Case Presentation
(Fast Forward to 2016)

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Izzy (2016)

- Izzy is now 61 y/o. In the past year, she has become a fan of having 2-3 alcoholic drinks with dinner.
- It has been 9 years since her lap band and she has gained back the weight, in fact she gained 20 more pounds.
- She began to complain of a dull RUQ pain.
- She had labs done and an U/S.
- ALT 145, AST 67, TB 0.9, Alk phos 177, albumin 3.6, Cr 1.1, WBC 4.6, Hb 13.5, Platelet count 145.
- RUQ U/S- increased liver echogenicity c/w fatty infiltration. Normal GB.

NAFLD/NASH: Workup and Diagnosis

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University of Texas Health Science Center, San Antonio
Chief, Hepatology
Vice President, Academic and Clinical Affairs
The Texas Liver Institute
San Antonio, Texas

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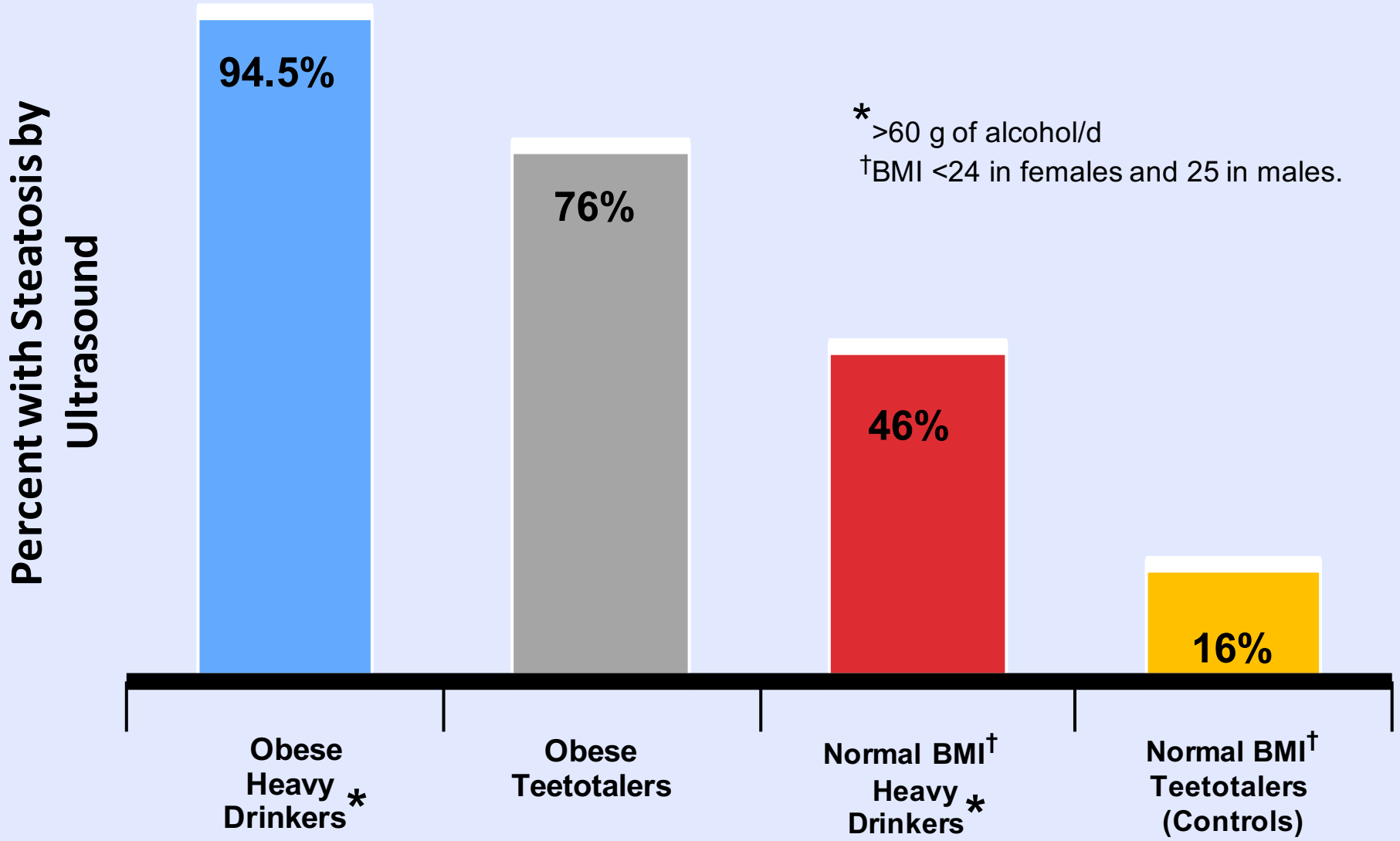
Is Food or Alcohol Worse for the Liver?

The Verona Study



Bellentani S, et al. *J Hepatol*. 2001;35:531-537.

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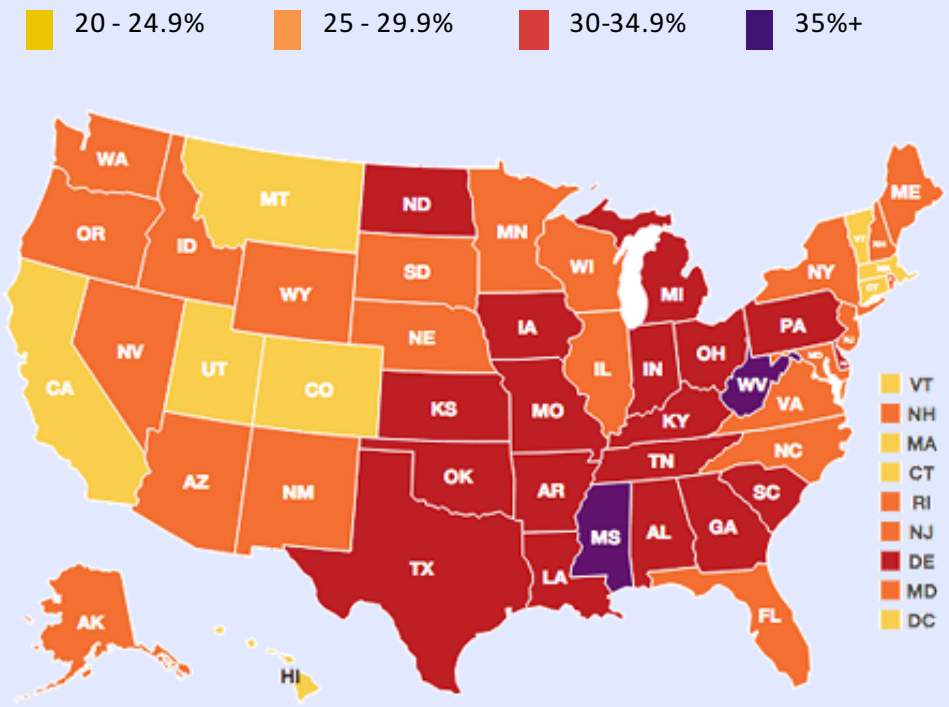
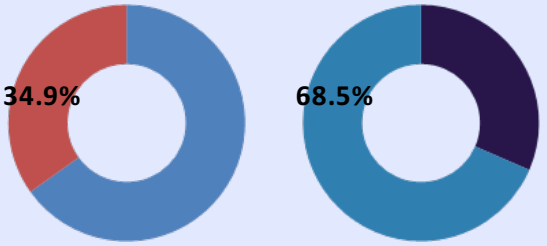
Adult Obesity in America 2014

Percent of Obese Adults (Body Mass Index of 30+)



TX ranks #11

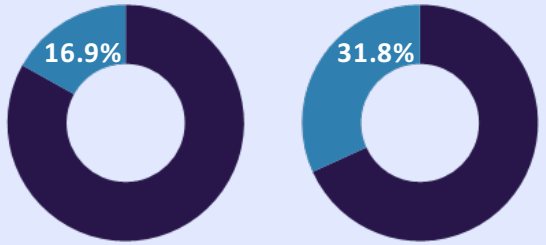
Adult Obesity in America 2011-12



Obese

Overweight or Obese

Childhood Obesity in America 2011-12



Obese

Overweight or Obese

http://stateofobesity.org/adult-obesity/

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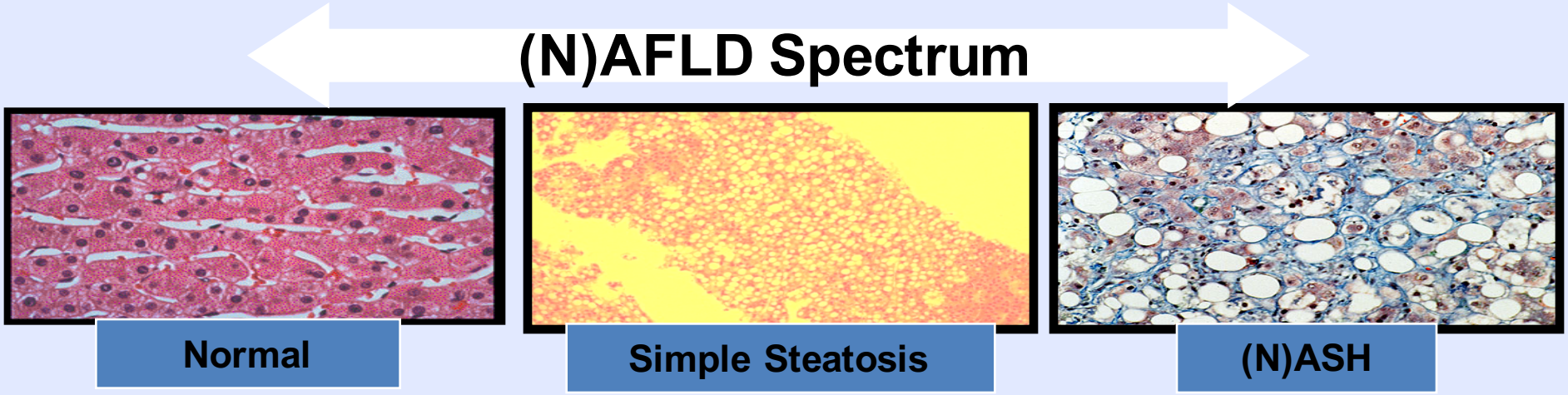
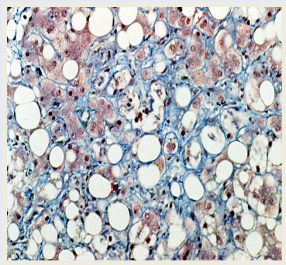
The Spectrum of NAFLD and AFLD



- Exclusion of liver diseases (HCV)
- Requires specific pathologic criteria for NASH
- Important for prognosis
- Some reversibility

Ludwig 1980; Diehl 1988; Lee 1989; Powell 1990; Bacon 1994; Younossi 1997; Matteoni/Younossi 1999; Angulo 1999; Caldwell 1999; Ponawala 2000; Contos 2001; Ong/Younossi 2001; Bugianesi 2002; Ratziu 2002; Saddeh/Younossi 2002; Harrison 2003; Marchesini 2003; Younossi 2004; Gramlich/Younossi 2004; Fassio 2004; Sanyal 2004; Ong/Younossi 2005; Adams 2005; Ong/Younossi 2008; Mishra/Younossi 2008; Rafiq/Younossi 2010; Hossain/Younossi 2009; Kim/Younossi 2010; Stepanova/Younossi 2010; Hossain/Younossi 2010; Stepanova, Younossi 2012; Younossi 2012; Chalasani, Younossi 2012.

Clinical Outcome of NASH Variable

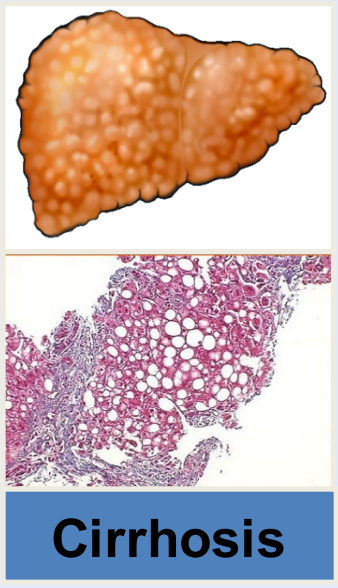


NASH

~20 million

Overall

10-15%

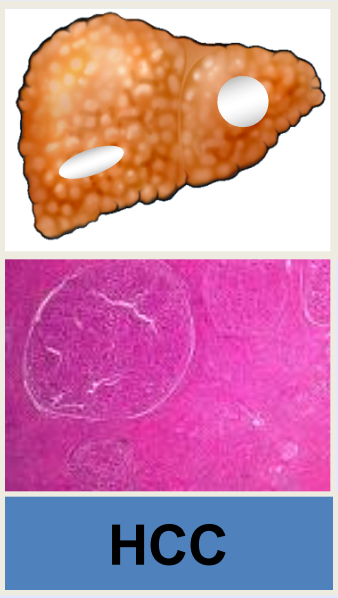


Cirrhosis

~3 million

Annual Incidence

2-3%



HCC

Ludwig 1980; Diehl 1988; Lee 1989; Powell 1990; Bacon 1994; Matteoni 1999; Angulo 1999; Caldwell 1999; Ponawala 2000; Contos 2001; Ong 2001; Bugianesi 2002; Ratziu 2002; Harrison 2003; Marchesini 2003; Younossi 2004; Fassio 2004; Sanyal 2004; Ong 2005; Adams 2005; Ong 2006; Rafiq 2008; Stepanova 2010; Younossi 2012.

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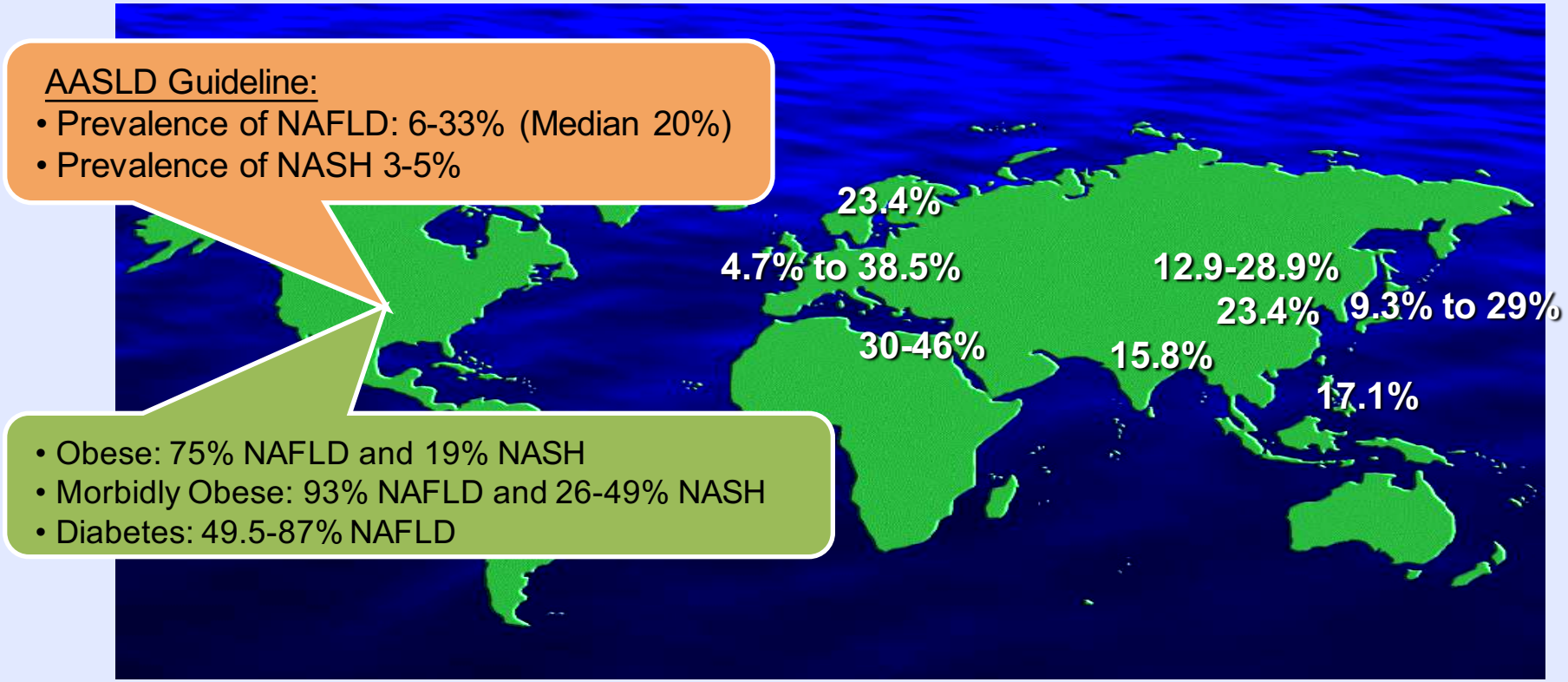
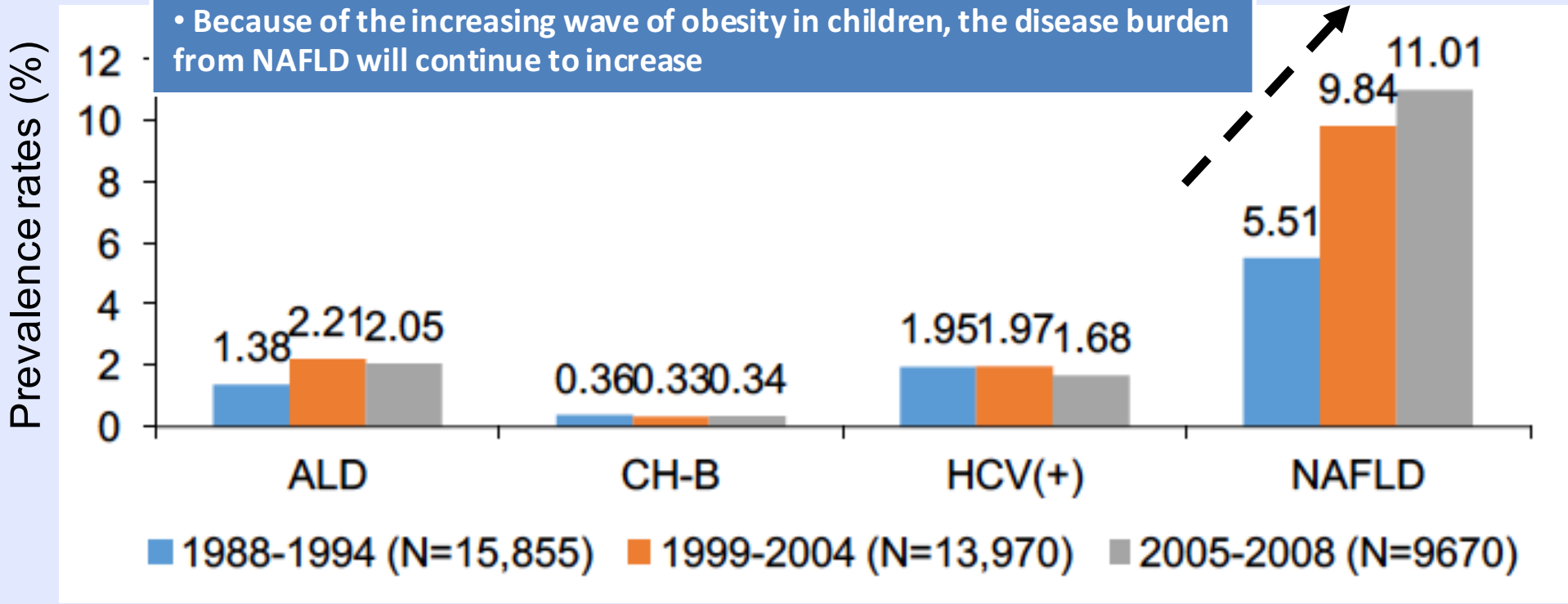
Epidemiologic Impact of NAFLD
Worldwide Prevalence of NAFLD



Hilden 1977; Ground 1982; Hultcrantz 1986; Nomura 1988; Nonomura 1992; El-Hassan 1992; Propst 1995; Lonardo 1997; Bellentani 2000; Clark 2001; Ruhl 2004; Browning 2004; Angelico 2005; Hamagushi 2005; Jimba 2005; Lin 2005; Fan 2005; Zelber 2006; Zhou 2007; Fan 2007; Targher 2007; Lazo 2008; Younossi 2011; Chalasani 2012.

Changes in the Prevalence of the Most Common Causes of Chronic Liver Diseases in the United States from 1988 to 2008 (N=39,750)

- In comparison to the prevalence of other etiologies, NAFLD is the most common cause of chronic liver disease
- Because of the increasing wave of obesity in children, the disease burden from NAFLD will continue to increase



Younossi Z, et al. *Clin Gastro and Hep*. 2011.

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Natural History of NAFLD

NAFLD

~70-75%

Isolated Fatty Liver

Fatty Liver with Mild Inflammation

1. None to very minimal progression to fibrosis
2. No ↑ risk of death compared with the general population

Possible sampling variability with some risk of progression

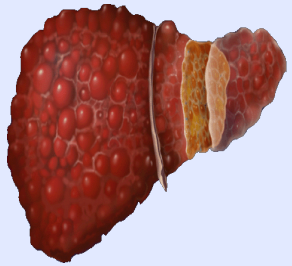
~20-25%

NASH

1. ↑ risk of death compared with general population
    1. Cardiovascular
    2. Malignancy
    3. Liver-related
2. NASH with fibrosis portends worse prognosis
    1. Fibrosis progression a/w DM, severe IR, weight gain >5kg, rising ALT, AST

~11% over 15 years, but significant variability

NASH Cirrhosis



~7.2% over 6.5 years

HCC

19-45% over 7-10 years

Decompensation

Modified from Torres DM, et el. *Clin Gastro Hepatol*. 2012;10:837-858.

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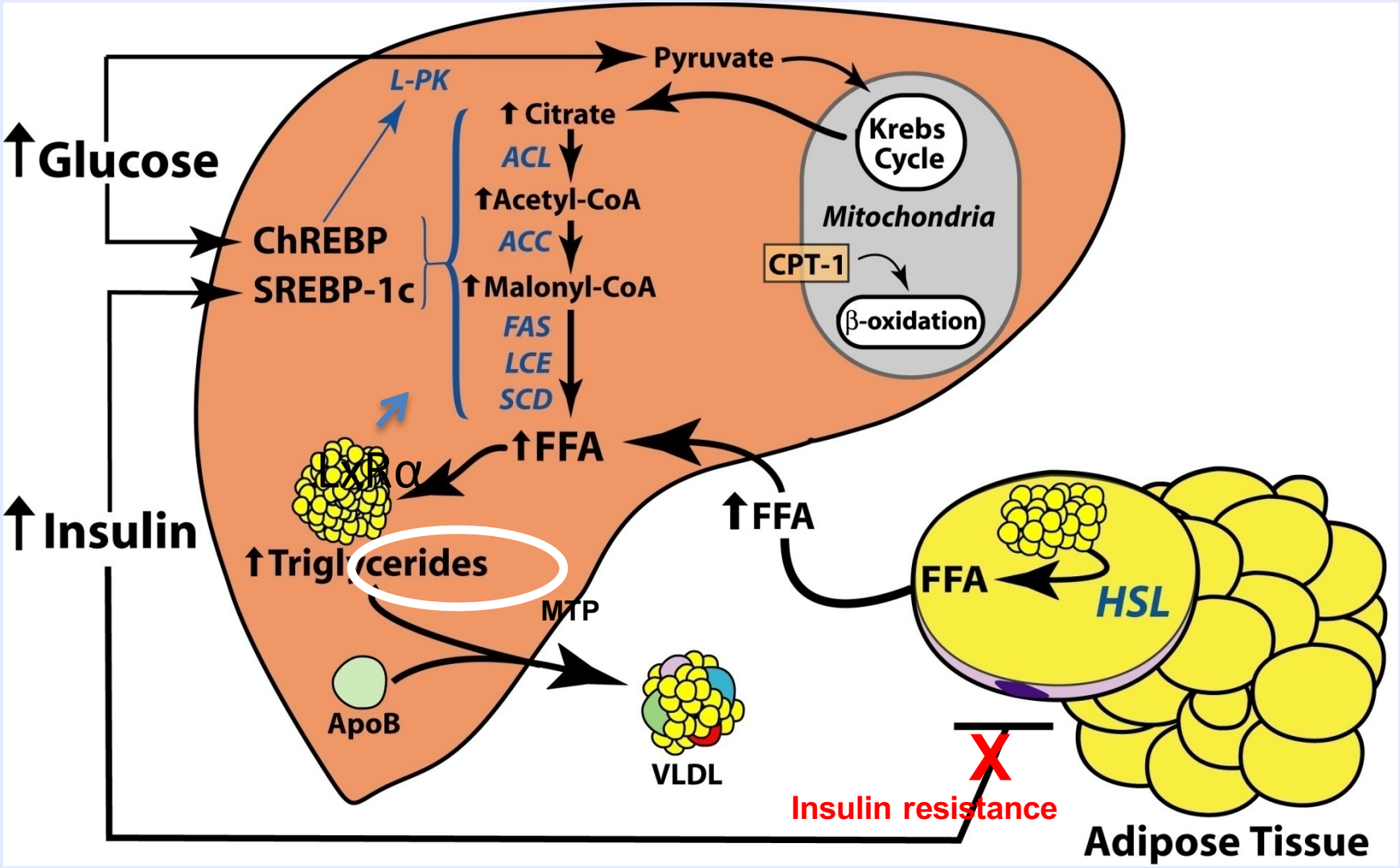
Metabolic Alterations That Lead to NAFLD



Browning and Horton. *J Clin Invest*. 2004;114:147-152.

