Alba Rocco

Gastroenterologia ed Epatologia
Dipartimento di Medicina Clinica e Chirurgia,
Università Federico II di Napoli

FATTORI DI RISCHIO NAFLD/NASH

Ospedale Evangelico Betania

IX Edizione

L'EPATOLOGIA NEL III MILLENNIO:
TRA BISOGNI DEL PAZIENTE
E SOSTENIBILITÀ DEL SISTEMA

NAPOLI
26 - 27
NOVEMBRE
2021

National Library of Medicine
National Center for Biotechnology Information

1993 2021

PubMed.gov

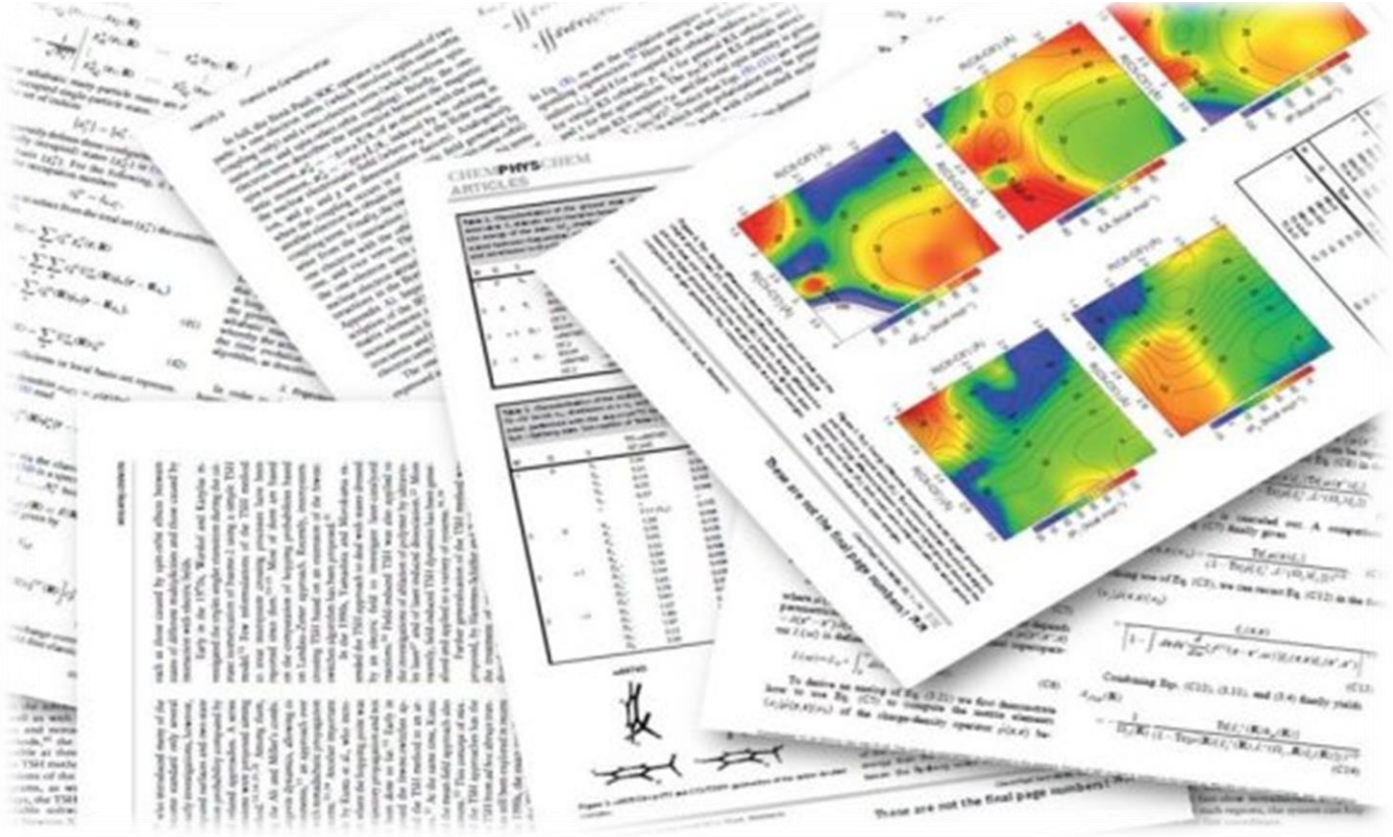
non alcoholic fatty liver disease risk factors

Search

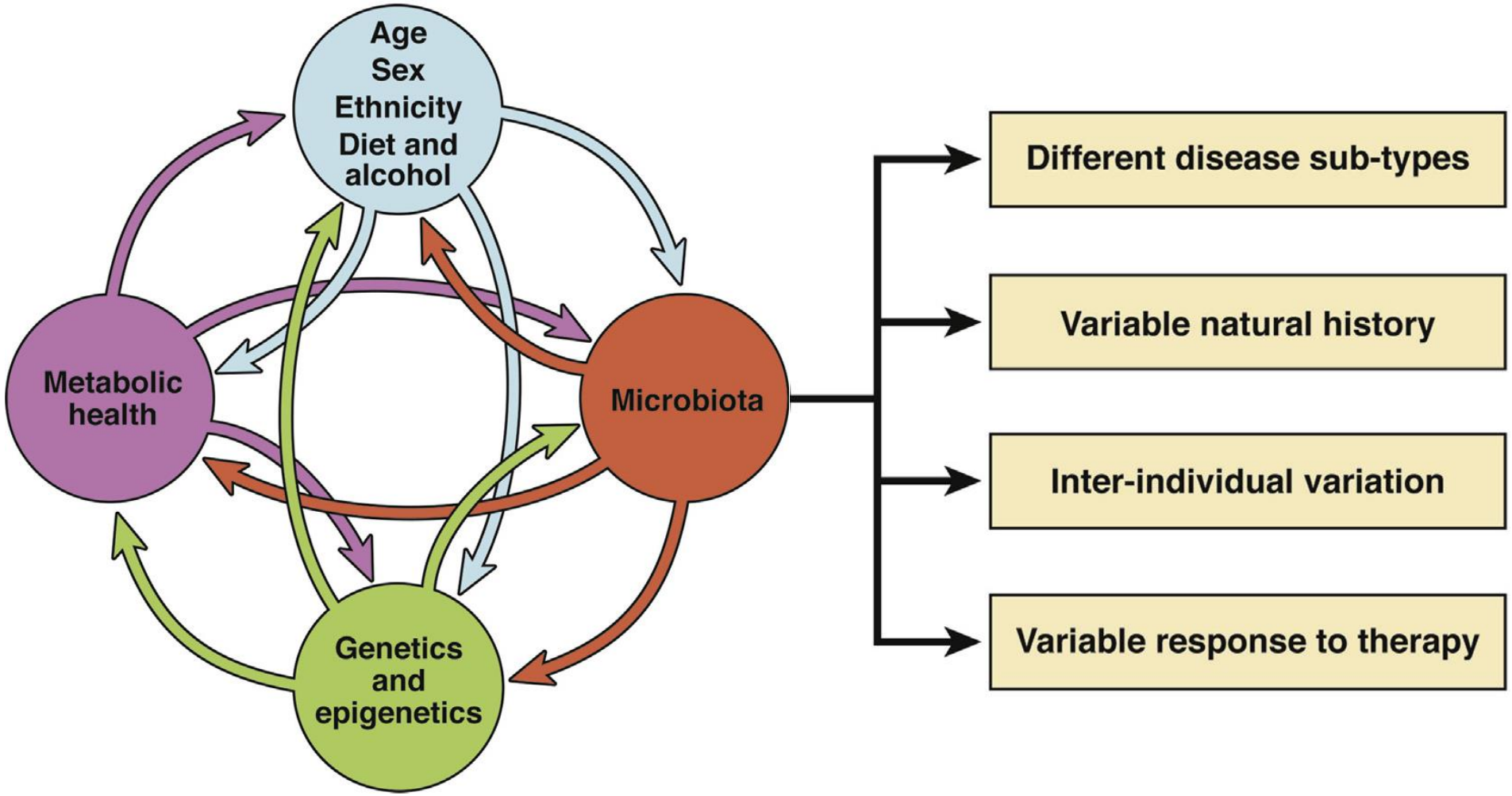
Advanced Create alert Create RSS User Guide

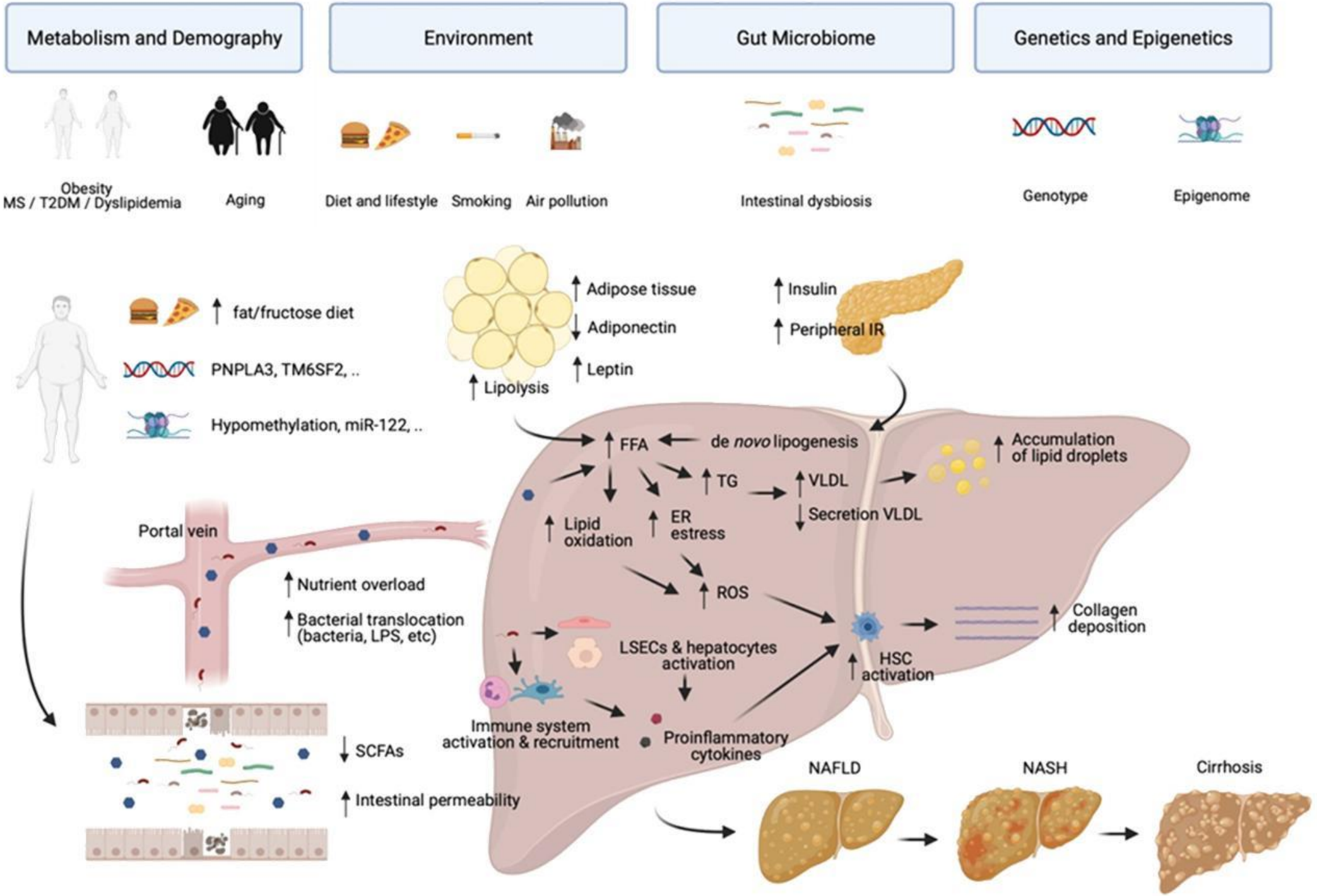
5,091 results

Page 1 of 510



The acronym nonalcoholic fatty liver disease (NAFLD) is an umbrella term including a complex multisystem disorder with significant pathogenic heterogeneity in terms of main drivers and coexisting disease modifiers




Metabolism and Demography
Environment
Gut Microbiome
Genetics and Epigenetics
Obesity
MS / T2DM / Dyslipidemia
Aging
Diet and lifestyle
Smoking
Air pollution
Intestinal dysbiosis
Genotype
Epigenome
fat/fructose diet
PNPLA3, TM6SF2, ..
Hypomethylation, miR-122, ..
Adipose tissue
Adiponectin
Leptin
Lipolysis
Insulin
Peripheral IR
FFA
de novo lipogenesis
TG
VLDL
Secretion VLDL
Accumulation of lipid droplets
Lipid oxidation
ER estress
ROS
Portal vein
Nutrient overload
Bacterial translocation (bacteria, LPS, etc)
LSECs & hepatocytes activation
HSC activation
Collagen deposition
Immune system activation & recruitment
Proinflammatory cytokines
SCFAs
Intestinal permeability
NAFLD
NASH
Cirrhosis

Obesity

Obesity has been present in humans, putatively, since the European upper Palaeolithic age

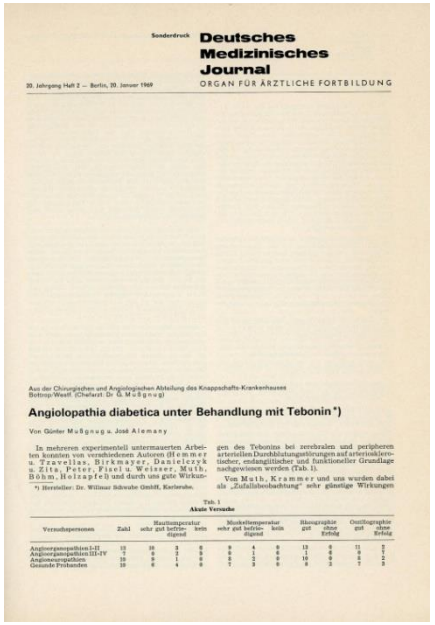
Venus of Willendorf (30 000 BC)

Neolithic Venus (5800-5300 BC)

In ancient Egypt, parts of sub-Saharan Africa, China and south Pacific islands, obesity has been considered a **sign of success**, **prosperity** and **good health**, and in women implied

Galen of Pergamon (129–216 AD) described obesity as an illness he termed ‘polysarcia’

With the emergence of overnutrition after World War II and increasing clinical relevance of obesity and related comorbidity, attention was finally directed to NAFLD



Sonderdruck **Deutsches Medizinisches Journal**

ORGAN FÜR ÄRZTLICHE FORTBILDUNG

Aus der Chirurgischen und Angiologischen Abteilung des Knappschafts-Krankenhauses Bottrop/Westf. (Chefarzt: Dr. G. Mußgnug)

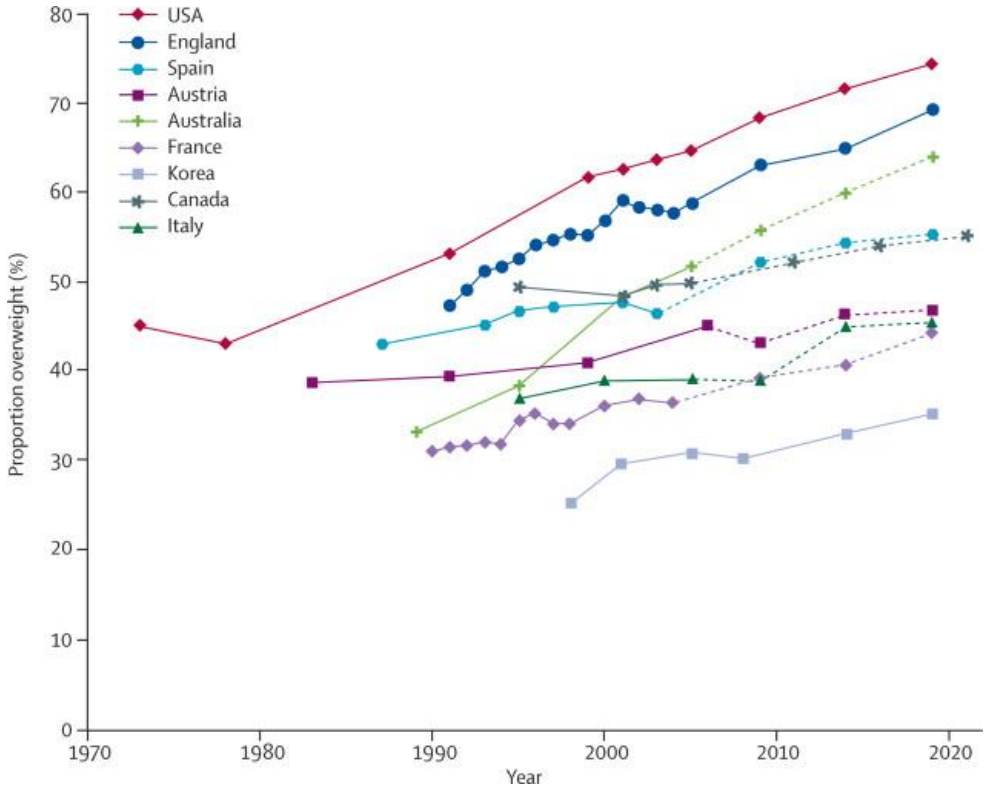
Angiolopathia diabetica unter Behandlung mit Tebonin*)

Von Günter Mußgnug u. José Alemany

Thaler in Vienna, Austria showed that steatosis affected half of obese subjects, whereas the frequency in a random series of 10 900 liver biopsies was 26.5%

Dtsch Med J. 1972 Nov 10; 23(11):648-53.

