NAFLD in Hispanic Children: Evidence for Gene (PNPLA3) by Environment (dietary sugars/fructose) Interaction

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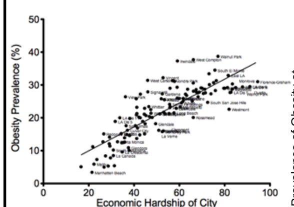
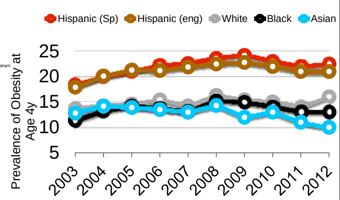
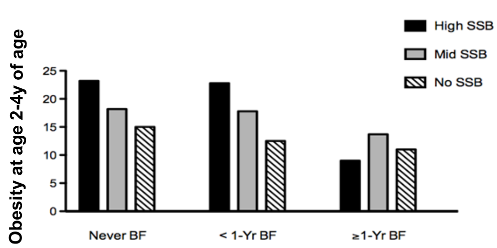
Hispanics Suffer from Dual Disparity to Obesity Starting in Early Life and Related to Early Exposure to Dietary Sugars

Shih et al; Pediatric Obesity 2013





The Main Dietary Driver of Obesity in Hispanic Children: High Sugar Sweetened Beverages

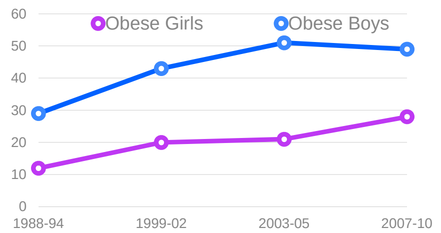


Link between high SSB and obesity established **early in life**

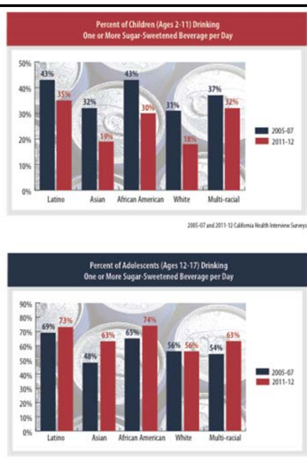
Davis et al; AJCN 2001

Trends in Adjusted Prevalence of Suspected NAFLD

Welsh et al; J Peds 2013

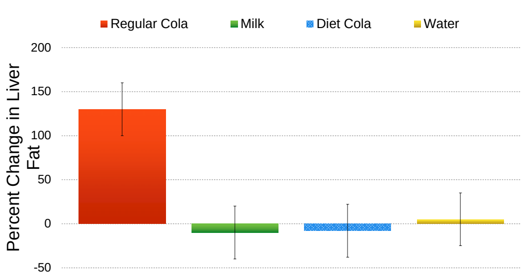


Among obese, odds of suspected NAFLD increased with age, BMI, Mexican American and male sex



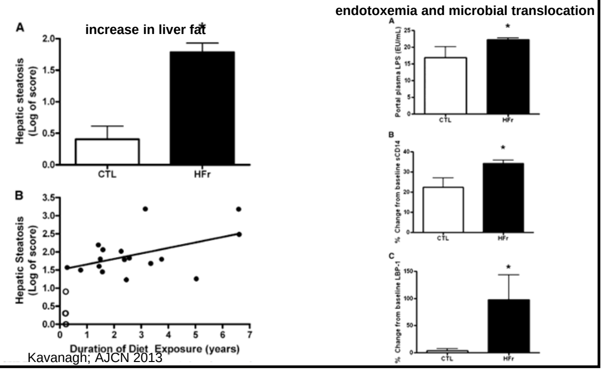
Dietary Sugars and Liver Fat

Maersk, et al. 2012 AJCN



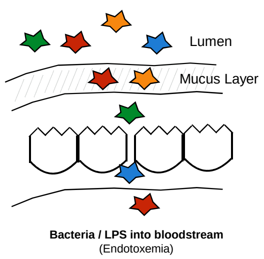
47 adults
1Liter per day for 6 months

Monkeys exposed to ad lib fructose diet (24% of kcal) in the absence of weight gain