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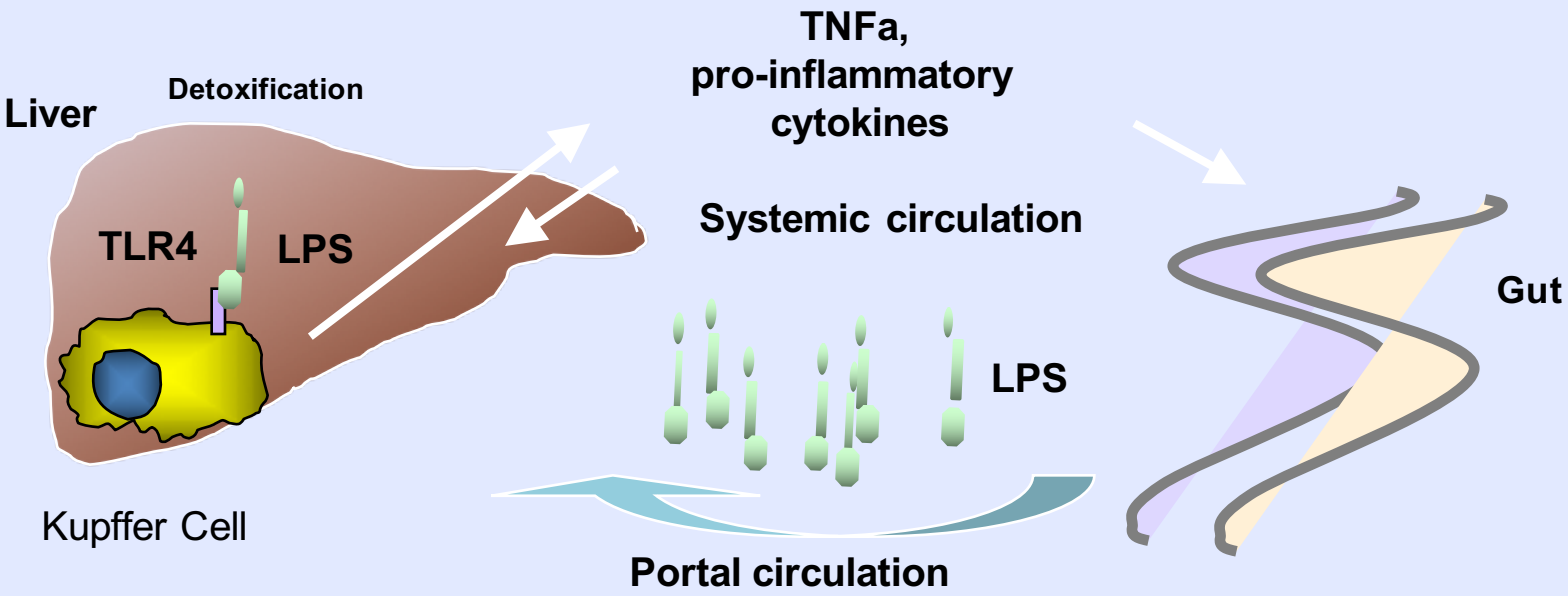
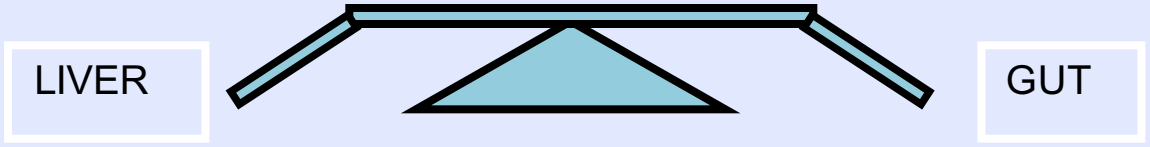
Gut - Liver Axis Microbial Translocation



- Detoxification
- Hepatocyte function
- Kupffer cell activation
- LPS sensitization

- Barrier dysfunction
- Permeability
- Bacterial overgrowth
- Microbial translocation



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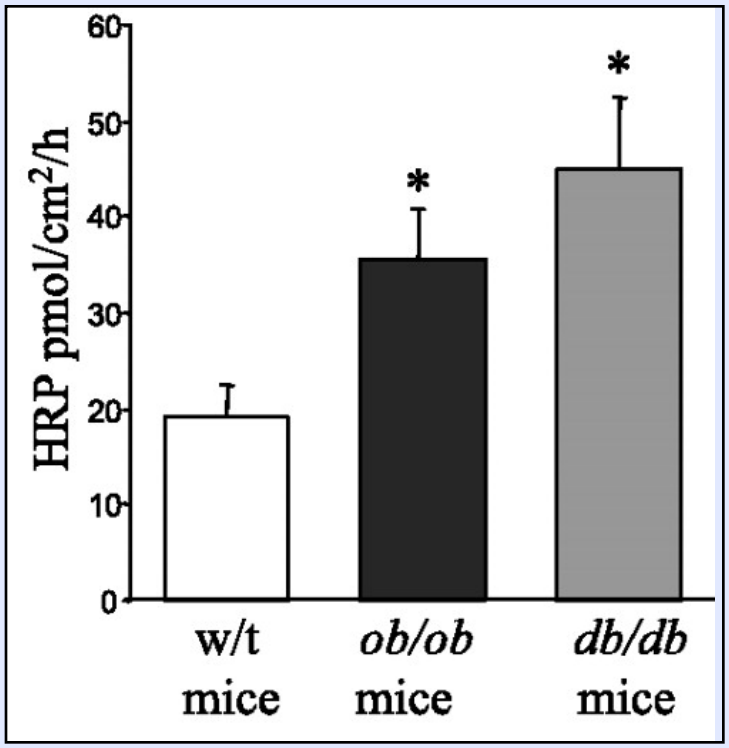
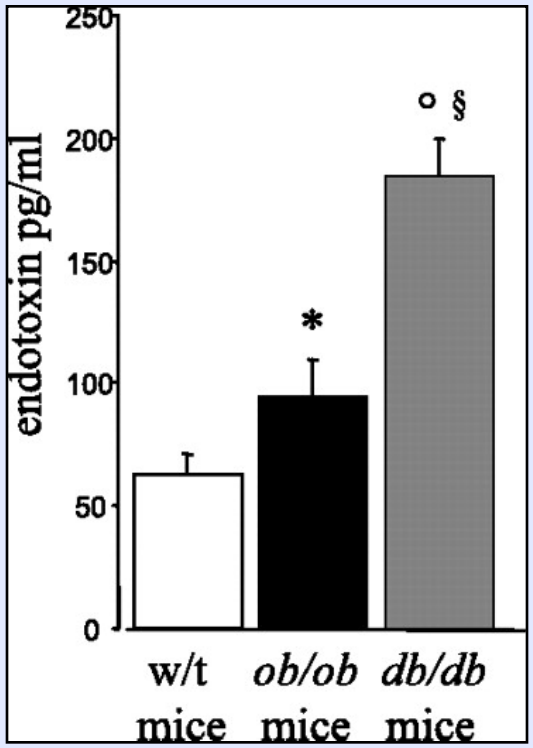
Genetically Obese Mice Have Increased Gut Permeability and Endotoxemia





Brun, et al. *Am J Physiol Gastrointest Liver Physiol.* 2007;292:G518.

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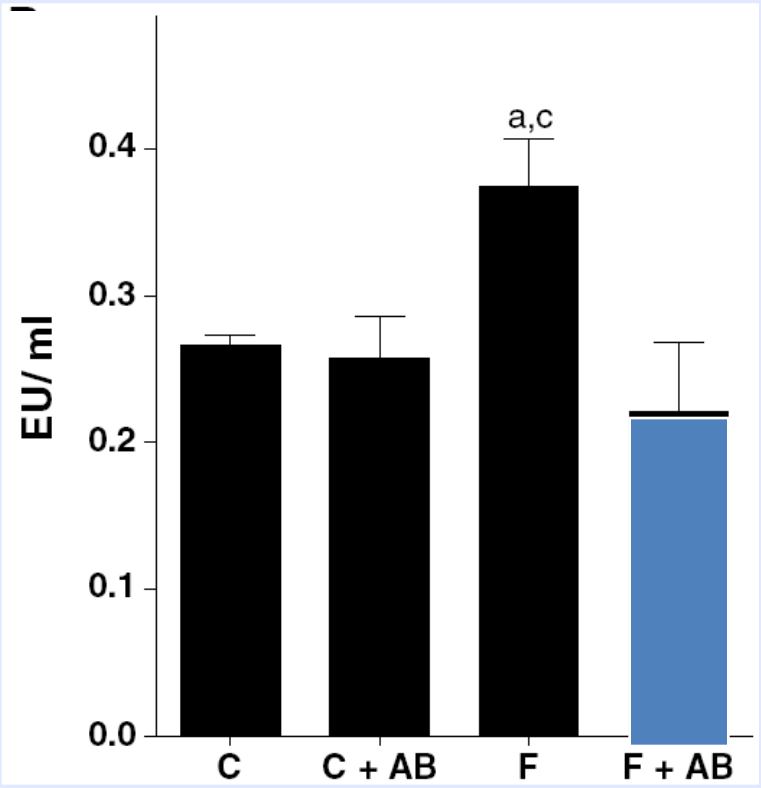
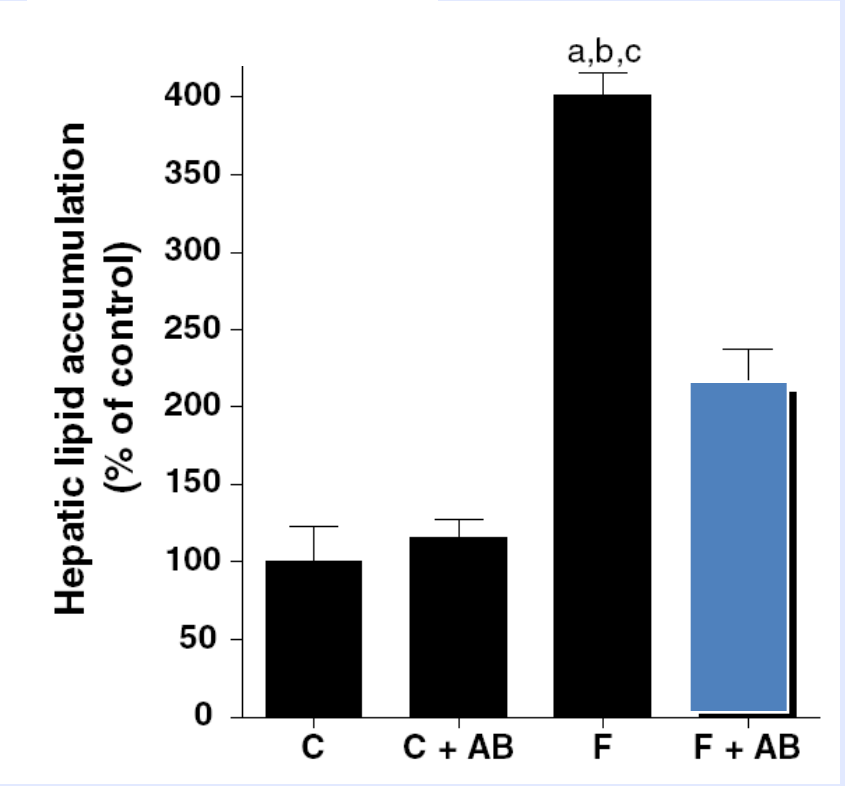
Antibiotics Protect Against High Fructose Diet-induced NAFLD

Endotoxin



Hepatic lipids



Bergheim, et al. *J Hep.* 2008;48(6):983-92.

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Genetic Predispositions to NAFLD

- Patatin like phospholipase containing domain 3 gene encodes (*PNPLA3*) for adiponutrin
- Adiponutrin is a lipogenic enzyme in the liver
- *PNPLA3* rs738409 single nucleotide polymorphism (SNP) is a non-synonymous variant, represented by a cytosine to guanosine substitution which encodes an isoleucine to methionine substitution at the amino acid position 148 (I148M)

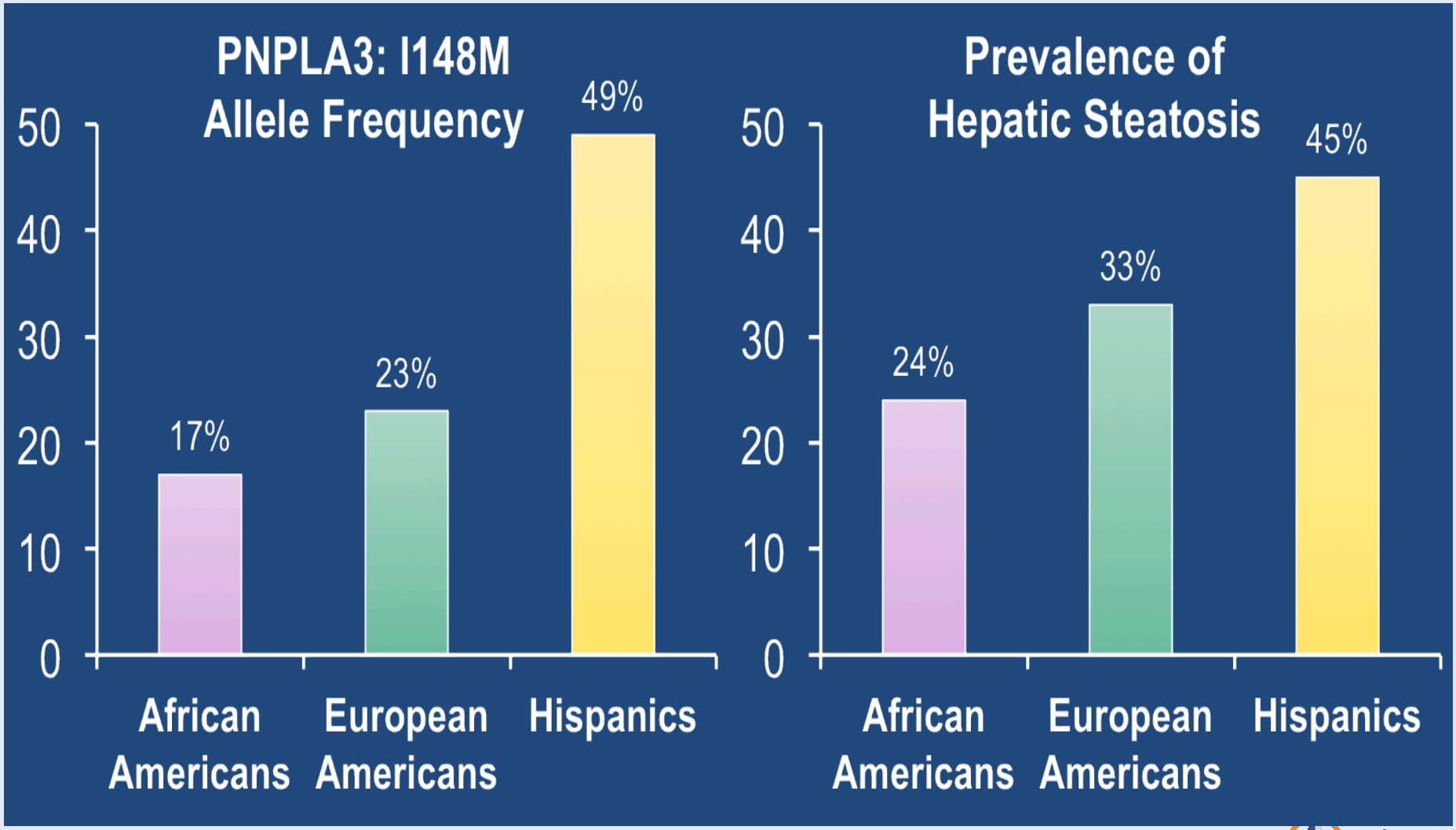
Romeo S. *Nat Genet*. 2008;40(12):1461-5.

Proposed Mechanism of Action of Each Genetic Variant Associated With Fatty Liver Disease

- ***PNPLA3*** - The *PNPLA3* encodes for the adiponutrin, an enzyme expressed in the liver and adipose tissue showing both a lipogenic and lipolytic activity. This variant could cause both a gain of function of the enzyme (which could have a lipogenic activity in the liver) and a loss of function (that could predispose to steatosis by decreasing triglyceride hydrolysis in hepatocytes)
- ***GCKR*** - The gene product is a regulatory protein that inhibits glucokinase in liver and pancreatic islet cells. The polymorphism could lead to increased hepatic glucokinase activity. This enhance the glycolytic flux and then promotes hepatic glucose metabolism and elevates the concentrations of malonyl coenzyme A, a substrate for de novo lipogenesis
- ***APOC3*** - *APOC3* variants could increase the plasma concentrations of apolipoprotein C3. The apolipoprotein C3 could then inhibit the lipoprotein lipase reducing the clearance of triglycerides. Consequence of reduced clearance of triglycerides is the increase of chylomicron-remnant particles that confer a predisposition to both fasting and postprandial hypertriglyceridemia. Higher circulating levels of chylomicron-remnant particles are then especially cleared by the liver through a receptor-mediated process, resulting in NAFLD and hepatic insulin resistance
- ***NCAN*** - *NCAN* encodes for a chondroitin sulfate proteoglycan thought to be involved in the modulation of cell adhesion and migration. *NCAN* is a risk factor for liver inflammation and fibrosis, suggesting that this locus is responsible for progression from steatosis to steatohepatitis
- ***LYPLAL1*** - *LYPLAL1* encodes for a lysophospholipase and it is associated with increased hepatic steatosis probably preventing breakdown of triglycerides
- ***PPP1R3B*** - This gene encodes the catalytic subunit of the serine/theonine phosphatase, protein phosphatase-1. The encoded protein is expressed in liver. It is associated with computer tomography–assessed liver attenuation but not histology-proven NAFLD
- ***GC*** - *GC* gene is expressed predominately in the hepatocytes where it encodes for VDBP. VDBP is the main vitamin D carrier, which has been implicated in the development of obesity and diabetes. In fact, low vitamin D concentrations could increase adipocyte intracellular calcium, stimulating lipogenesis, whereas vitamin D supplementation improves insulin resistance and down-regulates inflammatory cytokines such as tumor necrosis factor-a and interleukin-6 in cell models. Vitamin D levels are influenced by *GC* genetic polymorphisms

Marzuillo [illegible]

PNPLA3:I148M – Ethnic-specific Allele Frequencies in Dallas Heart Study



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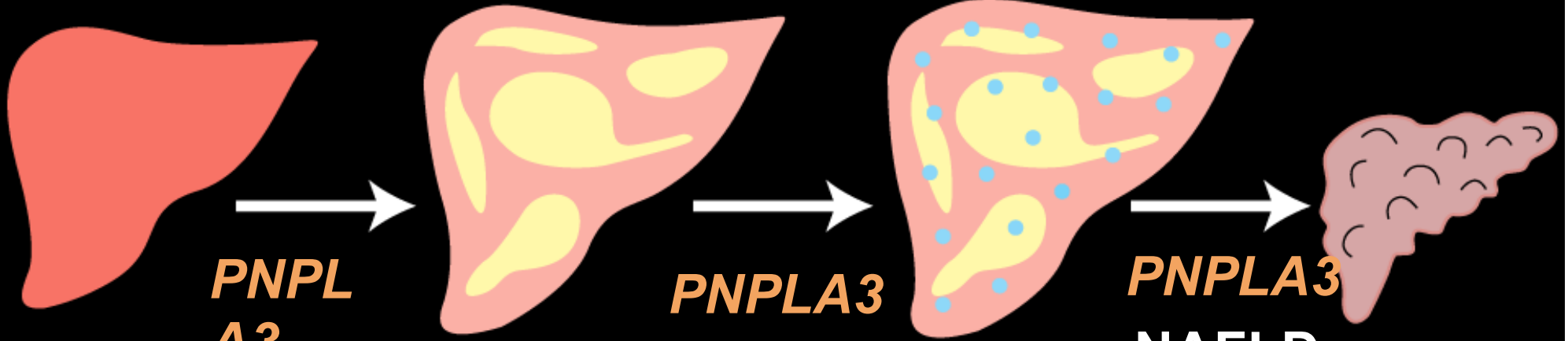
PNPLA3 and Disease Progression



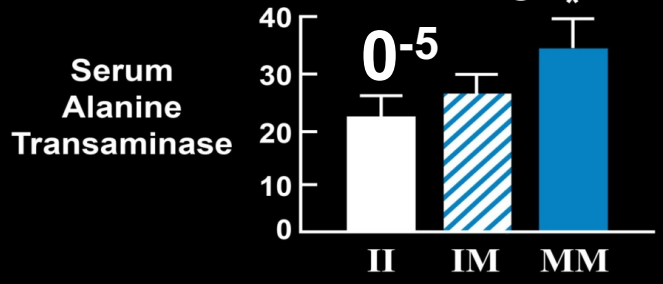
PNPLA3

PNPLA3

PNPLA3

Hispanics

P=1.3X10^{-5}



Yuan X, et al. *Am J Hum Genet.* 2008; ↑***LFTs***
Sookoian, et al. *J Lipid Res*. 2009. ***Pathology***

- **NAFLD**
- **Alcohol**

MM: 3.8 X risk cirrhosis

Tian C, et al. *Nat Genet*. 2010;
Muller, et al. *J. Hepatol.* 2011;
Trepo, et al. *J Hepatol*. 2011.

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What Are the Clinical Predictors of Advanced Fibrosis in NAFLD?

- NAFLD with liver biopsy (N=432)
- In multivariate analysis, elevated AST and ALT, presence of diabetes mellitus, male gender and Caucasian ethnicity were associated with moderate to severe fibrosis ($p<0.0001$)

Factors	Advanced Fibrosis OR (95% CI)	P-value
Hypertension	1.61 (1.21-2.01)	0.0374
Diabetes	1.64 (1.13-2.17)	0.0258
HTN and DM	1.69 (1.11-2.28)	0.0246
HTN+DM+Visceral Obesity	1.72 (1.13-2.31)	0.0205

Hossain N, et al. *Gastro and Hepatology*. 2009.

Diagnosis of NAFLD

Despite Being Very Common, NAFLD is Not Well Recognized in Clinical Practice

- Houston VA patients (2001–2011) with chronic elevation of ALT and no liver diseases (n = 19,692)
- Random sample (n = 450)
- Structured chart review to confirm the criteria for NAFLD and metabolic syndrome
- Data from the primary care providers' notes were abstracted for
  - Recognition of abnormal ALT levels
  - Mention of NAFLD as a possible diagnosis
  - Recommendations for diet or exercise
  - Referral to a specialist for NAFLD evaluation

NAFLD Case Definition
(n = 251)

- Persistently increased ALT
- No viral hepatitis
- No excessive alcohol (2 yr prior)
- Metabolic syndrome, BMI ≥30

Of patients with NAFLD (N=251)

- 39.4% recognition of ALT increase
- 21.5% diagnosed possible NAFLD
- 14.7% received recommendation for lifestyle changes
- 10.4% were referred to a specialist
- Of those at high risk for fibrosis, 3% were referred to specialists

Blais P, et al. *Am J Gastroenterol*. 2015;110:10-14.

Radiologic Assessment of Non-alcoholic Fatty Liver Disease

- **Liver Stiffness:** Ultrasound elastography or MR elastography (problems with reproducibility and inability to discriminate lower stages of fibrosis and no validity for longitudinal studies)
  - US elastography can fail in visceral obesity
- **Liver Fat Content:** MRI/MRS proton density fat fraction

Image Modality	N	Year	Author	Comment
US	165	2002	Mathiesen	• Echogenicity could not detect fibrosis
US, CT, MRI	25	2002	Saadeh	• Excellent to predict>30% steatosis • Unable to diagnose NASH and stage fibrosis
CT	30	2004	Brunt	• Hepatic fat content (L/S) not associated with steatohepatitis and fibrosis.

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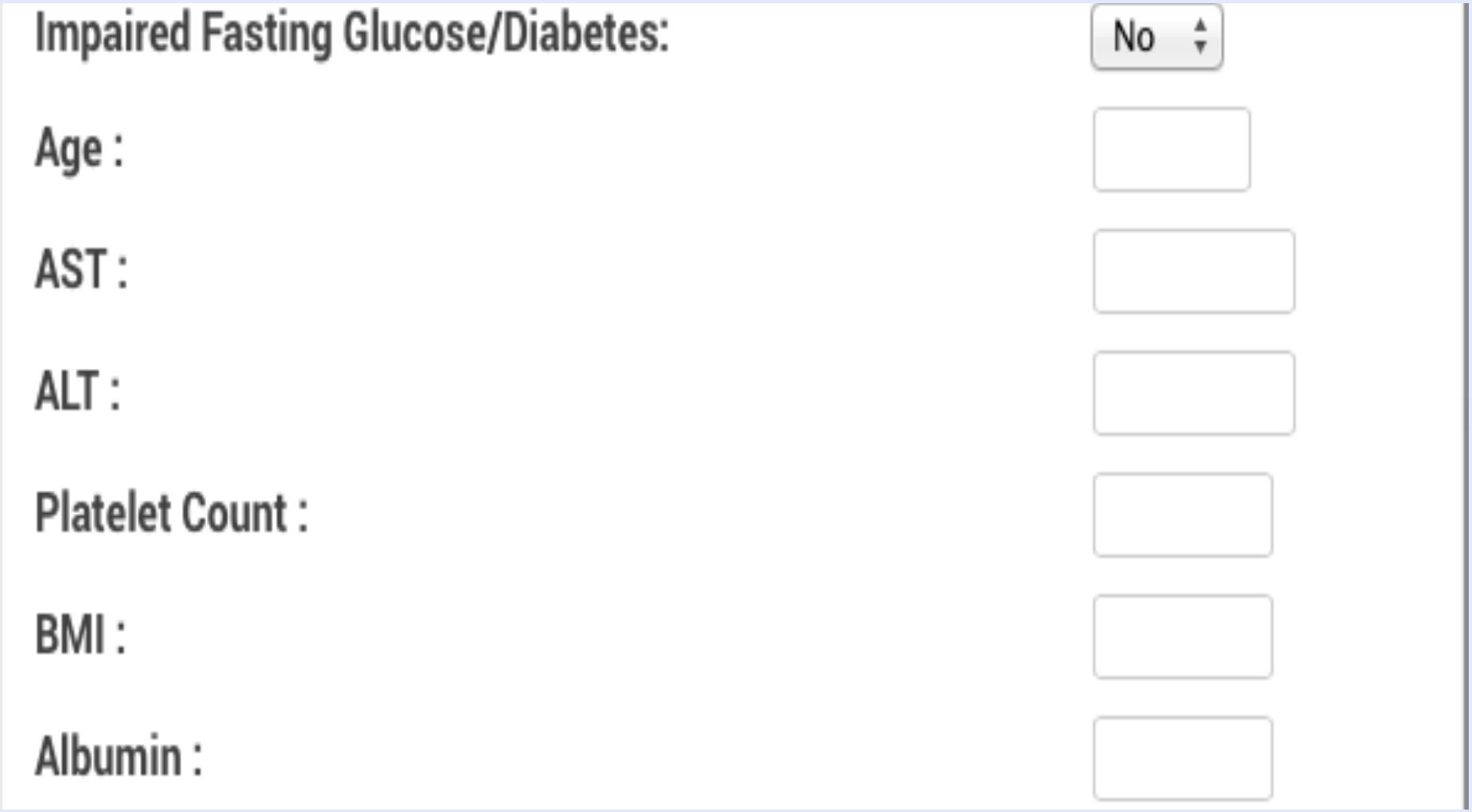
NAFLD Fibrosis Score



Angulo P. *Hepatology*. 2007;45(4):846-54.

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NAFLD Fibrosis Score

Formula

- 1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet (× 109/l) - 0.66 × albumin (g/dl)

Explanation of Result

- NAFLD Score < -1.455 = F0-F2
- NAFLD Score -1.455 – 0.675 = indeterminate score
- NAFLD Score > 0.675 = F3-F4

Angulo P. *Hepatology*. 2007;45(4):846-54.

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Pathology Scoring Systems Used in NASH

Ludwig, 1980 (Original)	• Steatosis, lobular inflammation, necrosis and Mallory bodies in zone 3, mild portal and periportal inflammation		
Younossi, 1999 (NAFLD Subtypes)	• Steatosis, ballooning degeneration	• Steatosis, ballooning with either Mallory's hyaline or fibrosis	
Brunt, 1999 (Brunt Criteria)	• Steatosis with mixed inflammation, occasional ballooned hepatocytes	• Steatosis, ballooning and disarray in zone 3	• Panacinar steatosis + ballooning + disarray + portal inflammation
Kleiner, 2005 (NAS Criteria)	• Steatosis (0-3), lobular inflammation (0-3), ballooning (0-3) • 0-2 not NASH → ≥ 5 usually NASH		

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Transient Elastography

- Allows painless and simultaneous measurement of two quantitative parameters:
- Liver stiffness expressed in kPa
  - Correlated to liver fibrosis[1]
- Controlled Attenuation Parameter (CAP™) expressed in dB/meter
  - Correlated to liver steatosis[2]
- Both quantitative parameters are assessed on the same volume of liver tissue
- 100 times bigger than liver biopsy

1. Rust F. et al. *Gastroenterology* 2008;134:960-74.
2. Sasso, et al. *Journal of Viral Hepatitis*. 2012;19:244-53.

Transient Elastography

- Measures velocity of a low-frequency (50 Hz) elastic shear wave propagating through the liver
  - Normal liver: ~5.5 kPa
- Good performance for excluding advanced stage disease (stage 3-4)
- User-friendly, short procedure time
- Problems still with severe obesity, ascites, operator experience
- False positives: acute hepatitis, extrahepatic cholestasis and congestion
- XL probe has ~25% unreliable results; cut-off concerns
- **Not very good in our hands at predicting fibrosis in NAFLD patients**

Castera L. *Nat Rev Gastroenterol Hepatol*. 2013;10:666-75.