Obesity is considered the **main risk factor for NAFLD**, since BMI and waist circumference correlate positively with both the presence of NAFLD and disease progression



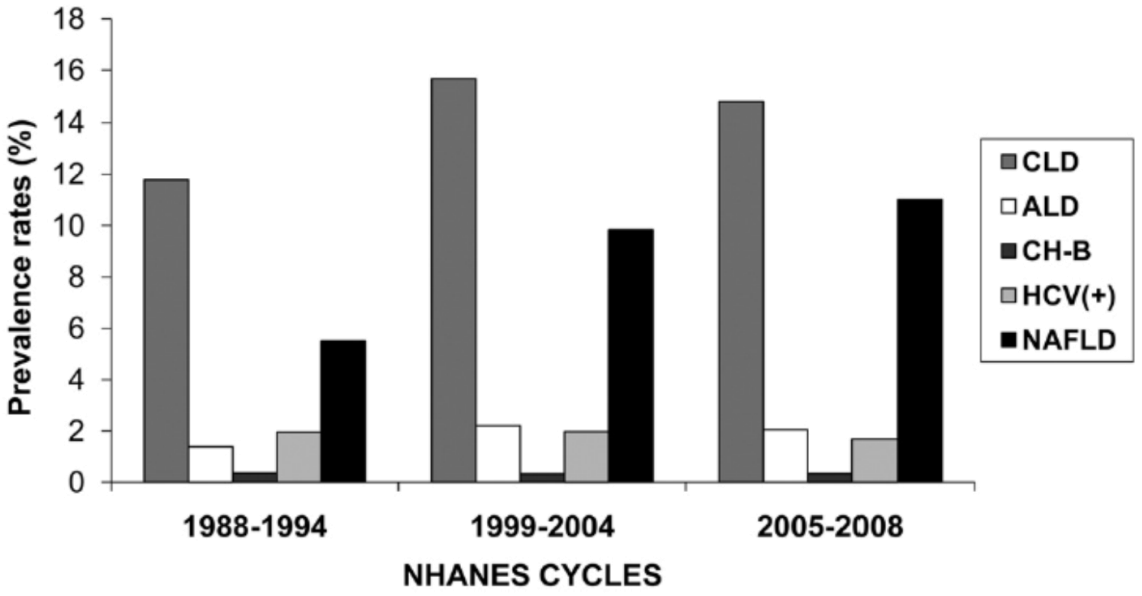


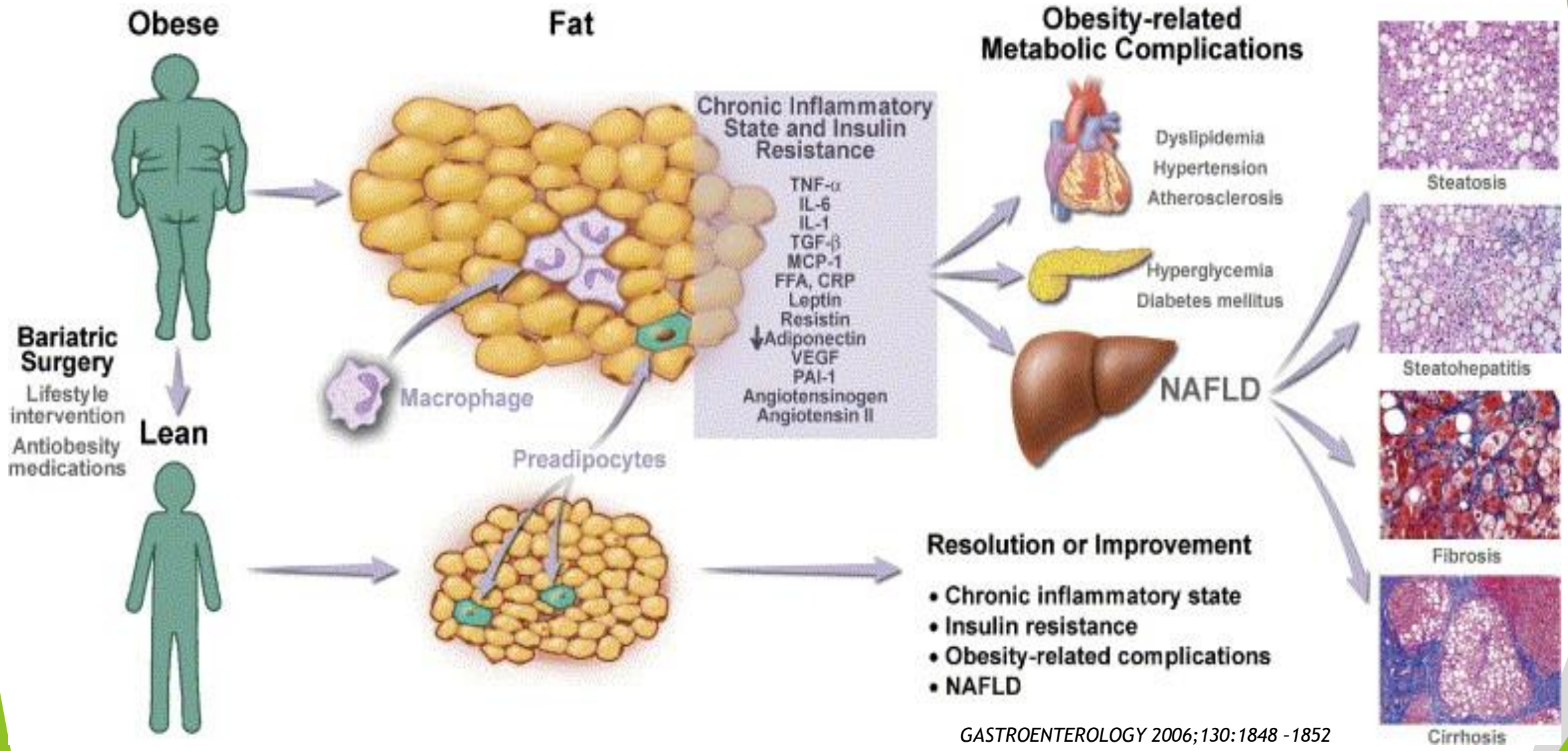
Figure 1. Prevalence rates for CLD over time.

The worldwide number of obese patients tripled from 1975 to 2018 and obesity likely accounts for the incremental changes in NAFLD over time in the USA

Population	Prevalence (%)
General population	20-30
Pediatric population	2,6-10
Morbid obese	75-90

Population	Prevalence (%)
General population	27-34
Pediatric population	13-14
Morbid obese	90-95

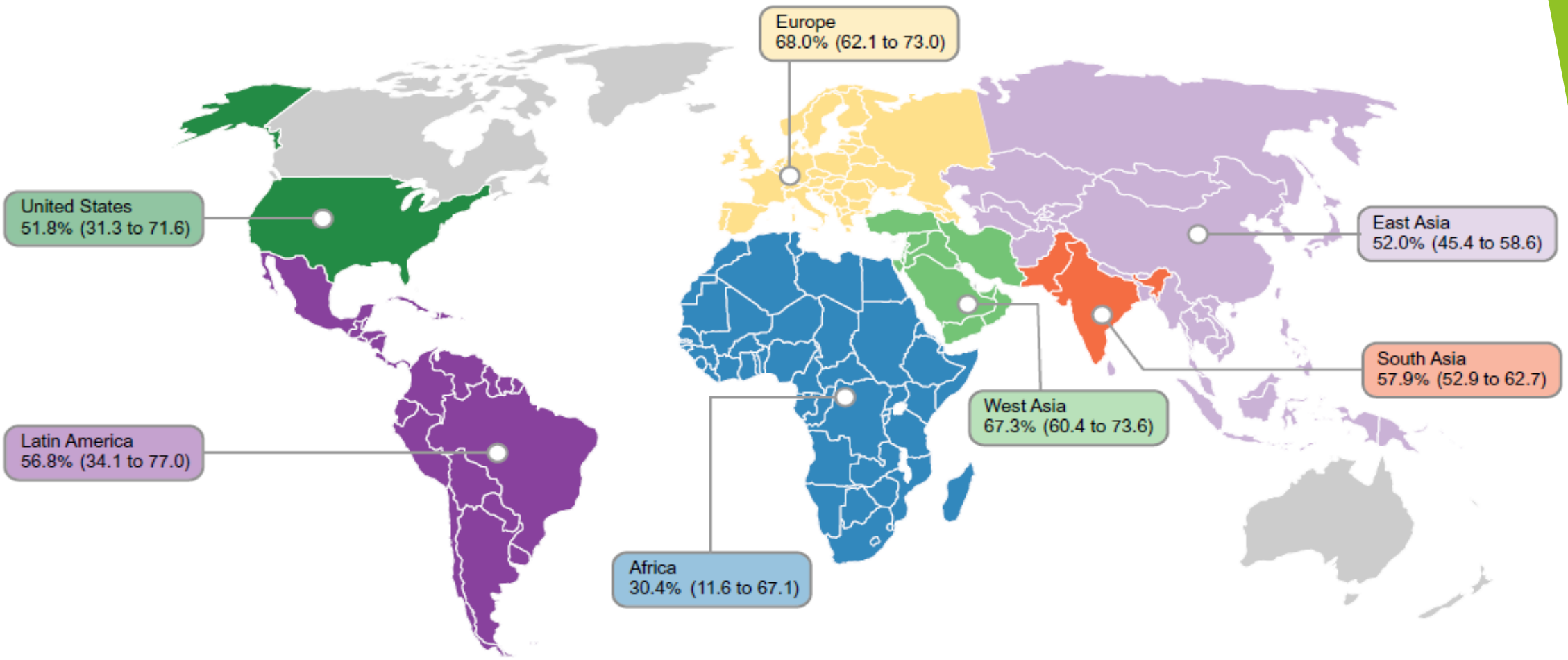
Population	Prevalence (%)
General population	15-18



GASTROENTEROLOGY 2006;130:1848 -1852

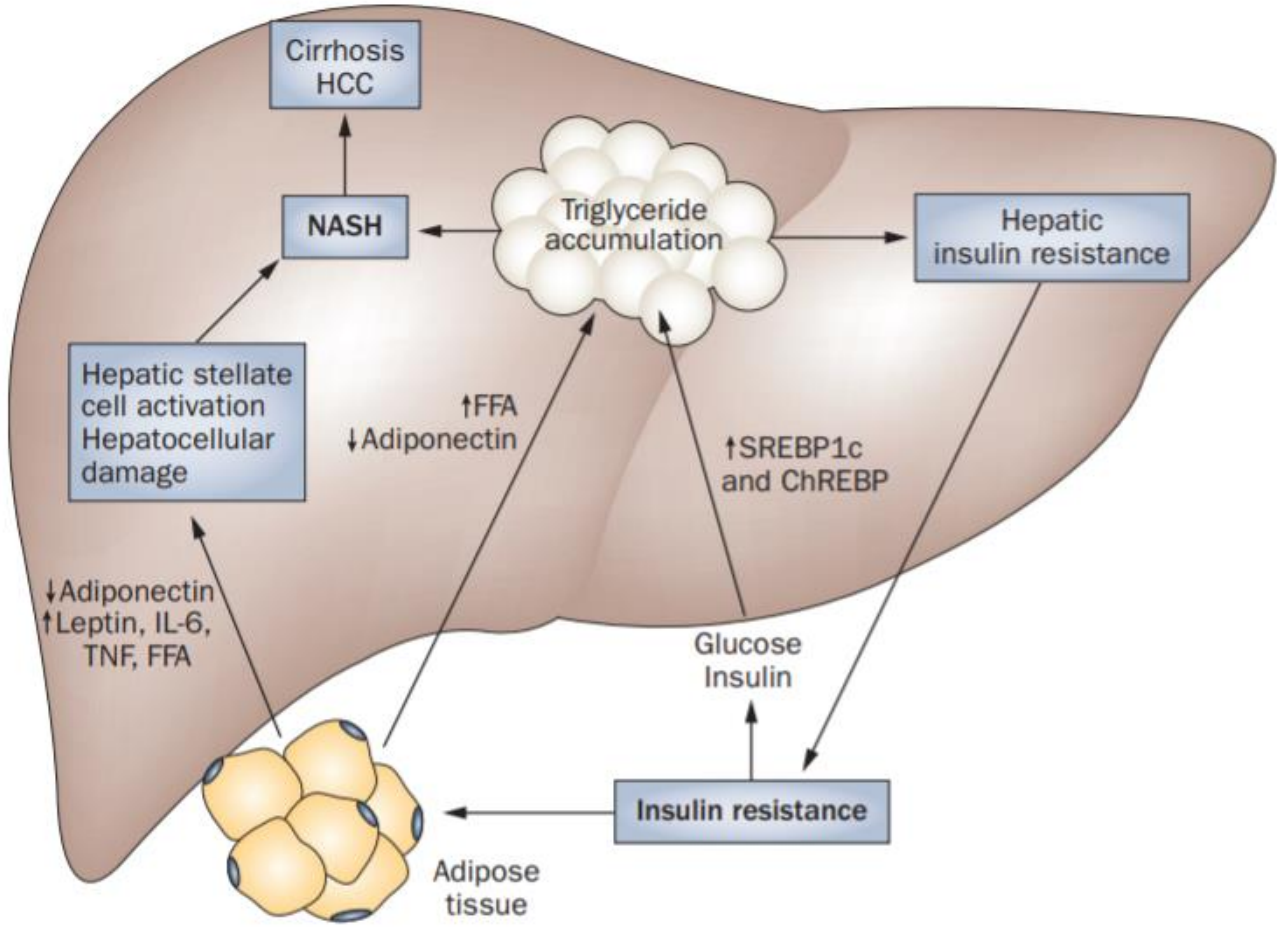
Type 2 Diabete mellitus

80 studies from 20 countries
49,419 individuals with T2DM



Global prevalence of NAFLD among T2DM patients 55.5%
(95% confidence interval: 47.3-63.7)

Insulin resistance promotes hepatic triglyceride accumulation by increasing peripheral adipose **lipolysis** and free fatty acid influx as well as upregulating levels of hepatic lipogenic transcription factors SREBP1c and ChREBP



Hepatic free fatty acids exacerbate hepatic insulin resistance via activation of JNK1 and nuclear factor κB pathways

This causes **a positive feedback loop**, which results in a self-perpetuating cycle of increased hepatic triglyceride content and hepatic insulin resistance

Published in final edited form as:
J Clin Gastroenterol. 2017 February ; 51(2): 160–166. doi:10.1097/MCG.0000000000000666.

