PanNASH initiative

TOWARDS A NASH SOLUTION

NASH Natural History

PanNASH initiative is supported by inventiva

The PanNASH initiative

PanNASH initiative
TOWARDS A NASH SOLUTION

The PanNASH initiative – contributing to NASH awareness and education

> The PanNASH initiative is led by an international, multidisciplinary expert committee

- Medical experts in areas related to NASH such as hepatology, diabetes and cardiology
- Scientific experts focused on promoting a better understanding of the pathophysiological mechanisms involved in NASH

> The objectives of the PanNASH initiative are to:

- Increase the visibility and contribute to a better understanding of non-alcoholic steatohepatitis (NASH)
- Share expertise and to establish best practices for the treatment of the disease
- Increase knowledge of pathological mechanisms ranging from metabolic disorders to fibrosis and co-morbidities, with a focus on the modulating role played by peroxisome proliferator-activated receptors (PPARs) α, δ and γ.

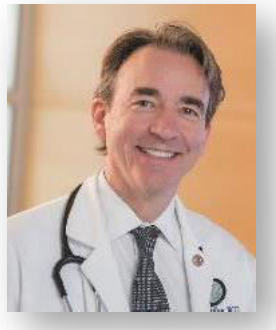
PanNASH initiative
TOWARDS A NASH SOLUTION

The PanNASH initiative expert committee

Sven Francque (Chair)
University of Antwerp
Antwerp, Belgium

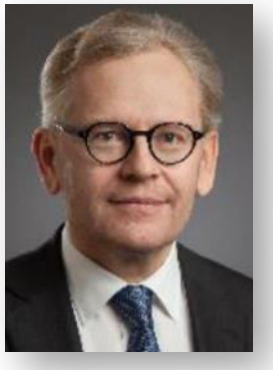
Manal F. Abdelmalek
Duke University
Durham, NC, USA

Christopher Byrne
University Hospitals Southampton
Southampton, UK



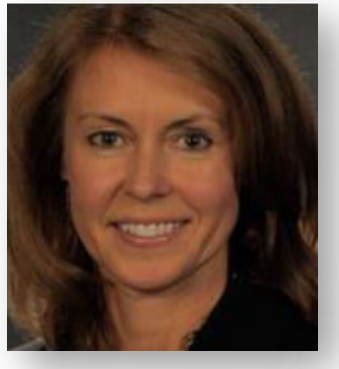
Kenneth Cusi
University of Florida
Gainesville, FL, USA

Jean-Francois Dufour
University of Bern
Bern, Switzerland



Michael Roden
Heinrich Heine University
Düsseldorf, Germany

Frank Sacks
Harvard Medical School
Boston, MA, USA



Gyongyi Szabo
University of Massachusetts
Worcester, MA, USA

Frank Tacke
Charité University Medical Center
Berlin, Germany

Definitions & epidemiology

PanNASH initiative
TOWARDS A NASH SOLUTION

NAFLD, NAFL, NASH?

Disease	Definition
NAFLD	> Entire spectrum of fatty liver disease in individuals without significant alcohol consumption > From fatty liver to hepatic steatosis to cirrhosis
NAFL	> Hepatic steatosis ≥5% > No evidence of hepatocellular injury (ballooning) > No evidence of fibrosis
NASH	> Hepatic steatosis ≥5% > Liver inflammation > Hepatocyte injury (ballooning) > With or without liver fibrosis

Notes NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis

References 1. Chalasani N et al. Hepatology 2018;67:328-57; 2. EASL, EASD, ESAO. J Hepatol 2016;64:1388-402

NAFLD or alcoholic liver disease?

- **Exclusion of secondary causes of hepatic fat accumulation**
- **Absence of significant alcohol consumption**

  daily consumption ≥30 g for men and ≥20 g for women, or

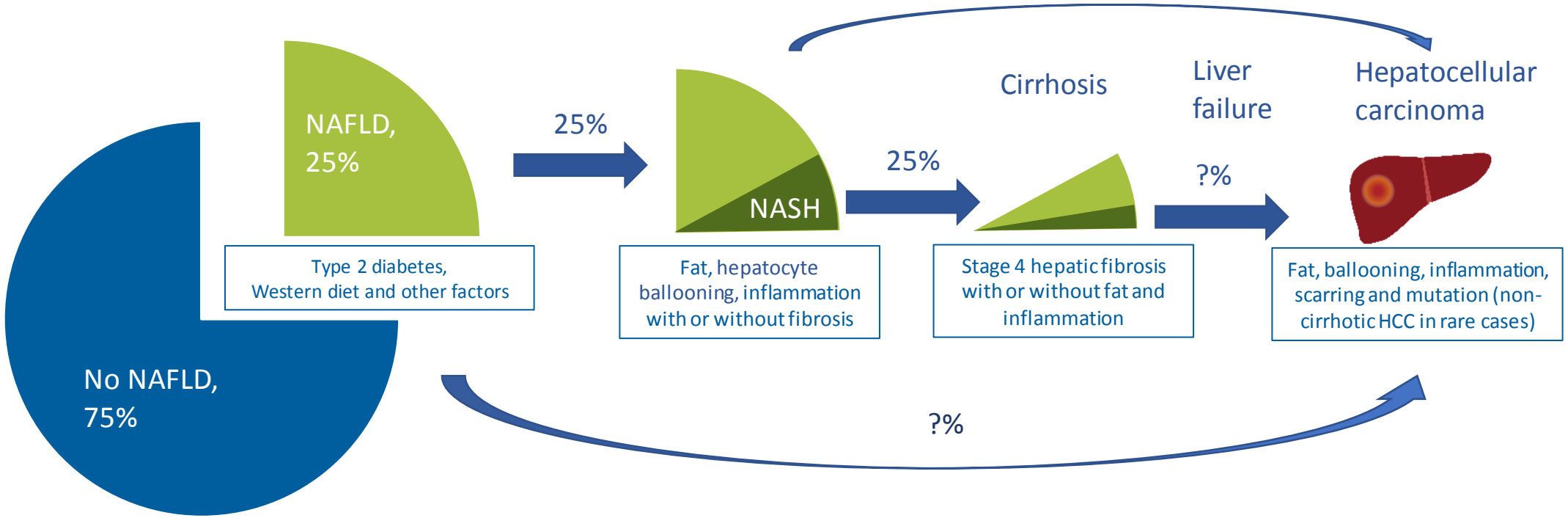
  >21 standard drinks on average per week in men and >14 in women

- **Moderate amounts of alcohol + metabolic risk factors may predispose to NAFLD**

References 1. Chalasani N et al. Hepatology 2018;67:328-57; 2. EASL, EASD, EASO. J Hepatol 2016;64:1388-402

Prevalence of NAFLD: 25% of the global adult population

- Increasing worldwide
- 25% of the global adult population
- Driven mainly by unhealthy lifestyles, obesity and diet



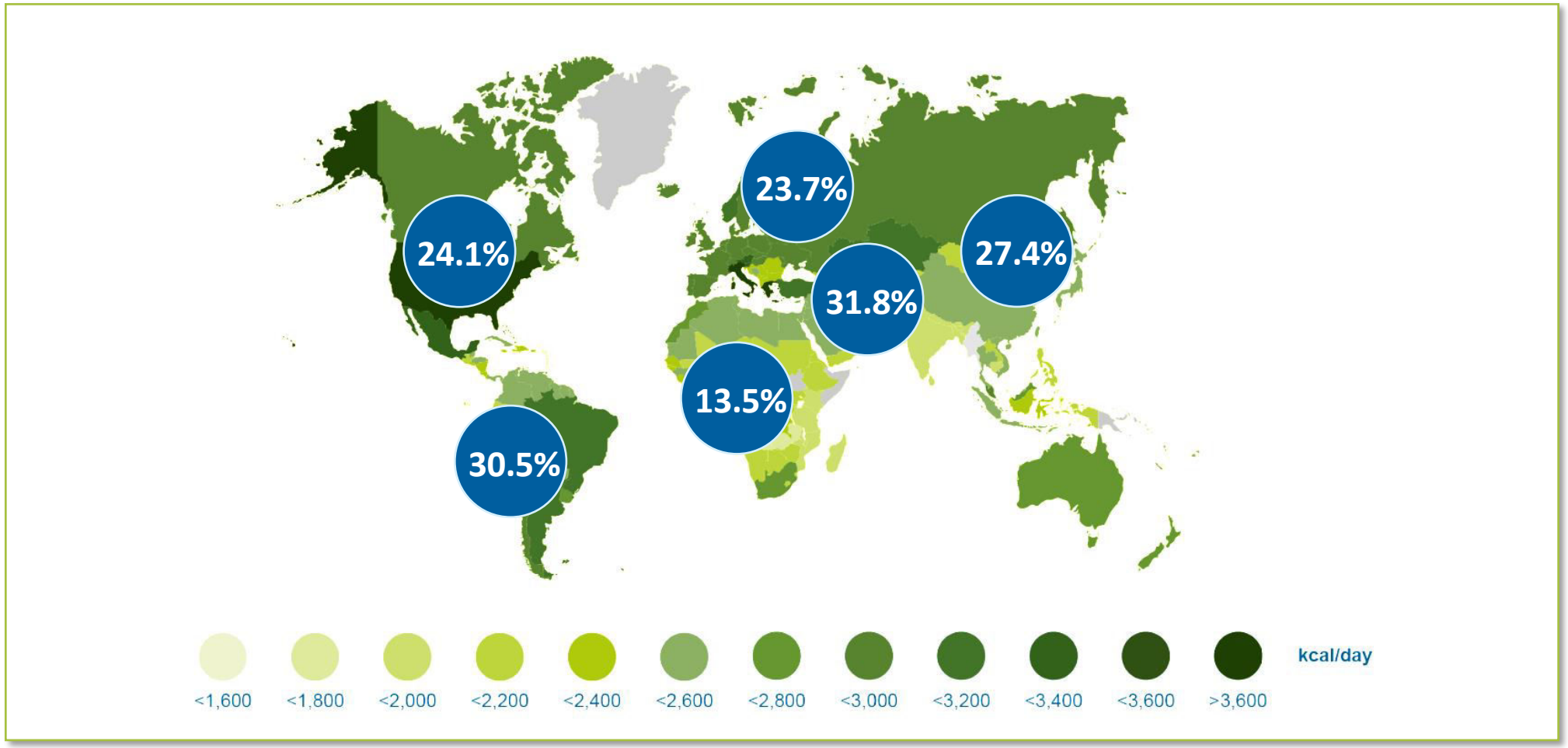
Progression: NAFLD: 1 stage fibrosis over 14 years; NASH: 1 stage fibrosis over 7 years

References

Adapted from Diehl AM, Day C. N Engl J Med 2017;377:2063-72; Singh S, et al. Clin Gastroenterol Hepatol. 2015;13:643-54

The globalisation of NAFLD

Geographic variation in the daily energy availability per capita and in the prevalence of NAFLD



References 1. Adapted from Rinella M et al. Hepatology 2016;64:19-22; 2. Younossi ZM et al. Hepatology 2016;64:73-84

Prevalence of NASH increases with BMI in children and adolescents

NAFLD prevalence by BMI population studies

	Prevalence (%) and 95% CI*	
	General population studies	Clinical obese population studies
Male	9.0 (6.5 to 12.5)	35.3 (26.0 to 45.8)
Female	6.3 (3.8 to 10.4)	21.8 (15.5 to 29.8)
Normal weight	2.3 (1.5 to 3.6)	-
Overweight	12.5 (9.2 to 16.7)	-
Obese	36.1 (24.6 to 49.4)	-

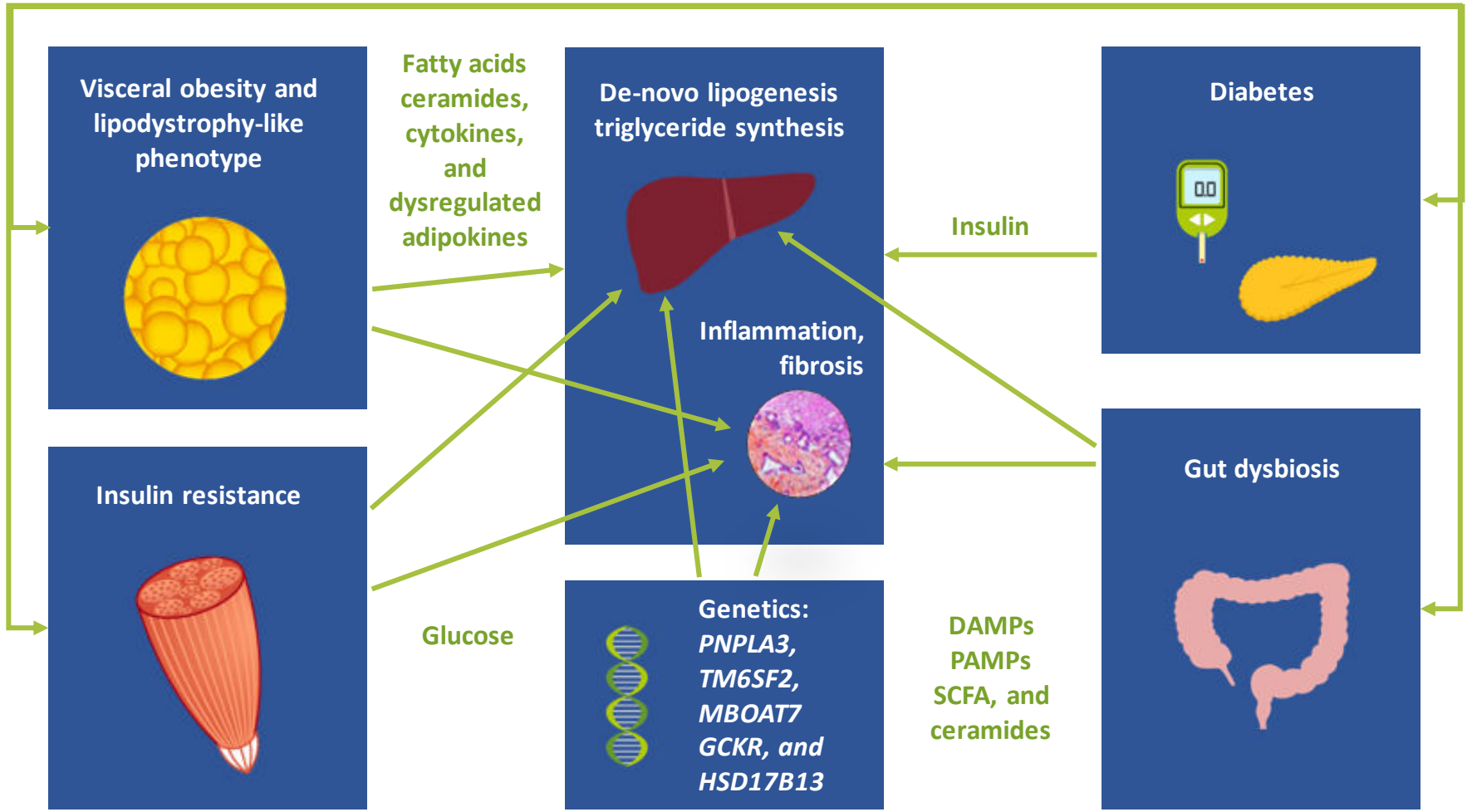
Across studies, prevalence of NAFLD increased considerably on average with increasing BMI category

Prevalence of NAFLD among children and adolescents affects approximately 3% to 10% of all children and over one-third of obese children in developed countries

Notes *Combines all diagnostic methods

References Anderson EL et al. PLoS One 2015;10:e0140908

NAFLD is largely driven by unhealthy lifestyles, ageing and genetics



DAMPs, damage-associated molecular patterns; GCKR, glucokinase regulator; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; MBOAT7, membrane-bound O-acyltransferase domain-containing 7; PAMPs, pathogen-associated molecular patterns; PNPLA3, patatin-like phospholipase domain-containing protein 3; SCFA, short-chain fatty acid; TM6SF2, transmembrane-6 superfamily member 2

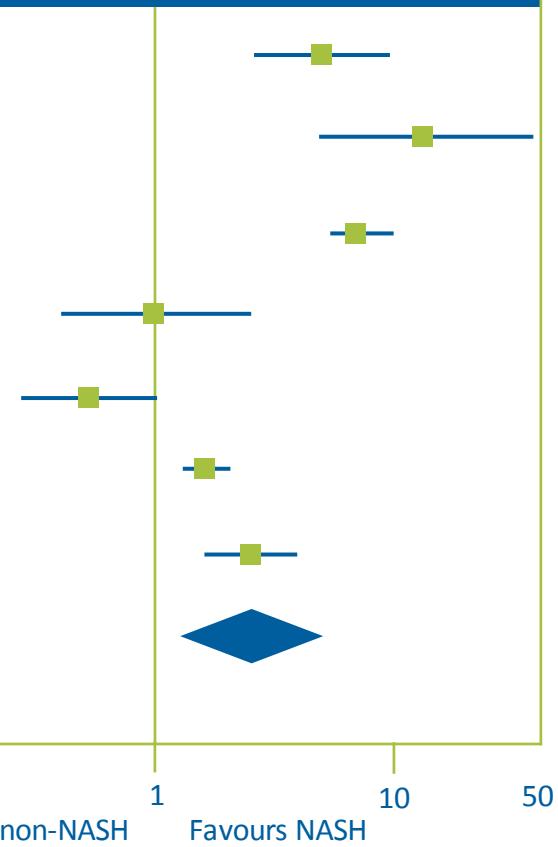
Notes

References

Adapted from Stefan N et al. Lancet Diabetes Endocrinol 2018; Aug 30 [Epub ahead of print]

NASH: 261% increased risk of HCC*
when compared to all other aetiologies of liver disease[1]

	NASH		Non-NASH		Odds ratio		Odds ratio
Study or subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	Year
Ertle et al.	31	59	19	103	14.0%	4.89 [2.40, 9.99]	2011
Reddy et al.	14	52	4	162	11.4%	14.55 [4.53, 46,71]	2012
Tokushige et al.	111	292	1092	14228	16.0%	7.38 [5.78, 9.42]	2013
Schutte et al.	6	43	87	621	13.0%	1.00 [0.41, 2.43]	2014
Rim et al.	12	35	406	724	14.0%	0.41 [0.20, 0.83]	2014
Tateishi et al.	228	590	1201	4640	16.1%	1.80 [1.51, 2.16]	2015
Mittal et al.	50	120	306	1380	15.5%	2.51 [1.71, 3.68]	2015
Total (95% CI)		1191		21868	100.0%	2.61 [1.27, 5.35]	
Total events	452		3115				



Heterogeneity $\tau^2 = 0.83$; $\chi^2 = 130.68$; $df = 6$ ($P<0.00001$), $I^2 = 95\%$
Test for overall effect: $Z = 2.61$ ($P = 0.009$)

