacilli
Epigenetic programming
Inflammation
Kupffer Cell activation
Steatosis
ER Stress
FXR Pathway
Bile Acids
Altered SCFA
Immune Cell Recognition
Increased energy extraction
Altered BA pathway
Inflammation, Appetite, Excess adiposity
Increased risk of:
Later life obesity
Immunological dysfunction
NAFLD→NASH


Thank-You !
Home Team:
Lynn Barbour, M.D.
Teri Hernandez, Ph.D.
Karim el Kasimi, Ph.D.
Stephanie Thorn, Ph.D.
Margaret Heerwagen, B.S.
BeckyDeLaHoussaye, M.S.
Rachel Van Pelt, Ph.D.
Sarah Borengasser, Ph.D.
Pete Baker, M.D.
Rachel Janssen, M.S.
Rebecca Aiken, M.S.
Allison Buti, M.S.
Sean Newsom, Ph.D.
Dominick Lemas, PhD.
Bridget Young, PhD.
Regina Reynolds, M.D.
Melanie Reece, PhD.
Nancy Krebs, M.D.
Dan Franks, PhD
Molly and the Nutrition sta
Oregon Health Sci Ctr & Novo-Nordisk
Kevin Grove, PhD., Dan Marks, M.D. PhD.
Kjersti Aagaard, M.D, PhD – Baylor Coll of Medicine
NIH-RO1 DK060685, RO1 DK074643,
R24 DK90964, P30 DK048520 , ADA
University of Colorado Nutrition & Obesity
Research -Center (NORC); University of Colorado
Maternal-Child Clinical Translational Research
Center , Bill and Melinda Gates foundation
NIH
American Diabetes Association.
BILL & MELINDA GATES foundation