Kavanagh; AJCN 2013

The Role of Fructose In Gut Permeability and Bacterial Translocation



Mice[1]

- Fructose increased portal blood endotoxin levels and liver fat (antibiotic treatment protective)
- *Suggests fructose and gut microbes play role in liver fat accumulation*

Primates[2]

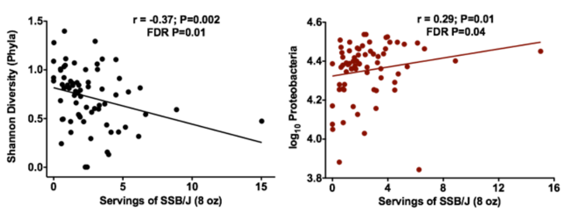
- Fructose increased liver fat and damage
  - Secondary to increased microbial translocation from gut and circulating bacterial-derived lipopolysaccharides (LPS)

Human

- Fructose may induce intestinal permeability, LPS in portal blood, and liver fat (NAFLD)
- Fructose readily taken up by liver[3]
  - Metabolism is unregulated
  - Contributes to formation of liver fat

[1]Bergheim I et al., J Hepatol, 2008; [2]Kavanagh K et al., Am J Clin Nutr., 2013; [3]Sum S et al., Nutr & Metab, 2012

Phyla Level: Diversity and Proteobacteria Associated with SSB/J Consumption

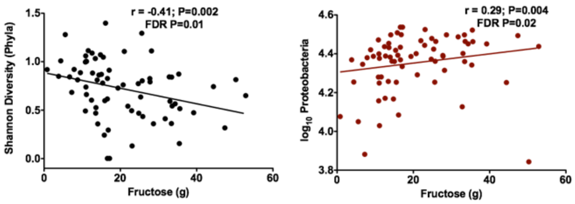


Proteobacteria:

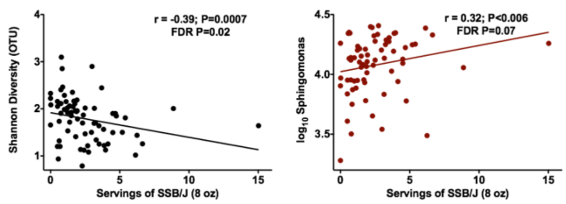
- In adults, proteobacteria has been shown to be the most abundant bacteria phylum in the blood[1] and predict cardiovascular disease[2]
- Greatest abundance in the gut of those with NASH (6%), followed by obese (3%) and lean (<1%) children 13-14 years of age[3]

[1]Amar et al., Diabetologia, 2011; [1]Amar et al., PLoS ONE, 2013; [3]Zhu et al., Hepatology, 2013

Phyla Level: Diversity and Proteobacteria Associated with FRUCTOSE Consumption in Obese Hispanic Children (Preliminary data)



OTU Level: Diversity and Sphingomonas Associated with SSB/J Consumption



Sphingomonas (Proteobacteria)[1-2]:

- Efficient carbohydrate scavenger, sugars used for growth
- Associated with infections and immune responses
- Elevated in obese rats (accompanied by low Bifidobacterium [Actinobacteria])

[1]Wakdran A et al., J of Proteome Res, 2008; [2]Aguirre M et al., Microorgan, 2015

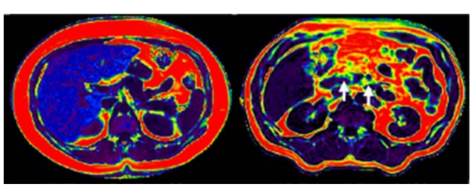
Methods Development: Liver and Pancreas Fat at 3T

