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Foundations of Cardiometabolic Health Certification Course

Certified Cardiometabolic Health Professional (CCHP)

NAFLD & NASH: Introduction and Disease Burden

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Professor, Harvard Medical School

Editor-in-chief, Metabolism, Clinical and Experimental

Novel Diagnostics and Therapeutics for the Unmet Clinical need NASH: from the Bedside to Bench and Back

The dawn of a new era for the epidemic of the 21st century

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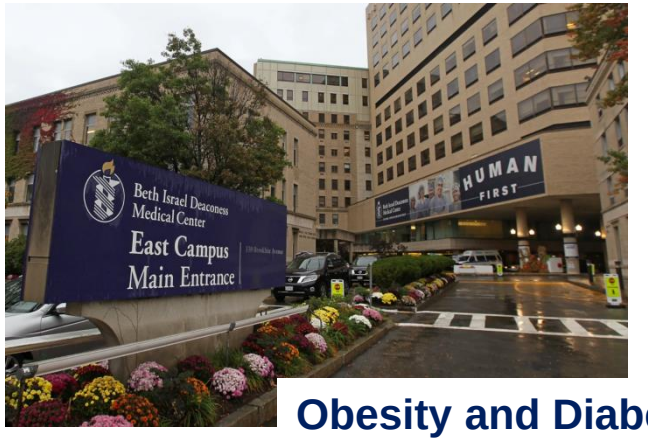
Beth Israel Deaconess Medical Center

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

Boston Veterans Affairs Healthcare System

Division of Endocrinology, Beth Israel Deaconess Medical Center, Harvard Medical School — MANTZOROS LABORATORY

Major Areas of Research in Metabolism



Obesity and Diabetes: Pathophysiology and Therapeutics

Artificial Intelligence / Machine Learning and Diabetes Technology

Adipokines (leptin and adiponectin)

Characterization of important metabolic effects of leptin in humans

a) Nature Communications 10/2020, and b) JACC 02/2021

Physiology and therapeutic potential of Gastrointestinal hormones

Identification of novel roles for Oxyntomodulin/Glicentin in appetite and weight regulation

a) Diabetes Obes Metab. 05/2021, b) Metabolism 10/2019; c) J Clin Endocrinol Metab. 03/2020

Physiology and therapeutic potential of Hepatokines & Muscle-acting hormones

Identification of novel roles for Follistatins and Activins in glucose homeostasis

a) Diabetes Obes Metab. 03/2019, b) Diabetes Res Rev 02/2020, c) J Clin Endocrinol Metab. 08/2018, d) Metabolism 05/2018

NAFLD – Non invasive diagnostic algorithms

Development of a novel tool for staging NAFLD in humans

a) Metabolism 10/2019; b) Metabolism 10/2020, c) J Clin Endocrinol Metab. 03/2020

NAFLD – Preclinical and clinical evaluation of treatments

Identification of effective treatments for NAFLD in preclinical models and in human trials

a) Hepatology Communications 07/2020, b) Liver Int. 03/2021, c) Int J Mol Sci. 01 and 06/2021

Definition and Aims

Nonalcoholic fatty liver disease (NAFLD): **excess fat** is stored in the liver not caused by heavy alcohol use.

- Non-alcoholic **simple steatosis**
- Non-alcoholic steatohepatitis (NASH):
  Steatosis + ballooning + inflammation ± fibrosis
  - ➢ *advanced NASH may lead to liver cirrhosis and finally to hepatocellular carcinoma (HCC)*

Most patients have **obesity** and the **metabolic syndrome** and will die from **metabolic complications** (Renal, Liver and CVD).

Thus…

Non-Alcoholic Fatty Liver Disease (NAFLD) **OR** Metabolism/Dysmetabolism Associated Fatty Liver Disease (MAFLD or DAFLD)?

Translational Approach to

T1: help advance science (novel Diagnostics and Therapeutics going from the question at the bedside to preclinical studies to observational studies to physiology to clinical studies)

T2: help ultimately change the guidelines and how we practice medicine

EASL/EASD/EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol, 2016.

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Current Gaps in NASH Awareness

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Professor, Harvard Medical School

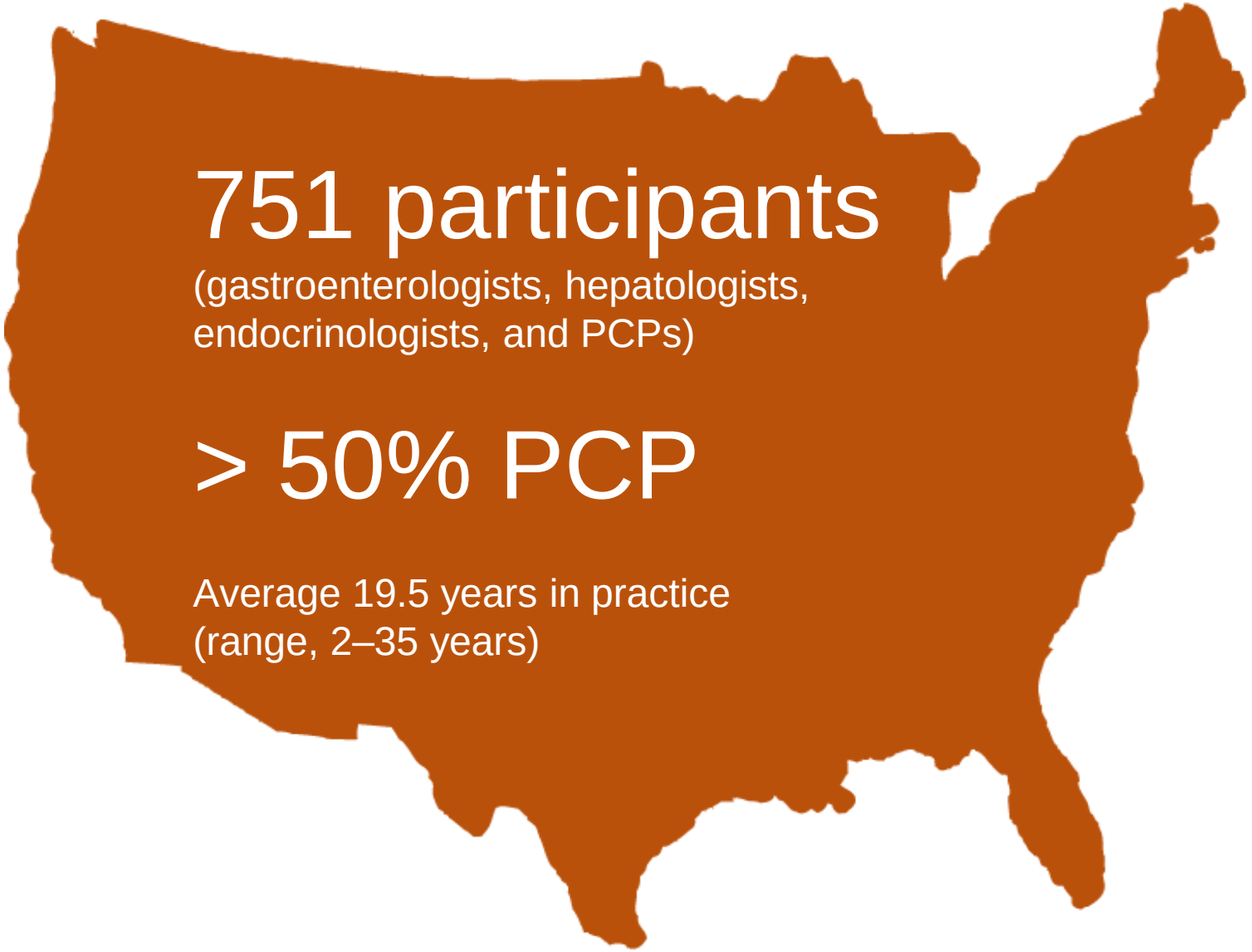
Editor-in-chief, Metabolism, Clinical and Experimental

NASH Needs Assessment Survey

To assess participants' knowledge

24 questions

screening, diagnosing, and managing NASH



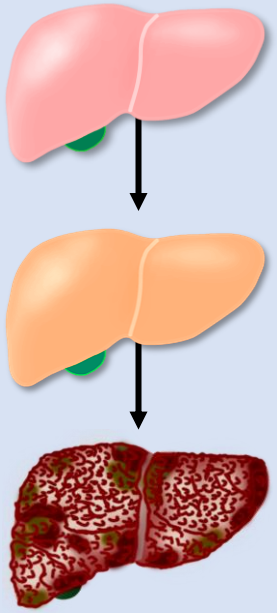
Preparing for the NASH Epidemic: A Call to Action

Kanwal F…Mantzoros C…Cusi K

aga American Gastroenterological Association
Metabolism Clinical and Experimental
Obesity A Research Journal

Delineating clinical care pathways for NAFLD/NASH patients,
to be widely implemented in primary care practices

37% of the population



Hepatic manifestations
Co-morbid metabolic traits
Increased cardiovascular risk

- Screening at the primary care / endocrinology level
- Diagnosing early
- Optimizing patient management
  - Consistently
  - Timely
  - Accessibly

Survey Results – Significant GAPS in Knowledge

Are almost all patients with severe obesity likely to have NAFLD?

Survey Results – Significant GAPS in Knowledge

Are almost all patients with severe obesity likely to have NAFLD?

YES

- only 35% of all respondents answered yes

Survey Results – Significant GAPS in Knowledge

Is NAFLD very common in patients with type 2 diabetes?

Survey Results – Significant GAPS in Knowledge

Is NAFLD very common in patients with type 2 diabetes?

YES

Table 1—Key results from the Nonalcoholic Steatohepatitis Needs Assessment Survey

Variable	All participants (*n* = 751)	Gastroenterologists/ hepatologists (*n* = 175)	Endocrinologists (*n* = 175)	Primary care (*n* = 401)
Proportions of the key patient groups likely to have NAFLD				
Patients with severe obesity	35	46	28	32
With T2D	50	62	49	45
With dyslipidemia	40	47	41	36
General population	67	79	65	62

- 49% of endocrinologists and 45% of PCPs recognized it

Survey Results – Significant GAPS in Knowledge

Should initial evaluation of patients with suspected NAFLD include cross-sectional abdominal imaging (e.g., contrast-enhanced computed tomography) to screen for HCC?

Survey Results – Significant GAPS in Knowledge

Should initial evaluation of patients with suspected NAFLD include cross-sectional abdominal imaging (e.g., contrast-enhanced computed tomography) to screen for HCC?

NO

Table 1—Key results from the Nonalcoholic Steatohepatitis Needs Assessment Survey

Variable	All participants (n = 751)	Gastroenterologists/ hepatologists (n = 175)	Endocrinologists (n = 175)	Primary care (n = 401)
Patient groups that should be screened for NAFLD				
Patients with abnormal liver chemistry	96	97	97	85
Patients with T2D	87	88	94	83
Patients older than 50 y who have hypertension and hyperlipidemia	70	81	73	67
Approaches to the initial evaluation of the patient with suspected NAFLD				
Exclude competing etiologies for steatosis and coexisting common chronic liver disease	96	95	95	97
Consider the presence of commonly associated comorbidities, such as obesity, dyslipidemia, insulin resistance, or diabetes	95	97	93	95
Cross-sectional abdominal imaging (such as contrast-enhanced CT scan) to screen for HCC	41	50	39	38

- Only 41% of participants answered correctly

Survey Results – Significant GAPS in Knowledge

Is pioglitazone or vitamin E recommended as treatment in select patients with NASH?

Survey Results – Significant GAPS in Knowledge

Is pioglitazone or vitamin E recommended as treatment in select patients with NASH?

YES

- Only 50% of participants answered correctly

Survey Results – Significant GAPS in Knowledge

Can abdominal ultrasound identify NAFLD patients with NASH?

Survey Results – Significant GAPS in Knowledge

Can abdominal ultrasound identify NAFLD patients with NASH?

NO

Table 1—Key results from the Nonalcoholic Steatohepatitis Needs Assessment Survey

Variable	All participants (*n* = 751)	Gastroenterologists/ hepatologists (*n* = 175)	Endocrinologists (*n* = 175)	Primary care (*n* = 401)
Knowledge about strategies for noninvasive diagnosis of steatohepatitis and advanced fibrosis in NAFLD				
NAFLD fibrosis score or Fibrosis-4 Index are useful tools for identifying NAFLD patients with high likelihood of advanced fibrosis	82	94	86	75
VCTE (FibroScan) or MRE (imaging) are useful tools for identifying advanced fibrosis in patients with NAFLD	81	93	85	74
Abdominal ultrasound is a useful tool for identifying NAFLD patients with steatohepatitis	16	29	18	9
Appropriateness of treatments for NASH				
GLP-1 agonists	16	21	15	15
Metformin	17	33	17	11
Obeticholic acid	15	33	13	9
Omega-3 fatty acids	23	37	23	16
Pioglitazone[a]	53	53	77	42
Ursodeoxycholic acid	22	49	17	12
Vitamin E for nondiabetic adults[a]	40	71	51	38

- 78% of participants think it can

Survey to determine physician awareness, familiarity, and practices in the diagnosis and management of NAFLD and NASH

A. Awareness

Q1. To the best of your knowledge, which of the following statements accurately defines nonalcoholic fatty liver disease (NAFLD)? (Please check one)

- Evidence of hepatic steatosis
- Evidence of hepatic steatosis and lack of secondary causes of hepatic fat accumulation
- Evidence of hepatic steatosis with secondary causes of hepatic fat accumulation
- Not sure, would like to receive more information

Q2. To the best of your knowledge, which of the following statements accurately defines nonalcoholic fatty liver (NAFL)? (Please check one)

- Presence of $\geq$5% hepatic steatosis
- Presence of $\geq$5% hepatic steatosis with hepatocellular injury
- Presence of $\geq$5% hepatic steatosis without hepatocellular injury
- Not sure, would like to receive more information

Q3. To the best of your knowledge, which of the following statements accurately defines nonalcoholic steatohepatitis (NASH)? (Please check one)

- Presence of $\geq$5% hepatic steatosis
- Presence of $\geq$5% hepatic steatosis with hepatocellular injury
- Presence of $\geq$5% hepatic steatosis without hepatocellular injury
- Not sure, would like to receive more information

https://doi.org/10.2337/figshare.14932179

Survey Results – Significant GAPS in Knowledge

- Who to screen?
- How to diagnose?
- How to treat patients at high risk for NASH?
- Disparities between published practice guidance and clinical practice

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NAFLD & NASH: Epidemiology & Risk Factors

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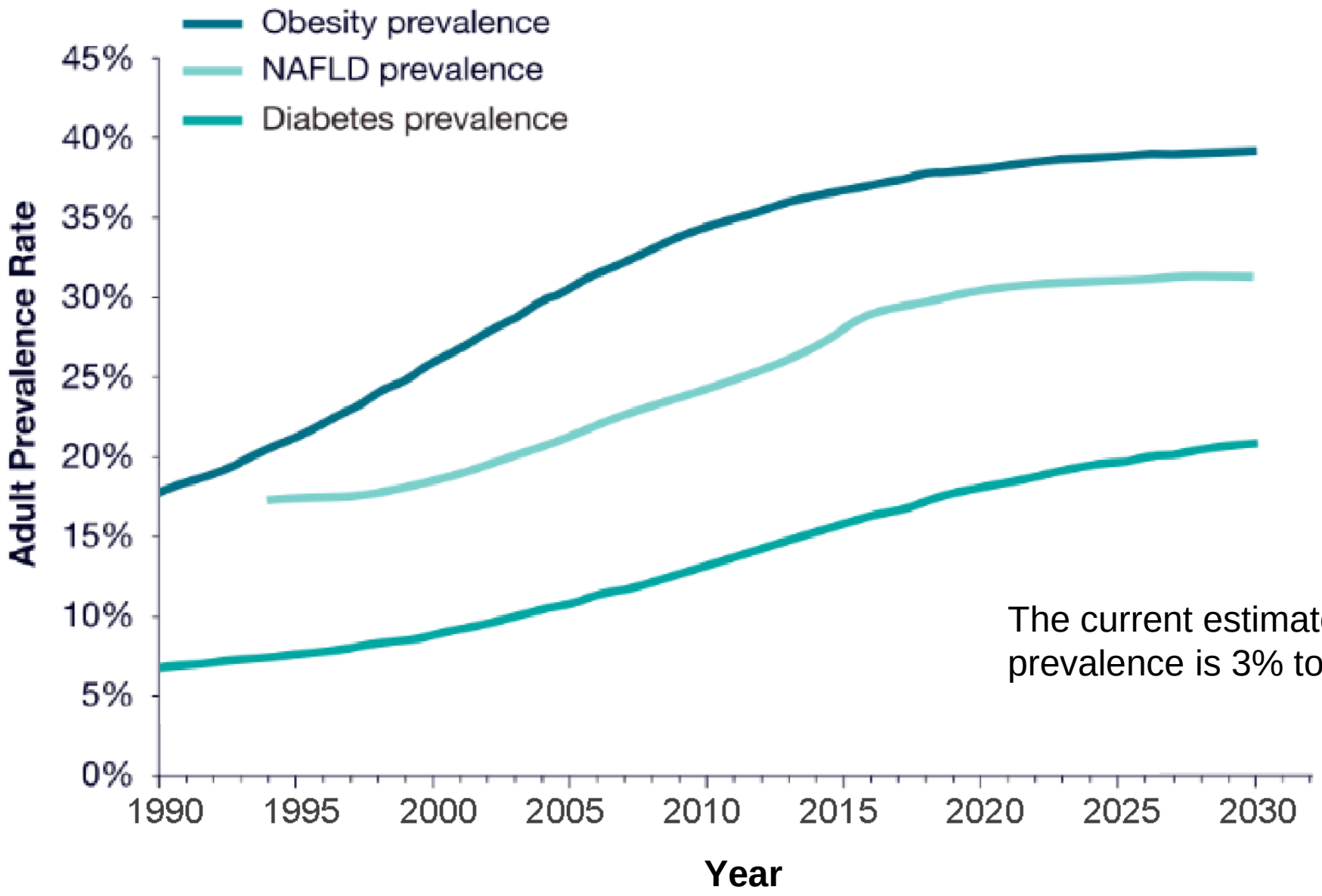
Epidemiology

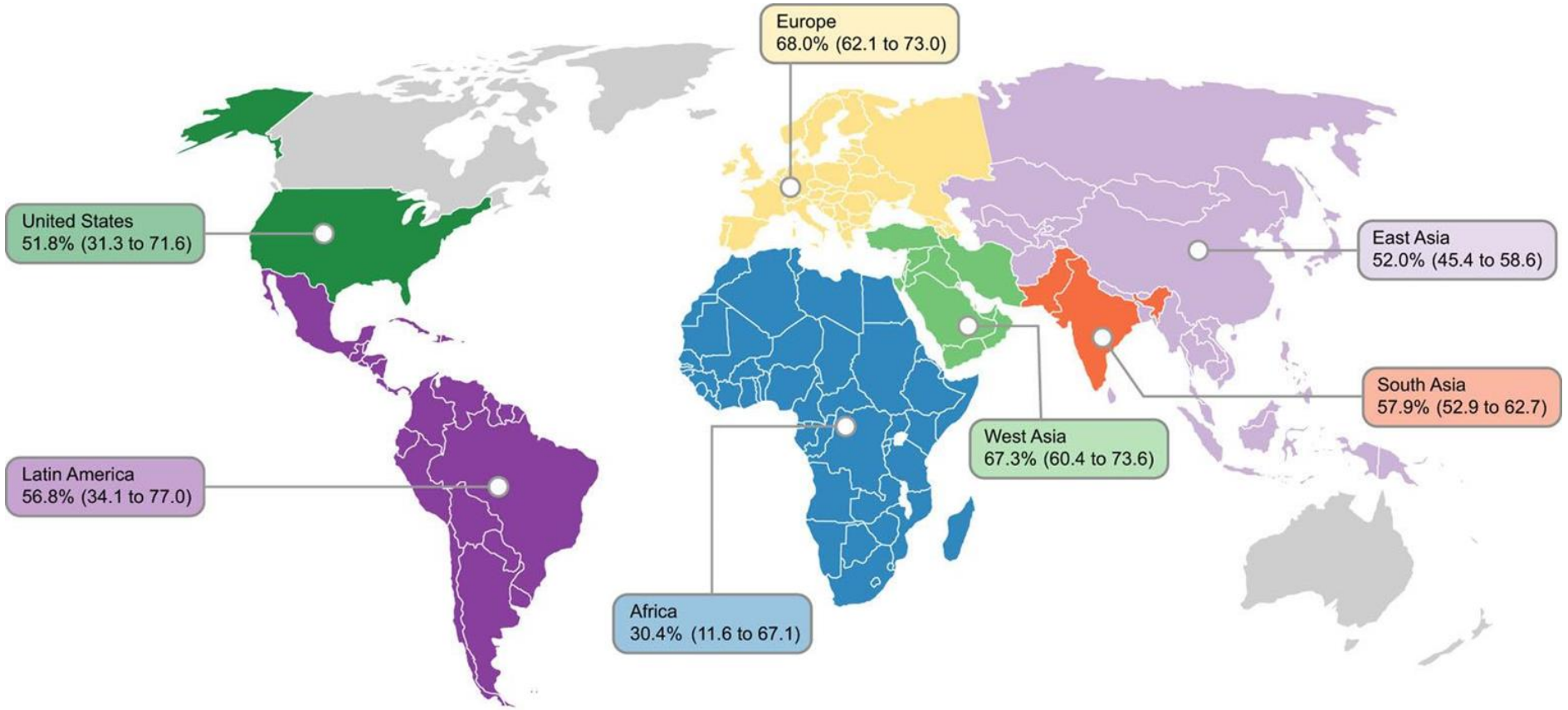
Adjusted Obesity, NAFLD and Diabetes Prevalence



The current estimate of NASH prevalence is 3% to 12%

Estes C et al. Hepatology, 2018.

Global NAFLD Prevalence among diabetics



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

Current direct medical cost for all incident and prevalent NAFLD cases in the United States is $908 billion. If, however, we assume the rate of increase in cost due to NAFLD parallels the growth in obesity prevalence, the 10-year projection for direct cost is **$1,005 trillion**.

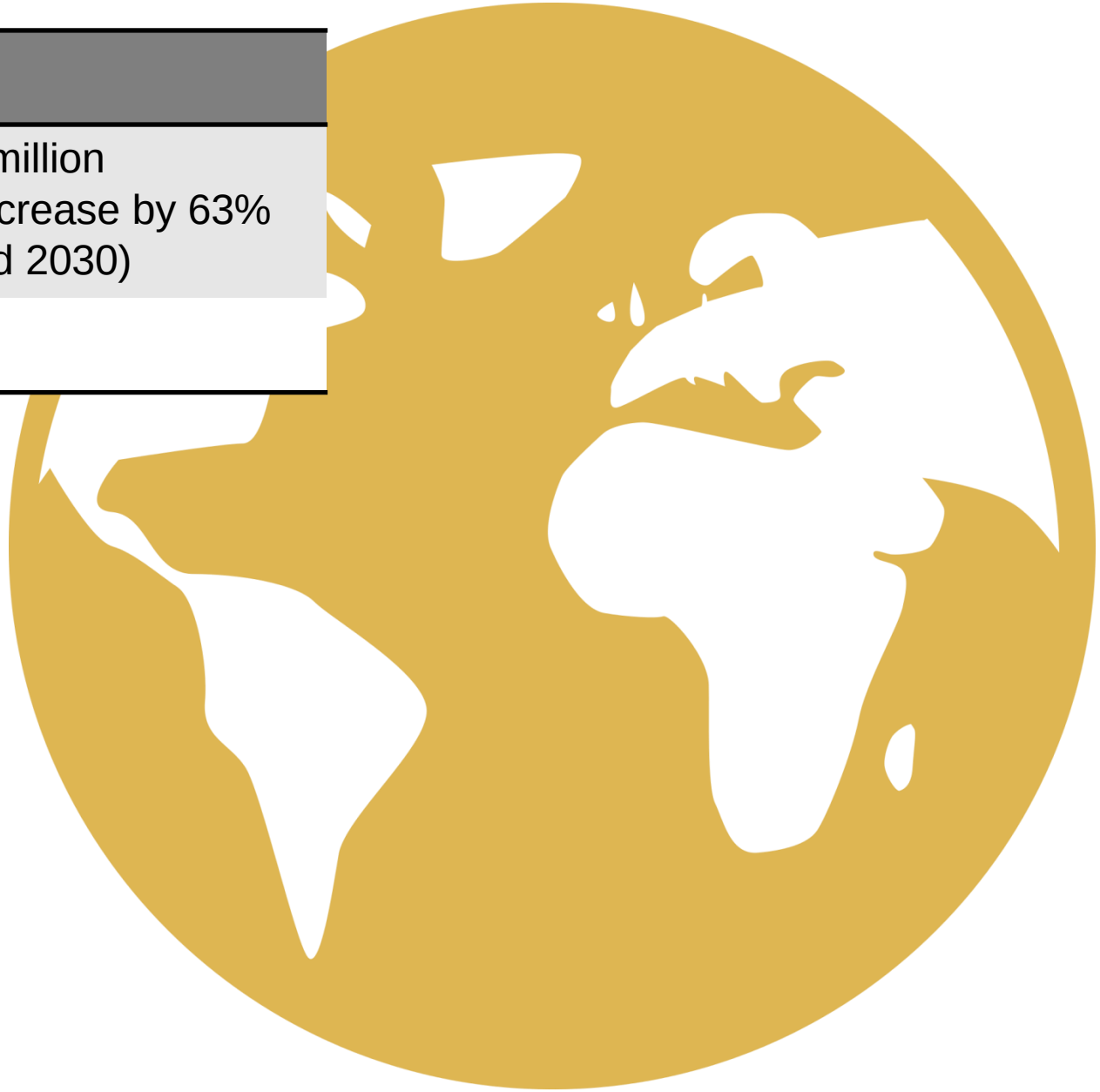
Targher G et al. N Engl J Med, 2010.
Younossi ZM et al. Hepatology, 2019.

Burden of NAFLD and NASH

	NAFLD	NASH
Global population	25%	1.5 – 6.5% = 100 million (Prevalence will increase by 63% between 2015 and 2030)
Patients with T2DM	> 60%	37%

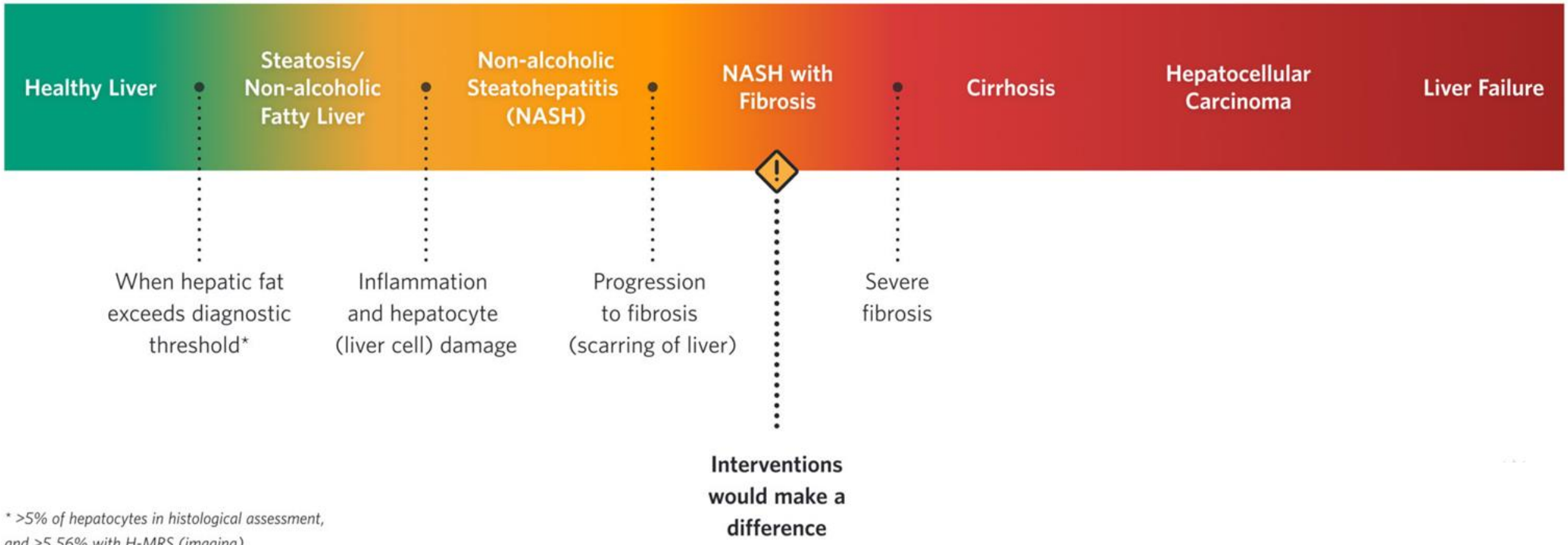
Estimated overall lifetime costs in 2017:
-All NASH patients in the U.S.: $222.6 billion
-Cost of the advanced NASH population: $95.4 billion

Estimated cost between 2017 and 2060:
Expected 10.8 million new cases, total lifetime costs of these cases will amount to $359 billion.



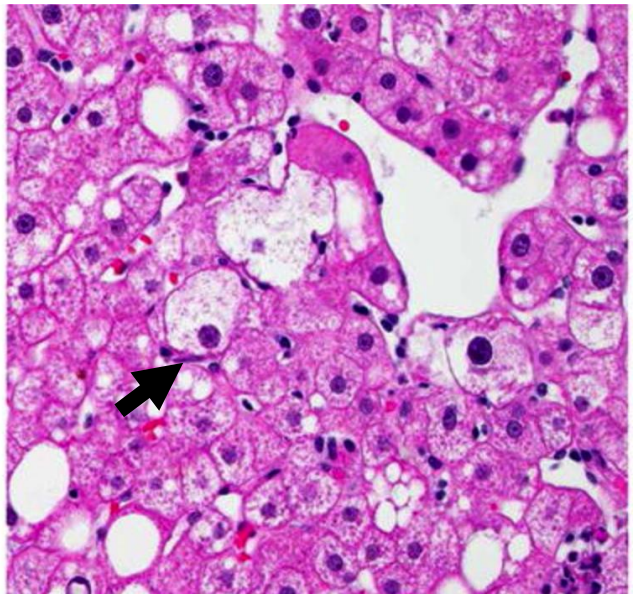
Younossi ZM et al. Hepatology, 2019.

NAFLD Spectrum



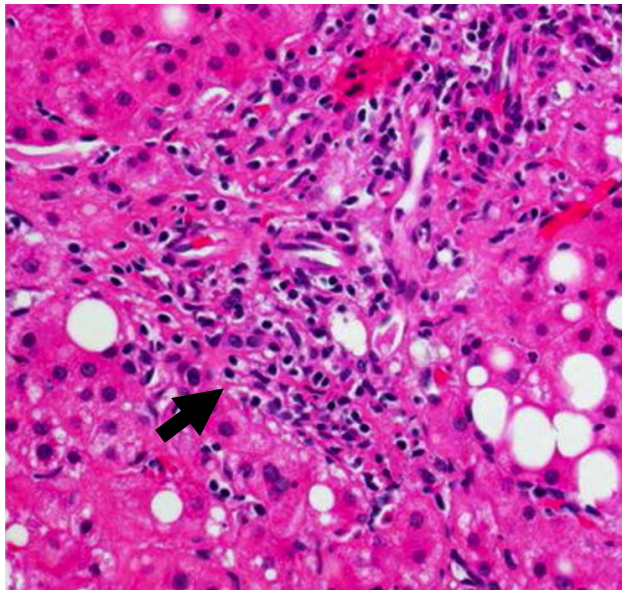
EASL/EASD/EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol, 2016.