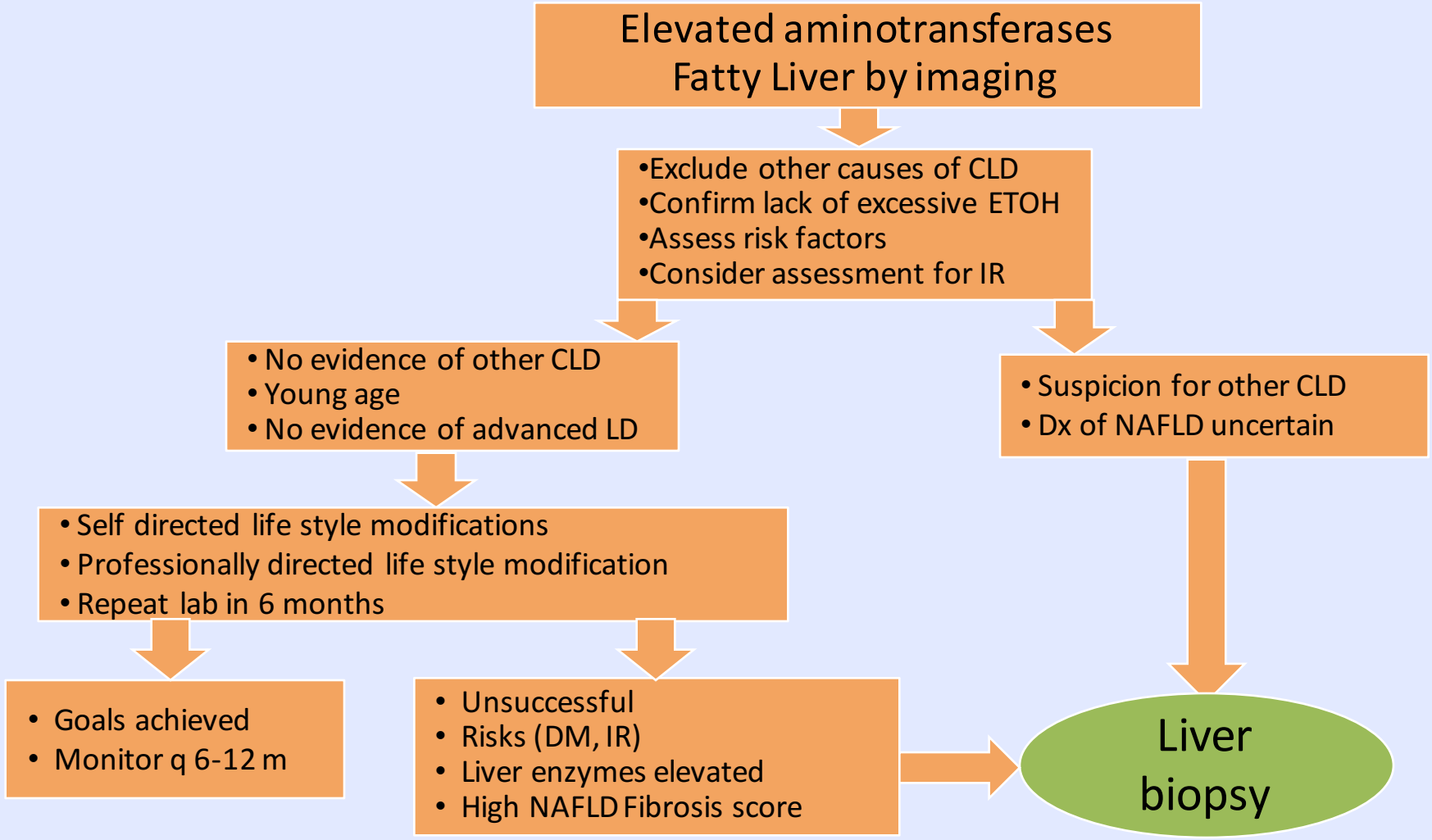
Liver Biopsy

- Identify NASH (ballooning, inflammation, etc)
  - Establish diagnosis
  - Clinical trials
- Stage fibrosis
- Rule out concomitant liver disease (iron loading, etc)
- Prognosis

Liver Biopsy Results

- Microscopic description
  - Sections obtained after processing show a 2.7 mm long liver biopsy containing more than 18 portal tracts.
  - The biopsy shows steatosis with scattered lobular and portal inflammation and prominent hepatocellular ballooning with a number of Mallory bodies, indicating an active steatohepatitis.
  - The trichrome stain shows extensive centrilobular pericellular fibrosis as well as several areas of bridging fibrosis.

How Do We Diagnose NAFLD Patients in 2016?



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NAFLD and NASH

- NAFLD is a complex disease tied closely to obesity and diabetes
- NASH patients with fibrosis most likely to progress
  - NAFLD/NASH in the setting of DM/MS has adverse outcomes
- Diagnosis needs to be individualized based on suspected risk of NASH and progression
- Non-invasive modalities are best approach with biopsy reserved for those with suspected aggressive disease

Back to Dr. Wells

Izzy

- A liver biopsy is performed 6 months after she quit drinking. She is diagnosed with NASH and fibrosis stage 3-4.
- What is Izzy's prognosis and how will we manage her?

Prognosis and Management of NASH

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Assistant Professor of Medicine
University of Texas Health Science Center, San Antonio
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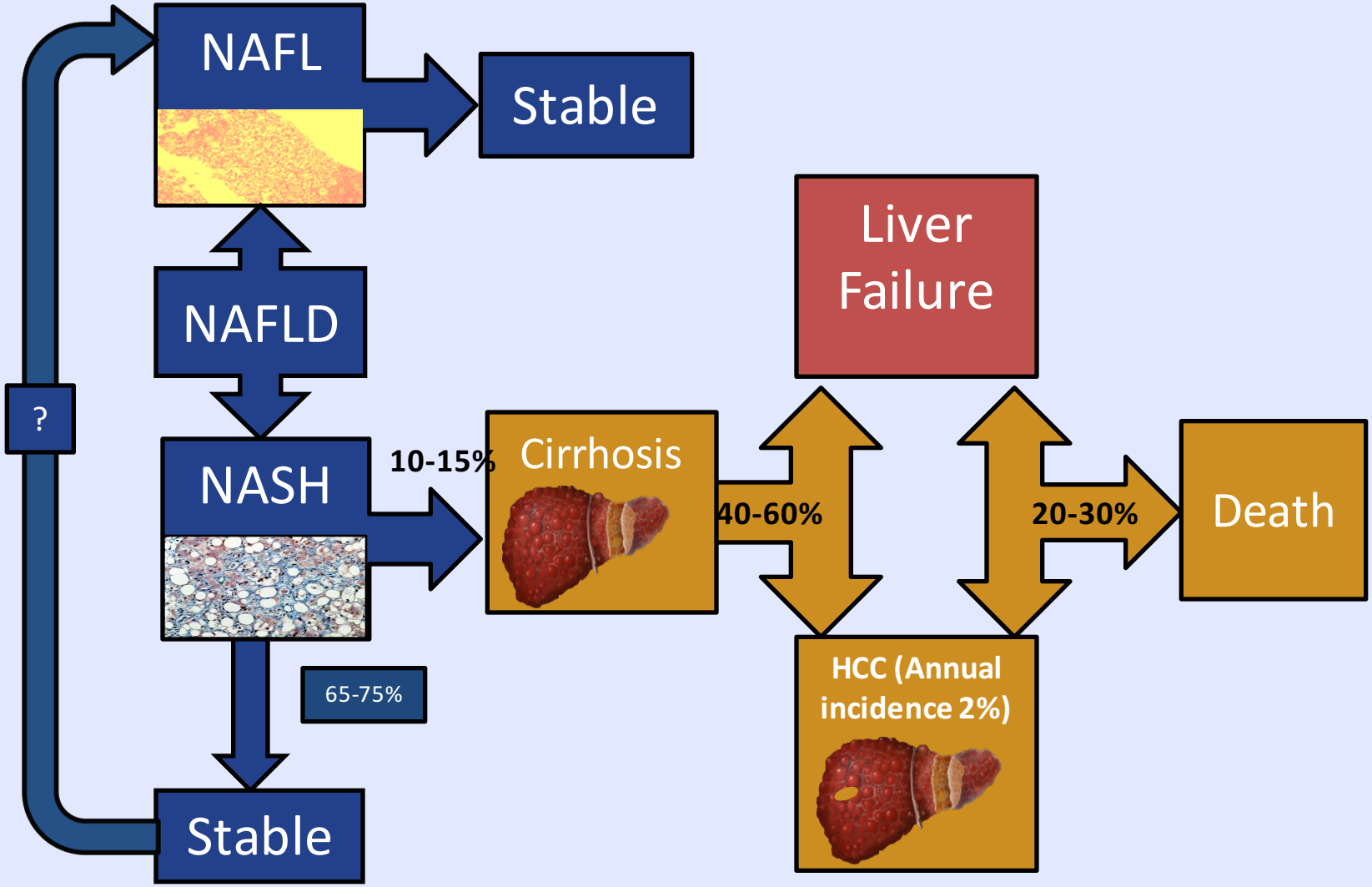
NASH is the Subtype of NAFLD that Primarily Progresses



Ludwig 1980; Diehl 1988; Lee 1989; Powell 1990; Bacon 1994; Matteoni 1999; Angulo 1999; Caldwell 1999; Ponawala 2000; Contos 2001;Ong 2001; Bugianesi 2002; Ratziu 2002; Harrisson 2003; Marchesini 2003; Younossi 2004; Fassio 2004; Sanyal 2004; Ong 2005; Adams 2005; Ong 2006; Rafiq 2008; Stepanova 2010; Younossi 2012.

What Are the Clinical Predictors of Mortality in NAFLD?

- Histologic NAFLD (N=289)
- Clinico-demographic data from biopsy date
- NASH (59.2%), non-NASH (40.8%)
  - **NASH patients were predominantly female, higher AST, ALT and serum glucose**
- Mortality: Median follow-up of 150 months
  - NASH had higher risk of liver-related mortality than non-NASH NAFLD (p= 0.002)

Risk factor	Overall mortality aHR (95% CI)	Liver-related mortality aHR (95% CI)
NASH	1.13 (0.74 - 1.71)	**9.16 (2.10 - 9.88)**
Age	**1.07 (1.05 - 1.10)**	**1.06 (1.02 - 1.10)**
Male gender	0.95 (0.62 - 1.47)	1.44 (0.62 - 3.34)
Caucasian race	1.67 (0.92 - 3.06)	1.85 (0.62 - 5.47)
Obesity	0.91 (0.60 - 1.40)	0.88 (0.38 - 2.04)
DM	**2.09 (1.39 - 3.14)**	**2.19 (1.00 - 4.81)**
Hyperlipidemia	1.01 (0.68 - 1.52)	0.48 (0.19 - 1.23)

Stepanova M, et al. *Dig Dis Sci*. 2013.

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What Are the Histologic Predictors of Mortality in NAFLD?

- NAFLD liver biopsy and mortality data (N=209)
- Biopsies were read centrally
- During follow-up (146 months), 31% of patients died with 9% dying of LRM

Univariate Survival Analyses [HR (95% CI) , p-value]	
Portal inflam (grade≥2)	[6.68 (2.20-20.3), p<0.001]
Ballooning (grade≥2)	[5.32 (1.89-14.9), p=0.001]
MD bodies (grade≥2)	[4.21 (1.66-10.7), p=0.002]
Portal fib (grade>2)	[14.1 (5.47-36.5), p<0.001]
Pericellular fib (grade>2)	[4.86 (1.73-13.7), p=0.003]

On multivariate analysis, only significant fibrosis (grade > 2) was an independent predictor of LRM

Younossi Z, et al. *Hepatology*. 2011.

- International study of NAFLD (N=619) diagnosed between 1975-2005
- All liver biopsies centrally ready
- Median follow-up 12.6 years
- 193 who died or had OLT

Multivariate Analysis		
Fibrosis Stage	**Hazard Ratio (95% CI)**	**P value**
0	1 (ref)	
1	2.4 (0.63, 8.91)	0.2
2	7.5 (2.26, 24.94)	0.01
3	13.8 (4.35, 43.65)	< 0.001
4	47.5 (11.94, 188.61)	< 0.001

Angulo P, et al. *Gastroenterology*. 2015.

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Non-Pharmacologic Management of NASH

Treatment of NAFLD: Weight Loss

Study	N	Intervention	Duration (months)	Design	ALT*	Histology*
Hickman	31	Diet	15	Open label	+	N/A
Huang	16	Diet	12	Open label	-	+
Palmer	39	Diet	2-111	Case series	+	N/A
Andersen	41	Diet	4-23	Open label	+	+/-
Kugelmas	8	Diet/Ex	3	Open- label	+	N/A
Ueno	15	Diet/Ex	3	Open label	+	+
Zhu	34	Diet/Ex	12	Open label	+	N/A
Harrison	10	Orlistat		Open label	+	+
Sabuncu	13/12	Sibutramine/Orlistat	6	Open label	+	N/A
Luyckx	69	Surgery	27	Case series	+	+/-
Silverman	91	Surgery	2-61	Case series	+	+
Dixon	36	Surgery	26	Case series	+	+
Mummadi	766	Surgery	12	Meta-analysis	+	+

***Improvement denoted by +; No change denoted by -**

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NAFLD Guideline Recommendations

- Weight loss generally reduces hepatic steatosis, achieved either by hypocaloric diet alone or in conjunction with increased physical activity

(Strength - 1, Evidence – A)

- Loss of at least 3-5% of body weight appears necessary to improve steatosis, but a greater loss (up to 10%) may be needed to improve necroinflammation

(Strength - 1, Evidence – B)

- Exercise alone in adults with NAFLD may reduce hepatic steatosis but its ability to improve other aspects of liver histology remains unknown

(Strength - 1, Evidence - B)

Bariatric Surgery and NAFLD

Study First Author	Year of publication	Sample size	Surgery type	Mean follow-up time	Change in Steatosis	Change in Inflammation	Change in Fibrosis	Comments
Ranlov	1990	15	RYGB or gastroplasty	12 months	↓	NR	NR	Improved liver enzymes
Silverman	1995	91	RYGB	18.4 months	↓	↓ / 0	↓	Improved lobular but no change in portal fibrosis
Luyckx	1998	69	Gastroplasty or LAGB	Not declared	↓	↓	0	NA
Dixon	2004	36	LAGB	25.6 ± 10 months	↓	↓	↓	Significant improvement in all liver panel enzymes.
Kral	2004	104	Biliopancreatic diversion (BPD)	74 ± 27 months	↓	NR	↑	Increase in fibrosis overall was small)
Clark	2005	16	RYGB	10 ± 4 months	↓	↓	↓	Improvement in lobular and portal fibrosis, ALT and AST
Keshishian	2005	78	BPD-duodenal switch (BPD-DS)	36 months	↓	↓	NR	NASH grade improved. No significant reduction in

- No RCTs
- Most show decrease in steatosis, inflammation and liver enzymes. Results highly variable.

Dixon. *J Clinic*

Pharmacologic Management of NASH

Vitamin E

Authors	N	Dose	Comparators	Outcomes
Arendt	80	1000 IU/d	Placebo	Improved steatosis (assessed by CT scan) vs placebo

- Vitamin E (a-tocopherol) administered at daily dose of 800 IU/day improves liver histology in non-diabetic adults with biopsy-proven NASH and therefore it should be considered as a first-line pharmacotherapy for this patient population. (Strength - 1, Quality - B)
- Until further data supporting its effectiveness become available, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis (US Guidelines: Strength - 1, Quality - C)

Lipid Lowering Agents

Study	Design	Meds	N	ALT	Hist
Laurin	Open label	Clofibrate	16	-	-
Fernández-Miranda C	Open label	Fenofibrate	16	+	+/-
Basaranoglu	RCT	Gemfibrozil	46	+	NA

- Given the lack of evidence to show that patients with NAFLD and NASH are at increased risk for serious drug-induced liver injury from statins, statins can be used to treat dyslipidemia in patients with NAFLD and NASH (US Guideline: Strength – 1, Quality – B)
- Until RCTs with histological endpoints prove their efficacy, statins should not be used to specifically treat NASH (US Guidelines: Strength – 1, Quality B)

PPAR-γ Agonist

Author	N	Drug	Time	DM?	Cirrhosis	ALT	Fat	Bal	Infl	Fibrosis
Caldwell 2001	10	Troglit 400 mg	3-6 months	1/10	Yes	Yes	?	No	?Yes	No
Promrat 2004	18	Pio	12	No	No	Yes	Yes	Yes	Yes	Yes

- Pioglitazone can be (?) used to treat steatohepatitis in patients with biopsy-proven NASH. However, it should be noted that majority of the patients who participated in clinical trials that investigated pioglitazone for NASH were non-diabetic and that long term safety and efficacy of pioglitazone in patients with NASH is not established. (US Guidelines: Strength – 1, Evidence- B)

Newer Agents

	Mechanism of Action	Effect on NAFLD
Glucagon-like peptide-1 (GLP-1) analogs	• Suppress postprandial glucagon production • Enhance satiety	• Improved steatosis and NASH histology in pre-diabetics
Dipeptidyl peptidase-4 (DPP-4) inhibitors	• Increase insulin synthesis/release	• Decreased liver enzymes • Improved liver histology

Bhatt B et al. *HepatoBiliary Surg Nutr* 2015;4(2):101-108; Eguchi Y et al. *Hepatol Res* 2015;45:269-78; Yilmaz Y et al. *Acta Gastroenterol Belg* 2012;75:240-4; Iwaski T et al. *Hepatogastroenterology* 2011;58:2103-5.

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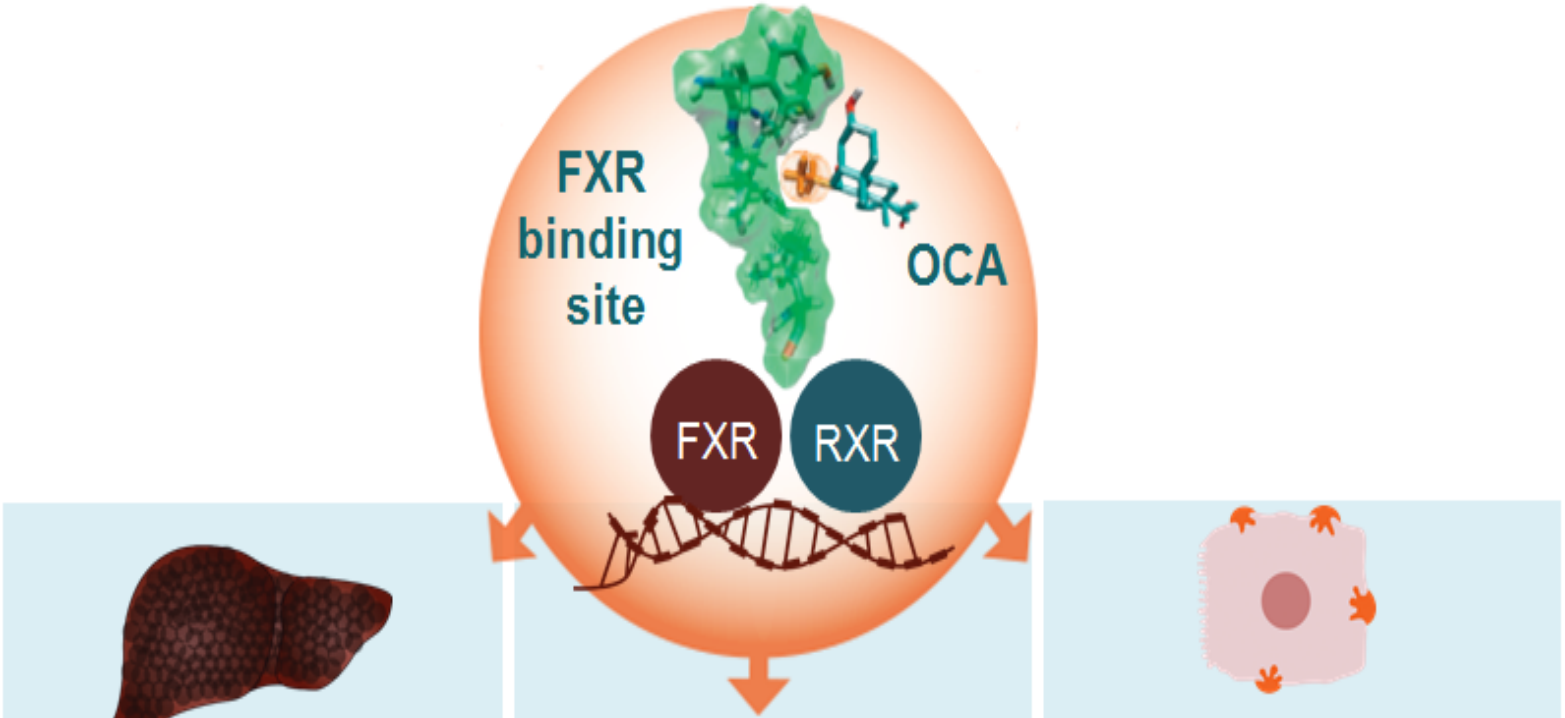
Treatment of NAFLD–New Regimens

Class	Drug
Farnesoid X Receptor (FXR) Agonist	Obeticholic Acid (OCA)
Anti-lysyl oxidase-like 2 monoclonal antibody	Simtuzumab
Fatty acid/bile acid conjugate	Aramchol
Dual inhibitor of CCR2 and CCR5	Cenicriviroc
Dual peroxisome proliferator-activated receptor alpha/delta agonist	GFT505
Probiotics	VSL#3

Obeticholic Acid (OCA): A Modified Bile Acid and FXR Agonist



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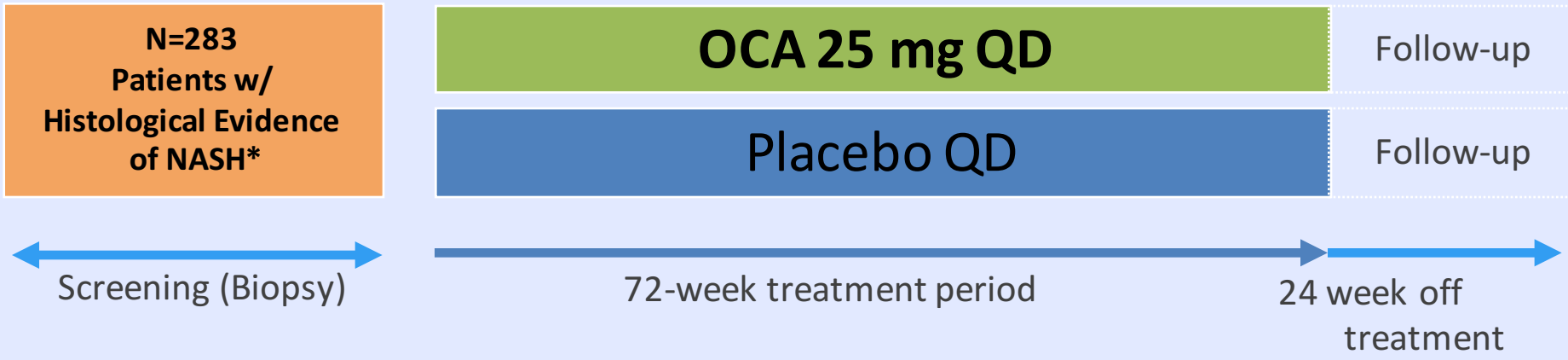
Phase 3 Trial Design- Obeticholic Acid (OCA) (FLINT Study)



Primary endpoint: liver histological improvement defined as decrease in NAFLD Activity Score (NAS) of ≥2 points with no worsening in fibrosis

*Entry was based upon histologic diagnosis of nonalcoholic steatohepatitis (NASH) based on local CRN site pathologist's read (end-of-study blinded central read of baseline biopsies revealed 80% of patients enrolled had definite NASH); interim analysis was conducted when ≥50% of patients completed treatment and had repeat liver biopsy; NAFLD: nonalcoholic fatty liver disease

Neuschwander-Tetri B, et al. *Lancet*. 2015, 385 (9972), 956-965.

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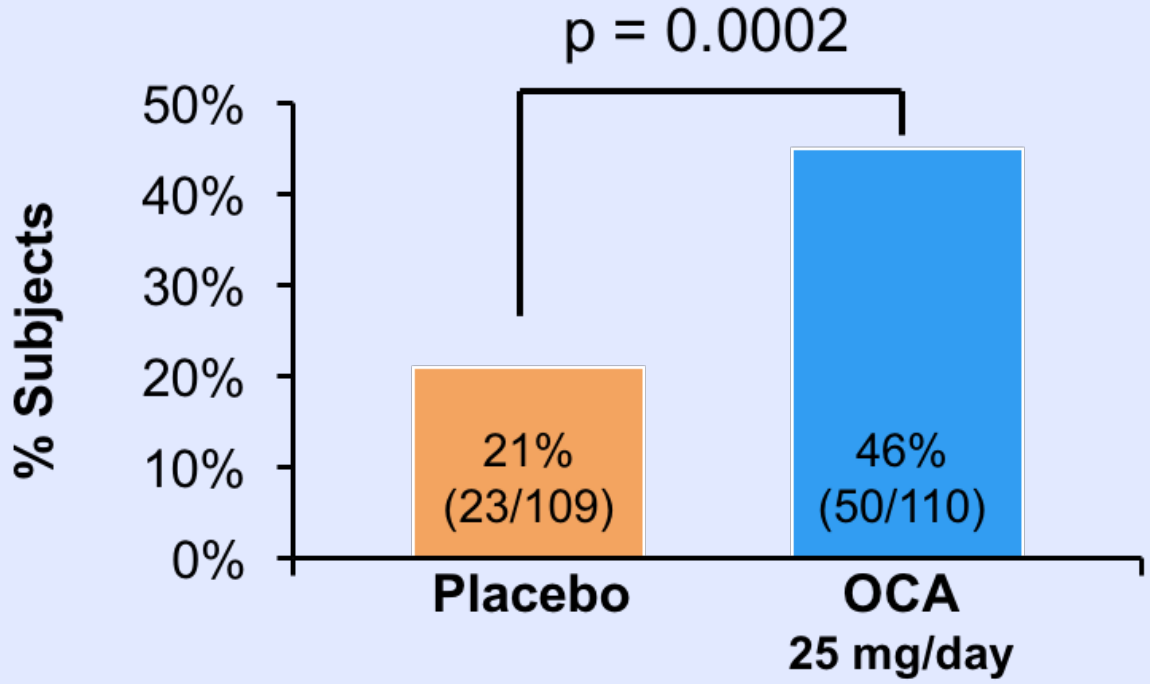
FLINT Primary Endpoint

- Improvement in NAFLD activity score* (NAS) ≥ 2 pts
  - *NAS = steatosis grade (0-3) + inflammation grade (0-3) + ballooning grade (0-2)
- No worsening of fibrosis



Neuschwander-Tetri B, et al. *Lancet*. 2015, 385 (9972), 956-965.

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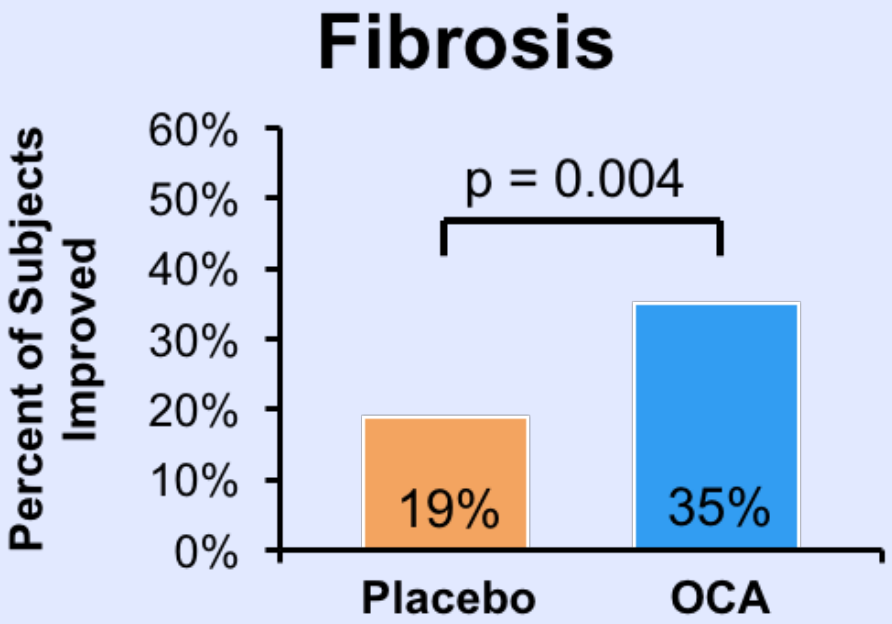
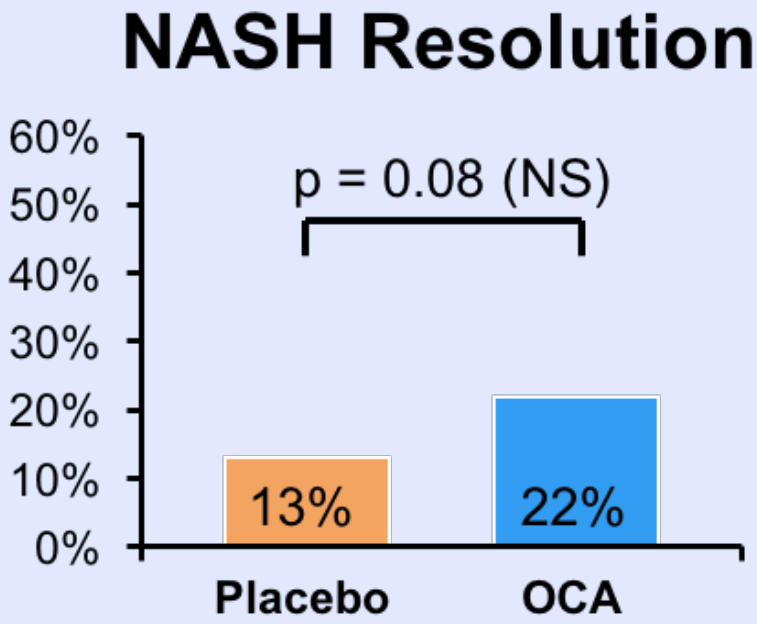
Improvement in Fibrosis and NASH Resolution (FLINT Study)





Neuschwander-Tetri B, et al. *Lancet*. 2015, 385 (9972), 956-965.