USC Viterbi School of Engineering

Krishna Nayak, PhD & Harry Hu, PhD

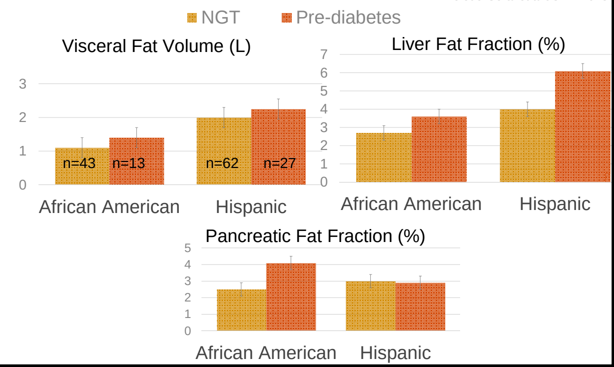
Iterative Decomposition using Echo-Asymmetry in the Least squares sense (IDEAL)

an optimal fat-water signal separation technique that utilizes knowledge of ^{1}H spectra in lipid and water - an extension of NMR except it is multi-voxel and 3-dimensional across entire liver



Visceral, Liver and Pancreatic Fat Fraction by Ethnicity Pre-diabetes Status

Toledo-Coral et al JCEM 2013

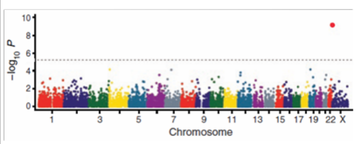


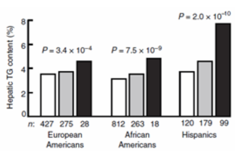
nature genetics

Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease

Stefano Romeo[1,8], Julia Kozlitina[2,3,8], Chao Xing[1,2], Alexander Pertsemlidis[1], David Cox[4], Len A Pennacchio[5], Eric Boerwinkle[6], Jonathan C Cohen[1] & Helen H Hobbs[1,7]

NATURE GENETICS | VOLUME 40 | NUMBER 12 | DECEMBER 2008 1461





Additional Genetic Risk Factors for NAFLD

Gene (first report)	Protein	Function	Allele variant	Minor allele frequency	Steatosis	NASH	Odds ratio NASH	Associations with other liver disorders
PNPLA3 (5)	Patatin-like phospholipase domain–containing protein 3	TG hydrolase?	rs738409 I148M	0.23 in EA 0.49 in Hispanics 0.17 in AA	Yes	Yes	3.26	Alcohol-related cirrhosis Hepatocellular carcinoma
PPP1R3B (6)	Glycogen binding subunit of protein phosphatase 1	Enhances protein phosphatase 1 activation of glycogen synthase	rs4240624	0.08	Yes	No	0.93	ND
NCAN (6)	Neurocan	Unknown	rs2228603 P92S	0.07	Yes	Yes	1.65	ND
GCKR (6)	Glucokinase regulatory protein	Negative regulator of glucokinase	rs780094 P446L	0.39	No	Yes (not replicated)	1.45	ND
LYPLAL1 (6)	Lysophospholipase-like 1	Unknown	rs12137855	0.21	No	Yes (not replicated)	1.37	ND
APOC3 (25)	Apolipoprotein C3	Limits hydrolysis of TG in circulating lipoproteins	rs2854116 rs2854117	0.38 in EA 0.38 in Hispanics 0.71 in AA 0.26 in EA 0.32 in Hispanics 0.66 in AA	Yes*	ND	ND	ND

*The association was found in Asian-Indian men and replicated in a group of mixed ethnicity (25). The association was not observed in (26, 27).

ethnic difference in liver fat: role of genes and dietary sugar

BRIEF REPORT

Effects of *PNPLA3* on Liver Fat and Metabolic Profile in Hispanic Children and Adolescents

Michael I. Goran, Ryan Walker, Kim-Anne Le, Swapna Mahurkar, Susanna Vikman, Jaimie N. Davis, Donna Spruijt-Metz, Marc J. Weigensberg, and Hooman Allayee

Diabetes, 2010

Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in *PNPLA3* and high dietary carbohydrate and sugar consumption[1–4]