Fibrogenesis



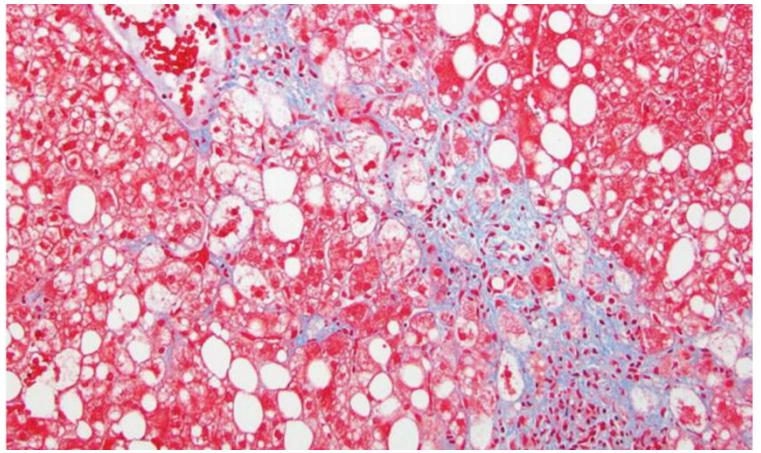
Hepatocyte Ballooning

+



Liver Inflammation

→

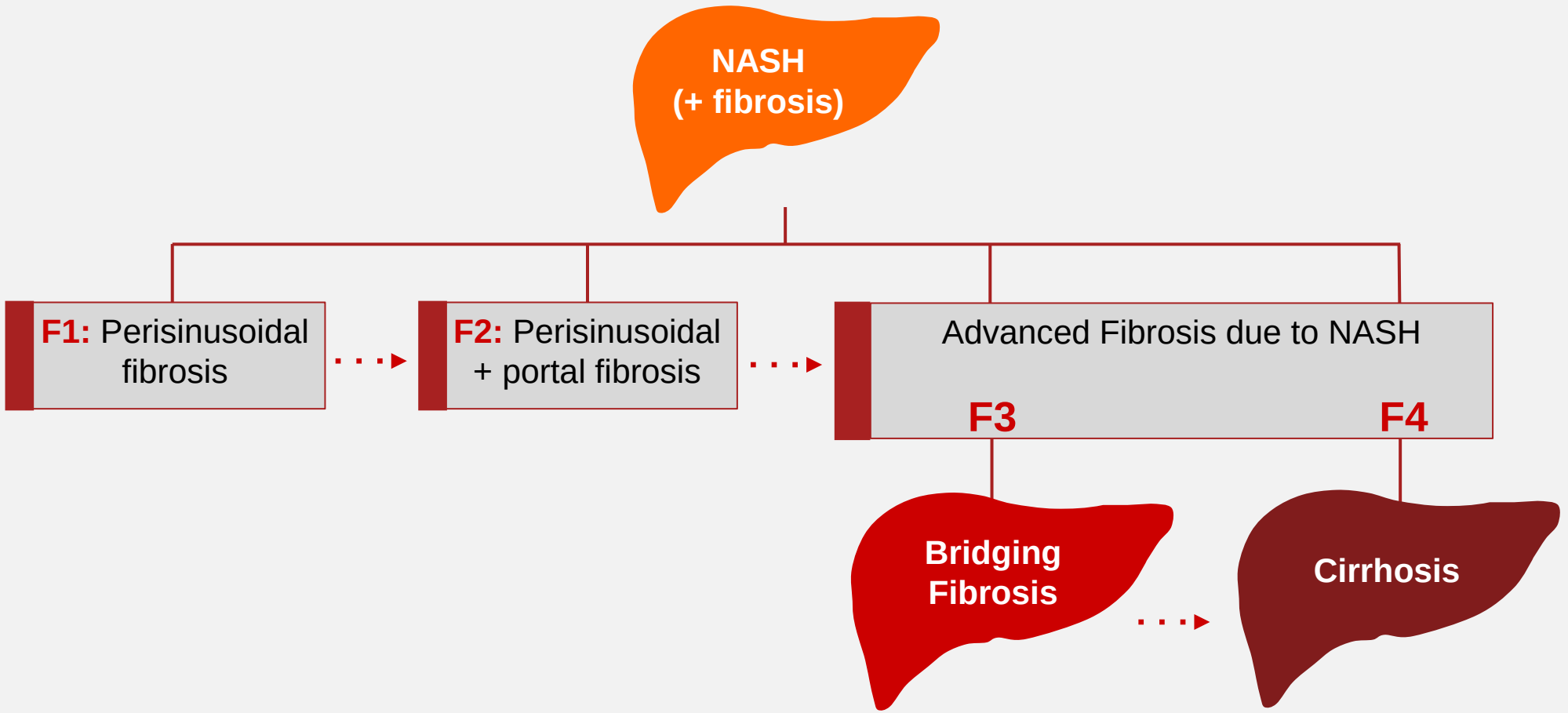


Fibrosis

- Fibrogenesis is not a linear process (e.g. from NALFD to NASH to Cirrhosis) but progresses or regresses in up to 30% of patients during a mean period of 5 years.
- Typically, NASH and NAFLD patients progress 1 stage of fibrosis every 7 and 14 years respectively
- The presence and stage of fibrosis is the strongest histologic determinant of hepatic and overall outcomes in patients with NAFLD.

1) Kleiner DE. Clin Liver Dis, 2016. 2)Brown TG et al. Metabolism, 2016. 3)Kleiner DE et al. JAMA Netw Open, 2019. 4)Singh S et al. Clin Gastroenterol Hepatol, 2015. 5)Cotter TG et al. Gastroenterology, 2020.

Advanced Fibrosis due to NASH is defined as Stages F3 and F4



Chalasani N et al. Hepatology, 2018.

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NAFLD & NASH: Pathophysiology, Disease Progression & Complications

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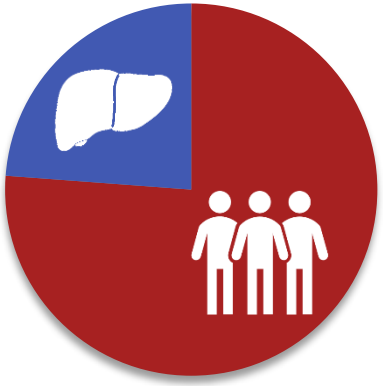
Professor, Harvard Medical School

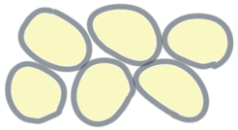
Editor-in-chief, Metabolism, Clinical and Experimental

Non-Alcoholic Fatty Liver Disease (NAFLD/ MAFLD) the Ultimate Unmet Clinical Need

~ 30%

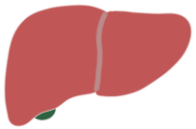


Genetic predisposition



Lipolysis
FFA

Dietary TG
Fructose



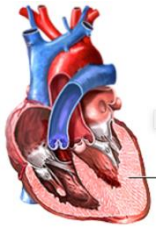
De novo lipogenesis

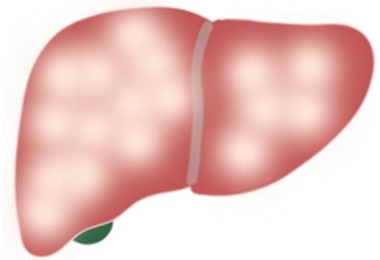
Obesity
T2DM
MetS
Hyperlipidemia

Inflammatory cytokines
Lipotoxicity
Oxidative stress
Adipokine dysregulation

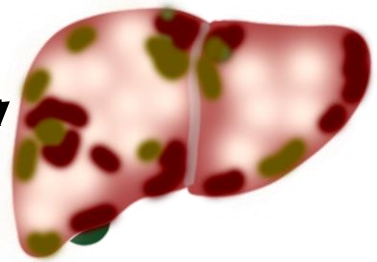
Stellate cell activation
Hepatocellular death
Extracellular matrix remodeling

CVD– Metabolic Complications

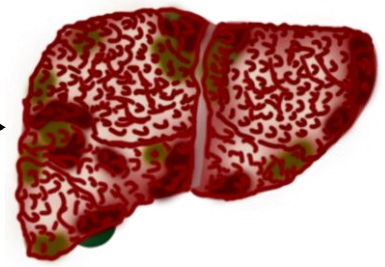




10-20%

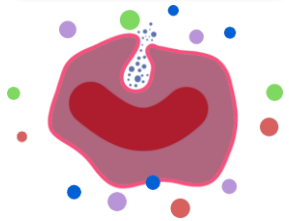


20%

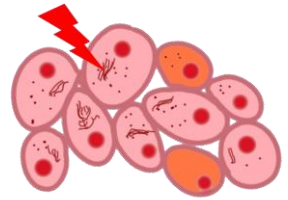


Steatosis

NASH

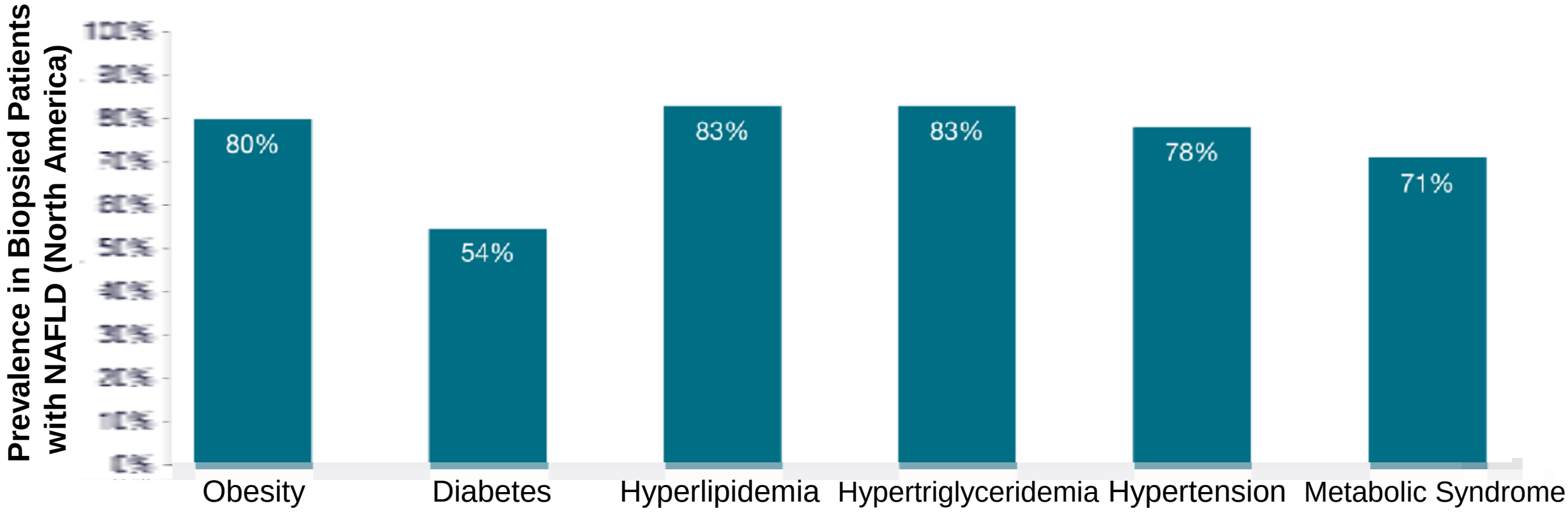


Fibrosis



Cirrhosis
Liver failure
Liver cancer

Risk Factors for NASH – Prevalence in patients with NAFLD



The incidence of NAFLD is higher in patients with more components of MS, and NAFLD is considered the hepatic manifestation of MS. Diabetes is closely associated with the risk for NASH, fibrosis, and advanced fibrosis.

Golabi P et al. Medicine (Baltimore), 2018.
Kanwar P et al. Clin Liver Dis, 2016.
Younossi ZM et al. Hepatology, 2016.

Global Prevalence, Incidence, and Outcomes of Non-obese or Lean non-alcoholic fatty liver disease: a Systematic review and Meta-analysis

Lean NAFLD Prevalence among the NAFLD, general, and Lean populations

	Studies (n)	Participants (n)	Lean NAFLD (n)	Prevalence (95% CI)	I^{2}*
NAFLD population	35	36 529	5387	19·2% (15·9–23·0)	98·0%
General population	23	113 394	4575	5·1% (3·7–7·0)	99·0%
Lean population	19	49 503	4211	10·6% (7·8–14·1)	99·0%

NAFLD=non-alcoholic fatty liver disease. *All p values for I^{2} are lower than 0·05.

NAFLD incidence among Non-Obese, Lean, and Obese populations

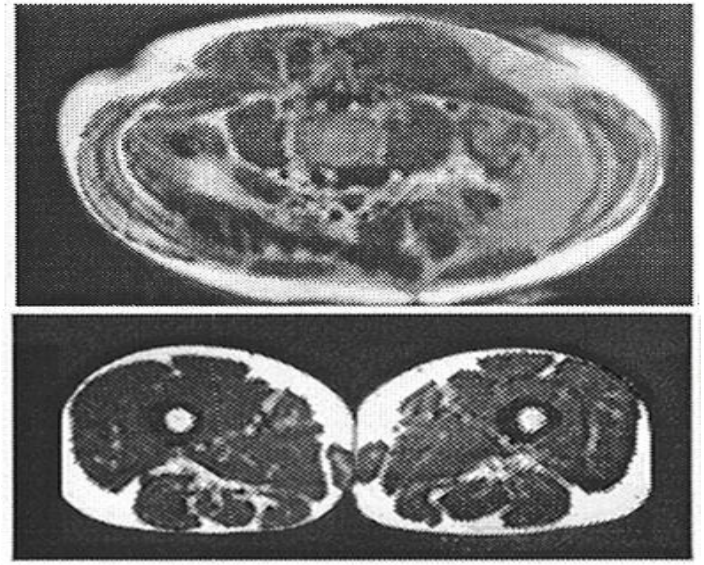
	Studies (n)	Non-NAFLD participants at baseline (n)	Incident patients with NAFLD (n)	Follow up (person-years)	Incidence per 1000 person-years (95% CI)	I^{2}*
Non-obese population	4	8827	678	50 234·9	24·6 (13·4–39·2)	97·7%
Lean population	4	3925	187	10 423·5	23·2 (7·3–48·0)	97·5%
Obese population	4	1969	433	10 033·8	77·5 (28·3–150·6)	98·6%

NAFLD=non-alcoholic fatty liver disease. *All p values for I^{2} are lower than 0·05.

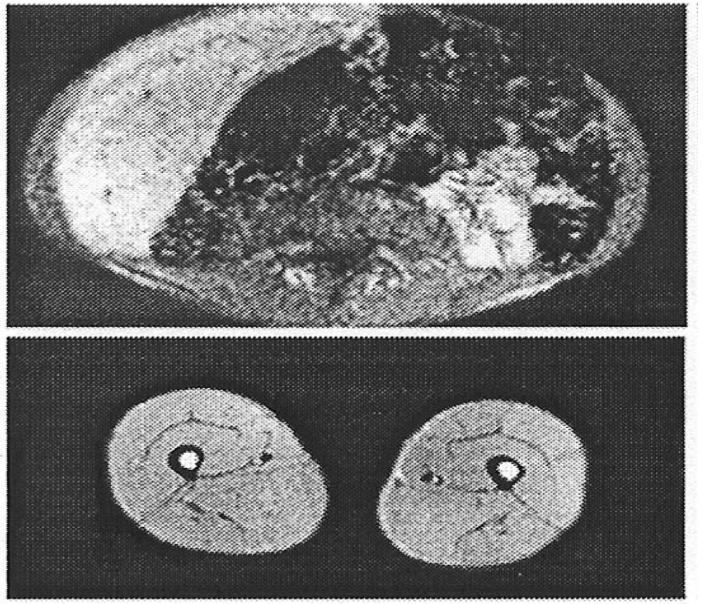
Qing Ye et al. Lancet 2020

Adipose Tissue Stores in Healthy and Insulin Resistant Subjects

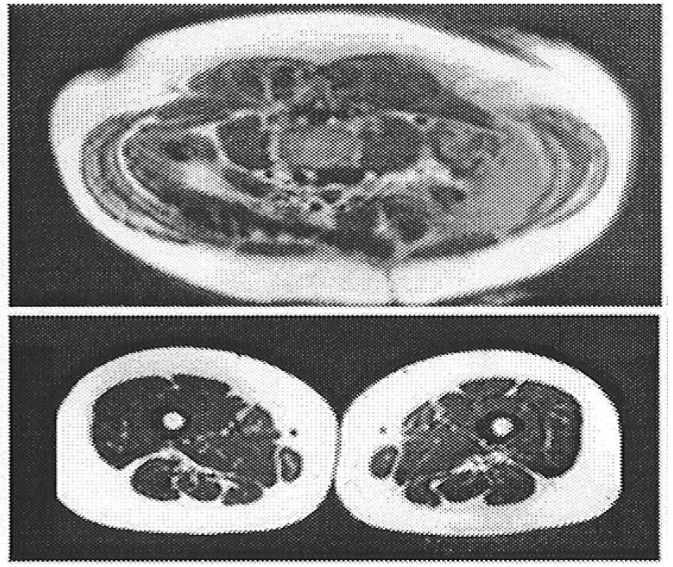
Exercise induced HA



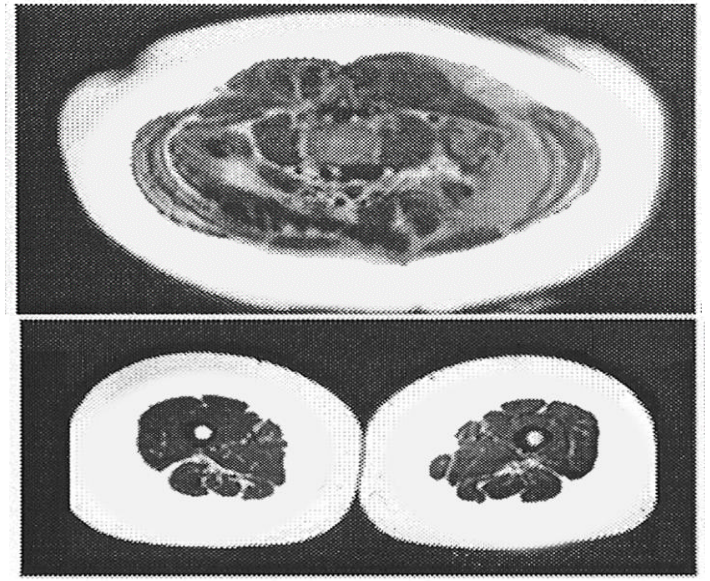
Generalized Lipodystrophy



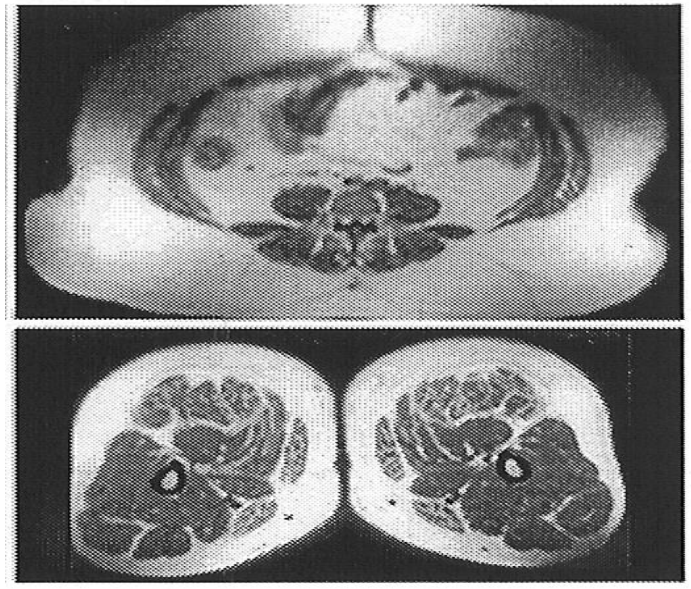
Normal weight healthy subject



Metabolically healthy obese subject



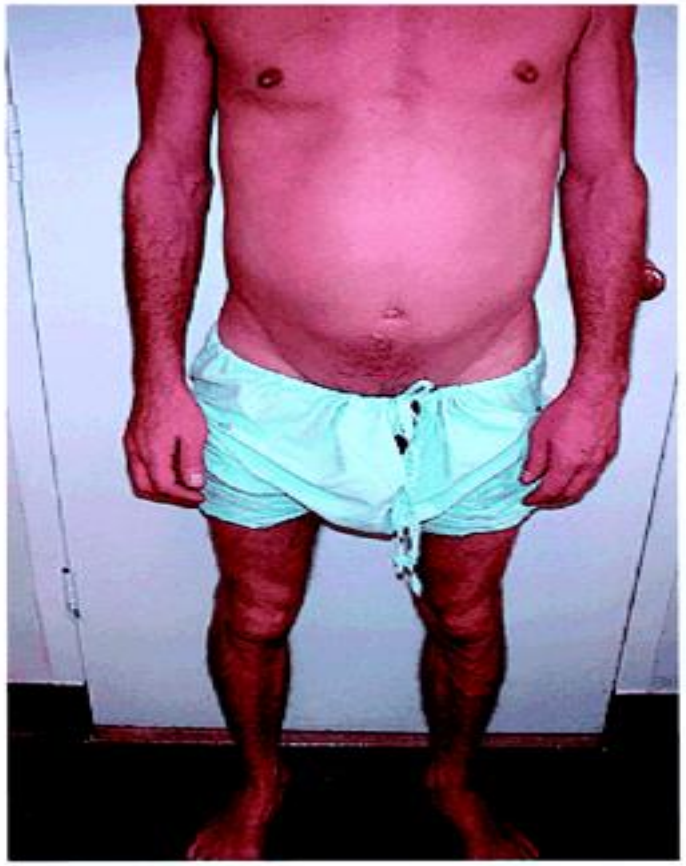
Morbid Obesity

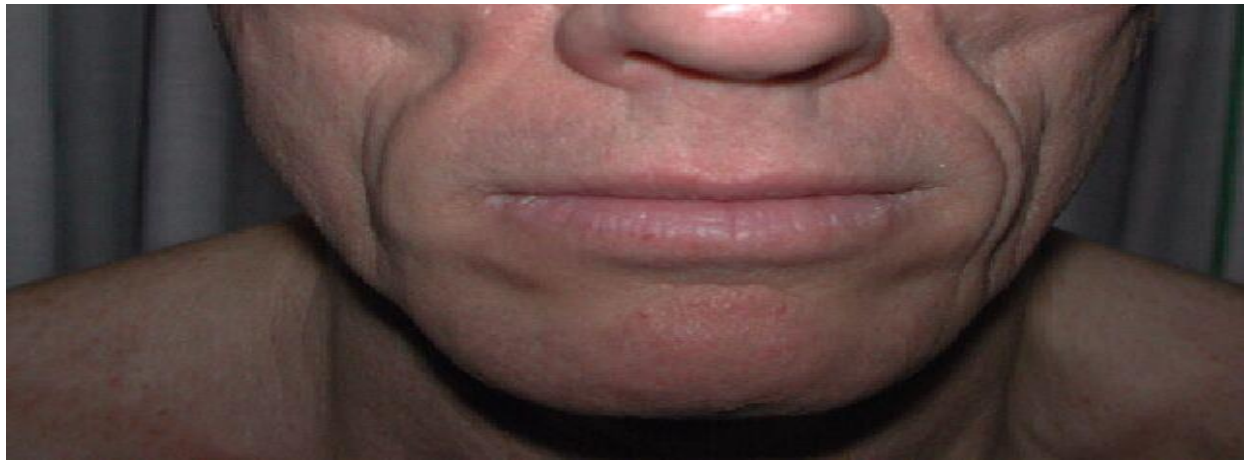


Boutari C … Mantzoros C. Metabolism, 2019.

Patients with Complete Congenital Lipodystrophies
Partial and HIV-Associated Lipoatrophy

- Leptin levels are low and adiponectin levels are high
- Leptin has been approved for complete lipodystrophies with metabolic abnormalities
- Leptin receptor analogs in phase II clinical trials

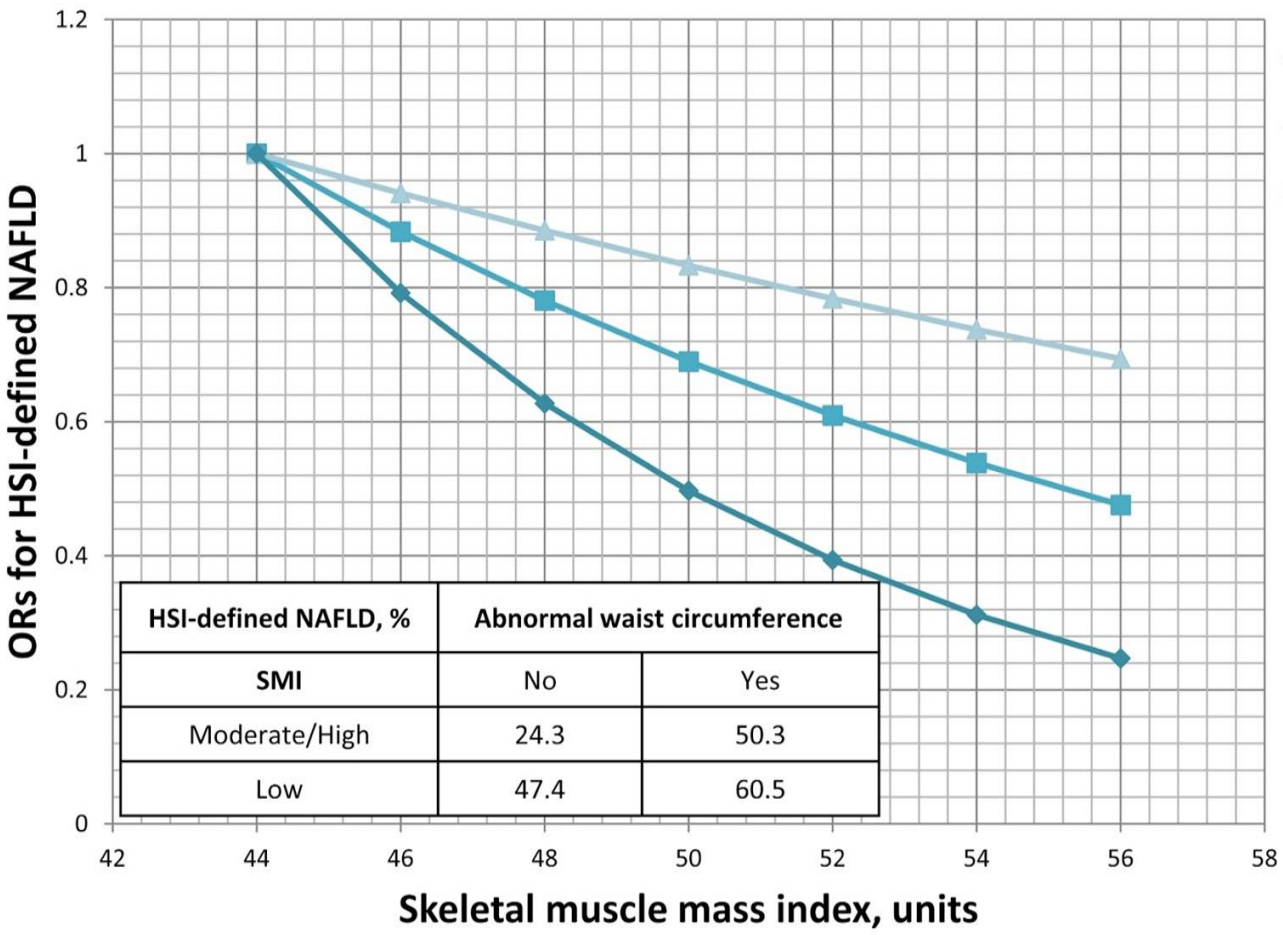




Leow MKS ... Mantzoros C. JCEM, 2003.

Chrysafi P, Perakakis N* ... Mantzoros C. Nature Communications, 2020. * equal contribution*

Epidemiology of Sarcopenia in NAFLD



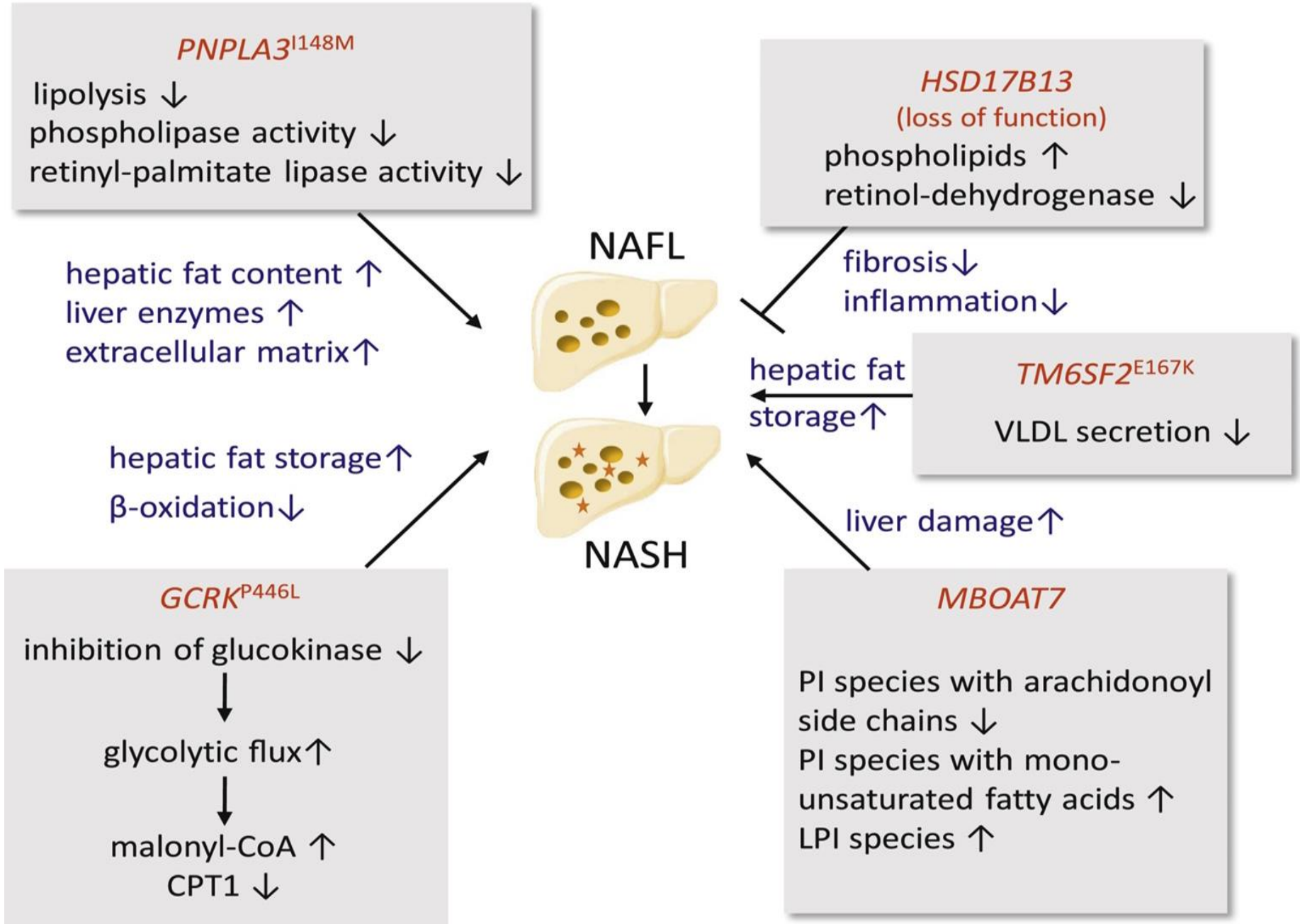
HSI-defined NAFLD, %	Abnormal waist circumference	
SMI	No	Yes
Moderate/High	24.3	50.3
Low	47.4	60.5

ORs and 95%Cis of SMI in relation to HIS-defined NAFLD

SMI, per 1 unit increase	OR (95%CI)
Total sample (n=3,042)	0.94 (0.92, 0.95)
Sample with normal waist circumference (n=1,549)	0.89 (0.84, 0.95)
Sample with abnormal waist circumference (n=1,493)	0.97 (0.94, 1.00)

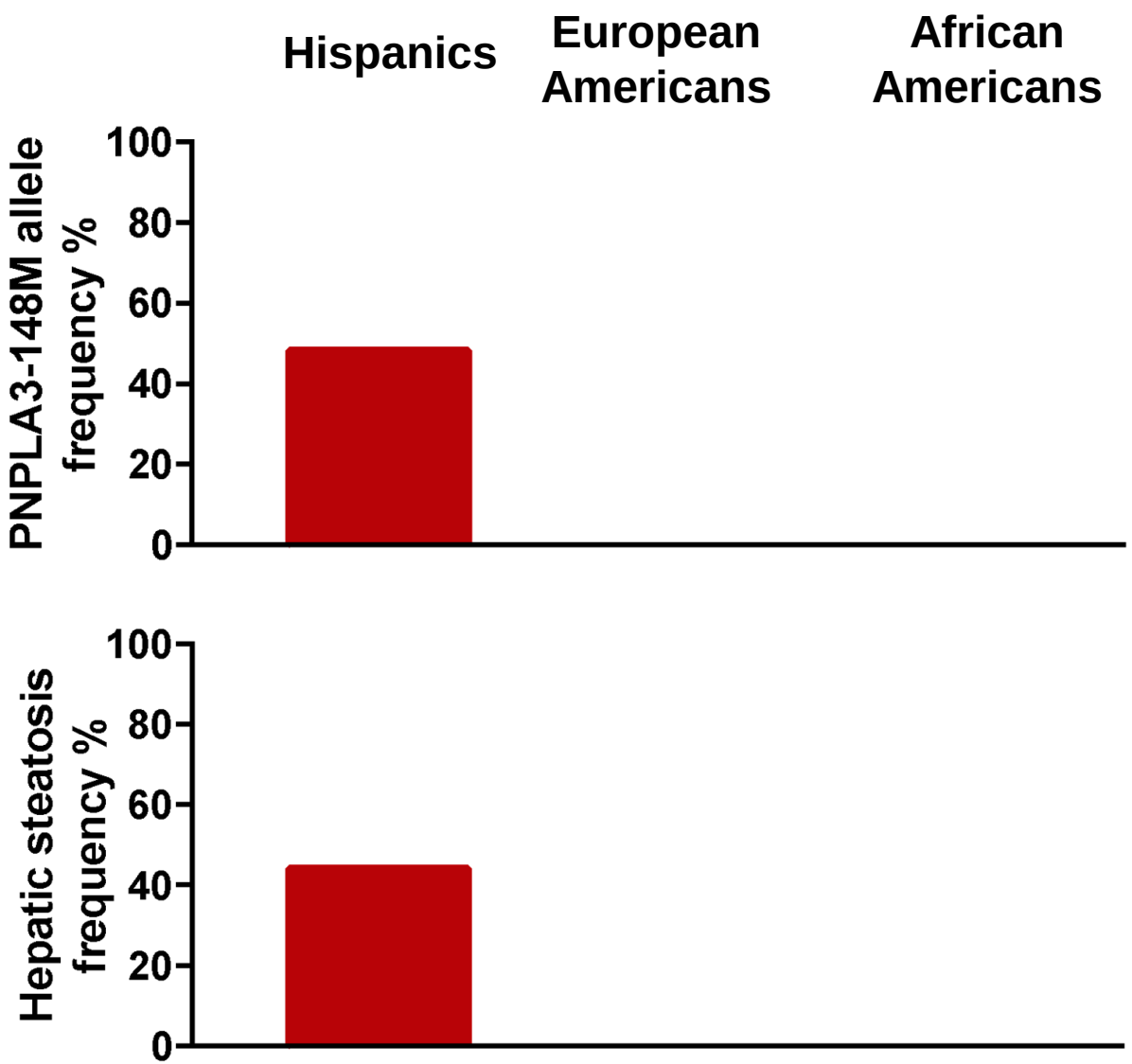
Mantzoros et al, 2021.