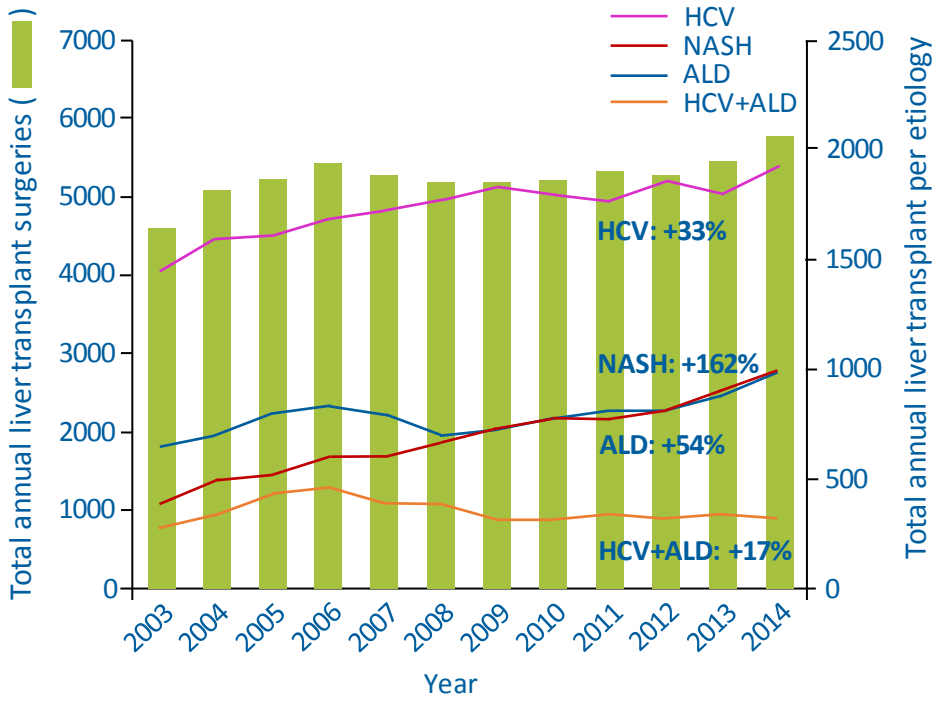
NASH: the most common predisposing factor to HCC in the upcoming decades[2]

Notes *HCC, hepatocellular carcinoma

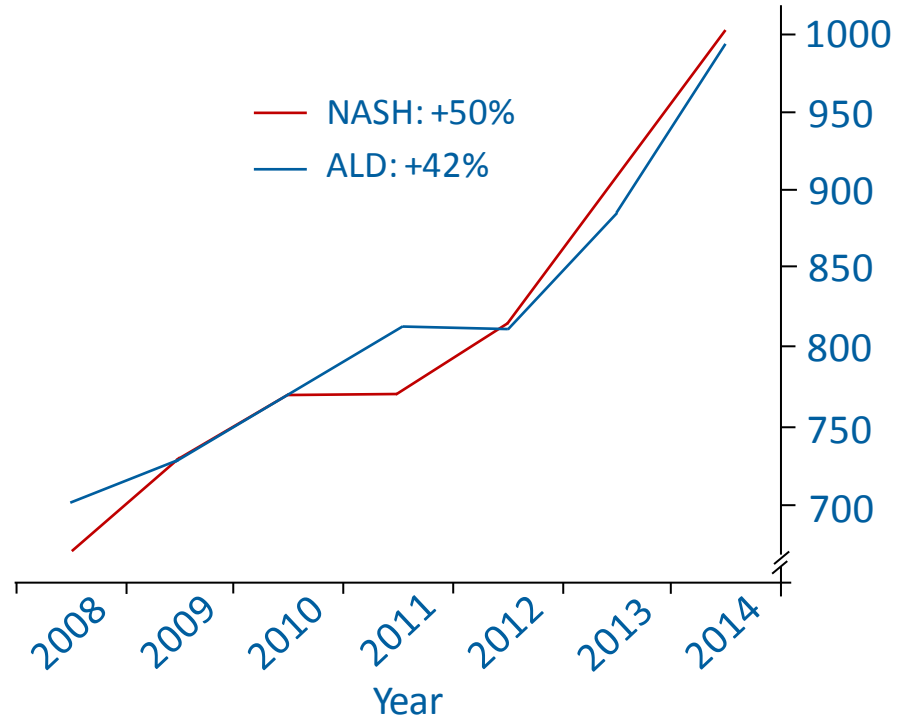
References 1. Adapted from Stine JG et al. Aliment Pharmacol Ther 2018;48:696-703; 2. Adapted from Massoud O et al. Clin Liver Dis 2018;22:201-11

NASH: the most rapidly growing indication for liver transplantation

Trends in liver transplantation by aetiology of liver disease[1]



Trends in liver transplantation for NASH and ALD between 2008 and 2014[2]



By 2020, NASH is expected to be the leading cause of liver transplantation in the US[3]

References

Adapted from: 1. Tsochatzis EA et al. Lancet Gastroenterol Hepatol 2018;3:509-17; 2. Singh S et al. Clin Gastroenterol Hepatol 2015;13:643-54.e1-9; 3. Cholankeril G et al. Dig Dis Sci 2017;62:2915-22

NAFLD is associated with a higher risk for CVD

- Prevalence and incidence of CVD is higher in NAFLD than in matched controls and driven by the association between NAFLD and metabolic syndrome components (Prevalence and incidence of CVD > NAFLD / matched controls and driven by NAFLD + MetS components)
- CVD is a more common cause of death than liver disease in NAFLD
- Biochemical markers of atherosclerosis (low HDL cholesterol, high triacylglycerol) or inflammation (high-sensitive C reactive protein [CRP]) and increased levels of procoagulant/prothrombotic factors are more common in NAFLD than in persons without steatosis
- Pre-atherogenic lesions* wall are more prevalent in NAFLD

Notes *such as increased carotid intima-media thickness; coronary artery, abdominal aortic and aortic valve calcifications; endothelial dysfunction and functional unresponsiveness of the artery

References EASL-EASD-EASO. J Hepatol. 2016;64:1388-402

Co-morbidities

PanNASH initiative
TOWARDS A NASH SOLUTION

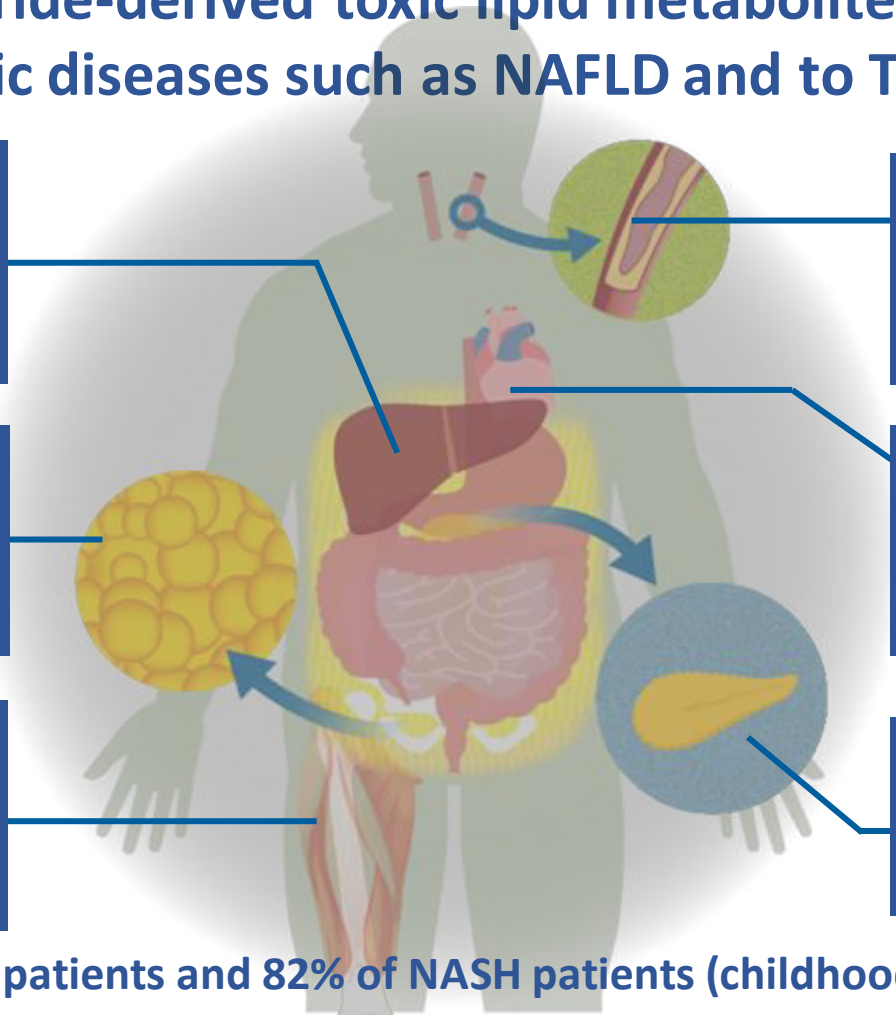
NAFLD is associated with obesity and T2DM

Obesity and triglyceride-derived toxic lipid metabolites lead to common chronic metabolic diseases such as NAFLD and to T2DM and CVD

LIVER
- ↑ Insulin resistance
- ↑ Glucose production
- ↑ VLDL production
- NAFLD or NASH → cirrhosis

DYSFUNCTIONAL ADIPOSE TISSUE
- ↑ Visceral fat
- ↑ Portal FFA → NAFLD
- ↑ Cytokine production
- ↓ Adiponectin

MUSCLE
- ↓ Mitochondrial function
- ↓ VO2 max
- Insulin resistance
- Sarcopenia?



ATHEROSCLEROSIS
- Endothelial dysfunction
- Plaque formation
- CV events

HEART
- Impaired energy metabolism
- Diastolic dysfunction
- ↑ Risk of CAD?

PANCREAS
- ↑ ß-cell apoptosis
- ↓ Insulin secretion
- ↑ T2DM

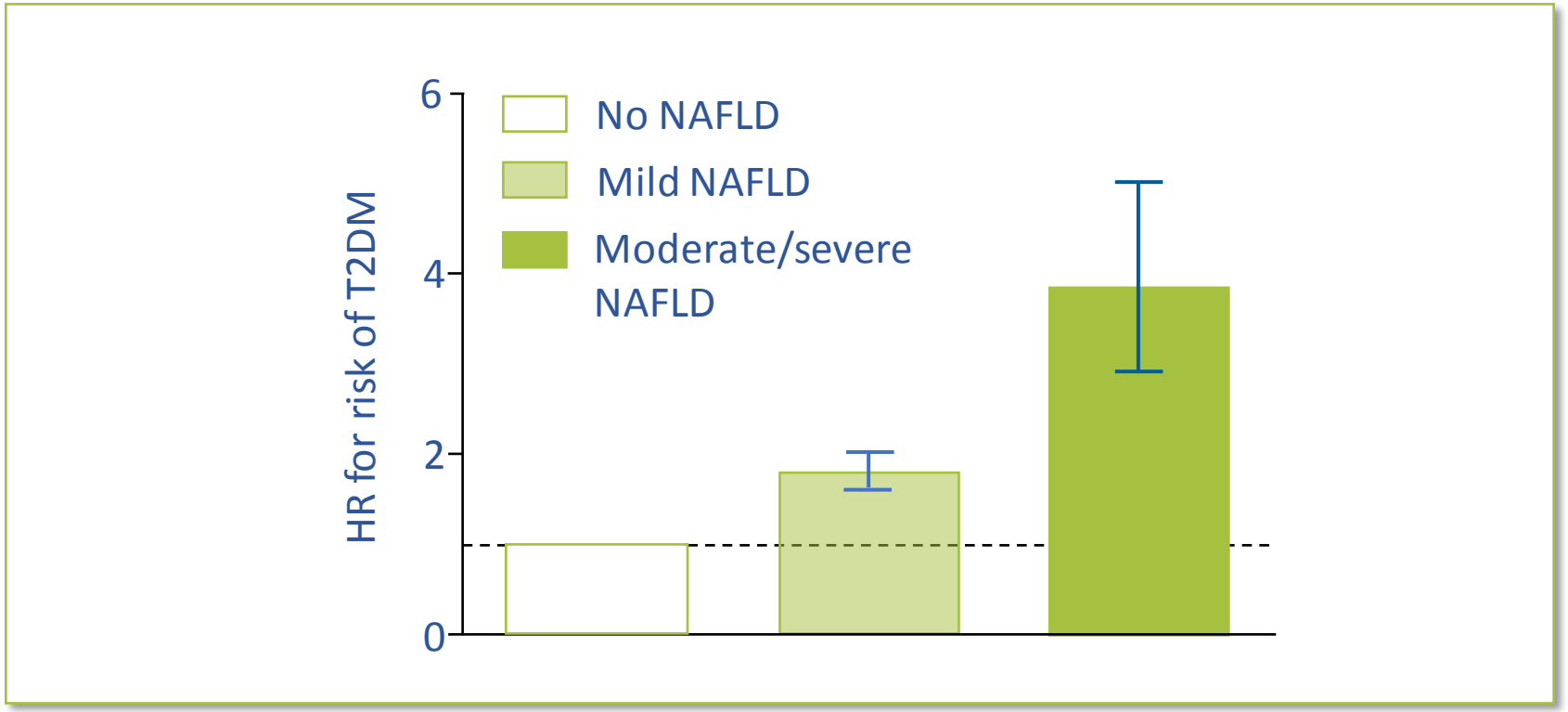
Obesity is present in 51% of NAFLD patients and 82% of NASH patients (childhood obesity is of particular concern)

Notes CAD, coronary artery disease CVD, cardiovascular disease; FFA, free fatty acid; T2DM, type 2 diabetes mellitus; VLDL, very-low-density lipoprotein

References Adapted from Cusi K et al. Gastroenterology 2012;142:711-25; Younossi ZM et al. Hepatology. 2016;64:73-84

NAFLD: increased risk of T2DM

T2DM is present in about 23% of NAFLD patients and about 44% of NASH patients



NAFLD was diagnosed by ultrasonography
Severity of NAFLD was defined based on NAFLD fibrosis score

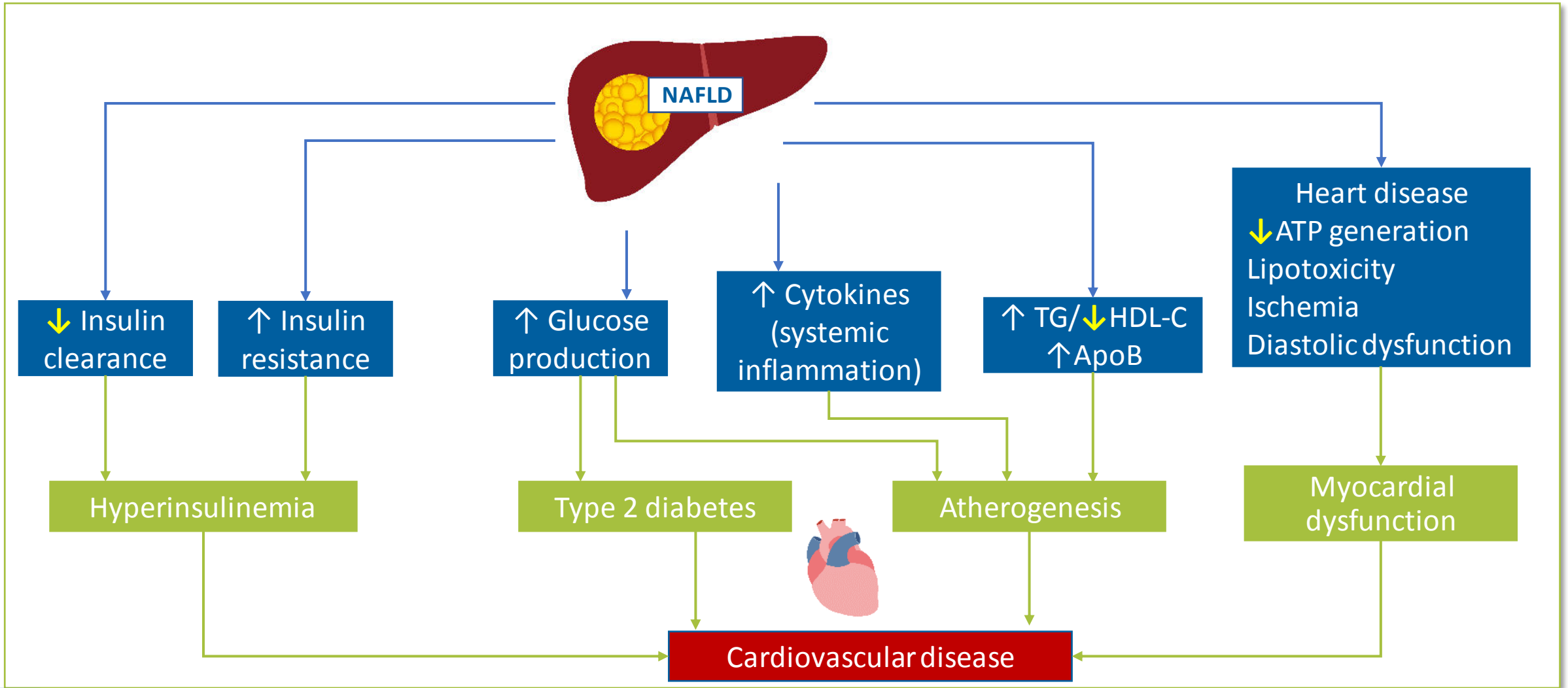
References Adapted from Lallukka S et al. Best Pract Res Clin Endocrinol Metab 2016;30:385-95; Younossi ZM et al. Hepatology. 2016;64:73-84.

Hyperlipidaemia/dyslipidaemia in NAFLD

- **Overall prevalence of hyperlipidaemia/dyslipidaemia:** NAFLD: 70% - NASH: 72%
- **Hypertriglyceridemia prevalence:** NAFLD: 41% - NASH: 83%
- **Patients with NAFLD have a proatherogenic lipid profile characterised by:**
  - high triglycerides
  - increased very-low density lipoprotein (VLDL)
  - high apolipoprotein B to apolipoprotein A-1 ratio
  - higher concentration of small dense LDL
  - low high-density lipoprotein (HDL) concentration
- **As for other commonly associated comorbidities, the presence of dyslipidaemia should be carefully considered when evaluating patients with suspected NAFLD**

References Younossi ZM et al. Hepatology. 2016;64:73-84; Corey KE et al. Lipids Health Dis. 2014;13:100; Chalasani N et al. Hepatology. 2018;67:328-57.