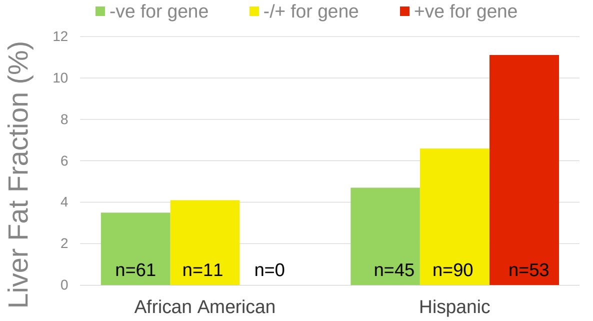
Jaimie N Davis, Kim-Anne Lê, Ryan W Walker, Susanna Vikman, Donna Spruijt-Metz, Marc J Weigensberg, Hooman Allayee, and Michael I Goran

AJCN, 2010

Clinical Characteristics

Trait	All Participants (n=223)	Non-NAFLD (n=126)	NAFLD (n=97)	p value
Age (year)	13.5 ± 2.9	13.5 ± 3.1	13.5 ± 2.8	NS
Male/Female (n)	96/137	43/87	53/50	0.005
Height (cm)	157.3 ± 16.6	157.4 ± 18.2	157.2 ± 11.6	NS
Weight (kg)	77.9 ± 28.2	72.1 ± 27.4	85.4 ± 27.6	**3.4×10^{-4}**
BMI (kg/m²)	30.5 ± 7.6	28.2 ± 7.1	33.4 ± 7.4	**1.7×10^{-57}**
BMI percentile	94.5 ± 10.3	92.3 ± 10.8	97.9 ± 3.6	**1.5×10^{-6}**
SAT (L)	12.1 ± 7.1	10.4 ± 6.6	14.2 ± 7.2	**2.75×10^{-5}**
VAT (L)	1.8 ± 1.3	1.4 ± 0.9	2.2 ± 1.4	**3.8×10^{-8}**
Total Fat (kg)	29.1 ± 12.1	26.1 ± 11.9	32.9 ± 11.5	**5.0×10^{-5}**
Liver fat (%)	8.8 ± 8.5	3.7 ± 1.1	15.2 ± 9.4	**5.4×10^{-32}**
ALT (IU/L)	14.9 ± 10.2	10.8 ± 5.4	20.7 ±12.3	**2.2×10^{-13}**
AST (IU/L)	20.8 ± 8.2	17.9±4.4	24.8±10.3	**4.4×10^{-10}**
TAG (mg/dL)	107.6 ± 52.7	96.6 ± 45.5	121.8 ± 58.1	**0.001**
Total Cholesterol (mg/dL)	140.9 ± 29.6	138.4 ± 28.6	143.9 ± 30.7	NS
HDL (mg/dL)	37.7 ± 9.4	39.0 ± 10.1	35.9 ± 8.3	**0.024**
LDL (mg/dL)	85.2 ± 28.4	84.4 ± 28.8	86.2 ± 28.1	NS