The Association between Nonalcoholic Fatty Liver Disease and Metabolic Abnormalities in United States Population

Third National Health and Nutrition Examination Survey (NHANES III) coordinated by National Center for Health Statistics and Centers for Disease Control and Prevention

Nationwide probability sample of 39,695 persons from 1988-1994

11,674 adults (20-74 years old)

- liver ultrasound examination
- NO chronic hepatitis B, chronic hepatitis C, and excessive alcohol use or elevated transferrin
- NO steatogenic medications (amiodarone, methotrexate, tamoxifen, corticosteroids, valproate, and antiretrovirals)

NAFLD among US population — **18.2%** (2124/11674)

NAFLD + ↑ serum transaminases — **15%** (319/2124)

- 60% (191) no fibrosis
- 33.6% (107) indeterminate range
- 6.6% (21) advanced fibrosis

Prevalence MetS — **21%** (2817/11674)

- 61% (1,715) 3 metabolic abnormalities
- 30.5 % (862) 4 metabolic abnormalities
- 8.5% (240) 5 metabolic abnormalities

Prevalence NAFLD among Mets — **43%** (1211/2817)

- 37% of those with MetS3
- 49% of those with MetS4
- 67% of those with MetS5

20% ↑ serum transaminases

24% moderate steatosis
19% severe steatosis

NAFLD among individuals with metabolic abnormalities

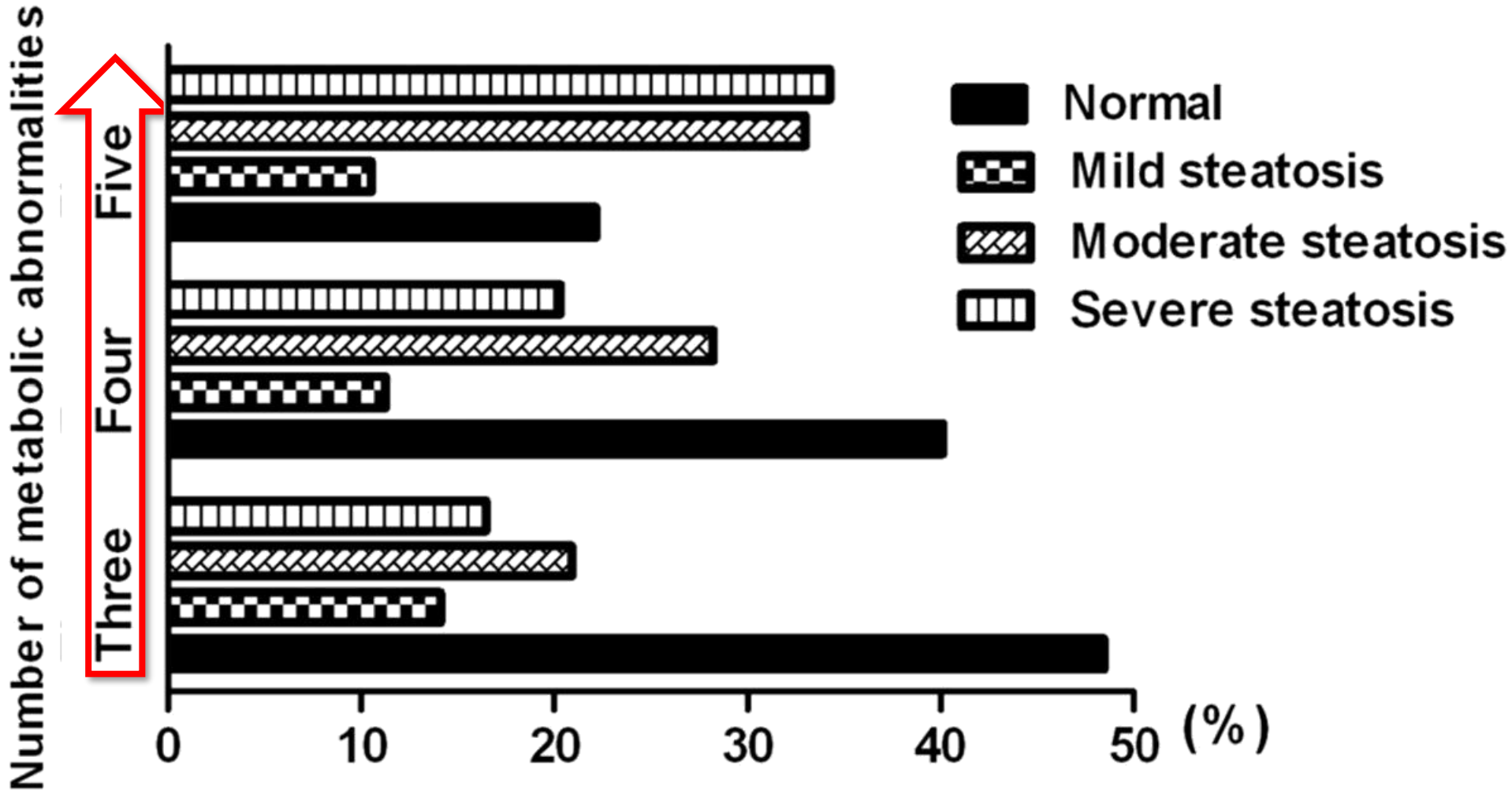
11,674 subjects enrolled in III **National Health and Nutrition Examination Survey**
(NHANES III 1988 -1994)

	Prevalence of NAFLD % (95% CI)	Percentage of NAFLD with elevated liver enzymes	Hepatic steatosis compared to controls	
			aOR* (95% CI)	*p* value
Metabolic syndrome	43.2 (39.4 – 46.9)	19.9% (1.9)	11.5 (8.9 – 14.7)	<0.001
Three abnormalities	37.3 (33.7 – 41.0)	18.1% (2.4)	9.7 (7.6 – 12.5)	<0.001
Four abnormalities	48.5 (43.9 – 53.1)	21.6% (3.8)	16.9 (12.0 – 23.8)	<0.001
Five abnormalities	67.3 (56.7 – 77.8)	22.8% (4.5)	37.6 (25.0 – 56.3)	<0.001
Increased WC†	31.2 (28.6 – 33.9)	17.1% (1.6)	2.9 (2.2 – 3.8)	<0.001
IFG/Diabetes†	41.2 (36.6 – 45.9)	20.3% (2.8)	2.0 (1.2 – 3.4)	0.007
High Triglyceride level†	34.7 (31.8 – 37.6)	19.0% (2.1)	2.2 (1.5 – 3.0)	<0.001
Low HDL level†	27.8 (25.1 – 30.4)	17.6% (2.0)	1.5 (1.2 – 1.9)	<0.001
High BP†	29.2 (26.5 – 31.9)	17.9% (1.9)	1.3 (0.9 – 1.8)	0.060

aOR – adjusted odds ratio (adjusted for age, sex, race, smoking, alcohol use, and education level), CI – confidence interval.

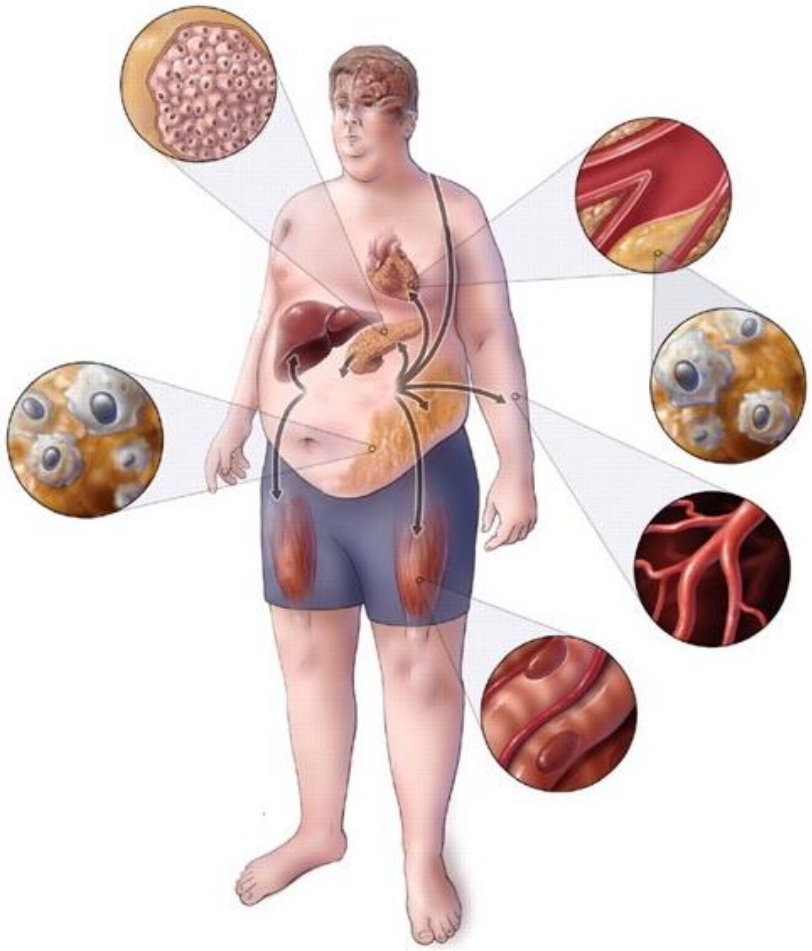
J Clin Gastroenterol. 2017;51:160-6.

Number of metabolic abnormalities and severity of hepatic steatosis

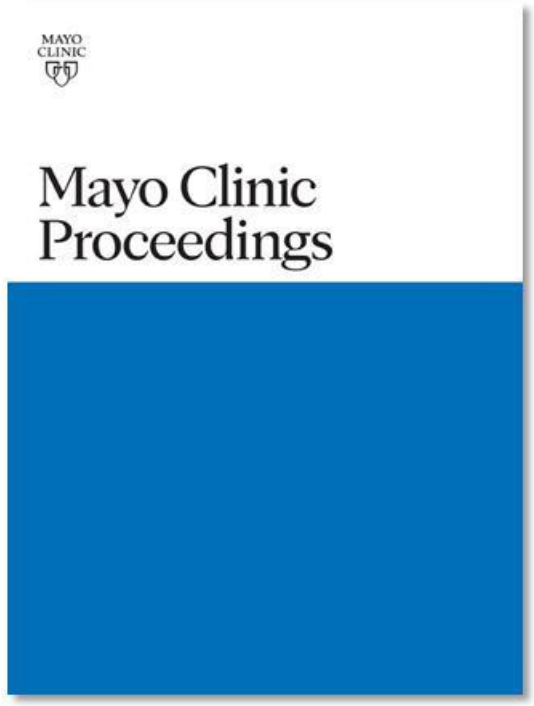


J Clin Gastroenterol. 2017;51:160-6.

Metabolic Risk Factors



A strong association between NAFLD and each of the risk factors associated with **metabolic syndrome**, especially **obesity**, **T2DM**, and **dyslipidemia** has been demonstrated



Ludwig J, Viggiano TR, McGill DB, et al.

Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease.

Mayo Clin Proc 1980;55:434–438.

Presence of **steatosis in >5% of hepatocytes** in the **absence of significant ongoing or recent alcohol consumption** and other known causes of liver disease

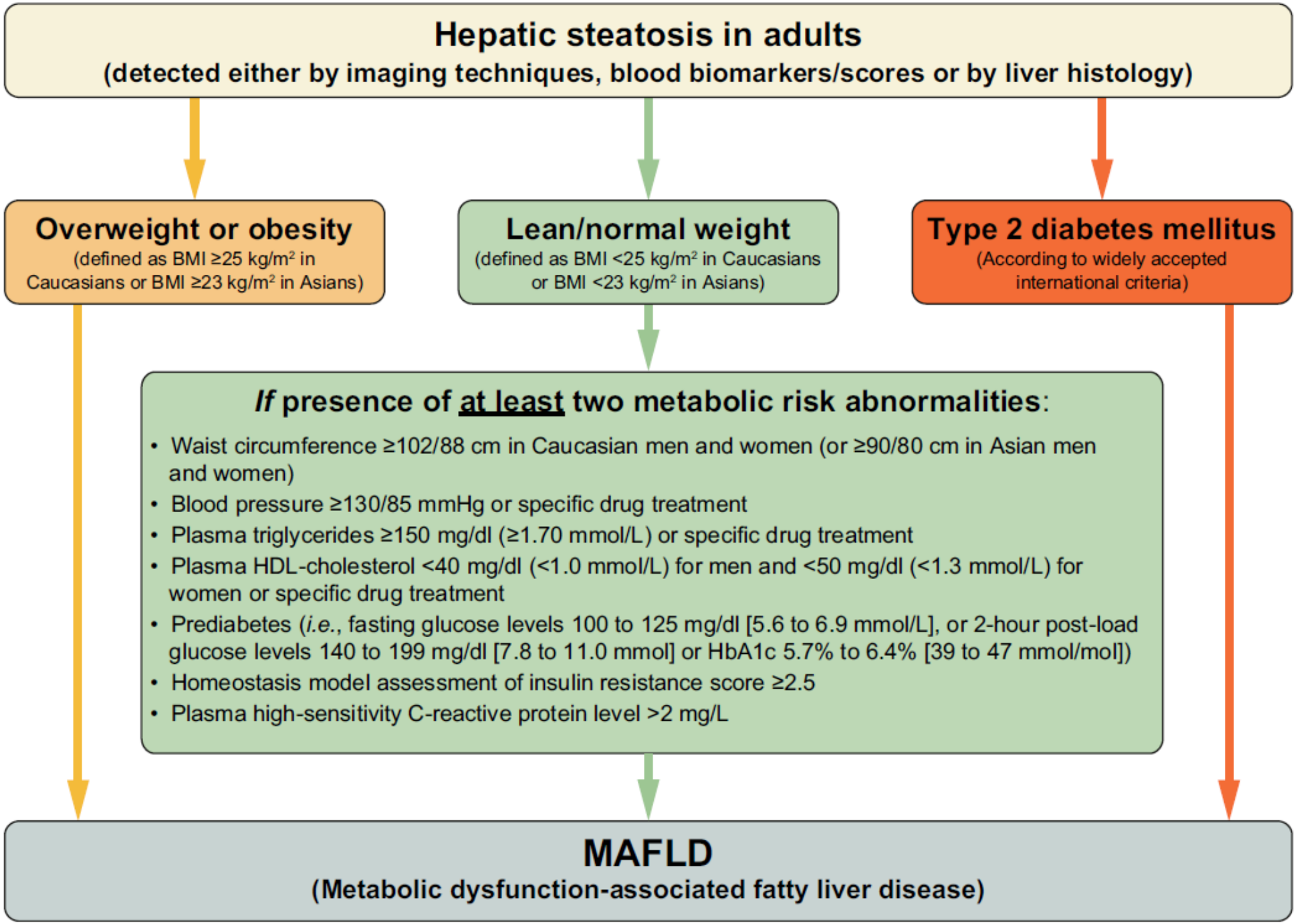
Expert Opinion

JOURNAL OF HEPATOLOGY

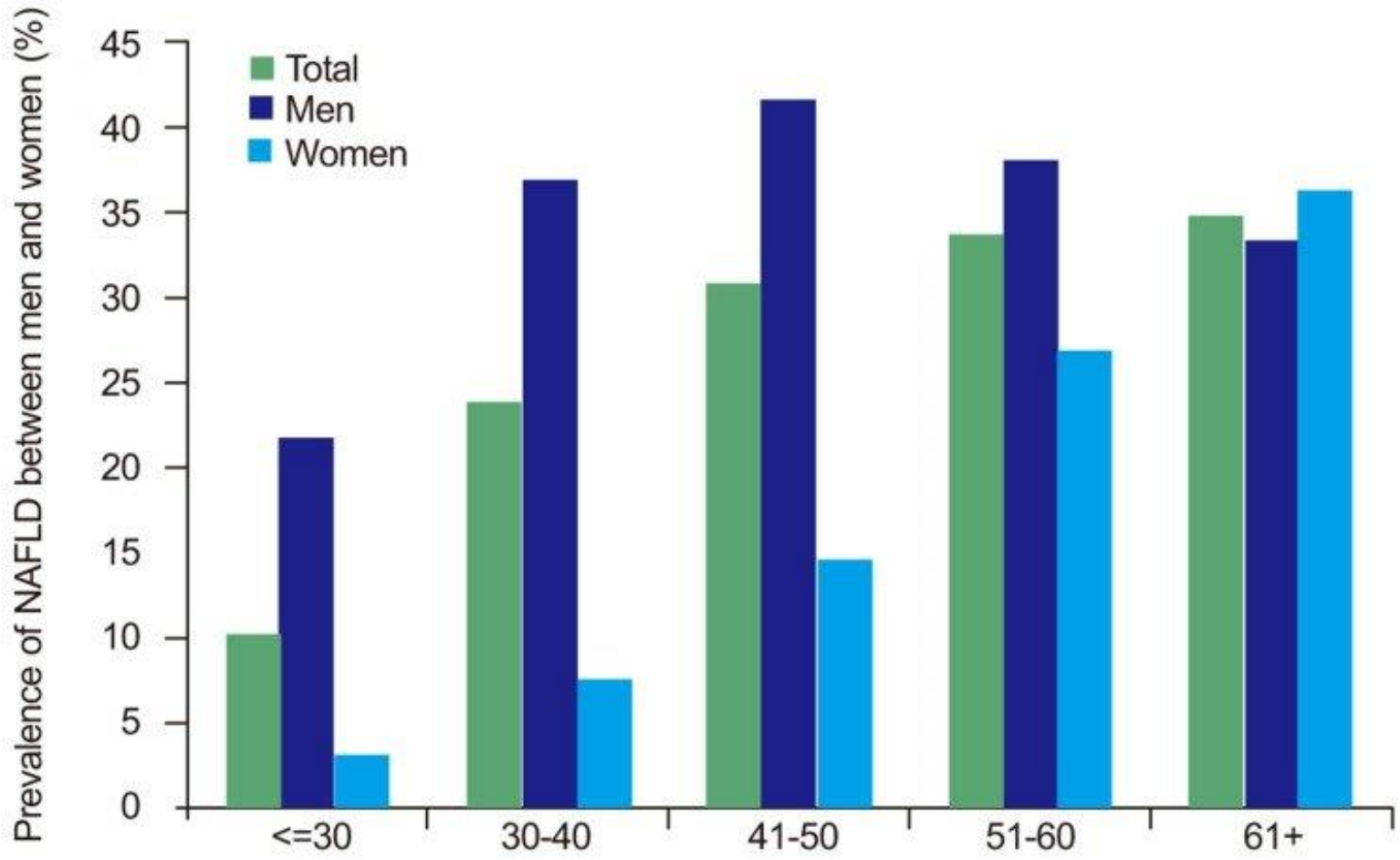
A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement

