CMHC
Cardiometabolic Health Congress

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Foundations of Cardiometabolic Health Certification Course

Certified Cardiometabolic Health Professional (CCHP)

NASH: Diagnosis & Risk Stratification

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

Editor-in-chief, Metabolism, Clinical and Experimental

Initial Evaluation in Patients with Suspected NALFD

History and medical review	Investigations
Obesity	Liver biochemistries (ALT, AST)
T2D	Exclude/identify other liver diseases[a]
Metabolic syndrome	HBV and HCV serology (and viral load)
Alcohol intake	Auto antibodies (ANA, AMA, ASMA)
<14 drinks/wk. for women	Serum ferritin, A1AT
<21 drinks/wk. for men	Liver ultrasound: increased echogenicity
No known pre-existing liver disease	–

A1AT, α1 antitrypsin; AMA, antimitochondrial antibody; ANA, antinuclear antibody; ASMA, anti–smooth muscle antibody; HBV, hepatitis B virus; HCV, hepatitis C virus.

aga American Gastroenterological Association

Metabolism Clinical and Experimental

Obesity A Research Journal

Fasiha K et al. Metabolism, 2021.

Diagnosis and Initial Risk Stratification

Liver US→ >80% accuracy for moderate or severe steatosis BUT suboptimal sensitivity for mild steatosis.

Risk stratification with noninvasive fibrosis scores (NAFLD fibrosis score or FIB-4 Index) to rule out advanced fibrosis.

If intermediate or high-risk further assessment may be required with elastography or direct fibrosis serum markers (e.g. propeptide of type III procollagen).

Hernaez R et al. Hepatology, 2011.
Bril F et al. Liver Int, 2015.
Bril F et al. Diabetes Care, 2020.

AASLD Guidelines Provide High Level Information on NITs

Noninvasive Tests (NITs)	
FIB-4 Index	Noninvasive scoring system based on several routine laboratory tests that help to estimate the amount of liver fibrosis.
NAFLD Fibrosis Score (NFS)	Based on 6 readily available variables and is calculated using a published formula.
ELF Test	An algorithm combining specific serum markers. Approved for commercial use in Europe but not available for clinical use in the US.
VCTE (FibroScan)	Assesses liver stiffness via measurement of shear-wave velocity. Approved by the FDA in 2013 for use in adults and children with liver disease.
MRE	Stiffness measurement through modified phase-contrast pulse sequence using magnetic resonance technology.

AASLD Guidance Statements

- **FIB-4** Index or **NFS** are clinically useful tools for identifying NAFLD patients with higher likelihood of having bridging fibrosis (F3) or cirrhosis (F4)
- **Vibration controlled transient elastography (VCTE)** or magnetic resonance elastography **(MRE**) are clinically useful tools for identifying advanced fibrosis in patients with NAFLD
- Clinical decision aids such as **FIB-4** or **NFS** or **VCTE** can be used to identify those at low or high risk for advanced fibrosis (bridging fibrosis or cirrhosis)

- **FIB-4** uses Age, AST, ALT, and Platelets
- **NFS** uses Age, AST, ALT, platelets, BMI, albumin, and presence of diabetes or elevated fasting glucose
- **ELF** (enhanced liver fibrosis test) uses hyaluronic acid (HA), procollagen-3 N-terminal peptide (P3NP), and tissue inhibitor of metalloproteinase-1 (TIMP-1).

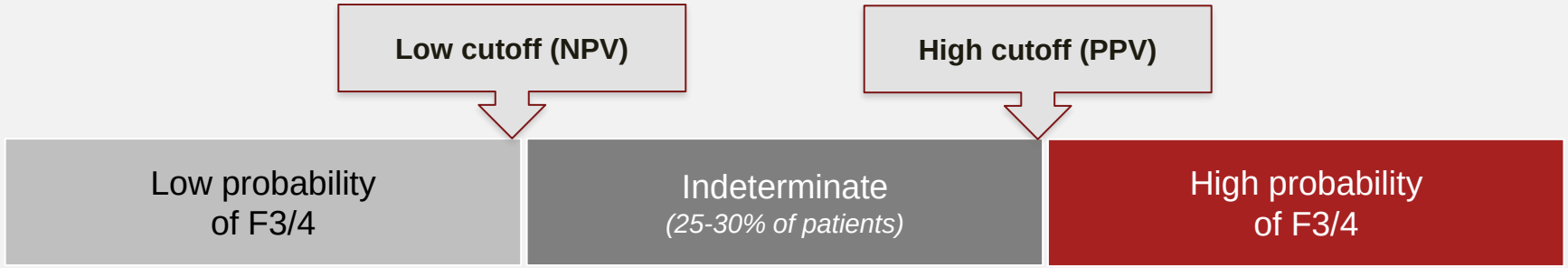
Chalasani N et al. Hepatology, 2018.