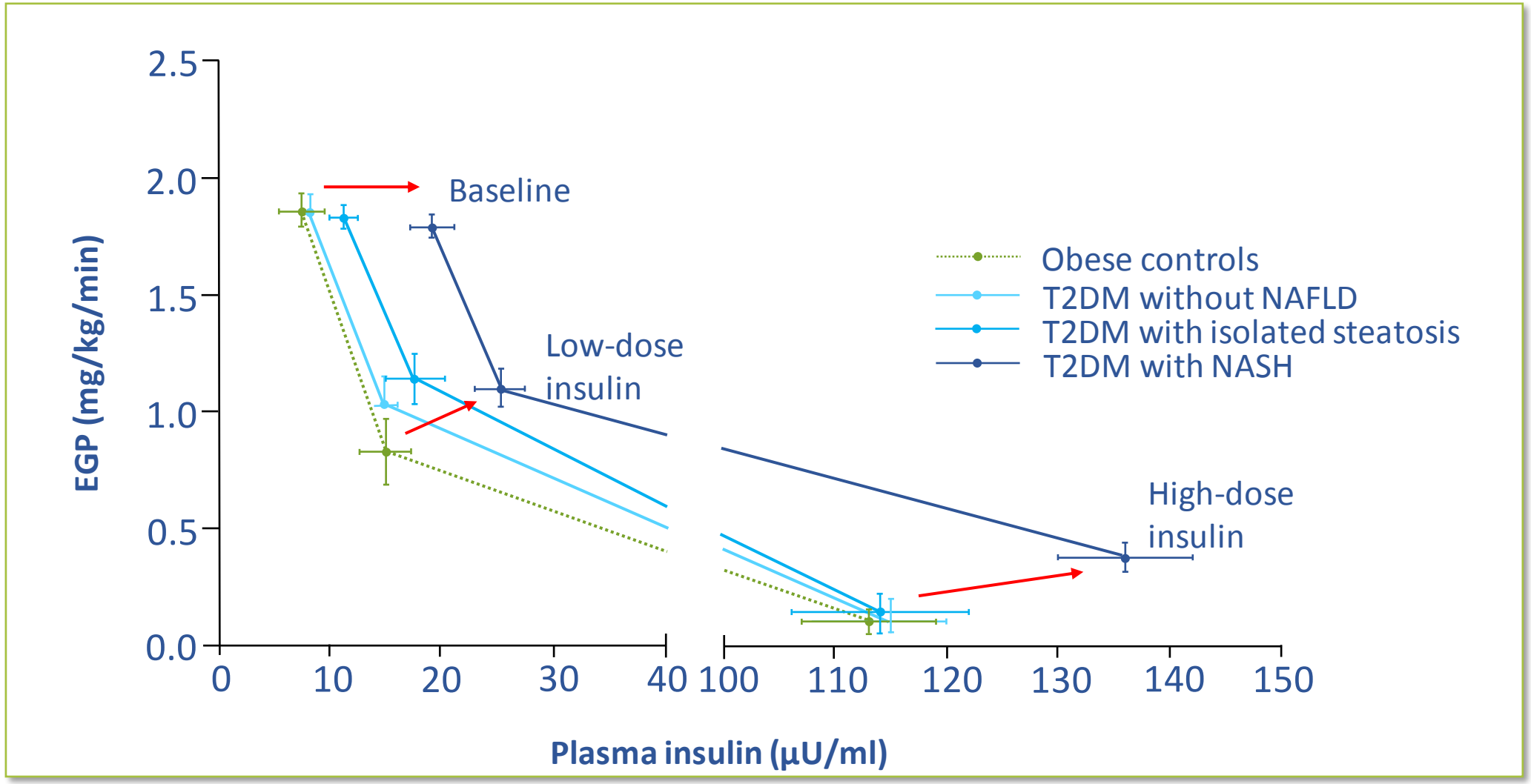
NAFLD may accelerate atherosclerosis



Notes ApoB, apolipoprotein B; ATP, adenosine triphosphate; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride

References Adapted from Cusi K et al. Gastroenterology 2012;142:711-25

NAFLD is associated with a worse insulin resistance and metabolic profile

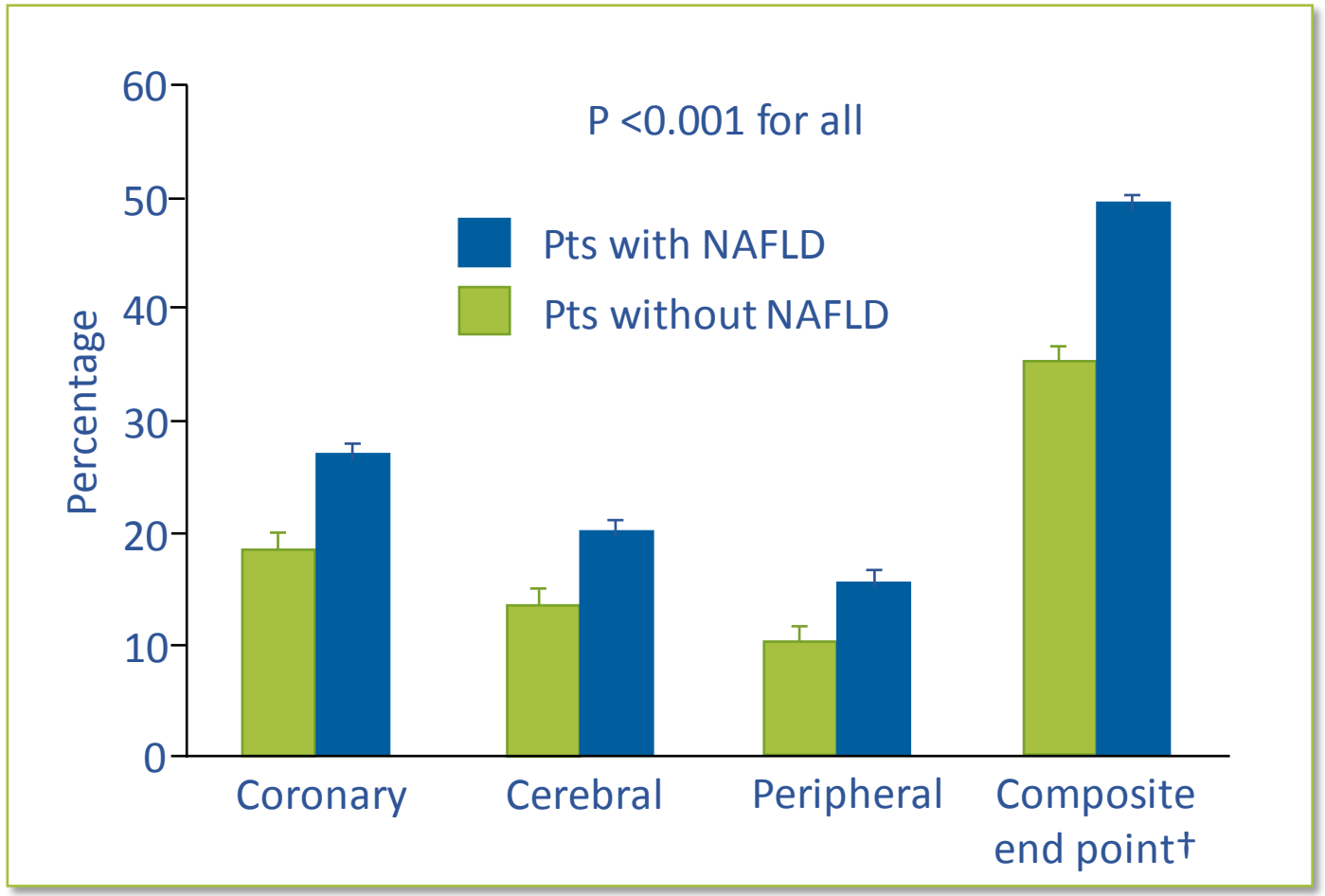


Notes: EGP, endogenous glucose production

References: Adapted from Lomonaco R et al. Diabetes Care 2016;39:632-8

NAFLD: worse micro-/macrovascular disease

Prevalence* of CVD in type 2 diabetic adults with and without NAFLD



† CVD was considered as the composite end point inclusive of those patients with coronary, cerebrovascular, or peripheral vascular disease; *Age- and sex-adjusted

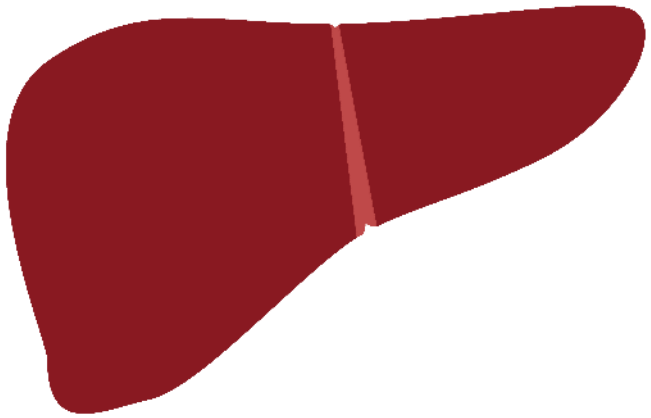
Notes

References

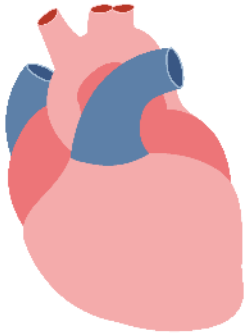
Adapted from Targher G et al. Diabetes Care 2007;30:1212-8

NAFLD: increased risk of cardiovascular disease-related mortality

NAFLD is a significant independent risk factor for CVD



- Endothelial dysfunction
- Insulin resistance
- Atherogenic dyslipidaemia
- Pro-inflammatory cytokines
- Unstable carotid plaque-remodelling

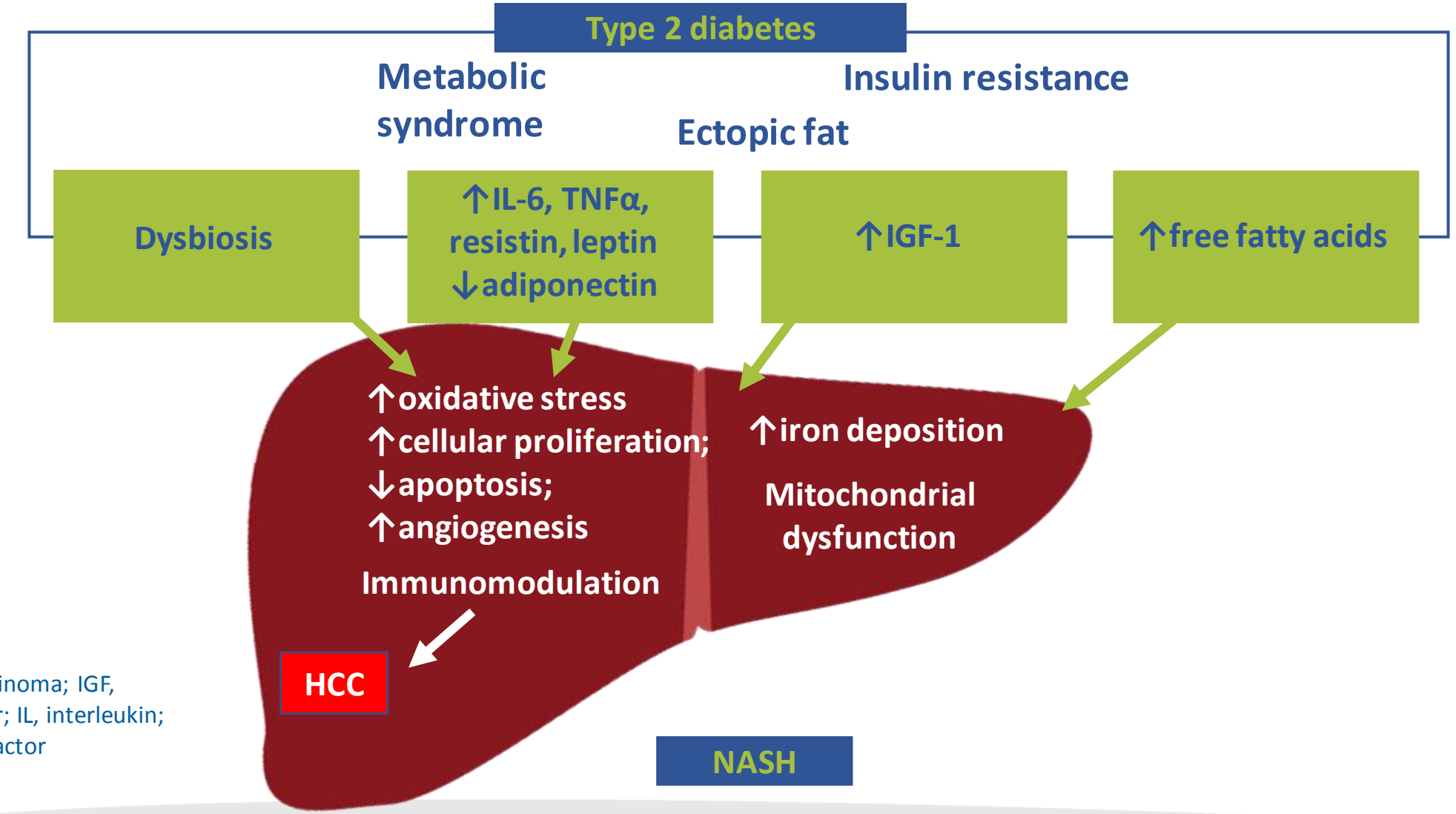


NAFLD (NASH) → **Cardiovascular disease**

References

Adapted from Rinella ME. Hepatology 2016;63:688-90

Processes underlying the development of hepatocellular carcinoma



HCC, hepatocellular carcinoma; IGF, insulin-like growth factor; IL, interleukin; TNFa, tumour necrosis factor

Notes

References

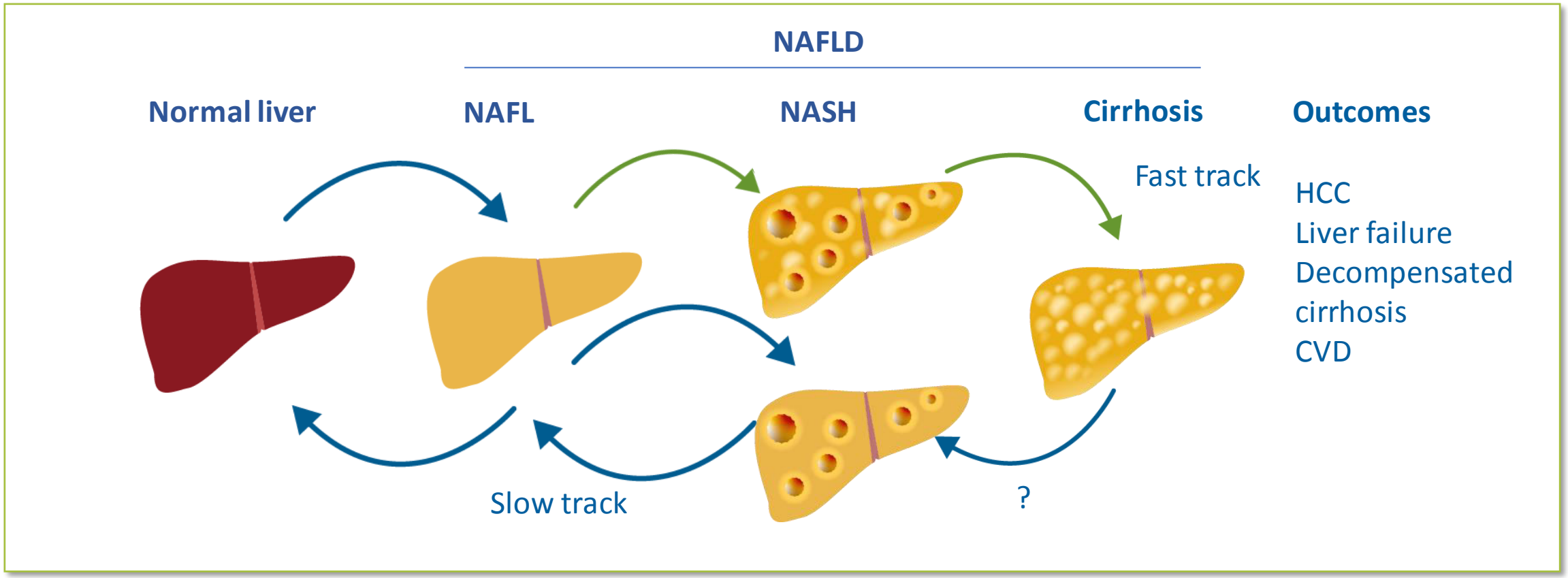
Adapted from Lallukka S et al. Best Pract Res Clin Endocrinol Metab 2016;30:385-95

Pathogenesis

PanNASH initiative
TOWARDS A NASH SOLUTION

The natural progression of NAFLD

Stages and liver conditions included in the clinical definition of NAFLD



References Adapted from Haas JT et al. Annu Rev Physiol 2016;78:181-205

Genetic and molecular factors in NAFLD

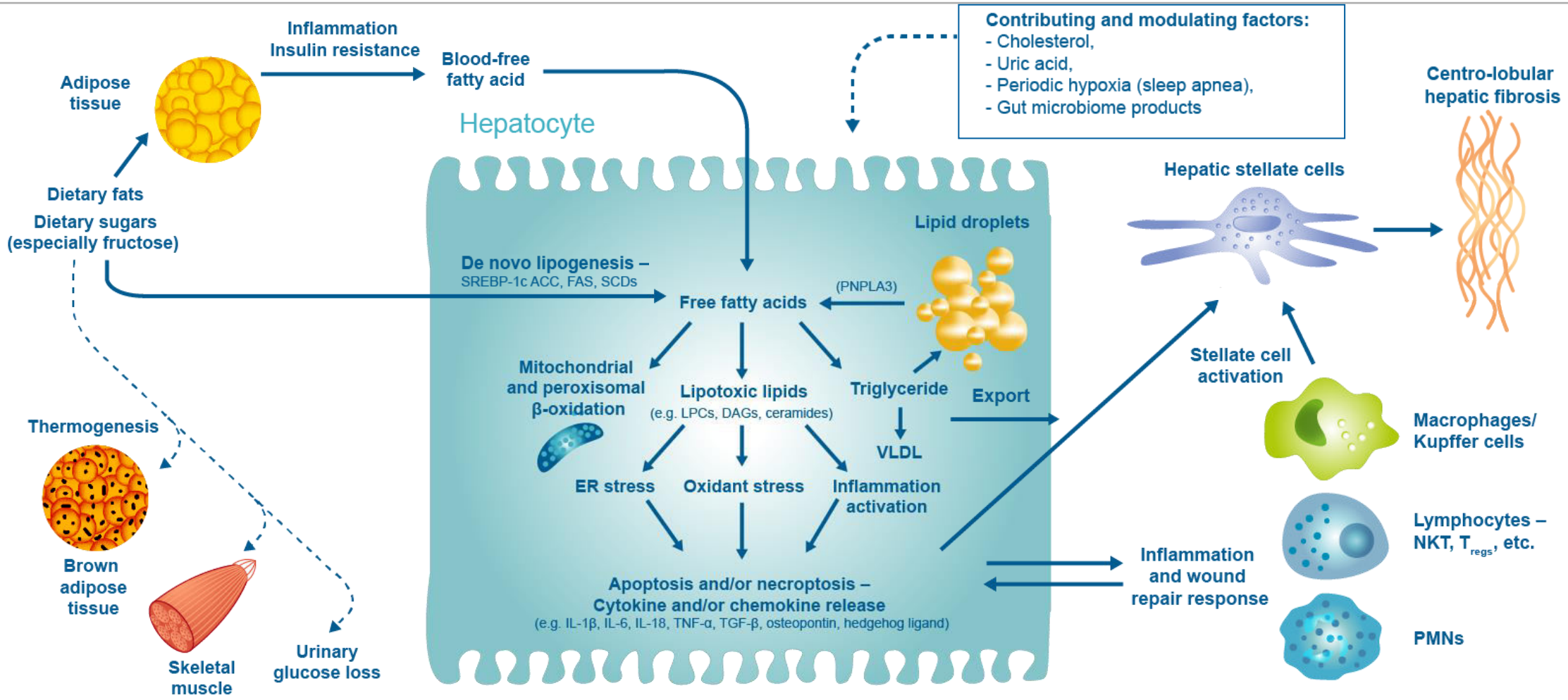
Single-nucleotide polymorphisms associated with NASH