Liver Fat Fraction by Ethnicity & Genotype



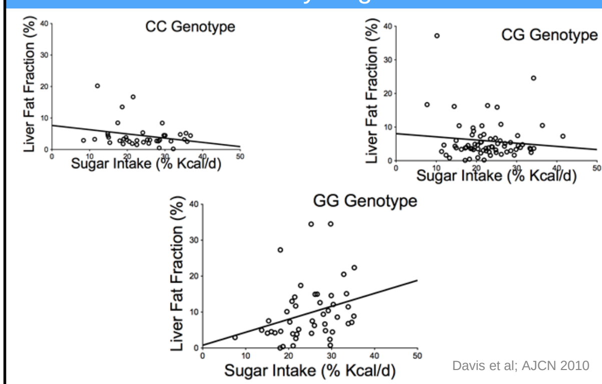
Goran et al; Diabetes 2010

Variants Associated with NAFLD in Hispanic Children and Adolescents

					Effect Allele Copy Number			
Gene	**SNP**	**Alleles[a]**	**EAF[b]**	**Reported EAF[b]**	**0**	**1**	**2**	**p[d]**
PNPLA3	rs738409	G/C	0.52	0.239	5.6±4.3 (n=53)	8.0±8.3 (n=109)	12.3±9.8 (n=60)	**4.2×10^{-6}**
APOC3	rs2854117	A/G	0.35	0.302	7.1±7.6 (n=91)	9.4±8.4 (n=103)	11.3±10.5 (n=26)	**0.01**
GCKR	rs780094	A/G	0.32	0.391	8.6±9.0 (n=101)	8.5±7.4 (n=102)	10.7±9.9 (n=22)	0.44
NCAN	rs2228603	T/C	0.03	0.09	8.7±8.5 (n=212)	9.2±7.8 (n=11)	NA	0.96
LYPLAL1	rs12137855	C/T	0.92	0.79	9.7±0 (n=1)	7.8±8.4 (n=36)	8.9±8.5 (n=184)	0.50
PPP1R3B	rs4240624	A/G	0.67	0.92	8.7±7.9 (n=101)	8.8±9.6 (n=88)	8.5±7.8 (n=28)	0.38

Data are shown as mean liver fat content (%) ± SD as a function of carrying 0, 1, or 2 copies of the effect alleles for selected SNPs. [a]Effect/Other allele. [b]EAF, effect allele frequency in Hispanics. [c]Reported effect allele frequency in Caucasians (HapMap-CEU). [d]p-values are obtained from multiple linear regression using natural log-transformed values, adjusted for age, sex and VAT.

Evidence of Interaction Between PNPLA3 and Dietary Sugar



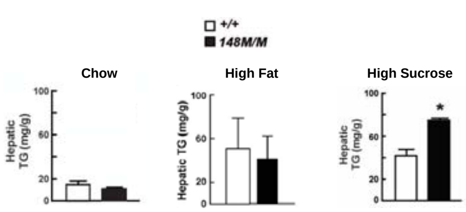
Davis et al; AJCN 2010

This Concept is Supported by a Recent Study from the Hobbs lab using PNPLA3 knockin

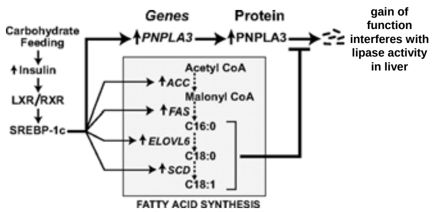
Pnpla3I148M Knockin Mice Accumulate PNPLA3 on Lipid Droplets and Develop Hepatic Steatosis

Eriks Smagris,[1] Soumik BasuRay,[1] John Li,[1] Yongcheng Huang,[1] Ka-man V. Lai,[2] Jesper Gromada,[2] Jonathan C. Cohen,[1] and Helen H. Hobbs[1,3]

HEPATOLOGY, Vol. 61, No. 1, 2015

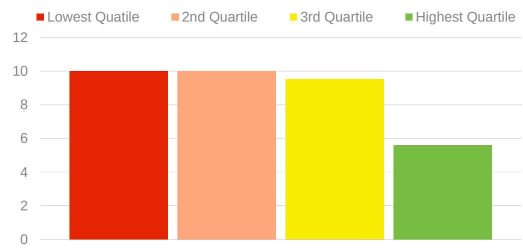


Plausible Biological Mechanism?



Huang Y et al. PNAS 2010;107:7892-7897

Other Dietary Factors Associated with Fatty Liver: Non-starchy Vegetable Intake



Servings (mean ± SD)
Q1: 0.1 ± 0.1, n=43; Q2: 0.4 ± 0.1, n=44;
Q3: 0.7 ± 0.1, n=44; Q4: 1.7 ± 1.0, n=44

no effects for fruits or fibre

Cook et al; JADA, in press

Obesity and NAFLD in Hispanics: The Perfect Storm

childhood obesity 7 times higher in low-income communities relative to affluent

Greater susceptibility to this environment in Hispanics starting in first 2y of life

From a dietary perspective, this effect is most related to high dietary sugars esp proliferation of HFCS in the diet

50% prevalence of PNPLA3 gene in Hispanics related to ~2-fold higher liver fat

Impact of PNPLA3 gene on fatty liver exacerbated by high sugar diet

Will Reducing Dietary Sugars in Hispanics be an Effective Intervention Strategy?