FIB-4: A Simple and Available Tool to Determine Likelihood of Advanced Fibrosis (F3/4)

Parameters
Age
AST
ALT
Platelet count

FIB-4 Score	Interpretation	NPV or PPV, %
<1.30	Rules out fibrosis	90
>2.67	Predicts advanced fibrosis	80

AUROC = 0.802



Advantages

- Good reproducibility
- Easily performed from readily available lab tests
- Low cost & wide availability (non-patented)

Disadvantages

- Performance to identify exact fibrosis stage is poor
- Patients <35 or >65 years of age must use alternate cutoffs

Majority (~60%) will fall into “low-risk” category when using predictive models to identify those at high risk for advanced fibrosis due to NASH

FIB-4 calculator available at http://gihep.com/calculators/hepatology/fibrosis-4-score/

ALT = alanine aminotransferase; AST = aspartate aminotransferase; AUROC = area under receiver operating characteristic; NPV = negative predictive value; PPV = positive predictive value

1. Shah AG et al. Clin Gastroenterol Hepatol, 2009. 2. Vilar-Gomez E et al. J Hepatol, 2018.

NAFLD Fibrosis Score (NFS): A Simple and Available Tool to Determine Likelihood of Advanced Fibrosis (F3/4)

Parameters
Age
AST
ALT
Platelet count
BMI
Albumin
Impaired fasting glucose/diabetes?

NFS	Interpretation	NPV or PPV, %
<-1.455	Rules out fibrosis	88 to 93
>0.676	Predicts advanced fibrosis	82 to 90

AUROC = 0.84

Low cutoff (NPV)

High cutoff (PPV)

Low probability of F3/4	Indeterminate *(25-30% of patients)*	High probability of F3/4

Advantages

- Good reproducibility
- Easily performed from readily available lab tests
- Low cost & wide availability (non-patented)

Disadvantages

- Performance to identify exact fibrosis stage is poor
- Patients <35 or >65 years of age must use alternate cutoffs

Majority (~60%) will fall into “low-risk” category when using predictive models to identify those at high risk for advanced fibrosis due to NASH

ALT = alanine aminotransferase; AST = aspartate aminotransferase; AUROC = area under receiver operating characteristic; BMI = body mass index; NFS= NAFLD Fibrosis Score;
NPV = negative predictive value; PPV = positive predictive value.

1. Angulo P et al. Hepatology, 2007. 2. Vilar-Gomez E et al. J Hepatol, 2018.

Accurate Fibrosis Staging

- Accurate fibrosis staging provides information regarding prognosis, need for pharmacotherapy, intensive lifestyle modification and/or bariatric surgery, and screening/surveillance for varices and HCC.

Vibration-controlled transient elastography

- Presence or absence of advanced fibrosis
- 92% specificity

OR

Magnetic resonance elastography

- Identifies intermediate stages of fibrosis
- Not widely available
- More costly

Wong VW et al. Hepatology, 2010.
EASL/EASD/EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol, 2016.