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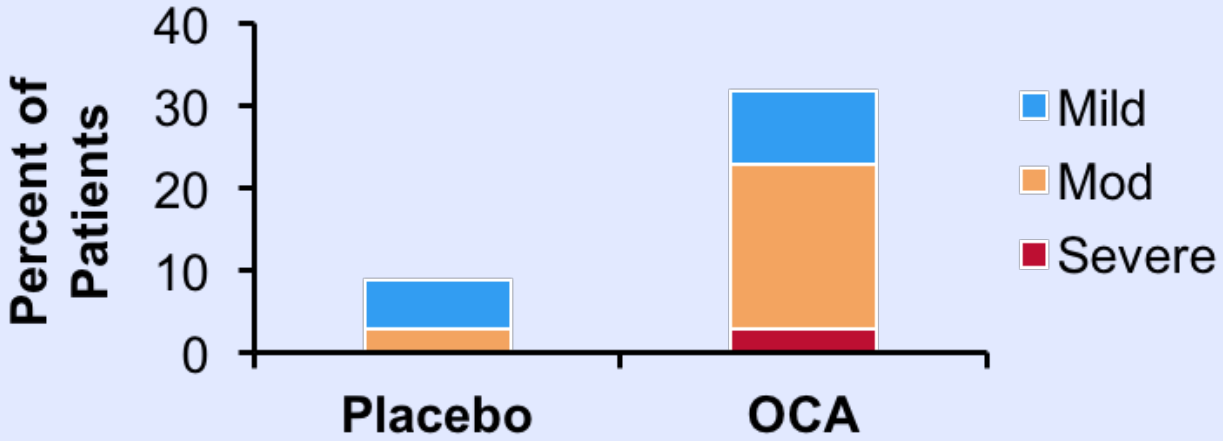
Adverse Events (FLINT Study)

- 6 severe adverse events in OCA group
  - 4 severe pruritus (1 stopped treatment)
  - 1 hypoglycemia
  - 1 possible cerebral ischemia (dysarthria and dizziness)
- Moderate or severe pruritus
  - 23% in obeticholic acid
  - 6% in placebo

P < 0.0001



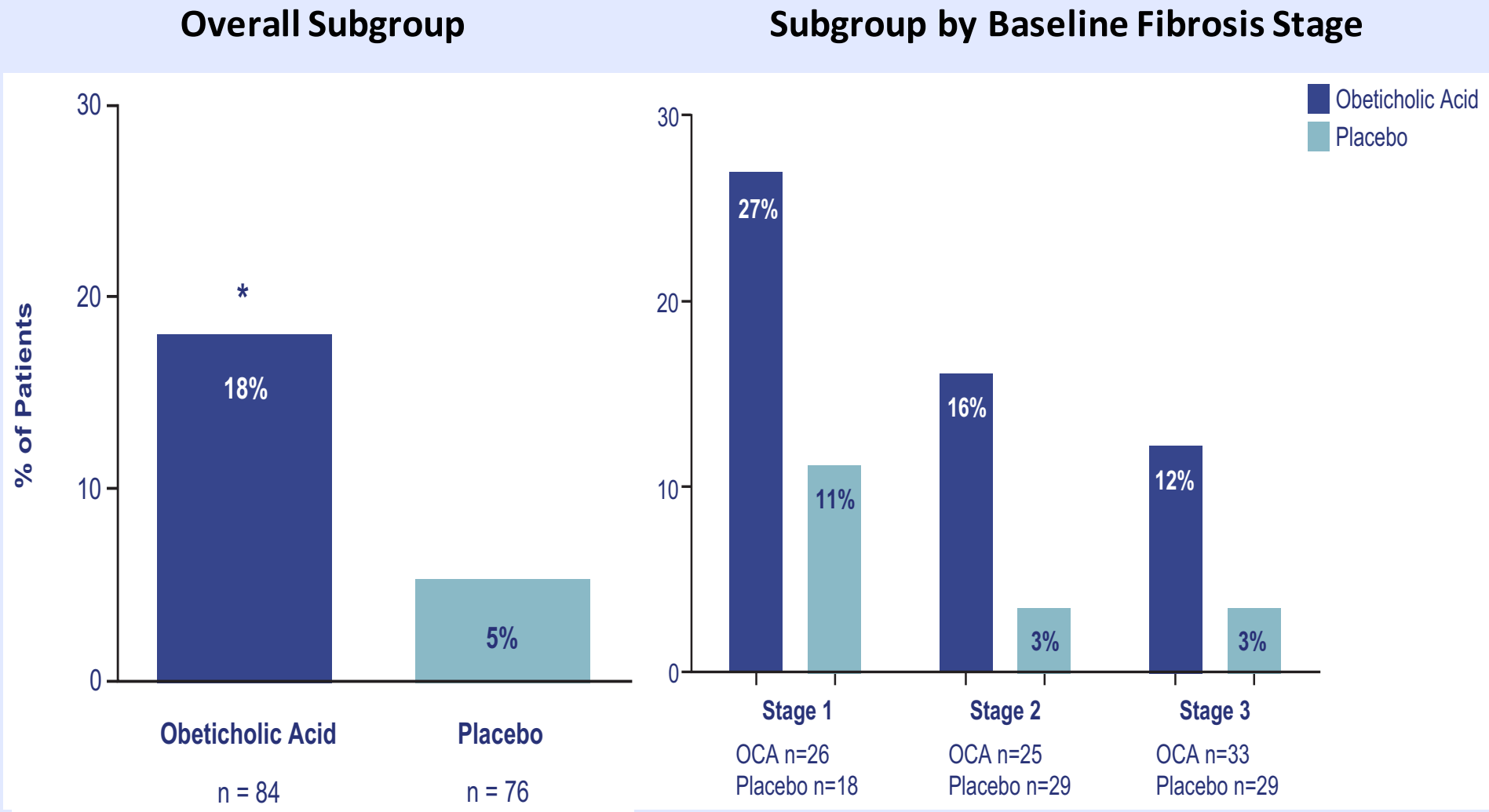
Neuschwander-Tetri B, et al. *Lancet*. 2015, 385 (9972), 956-965.

Patients at High Risk for Disease Progression

- Established fibrosis is the best predictor of liver-related mortality[1,2]
  - Hazard ratio (HR) = 20.4 for histologic documentation of fibrosis stage ≥2
- Various factors have been shown to be associated with higher rates of fibrosis progression[3-5]:
  - Diabetes
  - Elevated body mass index (BMI)
  - Elevated ALT
- Based on the literature, high-risk FLINT subgroup was defined:
  - Fibrosis stage 2 (perisinusoidal and portal/periportal) or stage 3 (bridging); or
  - Fibrosis stage 1 (perisinusoidal or periportal) if accompanied by one or more of:
    - Diabetes
    - Obesity (BMI ≥30 kg/m^2)
    - Elevated ALT (ALT ≥60 U/L)

1. Younossi ZM, et al. *Hepatology*. 2011;53(6):1874-82; 2. Ekstedt M, et al. *Hepatology*. 2014 Aug. doi:10.1002/hep.27368 [epub ahead of print]; 3. Adams LA, et al. *J Hepatol*. 2005;42(1):132-8; 4. Ekstedt M, et al. *Hepatology*. 2006;44(4):865-73; 5. Singh S, et al. *Clin Gastroenterol Hepatol*. 2015;13(4):643-654; Neuschwander-Tetri B, et al. *Lancet*. 2015, 385 (9972), 956-965.

NASH Resolution in High-Risk Subgroup†



*p=0.014; †NASH resolution as defined by NASH CRN pathologists; High-risk subgroup: patients with NAS ≥4 and fibrosis stage 2 or stage 3 or stage 1 with diabetes, BMI ≥30 kg/m2 or ALT ≥60 U/L; Intercept post hoc analyses.

Neuschwander-Tetri B, et al. *Lancet*. 2015, 385 (9972), 956-965.

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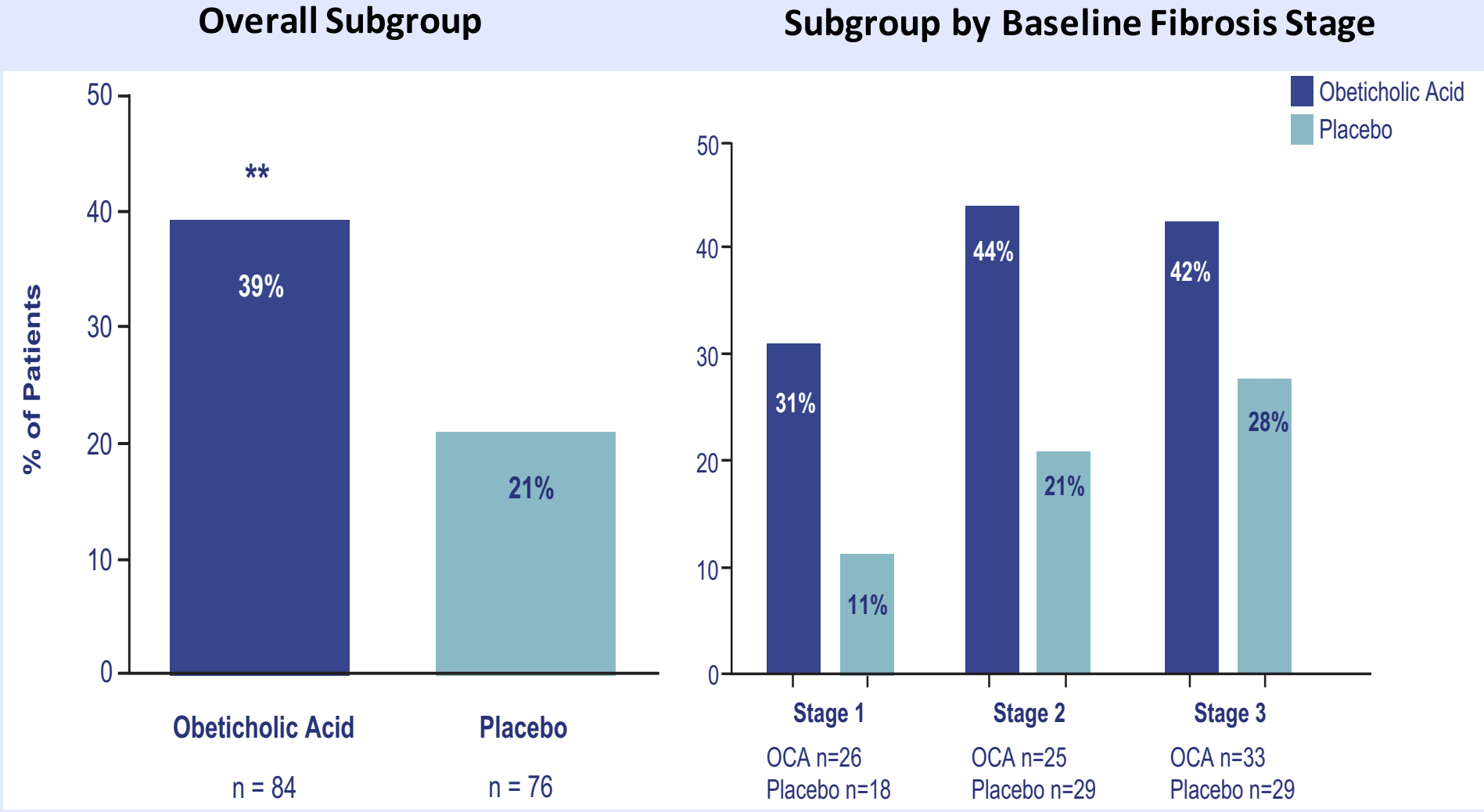
Fibrosis Improvement in High-Risk Subgroup†



*p=0.014; †NASH resolution as defined by NASH CRN pathologists; High-risk subgroup: patients with NAS ≥4 and fibrosis stage 2 or stage 3 or stage 1 with diabetes, BMI ≥30 kg/m2 or ALT ≥60 U/L; Intercept post hoc analyses.

Neuschwander-Tetri B, et al. *Lancet*. 2015, 385 (9972), 956-965.

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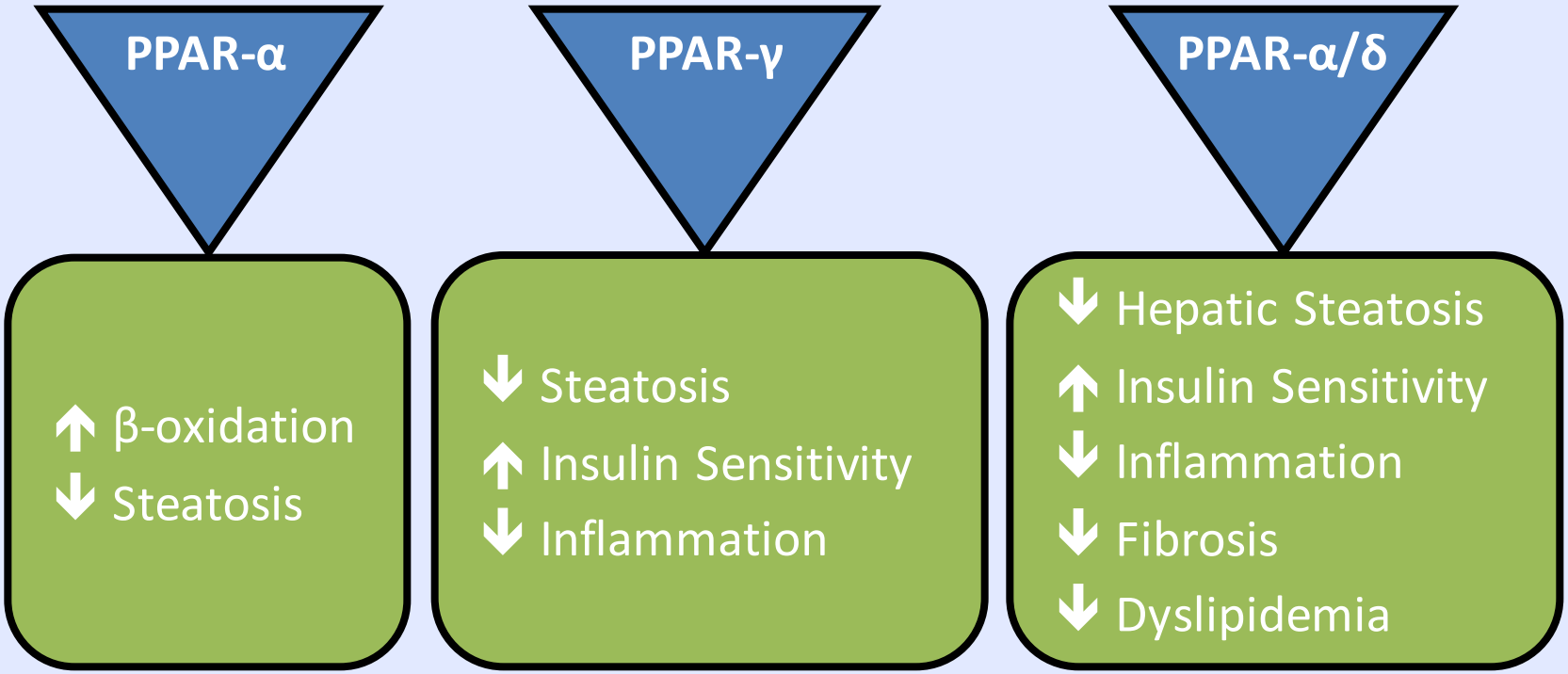
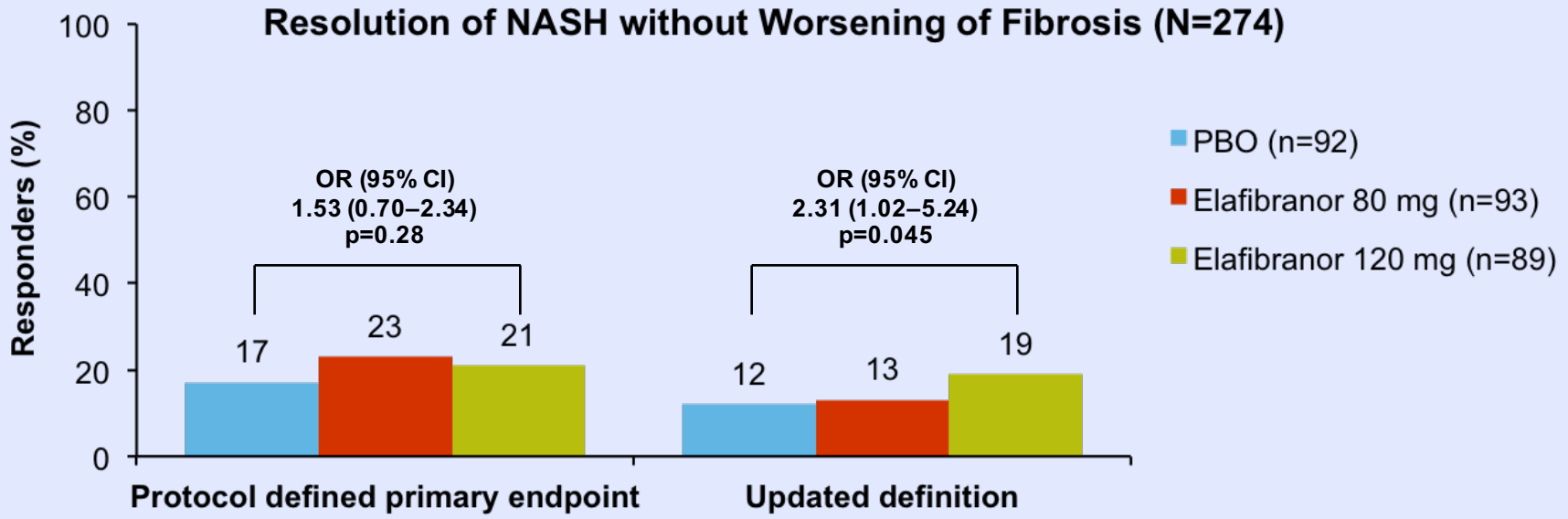
Peroxisome Proliferator-Activated Receptor (PPAR) Agonist



Anderson N, Borlak J. *Pharmacol Rev*. 2008;60:311-357; Ratziu V. *Nat Rev Gastroenterol Hepatol*. 2013;10;646-685.

Phase 2 RCT Dual PPAR α-δ Agonist GFT505 in Adults with NASH

- While no significant effect of elafibranor was observed on resolution of NASH without worsening of fibrosis as predefined in the protocol, in the global population, significant effect of elafibranor 120 mg was obtained with the new recommended definition



[a]Complete resolution of ballooning and either 0 or 1 for lobular inflammation
Ratziu V, et al. AASLD 2015, San Francisco. #105.

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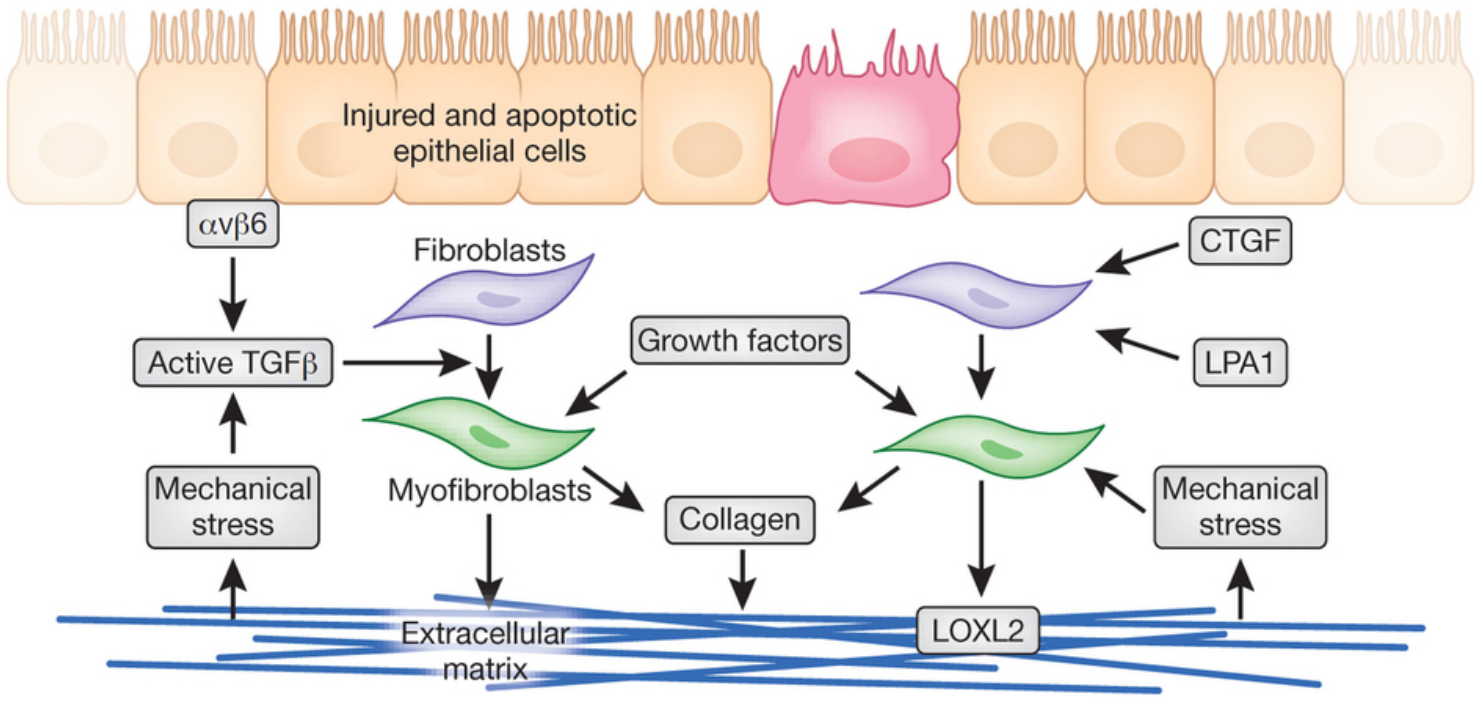
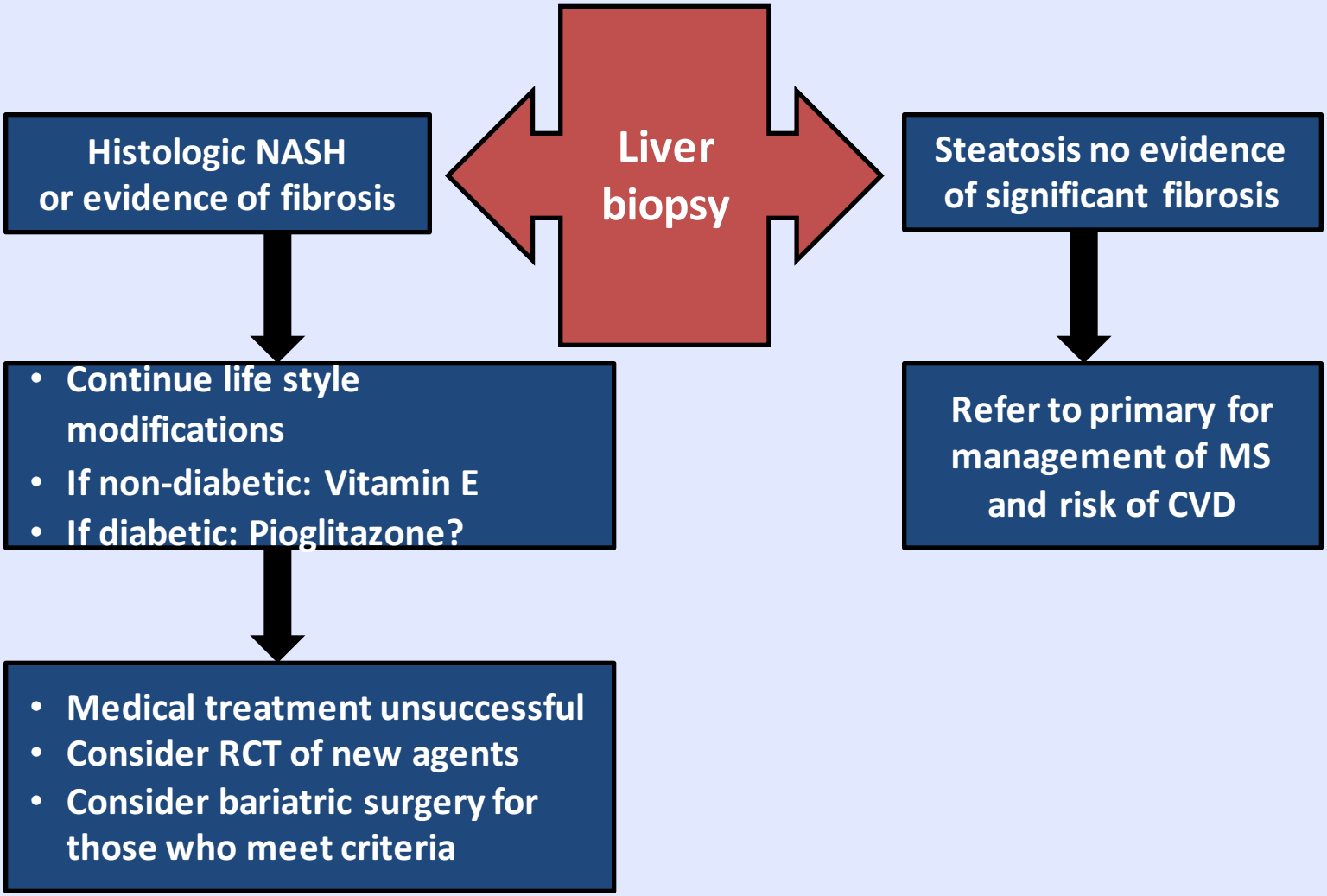
Simtuzumab-Mechanism of Action

- Phase 2 trials ongoing
- Humanised monoclonal antibody
- Inhibits cross-linking of collagen in pathologic stroma
- Lysyl oxidase-like 2 (LOXL2) levels may correlate with extent of fibrosis and clinically relevant endpoints for idiopathic pulmonary fibrosis



Ratziu V. *Nat Rev Gastroenterol Hepatol*. 2013;10;646-685; Barry-Hamilton V, et al. *Nat Med*. 2010;16:1009-1017.

How Do We Manage Our NAFLD Patients in 2016?