Mohammed Eslam[1,*,†], Philip N. Newsome[2,*,†], Shiv K. Sarin[3], Quentin M. Anstee[4], Giovanni Targher[5], Manuel Romero-Gomez[6], Shira Zelber-Sagi[7], Vincent Wai-Sun Wong[8], Jean-François Dufour[9], Jörn M. Schattenberg[10], Takumi Kawaguchi[11], Marco Arrese[12], Luca Valenti[13], Gamal Shiha[14], Claudio Tiribelli[15], Hannele Yki-Järvinen[16], Jian-Gao Fan[17], Henning Grønbæk[18], Yusuf Yilmaz[19], Helena Cortez-Pinto[20], Claudia P. Oliveira[21], Pierre Bedossa[22], Leon A. Adams[23], Ming-Hua Zheng[24], Yasser Fouad[25], Wah-Kheong Chan[26], Nahum Mendez-Sanchez[27], Sang Hoon Ahn[28], Laurent Castera[29], Elisabetta Bugianesi[30], Vlad Ratziu[31,*,‡], Jacob George[1,*,‡]

J Hepatol. 2020 Jul;73(1):202-209


Hepatic steatosis in adults
(detected either by imaging techniques, blood biomarkers/scores or by liver histology)
Overweight or obesity
(defined as BMI ≥25 kg/m² in Caucasians or BMI ≥23 kg/m² in Asians)
Lean/normal weight
(defined as BMI <25 kg/m² in Caucasians or BMI <23 kg/m² in Asians)
Type 2 diabetes mellitus
(According to widely accepted international criteria)
If presence of at least two metabolic risk abnormalities:
• Waist circumference ≥102/88 cm in Caucasian men and women (or ≥90/80 cm in Asian men and women)
• Blood pressure ≥130/85 mmHg or specific drug treatment
• Plasma triglycerides ≥150 mg/dl (≥1.70 mmol/L) or specific drug treatment
• Plasma HDL-cholesterol <40 mg/dl (<1.0 mmol/L) for men and <50 mg/dl (<1.3 mmol/L) for women or specific drug treatment
• Prediabetes (i.e., fasting glucose levels 100 to 125 mg/dl [5.6 to 6.9 mmol/L], or 2-hour post-load glucose levels 140 to 199 mg/dl [7.8 to 11.0 mmol] or HbA1c 5.7% to 6.4% [39 to 47 mmol/mol])
• Homeostasis model assessment of insulin resistance score ≥2.5
• Plasma high-sensitivity C-reactive protein level >2 mg/L
MAFLD
(Metabolic dysfunction-associated fatty liver disease)

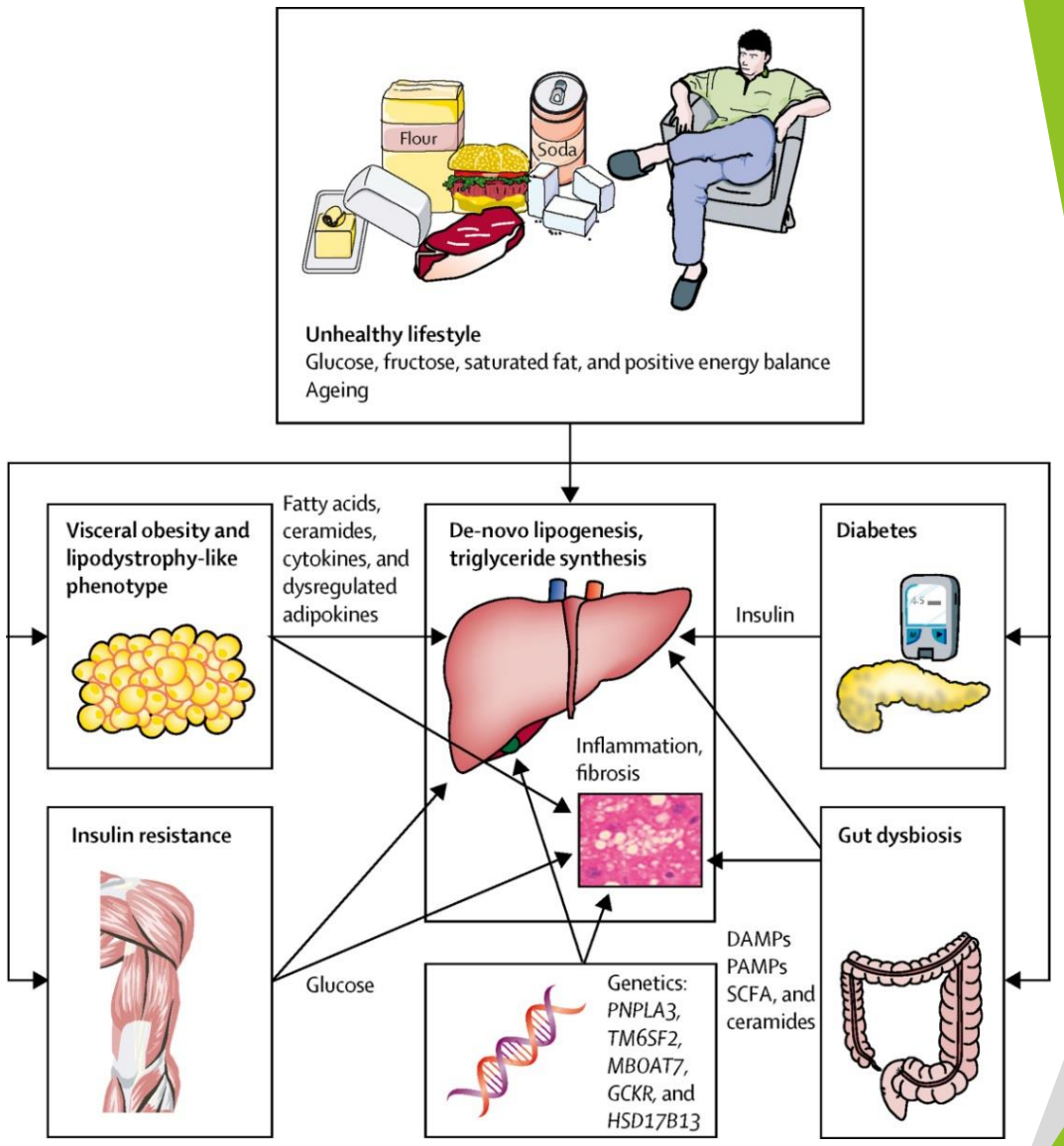
Age, sex and NAFLD

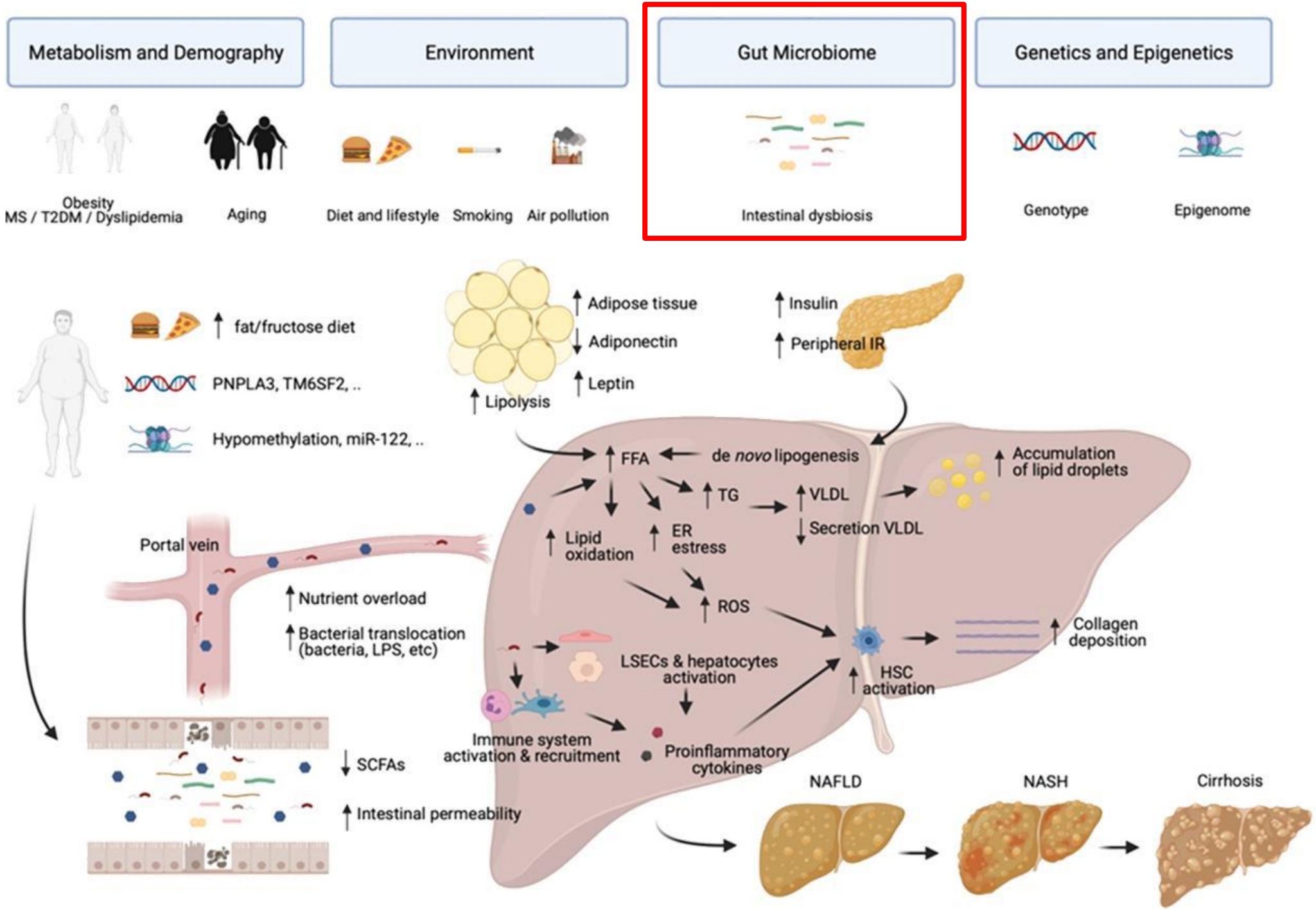


NAFLD and aging are known to be strongly correlated, with increasing age being one of the **strongest epidemiological factors for NAFLD, NASH, and fibrosis**

Lifestyle habit and NAFLD

- Increased options of food source including grocery stores, restaurants, and fast-food places
- Increased consumption of low-nutrient, high-sodium, and high -fat foods, especially high-fat diets derived from meat and lower amounts of fresh fruits
- Increased consumption of carbohydrates, animal proteins, refined sugars, and additives
- Rise in knowledge-based jobs and decrease in the physical activity




Metabolism and Demography
Environment
Gut Microbiome
Genetics and Epigenetics
Obesity
MS / T2DM / Dyslipidemia
Aging
Diet and lifestyle
Smoking
Air pollution
Intestinal dysbiosis
Genotype
Epigenome
fat/fructose diet
PNPLA3, TM6SF2, ..
Hypomethylation, miR-122, ..
Adipose tissue
Adiponectin
Leptin
Lipolysis
Insulin
Peripheral IR
FFA
de novo lipogenesis
TG
VLDL
Secretion VLDL
Accumulation of lipid droplets
Lipid oxidation
ER estress
ROS
Portal vein
Nutrient overload
Bacterial translocation (bacteria, LPS, etc)
LSECs & hepatocytes activation
HSC activation
Collagen deposition
Immune system activation & recruitment
Proinflammatory cytokines
SCFAs
Intestinal permeability
NAFLD
NASH
Cirrhosis

Gut Microbiome Composition

The human gut microbiome is made up of 10–100 trillion microorganisms, mainly bacteria, with this number about ten times higher than the number of eukaryotic human cells, susceptible to environmental and pathophysiological alterations

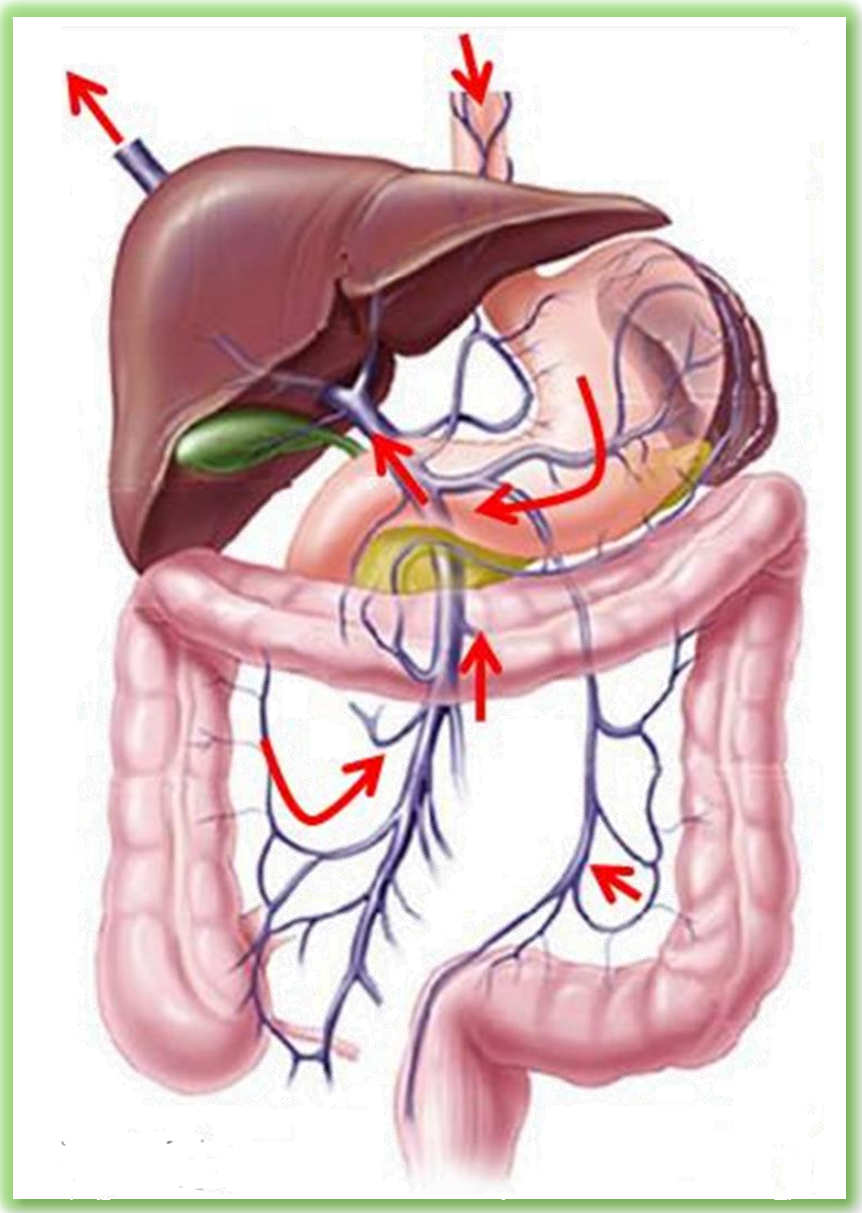
In physiological conditions, the most common bacterial phyla are ***Bacteroidetes*** and ***Firmicutes***, while the predominant prokaryotic microorganisms are *Euryarchaeota*