Gene and SNP(s)	Screening associated with	Follow-up findings
PNPLA3, rs738409, I148M	Hepatic fat content by MRS	Associated with NASH severity, fibrosis, and HCC
GCKR, rs780094	Histological NAFLD	For fibrosis [AUROC 0.85 (95%); CI 0.81-0.90]; many patients fall info an undetermined category
FDFT1, rs2645424	NAS in histological screen	For NASH; proprietary
LYPLAL1, rs12137855	Histological NAFLD	AUROC 0.90 for NASH
NCAN, rs2228603, P91S	Steatosis by CT and histological NAFLD	AUROC 0.87 for NASH
PPP1R3B, rs4240624	Steatosis by CT	AUROC 0.81 (95%; CI: 0.70-0.89)
TM6SF2, rs58542926, E167K	Hepatic fat content by MRS and identified by exome sequencing, rather than by SNP arrays	TM6SF2 mutation is associated with reduced CVD and reduced VLDL secretion

Notes AUROC, area under the receiver operating curve; MRS, magnetic resonance sounding; NAS, NAFLD Activity Score; SNP, single-nucleotide polymorphism; VLDL, very low density lipoprotein

References 1. Haas JT et al. Annu Rev Physiol 2016;78:181-205; 2. Naik A et al. Genomics 2013;102:84-95

The substrate-overload liver injury model of NASH pathogenesis

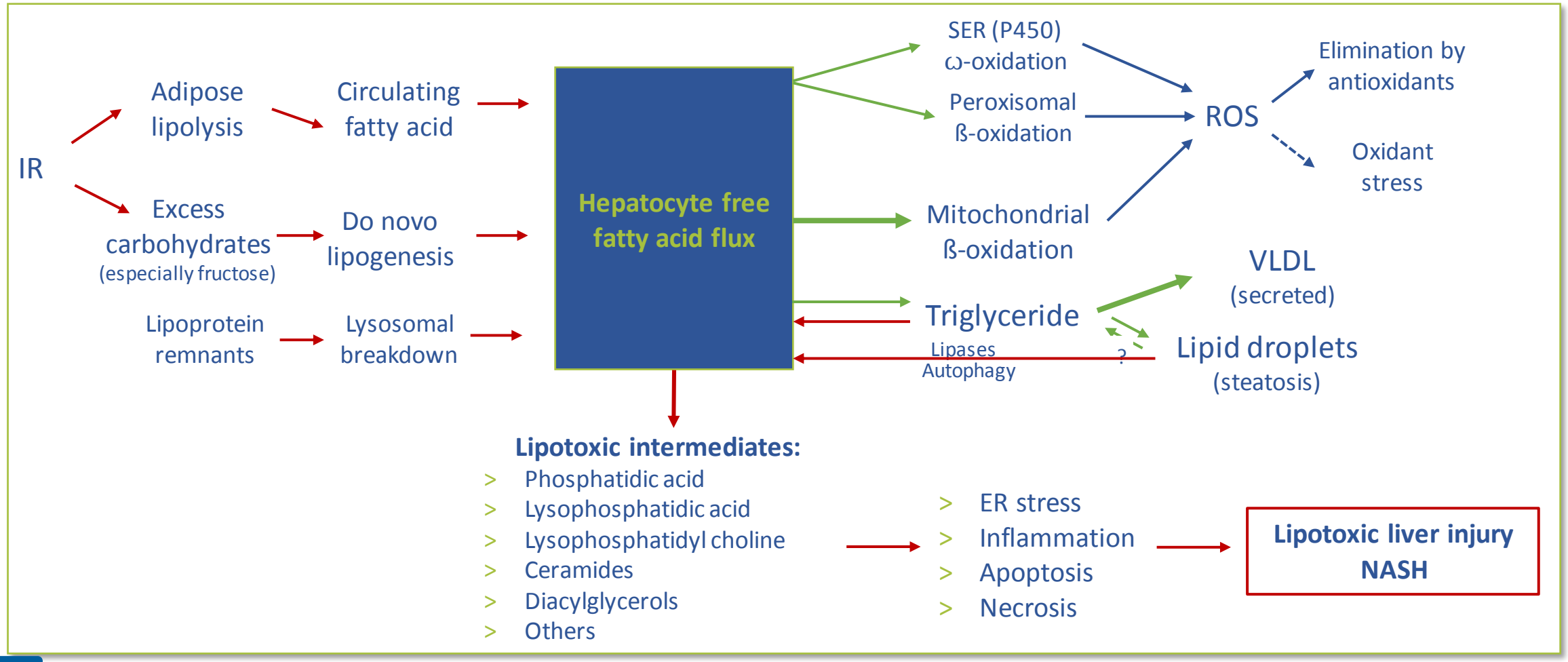


Notes ACC, acetyl-CoA carboxylase; DAG, diacylglycerol; FAS, fatty acid synthase; LPC, lysophosphatidylcholine; NKT, natural killer T cell; PMNs, polymorphonuclear leukocytes; SCD, steroyl CoA-desaturase; Tregs, regulatory T cells

References Adapted from Friedman SL et al. Nat Med 2018;24:908-22

Fatty acids: innocent bystanders?

Cellular injury and death caused by free fatty acids and their metabolites



Notes ER, endoplasmic reticulum; IR, insulin resistance; ROS, reactive oxygen species

References 1. Adapted from Neuschwander-Tetri BA et al. Hepatology 2010;52:774-88; 2. Lee Y et al. Proc Natl Acad Sci USA 1994;91:10878-82

Lipotoxicity, a driver of intrahepatic triglyceride accumulation

- IHTG accumulation is strongly associated with adipose tissue IR
- This supports the current theory of lipotoxicity as a driver of IHTG accumulation
- Once IHTG > 6 ± 2%, skeletal muscle IR, hypertriglyceridemia, low HDL-C become fully established
- Histological activity (inflammation, ballooning, and fibrosis) is not significantly influenced by IHTG accumulation

Notes IHTG, intrahepatic triglyceride; IR, insulin resistance

References Bril F et al. Hepatology 2017;65:1132-44

Hepatic fat content, a cardiovascular risk factor

- **Known association between hepatic fat content and NAFLD and risk of ischaemic heart disease (IHD)[1]**
- **Strong association between a variant in the *PNPLA3* gene and NAFLD[2]**
- **However, fatty liver due to *PNPLA3* variant is not causally linked to IHD[1]**
- **Caveats[3]:**
  - At least 2 distinct forms of NAFLD: obese/metabolic NAFLD and *PNPLA3*-associated NAFLD
  - They have different consequences for risk of IHD

References 1. Lauridsen BK et al. Eur Heart J. 2018;39:385–93. 2. Trepo E et al. J Hepatol. 2016;65:399-412. 3. Byrne CD, Targher G. Eur Heart J 2018;39:3398.

Mitochondrial antioxidant balance and NASH

- Oxidative stress, alterations in mitochondrial function: a significant role in NASH
- Important contribution to generation of reactive oxygen species (ROS)
- Evidence that a subtle balance among antioxidants, particularly in mitochondria, is necessary to avoid the generation of ROS and hence oxidative stress

References Garcia-Ruiz C, Fernandez-Checa JC. Hepatol Commun. 2018;2:1425-39.

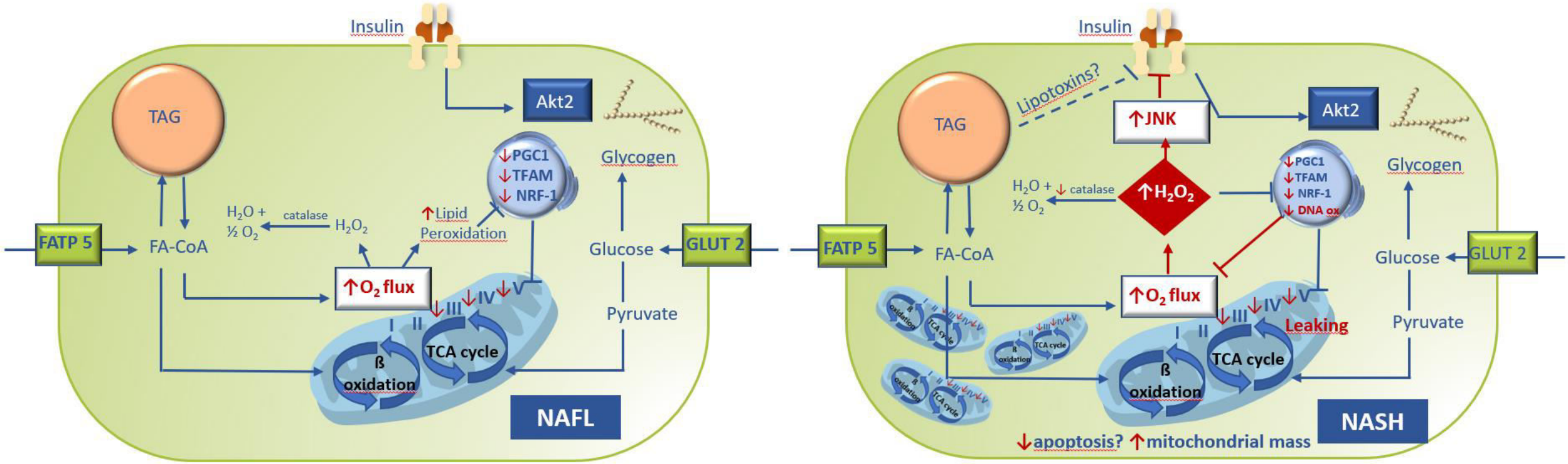
Major pathophysiological mechanisms involved in oxidative stress in NAFLD

- Mitochondrial dysfunction
- Endoplasmic reticulum stress
- Disturbance of iron metabolism
- Inappropriate inflammatory response mediated by GUT-liver axis
- Insulin resistance and endothelial dysfunction

References Masarone M et al. Oxid Med Cell Longev. 2018;2018:9547613.

NASH: loss of adaptation of hepatic mitochondrial function

- Evidence for a compensatory upregulation of hepatic mitochondrial respiration in obese insulin-resistant humans with and without NAFL
- Impaired respiratory capacity and proton leakage in obese humans with NASH
- Elevated oxidative stress coupled to reduced anti-oxidant capacity in NASH



Notes Akt2, protein kinase B; FA-CoA, fatty acyl coenzyme A; FATP, fatty acid transport protein; GLUT, glucose transporter; PGC1a, PPARg-coactivator 1a; NRF-1, nuclear respiratory factor 1; TAG, triacylglycerol; TCA, tricarboxylic acid; TFAM, mitochondrial transcription factor A

References Adapted from Koliaki C et al. Cell Metab. 2015;21:739-46

Macrophages: a role model of pathogenic immunometabolism

- Immunometabolism: an emerging field of basic and clinical research
- Influence of immune cells on the whole-body metabolism
- Link between inflammatory status and cell metabolic activity
- Liver macrophages: tissue-resident Kupffer cells and monocyte-derived macrophages

References Krenkel O, Tacke F. Semin Liver Dis 2017;37:189-97.

Triggering inflammation: outside and inside the liver

- **Outside the liver**
  - Adipose tissue
  - Gut
- **Inside the liver**
  - Lipotoxicity
  - Innate immune responses
  - Cell death pathways
  - Mitochondrial dysfunction
  - Endoplasmic reticulum stress

References Schuster S et al. Nat Rev Gastroenterol Hepatol 2018;15:349-364.

Kupffer cells: the resident hepatic macrophages

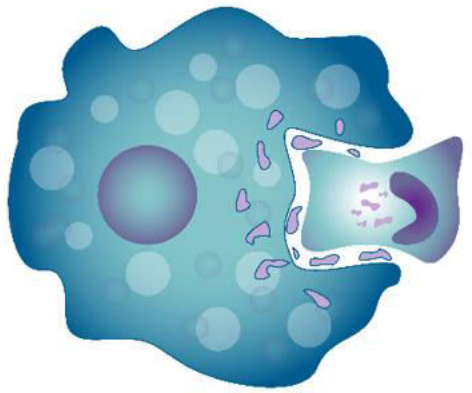
- **Important members of the innate and adaptive immune systems**
- **Lipopolysaccharides, free fatty acids and cholesterol can activate Kupffer cells**
  - Produce proinflammatory factors
  - Lead to progression from NAFL to NASH

References Li P et al. Mol Immunol 2017;85:222-9.

Macrophages in NAFLD

- **Liver-resident Kupffer cells**
  - Initiate the inflammatory response
  - Are instrumental in recruiting monocytes to the liver
- **Monocytes rapidly differentiate into pro-inflammatory macrophages**
- **Activation: not restricted to the liver**

Kupffer cell

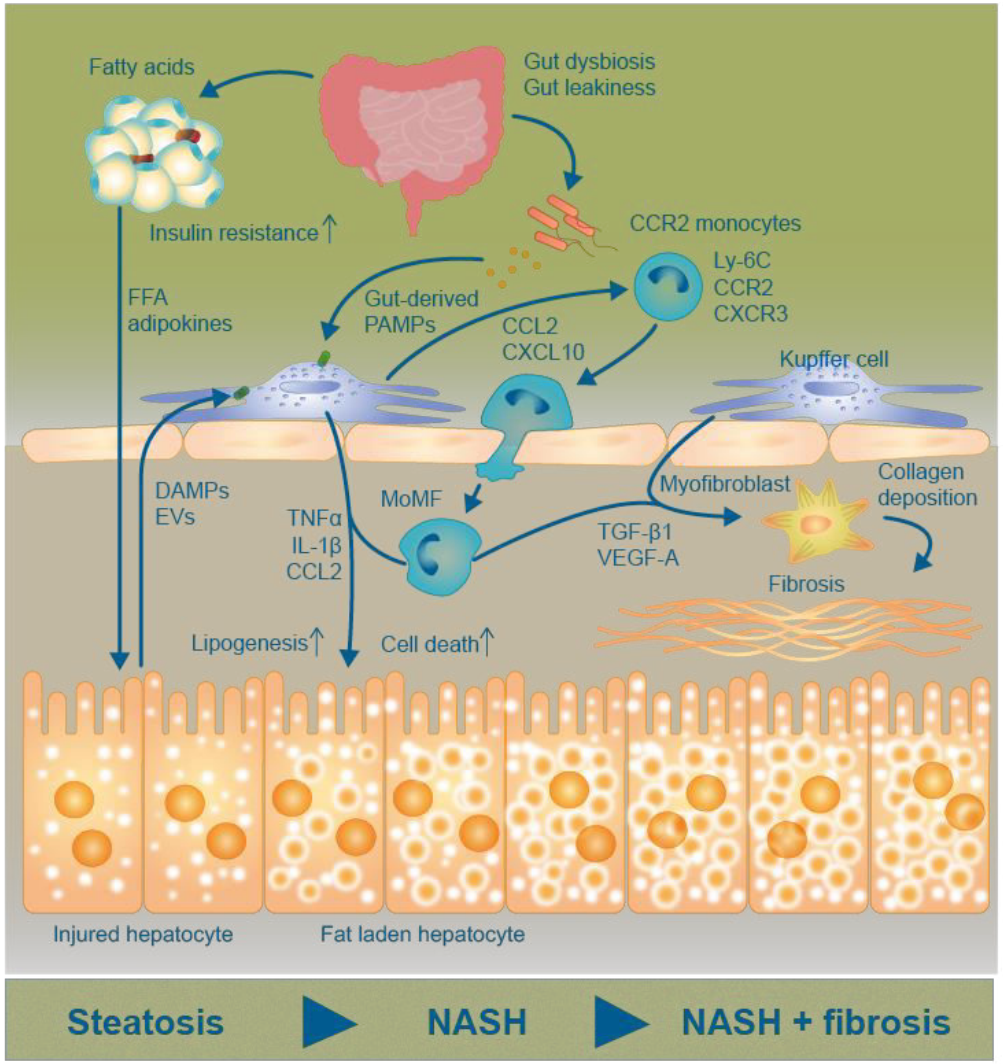


M1 macrophage

References

Adapted from Kazankov K et al. Nat Rev Gastroenterol Hepatol 2018 [doi: 10.1038/s41575-018-0082-x]

Role of hepatic macrophages in the development of NASH



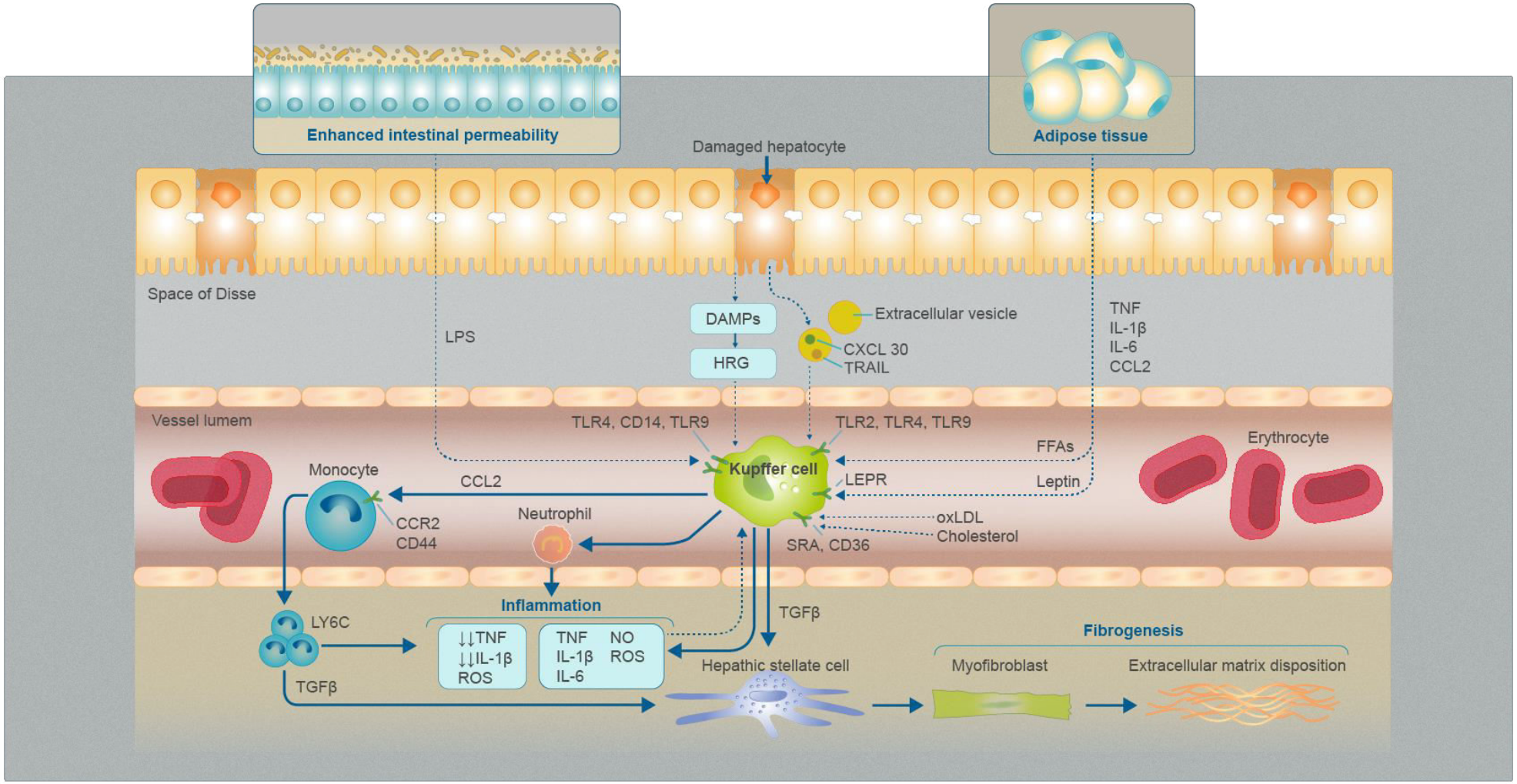
CCL, C–C motif chemokine; CXCL, CXC-chemokine ligand; DAMP, damage-associated molecular pattern; EV, extracellular vesicles; MoMF, monocyte-derived macrophages; PAMP, pathogen-associated molecular pattern; TNF, tumor necrosis factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor