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Concluding Thoughts

- NAFLD has tremendous clinical, economic and QoL burden to the patients and to the society and this burden is growing globally
- NASH is the progressive form of NAFLD
- Histologic fibrosis (stage 2 or more) predicts LRM
- Pathogenesis of NASH is complex (multiple hits)
- Current treatment for patients with NASH:
  - Life style modifications for all
  - Vitamin E for non-DM NASH
  - ??Pio for DM with NASH but be aware of safety concerns
  - Consider bariatric surgery for morbidly obese+/-DM with NASH
- Future treatment considerations:
  - Clinical trials of new agents are underway

Back to Dr. Wells

Izzy

- She was counseled on lifestyles modifications. She started to lose weight but could only lose 3% of her initial weight despite only eating 1 meal/day.
- She has become frustrated, she does know what else to do. She is always hungry and all the things she likes to eat, she can't eat.
- Her hepatologist recommended she start probiotics as there is emerging data on the benefits of probiotics in patients with NASH.
- She is also referred to a dietitian.
- Can diet and gut microbiome have any impact?

Metabolic Effects of Endotoxin Signaling

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Geriatric Research, Education and Clinical Center (GRECC)
Audie L Murphy VA Medical Center
Barshop Institute
University of Texas Health Science Center
San Antonio, TX

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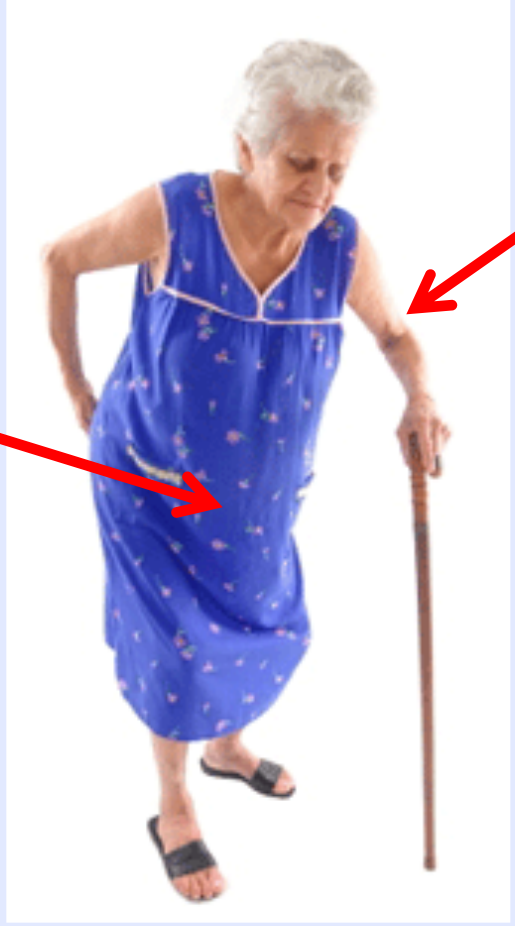
Inflammation and Metabolic Disease



Sarcopenia
Muscle Mass
Strength

Type 2 DM
Obesity
NAFLD

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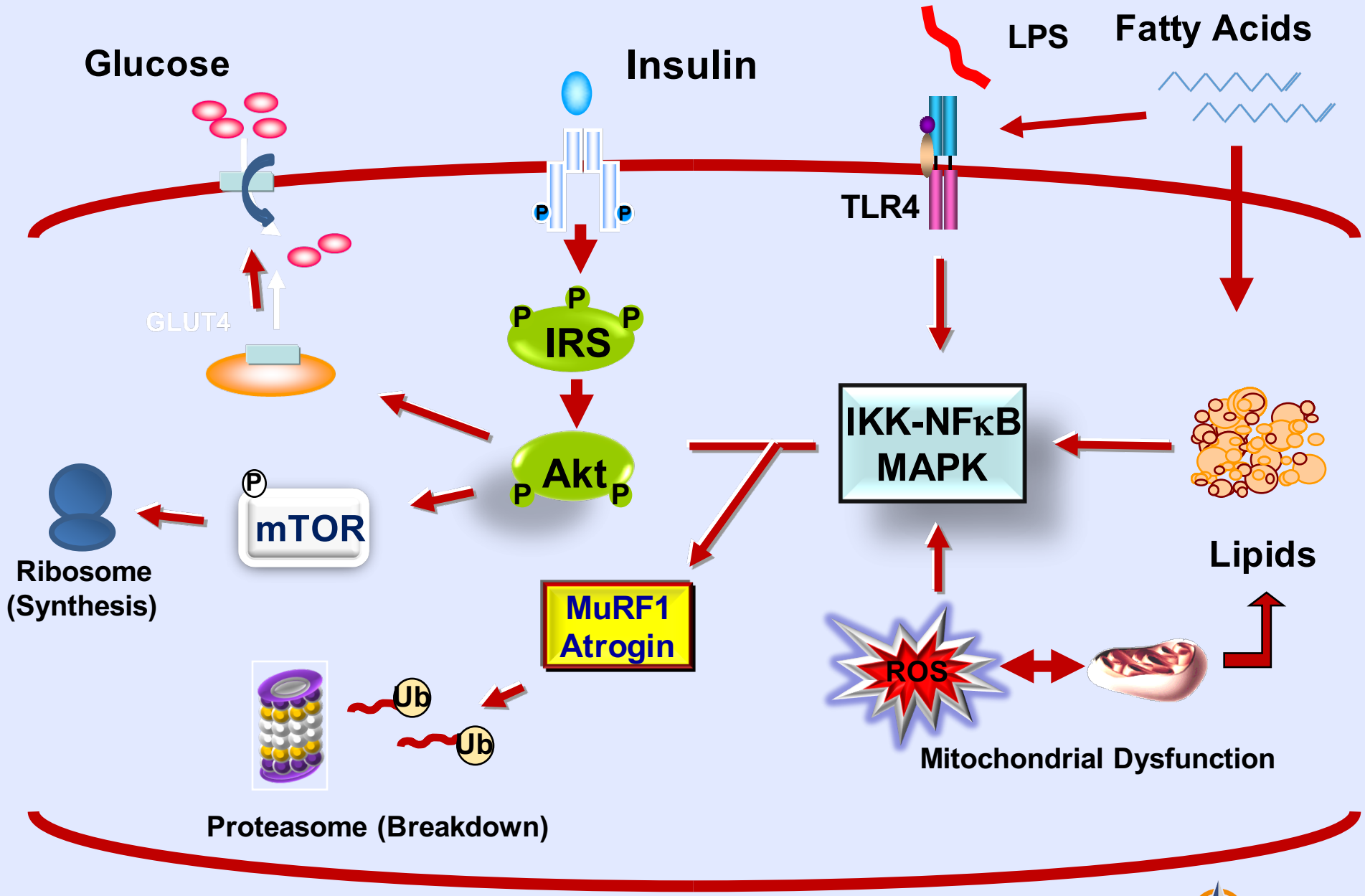

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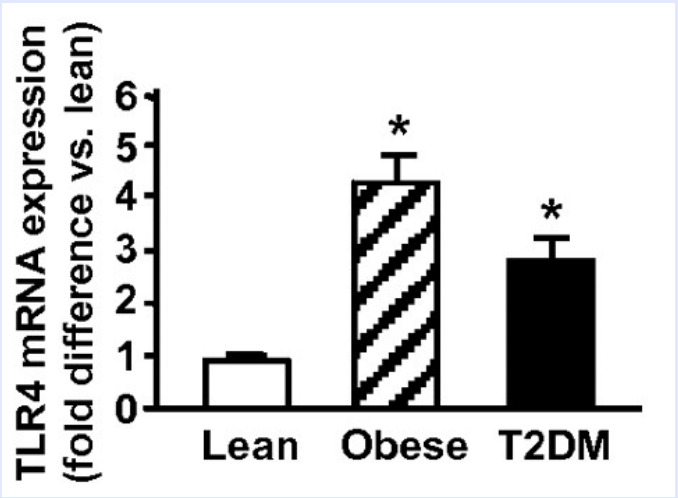
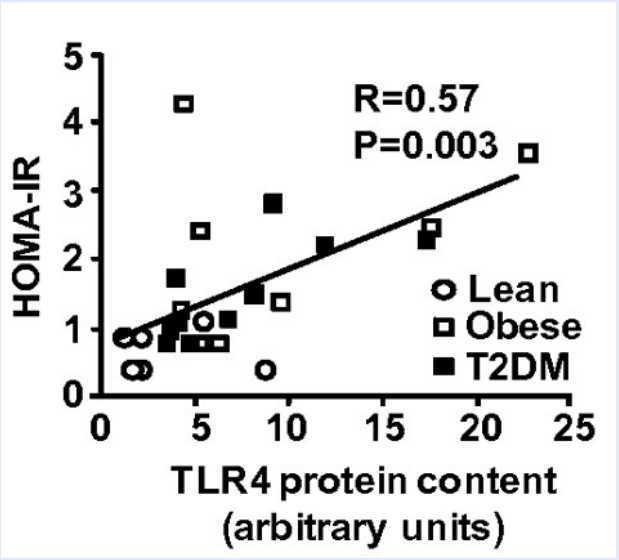
Elevated TLR4 Expression in Sk. Muscle from IR Subjects





Reyna et al. Diabetes 2008

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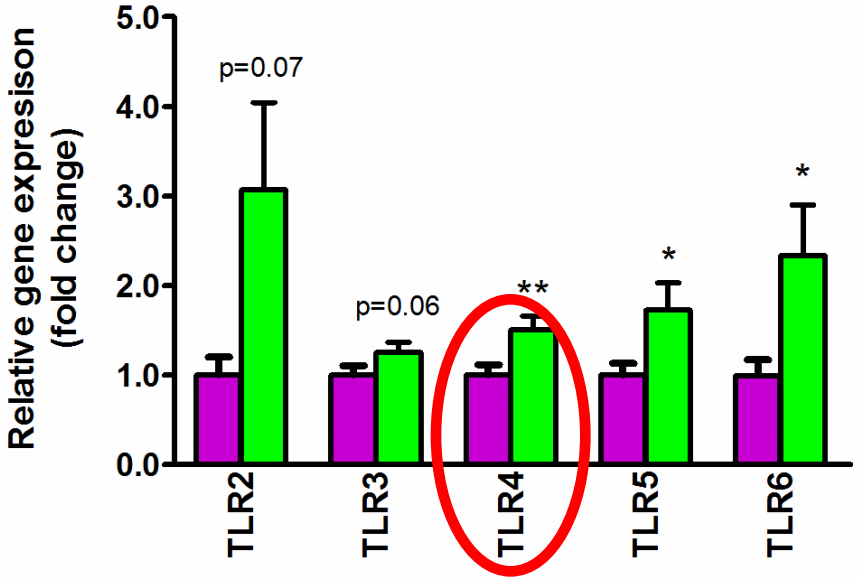
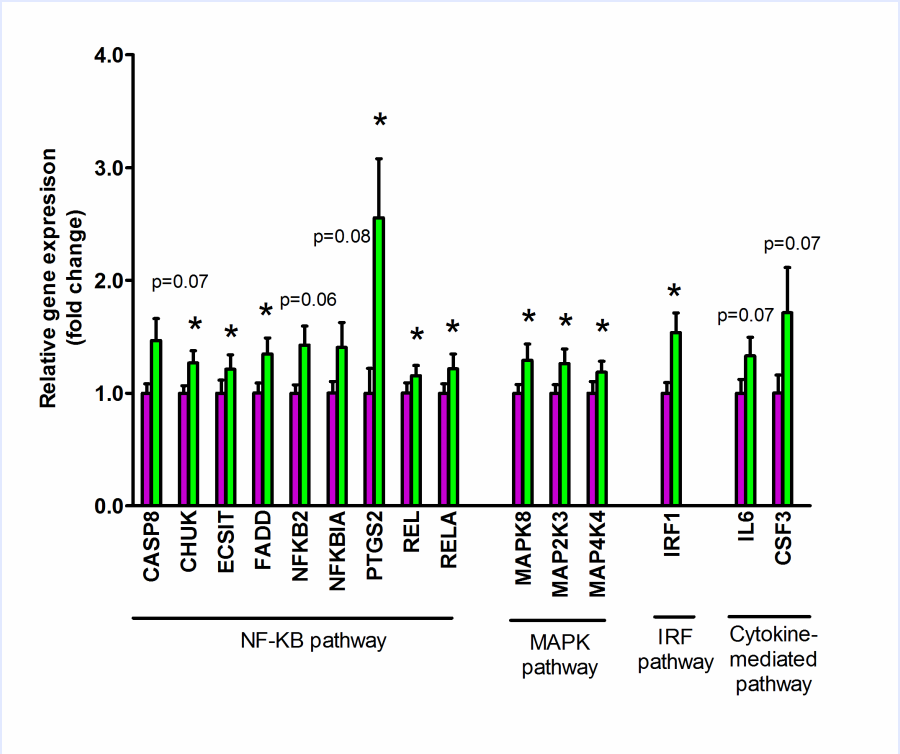
A Low-dose Lipid Infusion Increases Muscle TLR Expression and Signaling

- **Lean Healthy Volunteers (n=12, age= 40, BMI = 23)**

- **48 hour Intralipid infusion**





Hussey SE, Diabetologia. 2014

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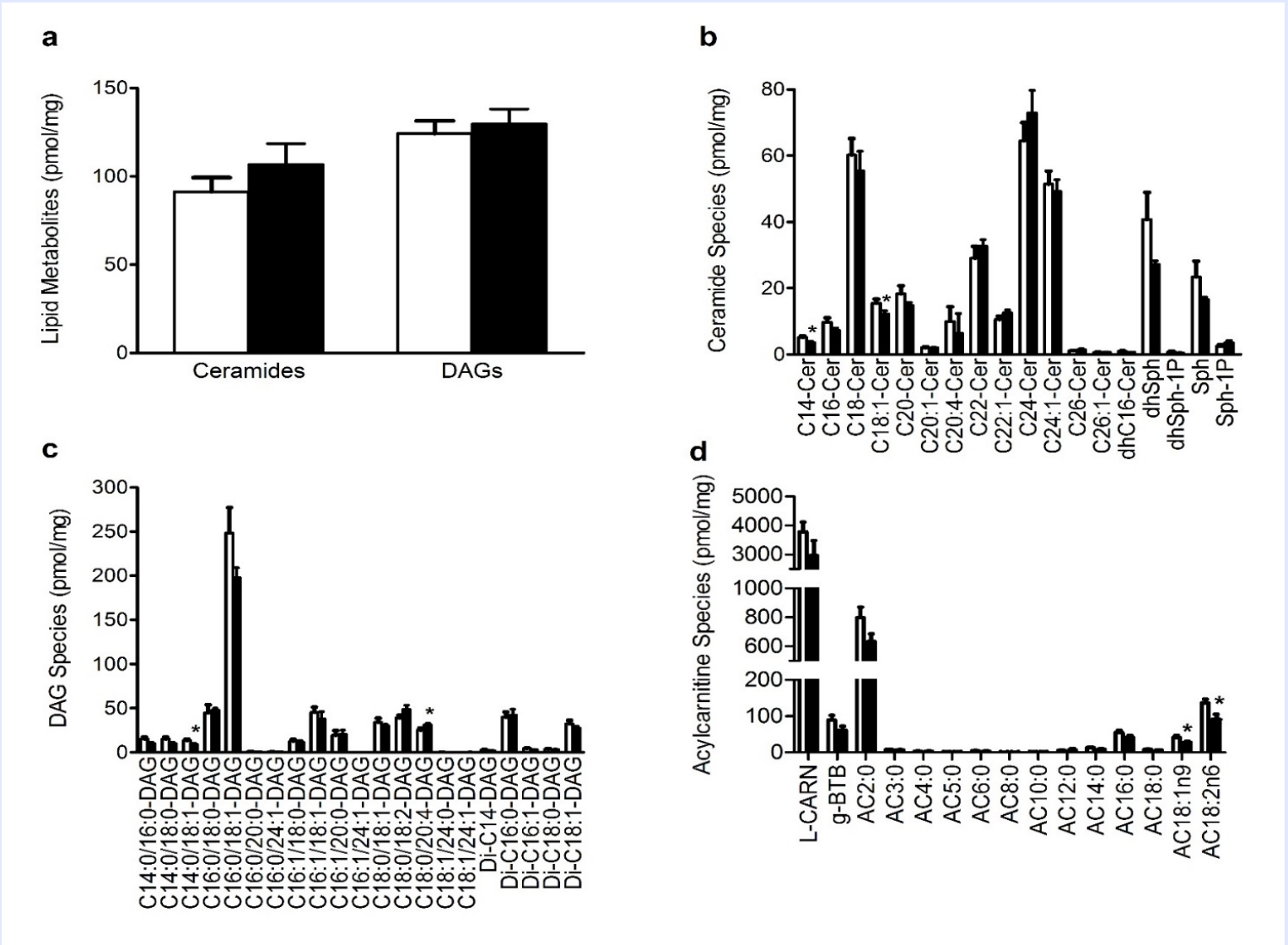
A Low-dose Lipid Infusion Does Not Increase Muscle Ceramides and DAGs



Hussey SE, Diabetologia. 2014

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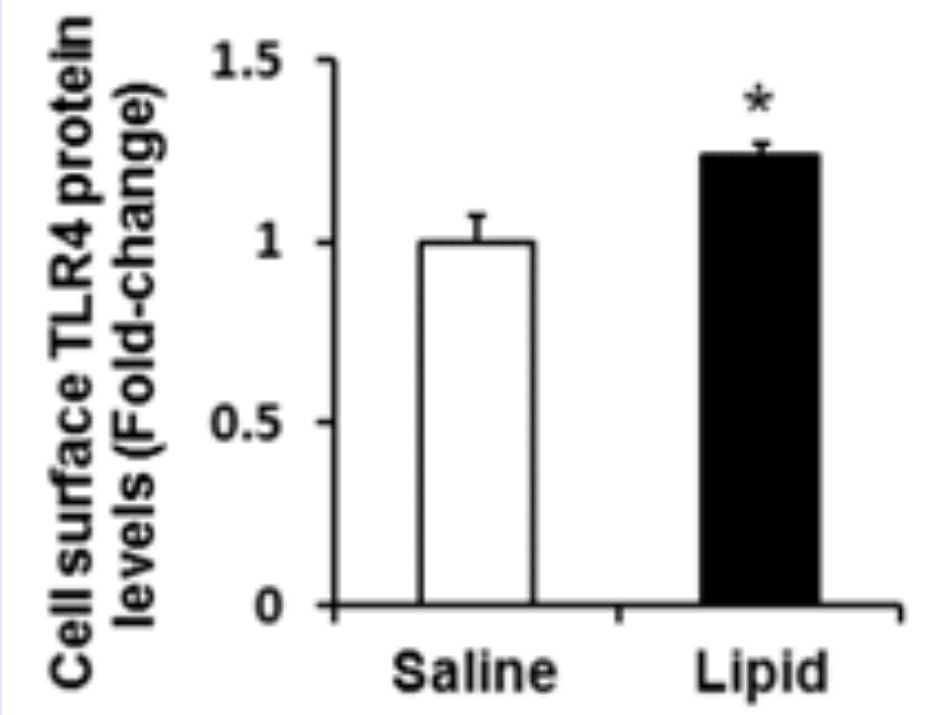
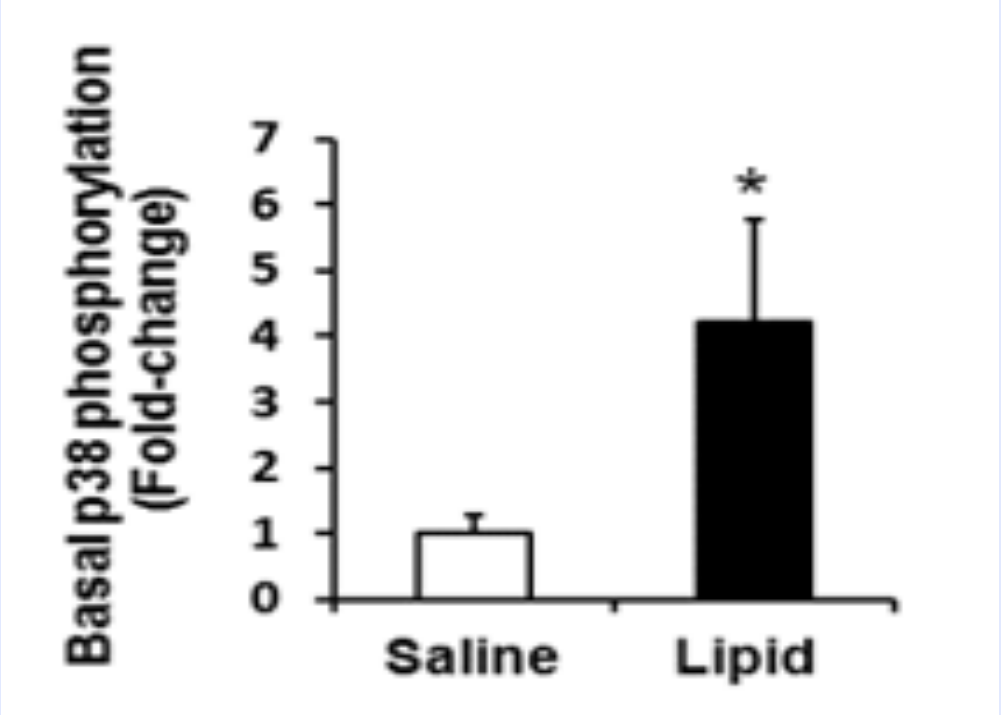
A Low-dose Lipid Infusion Increases TLR4 Expression/Signaling in Monocytes





Liang, Musi, unpublished

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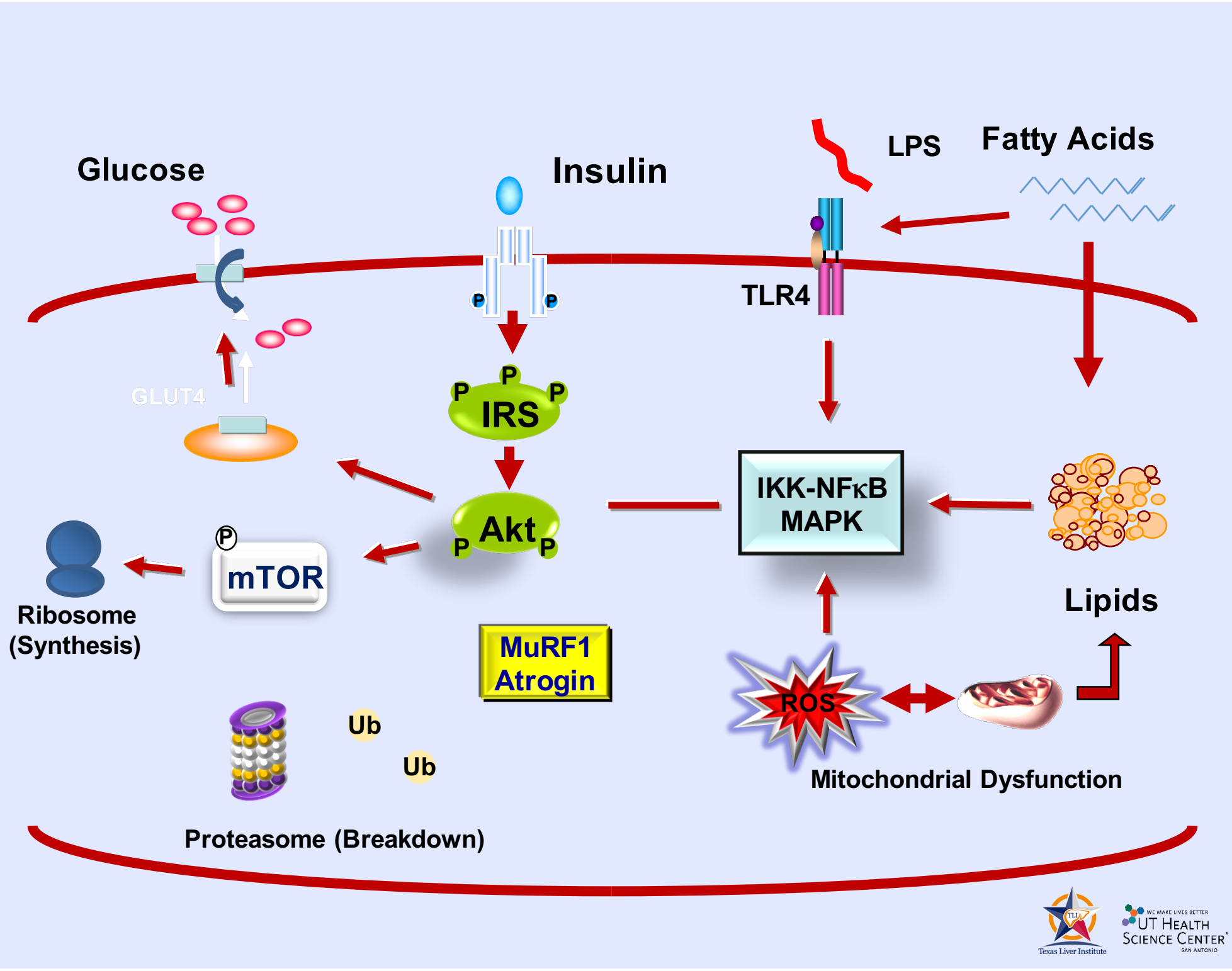
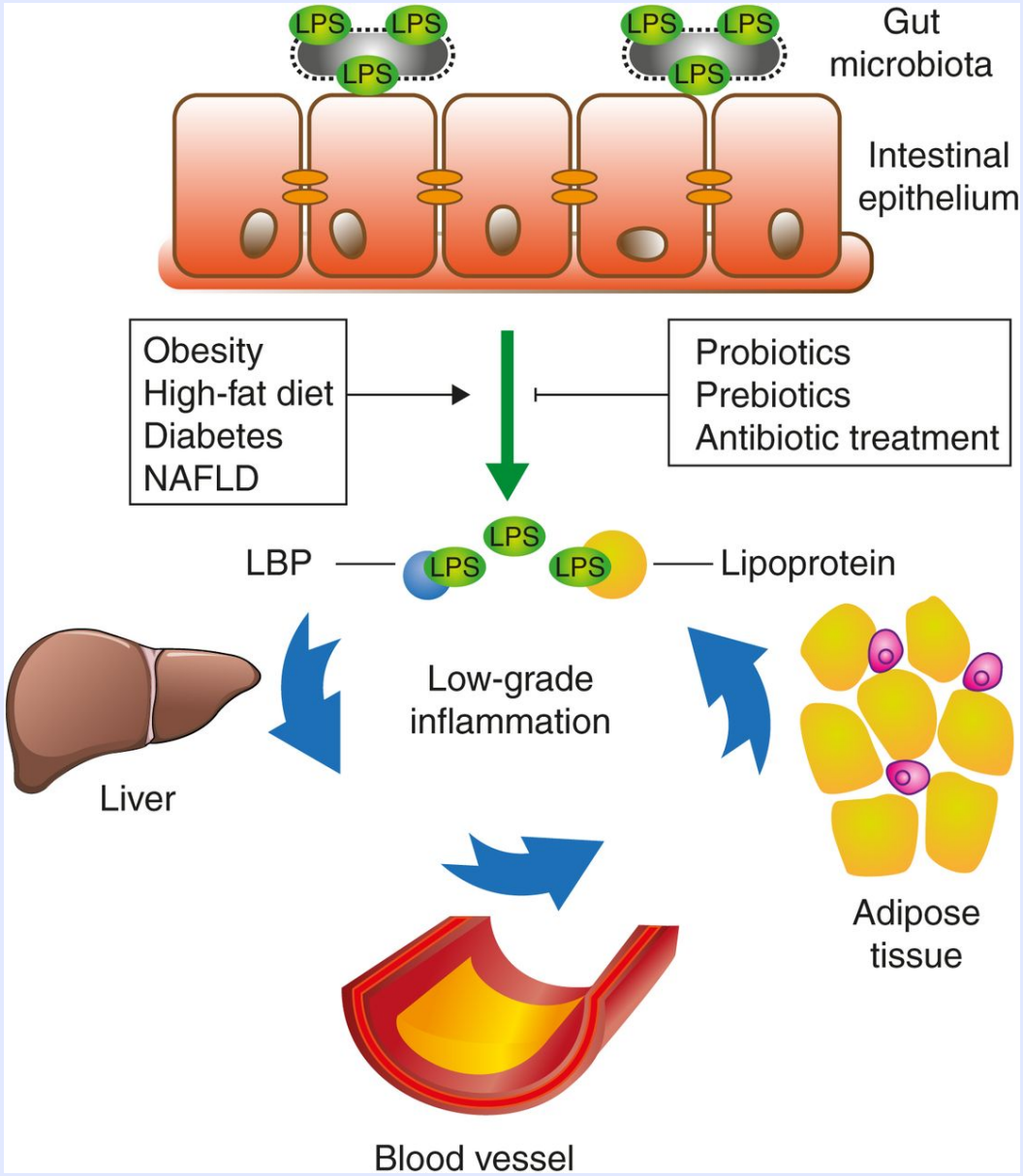

Glucose
Insulin
LPS
Fatty Acids
TLR4
GLUT4
P
IRS
Akt
mTOR
IKK-NFκB
MAPK
Ribosome
(Synthesis)
MuRF1
Atrogin
ROS
Lipids
Ub
Ub
Proteasome (Breakdown)
Mitochondrial Dysfunction

The gut epithelium is an efficient barrier that prevents absorption of LPS derived from Gram-negative gut microbiota.