The gut-liver axis

- Commensal mutualistic bacteria inhabit gut together with low concentration of pathogens

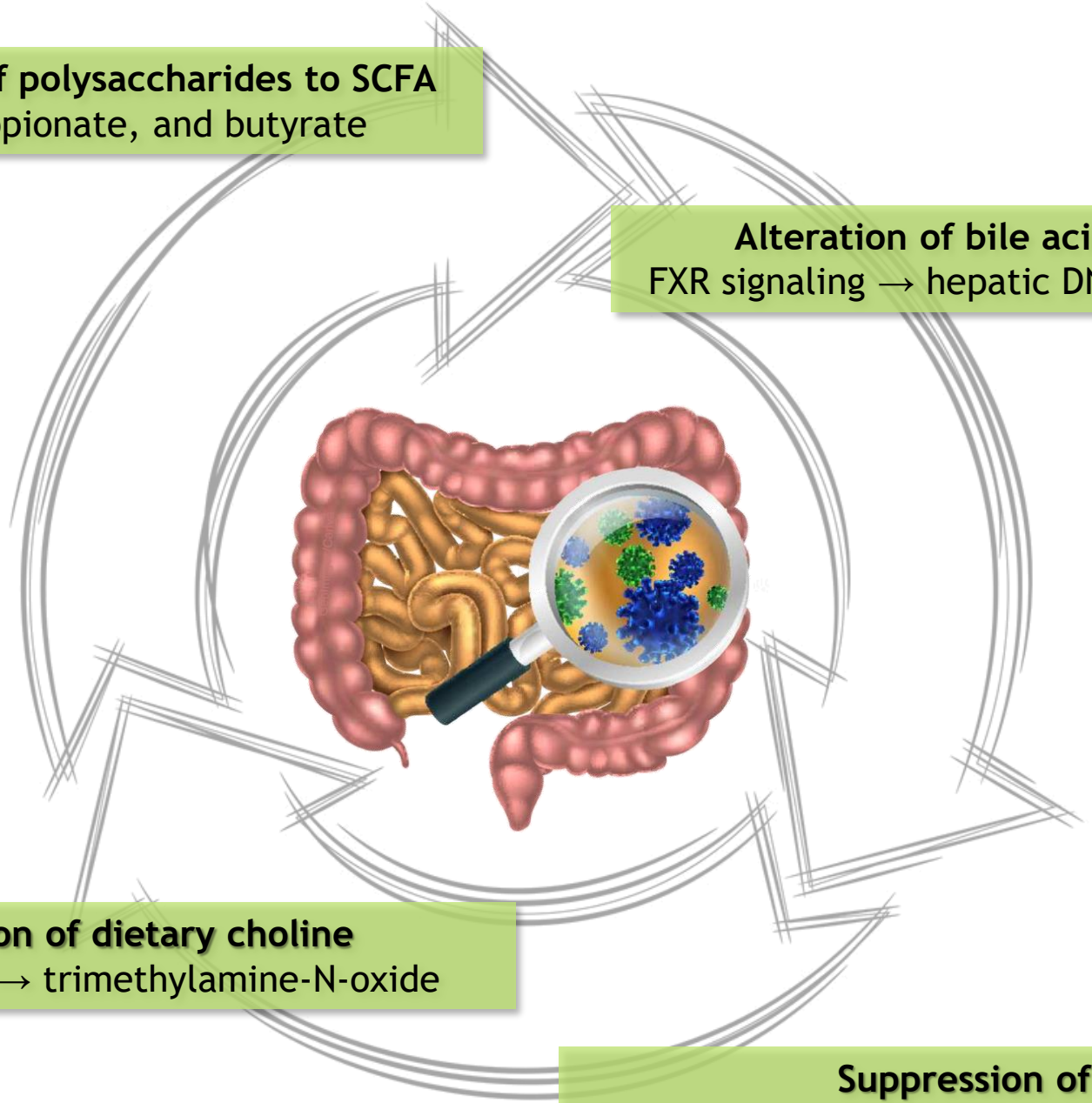
- 70% of liver blood supply is the direct venous outflow of the intestine

- Liver is chronically exposed to gut-derived bacteria and bacterial components



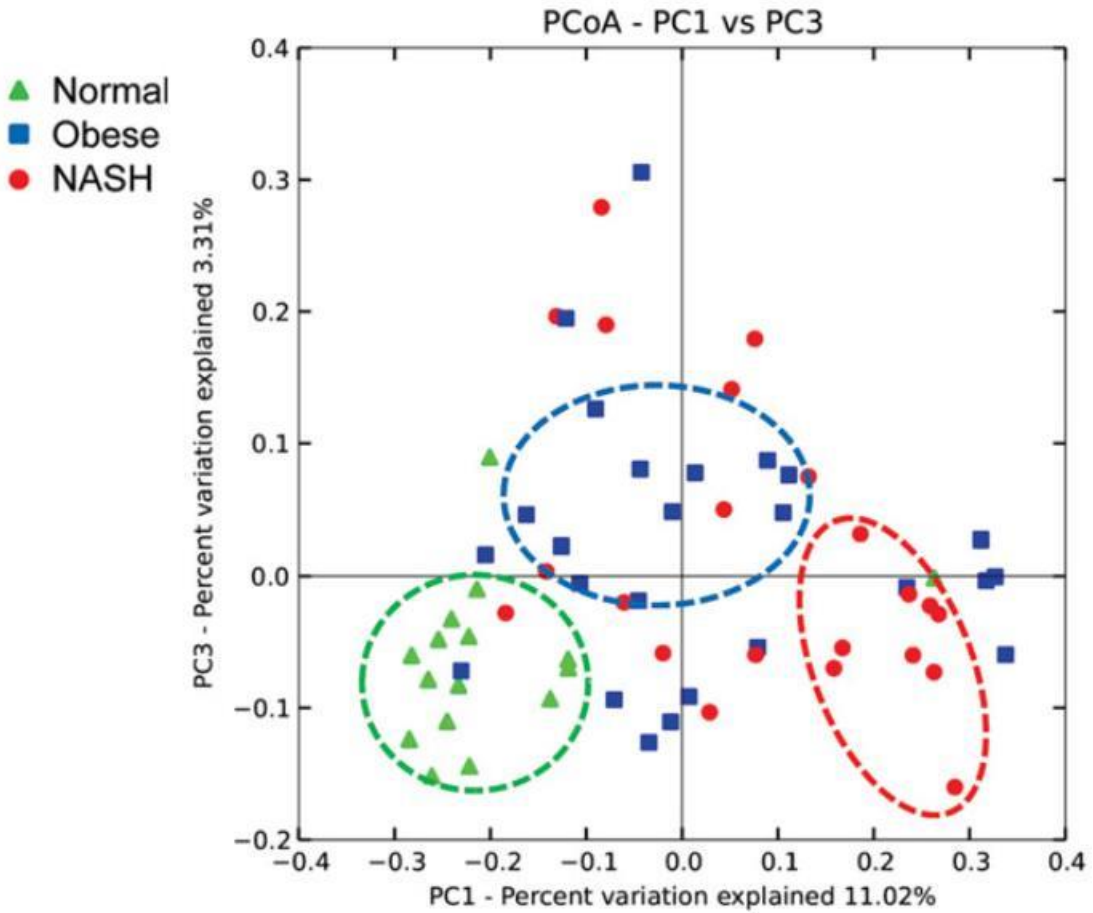
Fermentation of polysaccharides to SCFA
acetate, propionate, and butyrate

Alteration of bile acid metabolism
FXR signaling → hepatic DNL and VLDL export

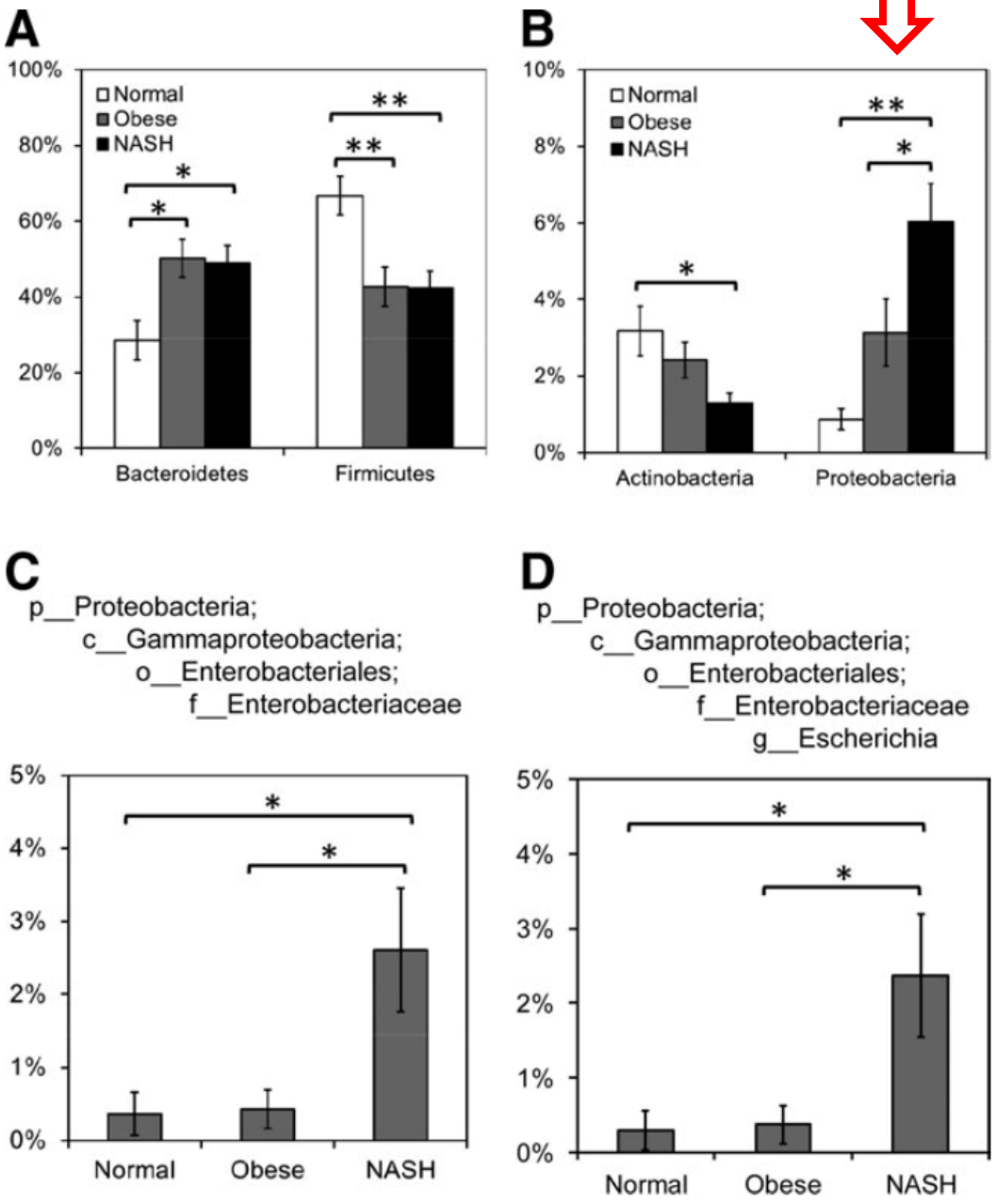


Conversion of dietary choline
Methylamines → trimethylamine-N-oxide

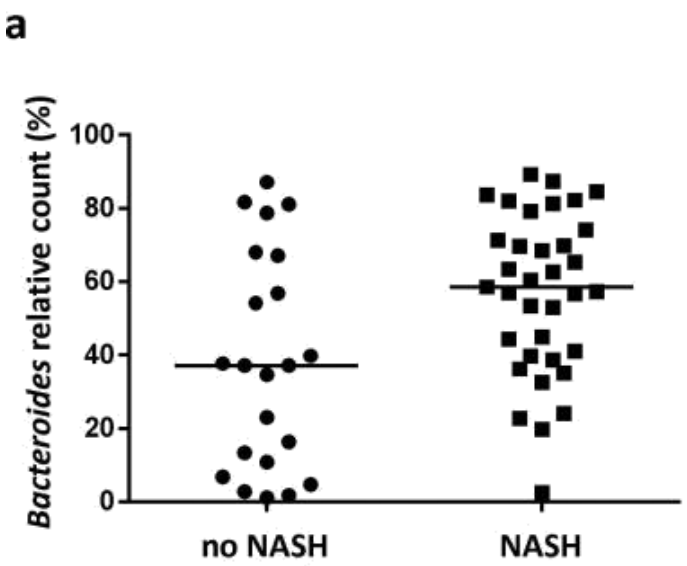
Suppression of Fiaf
↑ lipoprotein lipase activity and TG accumulation

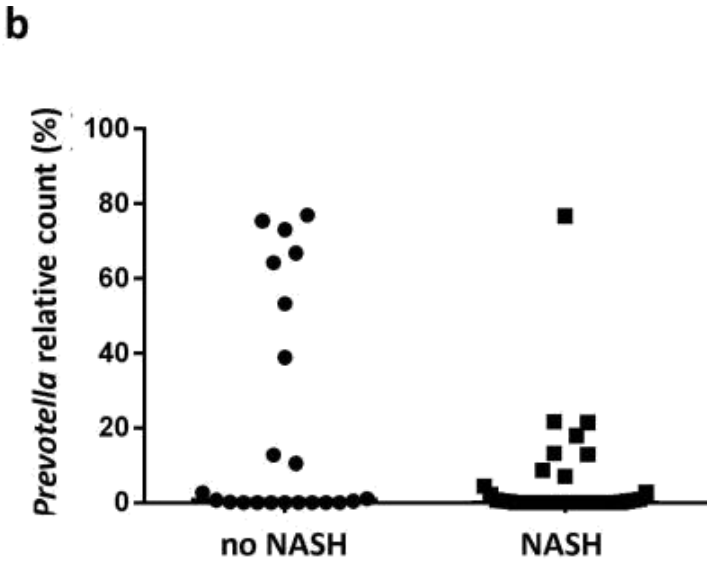


The **increased abundance of alcohol-producing bacteria in NASH microbiomes** and the role of alcohol metabolism in oxidative stress and liver inflammation suggest a **role for alcohol-producing microbiota in the pathogenesis of NASH**

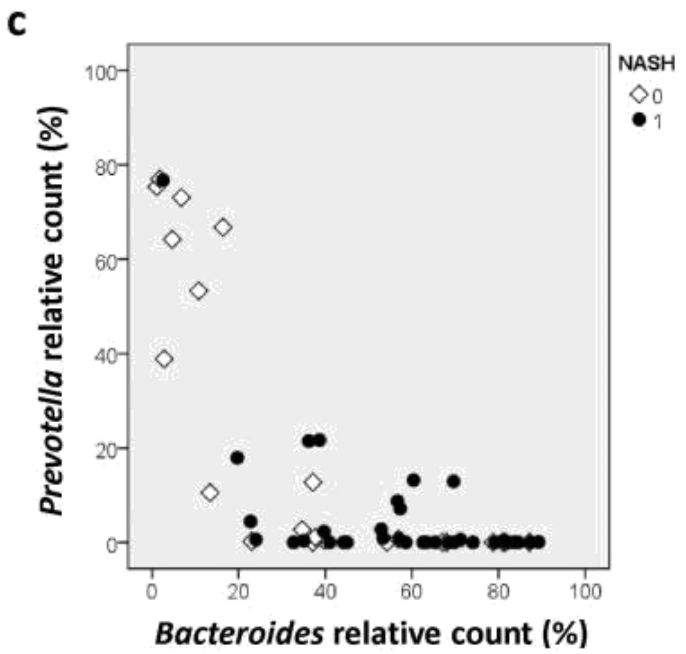


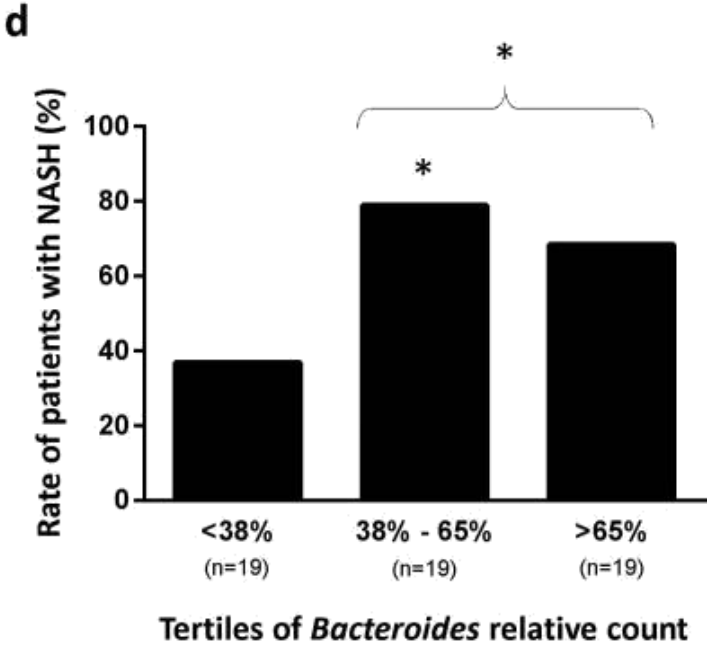
Zhu L, et al. Hepatology. 2013 Feb; 57(2):601-9.