Available Noninvasive Tests: Two Different but Complementary Approaches

Serum biomarkers

Non-specific

- FIB-4
- AST/ALT ratio
- APRI
- FibroTest®
- ELF®
- FibroMètre®

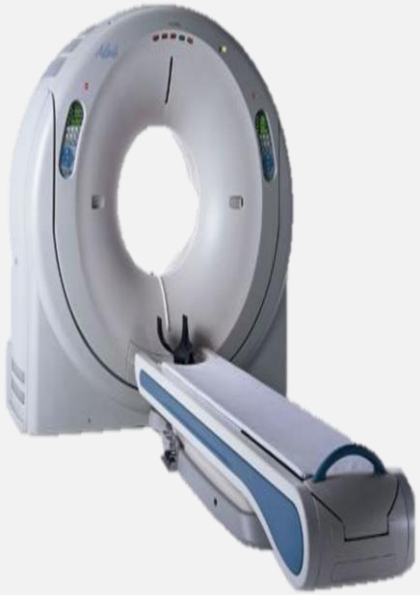
More Specific

- NAFLD score (NFS)
- BARD score

Imaging

CAP/TE

PDFF/MRE



AST = aspartate aminotransferase; ALT = alanine aminotransferase; APRI = aspartate aminotransferase to platelet ratio index; CAP = controlled attenuation parameter; FIB-4 = fibrosis-4 score; ELF = enhanced liver fibrosis; MRE = magnetic resonance elastrography; PDFF = proton density fat fraction; TE= transient elastography.

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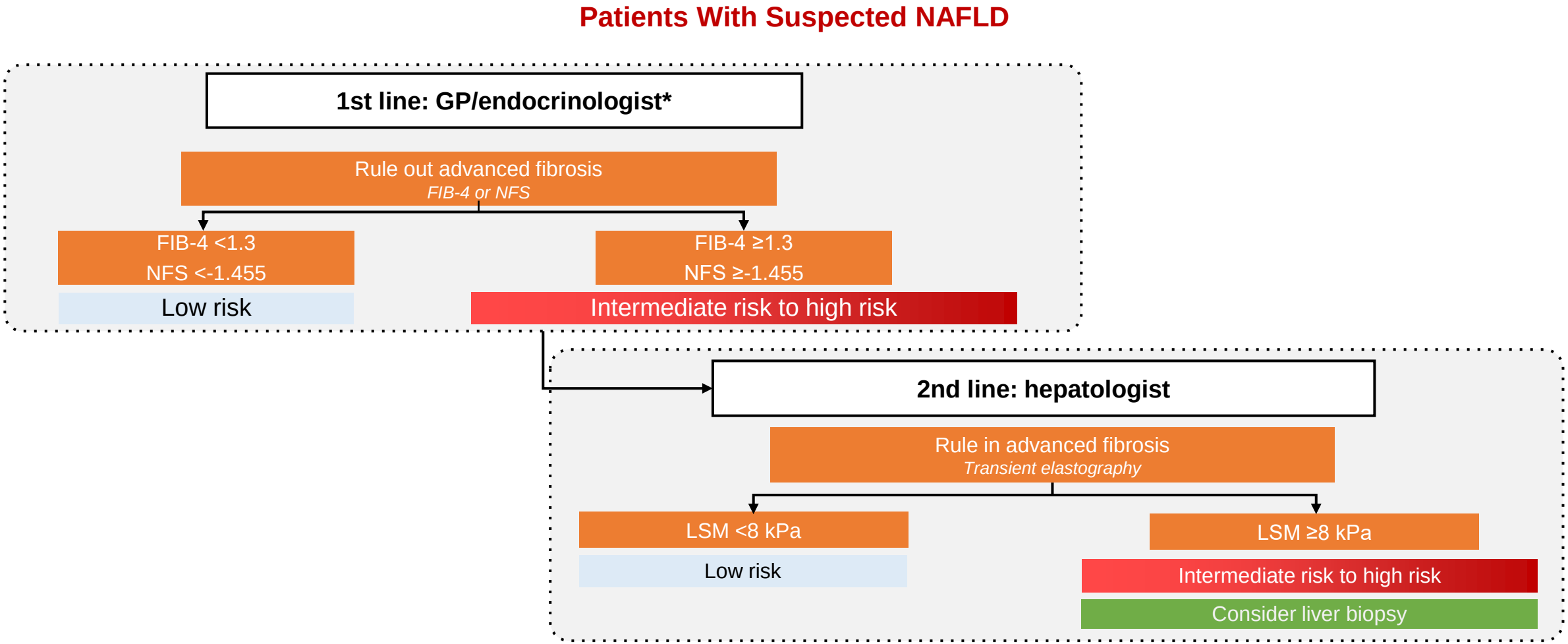
NASH & NAFLD: Emerging Non-Invasive Diagnostic Algorithms

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

Editor-in-chief, Metabolism, Clinical and Experimental

Clinical Algorithms Combining the Use of Several NITs Assist with Proper Identification and Referral of High-Risk Patients