Ana Luísa Neves et al. *J Mol Endocrinol* 2013;51:R51-R64

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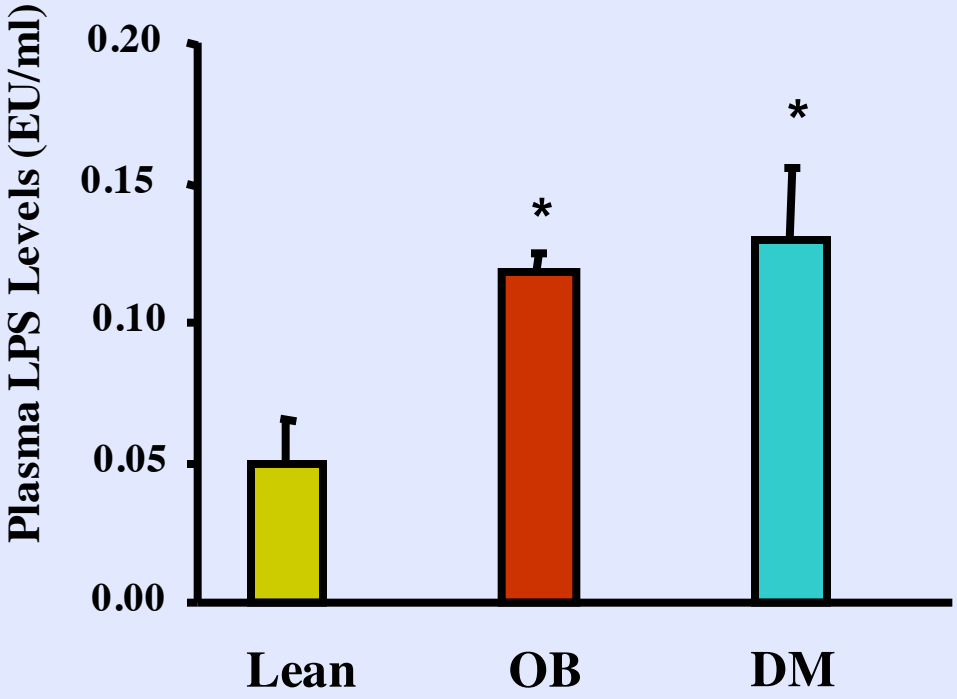
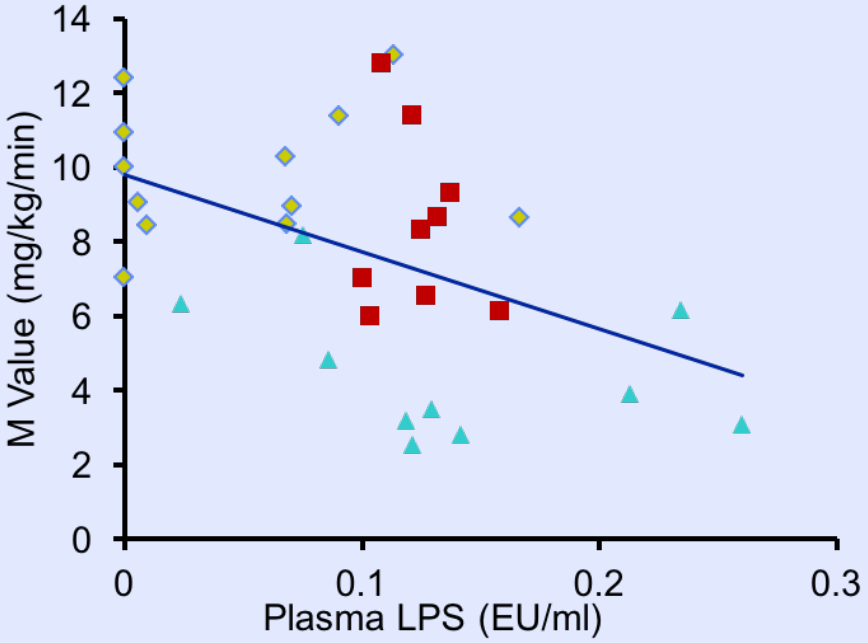
Elevated LPS Levels in Plasma of Obese and T2DM Subjects





Liang H, PLoS One. 2013

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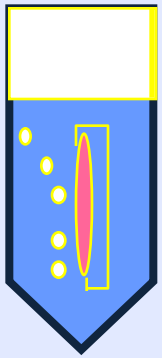
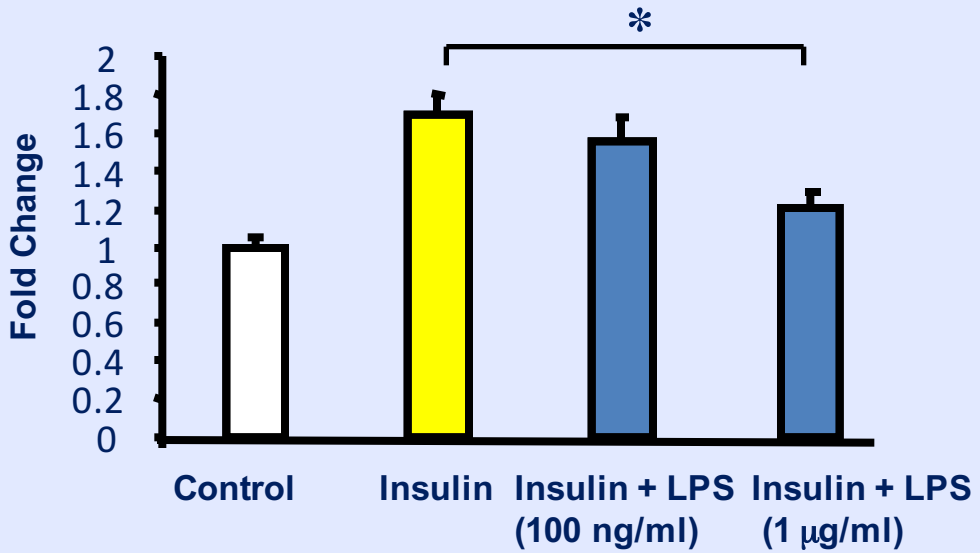
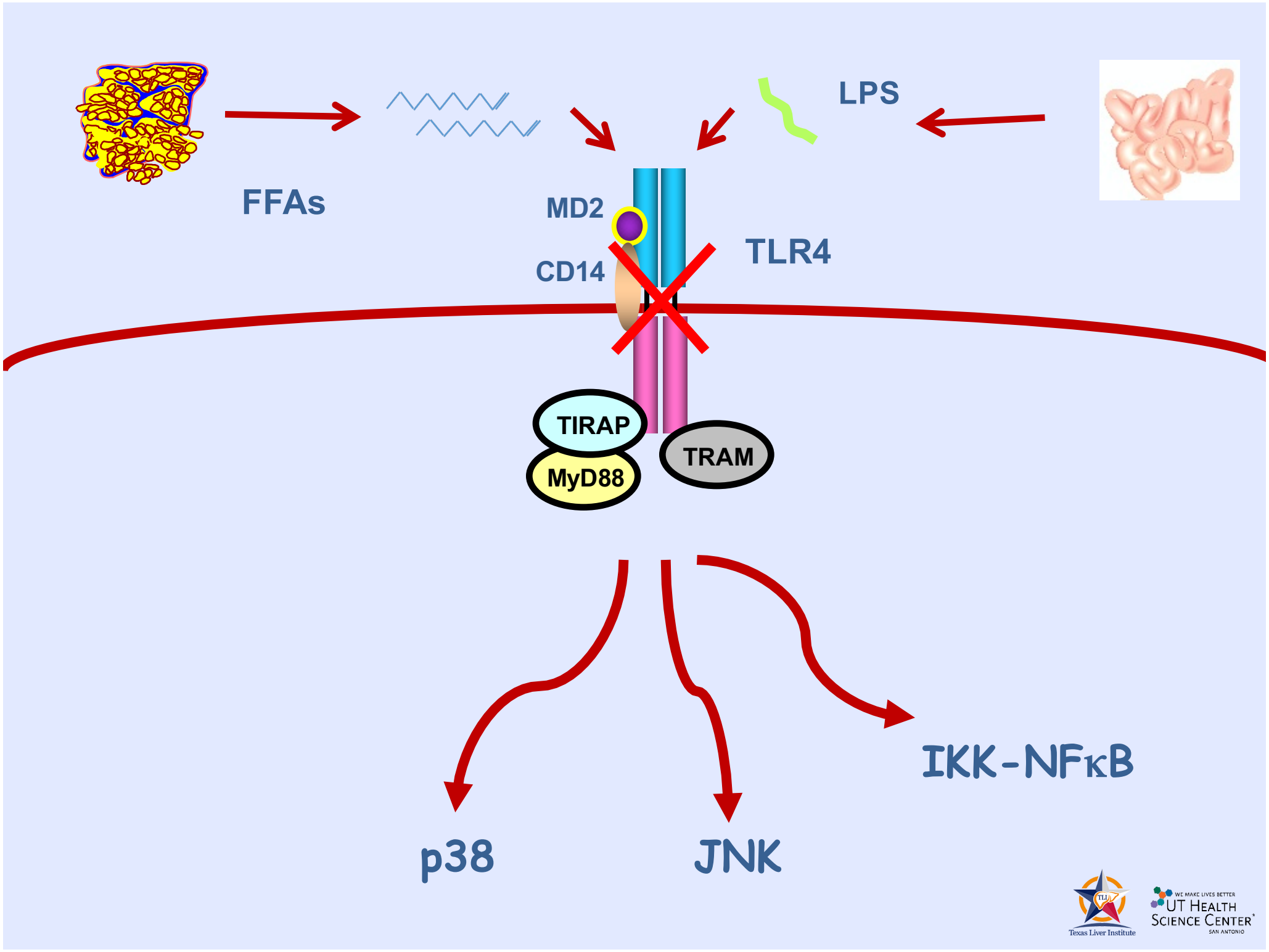
LPS inhibits insulin action in muscle



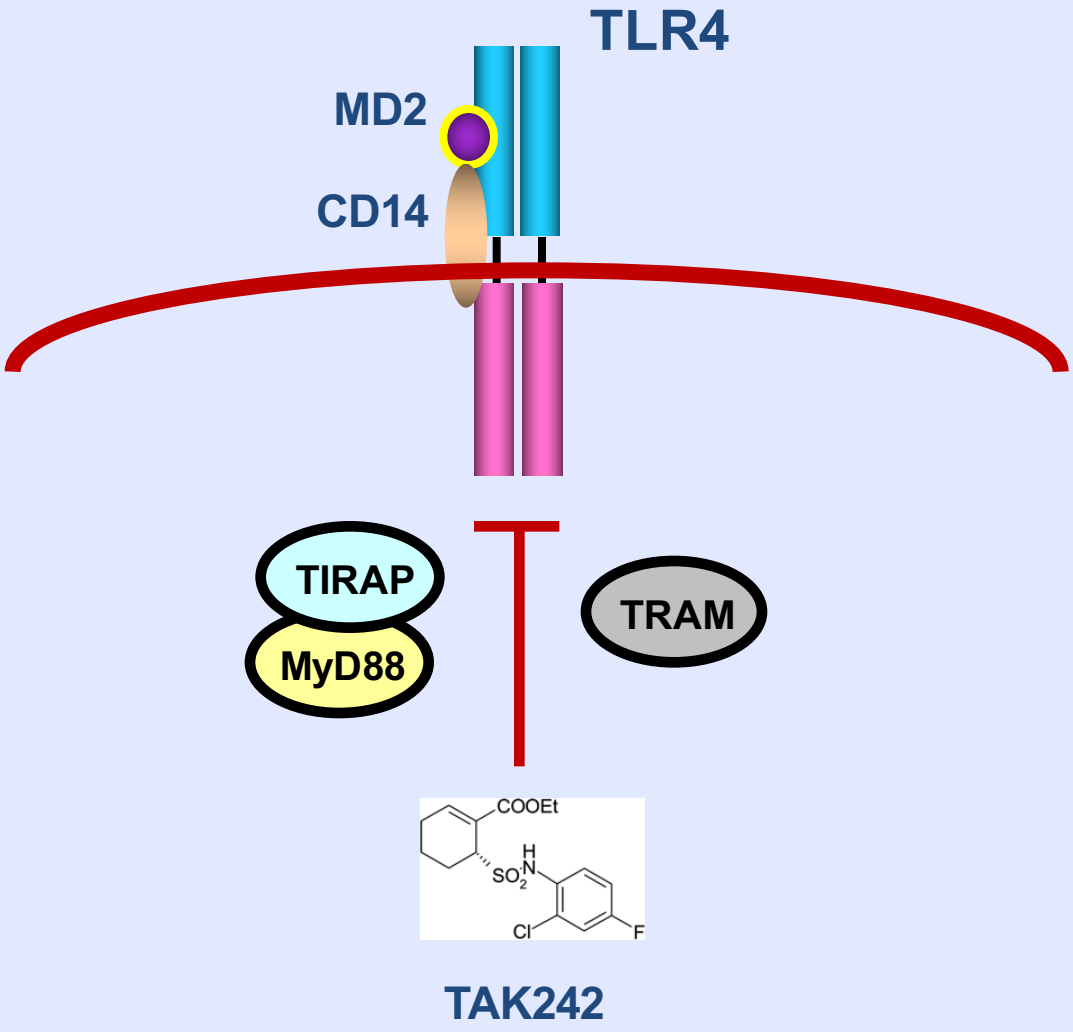
- Glucose transport assay
- Rat *epitrochlearis* muscle
- 4 h pre-incubation with LPS



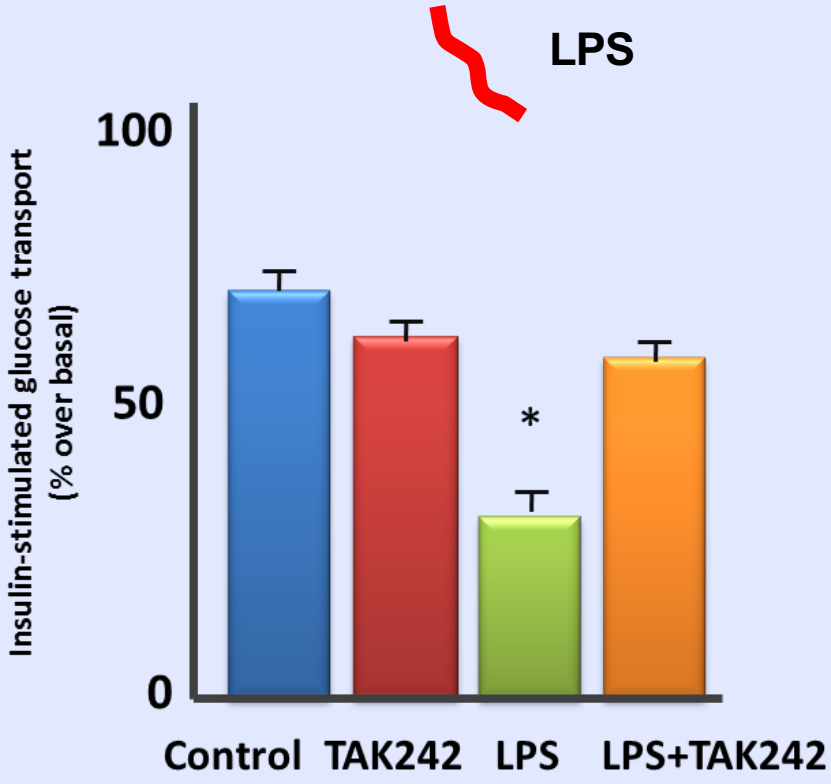
Zhang, Musi, unpublished

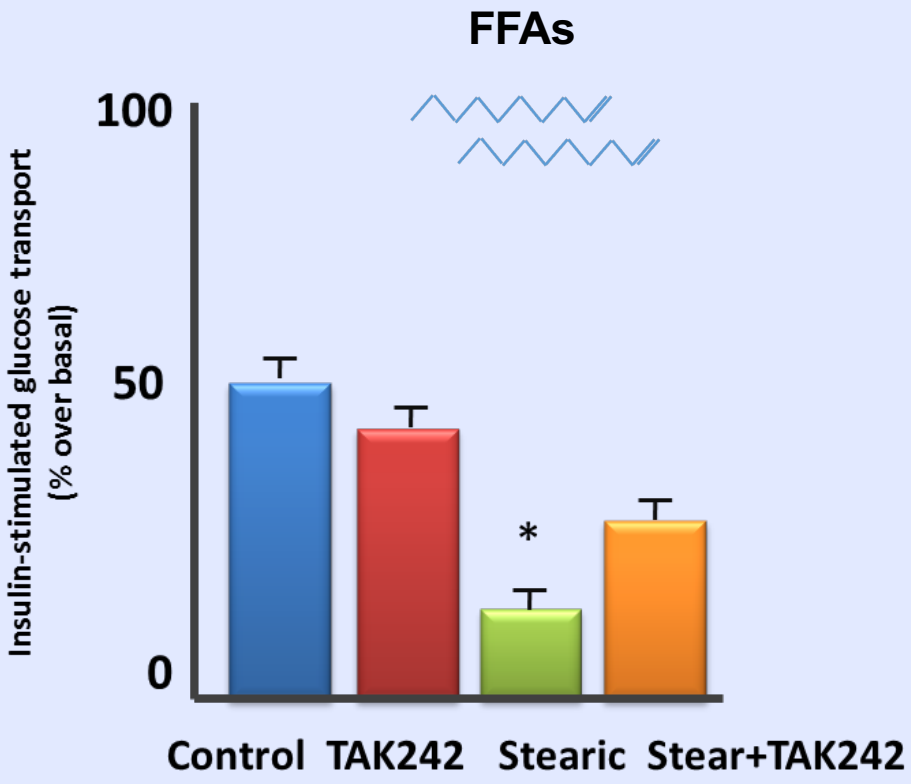

LPS
FFAs
MD2
CD14
TLR4
TIRAP
MyD88
TRAM
IKK-NFκB
p38
JNK

Pharmacological inhibition of TLR4 with TAK242



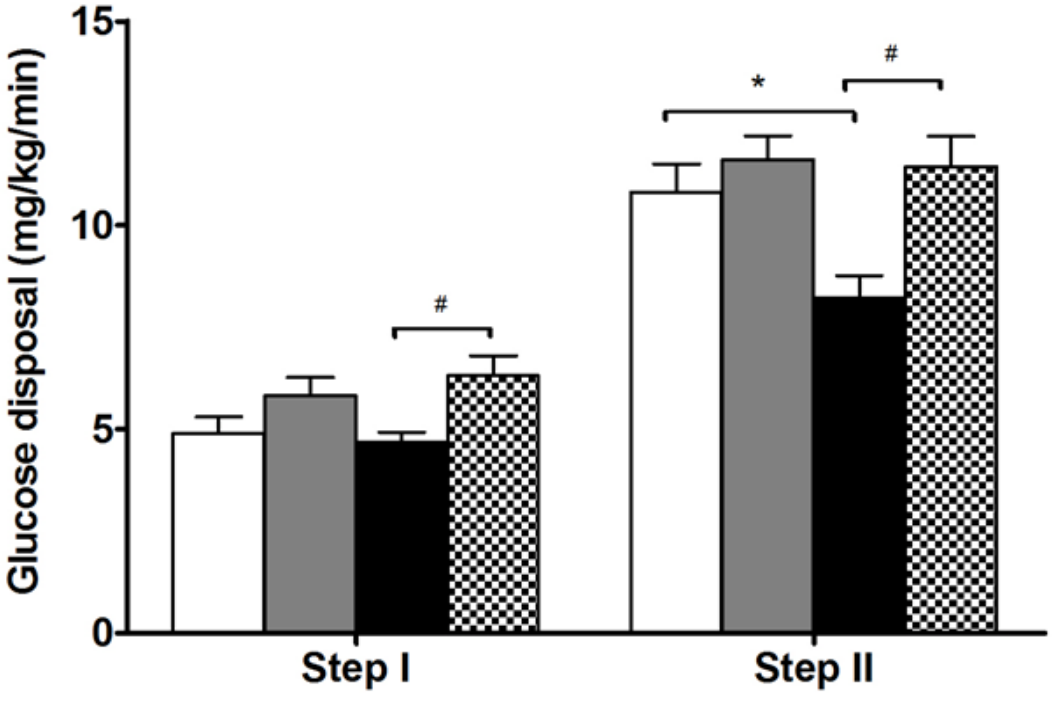
Effect of TAK242 on insulin-stimulated glucose transport





Hussey SE, Biosci Rep. 2012

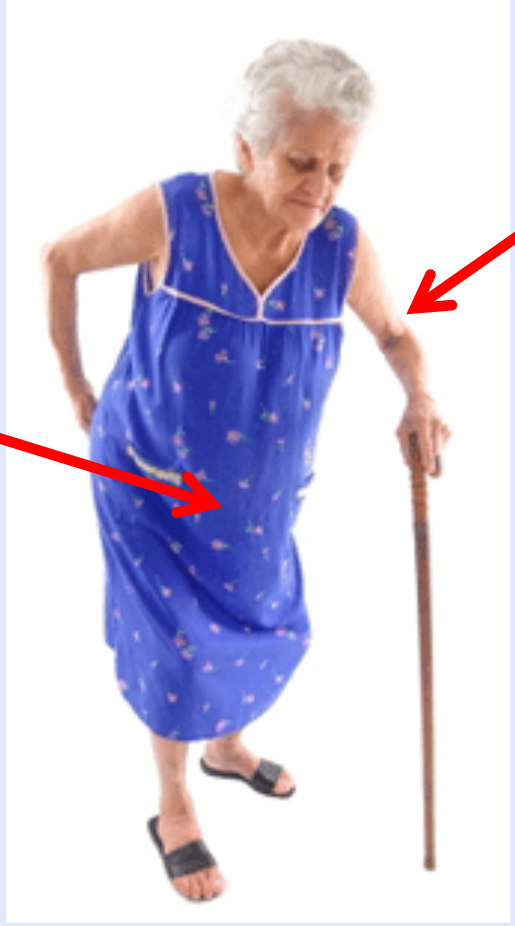
TAK242 protects against high fat diet-induced IR



LFD + Vehicle
LFD + TAK-242
HFD + Vehicle
HFD + TAK-242

Zhang N, PLoS one. 2015

Inflammation and metabolic disease



Sarcopenia
Muscle Mass
Strength

Type 2 DM
Obesity

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Clinical Characteristics

	Younger	Older
Age (yr)	25.5 ± 1.0	73.8 ± 2.1*
BMI (kg/m^2)	23.5 ± 0.7	24.1± 1.0
Fasting Glucose (mg/dl)	77.6 ± 3.6	88.4 ± 2.0*
Fasting Insulin (mU/ml)	4.4 ± 0.7	6.9 ± 1.1
$VO_{2\,max}$ (ml/kg.min)	27.6 ± 2.4	16.5 ± 0.7*
IL6 (pg/ml)	1.24 ± 0.26	2.1± 0.2*
TNF a (pg/ml)	1.14 ± 0.25	1.7± 0.3

*P<0.05 vs Younger group

Ghosh S, J Geront Biom Sci A. 2015

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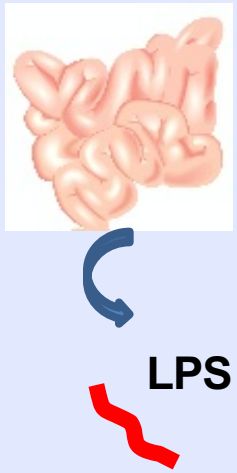
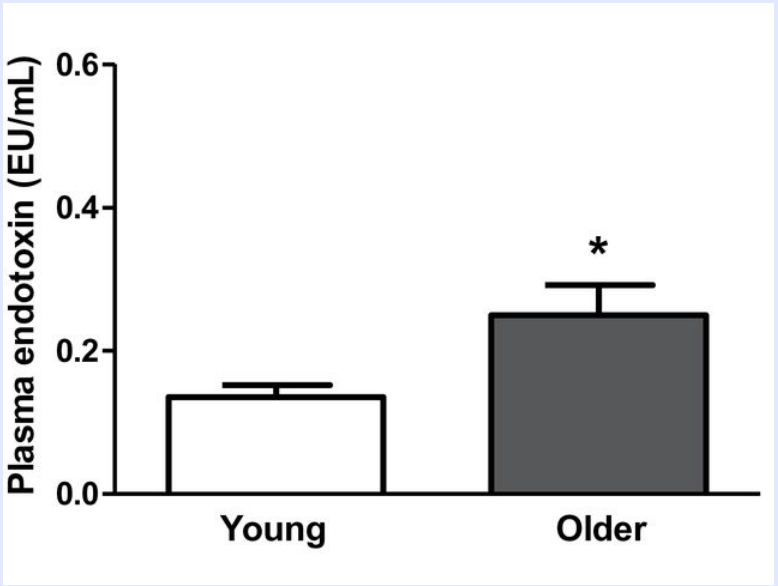
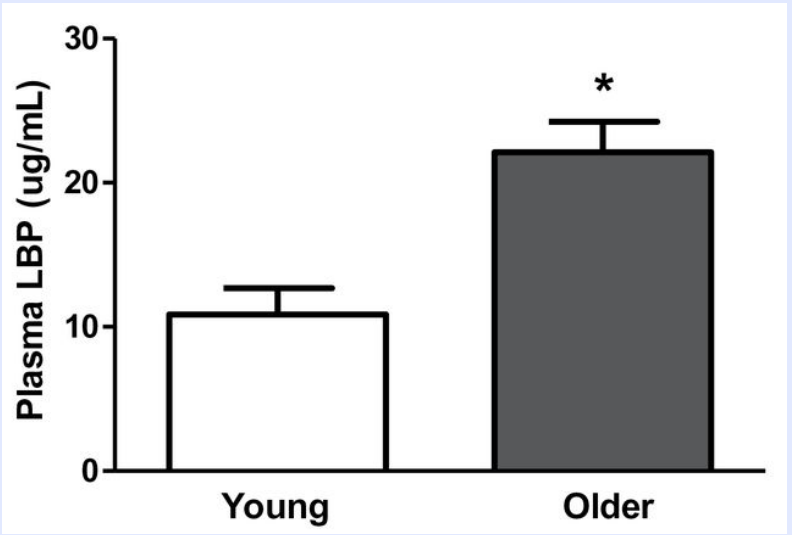
Metabolic endotoxemia in aging