Notes

References

Krenkel O, Tacke F. Semin Liver Dis 2017;37:189-197

Kupffer cell activation in NAFLD



CXL10, CXC-chemokine ligand 10; LY6C, lymphocyte antigen 6C; NO, nitric oxide; oxLDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; TRAIL, TNF-related apoptosis-inducing ligand

Notes

References

Kazankov K et al. Nat Rev Gastroenterol Hepatol 2018 [doi: 10.1038/s41575-018-0082-x]

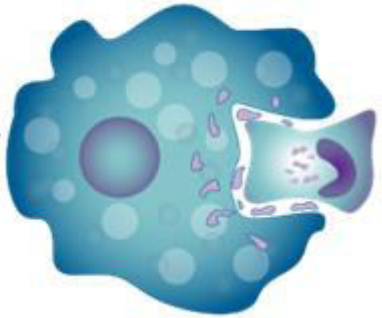
Macrophage polarization: an important mechanism of inflammatory response

Kupffer cell
Liver-resident macrophage, originating from erythromyeloid progenitors of the yolk sac

Monocyte
Blood-derived, originating from haematopoieitic stem cell

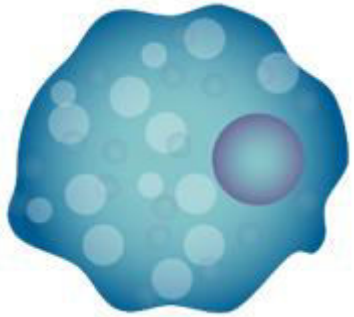
CSFR1
CCR2
IL4RA
IL13RA1
IL13RA2
STAT6

IL-12, IFNγ



Classically activated M1 macrophage (M1a-c)

IL-4, IL-13



Alternatively activated M2 macrophage (M2a-d)

Fibrolytic, immune-activating
> Phagocytosis of debris and apoptotic cells
> Generation of pro-inflammatory cytokines

Pro-resolution macrophage CD11bF4/80LY6C

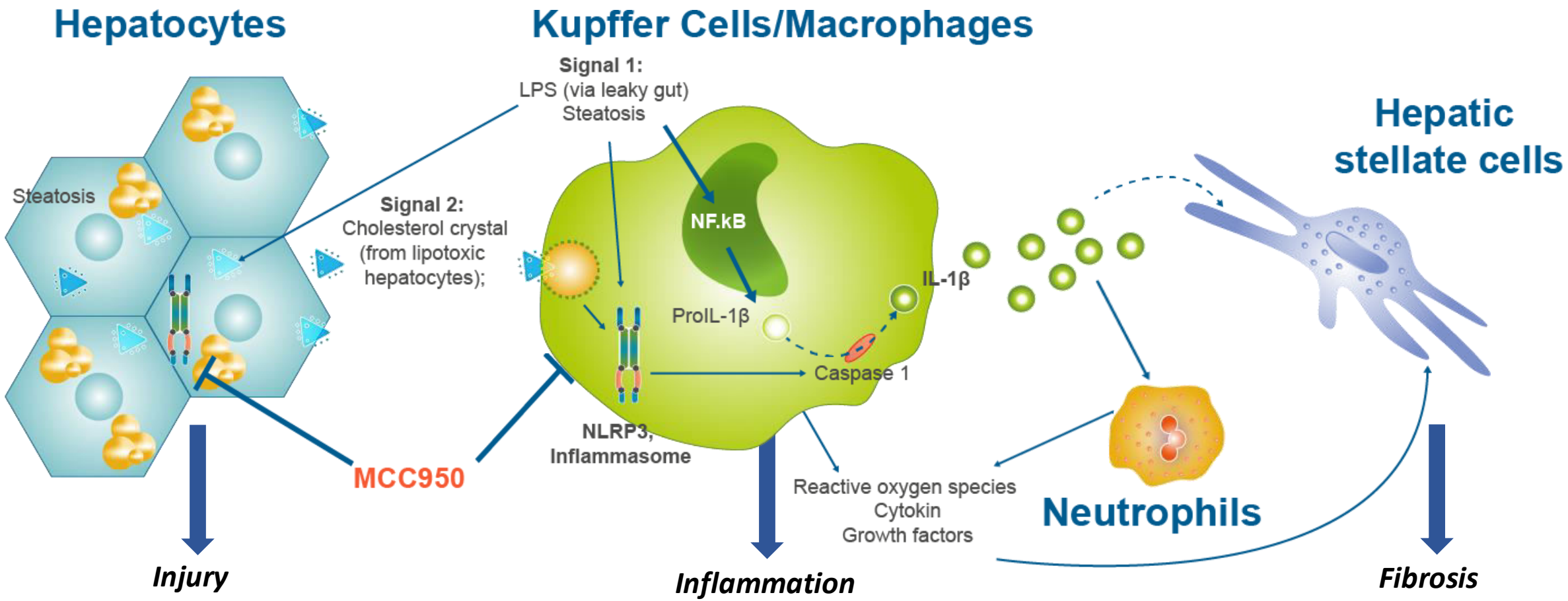
Targeted modulation?

Probiotic, immuno-suppressive
> Promotion of angiogenesis
> Generation of anti-inflammatory cytokines

Notes CCR2, CC-chemokine receptor 2; CSFR1, macrophage colony-stimulating factor receptor 1; IFNγ, interferon-γ; IL4RA, interleukin-4 receptor subunit-α; IL13RA, interleukin-13 receptor subunit-α; LY6C, lymphocyte antigen 6C; STAT6, signal transducer and activator of transcription 6

References Adapted from Kazankov K et al. Nat Rev Gastroenterol Hepatol 2018 [doi: 10.1038/s41575-018-0082-x]

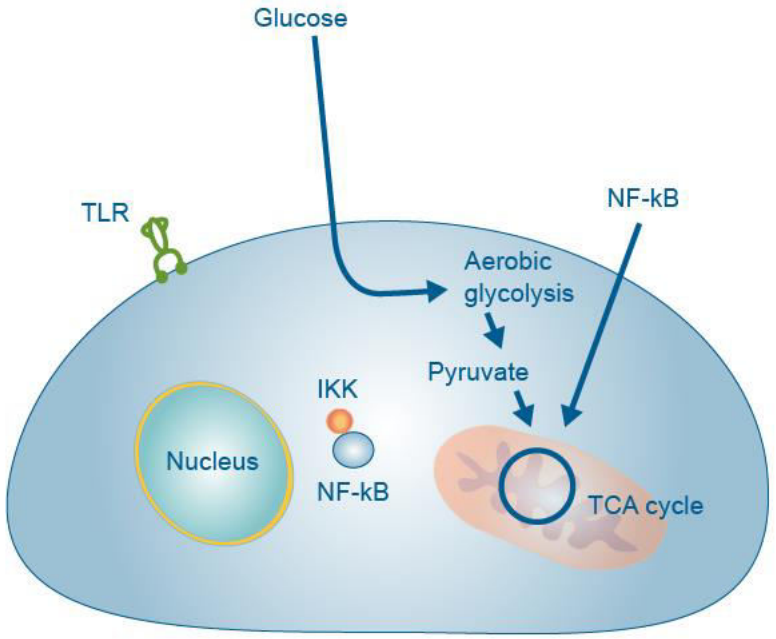
Activation of the inflammasome is important in NAFLD progression



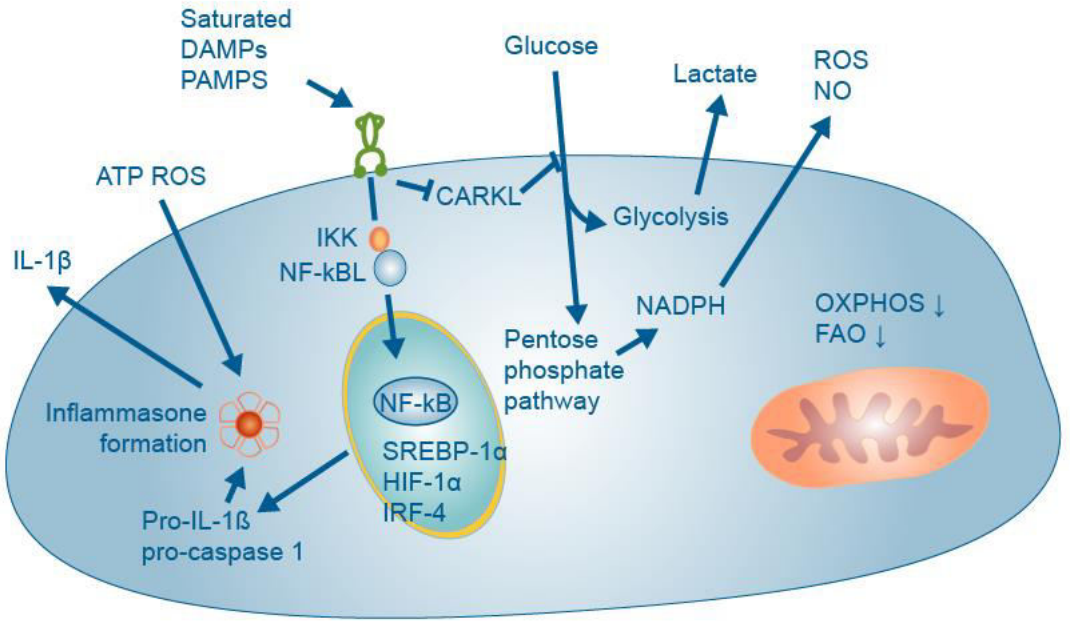
References Adapted from: 1. Thomas H. Nat Rev Gastroenterol Hepatol 2017;14:97; 2. Mridha AR et al. J Hepatol 2017;66:1037-104

Metabolic reprogramming of macrophages

Macrophage metabolism in homeostasis



Macrophage metabolism upon activation



Notes

CARKL, carbohydrate kinase-like protein; DAMP, damage-associated molecular pattern; FAO, fatty acid oxidation; HIF-1α, hypoxia inducible factor 1α; IRF-4, interferon regulatory factor 4; NO, nitrogen oxide; OXPHOS, oxidative phosphorylation; PAMP, pathogen-associated molecular pattern; ROS, reactive oxygen species; SREBP-1α, sterol regulatory element binding protein 1α; TCA, tricarboxylic acid; TLR, toll-like receptor

References

Adapted from Krenkel O, Tacke F. Semin Liver Dis 2017;37:189-97

Inflammation in NASH

- **Triggers of hepatic inflammation: origins outside and inside the liver**
- **Adipose tissue dysfunction and hepatic inflammatory response: a fundamental role during NASH development**
- **Abrogation of liver inflammation could be achieved by exploiting**
  - active, physiological pro-resolving mechanisms (a ‘pushing for’ strategy)
  - classical passive blockade of pro-inflammatory mediators (the ‘push back’ strategy)

References Schuster S et al. Nat Rev Gastroenterol Hepatol 2018;15:349-64

Fibrosis in NASH

- **NASH is associated with some degree of hepatic fibrosis and cirrhosis with some further progressing to HCC, and a small fraction of patients will develop progressive fibrosis**

- **Fibrosis progression is not necessarily linear and varies from patient to patient**
  - Liver biopsy studies suggest that fibrosis progresses at a rate of approximately one stage per decade,
  - suggesting that stage 2 fibrosis will progress to cirrhosis within 20 years

- **While NASH improvement or resolution leads to a reduction of fibrosis in some patients, in others fibrosis continues or worsens**

References Hardy T et al. Annu Rev Pathol. 2016;11:451-96

Fibrosis staging

F0	No fibrosis
F1	Periportal or perisinusoidal fibrosis
F2	Periportal and perisinusoidal fibrosis
F3	Bridging fibrosis
F4	Cirrhosis

References Derosa G et al. J Cell Physiol 2018;233:153-61

Diagnosis

PanNASH initiative
TOWARDS A NASH SOLUTION

Simplified algorithm for the diagnosis of NASH

Abnormal liver function test + **No significant alcohol consumption** (♂ ≤30 g; ♀ ≤20 g/day)

History and examination
- Exclude drug-induced liver injury
- Exclude other aetiologies of liver disease

Non-invasive liver screen
- Blood test (raised GGT, ALT, AST)
- Ultrasound confirmation of hepatic steatosis

NAFLD

Investigate severity of liver fibrosis
- Enhanced liver fibrosis (ELF) test
- Magnetic resonance elastography (MRE)
- Vibration-controlled transient elastography (VCTE)
- Shear-wave elastography
- Acoustic radiation force impulse imaging (ARFI)
- Fibrosis-4 (FIB-4) index, FibroTest, AST platelet ratio index (APRI)

Liver biopsy (NAS et SAF score)

NASH

ALT, alanine aminotransferase;
AST, aspartate aminotransferase;
GGT, γ-glutamyltransferase

Notes

References

https://www.bmj.com/content/bmj/suppl/2018/07/12/bmj.k2734.DC1/testing-NAFLD-v52-web.pdf

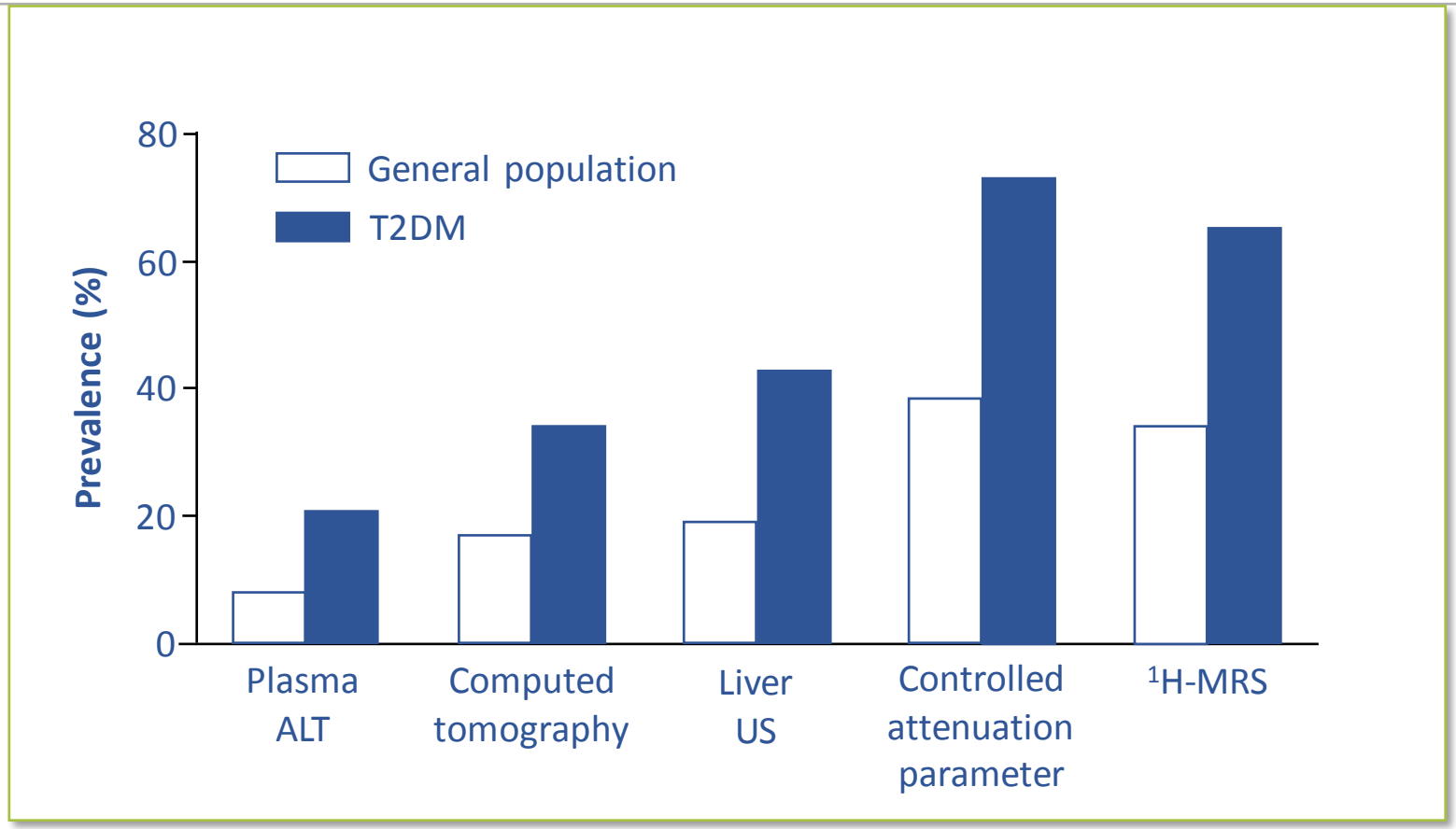
Diagnosis of NAFLD

Requires:

- **Hepatic steatosis by imaging or histology**
- **No significant alcohol consumption**
- **No competing aetiologies for hepatic steatosis**
- **No coexisting causes of chronic liver disease**
- **Exclusion of coexisting aetiologies for chronic liver disease**

References Chalasani N et al. Hepatology 2018;67:328-57

Prevalence of NAFLD varies depending on the tool used[1]



Furthermore, up to 50% of type 2 diabetes patients with normal ALT levels have been diagnosed with NAFLD using ^{1}H-MRS, suggesting that ALT is a poor marker of NAFLD[2]

Notes ALT, alanine aminotransferase; MRS, magnetic resonance spectroscopy; US, ultrasonography

References 1. Bril F et al. Diabetes Care 2017;40:419-30; 2. Portillo-Sanchez P et al. J Clin Endocrinol Metab. 2015;100:2231-8