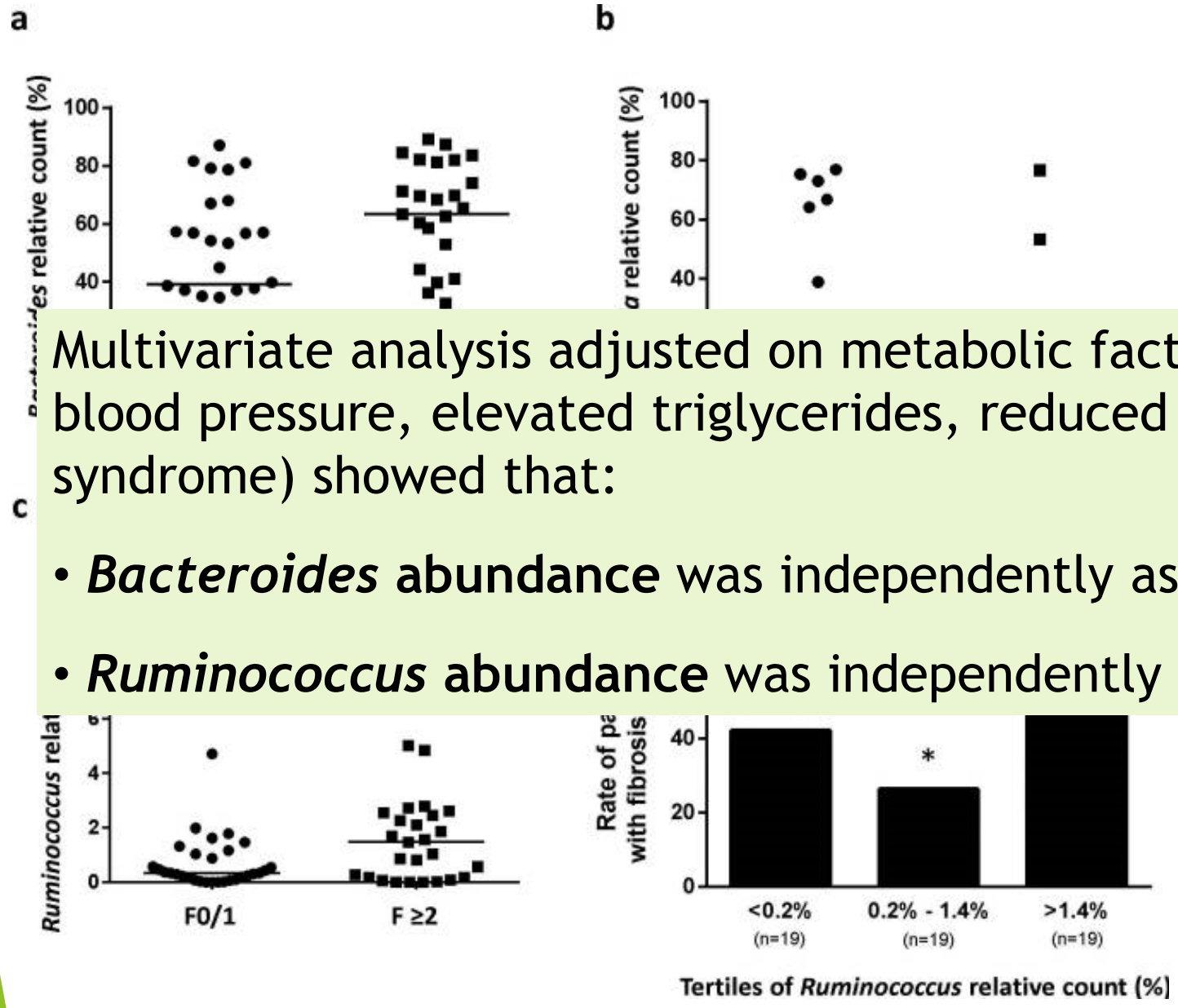
Patients with NASH had higher abundance of *Bacteroides* and lower abundance of *Prevotella*





Patients in the *Bacteroides* count tertiles 2 and 3 had a 2-fold increase in NASH compared to those in the first tertile (74% vs 37%, p=0.010)

Boursier J, et al. Hepatology. 2016 Mar; 63(3):764-75.



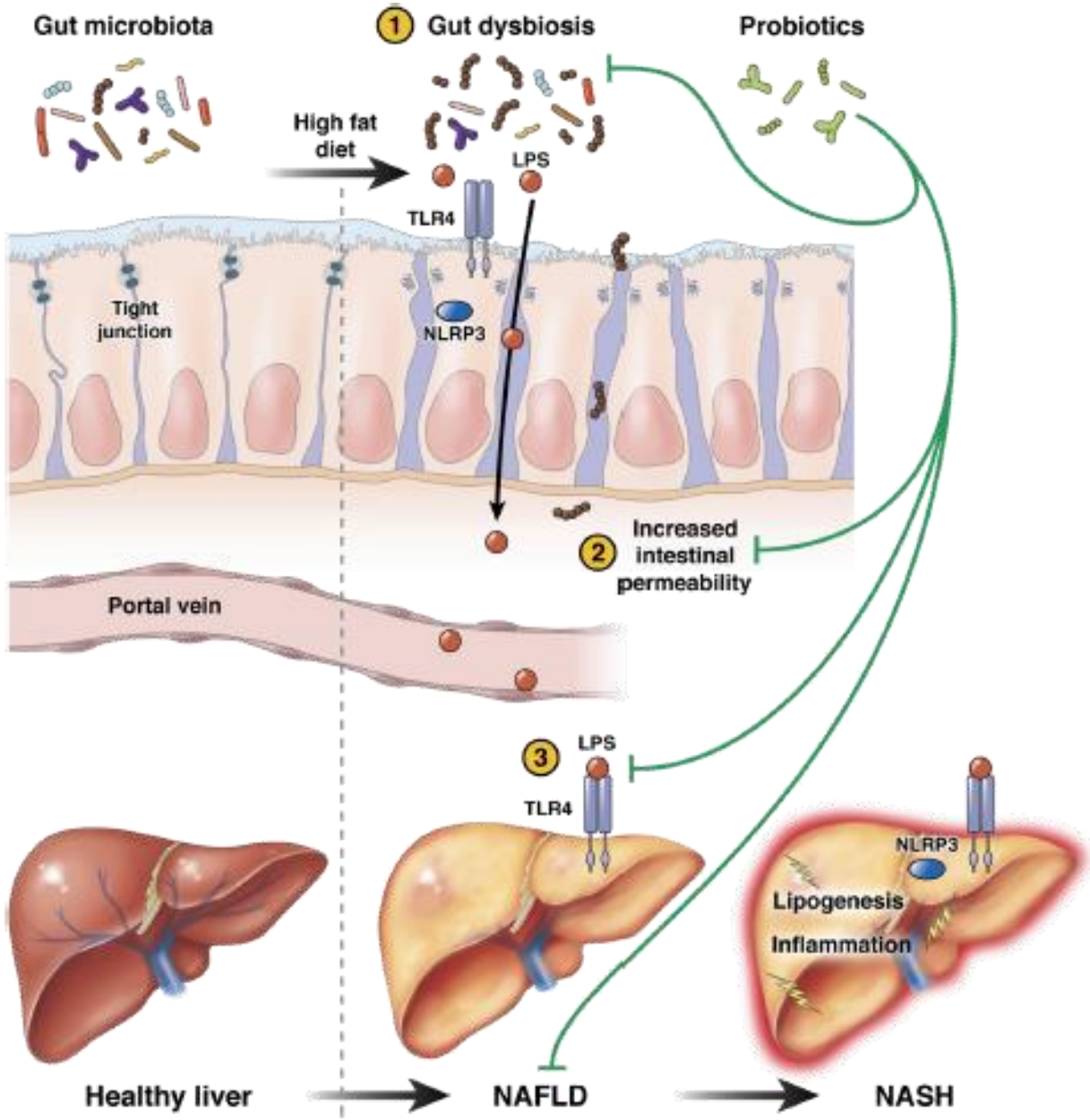
Bacteroides, *Prevotella*, and *Ruminococcus* genera significantly differed between patients with F0/1 fibrosis and those with significant F≥2

Multivariate analysis adjusted on metabolic factors (BMI, diabetes, elevated blood pressure, elevated triglycerides, reduced HDL-cholesterol, metabolic syndrome) showed that:

- ***Bacteroides* abundance** was independently associated with **NASH**
- ***Ruminococcus* abundance** was independently associated with **fibrosis F≥2**

abundancesof *Bacteroides* and *Ruminococcus*, and lower abundance of *Prevotella*

Boursier J, et al. Hepatology. 2016 Mar; 63(3):764-75.

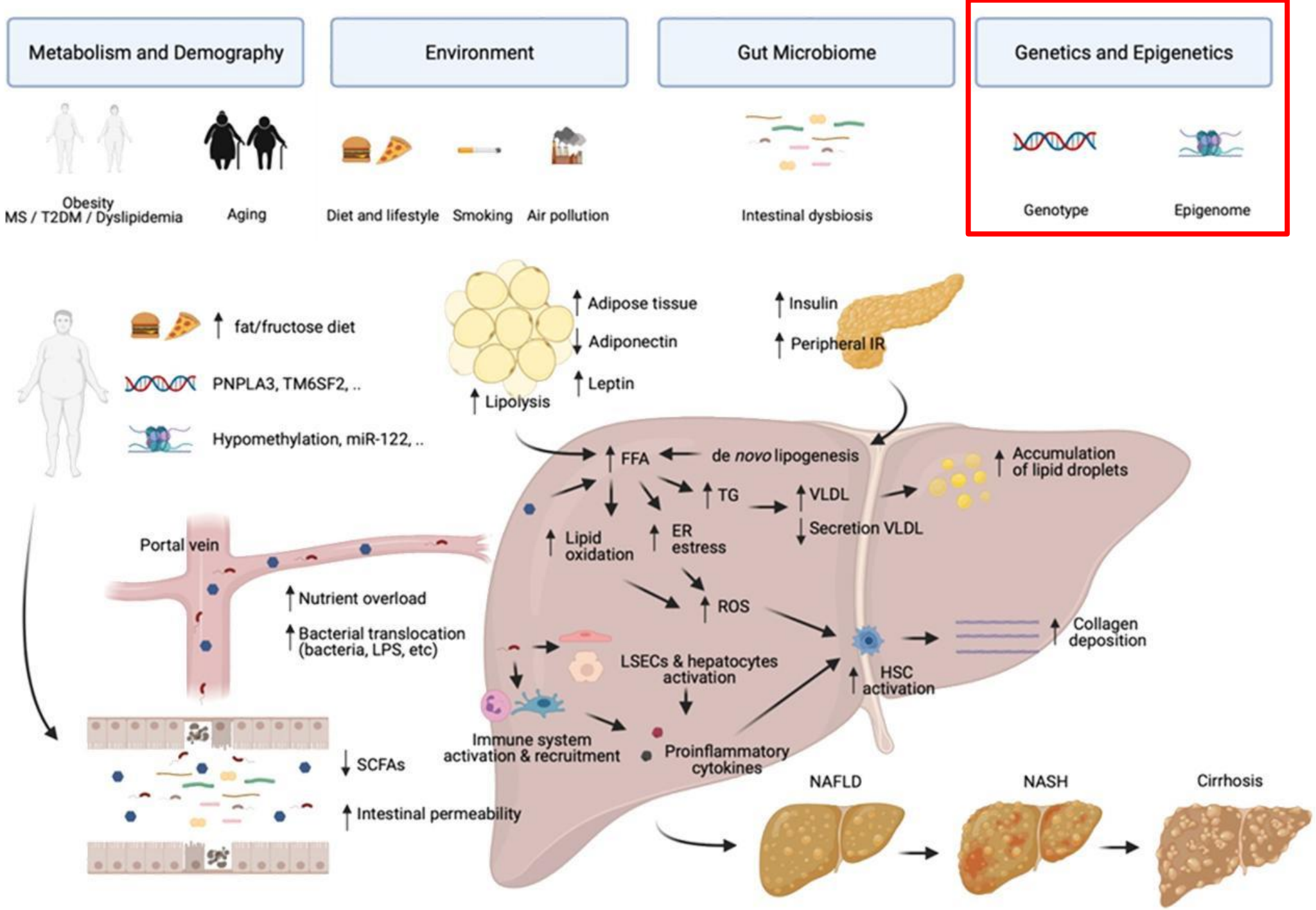


Gastroenterology. 2020 May;158(7):1881-1898

There may be correlations between intestinal bacterial composition and NAFLD or NASH

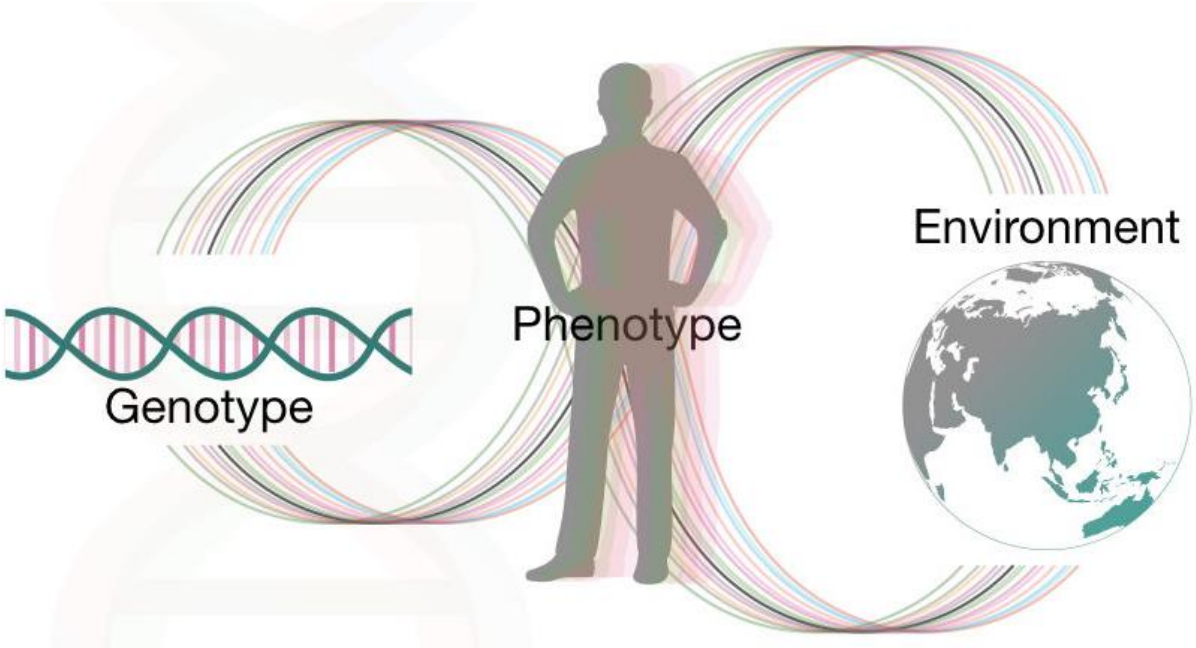
However, these observations are limited by the **lack of reproducibility of the studies**