Ghosh S, J Geront Biom Sci A. 2015

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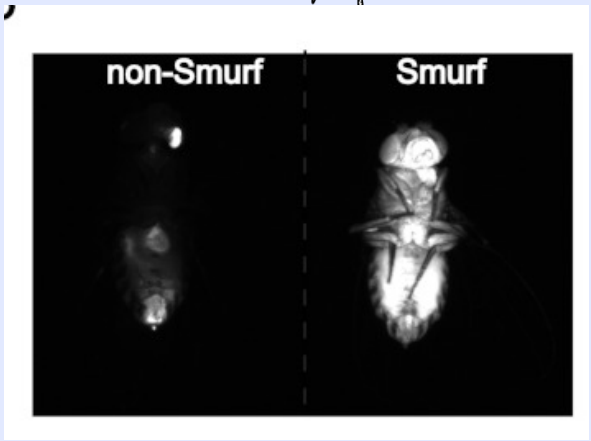
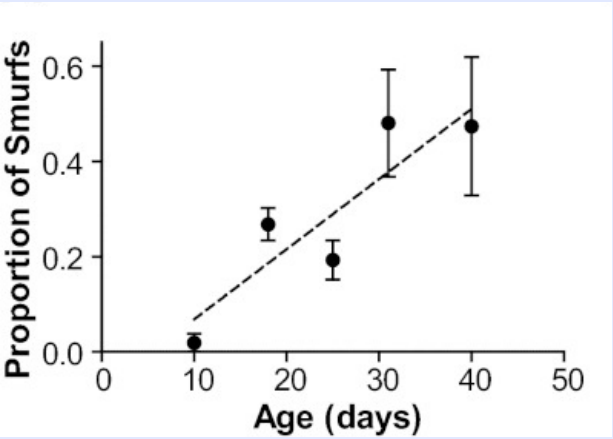
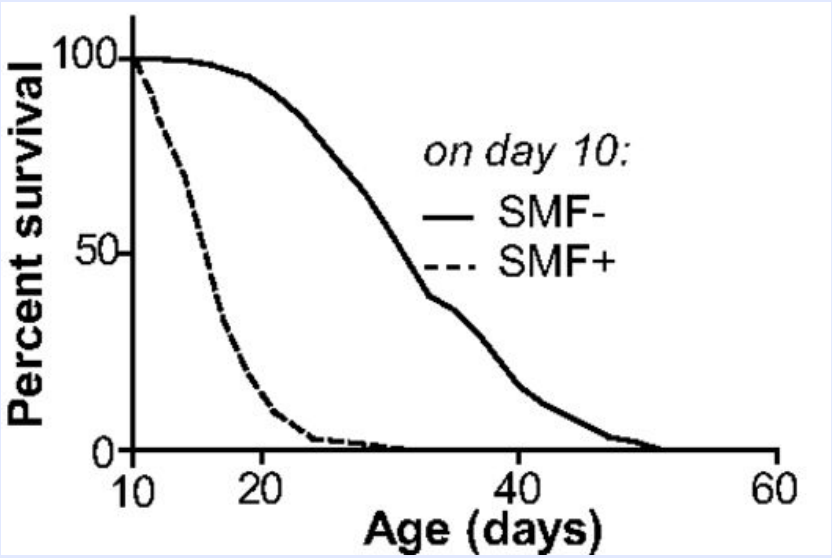
Intestinal Barrier Dysfunction and Aging







Intestinal Barrier Dysfunction Predicts Death

Rera M, PNAS. 2012

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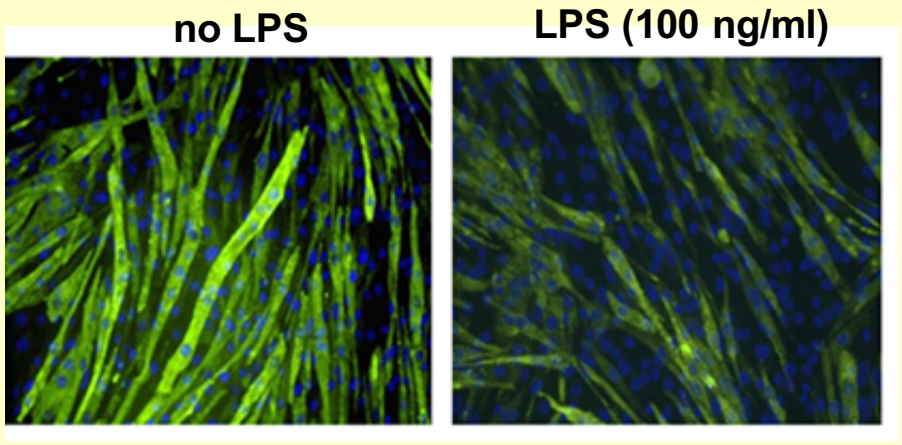
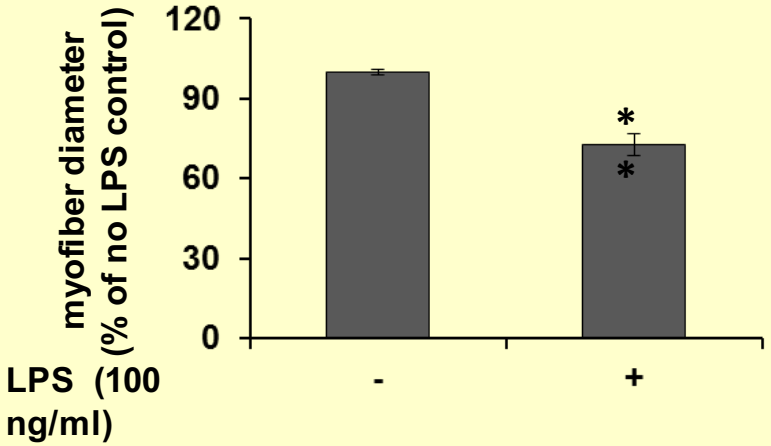
Effect of LPS on Myotube Diameter





Arun Bhattacharya, unpublished

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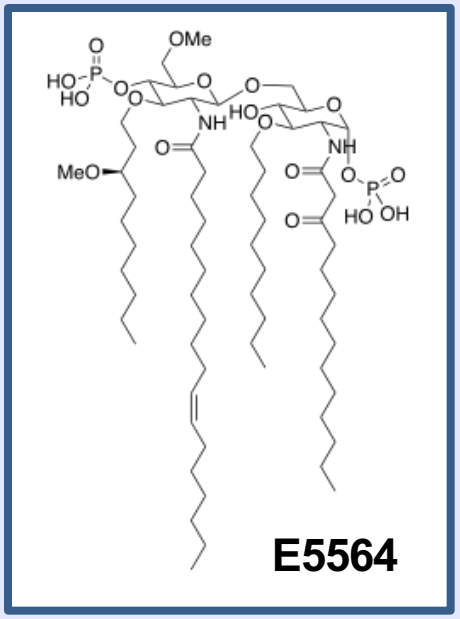
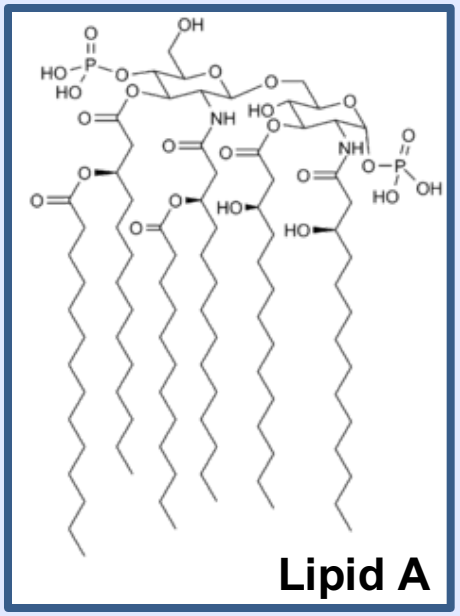
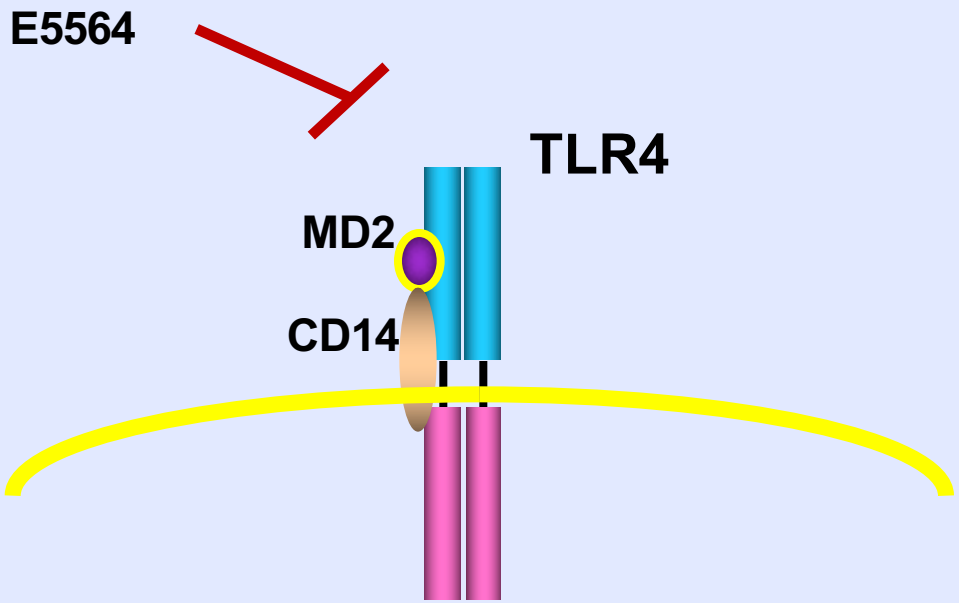
Human Interventions

Pharmacologic Inhibition of TLR4







Effect on:
1) Intralipid-induced IR (lean subjects)
2) Obese Subjects
3) T2DM Subjects

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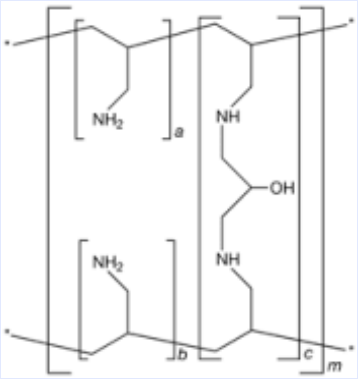
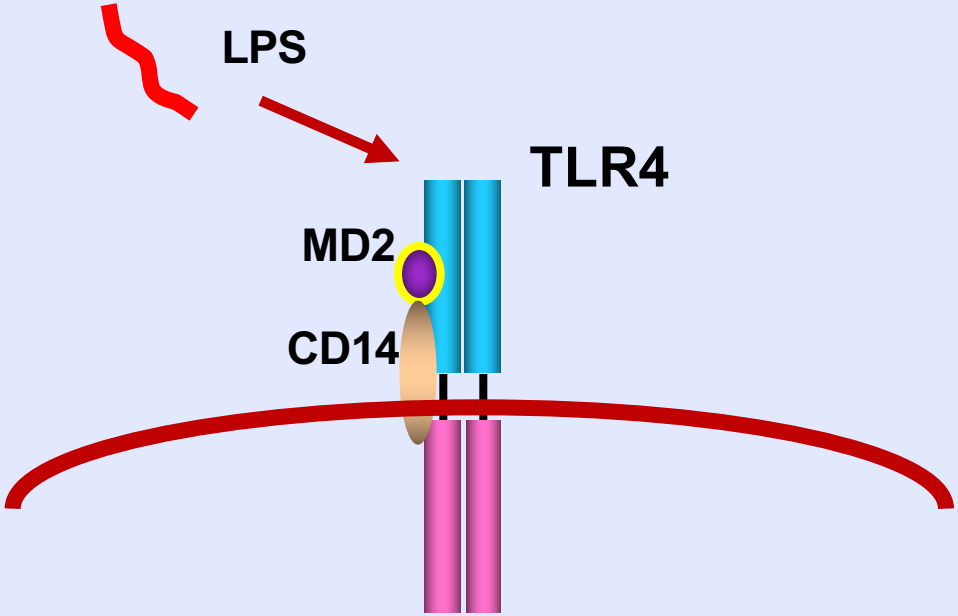
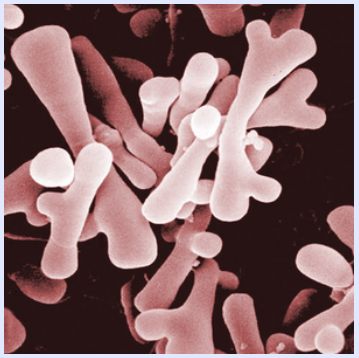
Reduction of plasma endotoxin concentration



Sevelamer





Bifidobacterium longum

Effect on:
1) HFD-induced IR (lean subjects)
2) Obese Subjects
3) T2DM Subjects

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Summary

- Increased plasma endotoxin concentration is associated with metabolic disease (obesity, T2DM, NAFLD) and aging.
- Endotoxin induces insulin resistance in muscle and liver.
- Genetic and pharmacological blockade of endotoxin (TLR4) action improves insulin sensitivity in cell culture and animal models.
- Human studies to elucidate the role of metabolic endotoxemia on metabolic disease (IR, diabetes, NAFLD etc) are ongoing.

Acknowledgments

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Andrea Alvarez
Sara Espinoza
Becky Powers
Ji Li
You Zhou

NIDDK
NIA
ADA
VA
AFAR

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Back to Dr. Wells

Izzy

- With the new dietary recommendations, she has lost 10% of her initial weight.
- She is ecstatic. She is now a volunteer Zumba instructor and has been reading up on what she can do to improve her prognosis.
- She goes back to see her hepatologist.
- She is concerned about her risk of liver cancer and wants to know how to be screened for this condition.

Obesity and the Link to Cancer

Jennifer T Wells, MD

Director

Regenerative Liver and Neoplasia

Texas Liver Institute

Assistant Professor, UTHSCSA

Austin, Texas

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Cancer Related Deaths

- Prospective study of 900,000 individuals followed for 16 years

- BMI > 40 % increase cancer related death
  - Men: (578/841) 52% RR 1.52
  - Women: (329/522) 62% RR 1.62

Calle, NEJM 2003

American Society of Clinical Oncology

Position statement 2014:

”Obesity is quickly overtaking tobacco as the leading preventable cause of cancer in the United States"

Ligibel et al. J. Clin. Oncol. 2014;32:3568

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The AICR 2015 Cancer Risk Awareness Survey Report

www.AICR.org

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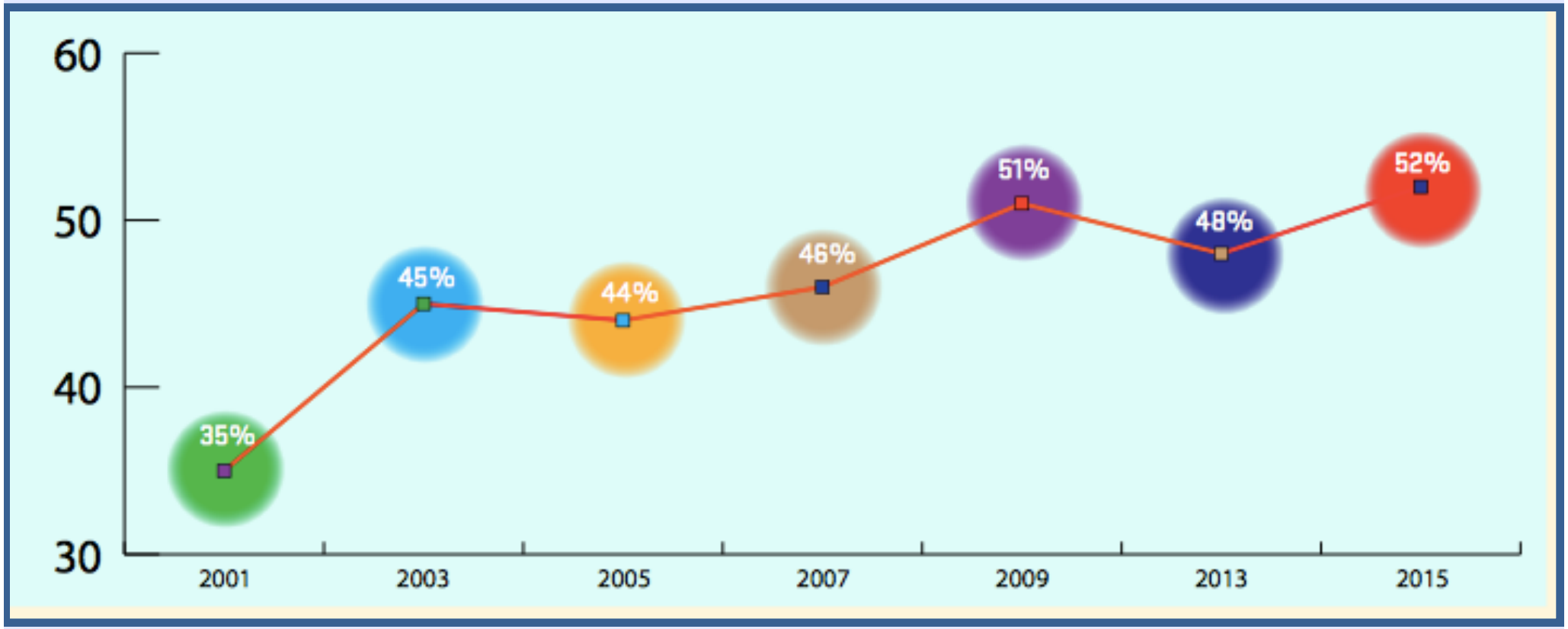
Public Awareness: Obesity and Cancer



www.AICR.org

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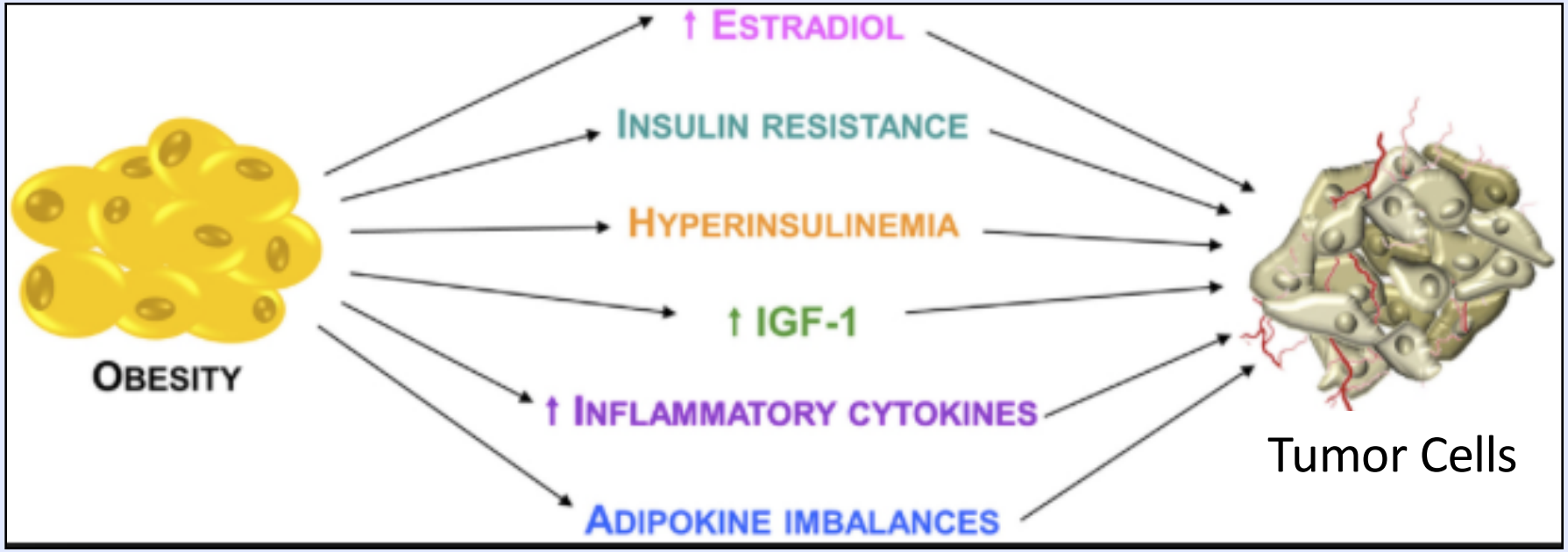
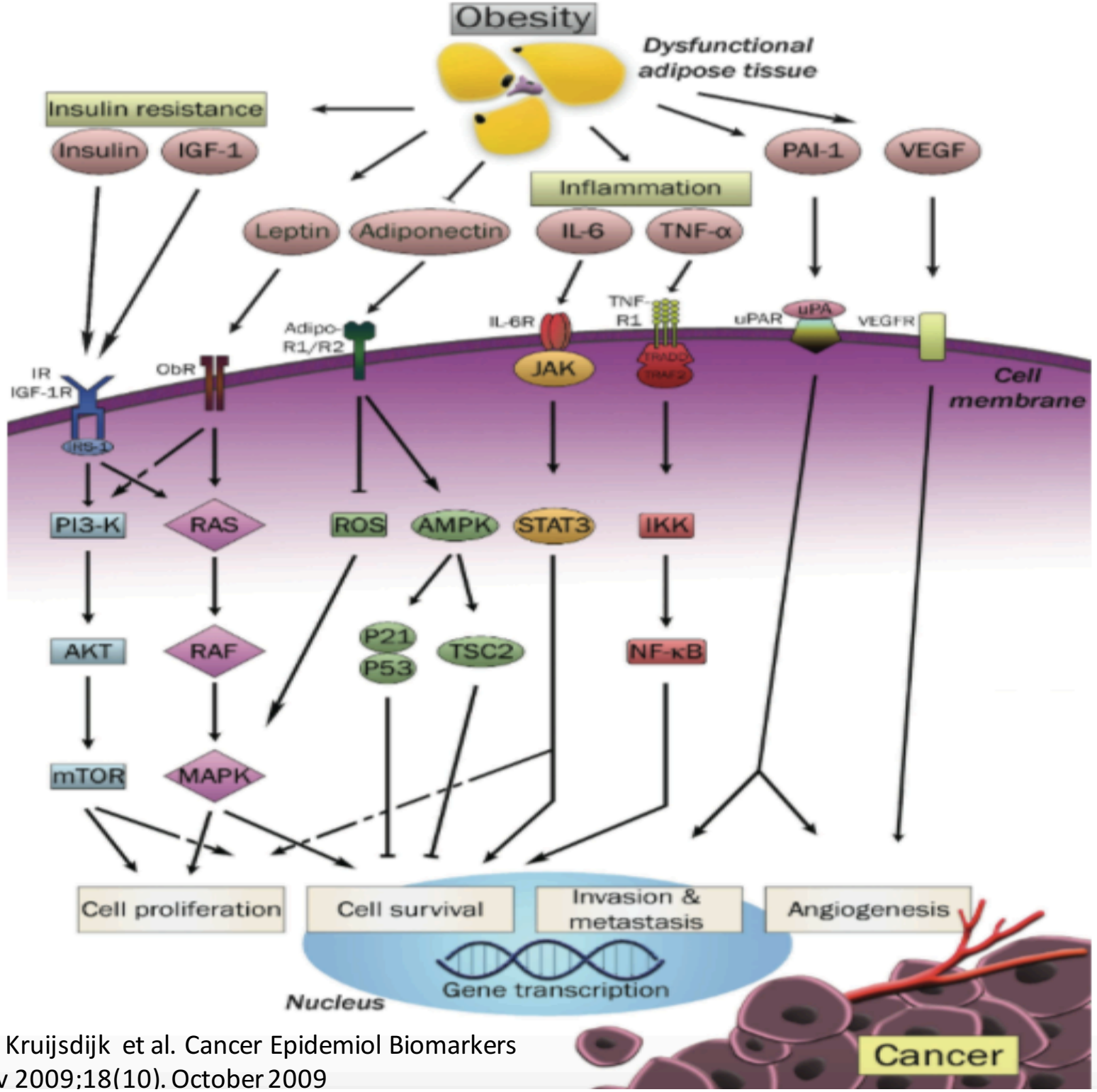
How Does Obesity Cause Cancer?

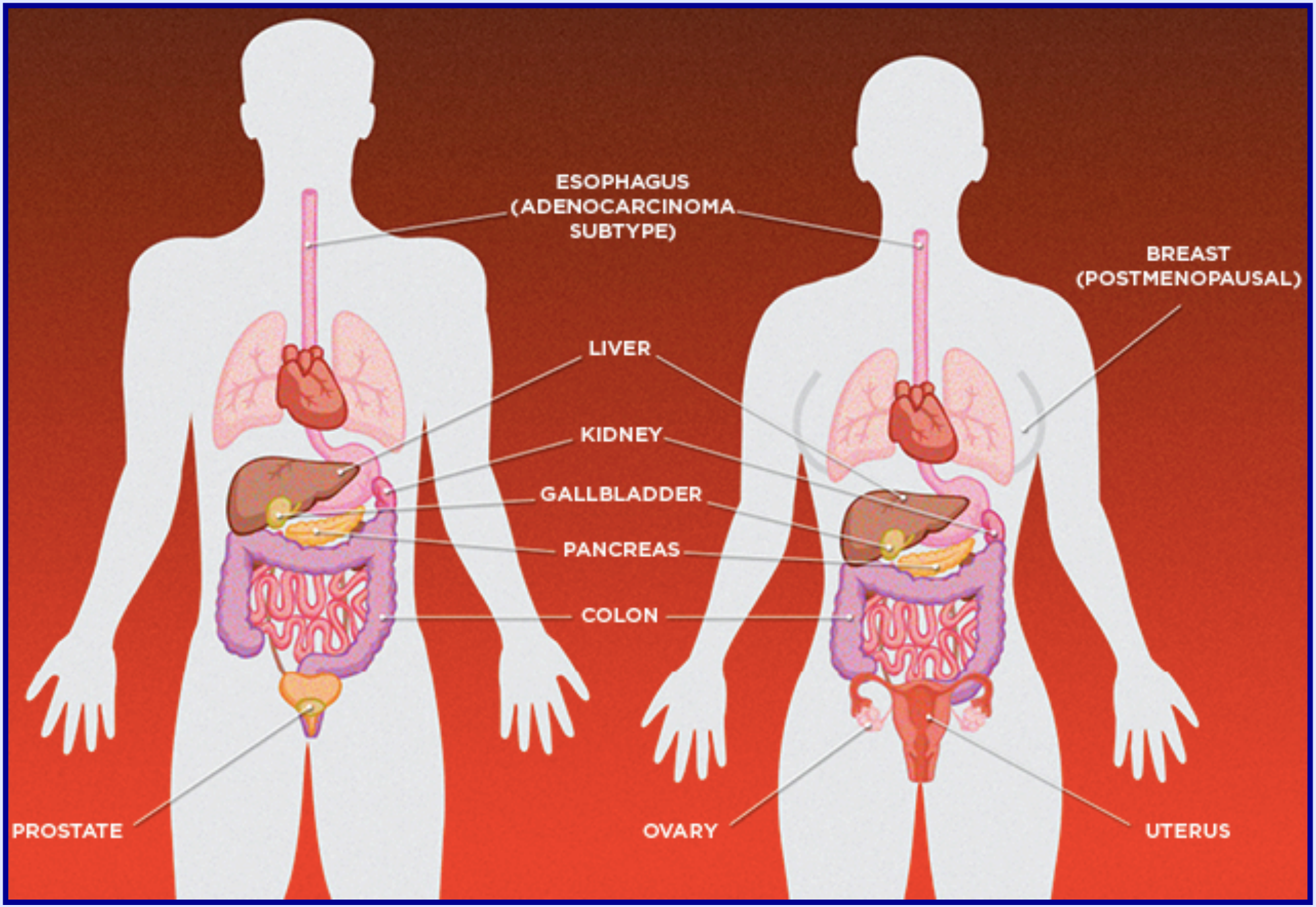
State of Hormone Imbalance



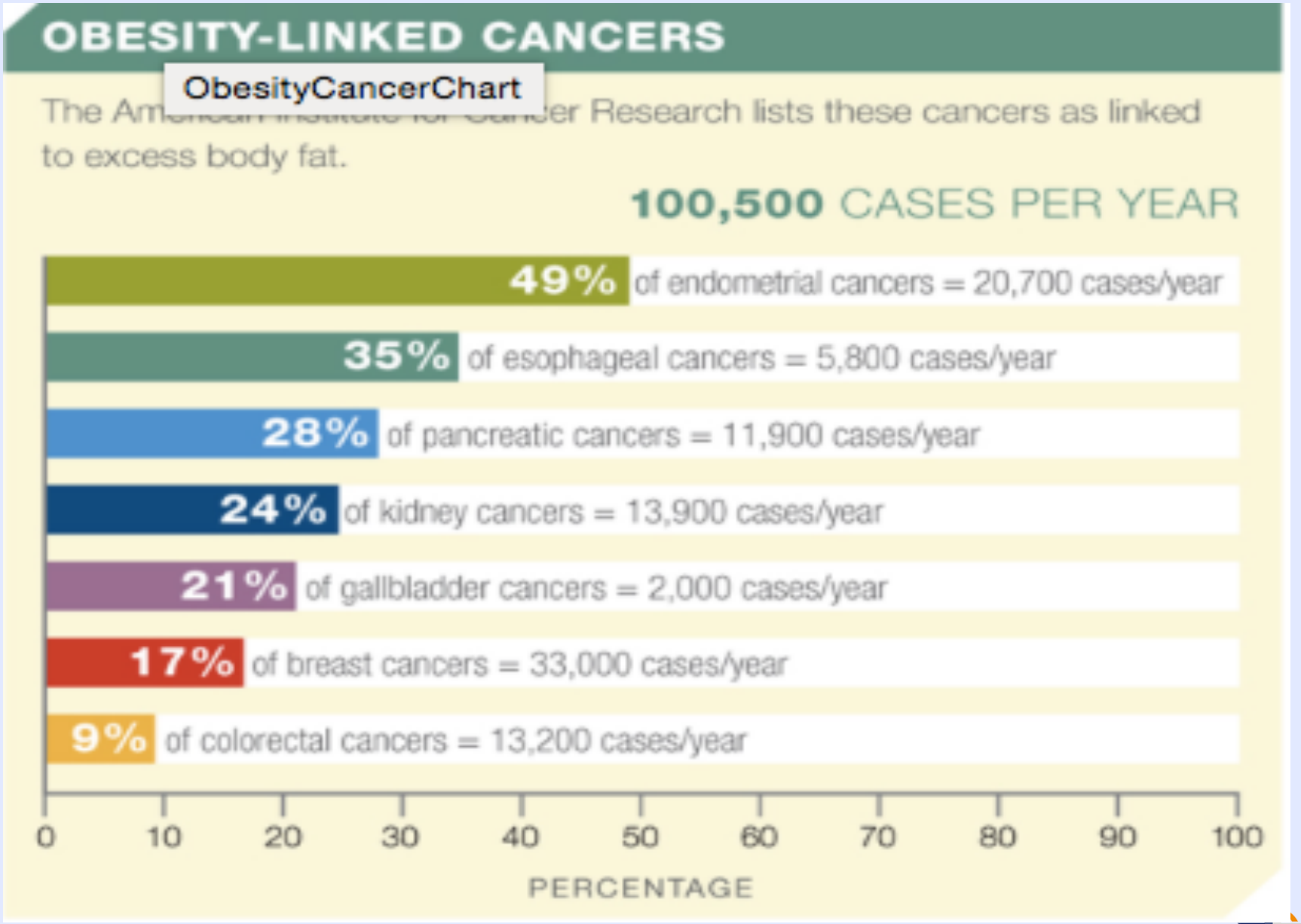
Mauro et al, *Front. Oncol.*, 2015



van Kruijsdijk et al. Cancer Epidemiol Biomarkers Prev 2009;18(10). October 2009



The American Institute for Cancer Research lists these cancers as linked to excess body fat