Liver ultrasonography: a pragmatic first-line test

After history and examination

- Non-invasive liver screen: is it NAFLD or something else?
- Ultrasound technique of choice for NAFLD screening (overall sensitivity 85%, specificity 94%)
- Liver ultrasound: features suggestive of NAFLD?
- Confirmed hepatic steatosis:
  fibrosis biomarker panels and/or vibration-controlled transient elastography
- Hepatic fibrosis: referral for specialist opinion

References Hernaez R et al. Hepatology. 2011;54:1082-90; Byrne CD et al. BMJ 2018;362:k2734

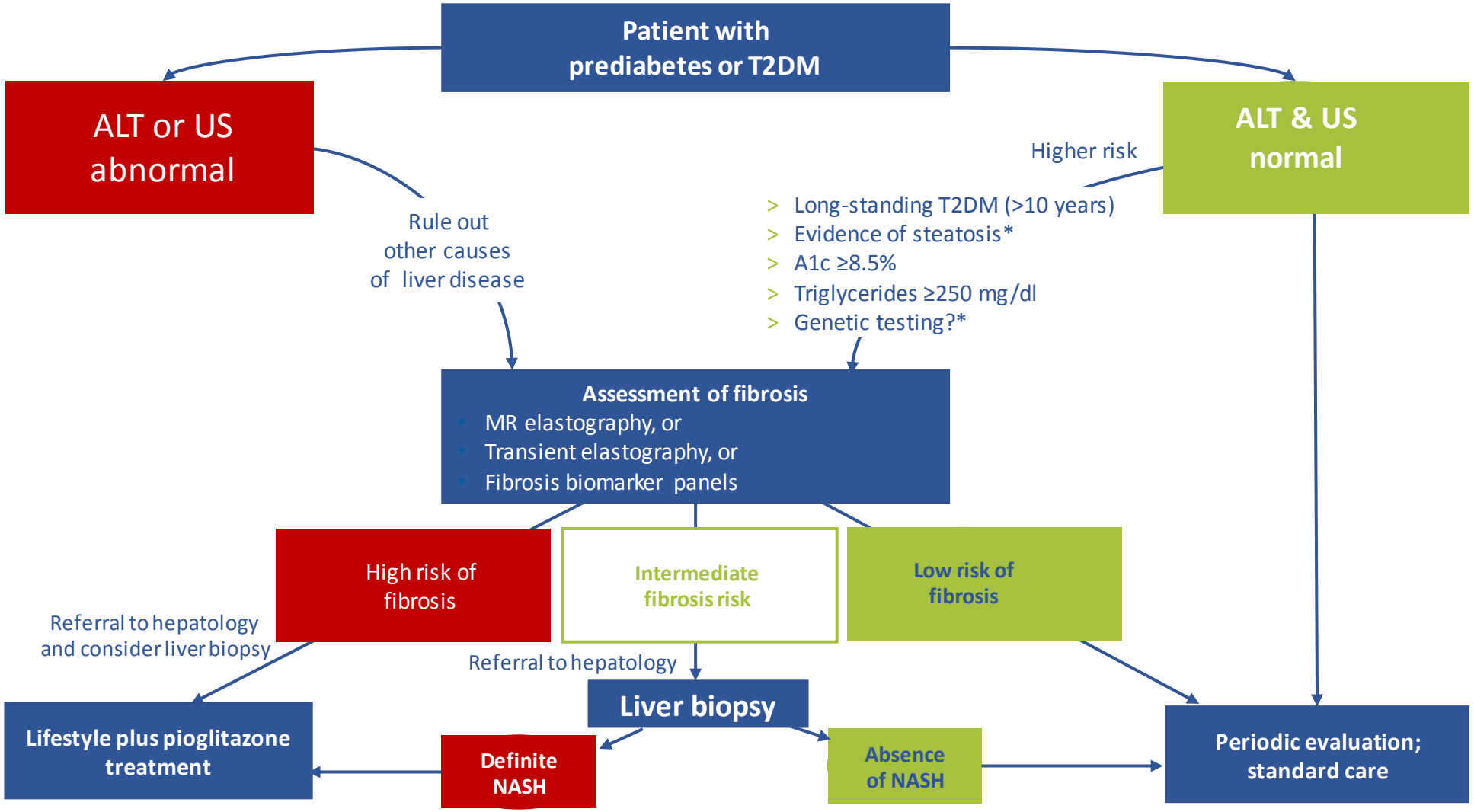
Fibrosis assessment: liver biopsy, elastography and scoring systems

- **Liver biopsy = gold standard to diagnose NAFLD and differentiate NAFL/NASH**
- **However, elastography and scoring systems can be used to assess fibrosis in patients with NAFLD**
  - Enhanced liver fibrosis (ELF) test
  - Vibration-controlled transient elastography (VCTE, FibroScan©)
  - Magnetic resonance elastography (MRE)
- **Combination scores + elastography: additional accuracy**
- **Patients with fibrosis are thought to have NASH**
- **Patients suspected of having NASH should undergo liver biopsy**

Notes ELF test, Enhanced liver fibrosis test; VCTE, Vibration-controlled transient elastography; MRE, Magnetic resonance elastography

References Gunn NT et al. Clin Liver Dis 2018;22:109-19; Byrne CD et al. BMJ 2018;362:k2734

Algorithm for the diagnosis of NAFLD and NASH in patients with prediabetes or T2DM



Notes *Based on results from more sensitive tests such as liver 1H-MRS, MRI-proton density fat fraction, or controlled attenuation parameter

References Adapted from Bril F et al. Diabetes Care 2017;40:419-30

Treatment

PanNASH initiative
TOWARDS A NASH SOLUTION

Lifestyle intervention improves liver histology in NASH

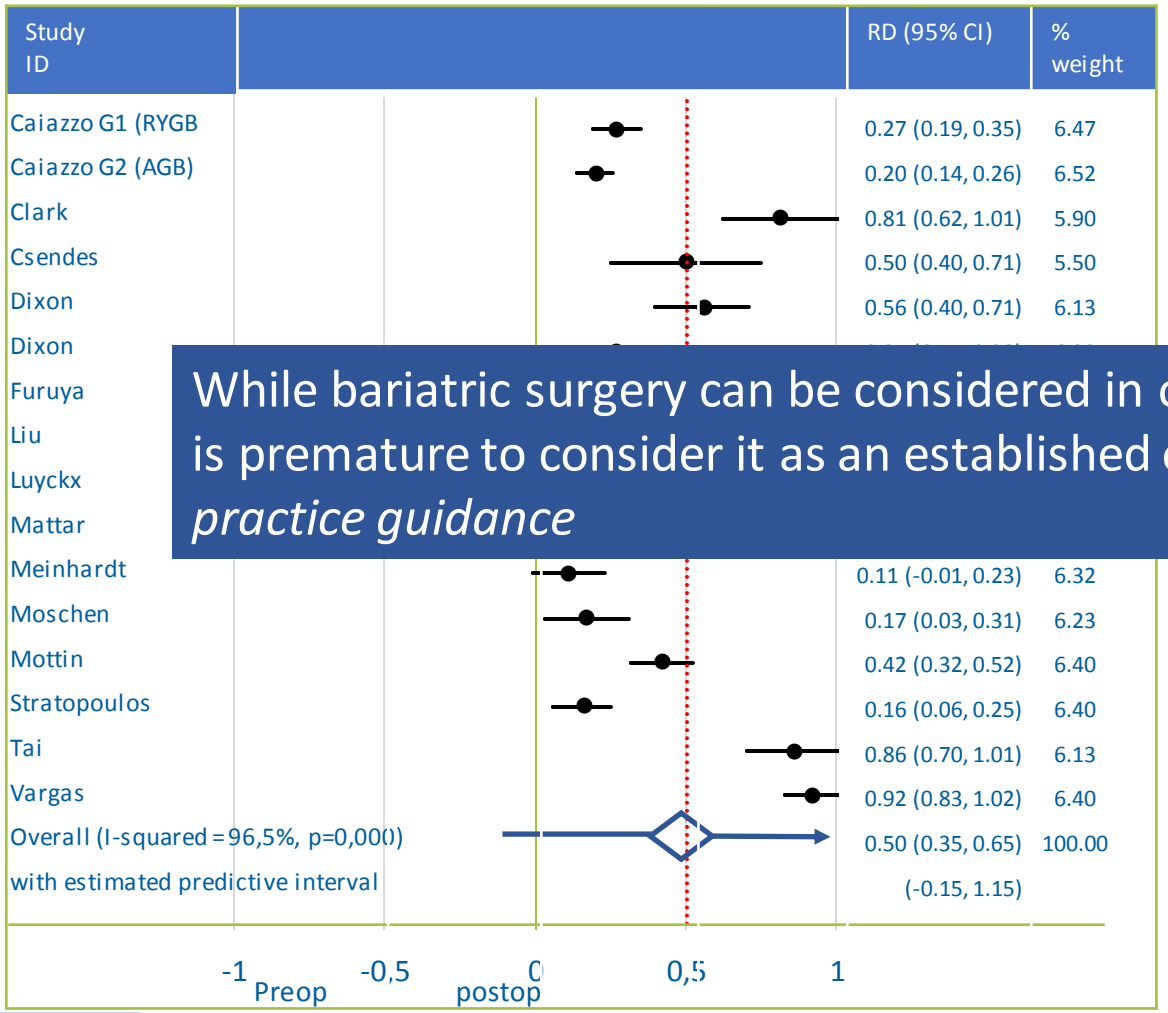
Diet and lifestyle changes are mandatory in all patients

Area	Suggested intervention
Energy restriction	> 500-1000 kcal energy defect > 7-10% total weight loss target > Long-term maintenance approach
Alcohol intake	> Strictly keep alcohol below the risk threshold (30 g, men; 20 g, women)
Exercise/physical activity	> Moderate intensity aerobic physical activities (150-200 min/week) > 3-5 sessions > Resistance training

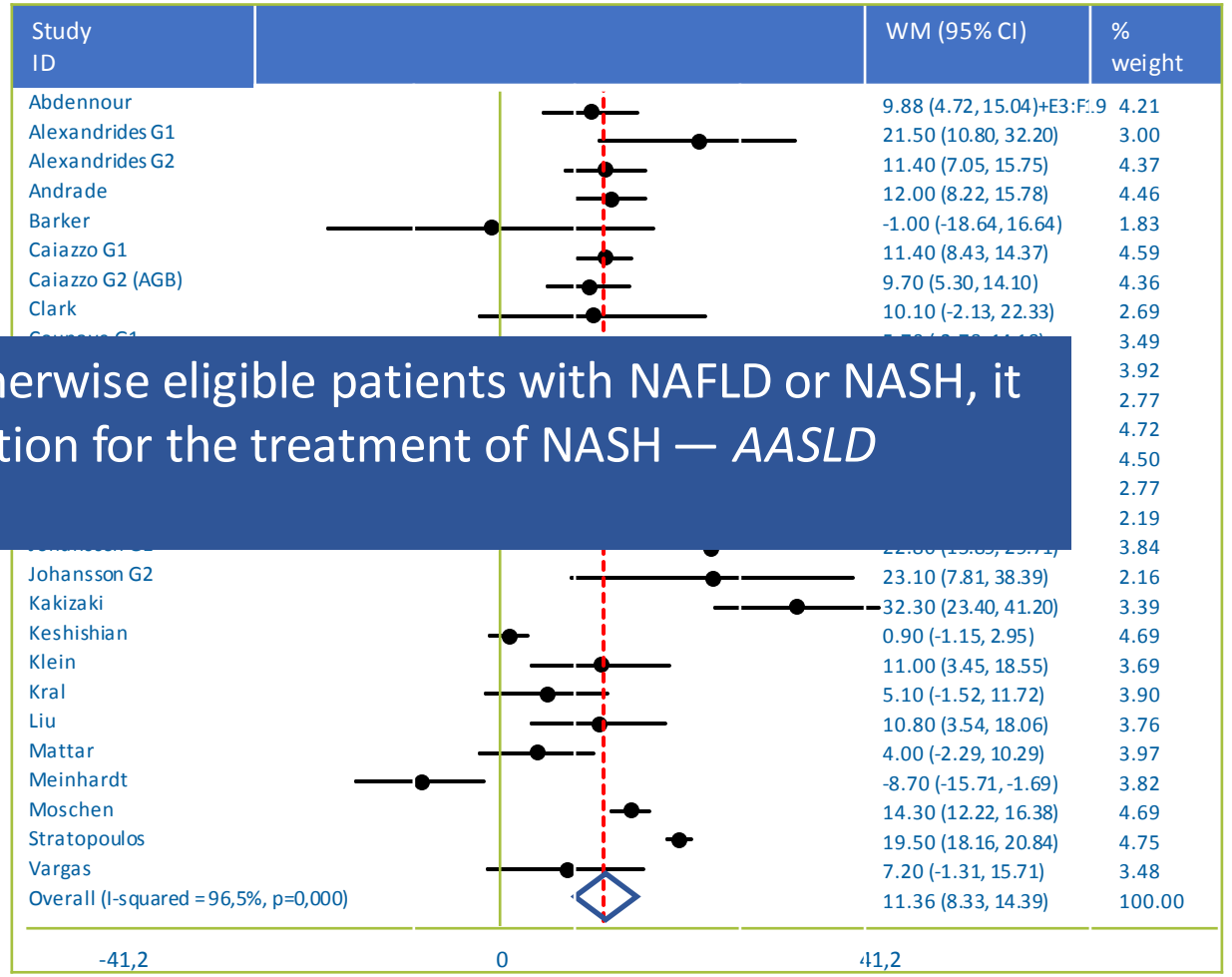
References EASL, EASD, EASO. J Hepatol 2016;64:1388-402; Chalasani N et al. Hepatology 2018;67:328-57

Bariatric surgery improves comorbid disease and improves long-term survival and death from CVD and malignancy

Changes in liver histology for steatosis



Changes in liver biochemistry for alanine aminotransferase (ALT)



While bariatric surgery can be considered in otherwise eligible patients with NAFLD or NASH, it is premature to consider it as an established option for the treatment of NASH — *AASLD practice guidance*

Notes Weights are from random effects analysis

References Bower G et al. Obes Surg 2015;25:2280-9

Pharmacotherapy: Lack of approved therapies

- **Pharmacotherapy: should be reserved for patients with biopsy-proven NASH**
- **Pioglitazone[1] or vitamin E[2] or their combination could be used for NASH according to European guidelines[3]**
  - Optimal duration of therapy: unknown
- **Statins may be confidently used to reduce LDL-cholesterol and prevent CV risk in NAFLD patients, with no increased risk of hepatotoxicity, may even significantly reduce aminotransferases (B1)**
- **N-3 PUFAs: reduce both plasma and liver lipids, but no data to support their use specifically for NASH (B1)**

Notes B1: Evidence of moderate quality; strong recommendation warranted; B2: Evidence of moderate quality; weaker recommendation; PUFA, polyunsaturated fatty acids
[1]most efficacy data, but off-label outside T2DM; [2]better safety and tolerability in the short-term; [3]B2 recommendation

References EASL, EASD, EASO. J Hepatol 2016;64:1388-402; Chalasani N et al. Hepatology 2018;67:328-57

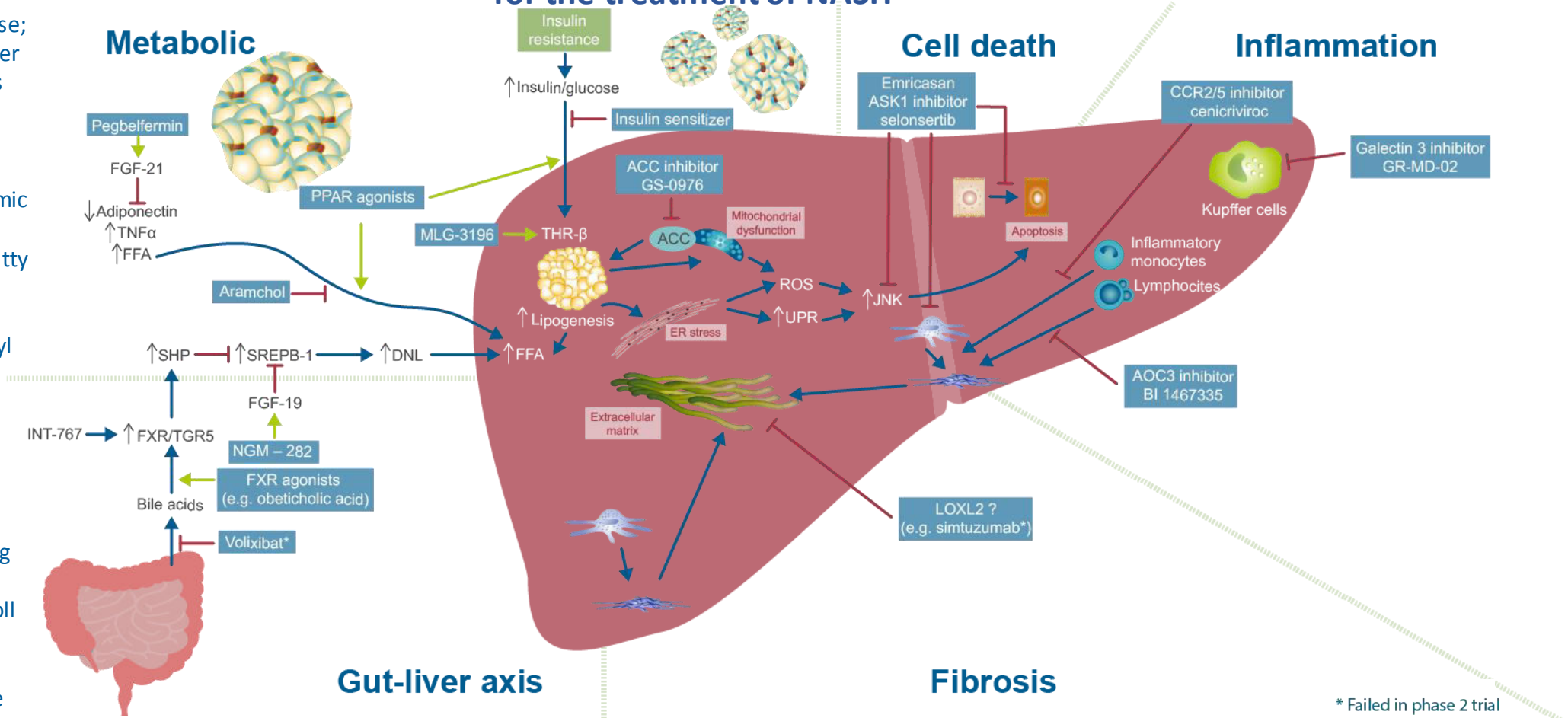
Pharmacotherapy: Points to consider

- Lack of approved therapies for NAFLD
- Any treatment for NASH should aim at improving ballooning, inflammation and/or fibrosis
- Numerous therapies under development
- Diversity of disease mechanisms and pathways
- Need for robust models for successful target identification, validation and assessment of therapies

References Cole BK et al. Expert Opin Drug Discov 2018;13:193-205

Overview of pathways being investigated as pharmacological targets in NASH

Pathways in metabolism, cell death, inflammation, fibrosis and the gut-liver axis proposed as pharmacological targets for the treatment of NASH