Genetics of NAFLD/NASH



Single Nucleotide Polymorphisms Associated with Increased Risk for NAFLD/NASH
PNPLA3 – 148M Gene Variant

Epidemiology



Pathophysiology

PNPLA3-148M Allele

↓

Decreased activity of adipose triglyceride lipase (ATGL) that normally mobilizes fatty acids

↓

Triacylglycerol accumulation in hepatocytes

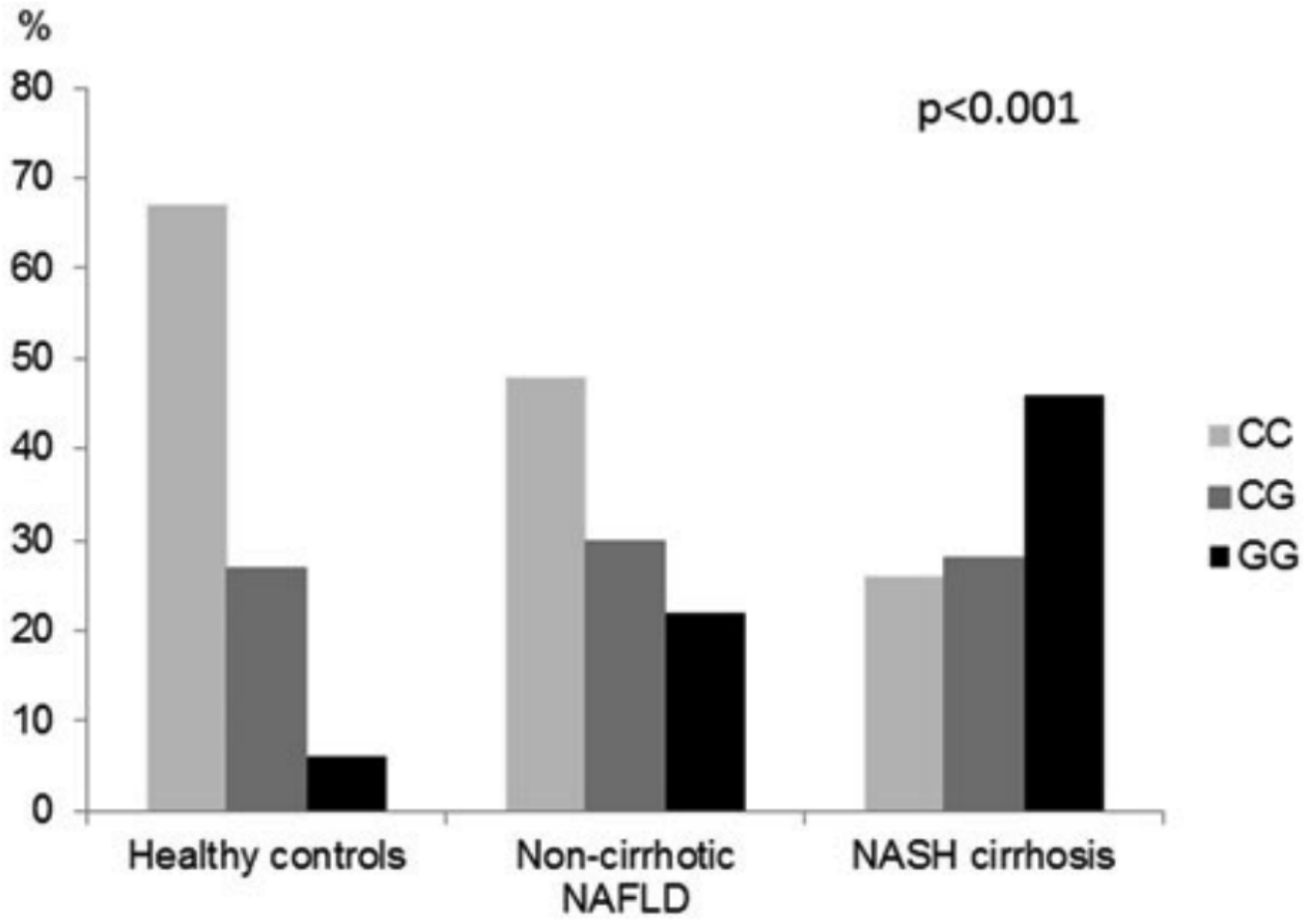
↓

Hepatic Steatosis
+ increased risk for cirrhosis and HCC evolution

Romeo S et al. Nat Genet, 2008.
Browning JD et al. Hepatology (Baltimore, Md.), 2004.
Jha P et al. Hepatology (Baltimore, Md.), 2014.
Dong XC et al. Front Med (Lausanne), 2019.

The PNPLA3-148M Polymorphism is Associated with the Risk of Progression to Cirrhosis in NAFLD Patients

PNPLA3 minor G allele associated with decreased ATGL activity



GG

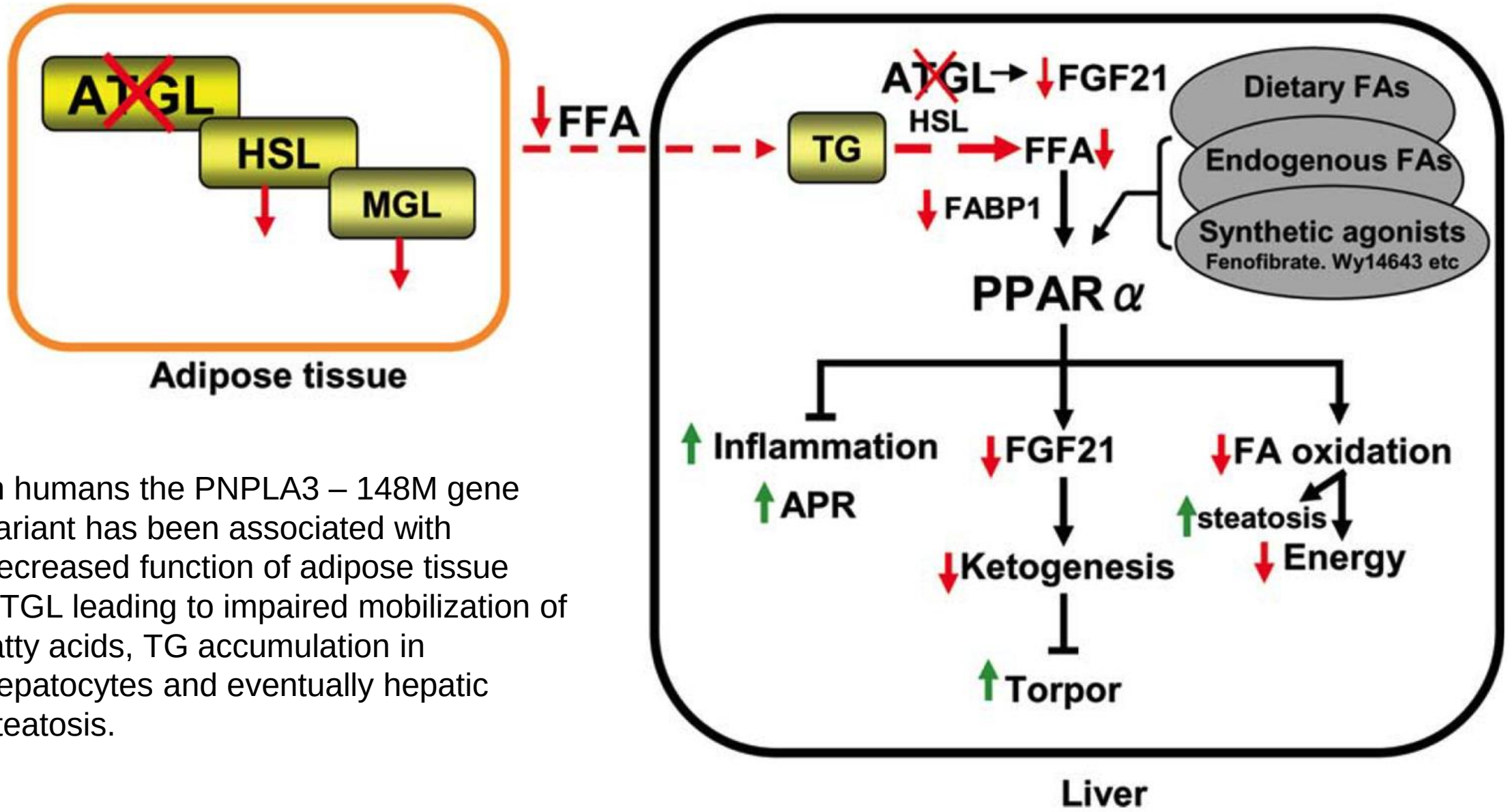
- × 2 increased risk for NASH compared to GC
- × 4 increased risk for NASH compared to CC

Each G allele

- × 2 increased risk for HCC

Vespasiani-Gentilucci, U et al. Scand J Gastroenterol, 2016.
Liu YL et al. J Hepatol, 2014.

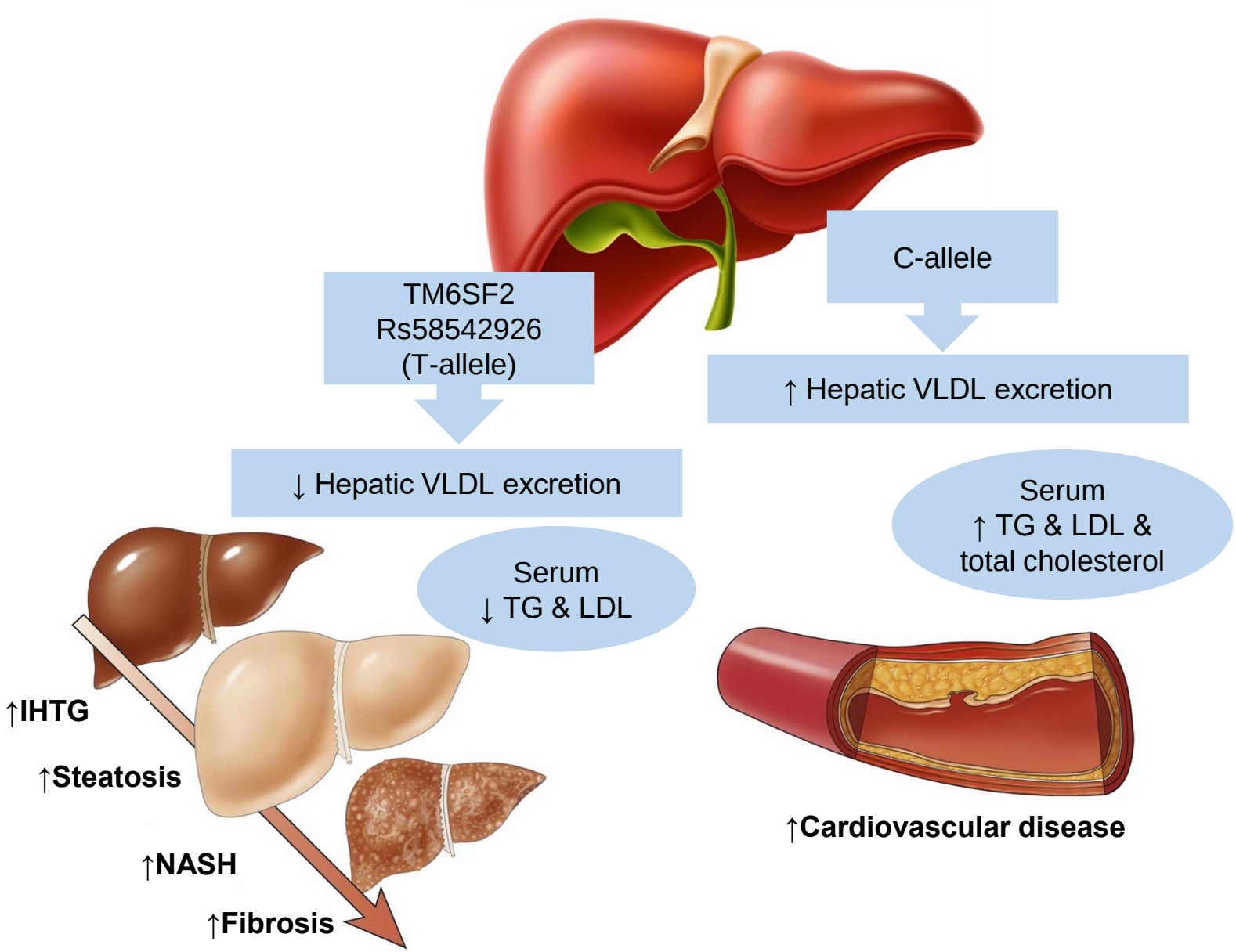
Proposed Mechanism for Enhanced Susceptibility to Hepatic Inflammation in ATGL-KO Mice upon MCD or LPS Challenge.



- In humans the PNPLA3 – 148M gene variant has been associated with decreased function of adipose tissue ATGL leading to impaired mobilization of fatty acids, TG accumulation in hepatocytes and eventually hepatic steatosis.

Jha P et al. Hepatology (Baltimore, Md.), 2014.

Single Nucleotide Polymorphisms Associated with Increased Risk for NAFLD/NASH
TM6SF2 rs58542926 Polymorphism



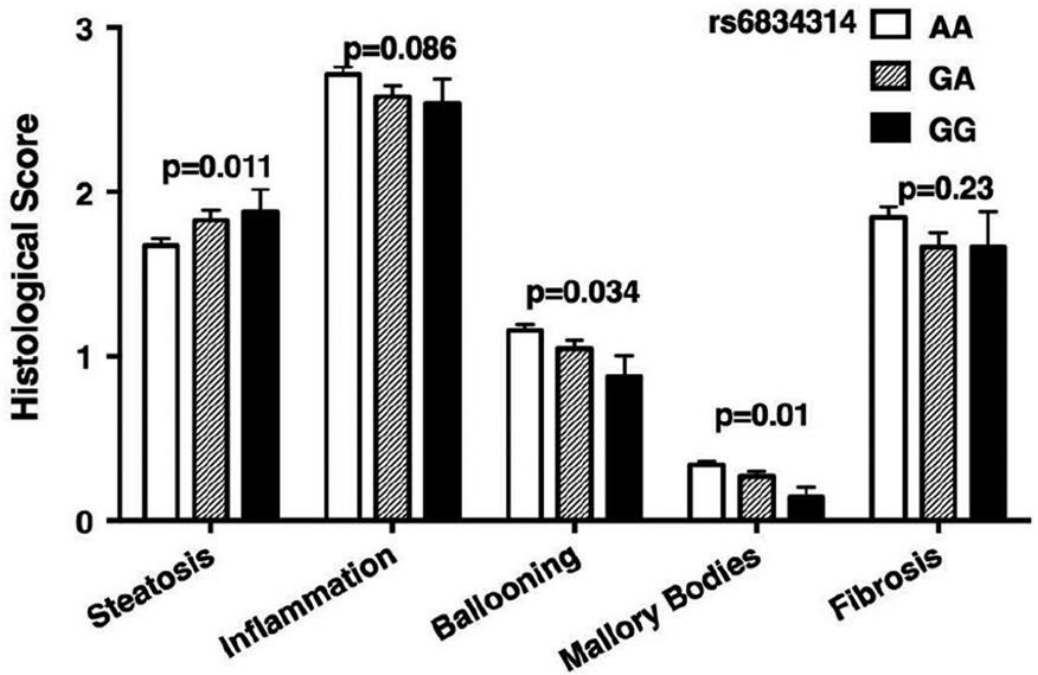
- The *TM6SF2* rs58542926 variant has subsequently been associated with **severity of NAFLD-associated hepatic fibrosis/cirrhosis** (OR, 1.88 [95% CI, 1.41–2.5] for advanced fibrosis per each copy of the minor allele carried), independent of confounding factors including age, diabetes, obesity, or PNPLA3 genotype in a cohort of >1000 histologically characterized patients

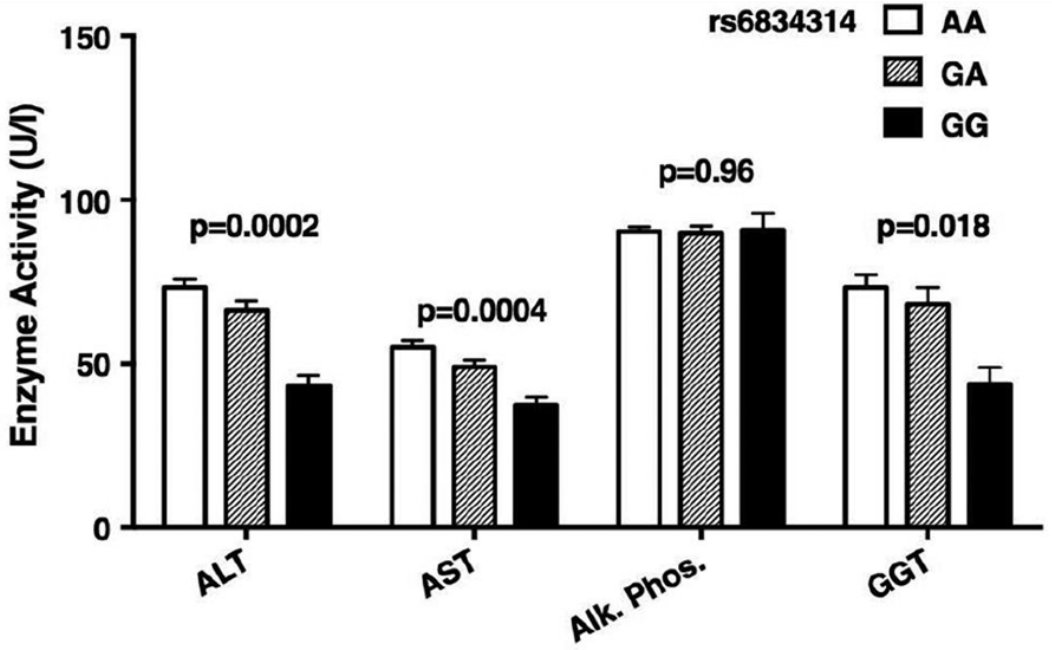
Kahali B et al. Gastroenterology, 2015.
Liu YL et al. Nat Commun, 2014.

HSD17B13 Gene Variants Associated with Histological Features of Non-Alcoholic Fatty Liver Disease

rs6834314 minor G allele is associated with:

- ↑ Steatosis: mean steatosis grade in G/G was 1.88±0.87 vs.1.68±0.89 in A/A
- ↓ Inflammation (OR=0.77 for total inflammatory score ≥3, CI 0.60–0.99)
- ↓ Ballooning (OR=0.67 for ballooning score >1, CI 0.51–0.87)
- ↓ Mallory-Denk bodies (OR=0.68, CI 0.51–0.91)
- Trend for ↓ fibrosis (OR=0.79, CI 0.60–1.05)
- ↓ Serum transaminases and GGT





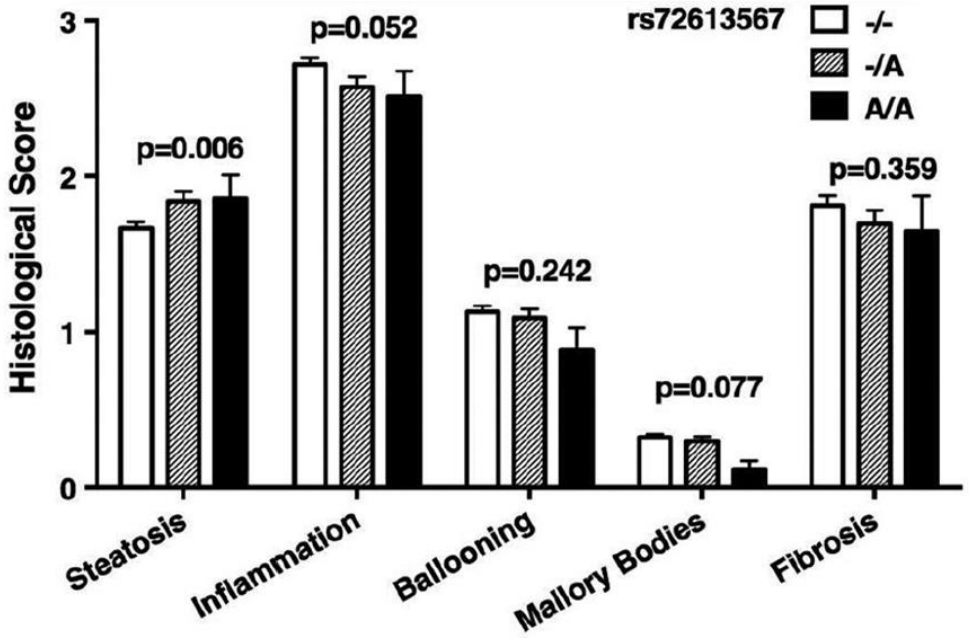
P-values for histology from multivariate ordinal regression adjusted for age, gender, and BMI. P-values for enzymes from linear regression of log-transformed enzyme activity, adjusted for age, gender, and BMI.

Ma Y et al. Hepatology, 2019.

HSD17B13 Gene Variants Associated with Histological Features of Non-Alcoholic Fatty Liver Disease

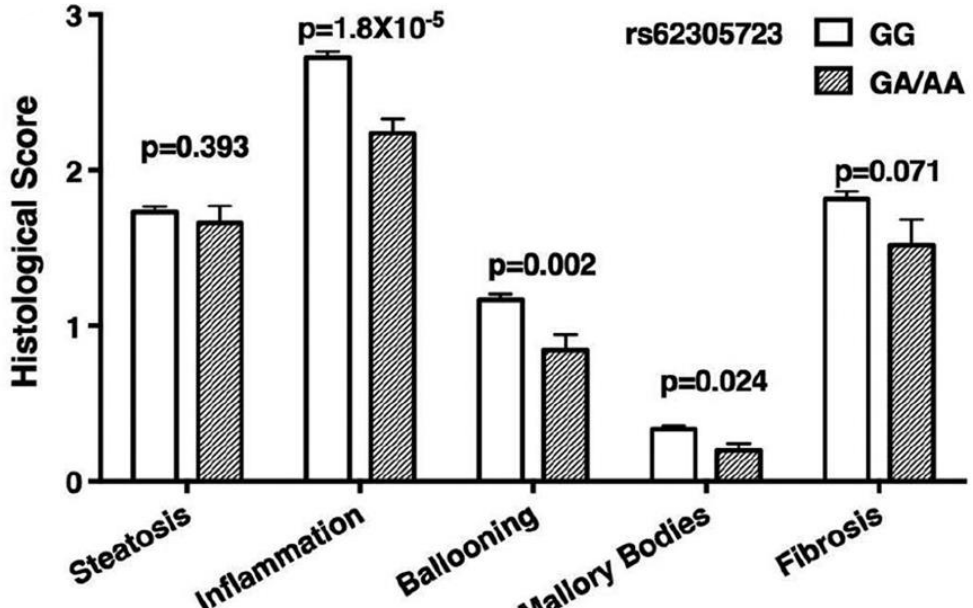
rs72613567 minor A allele is associated with:

- ↑ Steatosis (OR=1.36, CI 1.04–1.77)
- ↓Inflammation (OR=0.74, CI 0.57–0.96)
- Trend for ↓ ballooning (OR=0.78, CI 0.60–1.03)
- Trend for ↓ Mallory-Denk bodies (OR=0.77, CI 0.57–1.03)
- Trend for ↓ fibrosis (OR=0.77, CI 0.58–1.03)



rs62305723 minor A-allele is associated with:

- ↓ Inflammation (OR=0.46, CI 0.28–0.74)
- ↓ Ballooning (OR=0.48, CI 0.30–0.76)
- ↓ Mallory-Denk bodies (OR=0.51, CI 0.28–0.91)

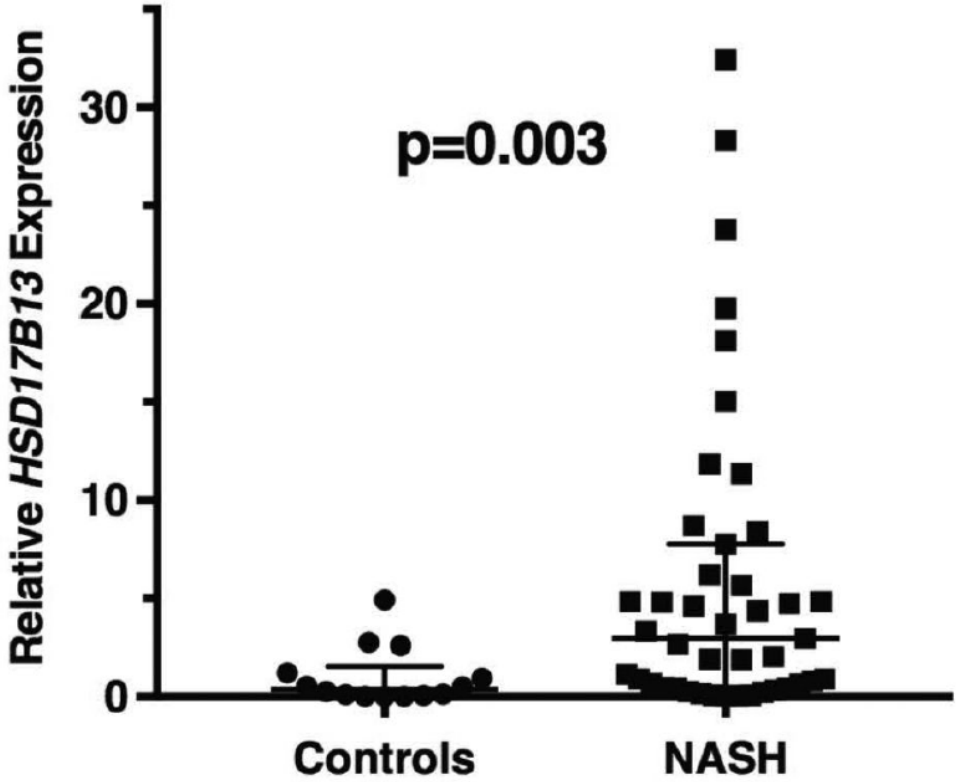


Ma Y et al. Hepatology, 2019.

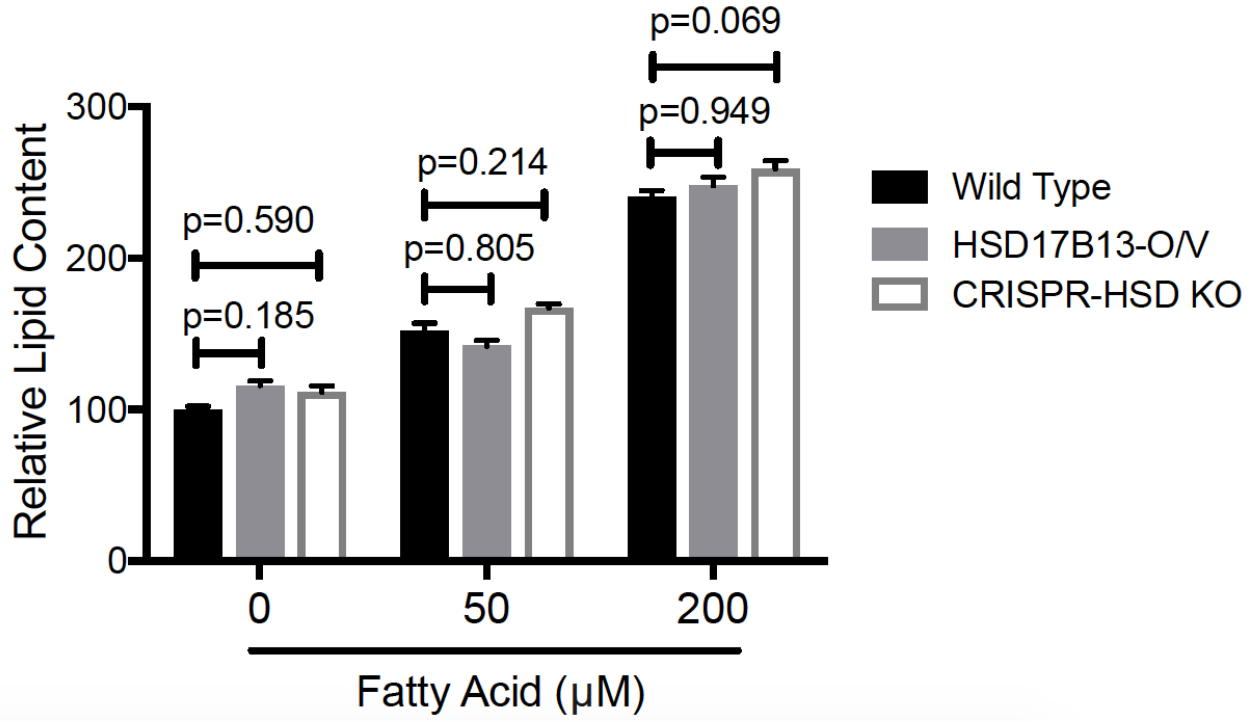
HSD17B13 Gene Variants Associated with Histological Features of Non-Alcoholic Fatty Liver Disease

➢ HSD17B13 is a retinol dehydrogenase

Hepatic HSD17B13 expression is elevated in NASH patients



Stable HSD17B13 overexpression or knockout in HepG2 cells incubated in different concentrations of fatty acids does not affect their lipid content, suggesting HSD17B13 does not regulate hepatocyte lipid content in a direct manner.



Ma Y et al. Hepatology, 2019.

Other Single Nucleotide Polymorphisms Associated with Increased Risk for NAFLD/NASH

- **rs641738 MBOAT7 Gene Variant**

↓expression → remodeling of the phosphatidylinositol acyl-chain → ↑ liver fat content

- **rs368234815 Interferon-λ3 Gene Variant**

↑ interferon-λ3 production which is connected with liver inflammation and fibrosis in patients with NAFLD, especially in non-obese patients

- **rs780094 Glucokinase Regulatory Gene (GCKR) Variant**

affects the ability of regulation of glucokinase in response to fructose-6-phosphate → ↑ hepatic glucose uptake → ↑ malonyl-CoA → favors lipogenesis

- **rs13412852 Lipin-1 Gene Variant**

In children: Associated with ↑ serum lipid levels, NASH progression and fibrosis

In adults: Associated with ↑ but not severity of hepatic pathology in NAFLD

Petta S et al. PloS one, 2014.
Petta S et al. J Hepatol, 2012.
Luukkonen PK et al. J Hepatol, 2016.
Mancina RM et al. Gastroenterology, 2016.
Valenti L et al. J Pediatr Gastroenterol Nutr, 2012.