Furthermore, in most studies, the **microbiome is sampled from stool samples**, being the bacterial composition different from the communities present in the most proximal areas of the intestine


Metabolism and Demography
Environment
Gut Microbiome
Genetics and Epigenetics
Obesity
MS / T2DM / Dyslipidemia
Aging
Diet and lifestyle
Smoking
Air pollution
Intestinal dysbiosis
Genotype
Epigenome
fat/fructose diet
PNPLA3, TM6SF2, ..
Hypomethylation, miR-122, ..
Adipose tissue
Adiponectin
Leptin
Lipolysis
Insulin
Peripheral IR
FFA
de novo lipogenesis
TG
VLDL
Secretion VLDL
Accumulation of lipid droplets
Lipid oxidation
ER estress
ROS
Portal vein
Nutrient overload
Bacterial translocation (bacteria, LPS, etc)
LSECs & hepatocytes activation
HSC activation
Collagen deposition
Immune system activation & recruitment
Proinflammatory cytokines
SCFAs
Intestinal permeability
NAFLD
NASH
Cirrhosis

Genetic factors

The interaction between the genetic status and the environmental factors may, at least in part, explain inter-individual variability observed in the manifestation of the phenotype and severity of NAFLD



Clinical studies using family members demonstrate that **first-degree relatives are at higher risk for NAFLD**, suggesting a genetic predisposition to the disease

Heritability of Nonalcoholic Fatty Liver Disease

JEFFREY B. SCHWIMMER,[*,‡] MANUEL A. CELEDON,[*] JOEL E. LAVINE,[*,‡] RANY SALEM,[§] NZALI CAMPBELL,[§] NICHOLAS J. SCHORK,[§] MASOUD SHIEHMORTEZA,[‖] TAKESHI YOKOO,[‖] ALYSSA CHAVEZ,[‖] MICHAEL S. MIDDLETON,[‖] and CLAUDE B. SIRLIN[‖]

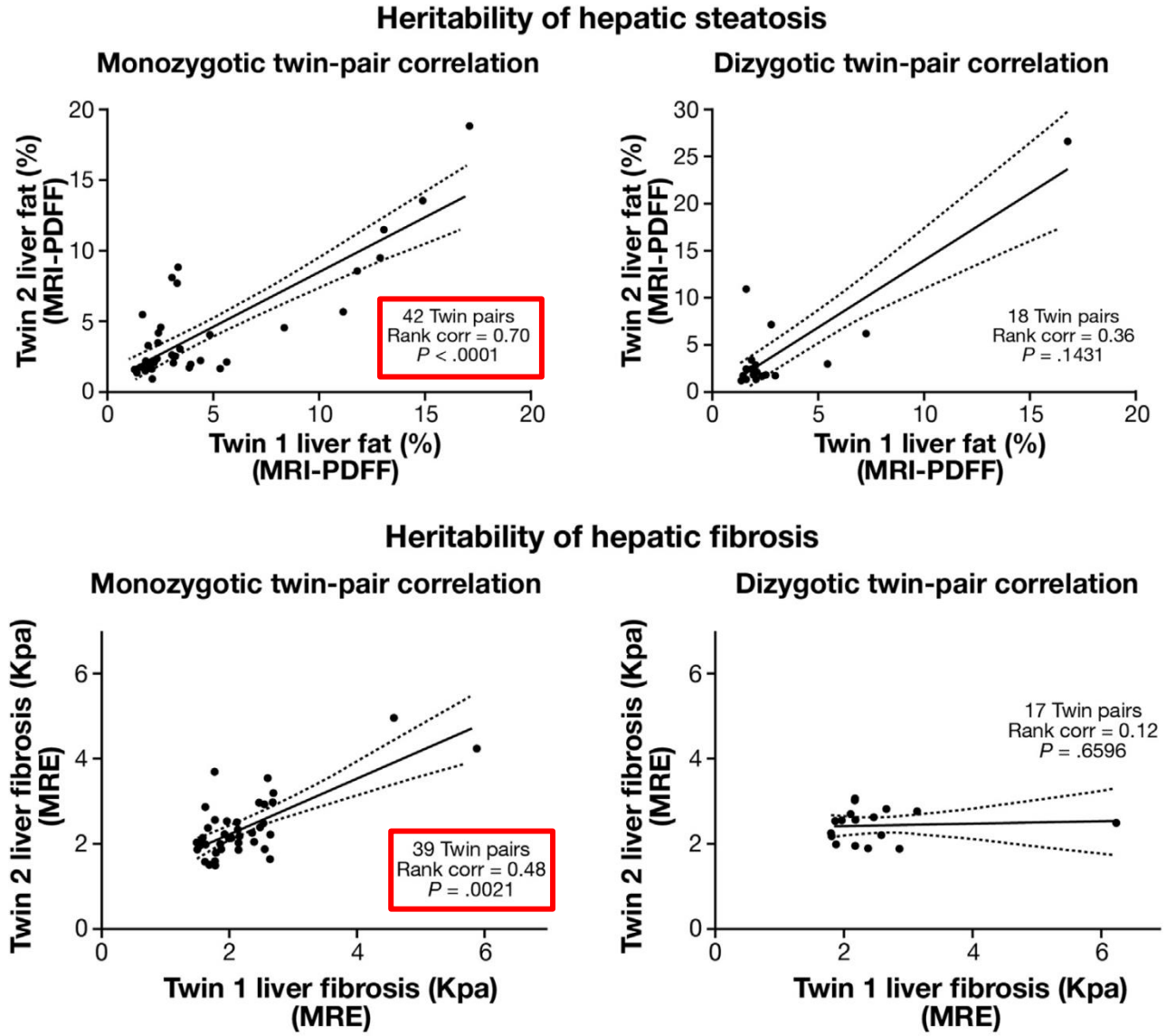
GASTROENTEROLOGY 2009;136:1585–1592

33 overweight children with biopsy-proven NAFLD and 11 children without NAFLD matched for age and BMI
152 nonproband family members

Characteristic	Probands		Siblings		Parents	
	Normal (N = 11)	NAFLD (N = 33)	Normal proband (N = 12)	NAFLD proband (N = 29)	Normal proband (N = 19)	NAFLD proband (N = 55)
Mean age (SD), *y*	13.4 (4.3)	13.5 (2.7)	17.4 (6.5)	18.3 (6.0)	43.1 (5.2)	42.9 (10.1)
Sex, N (%)						
Male	6 (55)	22 (67)	5 (42)	15 (52)	8 (42)	23 (42)
Female	5 (45)	11 (33)	7 (58)	14 (48)	11 (58)	32 (58)
Mean weight (SD), *kg*	82.9 (19.0)	83.2 (24.7)	65.6 (12.4)	75.5 (22.4)	83.7 (16.9)	87.3 (20.2)
Mean height (SD), *cm*	160.4 (17)	159.3 (15)	158.9 (10)	162.1 (14)	167.5 (11)	162.7 (10)
Mean BMI (SD), kg/m^2	32.0 (5.4)	32.1 (5.8)	26.0 (4.5)	28.0 (5.7)	30.0 (6.3)	32.9 (6.6)
Obese, N (%)	9 (82)	27 (82)	6 (50%)	13 (45)	10 (53)	32 (58)
Mean ALT level (SD), *U/L*	26 (14)	71 (38)[a]	30 (24)	51 (59)	39 (37)	40 (28)
Mean AST level (SD), *U/L*	34 (16)	41 (21)	30 (14)	37 (23)	28 (13)	31 (15)
Mean HDL cholesterol level (SD), *mg/dL*	48 (20)	40 (9)	45 (8)	42 (10)	44 (13)	44 (11)
Mean triglyceride level (SD), *mg/dL*	122 (95)	138 (105)	101 (48)	114 (79)	103 (67)	152 (113)[b]
Mean glucose level (SD), *mg/dL*	90 (7)	96 (20)	93 (12)	92 (13)	100 (23)	102 (37)
Mean liver fat fraction (SD), *%*	1.6 (1.4)	18.1 (8.7)[a]	2.7 (5.9)	9.3 (7.6)[c]	7 (8.3)	14.0 (10.0)[c]
Liver fat fraction >5%, N (%)	0 (0)	33 (100)[a]	2 (17)	17 (59)[b]	7 (37)	43 (78)[c]

Adjusted for age, sex, race, and BMI, the heritability of fatty liver was 1.000 and of liver fat fraction was 0.386

Cross sectional analysis of a cohort of 60 pairs of twins (42 monozygotic and 18 dizygotic; average age, 45.7 ± 22.1 y; average body mass index, 26.4 ± 5.7 kg/m2) residing in Southern California



In multivariable models adjusted for age, sex, and ethnicity, the heritability of hepatic steatosis (based on MRI-PDFF) was 0.52 and the heritability of hepatic fibrosis (based on liver stiffness) was 0.5

Both hepatic steatosis and hepatic fibrosis in NAFLD are heritable

Both hepatic steatosis and hepatic fibrosis are highly correlated in MZ twins but not in DZ twins

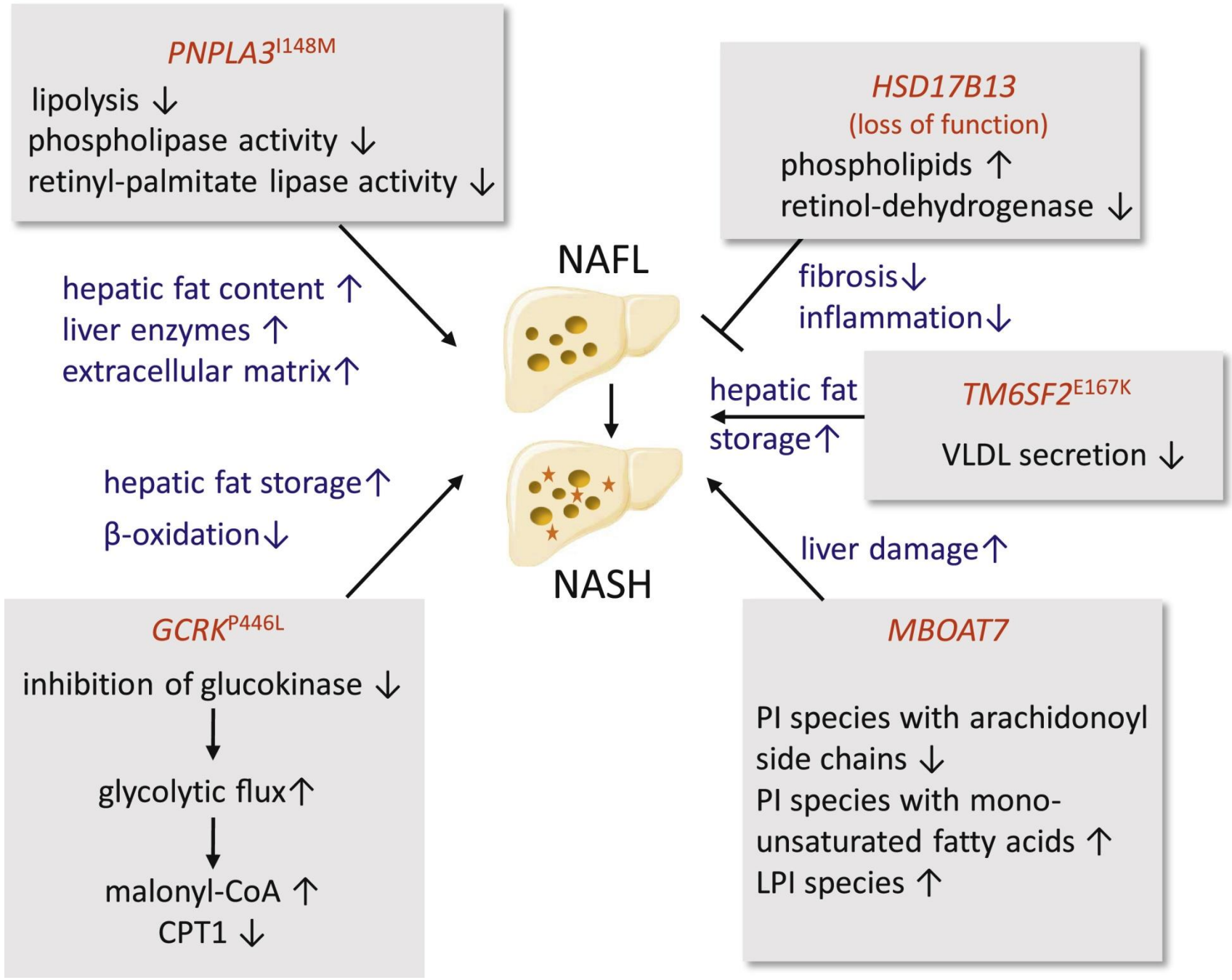
Loomba R et al. Gastroenterology 2015;149:1784-1793

In 2008, a GWAS by Romeo et al. firstly identified the most prominent fatty liver gene patatin-like phospholipase domain containing 3 (PNPLA3), also known as adiponutrin and demonstrated the association of the SNP rs738409 (C > G) in PNPLA3 with NAFLD

Gene	Tissue expression [145,146]	Liver cell type	Function
PNPLA3	*Liver and kidney*	Hepatocytes [43] Stellate cells [43]	Lipid droplet remodeling [40,42] Modulation of retinol production and release [43,147]
GCKR	*Liver, smooth muscle, and stomach*	Hepatocytes	Increasing glycolytic flux [51], regulation of *de novo* lipogenesis [48,148]
TM6SF2	*RNA enriched in intestine and liver* [57]	Hepatocytes [60]	VLDL secretion [53,57,58]
HSD17B13	*Ubiquitous (RNA enriched in liver)* and liver [64]	Hepatocytes [149]	Lipid droplet remodeling [63,64,149], involved in retinol metabolism [64]
MBOAT7	*Ubiquitous and RNA low tissue specificity* [69]	Hepatocytes, hepatic sinusoidal cells, and stellate cells [69]	Remodeling of phosphatidylinositol [69,150]
PPP1R3B	*Ubiquitous and low tissue specificity*	Hepatocytes [79]	Hepatic glycogen storage [77,79]
IRGM	Liver [82]	Hepatocytes [82]	Modulation of lipophagy [39] via interaction with lipase ATGL [82]
LPIN1	*Ubiquitous, adipose tissue,* and liver [84,151]	Hepatocytes [152]	Regulation of fatty acid metabolism [153,154]

ATGL, adipose triglyceride lipase; VLDL, very low-density lipoproteins.