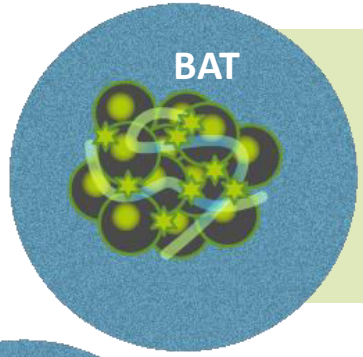
ACC, acetyl-coA carboxylase; AOC, amine oxidase, copper containing; ASK, apoptosis signal-regulating kinase; CCR, C-C motif chemokine receptor; DNL, de novo lipogenesis; ER, endoplasmic reticulum; FGF, fibroblast growth factor; FFA, free fatty acids; FXR, farnesoid X receptor; JNK, Jun N-terminal kinase; LOXL, lysyl oxidase-like; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SHP, small heterodimer partner; SREBP, Sterol regulatory element binding proteins; THR, thyroid hormone receptor; TLR, toll like receptor; TNF, tumour necrosis factor; UPR, unfolded protein response

Notes

References

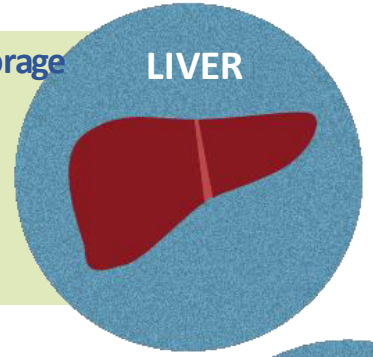
Adapted from Tacke F et al. Expert Rev Gastroenterol Hepatol 2018:1-10

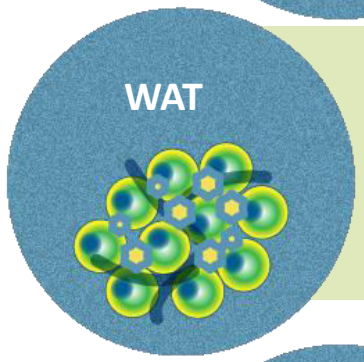
PPARs: sensors of key metabolic pathways in different organs



PPARγ – Differentiation, lipid storage
PPARα – FAO, energy uncoupling
PPARδ – FAO, energy uncoupling

PPARα – FAO, ketogenesis, lipid storage
PPARδ – Glc oxidation,
↓ inflammation, lipoprotein uptake
PPARγ – Lipid storage, oxidation, inflammation & fibrogenesis

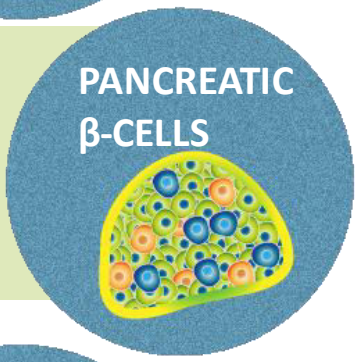


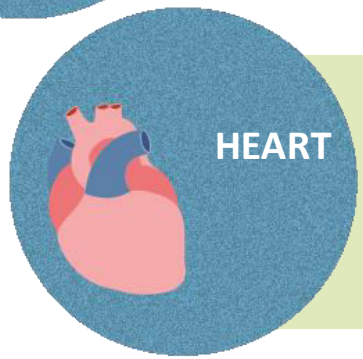


PPARγ – Adipogenesis, lipid storage
PPARδ – FAO, glc + BCAA oxidation
PPARα – FAO

PPARs

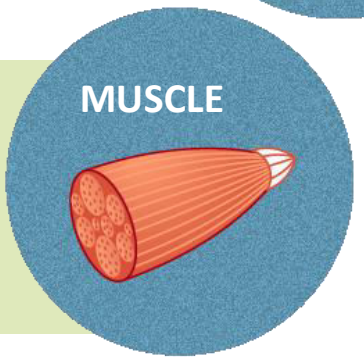
PPARδ – FAO, GSIS
PPARα – FAO, GSIS
PPARγ – Lipogenesis





PPARδ – FAO, glc oxidation
PPARα – FAO, ↓ gl; uptake
PPARγ – ↓ growth,
↓inflammation

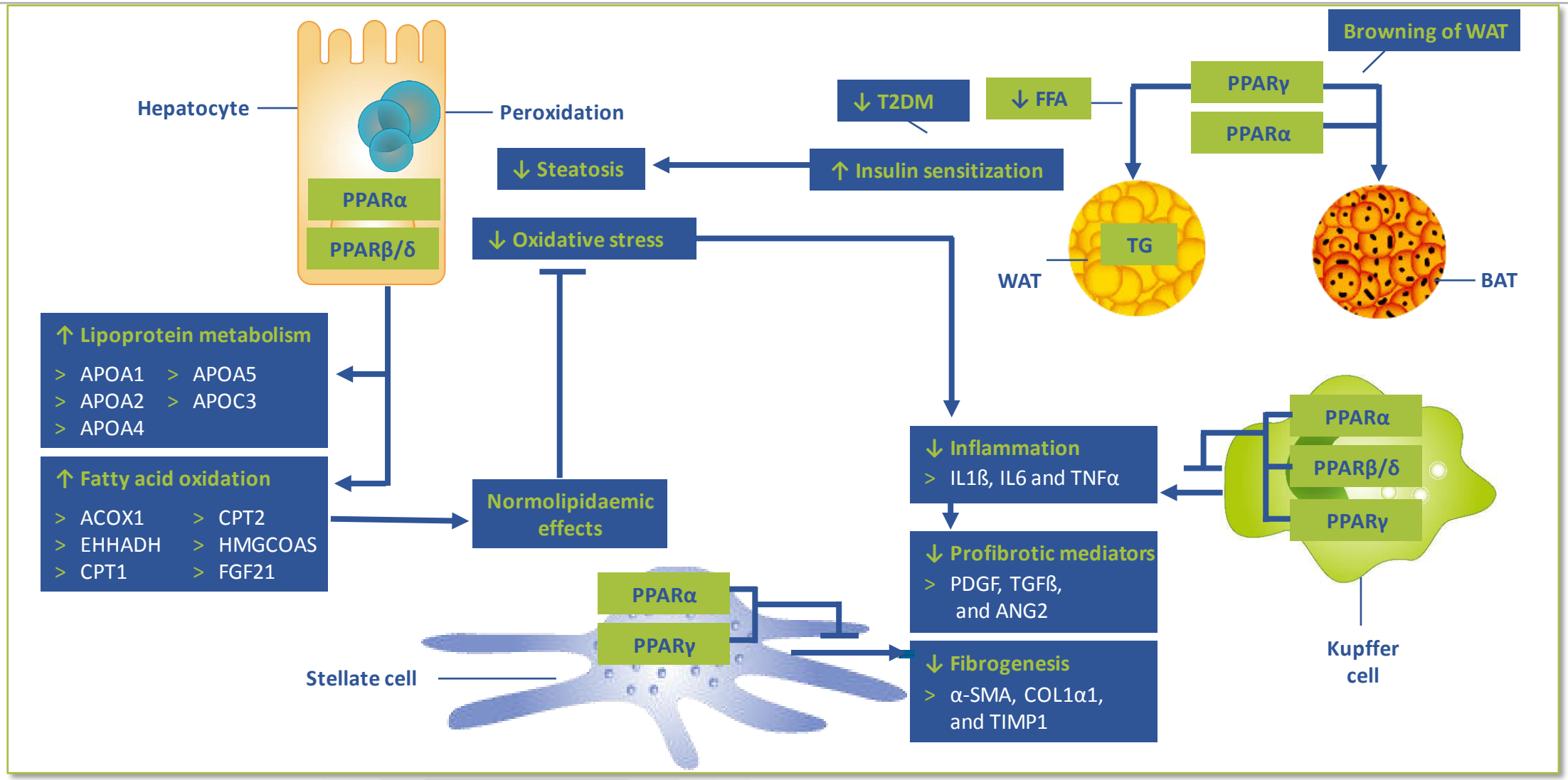
PPARδ – FAO, glc uptake + storage
↑ type I fibers
PPARα – FAO, ↓ glc uptake
PPARγ – Energy expenditure



Notes BAT, brown adipose tissue; FAO, fatty acid oxidation; glc, glucose; GSIS, glucose stimulated insulin secretion; PPAR, peroxisome proliferator-activated receptor; WAT, white adipose tissue

References Adapted from Poulsen LI et al. Semin Cell Dev Biol 2012;23:631-39

Differential PPAR signalling in fatty liver disease



α-SMA, alpha-smooth muscle actin; ANG2, angiopoietin-2; APOA, apolipoprotein A; APOC3, apolipoprotein C3; ACOX1, acyl-CoA oxidase 1; BAT, brown adipose tissue; COL1α1, collagen type I alpha 1; CPT, carnitine palmitoyltransferase; EHHADH, enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase; FFA, free fatty acid; FGF, fibroblast growth factor; HMGCOAS, 3-hydroxyl-3-methylglutaryl-coenzyme A synthase; IL, interleukin; PDGF, platelet-derived growth factor; PPAR, peroxisome proliferator-activated receptor; TG, triglyceride; TGFß, transforming growth factor beta; TIMP1, metalloproteinase type 1; TNFa, tumour necrosis factor alpha; WAT, white adipose tissue

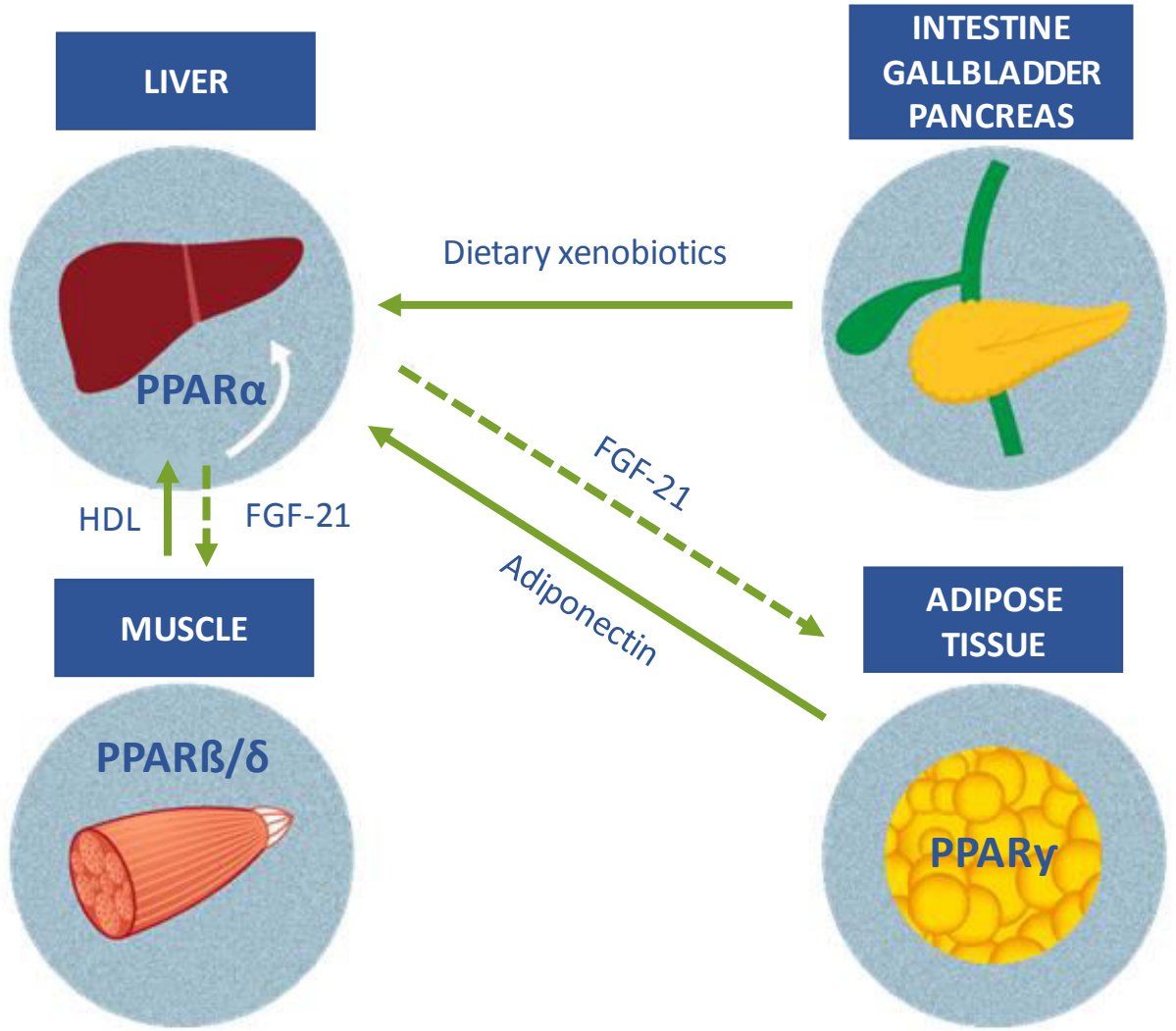
Notes

References

Adapted from Gross B. Nat Rev Endocrinolo. 2017 Jan;13(1):36-49

PPARs and metabolic improvement in NASH patients

↓ Steatosis
↓ Lipogenesis
↑ ß-oxidation
↔ Xenobiotic detoxification
↑ CYP4A
Δ Bile acid metabolism



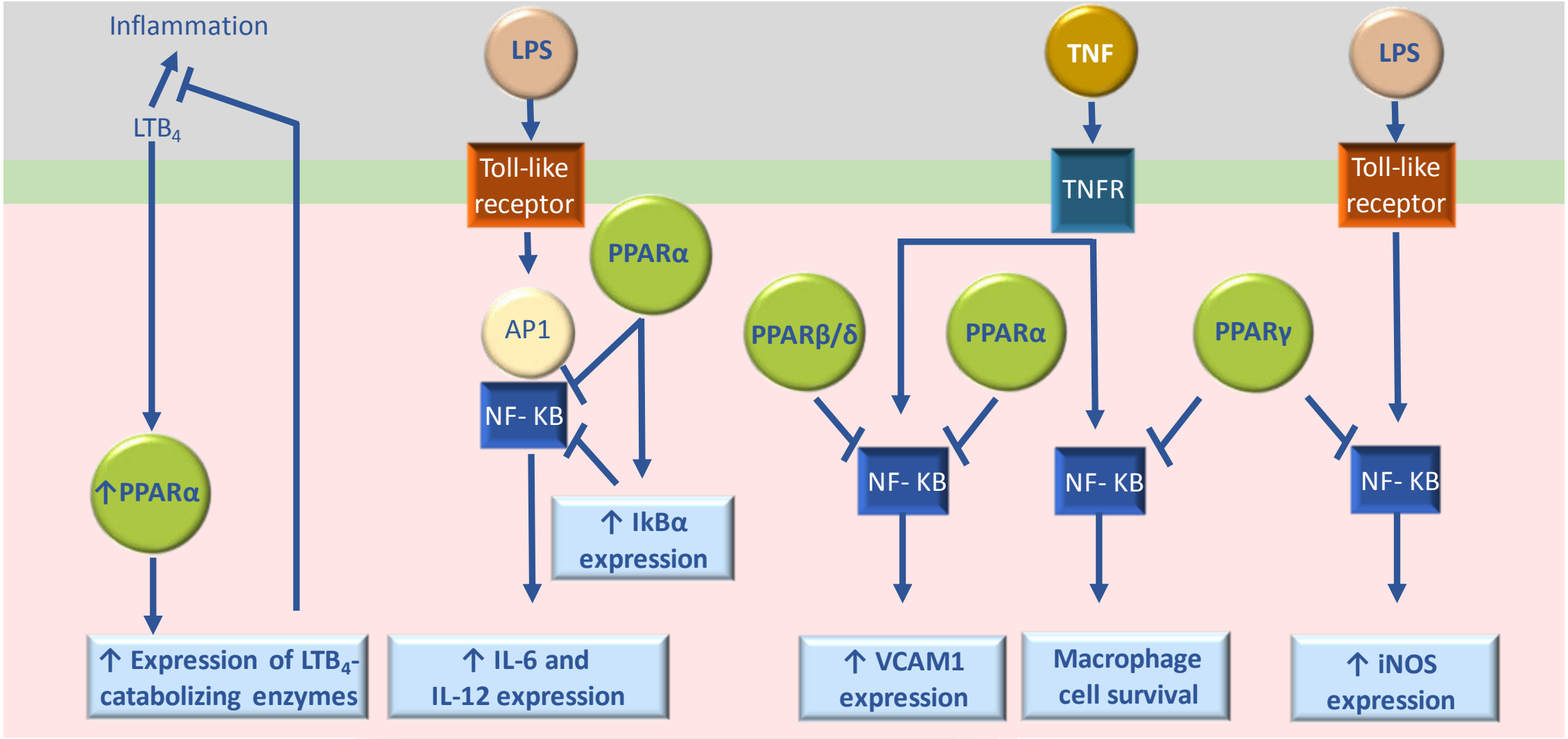
↓ Gut permeability
↓ Insulin resistance
↓ Diabetes

↑ Lipid uptake
↑ ß-oxidation
↑ Cholesterol efflux (HDL)

↑ Lipid uptake
↑ Body weight
↑ Adiponectin
↓ FFA flux to liver

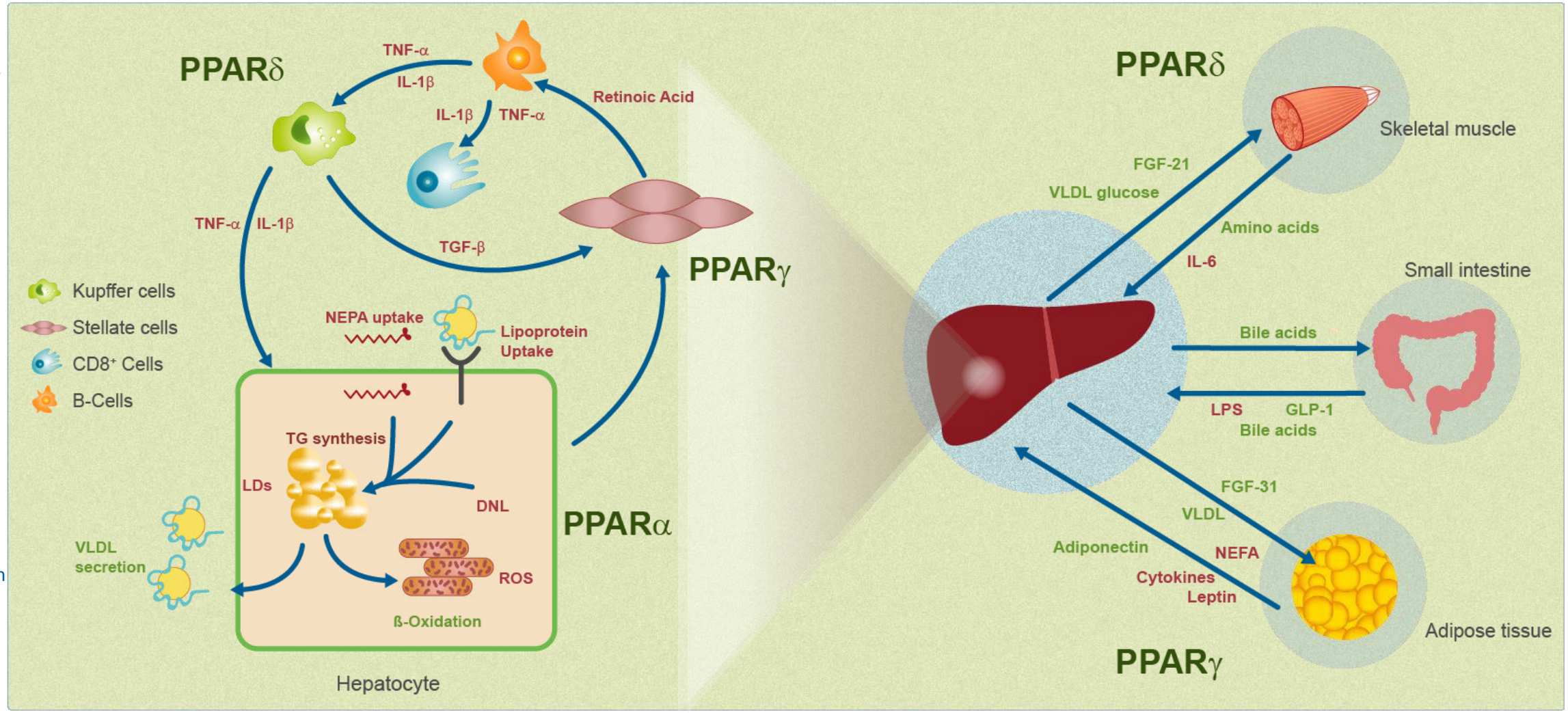
References Adapted from Cave MC, et al. Biochim Biophys Acta. 2016;1859:1083-99