Transcriptomic profiling across the nonalcoholic fatty liver disease spectrum reveals gene signatures for steatohepatitis and fibrosis

1 Unsupervised clustering to stratify NAFLD on the basis of fibrosis and disease activity

Cluster A was characterized by more advanced fibrosis, ballooning and inflammation compared to Cluster B

1292 differentially expressed genes in Cluster A vs Cluster B correlating to pathways including:

- extracellular matrix interaction
- focal adhesion
- phosphatidylinositol 3-kinase (PI3K)–Akt signaling
- Wnt signaling
- Cytokines
- Hepatic progenitor cells.

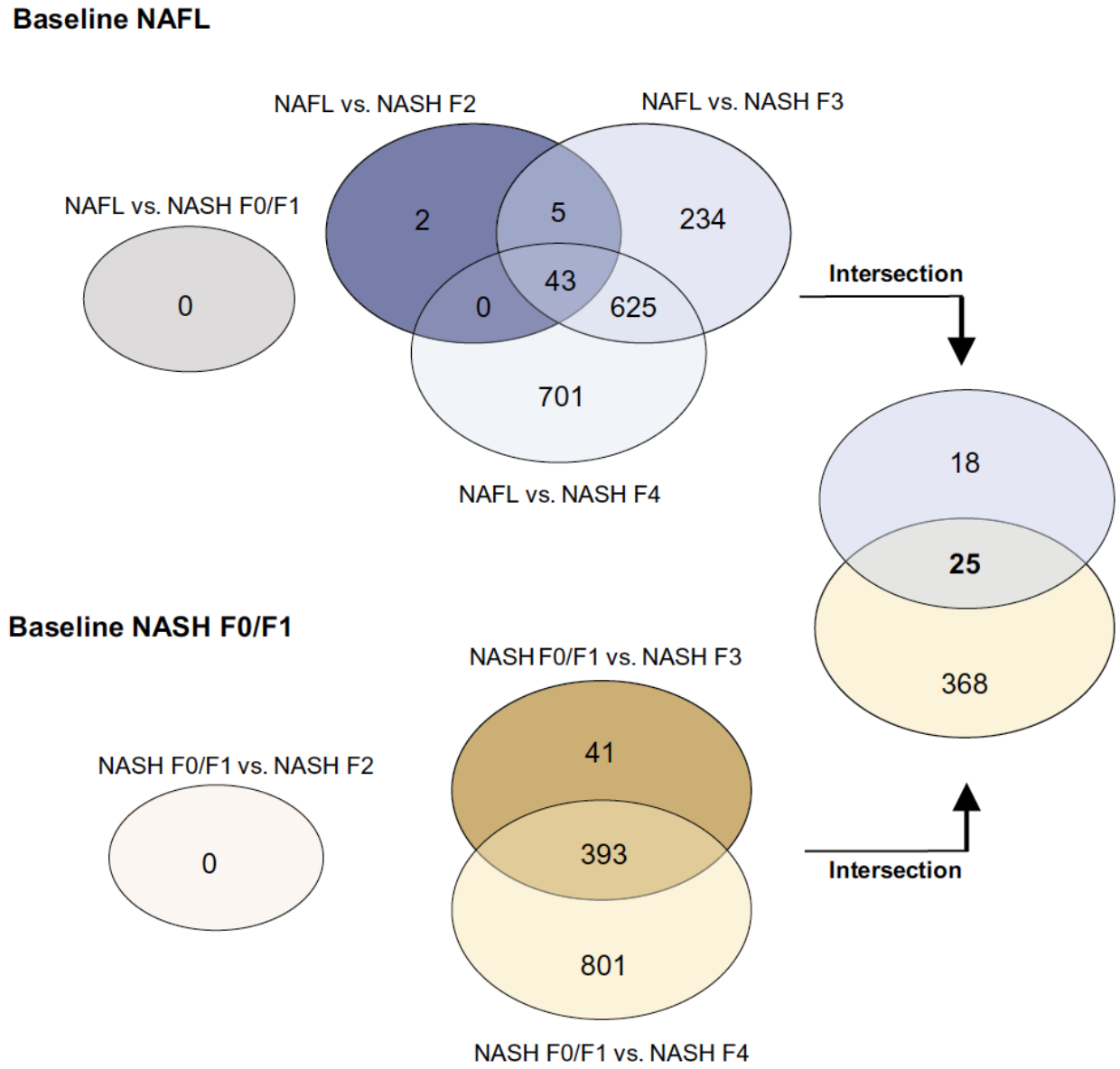
Govaere O et al. Sci Transl Med, 2020.

Transcriptomic profiling across the nonalcoholic fatty liver disease spectrum reveals gene signatures for steatohepatitis and fibrosis

2

To identify modifiers of steatohepatitis → comparison of different NASH stages with NAFL

To identify modifiers of fibrosis → comparison of different fibrosis stages within NASH.

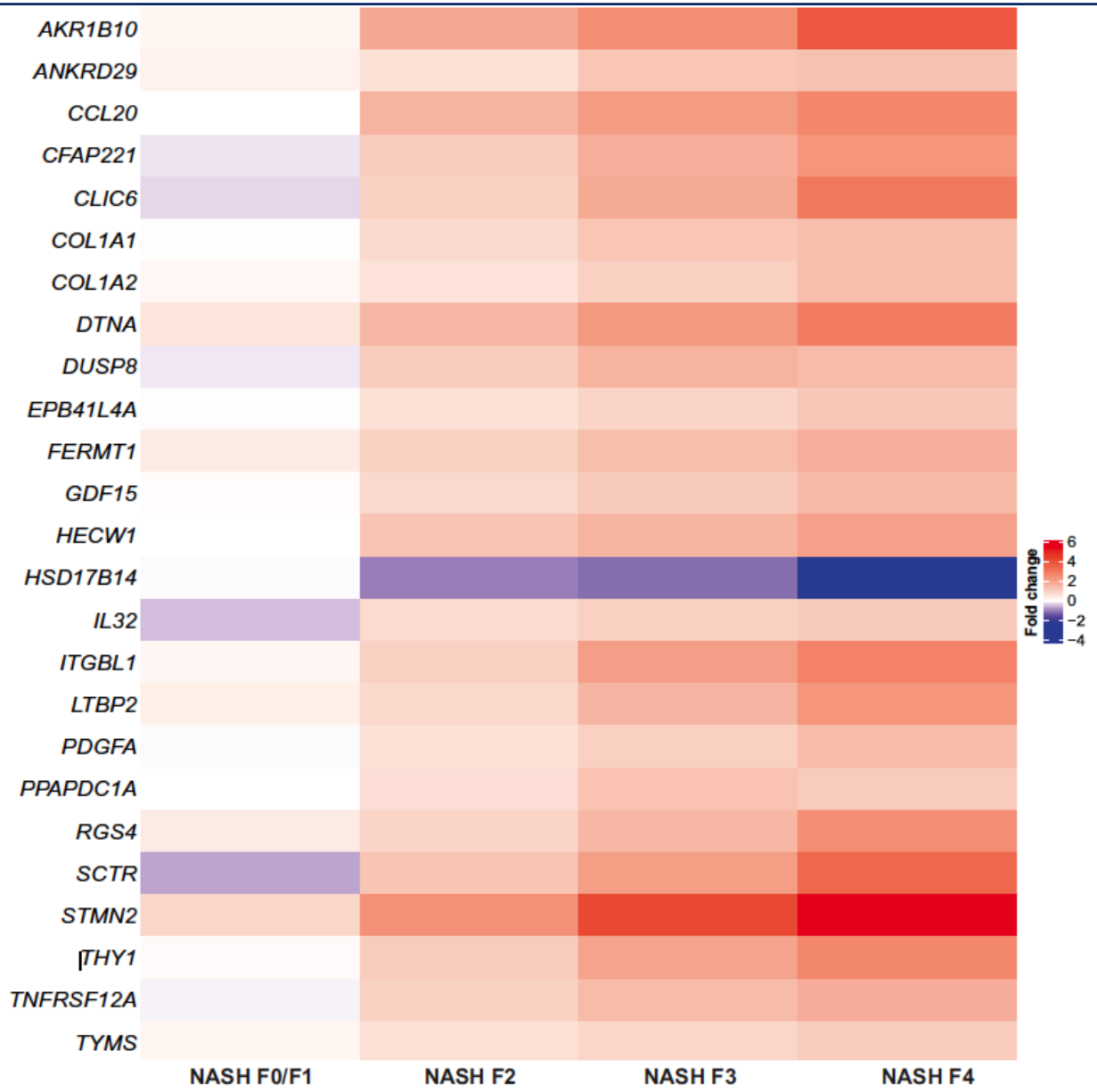


Govaere O et al. Sci Transl Med, 2020.

Transcriptomic profiling across the nonalcoholic fatty liver disease spectrum reveals gene signatures for steatohepatitis and fibrosis

3 Heatmap of the 25-gene signature associated with advanced NAFLD identified by using NAFL or NASH F0/F1 as a baseline. Expression fold change is compared with NAFL.

Expression fold change is compared with NAFL.



Govaere O et al. Sci Transl Med, 2020.

Transcriptomic profiling across the nonalcoholic fatty liver disease spectrum reveals gene signatures for steatohepatitis and fibrosis

4

Proteomics to assess whether circulating protein concentrations of the 25 differentially expressed gene set accurately reflected histological disease severity as an exemplar for future potential biomarker development.

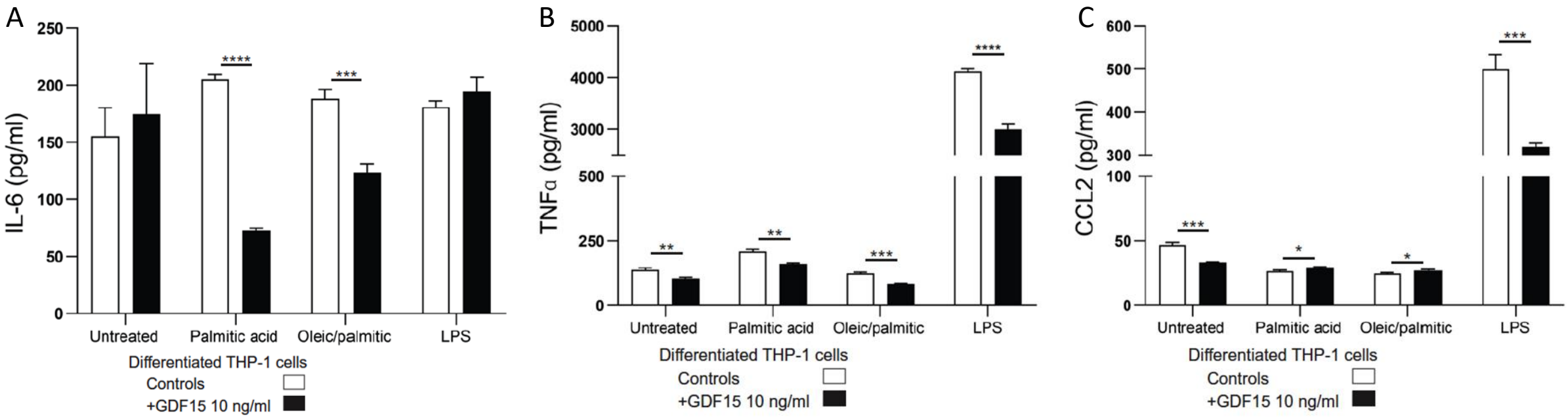
Serum AKR1B10 and GDF15 positively correlate with disease stage and histological activity score

	AKR1B10	GDF15
Steatosis Grade	✔✔✔✔✓	
Ballooning	✔✔✔✔✓	✔✔✔✔✓
Kleiner inflammation score	✔✔✔✔✓	✔✔✔✔✓
SAF inflammation score	✔✔✔✔✓	✔✔✔✔✓
Fibrosis stage ✔✔	✔✔✔✔✓	✔✔✔✔✓

Govaere O et al. Sci Transl Med, 2020.

Endoplasmic reticulum stress–induced GDF15 reduces the inflammatory response in vitro

- ↑ in AKR1B10 and GDF15 protein expression in Hep G2 cells after endoplasmic reticulum stress induced with tunicamycin and thapsigargin treatment but not after lipid loading.
- In macrophages GDF15 supplementation decreases IL-6, TNFα and C-C motif chemokine ligand 2 (CCL2) compared to controls.



Govaere O et al. Sci Transl Med, 2020.

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The Role of Insulin Resistance in NASH

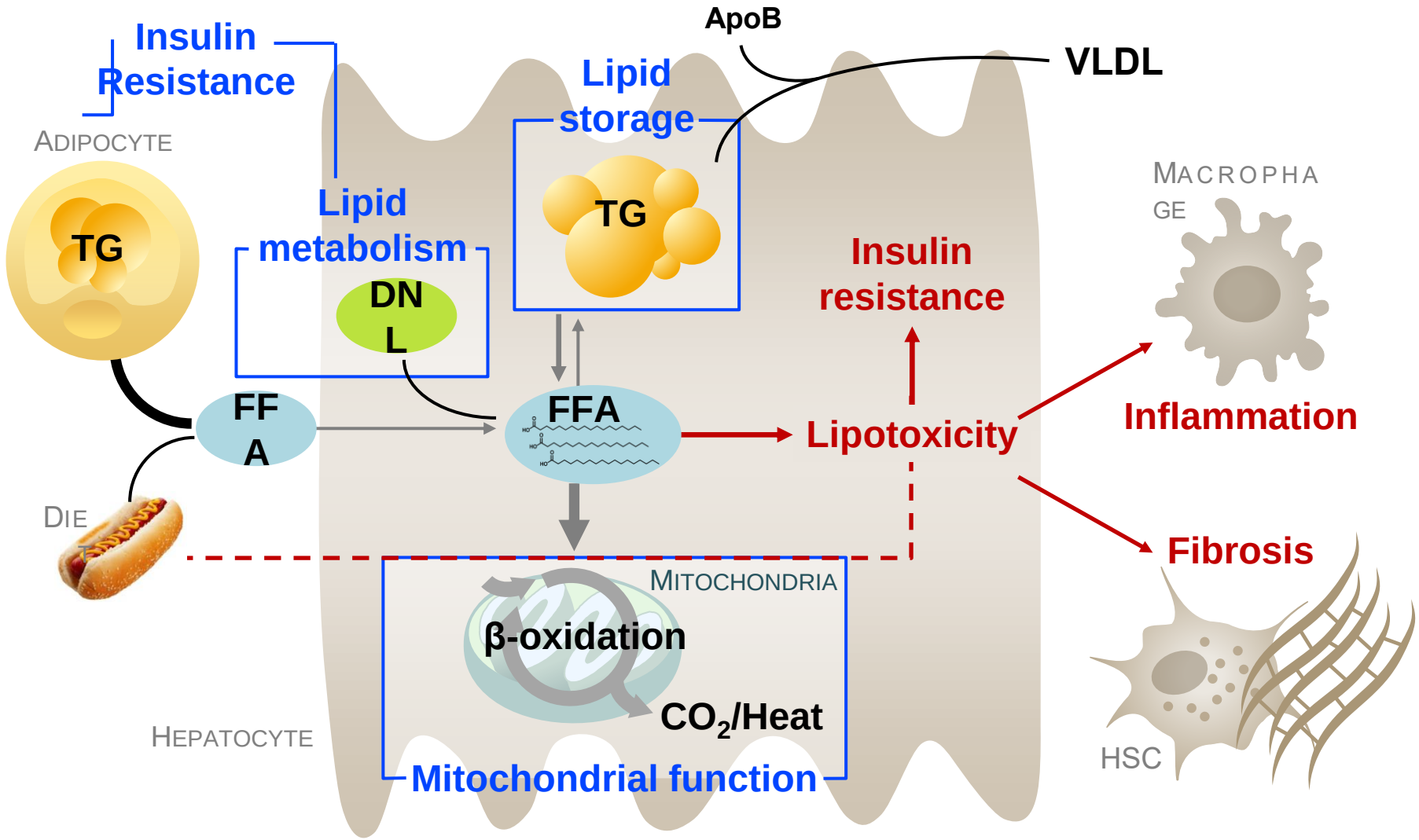
Christos S. Mantzoros, MD, DSc, PhD, h.c. mult.
Professor, Harvard Medical School

Editor-in-chief, Metabolism, Clinical and Experimental

Proposed Pathophysiologic Mechanisms for NAFLD/NASH

GAFLD

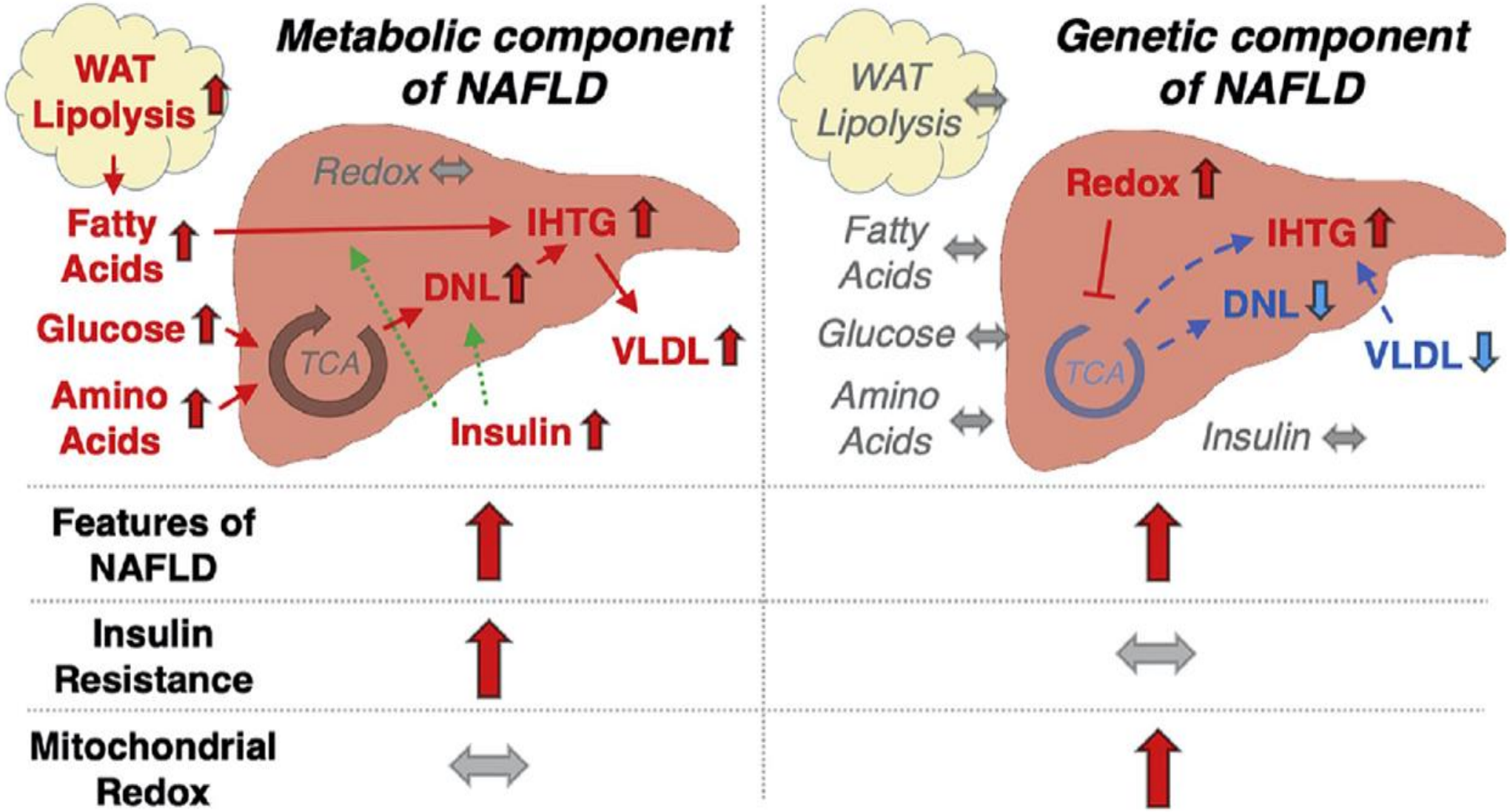
MAFLD
Central Obesity
Lipodystrophy
Sarcopenia



ApoB = apolipoprotein B; DNL = de novo lipogenesis; FFA = free fatty acid; VLDL = very low density lipoprotein.

1. *Browning JD et al. J Clin Invest, 2004.* ***2.*** *Samuel VT et al. J Clin Invest, 2016.* ***3.*** *Ramos-Roman MA et al. Arterioscler Thromb Vasc Biol, 2012.* ***4.*** *Sanders FW et al. Biol Rev, 2016.* ***5.*** *Neuschwander-Tetri et al. Hepatol, 2010.* ***6.*** *Peverill W et al. In J Mol Sci, 2014.*

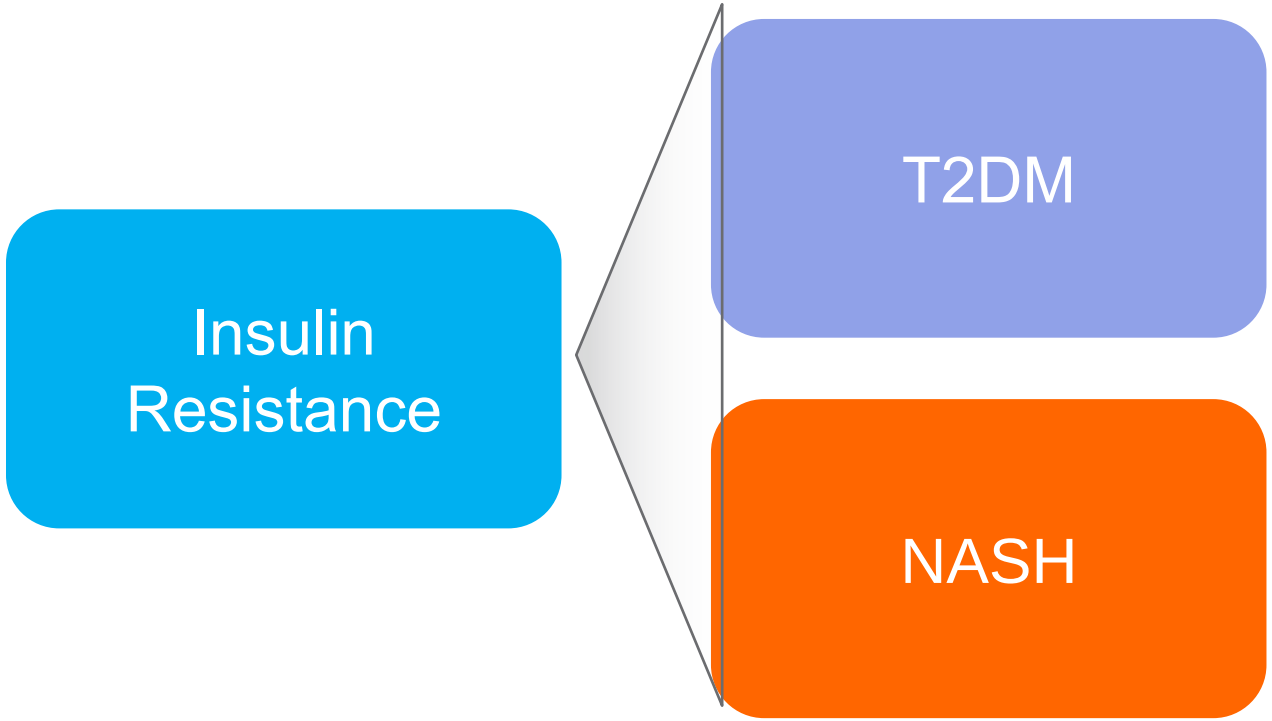
Distinct Contributions of Metabolic Dysfunction and Genetic Risk Factors in the Pathogenesis of NAFLD



Luukkonen PK et al. J Hepatol, 2021.

T2DM and NASH are Both Consequences of Insulin Resistance

NASH is a largely unrecognized complication of insulin resistance



Insulin resistance contributes to NASH

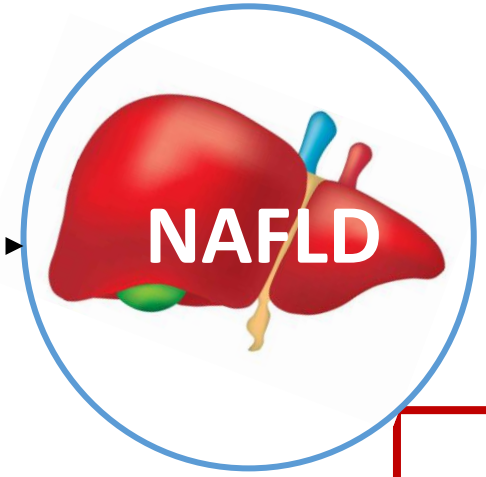
- Insulin resistance induces triglyceride lipolysis in adipocytes and results in excess FFA delivery to the liver
- Hyperinsulinemia enhances FFA uptake and activates de novo lipogenesis (DNL)
- Hyperglycemia can activate DNL

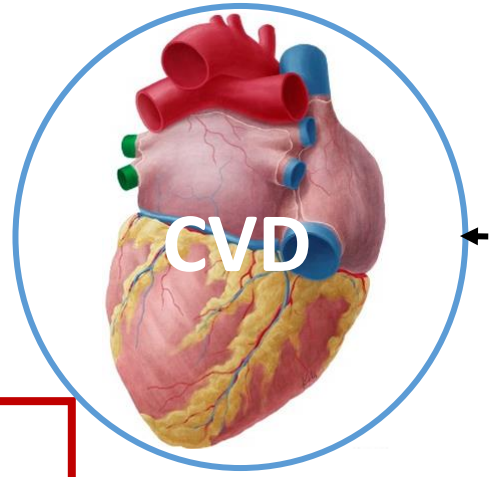
FFA = free fatty acids

1. Ballestri S et al. J Gastroenterol Hepatol, 2015. 2. Bril F and Cusi K. Endocrinol Metab Clin N Am, 2016. 3. Lee CC et al. Diabetes Care, 2015. 4. Melsom T et al. Am J Kidney Dis, 2016. 5. Nguyen TT et al. Diabetes Care, 2007. 6. Smith AG et al. Diabetes Care, 2006.

A new Challenge in Medicine – Obesity and its Comorbidities

Lifestyle intervention/exercise
Pioglitazone
? GLP-1 RAs
? SGLT2i
Statins

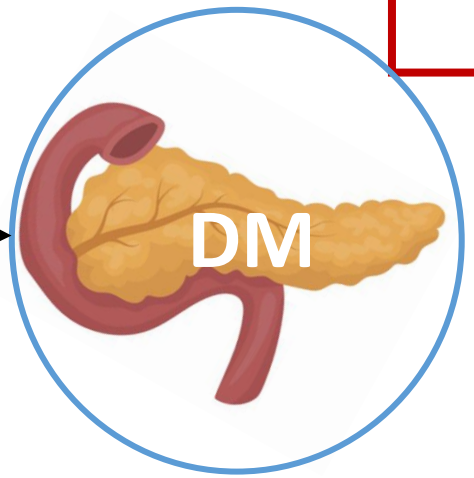


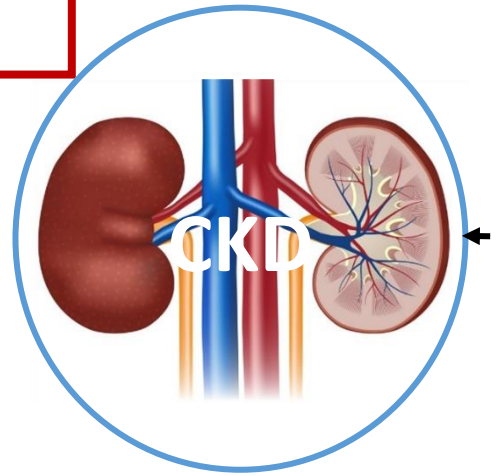


Lifestyle intervention/exercise
GLP-1 RAs
SGLT2i
Pioglitazone
Metformin
Statins

AT storage exceeded
IR
MetS
Microinflammation

Lifestyle intervention/exercise
Bariatric surgery
Pioglitazone
GLP-1 RAs
SGLT2i
Statins





Lifestyle intervention/exercise
GLP-1 RAs
SGLT2i
Statins

Abbreviations: AT, adipse tissue; CVD, cardiovascular disease; DKD, diabetic kidney disease; GLP-1 RA, glucagon-like peptide-1 receptor agonist; IR, insulin resistance; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NAFPD, non-alcoholic fatty pancreas disease; SGLT2i, sodium-glucose co-transporter-2 inhibitor; T2DM, type 2 diabetes mellitus.

Thank you!

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Foundations of Cardiometabolic Health Certification Course

Certified Cardiometabolic Health Professional (CCHP)

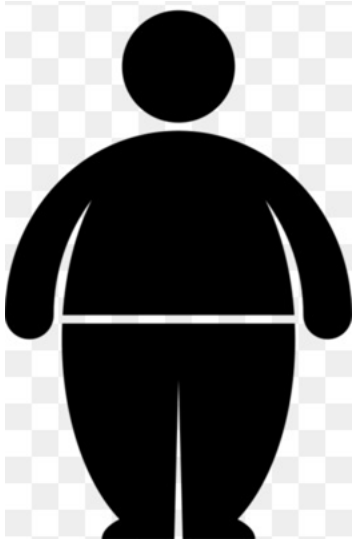
NAFLD & NASH: Epidemiology & Risk Factors

Christos S. Mantzoros, MD, DSc, PhD, h.c. mult.
Professor, Harvard Medical School

Editor-in-chief, Metabolism, Clinical and Experimental

Risk Factors for NAFLD and NASH

Obesity



- Obesity is linked not only to diabetes, Metabolic Syndrome and CVD, but also to NAFLD and NASH and related comorbidities.
- The close link between obesity and NAFLD/NASH is supported by treatment via weight loss, which can even result in resolution of NASH and fibrosis regression.

Pathophysiology

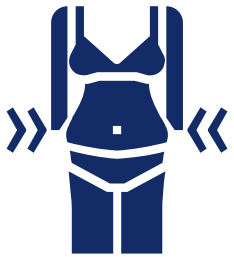
- Obesity is a key contributor to lipotoxicity, as well as subsequent liver inflammation and fibrosis, through the release of FFAs.
- Patients with obesity have a higher prevalence of NAFLD and NASH and a higher rate of fibrosis progression than patients who are not obese.

Calzadilla Bertot L et al. Int J Mol Sci, 2016.
Benedict M et al. World J Hepatol, 2017.
Romero-Gomez M et al. J Hepatol, 2017.

Multiple Risk Factors can Help Identify Patients with Advanced NASH

RISK FACTORS

T2DM	Obesity	Metabolic Syndrome >features = >risk	Advanced Age (>50 years)	Others



- Hispanic
- High ALT/AST
- Low platelets
- Low Albumin

1) Adams LA et al. Am J Gastroenterol, 2010. 2) Chalasani N et al. Hepatol, 2012. 3) Chalasani N et al. Hepatol, 2018. 4) Doycheva I et al. J Diabetes Compl, 2013. 5) EASL, EASD, EASO. J Hepatol, 2016. 6) Hamaguchi M, et al. Ann Intern Med, 2005. 7) Loomba R et al. Hepatol, 2012. 8 Neuschwander-Tetri BA et al. Hepatol, 2010. 9) Noureddin M et al. Clin Liver Dis, 2012. 10) Ong JP et al. Obes Surg, 2005. 11. Stephanova M et al. Ailment Pharmacol Ther, 2010. 12) Suzuki A et al. Hepatol, 2005.