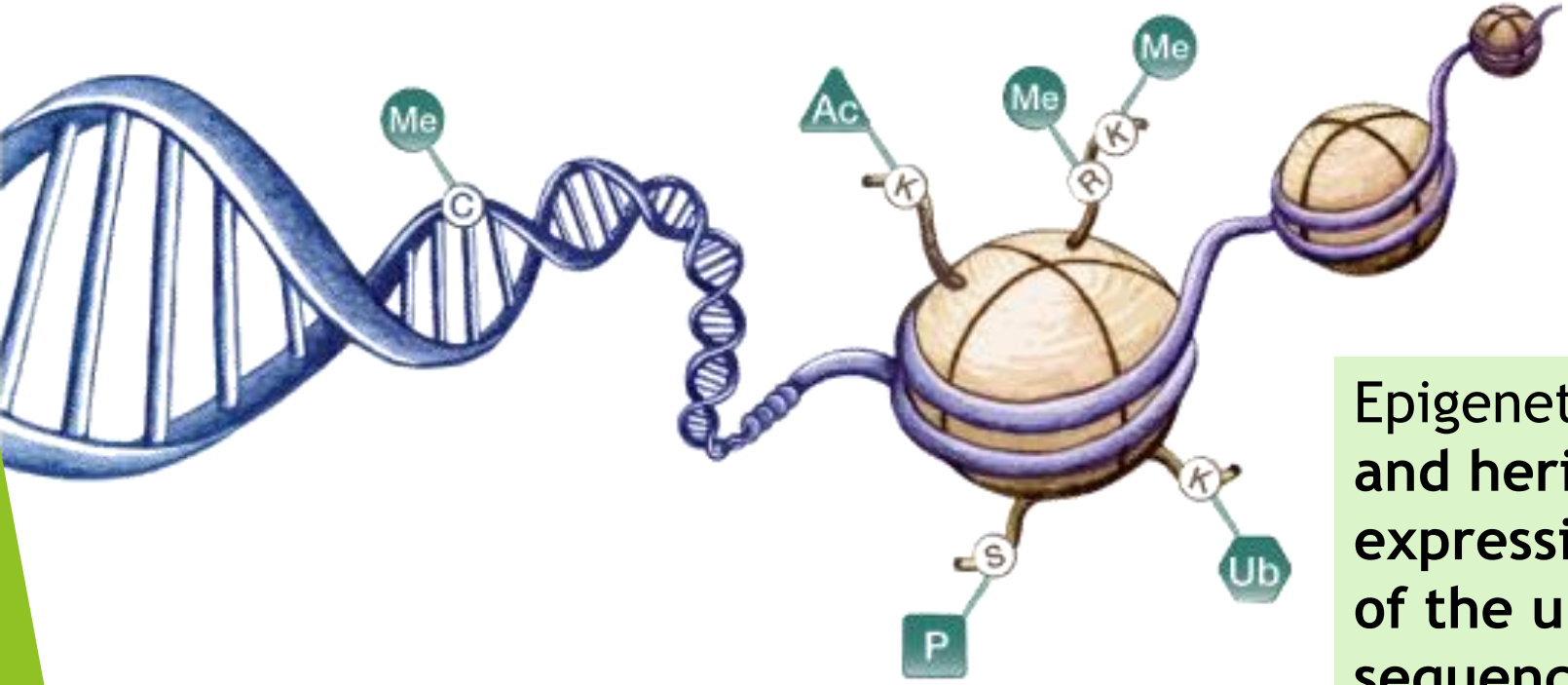
Unbiased genome-wide association studies show **strong evidence for the heritability of characteristic traits of NAFLD** and have identified gene *loci* associated with pathogenesis and progression of the disease



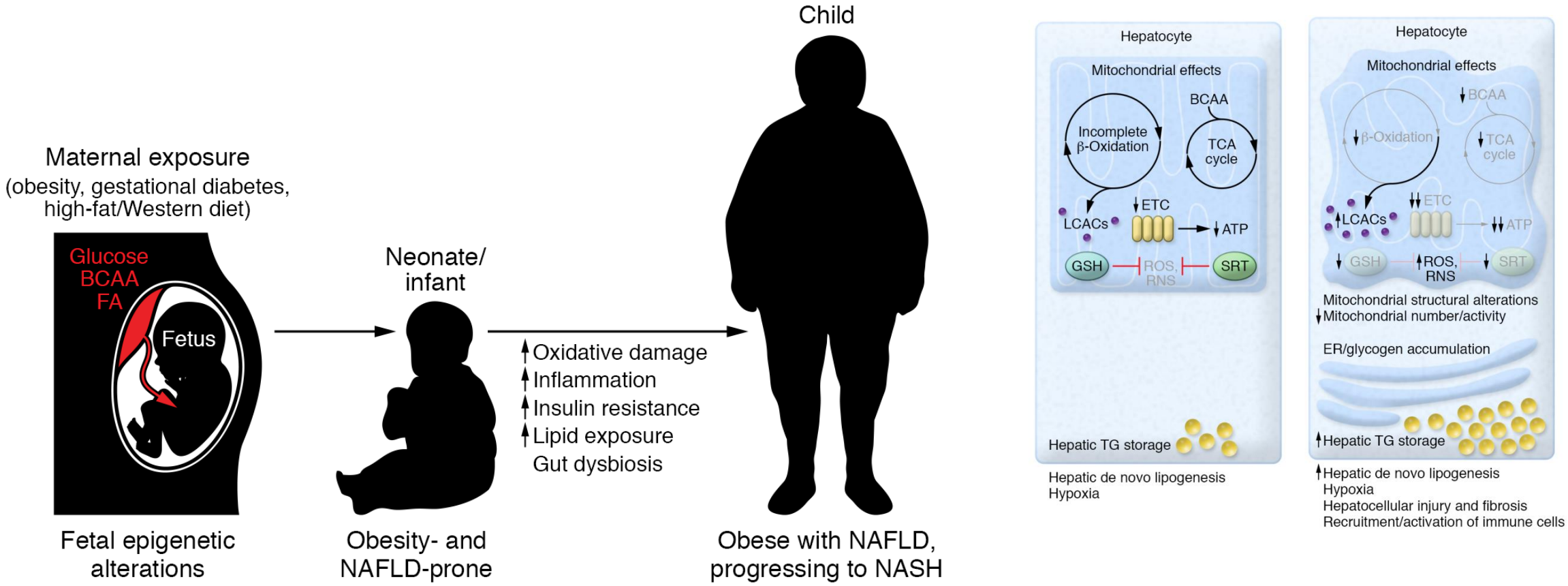
Jonas W et al. Mol Metab. 2021 Aug;50:101111

Epigenetic factors

Although the causal role of certain loci and NAFLD pathogenesis is undeniable, it is becoming clear that the rising prevalence of NAFLD cannot be explained exclusively by the contribution of environmental and genetic factors



Epigenetic, that is the **reversible and heritable change in gene expression without modification of the underlying nucleotide sequence**, can be the mechanistic bridge between genetic and environment



Maternal obesity, diabetes, or dietary habits and lifestyle lead to an “unfavorable intrauterine” environment in which hepatic mitochondrial function in fetal liver is susceptible to damage

The fetal metabolic reprogramming through epigenetic mechanisms, contributes to the lifetime risk of NAFLD, and likely to the severity and early onset of the disease in children

The amount of data available on the relationship between DNA methylation or histone modifications in NAFLD has increased rapidly over the years

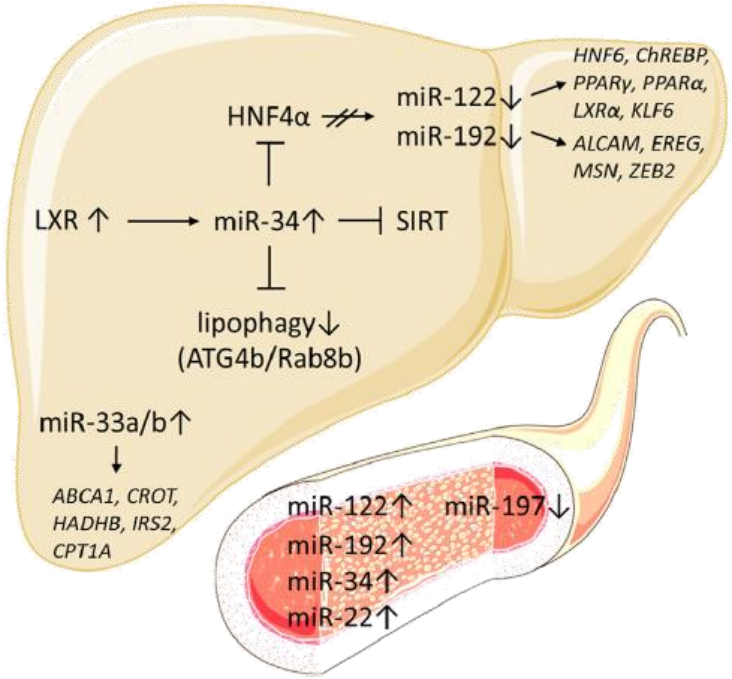
IGFBP2	Mainly liver and kidney	IGF factors transportation [116]	Hypermethylation. Reduced expression. [116]	↑ NAFLD [116]
PGC1α	Muscles, liver, adipose tissue and kidney	Energy metabolism and mitochondrial biogenesis [117]	Hypermethylation. Histone hypoacetylation. Reduced expression [117]	↑ NAFLD, NASH [117]
SIRT1	Ubiquitous	Histone deacetylase. Regulates several genes involved in metabolism control [118]	Reduced expression [119]	↑ NAFLD [119,120]

NAFLD has mainly been associated with hypomethylation, most probably caused by an imbalance in methyl donor supply although several interesting examples of hypermethylated genes with reduced expression can be found

Histone modifications are less understood in the context of fatty liver disease; nevertheless, some examples exist relating these epigenetic marks and NAFLD

miRNAs are known to regulate multiple biological pathways involved in the pathogenesis of NAFLD such as lipid uptake, *de novo* lipogenesis, lipid oxidation, and hepatic lipid export, apoptosis, cell proliferation, or fibrosis

miR-122	Ubiquitous, Liver enriched	Regulation of lipid metabolism and fibrogenesis [121–123]	Reduced expression in the liver [124]	↑ NAFLD, NASH, fibrosis, HCC [125,126]
miR-34	Ubiquitous	Regulates lipophagy. Negatively regulates *SIRT1* [127,128]	Liver overexpression [127,128]	↑ NAFLD [127,128]



At least a dozen of miRNAs have been strongly associated with NAFLD development, although the exact mechanism by which some of them contribute to the disease is still poorly understood

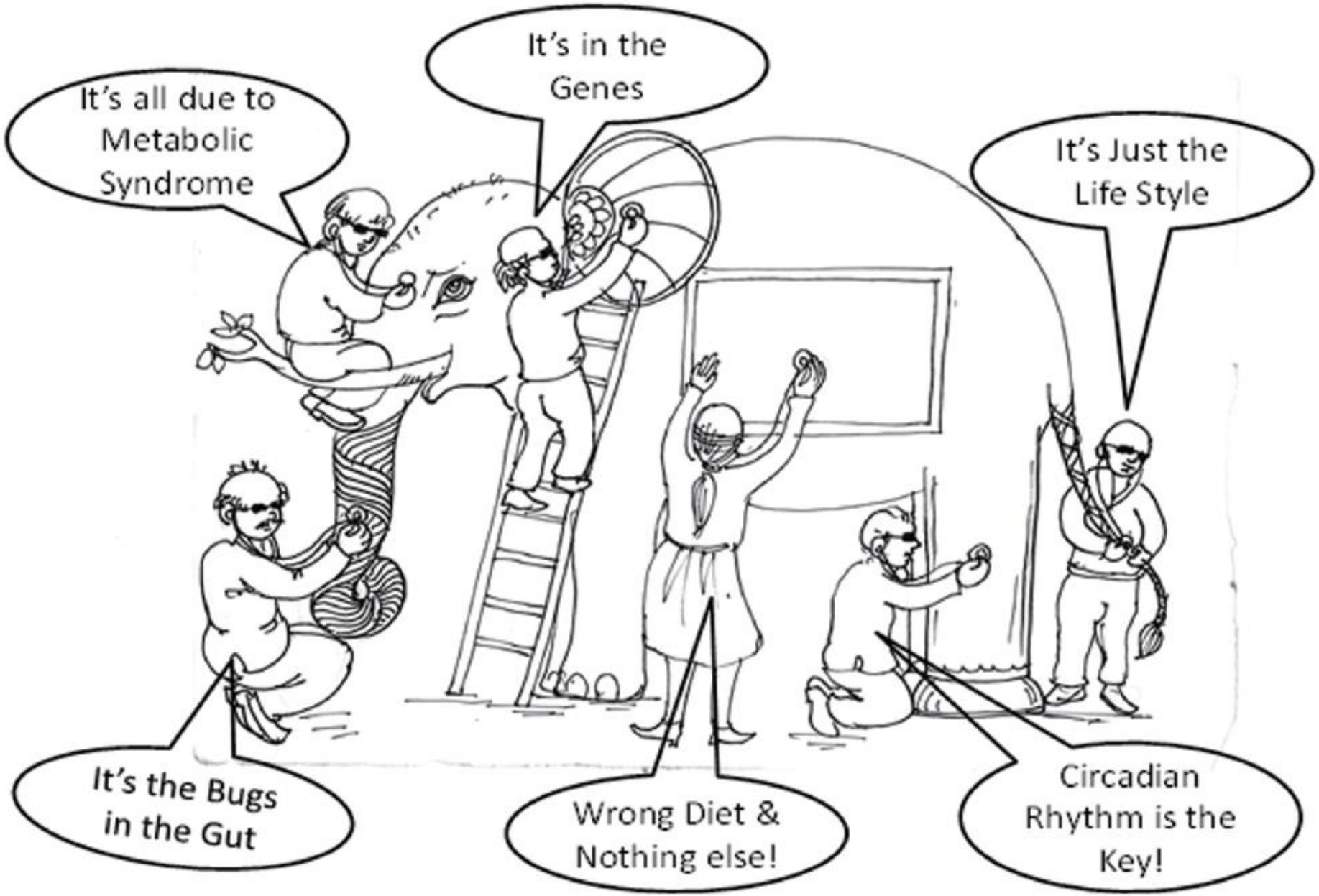
Conclusions

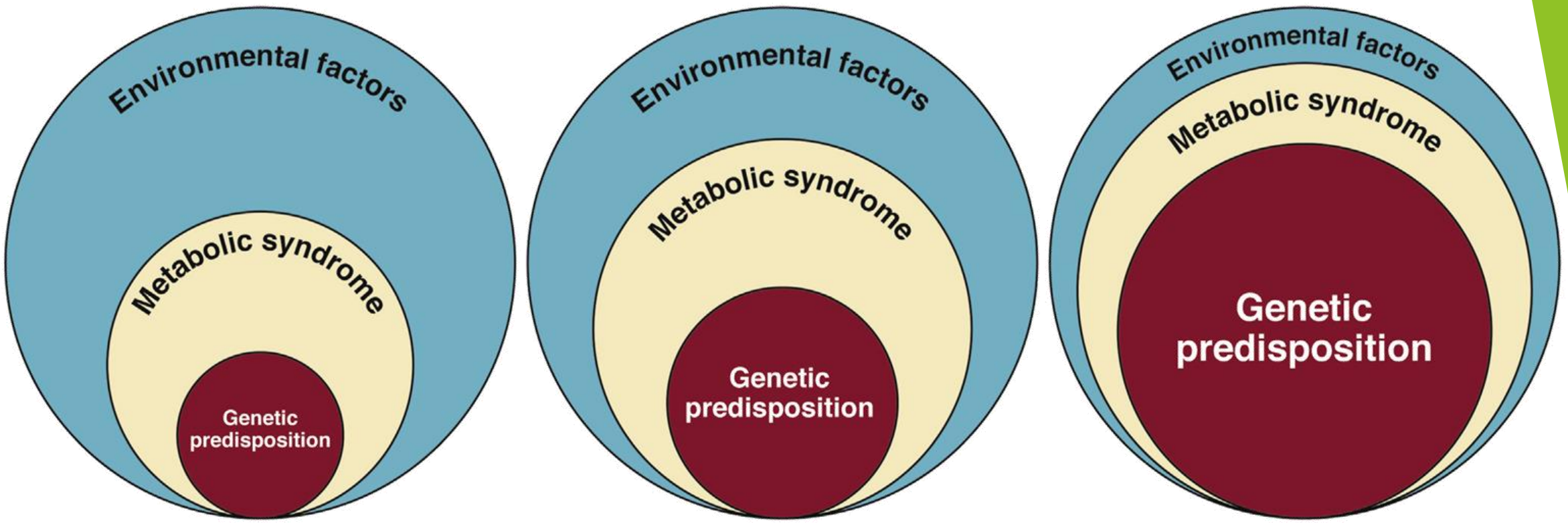
NAFLD is a complex phenotype shaped by the dynamic interaction of genetic predisposition with environmental factors and components of the metabolic syndrome

The effect size of genetic variants and the predominant drivers can exhibit marked interindividual variation

The exact contribution of each factor in the development of NAFLD is unknown and may vary by geographic location

The Six Blind Men of Indostan





The identification of the predominant drivers in every patient can help in personalization of medicine

What’s in a name? That which we call a rose by any other name would smell as sweet.

Romeo and Juliet

William Shakespeare