Good News and Bad News

The Bad News: Incredibly Difficult to Persuade Study Sections and NIDDK of Potential Impact of this Approach

R01 DK 091578-01A1; NIDDK - May 2011

NPLA3 Genotype for Reducing Liver Fat in Hispanics with Pediatric Non-alcoholic Fatt

Score = 25; 8th percentile

"Unfortunately, your application was deemed to be of low programmatic priority"

Other Grants Not Funded

R21: Omega-3 Fatty Acid Supplementation for Treatment of Fatty Liver in Children

RO1: Treatment of Insulin Resistance through Reduction of Liver Fat in Minorities

RO1: Improving obesity and metabolic outcomes in Hispanics through maternal-infant intervention

RO1: Improving obesity, liver fat and obesity outcomes in obese Hispanic teens through SSB reduction

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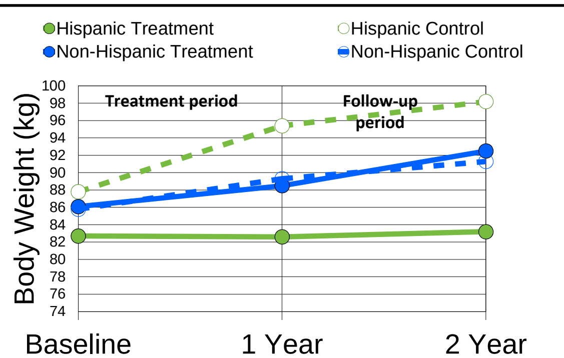

zero effect in Non-Hispanics

~10kg reduced weight gain in Hispanics sustained over 2 years

Ebbeling et al, NEJM 2012

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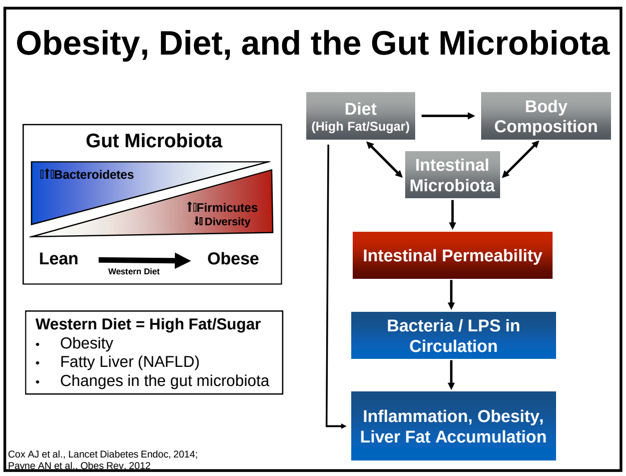
Krishna Nayak, PhD
Harry Hu, PhD
Kim-Anne Le
Postdoc
Hooman Allayee, PhD
Frank Sinatra, MD
Tanya Alderete, PhD
Ryan Walker, PhD
Jaimie Davis, RD, PhD
Marc Weigensberg, MD
Thanks!
www.goranlab.com


Obesity, Diet, and the Gut Microbiota
Gut Microbiota
Bacteroidetes
Firmicutes
Diversity
Lean
Obese
Western Diet
Western Diet = High Fat/Sugar
• Obesity
• Fatty Liver (NAFLD)
• Changes in the gut microbiota
Diet (High Fat/Sugar)
Body Composition
Intestinal Microbiota
Intestinal Permeability
Bacteria / LPS in Circulation
Inflammation, Obesity, Liver Fat Accumulation
Cox AJ et al., Lancet Diabetes Endoc, 2014;
Payne AN et al., Obes Rev, 2012