Risk Factors and Clinical Presentation of NAFLD/NASH

Risk Factors

- Obesity
- T2DM
- Hypertension
- Dyslipidemia

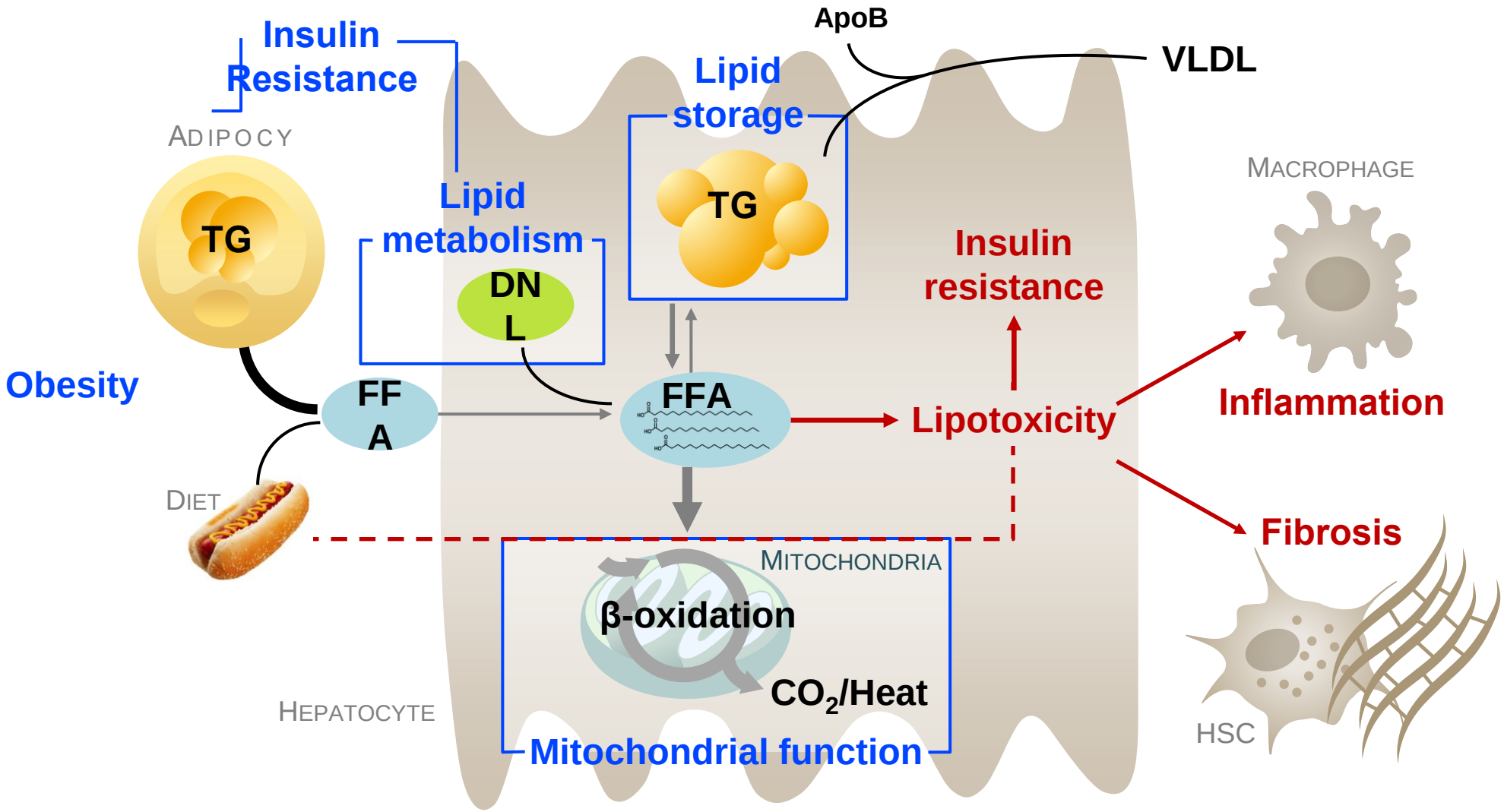
The pre-test probability of NAFLD may approach 75% or higher in these populations

Clinical Presentation

- Asymptomatic
- Symptomatic with non-specific symptoms like fatigue, malaise and RUQ discomfort

Chalasani N et al. Hepatology, 2018.
Ramesh S et al. J Hepatol, 2005.

Insulin Resistance Promotes an Increase in Free Fatty Acid Traffic to the Liver which can Trigger Hepatic Lipotoxicity

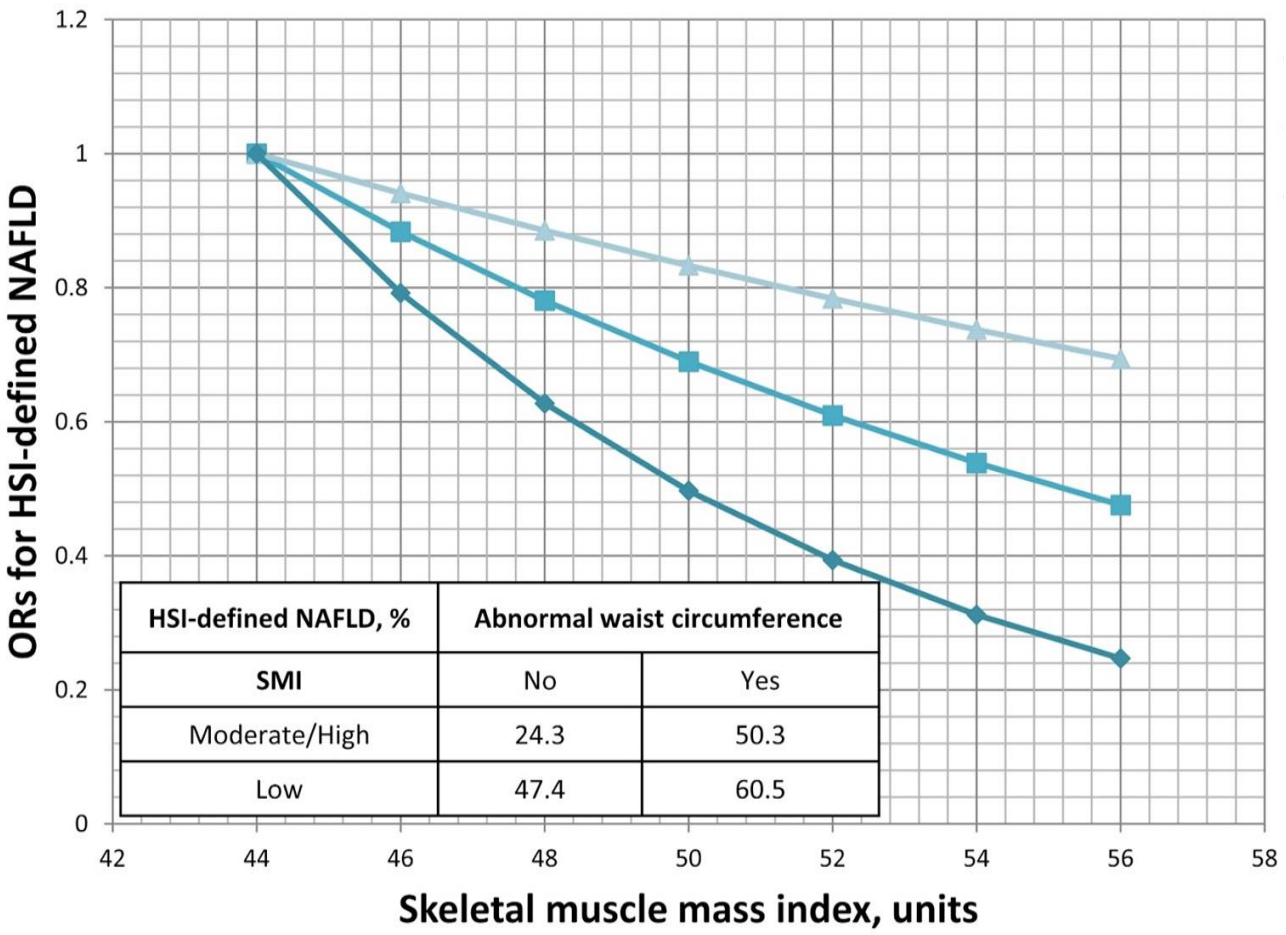


Slide courtesy of Jim Trevaskis

ApoB = apolipoprotein B; DNL = de novo lipogenesis; FFA = free fatty acid; VLDL = very low density lipoprotein.

1. *Browning JD et al. J Clin Invest, 2004.* ***2.*** *Samuel VT et al. J Clin Invest, 2016.* ***3.*** *Ramos-Roman MA et al. Arterioscler Thromb Vasc Biol, 2012.* ***4.*** *Sanders FW et al. Biol Rev, 2016.* ***5.*** *Neuschwander-Tetri et al. Hepatol, 2010.* ***6.*** *Peverill W et al. In J Mol Sci, 2014.*

Epidemiology



HSI-defined NAFLD, %	Abnormal waist circumference	
SMI	No	Yes
Moderate/High	24.3	50.3
Low	47.4	60.5

ORs and 95%Cis of SMI in relation to HIS-defined NAFLD

SMI, per 1 unit increase	OR (95%CI)
Total sample (n=3,042)	0.94 (0.92, 0.95)
Sample with normal waist circumference (n=1,549)	0.89 (0.84, 0.95)
Sample with abnormal waist circumference (n=1,493)	0.97 (0.94, 1.00)

Mantzoros et al, 2021.

HR and 95%CI of NAFLD in Relation to 10-year CVD Incidence According to SMI in the Total Sample, as well as Separately for Participants with Abnormal Waist Circumference (Indicating Central Obesity) (*n*=2,020)

	Total sample	Sample with low SMI	Sample with moderate/ high SMI	Sample with central obesity	Sample without central obesity	Sample with central obesity & low SMI	Sample with central obesity & moderate/ high SMI	Sample without central obesity & low SMI	Sample without central obesity & moderate/ high SMI
N/cases	2,020/317	672/153	1,348/164	1,084/211	936/106	586/131	498/80	83/22	853/81
p for interaction		$p_{(NAFLD*SMI)}$=0.03		$p_{(NAFLD*waist\ circumference)}$=0.05		$p_{(NAFLD * SMI * central\ obesity)}$=0.04			
HSI-defined NAFLD, yes vs. no	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	3.00 (2.28, 3.95)*	2.51 (1.62, 3.91)*	2.90 (2.00, 4.19)*	2.23 (1.57, 3.17)*	1.80 (0.72, 3.19)	2.85 (1.67, 3.90)**	2.29 (1.32, 3.98)**	2.40 (1.51, 7.80)**	1.97 (0.80, 3.23)
Multi-adjusted model	1.81 (1.31, 2.50)***	2.07 (1.30, 3.30)**	1.33 (0.87, 2.05)	1.67 (1.13, 2.46)**	1.10 (0.45, 2.60)	2.30 (1.31, 3.41)***	1.68 (0.90, 3.13)	1.85 (1.18, 6.69)***	1.21 (0.63, 2.95)
Multi-adjusted model + waist circumference	1.62 (1.19, 2.20)***	2.01 (1.24, 3.27)**	1.34 (0.88, 2.05)	-	-	-	-		-
Multi-adjusted model + SMI	1.38 (1.05, 2.07)***	-	-	1.55 (1.08, 2.35)***	1.10 (0.44, 2.61)	-	-		-

HRs and their 95%CIs were obtained from multiadjusted Cox regression analysis. Low SMI corresponded to the 1st SMI tertile and moderate/high SMI corresponded to 2nd and 3rd SMI tertiles. Multi-adjusted model was adjusted for age, sex, current smoking, physical activity, MedDietScore, daily ethanol intake, hypertension, hypercholesterolemia, diabetes mellitus and family history of cardiovascular disease. *p<0.001 **p<0.01 ***p<0.05

Mantzoros et al, 2021.

C-index of multiadjusted models to evaluate the discriminative ability of a standard definition of metabolic status in combination with or without waist circumference and NAFLD* against 10-year cardiovascular disease event in participants of ATTICA study.

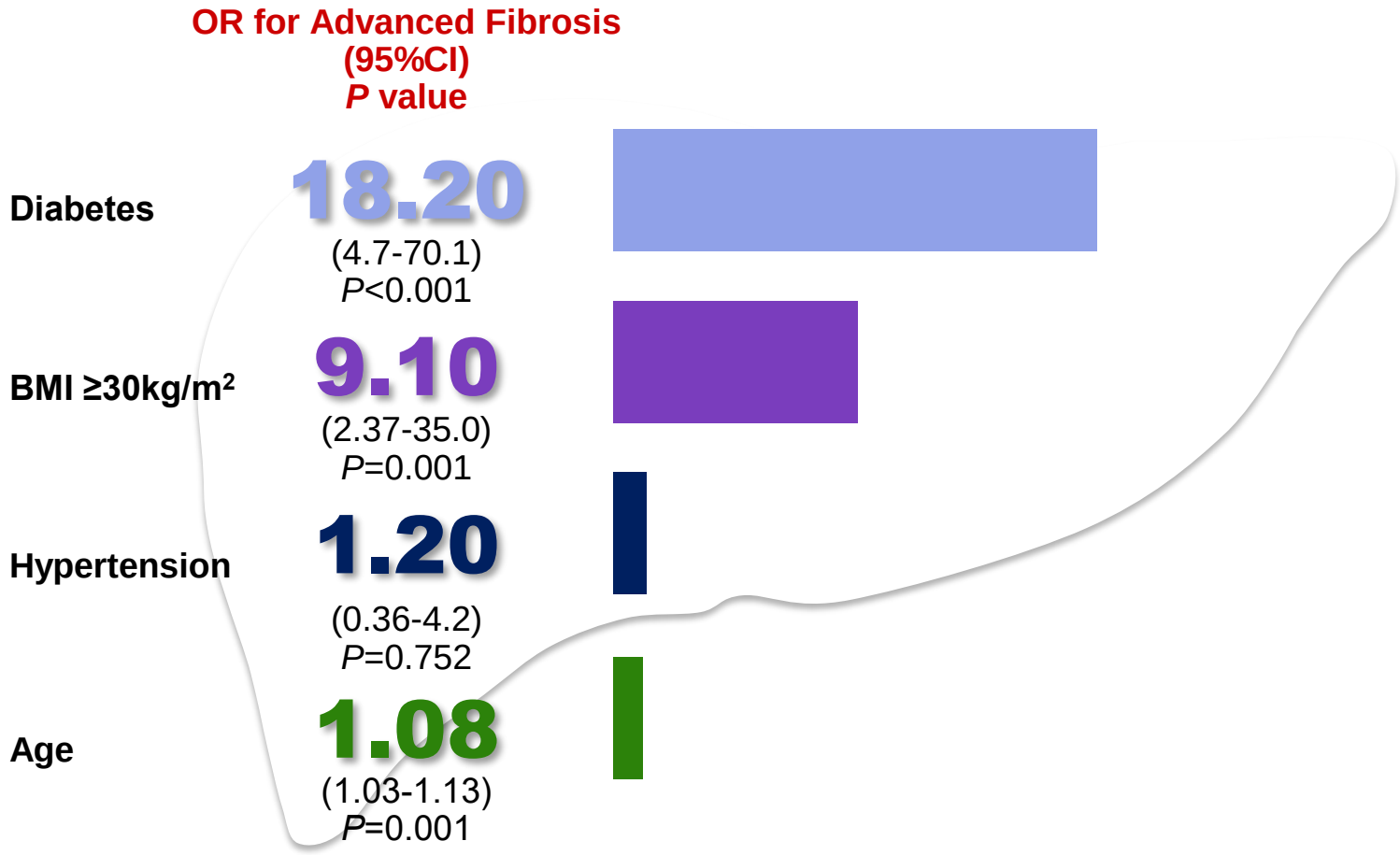
	C-index (95%CI)	p-value	C-index changes (95%CI)	p-value
TOTAL SAMPLE (n=1,890)				
Model 1: Predictive model using the standard definition of metabolically healthy vs. unhealthy status (i.e. defined as total absence all MetS components vs. presence of at least one of them)	0.699 (0.659, 0.711)	<0.001	-	-
Model 1 + waist circumference	0.710 (0.679, 0.720)	<0.001	0.011 (0.009, 0.020)	0.01
Model 1 + NAFLD status only	0.711 (0.689, 0.734)	<0.001	0.012 (0.008, 0.018)	0.002
Model 1 + joint evaluation of both waist circumference and NAFLD status	0.718 (0.681, 0.723)	<0.001	0.019 (0.012, 0.022)	0.002
SAMPLE WITH OBESE PARTICIPANTS (BMI≥30kg/m^2) (n=874)				
Model 1: Predictive model using the standard definition of metabolically healthy vs. unhealthy status (i.e. defined as total absence all MetS components vs. presence of at least one of them)	0.690 (0.618, 0.700)	<0.001	-	
Model 1 + waist circumference	0.702 (0.627, 0.714)	<0.001	0.012 (0.009, 0.014)	0.04
Model 1 + NAFLD status only	0.719 (0.640, 0.731)	<0.001	0.029 (0.022, 0.031)	0.003
Model 1 + joint evaluation of both waist circumference and NAFLD status	0.719 (0.641, 0.731)	<0.001	0.029 (0.023, 0.031)	0.003

All models were adjusted for traditional CVD risk factors i.e. age, sex, current smoking and family history of cardiovascular disease

Manztoros et al. In press

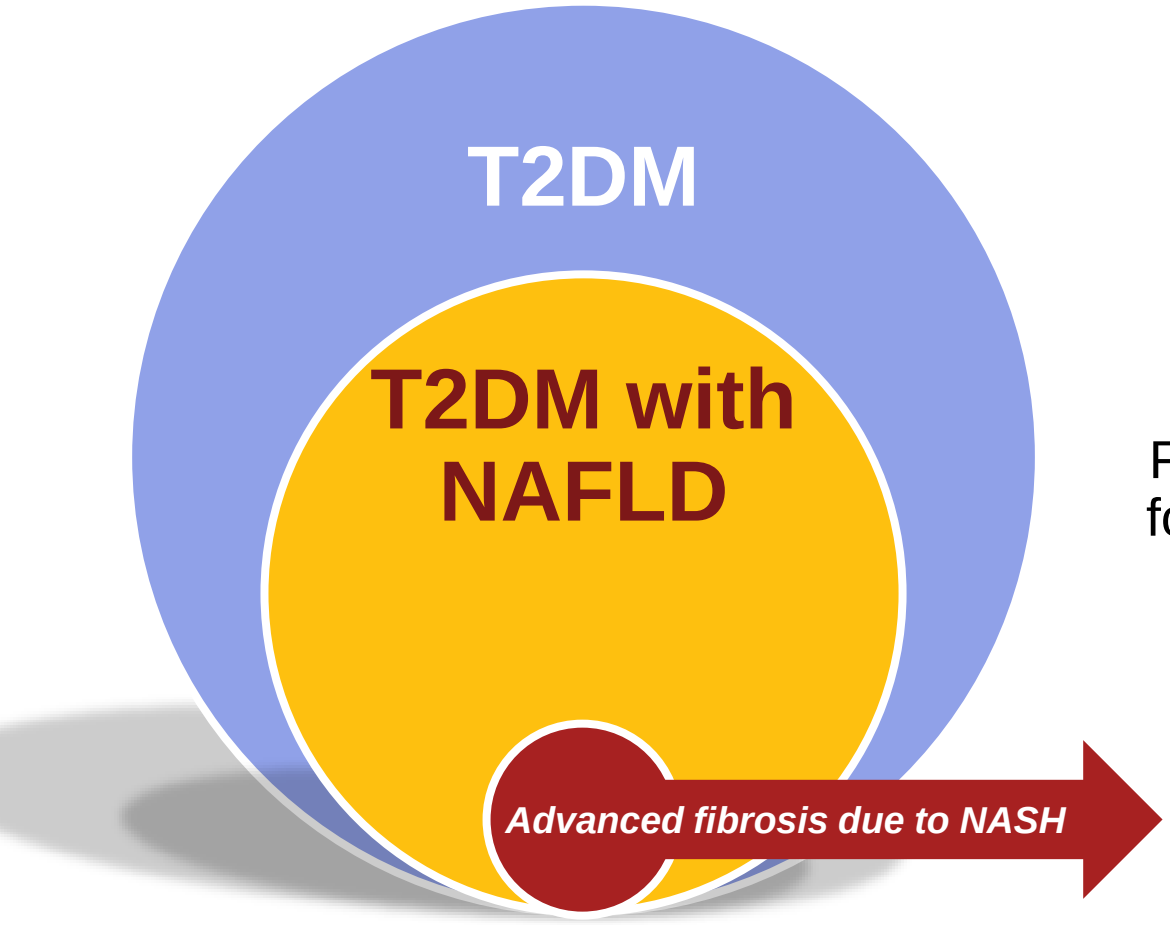
Diabetes was the Strongest Predictor of Advanced Fibrosis in Patients with NAFLD

- Cross-sectional study using 2011-2014 NHANES data to assess predictors of advanced fibrosis in NAFLD patients diagnosed by NAFLD fibrosis score (NFS)



NHANES = National Health and Nutrition Examination Survey.
Wong RJ et al. Aliment Pharmacol Ther, 2017.

Approximately 7% to 13% of those with T2DM have Advanced Fibrosis due to NASH



Four studies in 2,720 T2DM patients evaluated for NAFLD and advanced fibrosis due to NASH

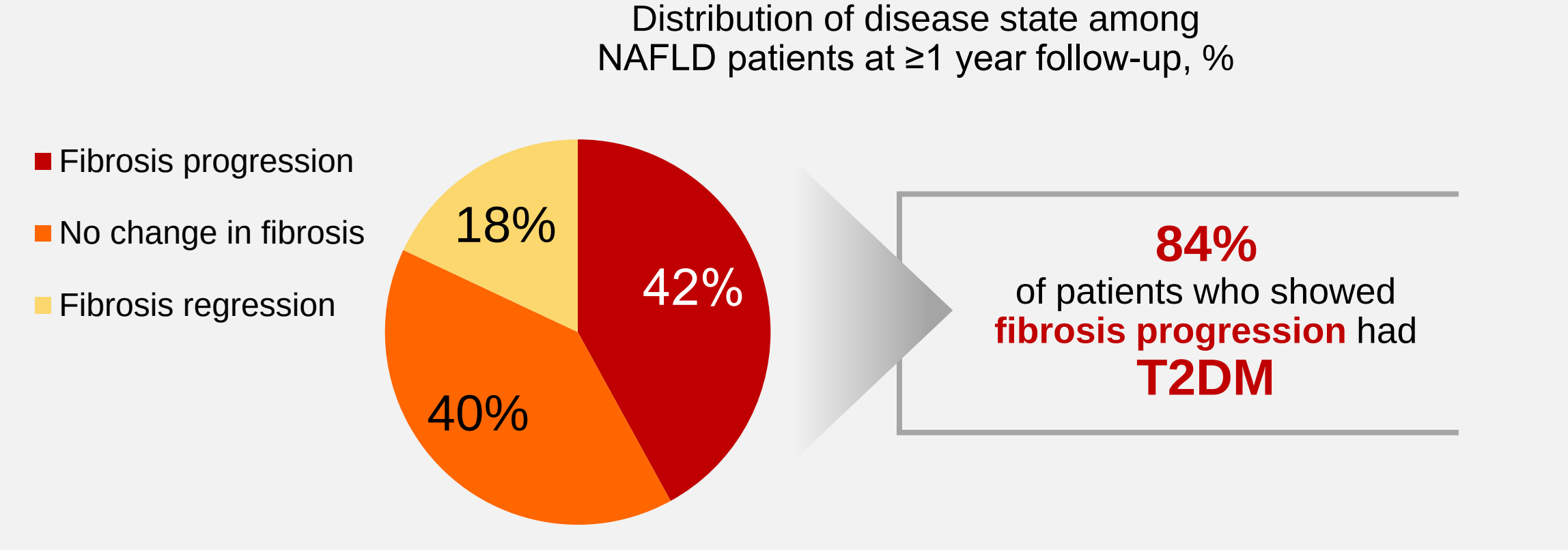
7% to 13%

of patients with T2DM may have
advanced fibrosis due to NASH

1. Arab JP et al. Ann Hepatol, 2016. 2. Doycheva I et al. Aliment Pharmacol Ther, 2016. 3. Kwok R et al. Gut, 2015. 4. Lai LL et al. J Gastroenterol Hepatol, 2018.

Patients with T2DM and NAFLD are at an increased Risk for Progression of Fibrosis

- The DELTA study cohort of 108 biopsy-proven NAFLD patients with at least two biopsy samples demonstrated the evolving natural history of NAFLD.

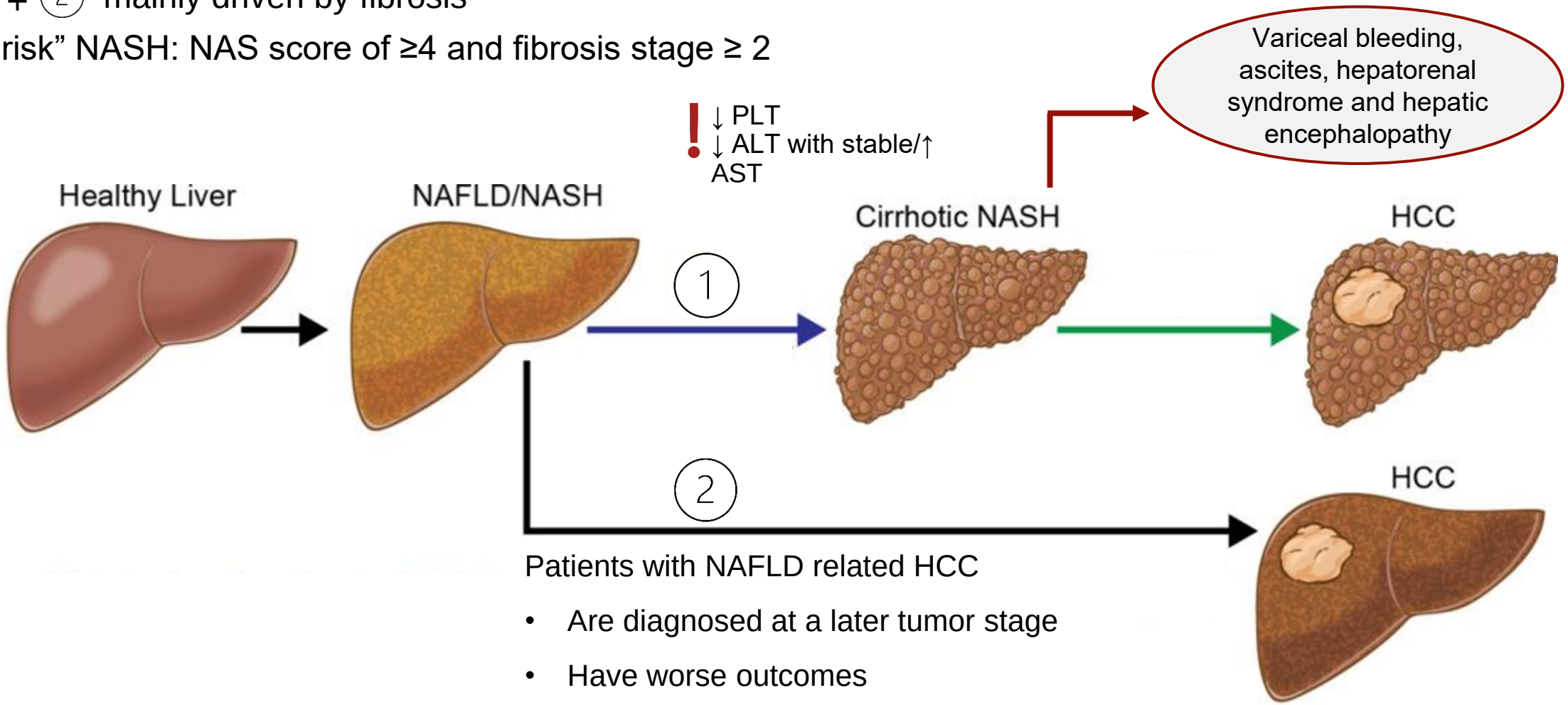


McPherson S et al. J Hepatol, 2015.

Hepatic Complications

① + ② mainly driven by fibrosis

"At risk" NASH: NAS score of ≥4 and fibrosis stage ≥ 2



Patients with NAFLD related HCC

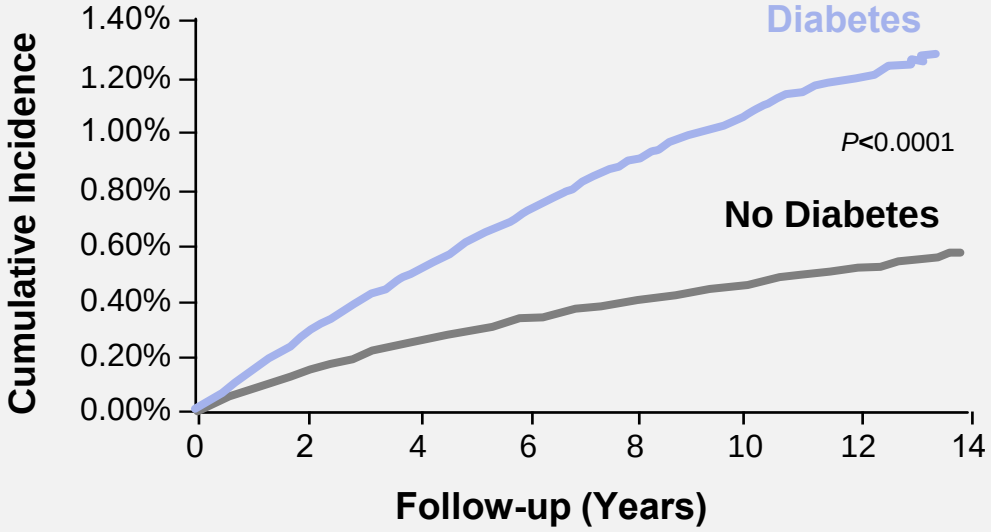
- Are diagnosed at a later tumor stage
- Have worse outcomes
- Potentially lower response to immune checkpoint inhibitor treatment

1) Garcia-Tsao G et al. Hepatology, 2017. 2) Kanwal F et al. Gastroenterology, 2018. 3) Younossi ZM et al. Hepatology, 2015. 4) Piscaglia F et al. Hepatology, 2016. 5) Pfister D et al. Nature, 2021. 6)Dulai PS et al. Hepatology, 2017. 6) D'Avola D et al. Clin Liver Dis, 2016.

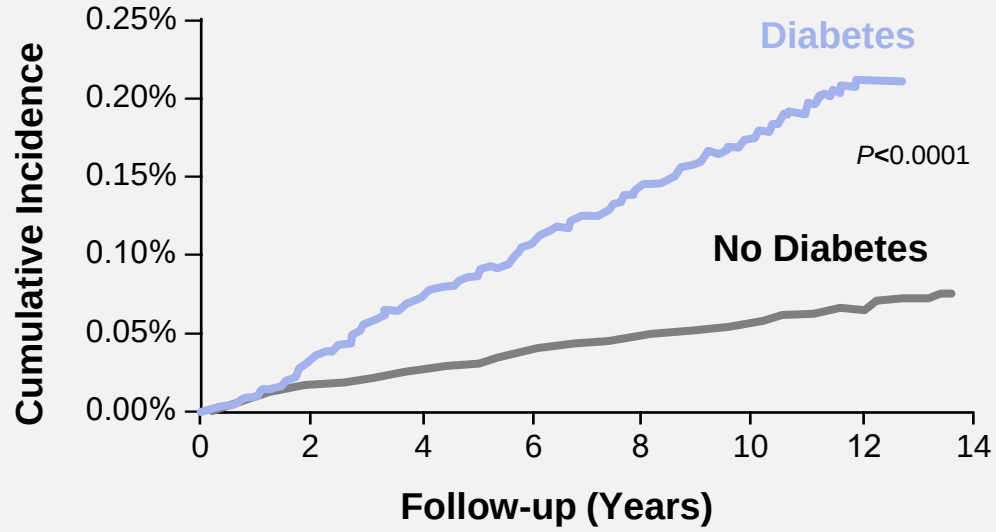
Patients with T2DM are at an increased Risk for Chronic Nonalcoholic Liver Disease and HCC

- US Department of Veterans Affairs national databases were used to evaluate the association between diabetes and risk for developing CNLD and HCC

CNLD[a]



HCC

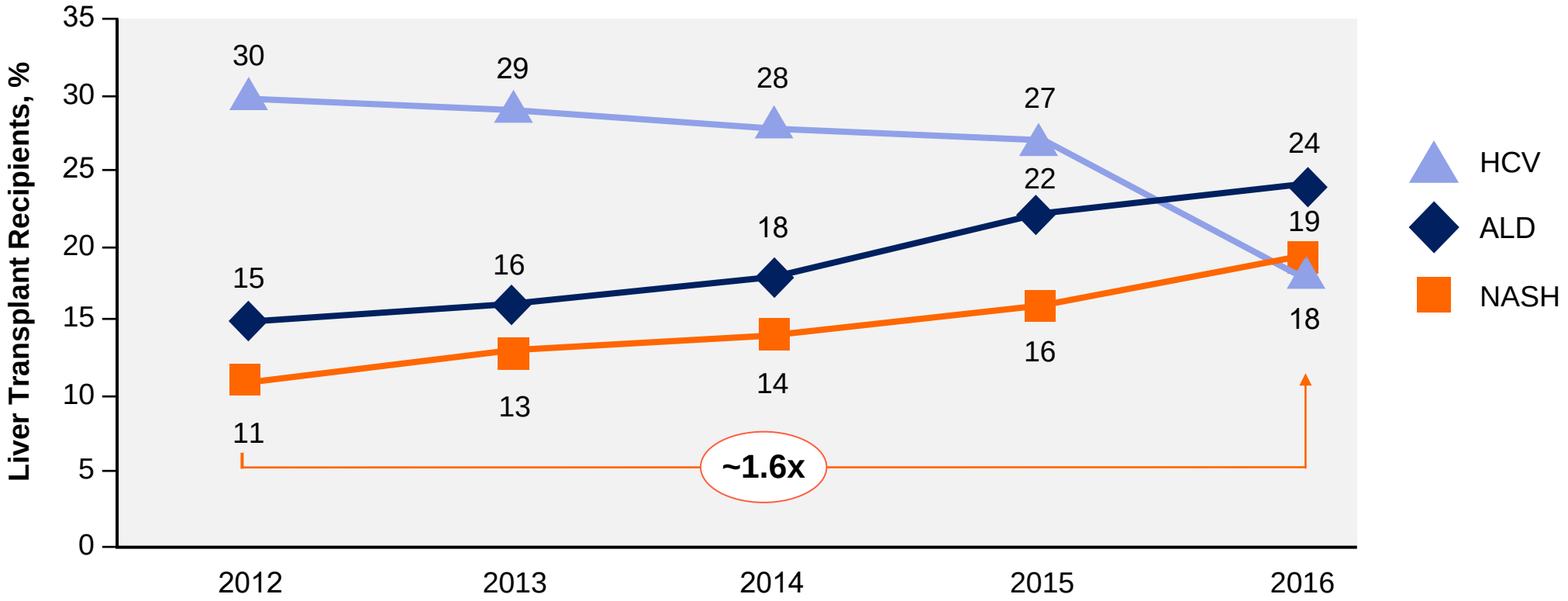


[a]Diagnosis of CNLD included chronic hepatitis (ICD-9 571.40, 571.41, 571.49), cirrhosis of liver without mention of alcohol (ICD-9 571.5), biliary cirrhosis (ICD-9 571.6), unspecified chronic liver disease without mention of alcohol (571.9), hepatic encephalopathy (ICD-9 572.2), portal hypertension (ICD-9 572.3), and other sequelae of chronic liver disease (ICD-9 572.8)
CNLD = chronic nonalcoholic liver disease.