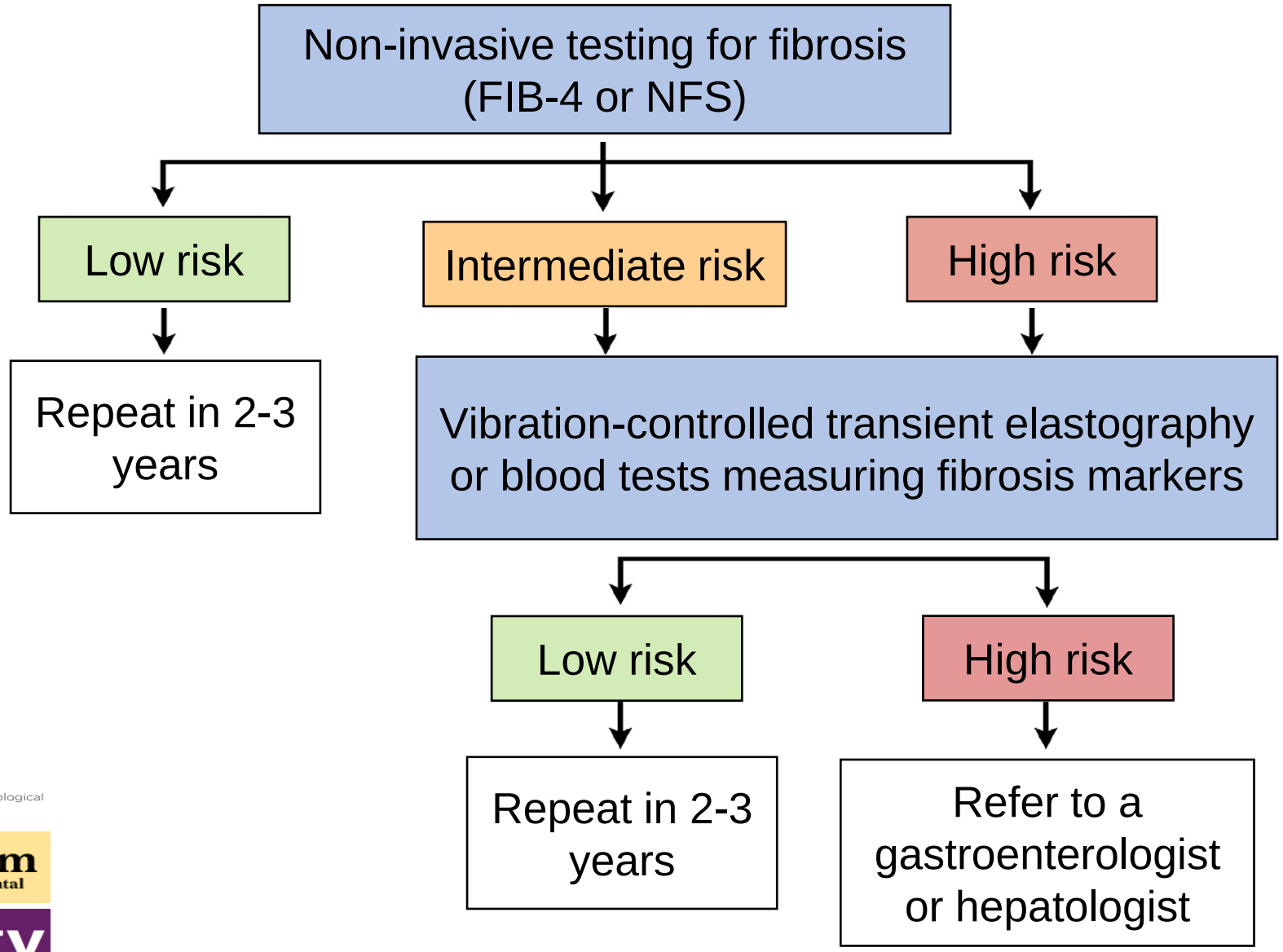


* Rule out other causes of liver disease (alcohol, HBV, HCV)

FIB-4 = Fibrosis-4 index; GP = general practitioner; HBV = hepatitis B virus; HCV = hepatitis C virus; LSM = liver stiffness measure; NAFLD = nonalcoholic fatty liver disease; NFS = NAFLD fibrosis score; NITs = noninvasive tests.