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Possible Links

- Non-Hodgkin lymphoma
- Multiple myeloma
- Cervical

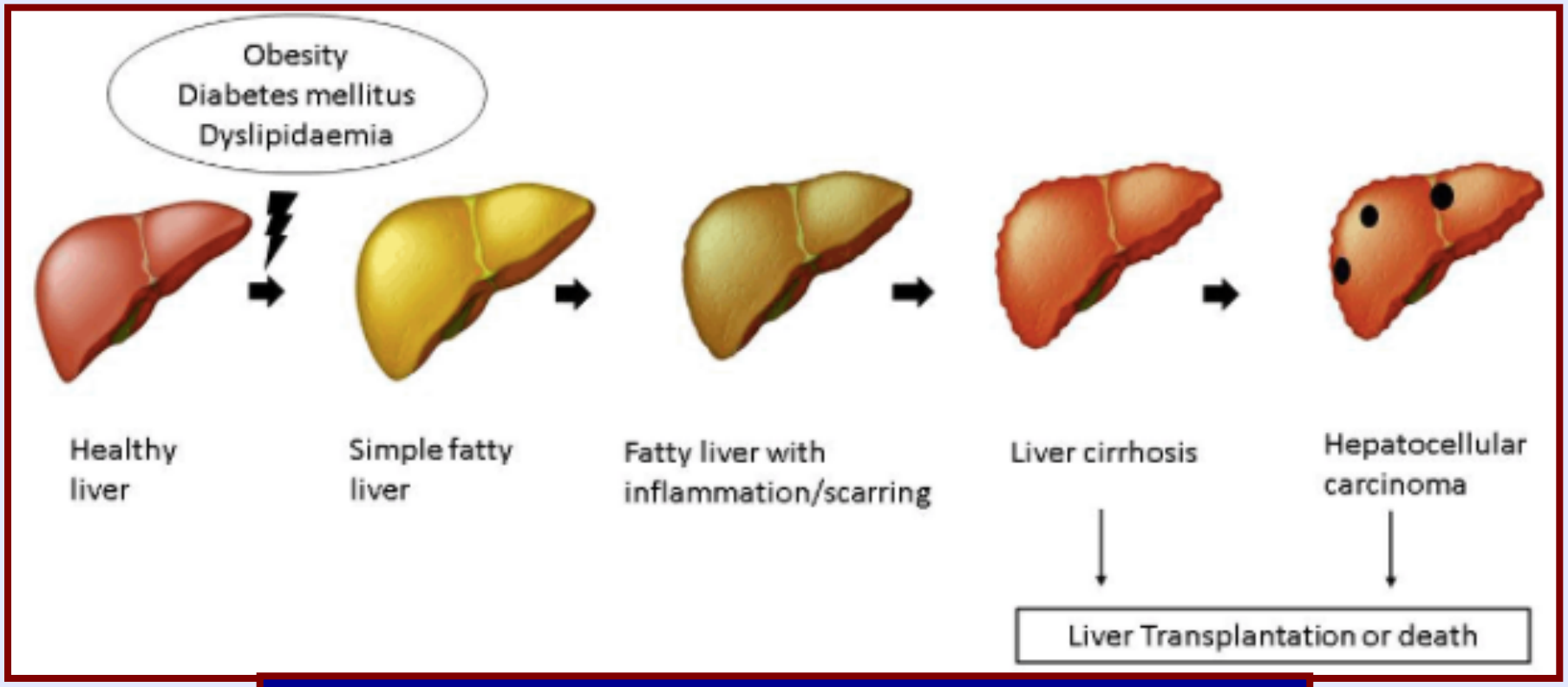
Obesity Worsens Cancer Outcomes

- Research has shown that a variety of cancers grow at faster rates in obese individuals versus in lean individuals
- Furthermore, obesity appears to increase the chances that a patient's cancer will metastasize.

Obesity Affects Cancer Treatment

- Decreased effective delivery of systemic cancer therapy
- Increased risk of growth/metastatic disease
- Morbidity from cancer treatment
- Poor wound healing, postoperative infections, and lymphedema
- Comorbid illnesses (eg, heart disease, cerebrovascular disease, and diabetes) in cancer survivors
- Increased risk of a 2nd primary

Hepatocellular Carcinoma

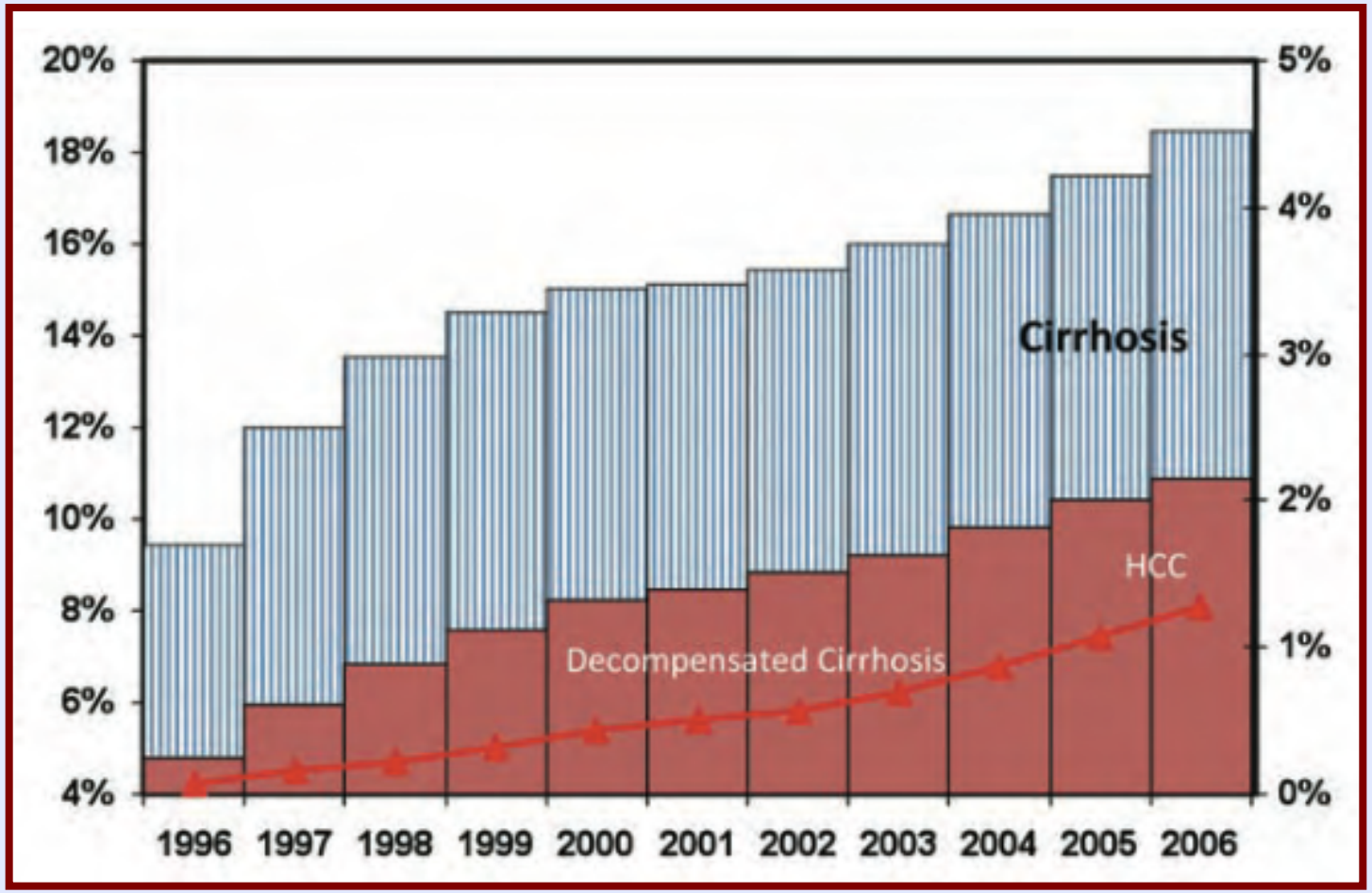


- Primary liver cancer: 90%
- 5th most common cancer worldwide
- 3rd cause of cancer death

- 5 year survival: 15%

HCC Incidence

HCC incidence during the past two decades has more than tripled in the United States, where it is the fastest increasing cause of cancer- related deaths.



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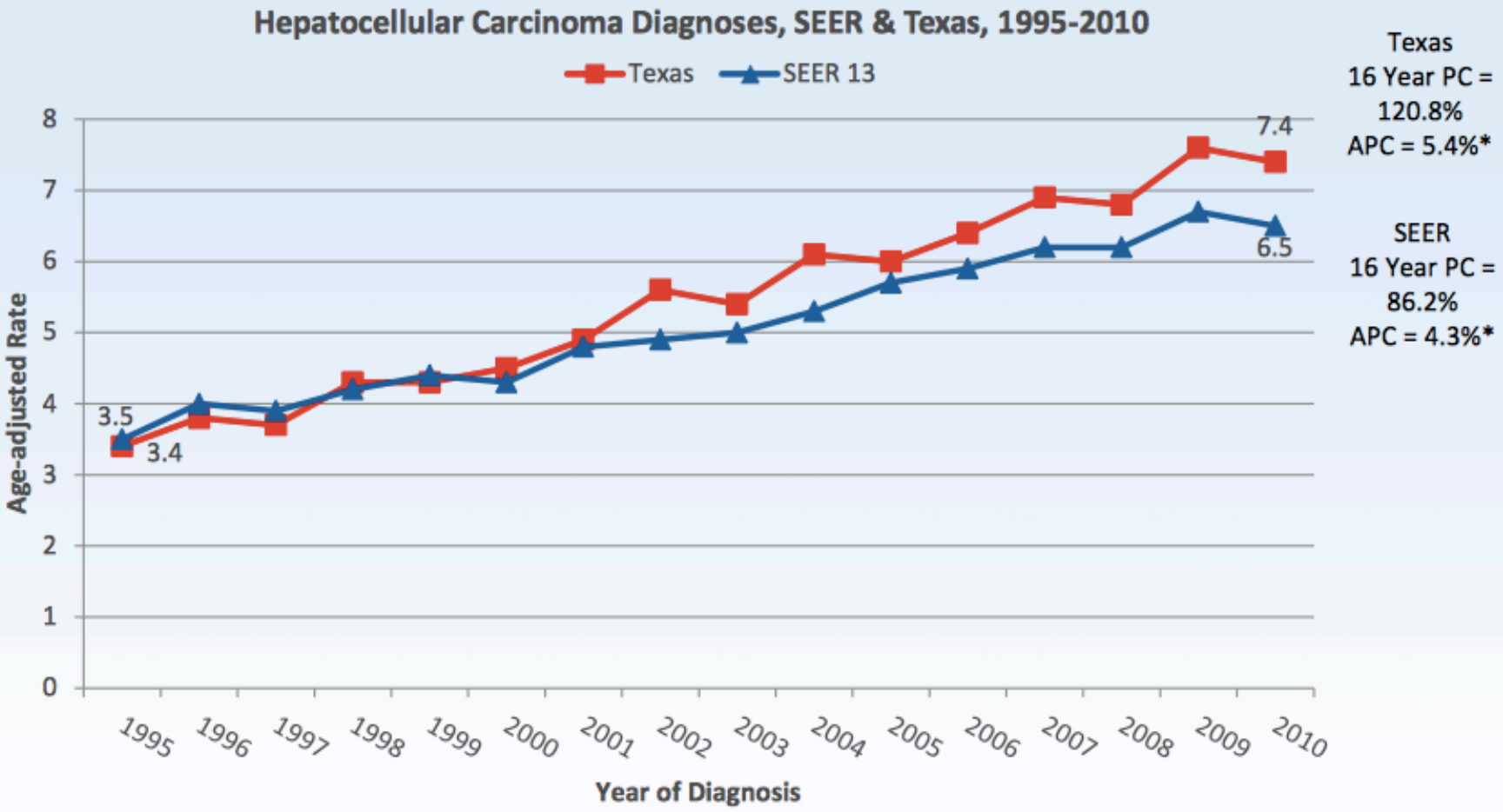
Hepatocellular Carcinoma Trends in Texas and the US (SEER)



*The APC is significantly different from zero ($p<0.05$)

PC = Percentage Change
APC = Annual Percentage Change

Rates are per 100,000 and age-adjusted to the 2000 US Standard Population (19 age groups - Census P25-1130) standard.
Percent changes were calculated using 1 year for each end point; APCs were calculated using weighted least squares method.

Data Sources: Texas Department of State Health Services, Cancer Epidemiology and Surveillance Branch, Texas Cancer Registry, Incidence – Texas, 1995-2010, Cut-off 11/30/12, SEER*Prep 2.5.2 and Incidence – SEER 13 Regs Research Data, Nov 2011 Sub.

Surveillance

- Important to follow and stage NAFLD so patients can be enrolled in surveillance
- Cirrhosis or advanced stage 3
  - US q 6 months
  - AFP not necessary
  - **Often cross-sectional imaging + AFP is used
- Fatty liver without advanced fibrosis: some risk however no official surveillance is recommended

Bruix et al *Hepatology*, Vol. 53, No. 3, 2011

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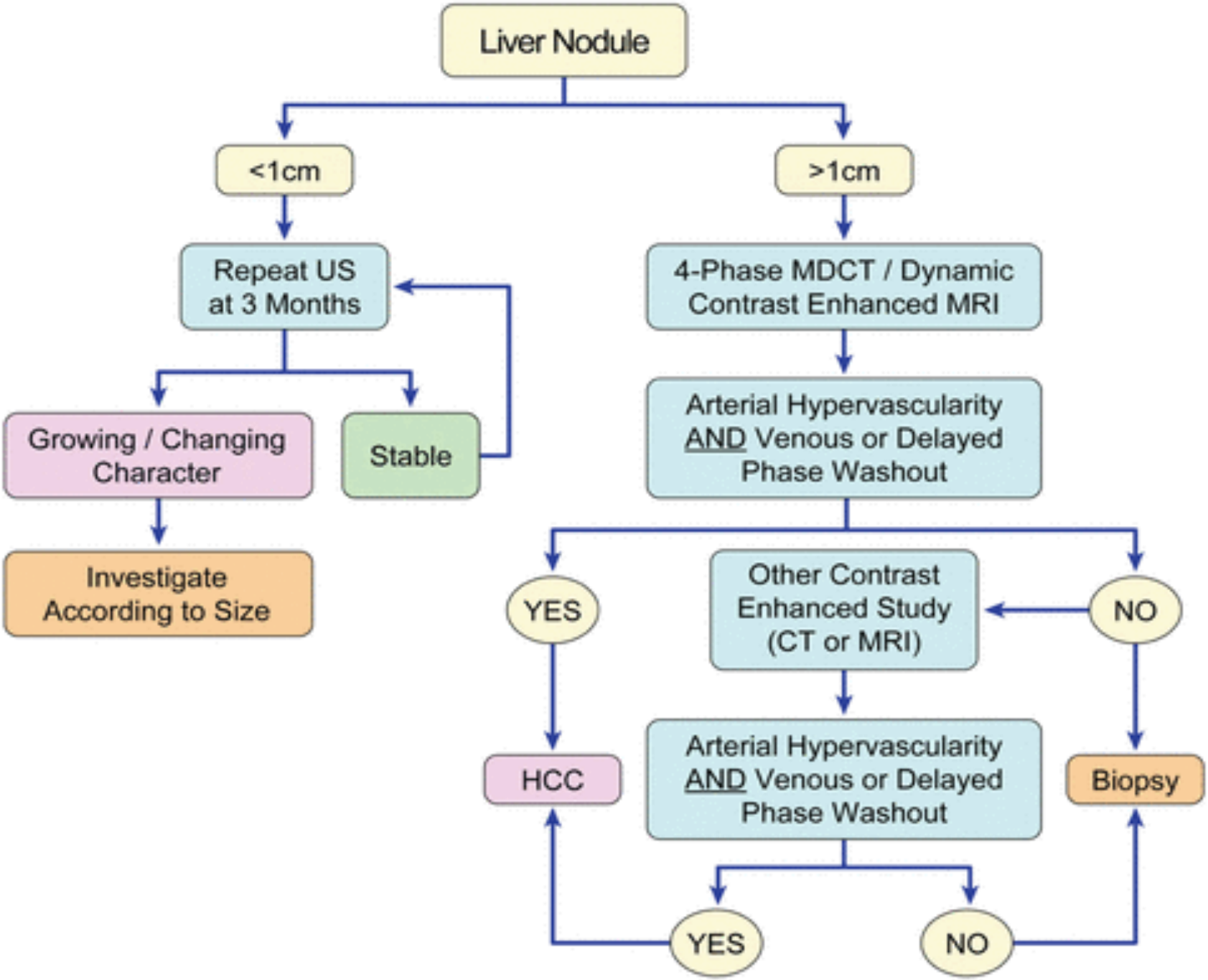
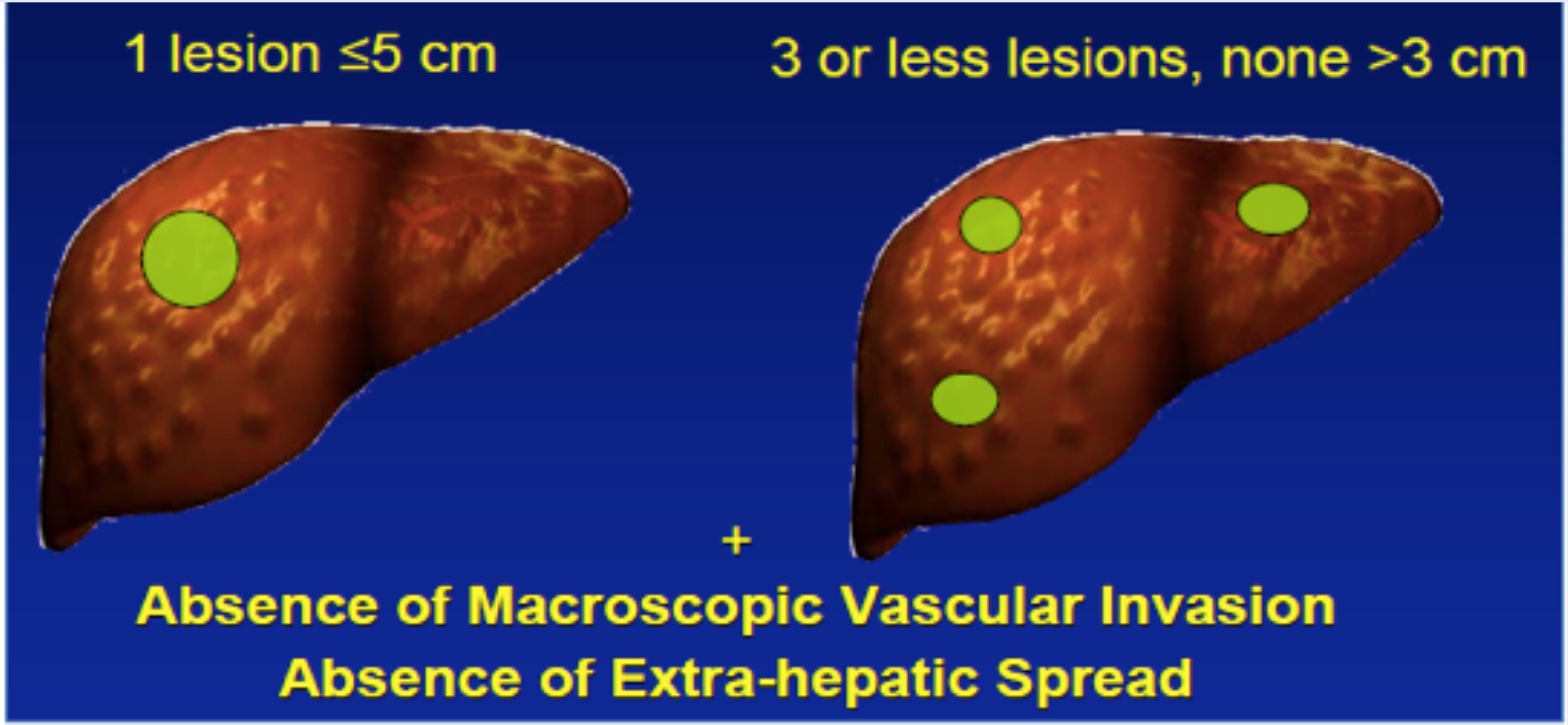


Bruix et al *Hepatology*, Vol. 53, No. 3, 2011

HCC Treatments

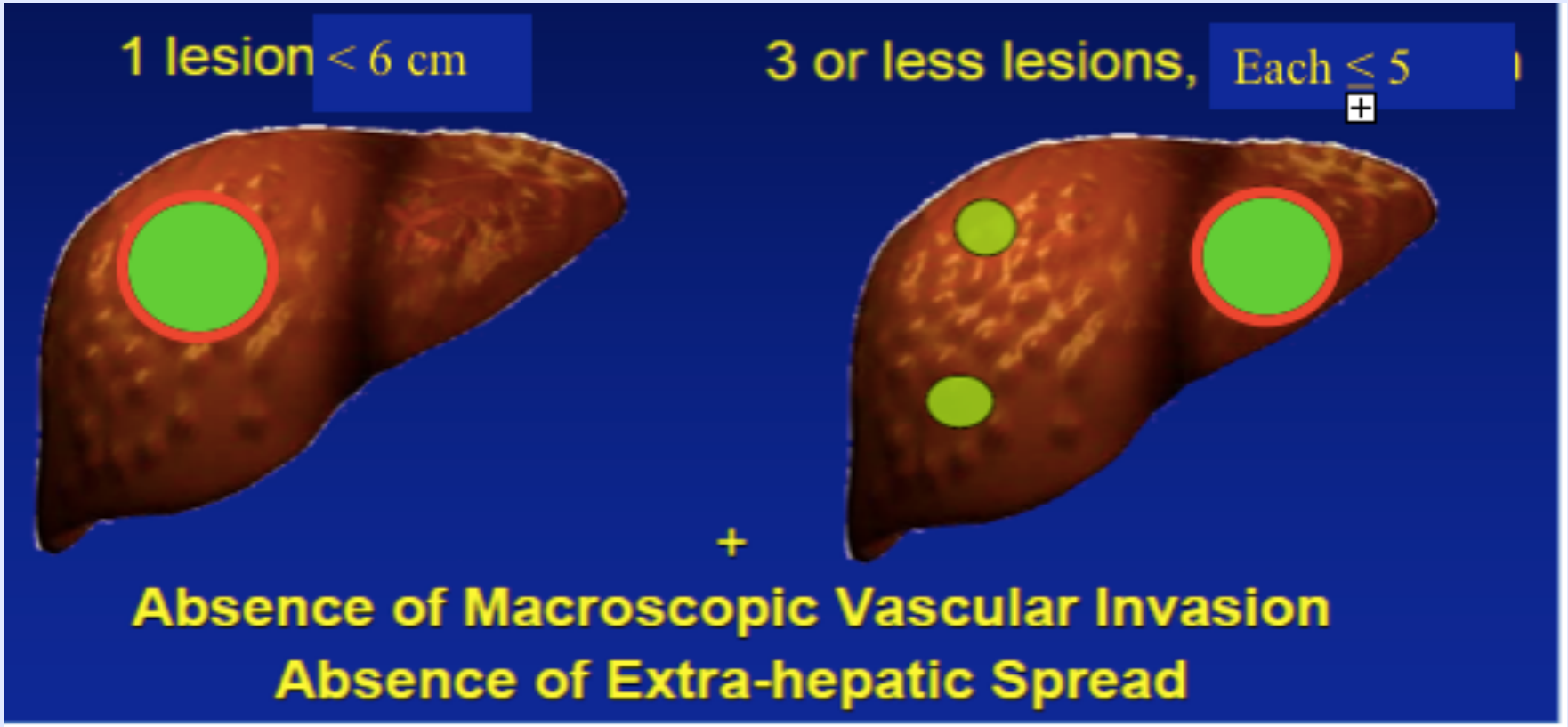
- Surgical resection: Rare
- Liver transplantation
  - Milan
  - Region 4 Criteria

Milan Criteria



Mazzeferro, *NEJM* 1996

Region 4



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HCC Treatments

- Surgical resection: Rare
- Liver Transplantation
  - Milan
  - Region 4 Criteria
- Radiofrequency Ablation
- Transarterial Chemo-embolization
- Radio-embolization
- Sorafenib

Summary

- Mass education
- Changing perceptions in large populations
- Appropriate evaluation with opportunity to improve risk factors
- Staging and enrollment in surveillance programs in those with advanced liver disease

Izzy

- She is diagnosed with HCC 2 years later. Treated with LRT.
- About a year later, she underwent liver transplantation.
- 3 years later, she is happy to be able to attend and dance at her son's wedding.

Panel Discussion/Q&A