El-Serag HB et al. Gastroenterology, 2004.

NASH Surpasses HCV as an Indication for Liver Transplant in 2016 in the US

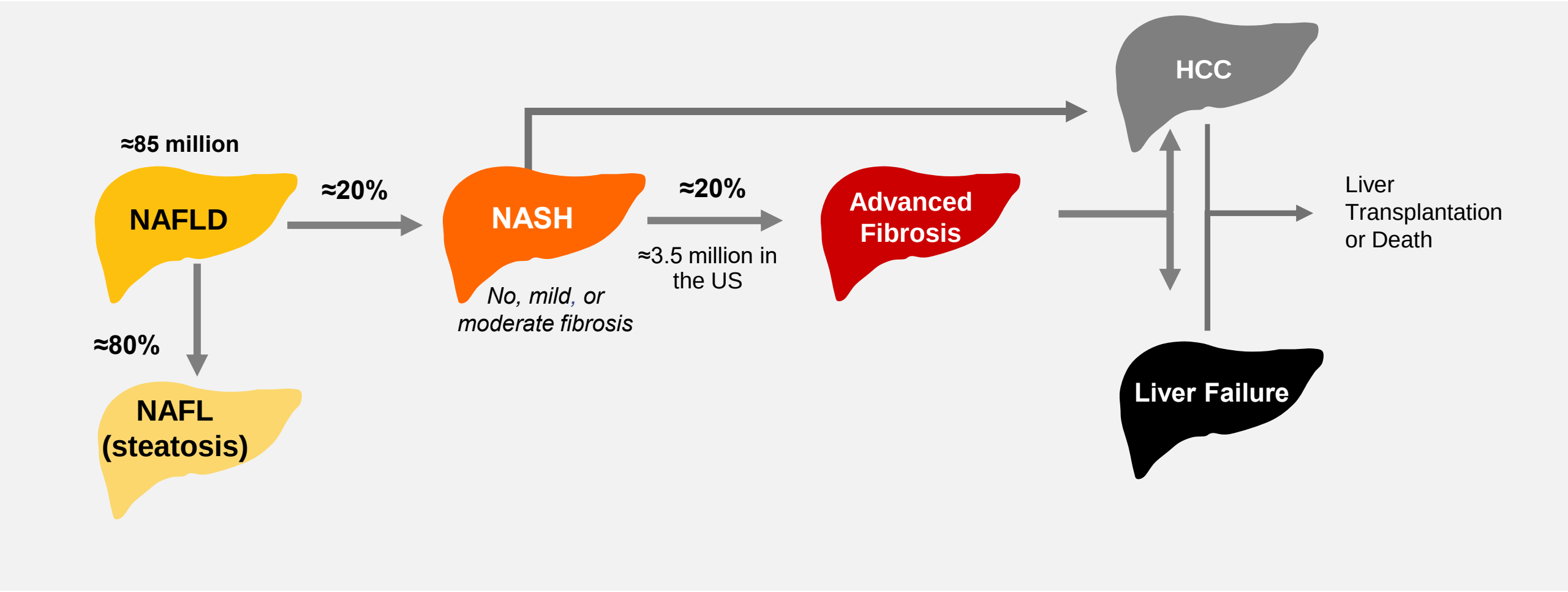
- UNOS database was used to analyze the leading indications for liver transplant among patients with hepatitis C virus infection (HCV), Alcoholic Liver Disease (ALD) and NASH from 2012 to 2016



- NASH now accounts for approximately 1/5 of all liver transplant listings and is expected to continue to increase substantially over next 10 years

Cholankeril G et al. *Clin Gastroenterol Hepatol*, 2018.

Progressing from NAFLD to Liver Related Morbidity and Mortality

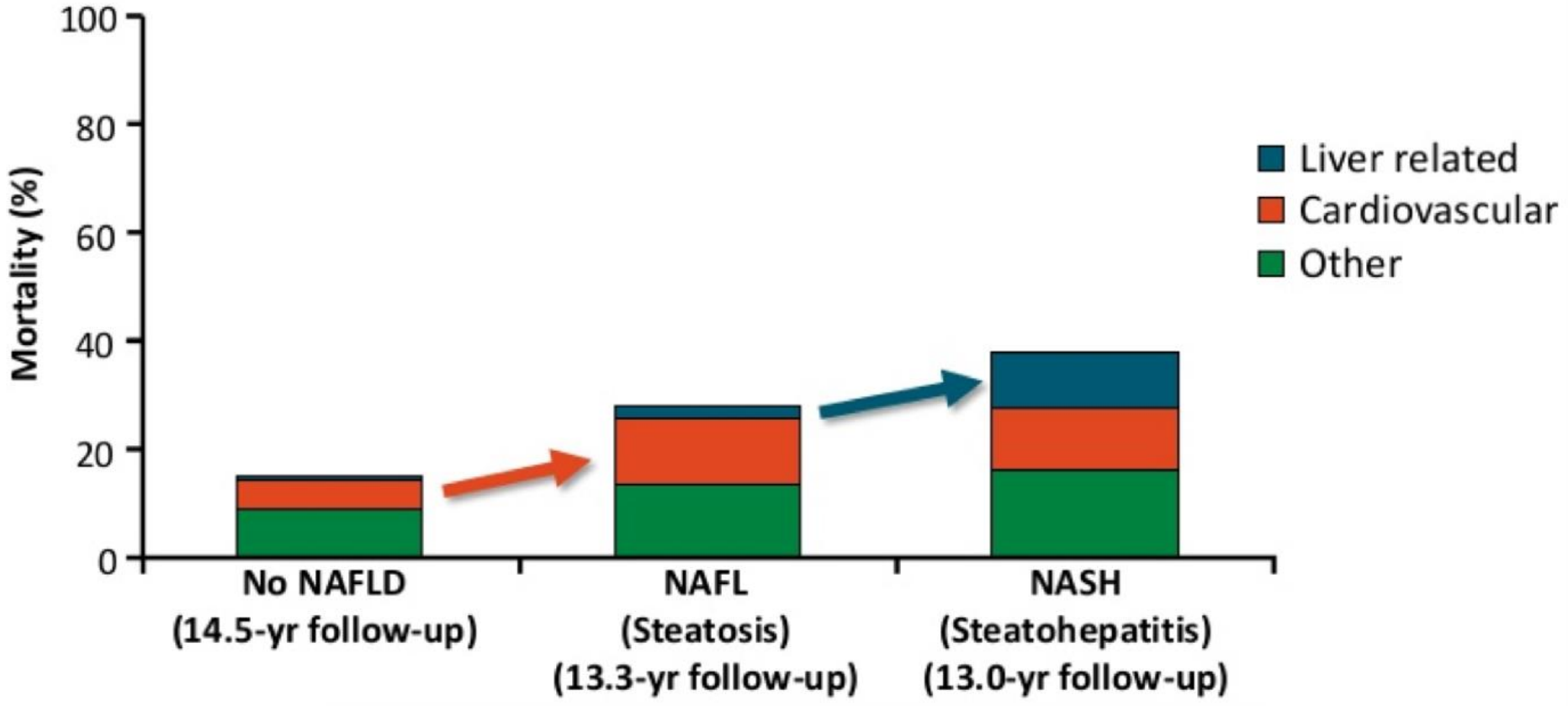


Angulo P et al. Gastroenterology, 2015.
Estes C et al. Hepatology, 2018.
Ahmed A et al. Clin Gastroenterol Hepatol, 2015.
deLemos A et al. EASL 2018. Abstract FRI-134.
Miller E. EASL 2018. SAT-483.

Mortality Associated with Isolated Steatosis and NASH

Analysis of all-cause mortality in 6 separate studies among patients without NAFLD vs with and without NASH

- NAFLD determined by ultrasound; NASH determined by liver biopsy



Bril F et al. Endocrinol Metab Clin North Am, 2016.

Is there an Association between Bariatric Surgery and long-term Major Adverse Liver / Cardiovascular Events in NASH and Obesity?

Retrospective Cohort. **1158** patients: biopsy-proven NASH.

650 Bariatric Surgery

508 Nonsurgical Controls

7 years Follow-Up

- Major adverse **Liver Outcomes:**

5 patients

-Cumulative incidence at 10 years:
2.3% (95%CI, 0%-4.6%).

- **MACE:**

39 patients.

-Cumulative incidence at 10 years:
8.5%
(95%CI, 5.5%-11.4%).

- Major adverse **Liver Outcomes:**

40 patients

-Cumulative incidence at 10 years:
(AARD, **12.4%** [95% CI, 5.7%-19.7%];
aHR, 0.12 [95%CI, 0.02-0.63];
p=.01)

- **MACE:**

60 patients.

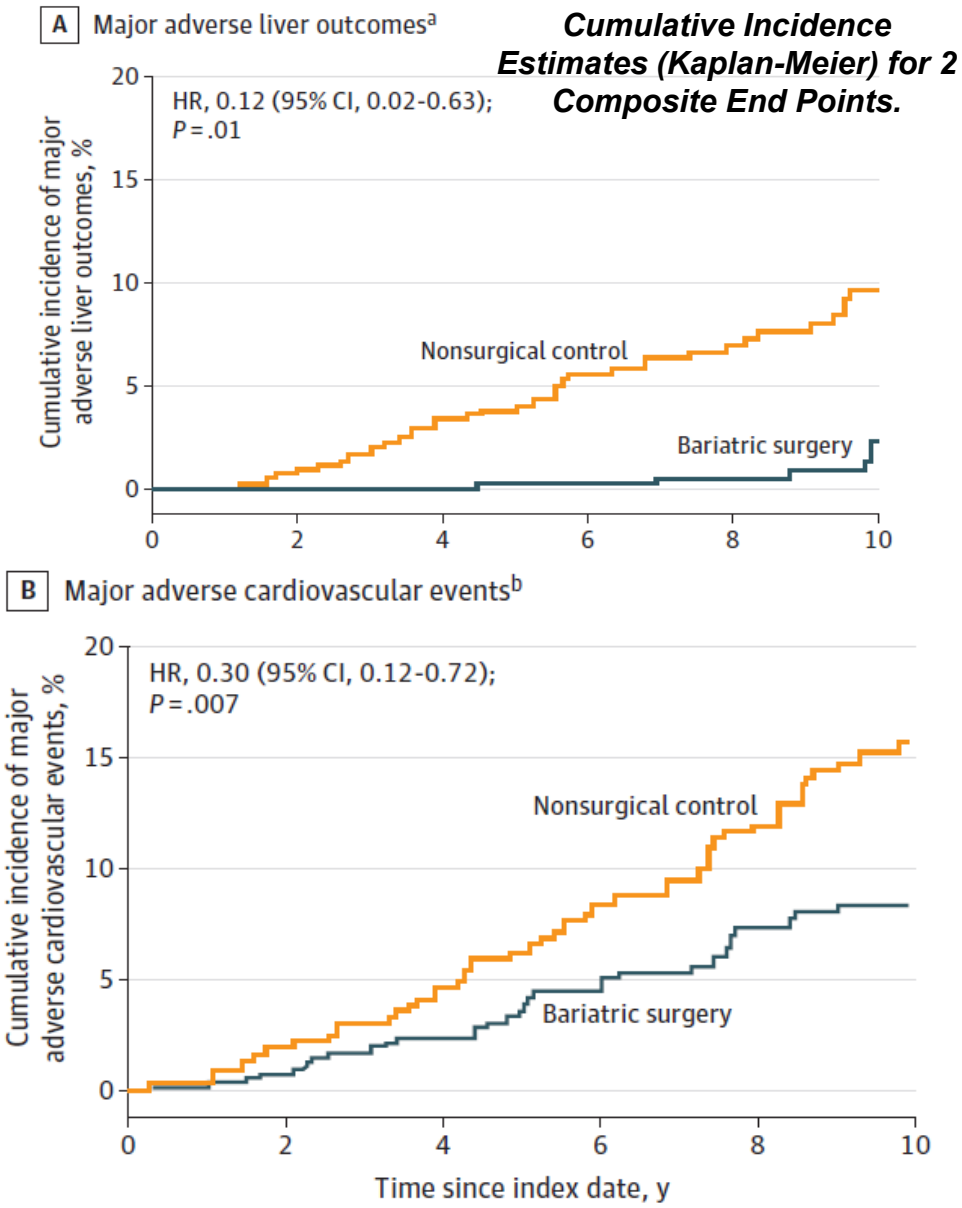
-Cumulative incidence at 10 years:
15.7%(95%CI, 11.3%-19.8%)

(AARD, 13.9% [95%CI, 5.9%-21.9%];
aHR, 0.30 [95%CI, 0.12-0.72]; p=.007.

AARD: adjusted absolute Risk Difference. aHR: adjusted Hazard Ratio

-Major adverse liver outcomes: first occurrence of progression to clinical (eg, development of esophageal varices, ascites, or hepatic encephalopathy) or histological (F4 on repeat liver biopsy) cirrhosis, development of hHCC, liver transplantation, or liver-related mortality after the index date

Cumulative Incidence Estimates (Kaplan-Meier) for 2 Composite End Points.



Bariatric Surgery was associated with a significantly lower risk of incident Major Adverse Liver Outcomes and MACE, compared with Nonsurgical management.

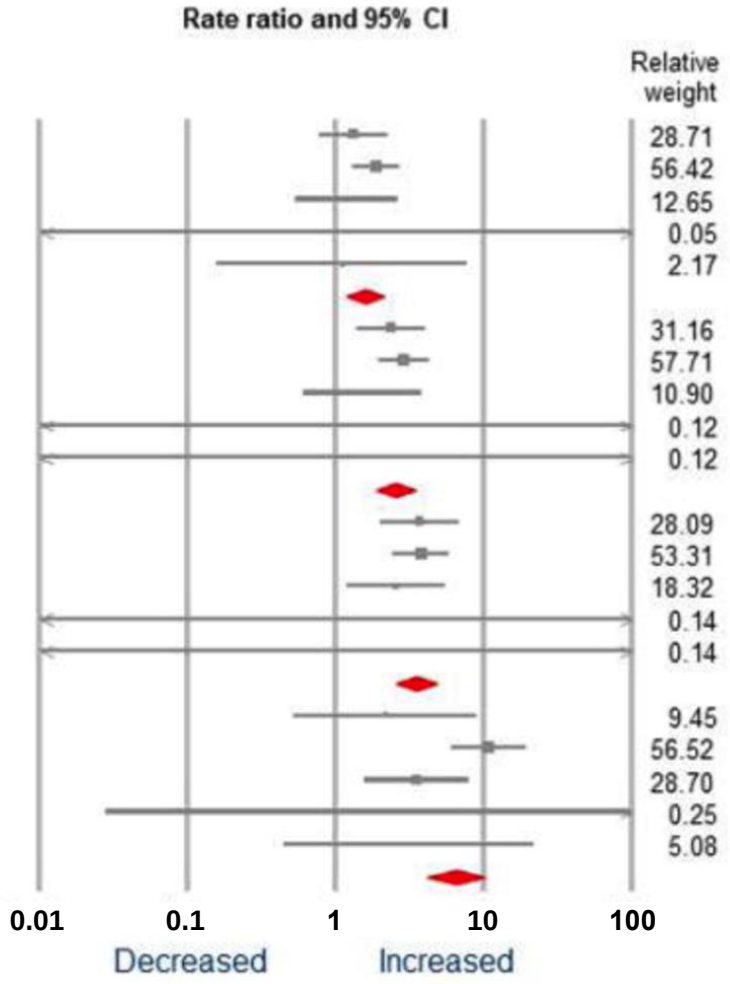
Aminian A et al.Jama, 2021

Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis

Incidence Rate of All-Cause Mortality in NAFLD

By Fibrosis Stage (vs. Stage 0 Fibrosis)

Study name	Fibrosis Stage	Rate ratio	Lower limit	Upper limit	Relative weight
Ekstedt	Stage 1	1.32	0.77	2.26	28.71
Angulo	Stage 1	1.88	1.28	2.76	56.42
Younossi	Stage 1	1.19	0.53	2.67	12.65
Sebastiani	Stage 1	0.44	0.00	107152.03	0.05
Leung	Stage 1	1.10	0.15	7.78	2.17
		1.58	**1.19**	**2.11**	
Ekstedt	Stage 2	2.35	1.36	4.08	31.16
Angulo	Stage 2	2.89	1.93	4.33	57.71
Younossi	Stage 2	1.49	0.59	3.79	10.90
Sebastiani	Stage 2	23.82	0.00	189525.41	0.12
Leung	Stage 2	0.12	0.00	842.82	0.12
		2.52	**1.85**	**3.42**	
Ekstedt	Stage 3	3.70	1.99	6.87	28.09
Angulo	Stage 3	3.76	2.40	5.89	53.31
Younossi	Stage 3	2.55	1.18	5.48	18.32
Sebastiani	Stage 3	57.84	0.01	398539.60	0.14
Leung	Stage 3	0.10	0.00	702.61	0.14
		3.48	**2.51**	**4.83**	
Ekstedt	Stage 4	2.17	0.52	9.13	9.45
Angulo	Stage 4	10.90	6.06	19.61	56.52
Younossi	Stage 4	3.54	1.55	8.07	28.70
Sebastiani	Stage 4	183.33	0.03	1227203.43	0.25
Leung	Stage 4	3.07	0.43	21.82	5.08
		6.40	**4.11**	**9.95**	



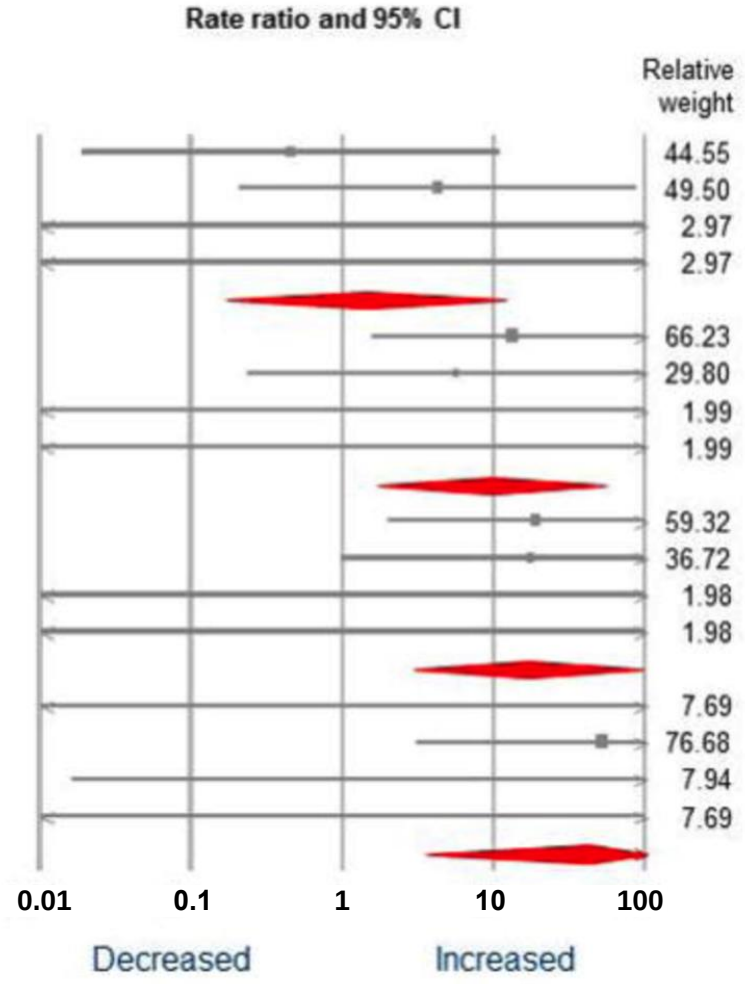
Dulai PS et al. Hepatology, 2017.

Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis

Incidence Rate of Liver-Related Mortality in NAFLD

By Fibrosis Stage (vs. Stage 0 Fibrosis)

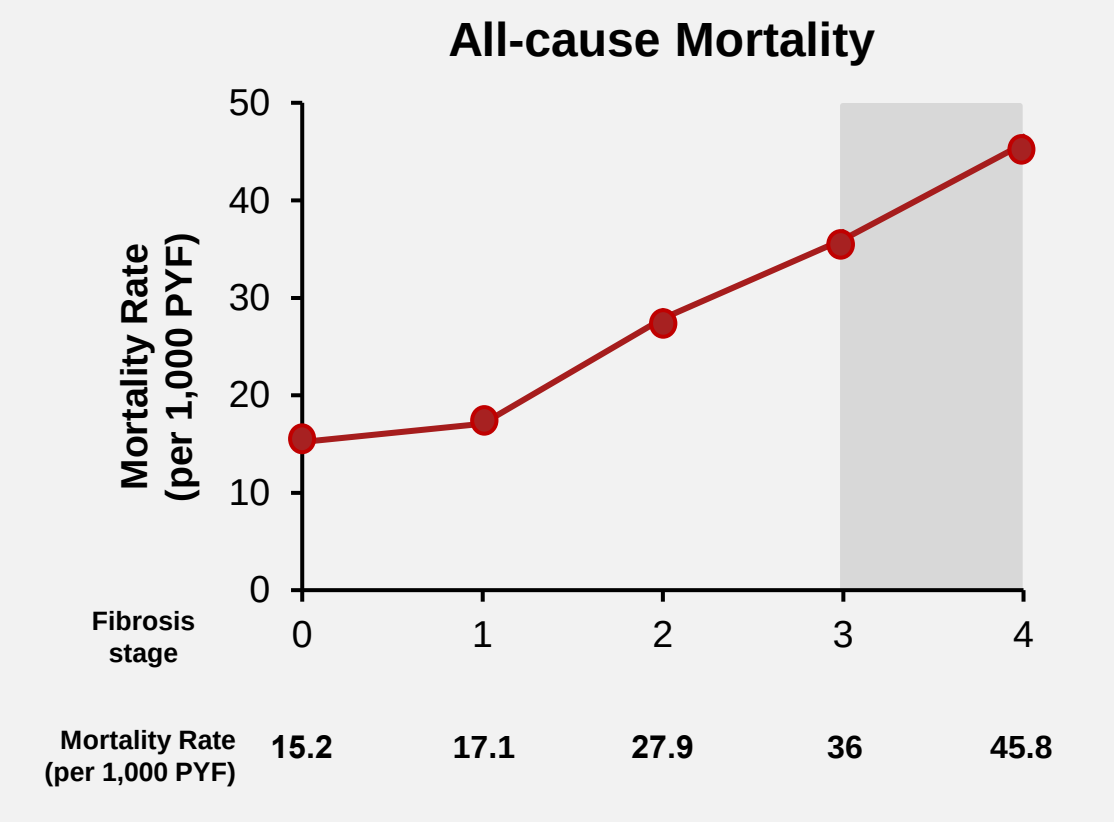
Study name	Fibrosis Stage	Rate ratio	Lower limit	Upper limit	Relative weight
Ekstedt	Stage 1	0.46	0.02	11.20	44.55
Younossi	Stage 1	4.24	0.20	88.35	49.50
Sebastiani	Stage 1	0.44	0.00	107152.03	2.97
Leung	Stage 1	1.10	0.00	264887.25	2.97
		1.41	**0.17**	**11.95**	
Ekstedt	Stage 2	13.24	1.55	113.36	66.23
Younossi	Stage 2	5.60	0.23	137.53	29.80
Sebastiani	Stage 2	1.19	0.00	288013.74	1.99
Leung	Stage 2	4.72	0.00	1141008.31	1.99
		9.57	**1.67**	**54.93**	
Ekstedt	Stage 3	18.74	1.95	180.13	59.32
Younossi	Stage 3	17.44	0.98	309.50	36.72
Sebastiani	Stage 3	0.96	0.00	233083.28	1.98
Leung	Stage 3	3.93	0.00	951186.02	1.98
		16.69	**2.92**	**95.36**	
Ekstedt	Stage 4	1.47	0.00	11663.29	7.69
Younossi	Stage 4	51.70	3.01	888.22	76.68
Sebastiani	Stage 4	110.00	0.02	758006.68	7.94
Leung	Stage 4	61.46	0.01	489000.99	7.69
		42.30	**3.51**	**510.34**	

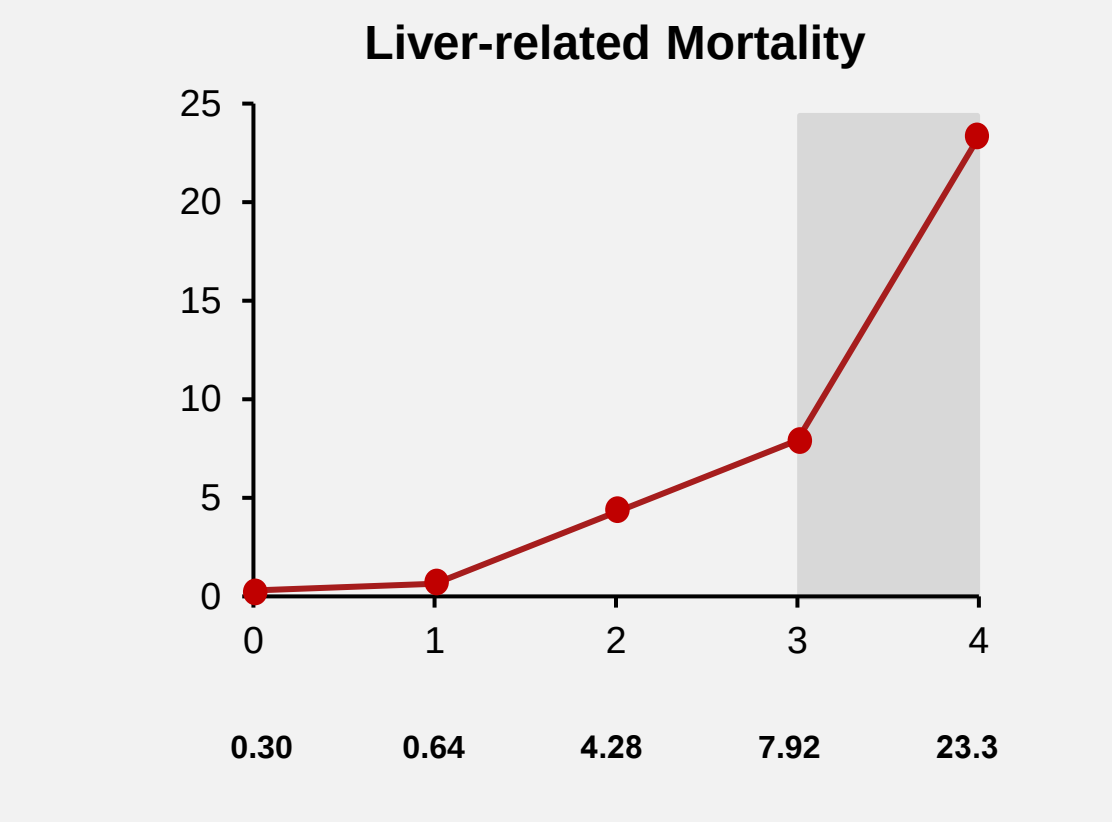


Dulai PS et al. Hepatology, 2017.

Mortality Risk Increases as Fibrosis Progresses

- Systematic review and meta-analysis of 5 studies in 1,495 NAFLD patients with 17,452 person-years follow-up





PYF = Patient Year Follow-up

Dulai P et al. Hepatology, 2017.

Fibrosis Stage is the Strongest Predictor for Disease-Specific Mortality in NAFLD after up to 33 Years of Follow-up

Hazard Ratios for Causes of Death in the Entire Cohort and in Histopathological Subgroups Compared With the Reference Population [HR (95% CI)]

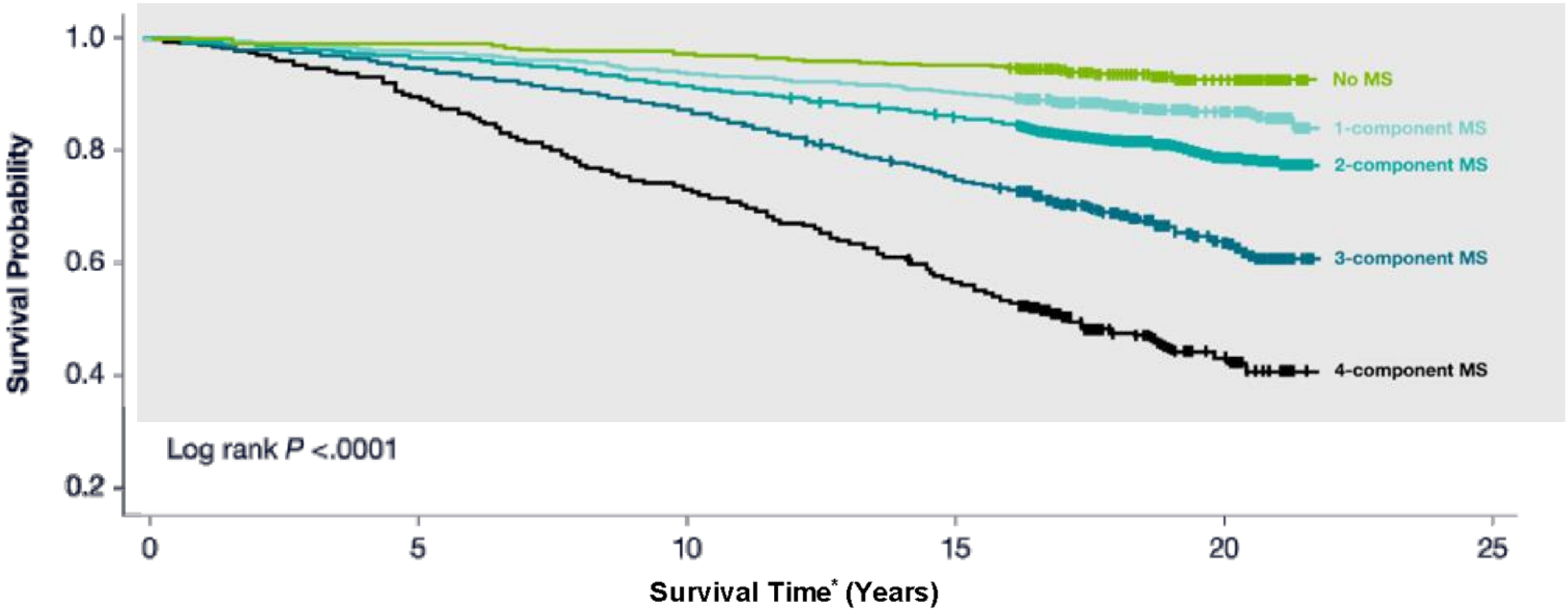
Cause of Death	Entire Cohort (n=229)	P	NAS 0-4, F0-2 (n=76)	P	NAS 5-8, F0-2 (n=57)	P	NAS 0-8, F3-4 (n=16)	P
Overall mortality	1.29 (1.04-1.59)	0.020	1.13 (0.79-1.60)	0.511	1.41 (0.97-2.06)	0.072	3.28 (2.27-4.76)	<0.001
Cardiovascular disease	1.55 (1.11-2.15)	0.01	1.19 (0.65-2.20)	0.557	1.38 (0.72-2.65)	0.335	4.36 (2.29-8.29)	<0.001
Hepatocellular carcinoma	6.55 (2.14-20.0)	0.001	No outcome	—	15.7 (4.1-59.9)	<0.001	16.9 (1.95-146)	0.01
Cirrhosis	3.2 (1.05-9.81)	0.041	4.86 (1.08-22.0)	0.04	No outcome	—	10.8 (1.38-83.9)	0.023
GI malignancy	0.60 (0.22-1.64)	0.322	1.26 (0.60-2.65)	0.546	0.54 (0.075-3.96)	0.548	No outcome	—
Non-GI malignancy	1.18 (0.70-1.98)	0.545	1.24 (0.55-2.76)	0.602	0.85 (0.27-2.65)	0.778	No outcome	—
Infectious disease	2.71 (1.02-7.26)	0.046	3.12 (0.72-13.5)	0.129	2.22 (0.31-16.4)	0.435	13.0 (3.13-54.5)	<0.001
Respiratory disease	1.01 (0.31-3.32)	0.979	No outcome	—	3.95 (1.22-13.0)	0.024	No outcome	—

NAS, NAFLD activity score; F, Fibrosis stage; HR, Hazard ratio; CI: confidence interval.
NAS is the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2).