PPARs: regulatory effects on inflammatory processes



References Adapted from Daynes RA et al. Nat Rev Immunol 2002;2:748-59; Ruzehaji N et al. Ann Rheum Dis 2016;75:2175-83

Coordinated activation of PPARs for NASH and fibrosis resolution



DNL, de novo lipogenesis; FGF-21, fibroblast growth factor 21; GLP-1, glucagon-like peptide 1; IgG, immunoglobulin G; IL, interleukin; LD, lipid droplet; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein 1α; NEFA, non-esterified fatty acid; NKT cell, natural killer T cell; ROS, reactive oxygen species; TG, triglyceride; TGF-β, transforming growth factor β; TNF-α, tumour necrosis factor α; VLDL, very low density lipoprotein

Notes

References

Haas JT, et al. Annu Rev Physiol. 2016;78:18.1–18.25

PPARγ: a master regulator of HSCs preventing their pro-inflammatory and profibrogenic effects

Activation of PPARg modulates different biological actions of HSCs that contribute to the process of liver inflammation and fibrogenesis

- **Inhibition of HSC proliferation**
- **Inhibition of HSC migration**
- **Inhibition of the chemokine expression, such as MCP-1 (stimulated by IL-1, TNF-a, and IFN-g)**
- **Inhibition of HSC differentiation into myofibroblasts**
- **Return of activated HSCs to their quiescent state**

Notes: HSC, hepatic stellate cell; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumour necrosis factor

References: Marra F et al. Gastroenterology 2000;119:466-78; Hazra S et al. J Biol Chem. 2004;279:11392-401

PPARγ: a master regulator of HSCs

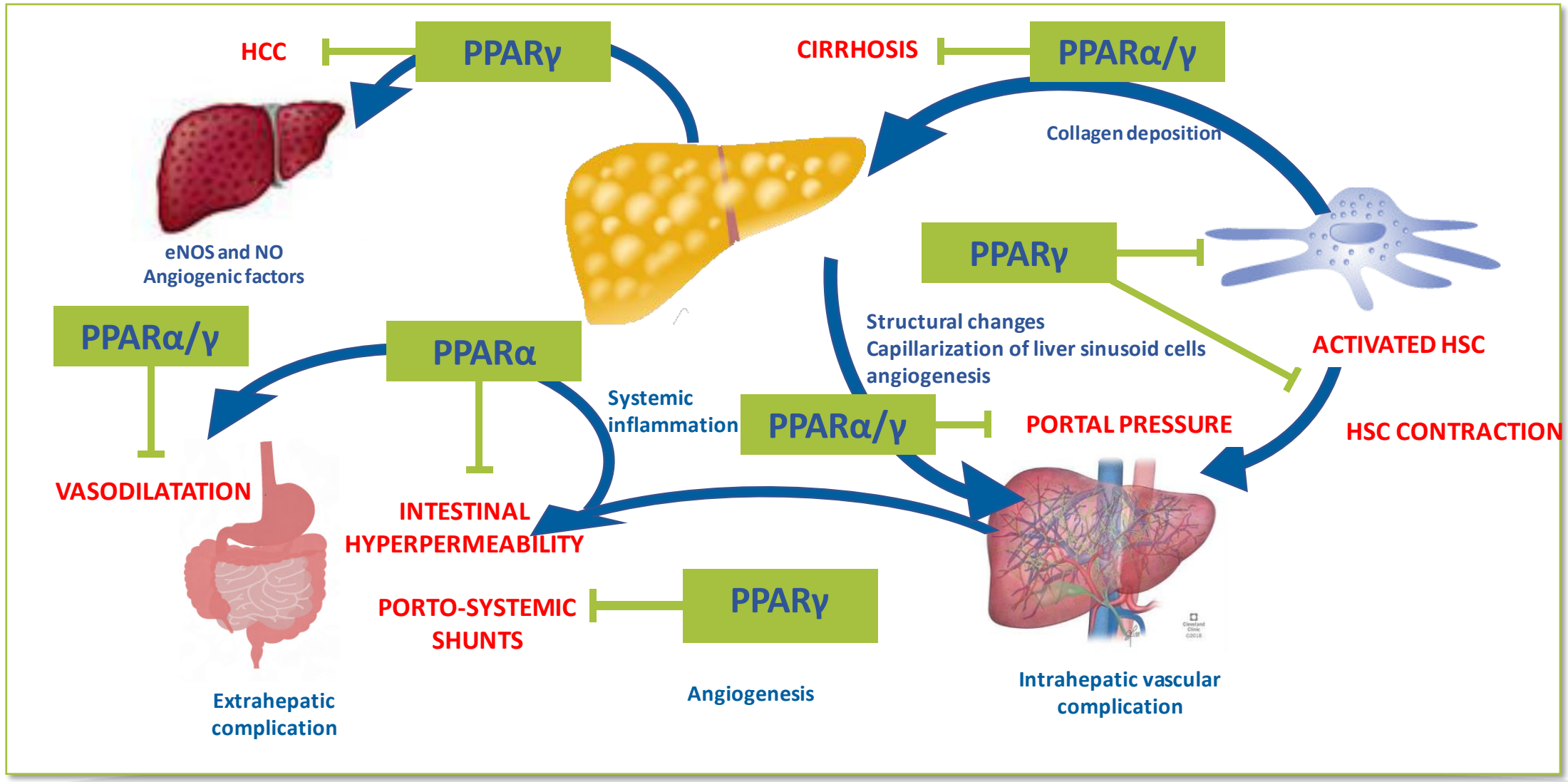
Activation of PPARg modulates different biological actions of HSCs that contribute to the process of liver inflammation and fibrogenesis

- **Inhibition of HSC:**
  - **Proliferation**
  - **Migration**
  - **Differentiation into myofibroblasts**
- **Inhibition of the chemokine expression, such as MCP-1 (stimulated by IL-1, TNF-a, and IFN-g)**
- **Inhibition of HSC Return of activated HSCs to their quiescent state**

Notes: HSC, hepatic stellate cell; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumour necrosis factor

References: Marra F et al. Gastroenterology 2000;119:466-78; Hazra S et al. J Biol Chem. 2004;279:11392-401

Cirrhosis interplay between intrahepatic and extrahepatic conditions



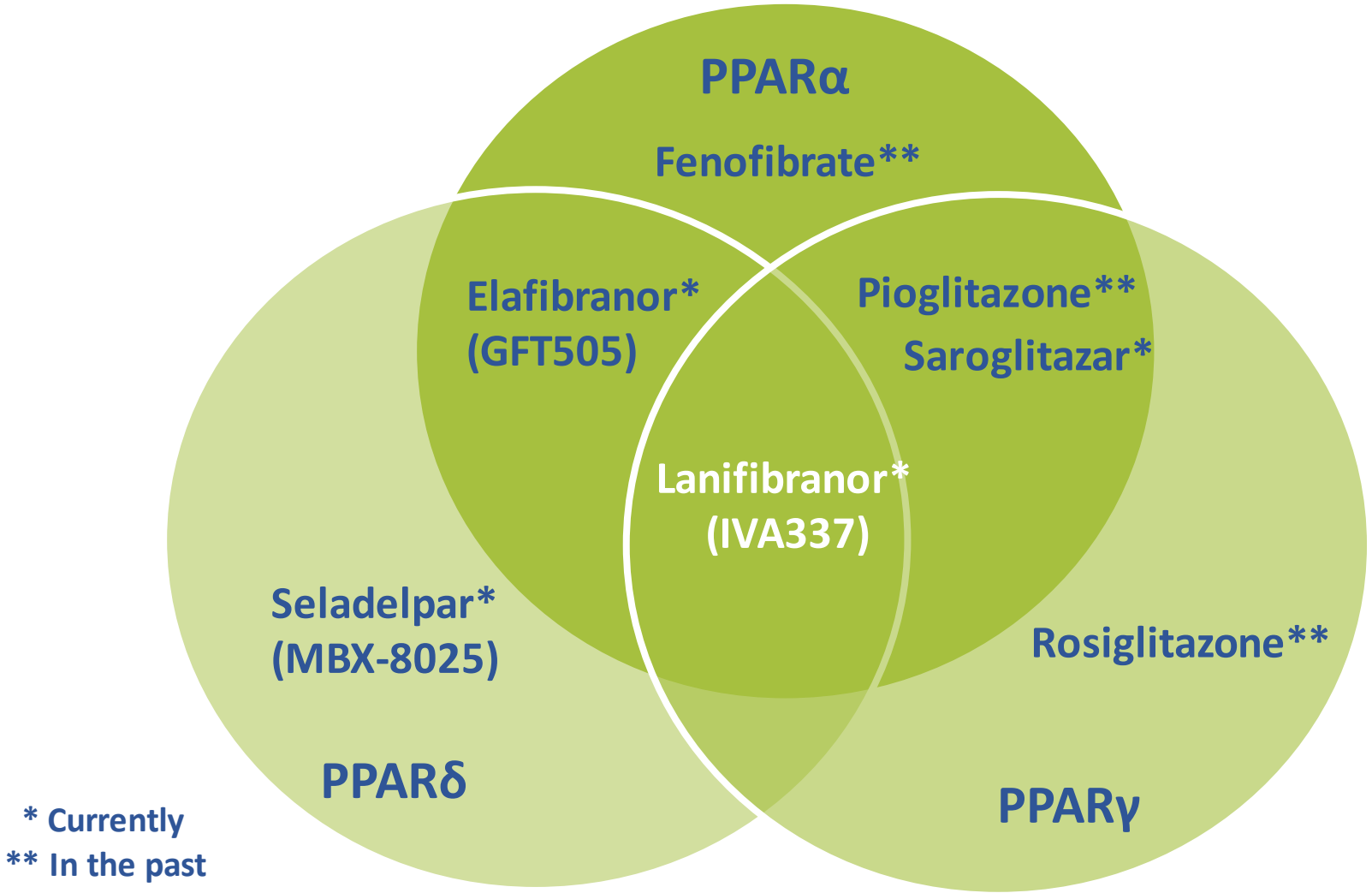
PPARs: application in clinical practice

PPAR	Action
PPARα	Reduction of triglycerides
PPARγ	Hypoglycaemic and hypocholesterolemic action
PPARβ/δ	Increase of free fatty acid consumption in skeletal muscle
PPARα/γ	Hypoglycaemic and hypocholesterolemic action
PPARα/δ	Reduction of steatohepatitis

Activation of all three isoforms by pan-PPAR agonists is expected to lead to greater improvement in therapeutic efficacy by targeting a larger array of disturbances and is expected to limit side effects

References Derosa G et al. J Cell Physiol 2018;233:153-61

PPAR agonists investigated currently or in the past in NASH



* Currently
** In the past

References Adapted from: Sumida Y, Yoneda M. J Gastroenterol 2018;53:362-76. Wettstein G et al. Hepatol Commun 2017;1:524-37; Weinstein D, et al. Neurology 2017;88 (16 Supplement)

The PPARγ agonist pioglitazone & vitamin E

- **EASL-EASD-EASO guideline states that pioglitazone** (off-label outside T2DM) **or vitamin E or their combination may be used for NASH**
- **PIVENS trial** (n=247; pioglitazone 30 mg daily, vitamin E 800 IU daily or placebo, for 96 weeks)**: vitamin E at a dose of 800 IU/day over 2 years improved steatosis, inflammation, ballooning, NAS score and NASH resolution in non-cirrhotic patients, but did not affect fibrosis**
- **However, in two meta-analyses, pioglitazone was associated with improved steatosis, inflammation, hepatocellular ballooning, NAS score, hepatic fibrosis and NASH resolution**

References EASL, EASD, EASO. J Hepatol 2016;64:1388-402; Sanyal AJ et al. N Engl J Med. 2010;362:1675-85; Boettcher E et al. Aliment Pharmacol Ther. 2012;35:66-75; Musso G et al JAMA Intern Med. 2017;177:633-40

Elafibranor a PPARα/δ dual agonist currently investigated in NASH

- **Phase 2b GOLDEN-505 trial[1]:** 274 non-cirrhotic patients with biopsy-proven NASH randomised to either oral elafibranor 80 or 120 mg daily or to placebo for 52 weeks
  - Primary endpoint, reduction of at least one of the NASH components to zero without worsening in fibrosis (progression to stage 3 or 4) not met
  - Secondary post-hoc endpoint (revised definition for the resolution of NASH, i.e. disappearance of ballooning and either disappearance of lobular inflammation or persistence of mild lobular inflammation (score of 0 or 1), without worsening in liver fibrosis (progression by ≥1 stage)) met by 19% of patients on 120 mg elafibranor vs. 12% on placebo (P=0.045)

- **RESOLVE-IT (NCT02704403)**: phase 3 international randomised, double-blind, placebo-controlled
  - Efficacy and safety of elafibranor 120mg once daily in patients with NASH and fibrosis
  - Primary endpoint: resolution of NASH without worsening of fibrosis after 72 weeks of treatment
  - Composite long-term outcome: all-cause mortality, cirrhosis, and liver-related clinical outcomes
  - Estimated primary completion date: December 2021

References
1. Ratziu V et al. Gastroenterology. 2016;150:1147-59 e5.

Lanifibranor a pan-PPAR agonist currently investigated in NASH in the Phase IIb NATIVE trial

Status
> Enrolling
Randomisation
> 1/1/1 stratification on T2DM patients
> Study powered with 75 patients per group
Clinicaltrials.gov identifier
> NCT03008070

Inclusion criteria
> Liver biopsy (SAF score)
> Moderate to severe patients with an inflammation or ballooning score of 3 or 4
> Steatosis score ≥1 and fibrosis score <4 (no fibrosis)
Primary endpoint
> Decrease from baseline ≥2 points of inflammation or ballooning score without worsening of fibrosis
> Central reading for pre- (before randomisation) and post treatment biopsy

225 patients

24 week treatment

Double blind randomized placebo controlled

Screening
> Liver biopsy

- **Placebo, 75 patients**
- **Lanifibranor, 800 mg once daily, 75 patients**
- **Lanifibranor, 1200 mg once daily, 75 patients**

End of treatment
> Liver biopsy

NAS vs. SAF score

NAFLD Activity Score (NAS)

Steatosis grade Low- to medium-power evaluation of parenchymal involvement by steatosis	**<5%**	**0**
	5-33%	+1
	34%-66%	+2
	>66%	+3
Lobular inflammation Overall assessment of all inflammatory foci	**No foci**	**0**
	1 focus per 200× field	+1
	2-4 foci per 200× field	+2
	>4 foci per 200× field	+3
Liver cell injury Ballooning	**None**	**0**
	New balloon cells	+1
	Many cells/prominent ballooning	+2

SAF score

Steatosis (0-3)	0 = <5%, 1 = 5-33%, 2 =3 4-66%, 3 = >66%
Activity (0-4)	Ballooning (0-2) + Lobular inflammation (0-2)
Fibrosis (0 – 4)	1a,b,c = perisinusoidal or periportal fibrosis, 2 = both perisinusoidal and periportal fibrosis, 3 = bridging fibrosis, 4 = cirrhosis

- Compared to NAS, SAF allows a comprehensive, complete and simple overview of the main liver lesions in NAFLD.
- It is easy to understand, simple to use and mirrors the continuous spectrum of the histopathologic features in NAFLD.
- The dynamic scale of the SAF score is adapted to clinical trials.

References Kleiner DE et al. Hepatology. 2005;41:1313-21; Bedossa P et al. Hepatology 2012;56:1751-9

Lanifibranor, a mechanism of action addressing all the key features of NASH

PPARα, γ

Metabolism
↑ Insulin sensitivity
↑ HDLc
↓ TG

PPARα, γ

Steatosis
↓ FA uptake
↑ FA catabolism
↓ Lipogenesis

Lanifibranor

Moderate and balanced panPPAR agonist activity regulating genes in:
> PPARα: hepatocytes
> PPARδ: kuppfer cells
> PPARγ: hepatic stellate cells

PPARα, δ, γ

Necroinflammation
↓ NFkB-dependant gene activation
↓ Inflammasome
↓ Ballooning

PPARγ

Fibrosis
↓ Stellate cell proliferation and activation
↓ Collagen and fibronectin production

PPARα, γ

Vascular
↓ Portal pressure
↓ Angiogenesis
↓ Portosystemic shunting

References Wettstein G et al. Hepatol Commun 2017;1:524-373

Take-home messages

- The prevalence of NAFLD is increasing worldwide in parallel with the rising epidemics of obesity and T2DM
- NASH is rapidly becoming one of the main causes of cirrhosis and HCC and the main indication for liver transplantation
- Except for lifestyle modification through diet and exercise, there are currently no approved treatments for NASH
- While bariatric surgery can be considered in otherwise eligible patients with NAFLD or NASH, it is premature to consider it as an established option for the treatment of NASH
- Numerous novel treatments for NASH are currently in development targeting metabolism, cell death, inflammation, fibrosis and the gut-liver axis. However, drugs focusing on just one target may not be sufficiently efficacious and might have to be used in combination
- Pan-PPAR agonists that act on multiple targets may be a promising new therapeutic option for NASH

Take-home messages for NASH

- Prevalence of NAFLD increases worldwide in parallel with obesity and T2DM
- NASH = one of main causes of cirrhosis and HCC, and indication for liver transplantation
- Except lifestyle modification (diet and exercise), currently no approved treatments
- While bariatric surgery can be considered, premature as an established option
- Numerous treatments in development targeting metabolism, cell death, inflammation, fibrosis and gut-liver axis. However, are drugs focusing on just one target sufficiently efficacious ? Or to be used in combination ?
- Pan-PPAR agonists acting on multiple targets = promising new option for NASH