Ekstedt M et al. Hepatology, 2015.

Risk Factors for NASH

Association between metabolic syndrome and mortality in NAFLD



* Kaplan-Meier survival curves for 23 year follow-up of 3613 participants with NAFLD.

Golabi P et al. Medicine (Baltimore), 2018.

An **Overweight** 58-year-old man,
with a past medical history of Controlled Hypertension, non-insulin-dependent **Type 2 Diabetes** Mellitus,
presented to your office for a check-up...

What else do you need to be aware of?

Which of the following is a stronger predictor of advanced fibrosis in patients with NAFLD ?

A. BMI ≥30kg/m2

B. Hypertension

C. Age

D. Diabetes Mellitus

Which is the crucial histological element for considering NASH?

A. When hepatic fat exceeds the diagnostic threshold

B. Inflammation and liver cell damage

C. Severe fibrosis

Which of the following is correct?

A. Fibrogenesis is not a linear process but progresses or regresses in up to 30% of patients during a mean period of 5 years.
B. NASH and NAFLD patients progress 1 stage of fibrosis every 7 and 14 years, respectively.
C. The presence and stage of fibrosis is the strostrongest histologic determinant of hepatic and overall outcomes in patients with NAFLD.
D. All of the above.

Prospective Study of Outcomes in Adults with NAFLD

1773 NAFLD patients followed for a median of 4 years

The NEW ENGLAND JOURNAL of MEDICINE

Mortality and New-Onset Nonfatal Outcomes According to Fibrosis Stage at Enrollment: rate per 1000 person-yr

	All-cause / Liver related Mortality	Variceal Bleeding / Ascites	Any Hepatic decompensation	Encephalopathy	HCC	<60 eGFR / DM2	CVD / Non-HCC.
F0 F1 F2	3.2/1000 0.4/1000	0/1000 0.4/1000	0.5/1000	0.2/1000	0.4/1000	21.7/1000 44.5/1000	8/1000 7.3/1000
F3	8.9/1000 HR 1.9 (0.9–3.7 95% CI) 2.8/1000 HR 5.8 (0.9–38.4 95% CI)	0.6/1000 5.2/1000 HR 18.9 (3.2–112.6 95% CI)	9.9/1000 HR 18.7 (4.8–73.1 95% CI)	7.5/1000 HR 40.8 (4.7–350.6 95% CI)	3.4/1000 HR 9.3 (1.4–61.8 95% CI)	29.7/1000 HR 1.0 (0.7–1.4 95% CI) 62.4/1000 HR 1.3 (0.9–2.0 95% CI)	9.3/1000 HR 0.7 (0.2–2.0 95% CI) 10.3/1000 HR 1.2 (0.5–2.9 95% CI)
F4	17.6/1000 HR 3.9 (1.8–8.4 95% CI) 6.8/1000 HR 12.7 (1.8–88.6 95% CI)	7/1000 12/1000 HR 29.4 (4.5–190.7 95% CI)	26.9/1000 HR 36.1 (8.9–146.3 95% CI)	23.9/1000 HR 109.1 (18.5–926 95% CI)	1.4/1000 HR 4.9 (0.4–63.2 95% CI)	44.9/1000 HR 1.4 (0.9–2.2 95% CI) 75.3/1000 HR 1.7 (1.0–3.0 95% CI)	8.1/1000 HR 0.8 (0.5–1.5 95% CI) 10/1000 HR 1.4 (0.8–2.7 95% CI)

Hazard Ratio (95% CI) Stage F3 vs. F0–F2 and Stage F4 vs. F0–F2

Francque SM, NEJM et al. 2021

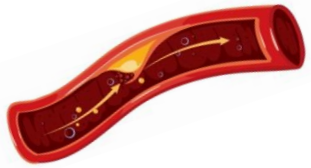
Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis

Long-term Outcomes of Non-obese, Lean and Obese NAFLD

	Studies (n)	Non-obese or lean and obese NAFLD (n)	Incident cases (n)	Follow up (person-years)	Incidence per 1000 person-years (95% CI)	I^{2}*
All-cause mortality (non-obese or lean NAFLD group)	3	35 707	590	194 805·8	12·1 (0·5–38·8)	99·6%
All-cause mortality (obese NAFLD group)	2	56 577	495	296 566·0	7·5 (0·0–33·6)	99·2%
Cardiovascular-related mortality† (non-obese or lean NAFLD group)	3	35 707	156	194 805·8	4·0 (0·1–14·9)	99·2%
Cardiovascular-related mortality† (obese NAFLD group)	2	56 577	105	296 566·0	2·4 (0·0–13·3)	98·3%
Liver-related mortality (non-obese or lean NAFLD group)	1	123	10	2447·7	4·1 (1·9–7·1)	..
Liver-related mortality (obese NAFLD group)	1	168	8	3343·2	2·4 (1·0–4·4)	..
New-onset diabetes (non-obese or lean NAFLD group)	3	771	67	5655·2	12·6 (8·0–18·3)	58·6%
New-onset diabetes (obese NAFLD group)	..	..	..	..	..	..
New-onset cardiovascular disease† (non-obese or lean NAFLD group)	2	141	12	640·2	18·7 (9·2–31·2)	..
New-onset cardiovascular disease† (obese NAFLD group)	1	235	32	959·6	33·3 (22·7–46·0)	..
New-onset hypertension (non-obese or lean NAFLD group)	1	84	33	588	56·1 (38·5–77·0)	..
New-onset hypertension (obese NAFLD group)	..	..	..	..	..	..

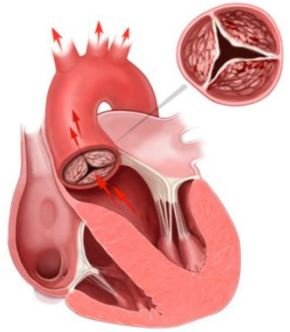
Qing Ye et al. Lancet 2020

Cardiac Morbidities Associated with NAFLD



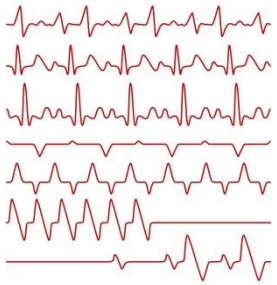
Atherosclerosis

- Increased risk of incident of MI



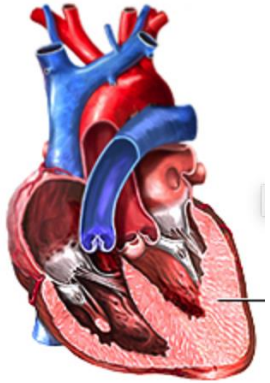
Valvular Disease

- Aortic valve stenosis
- Mitral annular calcification



Cardiac arrhythmias

- A. fib especially in patients with NAFLD and diabetes
- QT prolongation
- 1st degree AV block
- RBBB and LBBB

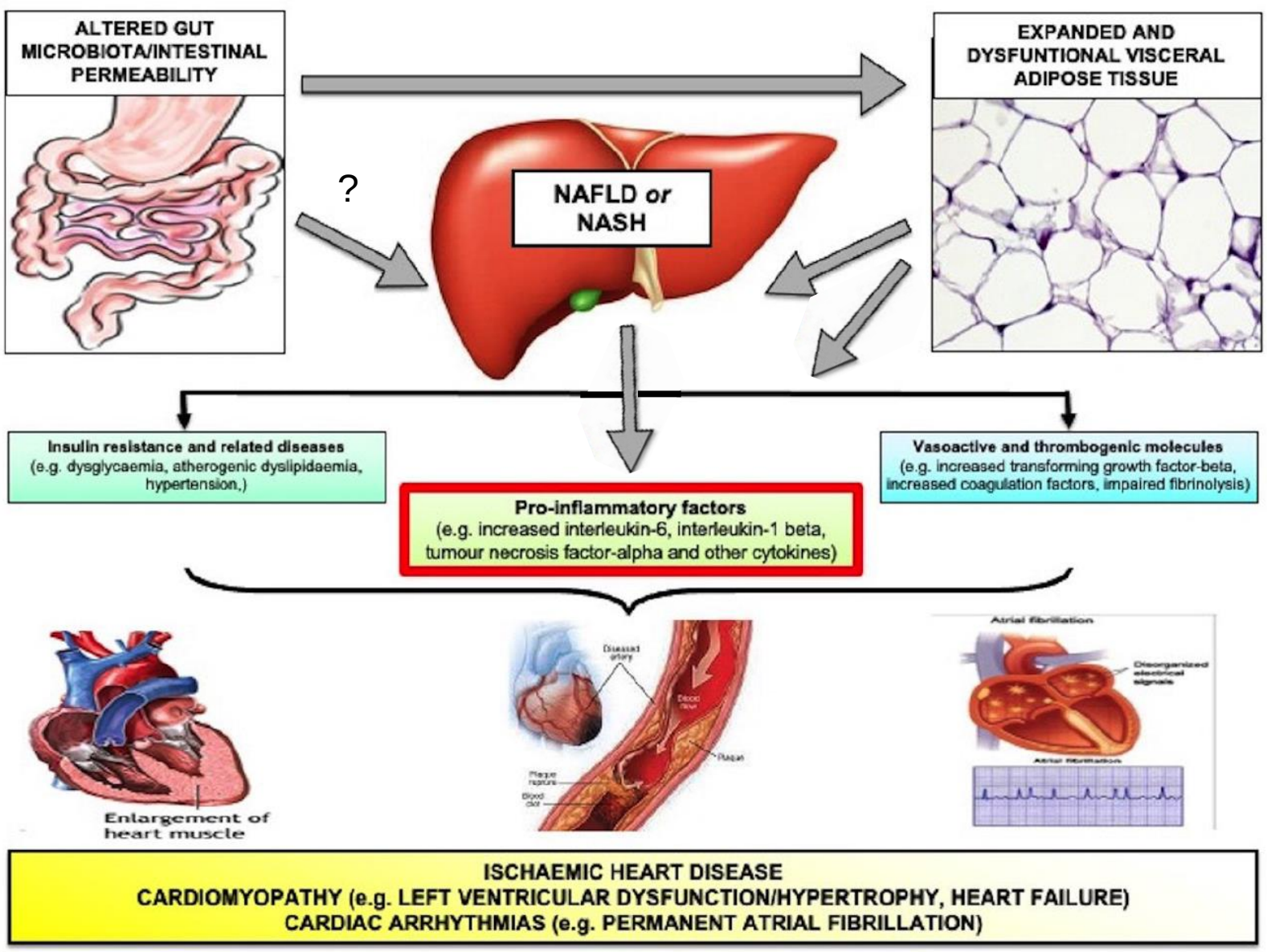


Enlargement of heart muscle

- Left ventricular diastolic dysfunction
- Might be independent of components of the metabolic syndrome
- Increases progressively with increased liver fibrosis

1) Sinn DH et al. J Gastroenterol Hepatol, 2020. 2) Lee SB et al. J Hepatol, 2018. 3) Di Minno MND et al. Int J Cardiol, 2019. 4) Mantovani A et al. Metabolism, 2015. 5) Mantovani A et al. Liver Int, 2019. 6) Targher G et al. Diabetes Metab, 2021. 7) VanWagner LB et al. Hepatology, 2015. 8) Kim NH et al. Heart, 2014. 9) Jung JY et al. Hepatol Res, 2017. 10) Canada JM et al. Am J Cardiol, 2019. 11) Chung GE et al. Atherosclerosis, 2018.

Cardiovascular Associations of NAFLD



Targher G et al. Gut, 2020.

Summary of potential pathophysiological mechanism responsible for increased CVD in NAFLD.

Adipokines
Adiponectin ↓
Leptin ↑

SNPs
PNPLA3 & TM6SF2

Bacterial dysbiosis??
TMA↑ → TMAO ↑

Incretins
GLP-1↓, GIP ↓

NAFLD

Endothelium
Dysfunction
Disruption

Aortic valve
Calcification
Stenosis

Vascular wall
Intima media ↑
Arterial stiffening

L. Ventricle remodeling

Conduction abnormalities
Arrhythmias

Inflammation
IL-6↑
hcCRP↑
IL-1β↑
TNF-α↑
M1/M2 balance↑

Redox status
Oxidative stress ↑
Homocysteine ↑
OxLDL↑

Paracrine cell signalling
Microvesicles ↑
MicroRNA profile

Hemostasis
Procoagulant imbalance
PAI-1 ↑
VIII, IX, XI, XII ↑
Protein C activity ↓
Viscosity ↑

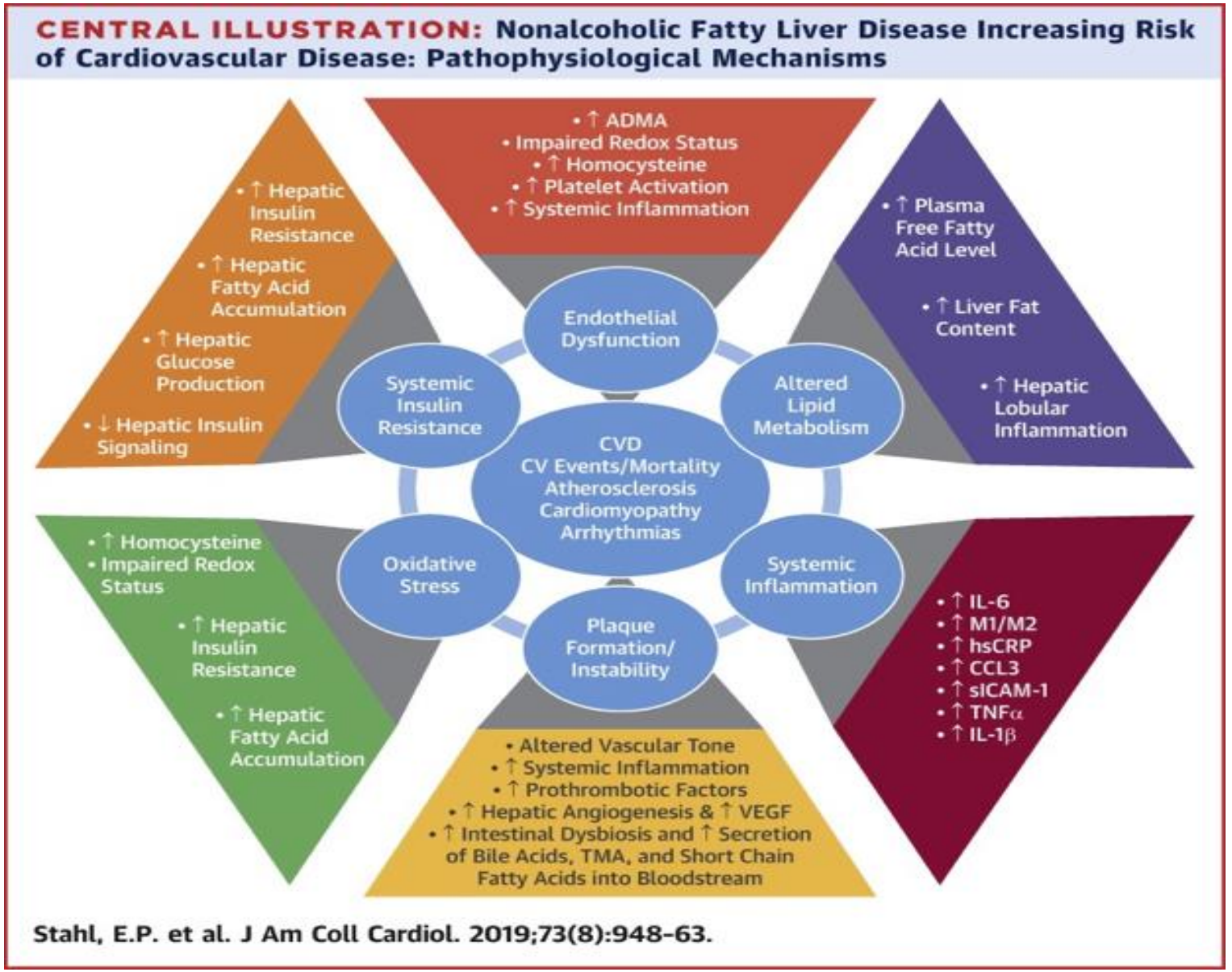
Angiogenesis
VEGF ↑
HMGB-1↓

Lipid profile
TG↑
HDL↓
sdLDL↑/large LDL↓
VLDL ↑
(Postprandial accentuation)

Hepatokines
FetA ↑
FGF21↑
SeP ↑
ANGPTL's ↑

Francque SM et al. J Hepatol, 2016.

NAFLD and Cardiovascular Disease are both Manifestations of End-Organ Damage of the Metabolic Syndrome

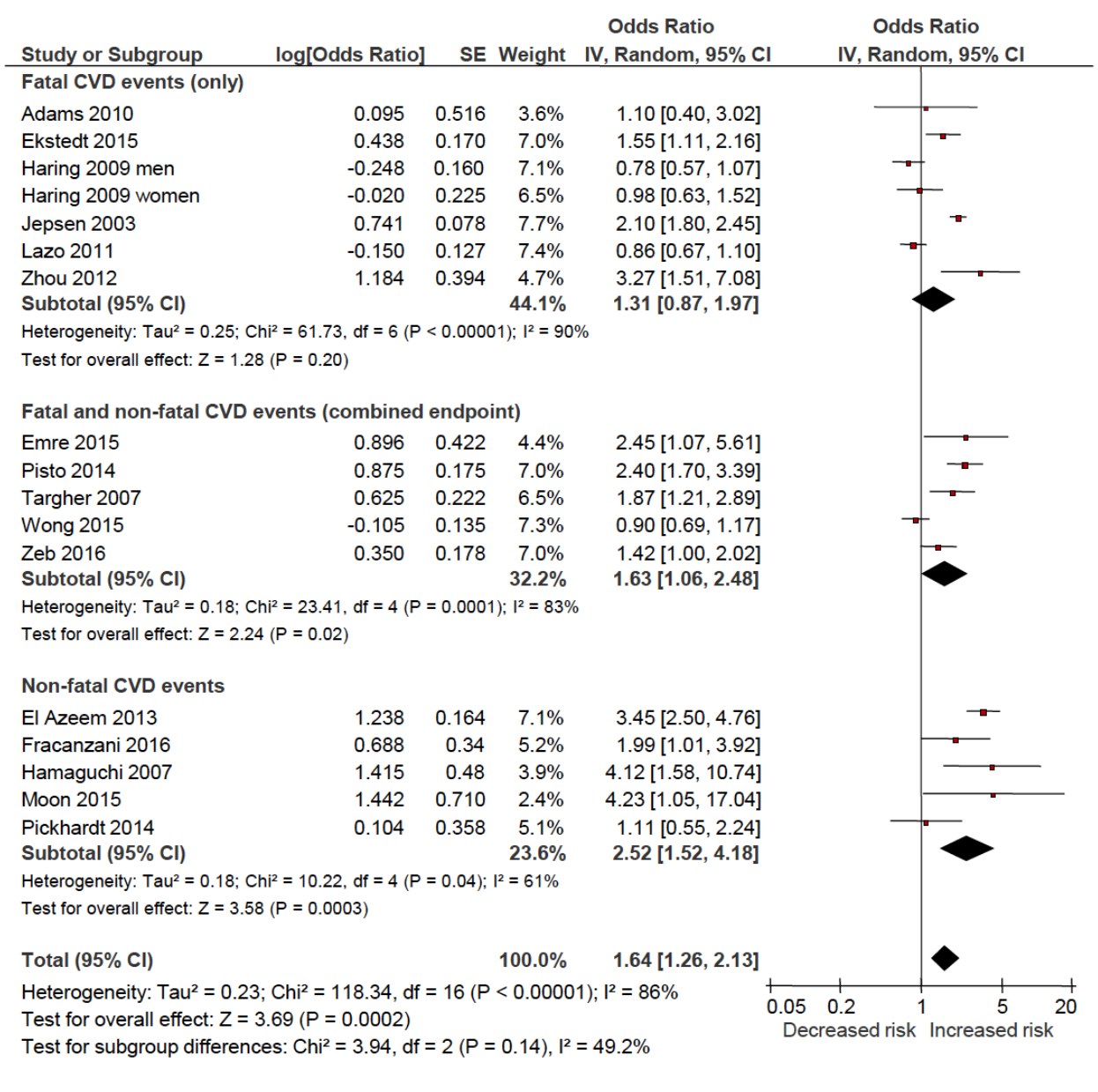


Stahl, E.P. et al. J Am Coll Cardiol. 2019;73(8):948-63.

Cardiovascular Mortality in NAFLD

Random-effects meta-analysis on the risk of incident CVD events associated with NAFLD.

Forest plot of comparison of patients with NAFLD versus those without NAFLD.

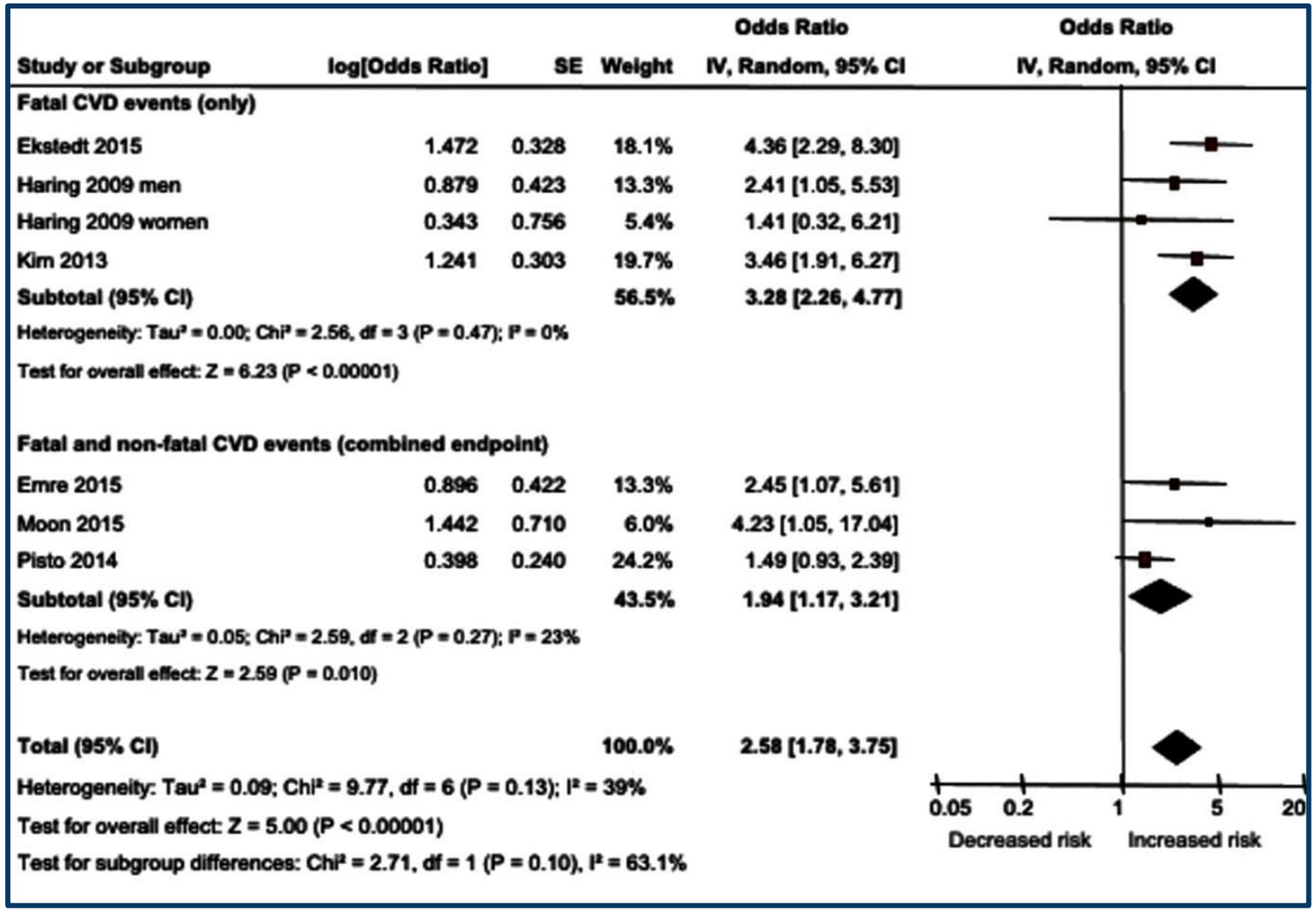


Targher G et al. J Hepatol, 2016.

Causes of Death HR and in Histopathological Subgroups vs Reference Population [HR (95% CI)]

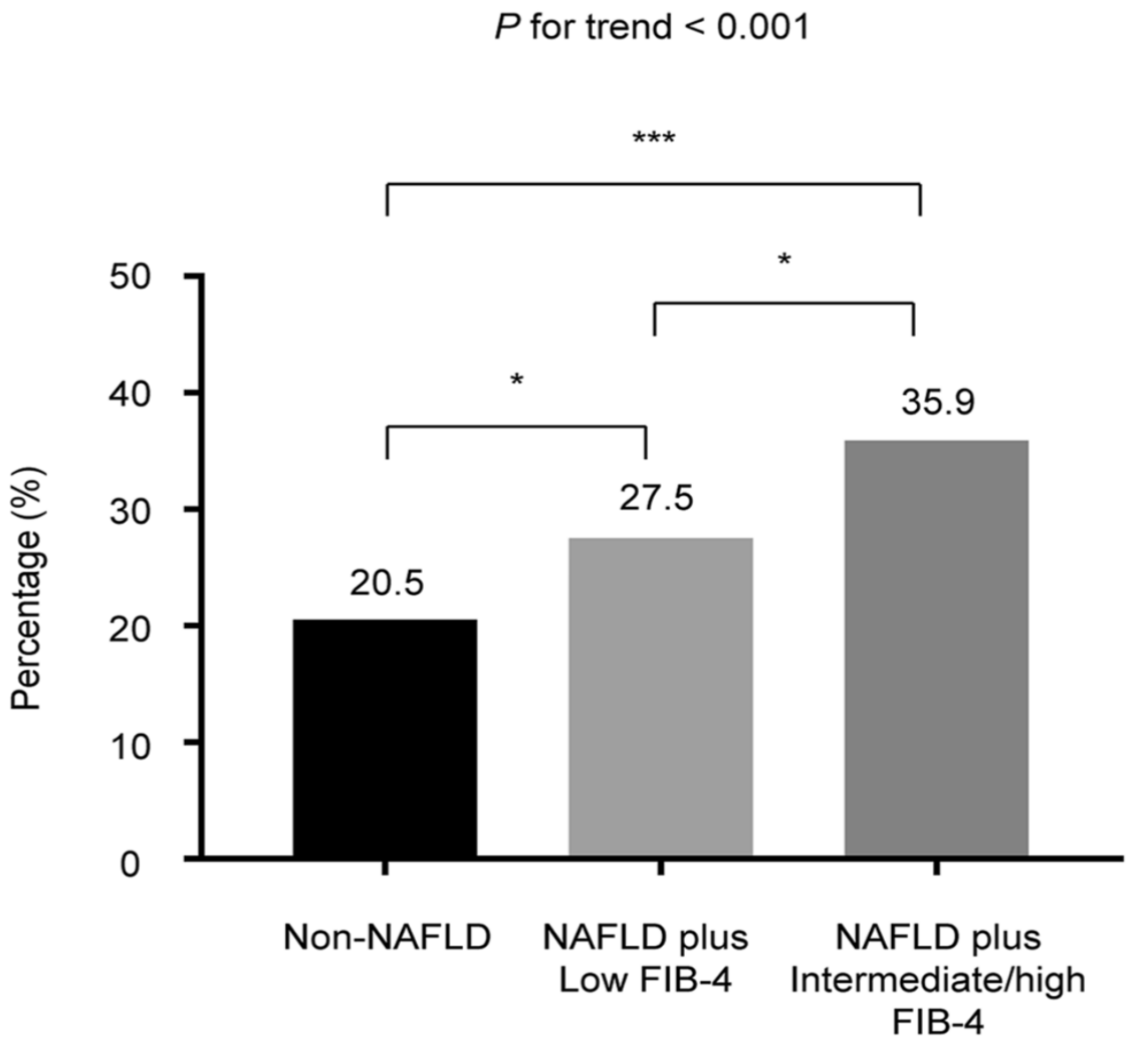
Cause of Death	Cardiovascular disease
Entire Cohort (n=229)	1.55 (1.11-2.15)
P	0.01
NAS 0-4, F0-2 (n=76)	1.19 (0.65-2.20)
P	0.557
NAS 5-8, F0-2 (n=57)	1.38 (0.72-2.65)
P	0.335
NAS 0-8, F3-4 (n=16)	4.36 (2.29-8.29)
P	<0.001

Random-effects meta-analysis on the risk of incident CVD events (fatal, non-fatal or both) associated with NAFLD. Forest plot of comparison of patients with NAFLD versus those without NAFLD.

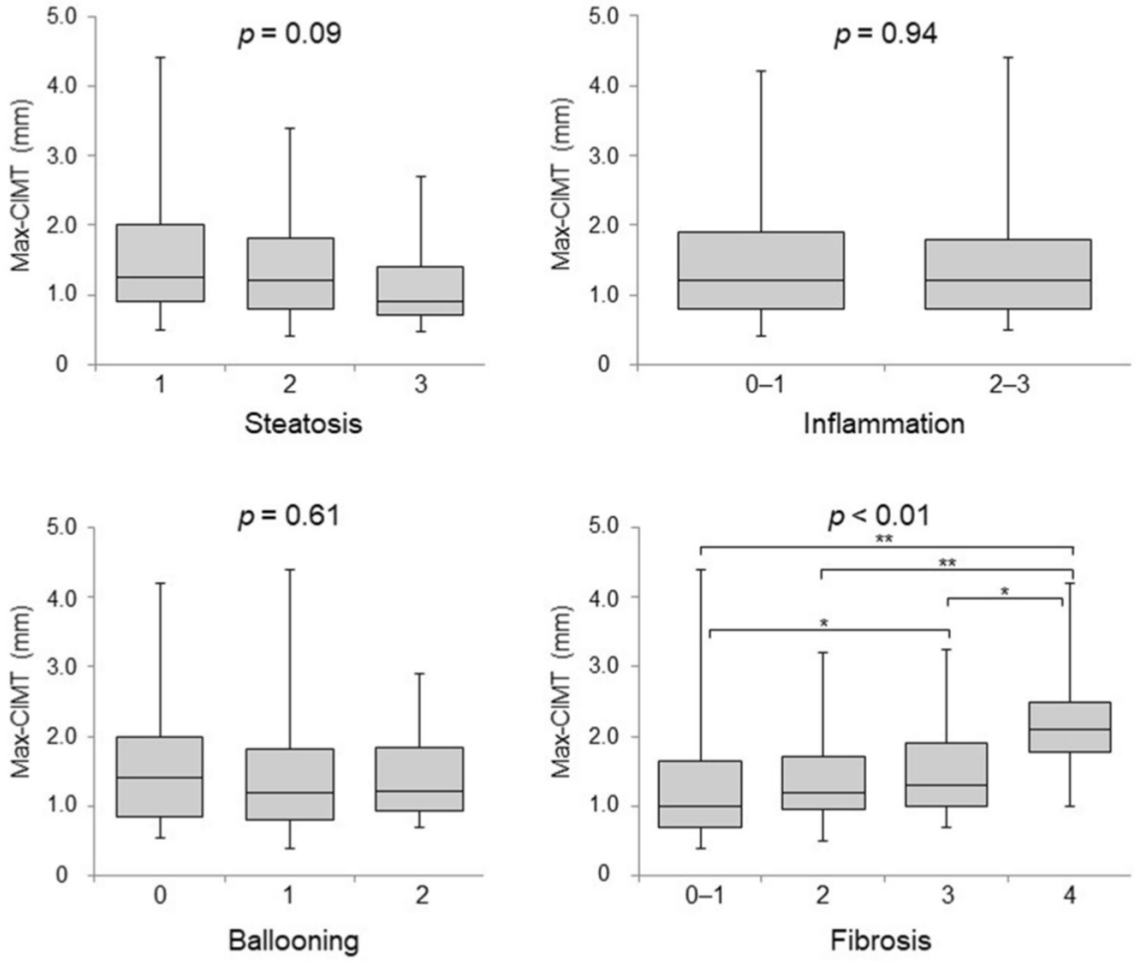


1.Ekstedt M et al. Hepatology, 2015. 2.Targher G, et al. Gut 2020

Proportion of coronary artery calcification score progression according to the baseline NAFLD status and liver fibrosis severity based on the FIB-4 score.



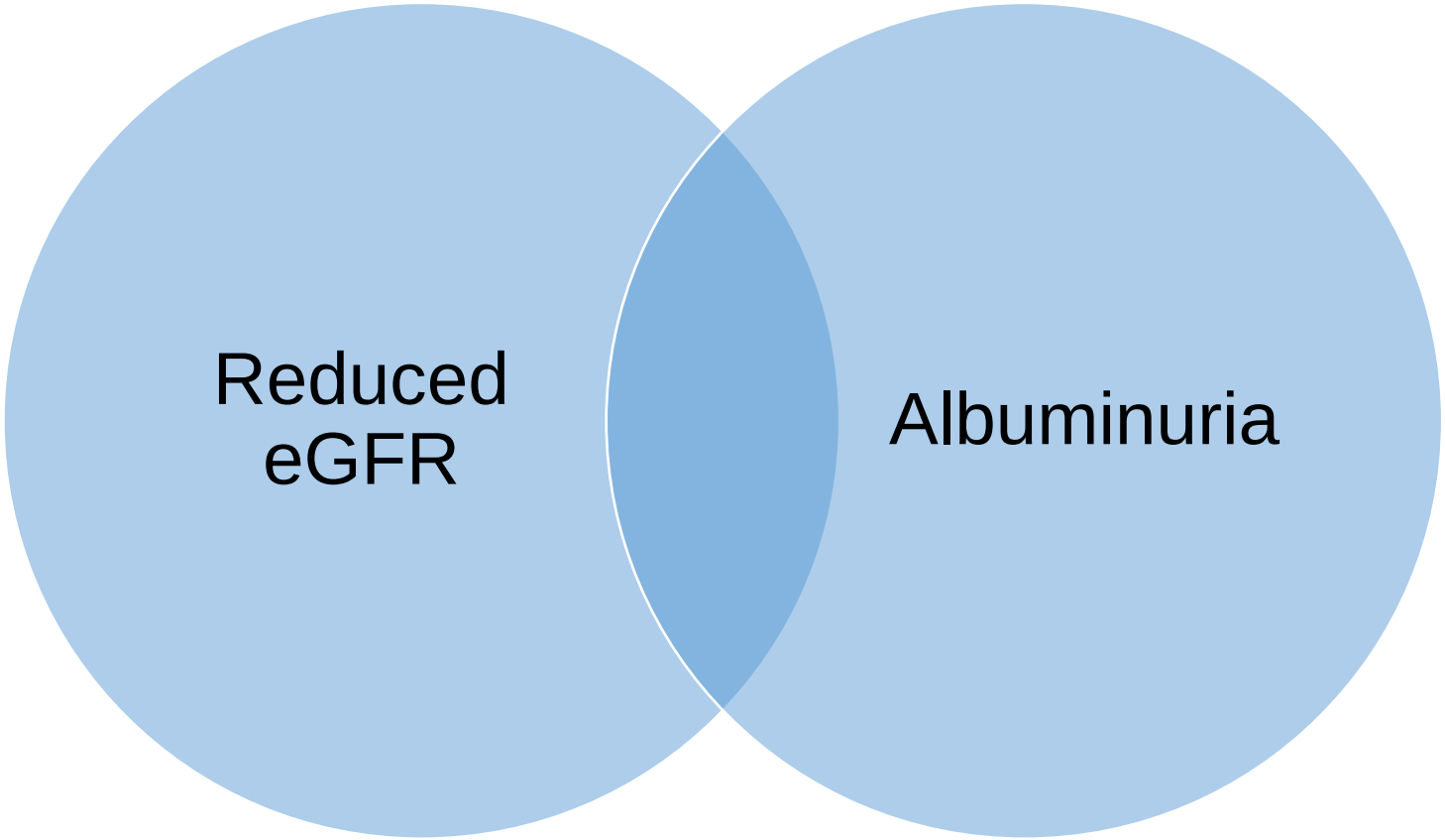
Max-CIMT values according to the severity of histological component in NAFLD patients.



1. Lee J, et al. Nature 2021. 2. Arai T, et al. Nature 2021

Manifestations of Kidney Disease in patients with NAFLD

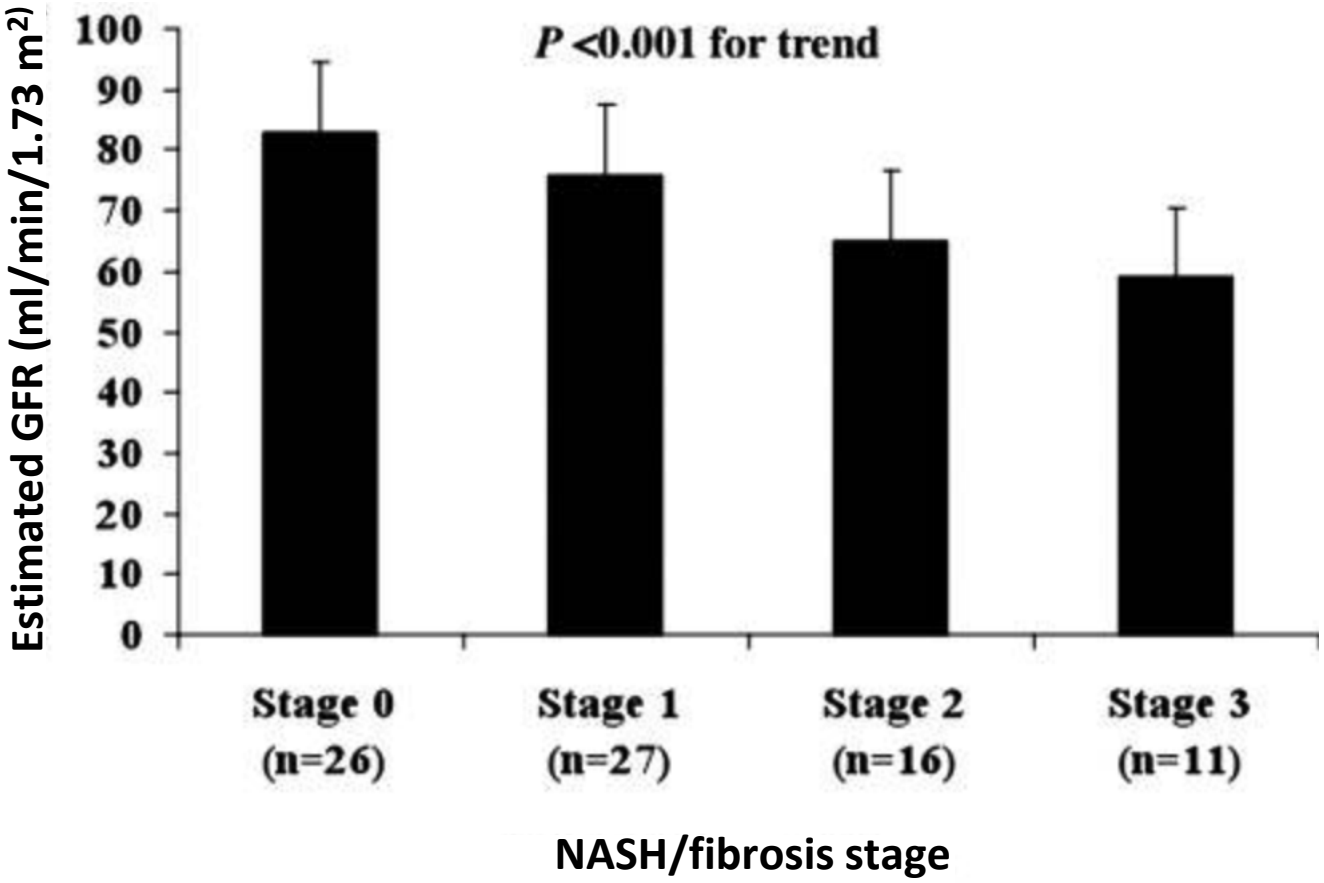
- When CKD occurred in patients with NAFLD, they had a higher mortality, but this was driven by metabolic comorbidities.
- Whether these represent common shared biology alone or if there are causal linkages between these end organ diseases remains an open question



Yilmaz Y et al. Metabolism, 2010.
Yasui K et al. Metabolism, 2011.
Machado MV et al. Liver Int, 2012.
Önnerhag K et al. Clin Res Hepatol Gastroenterol, 2019.

Relationship Between Kidney Function and Liver Histology in Subjects with NASH

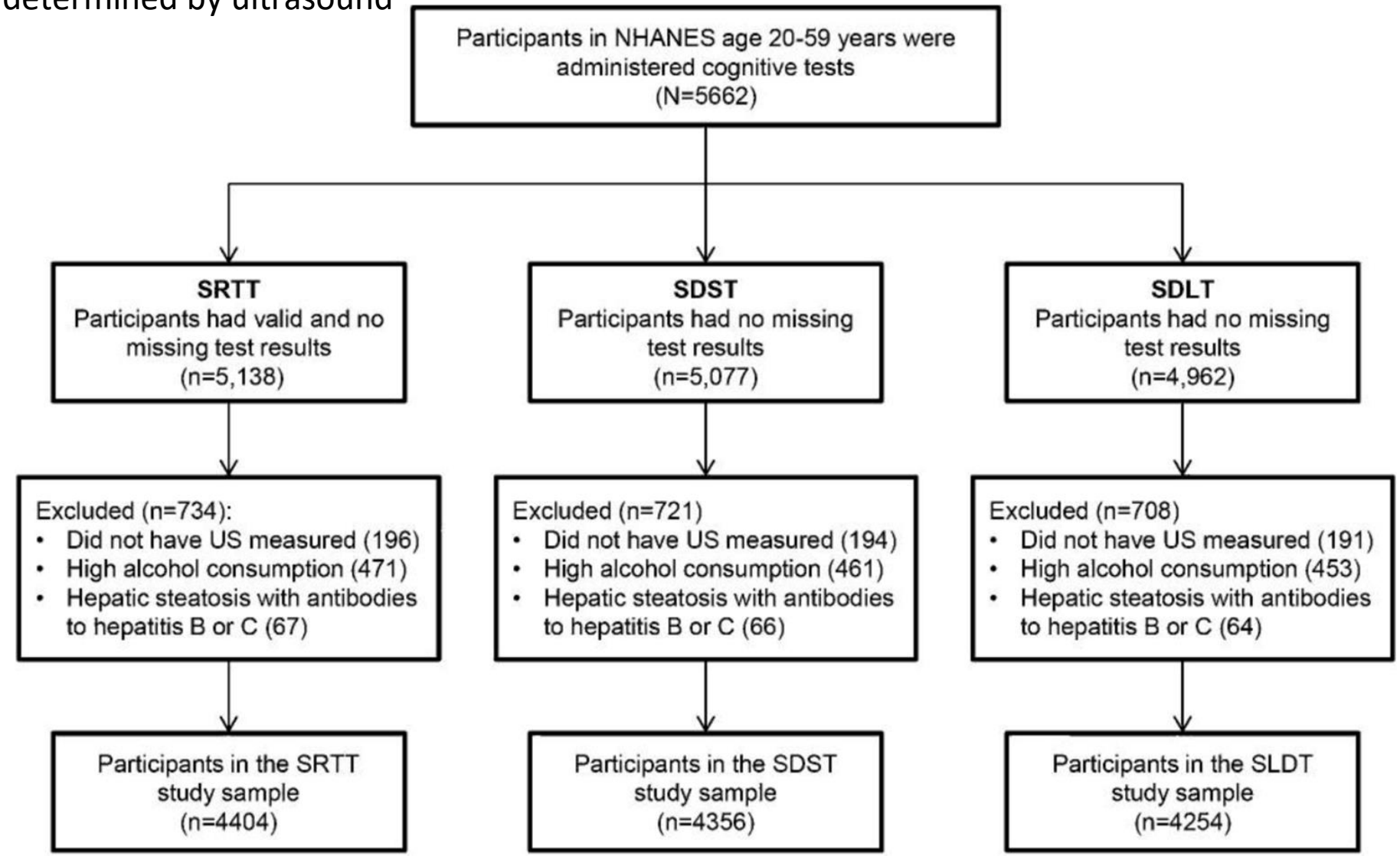
- Histologic severity of NASH (*i.e.,* fibrosis stage) was associated with decreasing mean (SD) values of eGFR even after adjustment for age, gender, waist circumference, HOMA-IR score, systolic BP, and plasma triglycerides ($P < 0.001$ for the trend by analysis of covariance).
- Results remained essentially unchanged after additional adjustment for albuminuria or when patients with diagnosed diabetes ($n = 10$) were removed from analysis.



Targher G et al. Clin J Am Soc Nephrol, 2010.

NAFLD is associated with cognitive function in adults

- NAFLD was determined by ultrasound



SRTT = Simple Reaction Time Test; SDST = Symbol Digit Substitution Test; SDLT = Serial Digit Learning Test

Seo SW et al. Neurology, 2016.

NAFLD is associated with cognitive function in adults

- Compared to participants without NAFLD, participants with NAFLD had lower performances on Serial Digital Learning Test even after controlling for CV risk factors.

	β	SE	95% CI
SRTT			
Model 1	7.827	3.496	0.975 to 14.679
Model 2	6.658	3.650	−0.496 to 13.812
SDST			
Model 1	0.110	0.054	0.004 to 0.216
Model 2	0.101	0.056	−0.009 to 0.211
SDLT			
Model 1	0.880	0.287	0.317 to 1.443
Model 2	0.726	0.317	0.105 to 1.347

Model 1: adjusted for age, sex, race, education
Model 2: model 1 and **further adjusted for BMI, waist circumference, hypertension, diabetes, hypercholesterolemia, acute MI and stroke**

SDLT = Serial Digit Learning Test; SDST = Symbol Digit Substitution Test; SRTT = Simple Reaction Time Test;

Seo SW et al. Neurology, 2016.

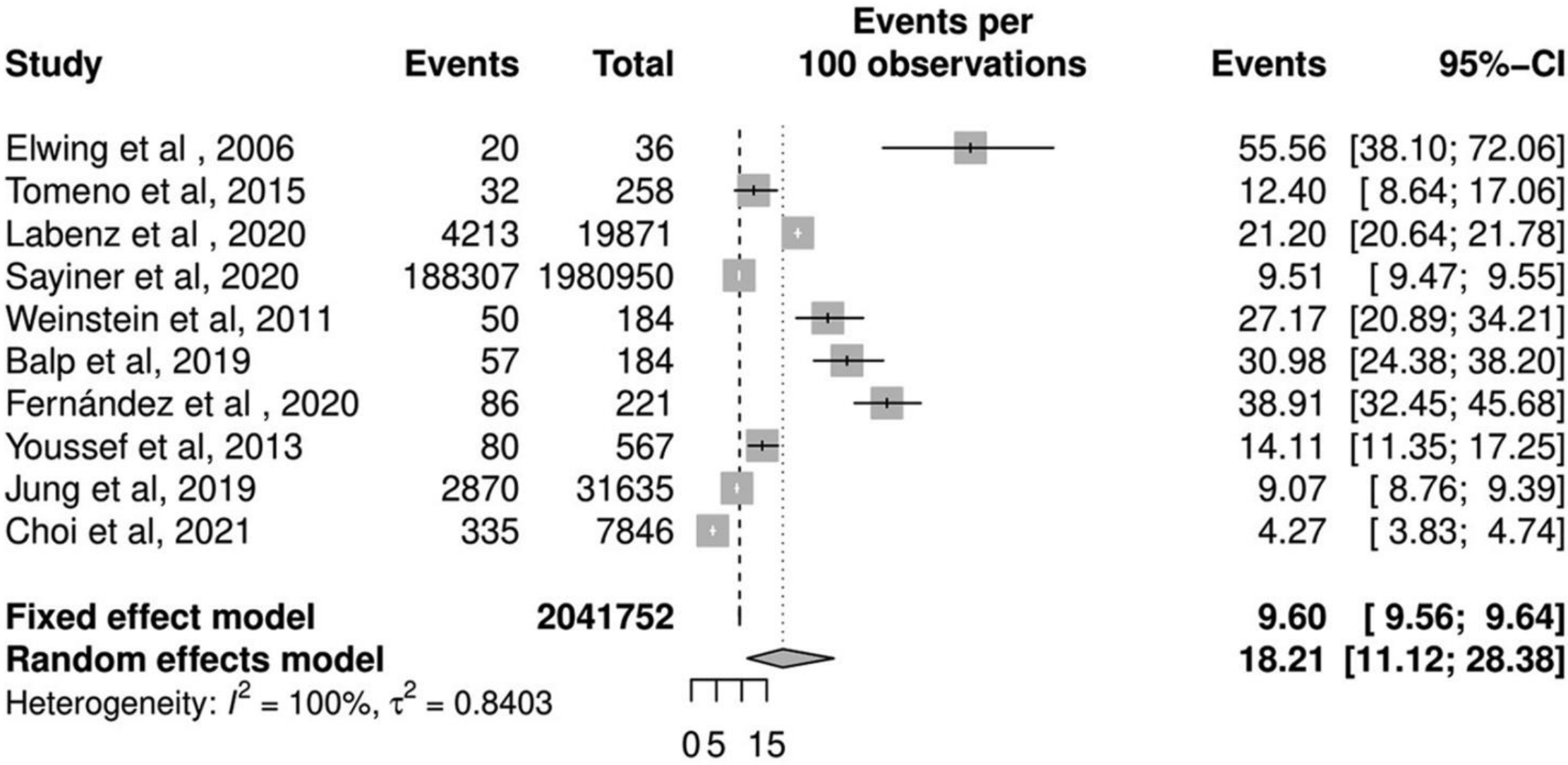
NAFLD is associated with cognitive function in adults

- NAFLD associated cognitive impairments may be localized to the prefrontal cortex (visuospatial and executive functioning domains)

- It is questionable whether NAFLD itself causes the cognitive defects, or it is due to cardiovascular risk factors.

Celikbilek A et al. Eur J Gastroenterol Hepatol, 2018.

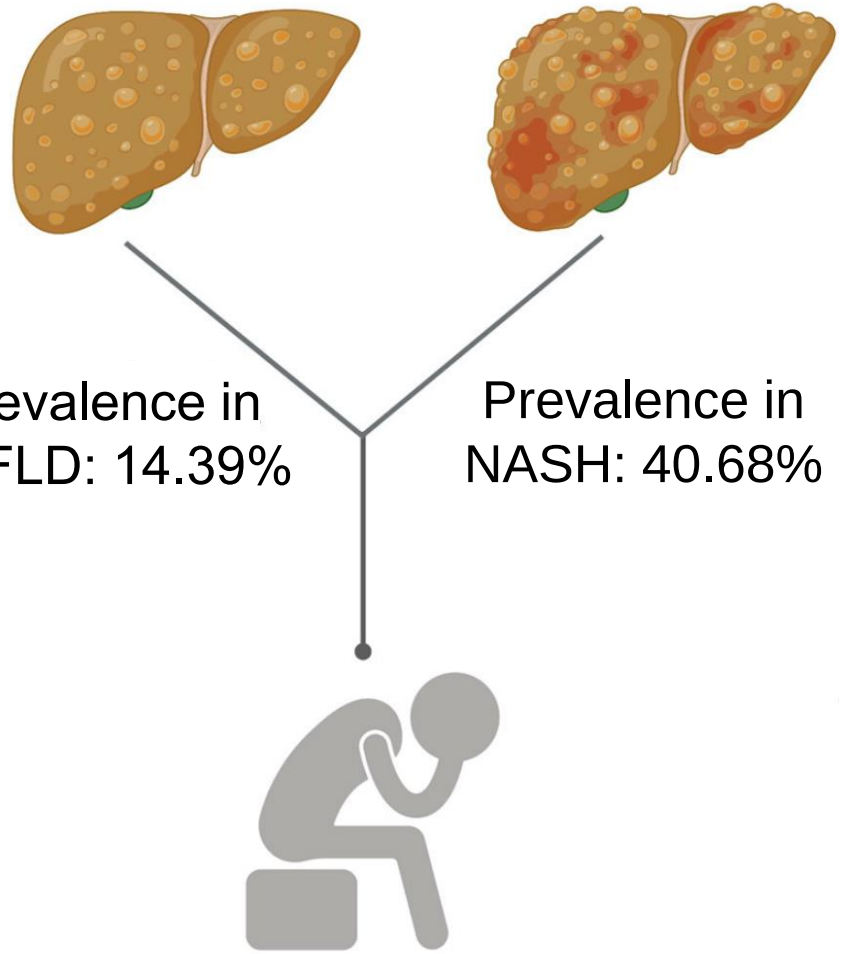
Is Fatty Liver Associated With Depression? A Meta-Analysis and Systematic Review on the Prevalence, Risk Factors, and Outcomes of Depression and Non-alcoholic Fatty Liver Disease.



Xiao J et al. Front Med (Lausanne), 2021.

Is Fatty Liver Associated With Depression? A Meta-Analysis and Systematic Review on the Prevalence, Risk Factors, and Outcomes of Depression and Non-alcoholic Fatty Liver Disease

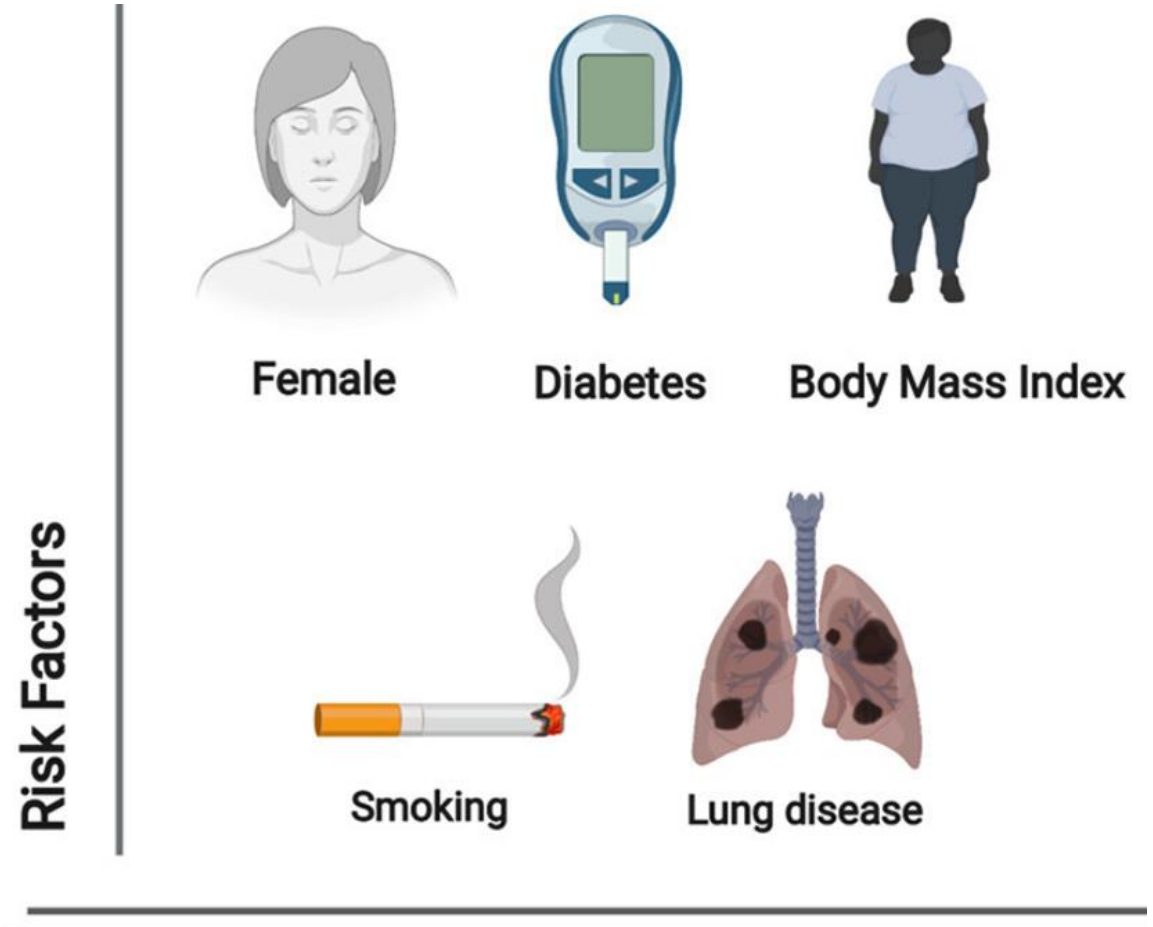
- Depression in NAFLD may be mediated by inflammatory cytokines



Prevalence in NAFLD: 14.39%

Prevalence in NASH: 40.68%

Relative Risk:
2.83 (CI: 2.41– 3.3.2), p<0.001



1) Xiao J et al. Front Med (Lausanne), 2021. 2)Colognesi M et al. Biomedicines, 2020.

Screening

- Clinical practice guidelines do not recommend screening for NAFLD in the general population

Who to screen for NASH and fibrosis?

- Moderate to severe obesity (BMI>35 kg/m^2)
- Metabolic syndrome
- T2DM of > 10 years' duration or in people older than 50 years
- Prediabetes or T2DM with steatosis or elevated ALT

EASL/EASD/EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol, 2016.
American Diabetes Association. Standards of medical care in diabetes-2020 abridged for primary care providers. Clin Diabetes, 2020.

Recent Guidelines in the Management of Liver Disease and Diabetes Recognize the Increased Association between Diabetes NAFLD and NASH

NAFLD

NASH/Liver Fibrosis

AASLD: There should be a high index of suspicion for NAFLD and NASH in patients with **T2DM**.

EASL: Patients with insulin resistance and/or metabolic risk factors (i.e., **obesity or metabolic syndrome [MetS]**) should undergo diagnostic procedures for the diagnosis of NAFLD.

ADA: Evaluation for nonalcoholic fatty liver disease (by measuring **aspartate aminotransferase and alanine aminotransferase**) should be done at diagnosis and annually thereafter.

AACE/ACE: Screening for NAFLD should be performed in all patients with **overweight or obesity, T2DM, or metabolic syndrome**; all patients with nonalcoholic fatty liver disease should be evaluated for the presence of overweight or obesity.

EASL: In high-risk individuals **[age >50 years, T2DM, MetS]**, case finding of advanced disease (i.e., NASH with fibrosis) is advisable.

ADA: Patients with **T2DM or prediabetes** and elevated ALT or fatty liver on ultrasound should be evaluated for presence of nonalcoholic steatohepatitis and liver fibrosis.

1. Chalasani N et al. Hepatology, 2018. 2. European Association for the Study of the Liver (EASL) et al. J Hepatol, 2016. 3. Riddle MC et al. Diabetes Care, 2019. 4. Garvey WT et al. Endocr Pract, 2016.

Facts Important for Recognizing NAFLD

- NAFLD is the one of the most common causes of abnormal liver enzymes. However, the degree of elevation of liver enzymes does not correlate with the severity of the disease and in many cases **ALT and AST can be normal** at all stages (including fibrosis).

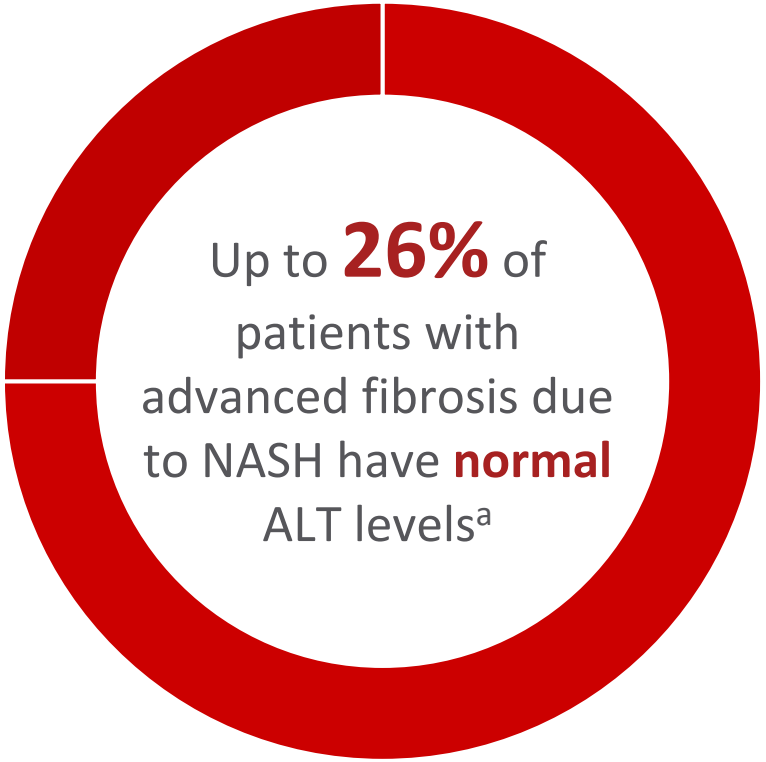
- Liver **fibrosis** has been linked to morbidity and reduced overall patient survival

- NAFLD and fibrosis are **reversible** with weight loss

- To differentiate alcoholic vs nonalcoholic fatty liver, the **AST/ALT** can be used which is ≥ 2 in alcohol induced fatty liver

Gawrieh S et al. Am J Gastroenterol, 2019.
Taylor RS et al. Gastroenterology, 2020.
Vilar-Gomez E et al. Gastroenterology, 2015.
Verma S et al. Liver International, 2013.

A Substantial Number of Patients with Advanced Fibrosis have normal ALT levels



- Approximately 80% of those with NAFLD have normal ALT
- Persistently high ALT levels can be associated with disease progression
- Individuals with normal ALT levels can have advanced disease: ALT typically falls (and AST may rise) as fibrosis progresses to cirrhosis
  - ALT is found abundantly in the cytosol of the hepatocyte and hepatocellular injury or death causes the initial increase in the measured serum ALT
  - During fibrosis progression, hepatocytes are replaced by scarring and the declining hepatocyte population is reflected by an eventual decrease in serum ALT

[a]Advanced fibrosis identified by VCTE and then liver biopsy.[1]

1. Hejazifar N et al. AASLD, 2018. Poster 1756. 2. Verma S et al. Liver Int, 2013. 3. Koehler EM et al. Hepatology, 2016. 4. Kutala B et al. AASLD, 2018. Poster 2343. 5. Portillo-Sanchez P et al. J Clin Endocrinol Metab, 2015. 5. Browning JD et al. Hepatology, 2004. 6. Dowman JK et al. Aliment Pharmacol Ther, 2011. 7. Ekstedt M et al. Hepatology, 2006. 8. Dyson JK et al. Frontline Gastroenterol, 2014. 9. Kim WR et al. Hepatology, 2008.

A 50-year-old man,
with a past medical history of Obesity
was presented to your office for a check-up...

Which screenings test would you look for?

Who to screen for NASH and fibrosis?

A. Moderate to severe obesity BMI ≥35 kg/m2
B. Patients with metabolic syndrome
C. T2DM of >10 years duration, steatosis or elevated ALT
D. All of the above.

Which of the following Genes are associated with NAFLD-NASH development?

A. PNPLA3

B. HSD17B13

C. GCRK

D. MBOAT7

E. All of the above

Which Population more frequently present the Single Nucleotide Polymorphism PNPLA3?

A. African Americans
B. Hispanics
C. Europeans Americans