Adapted from Castera L et al. Gastroenterology, 2019.

Algorithm for Risk Stratification in Patients with NAFLD/NASH



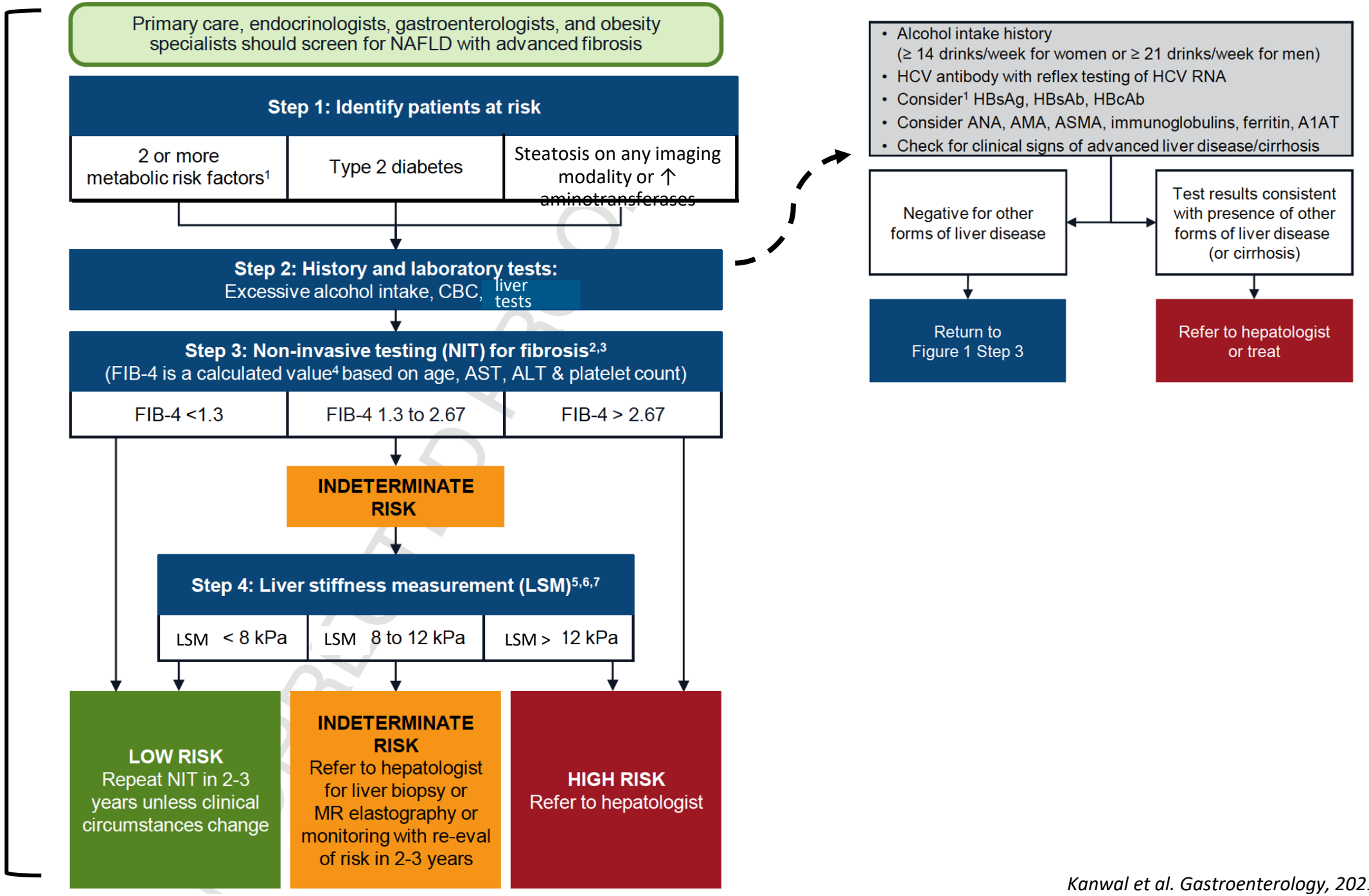
aga American Gastroenterological Association

Metabolism Clinical and Experimental

Obesity A Research Journal

Fasiha K et al. Metabolism, 2021.

Screening for Advanced Fibrosis



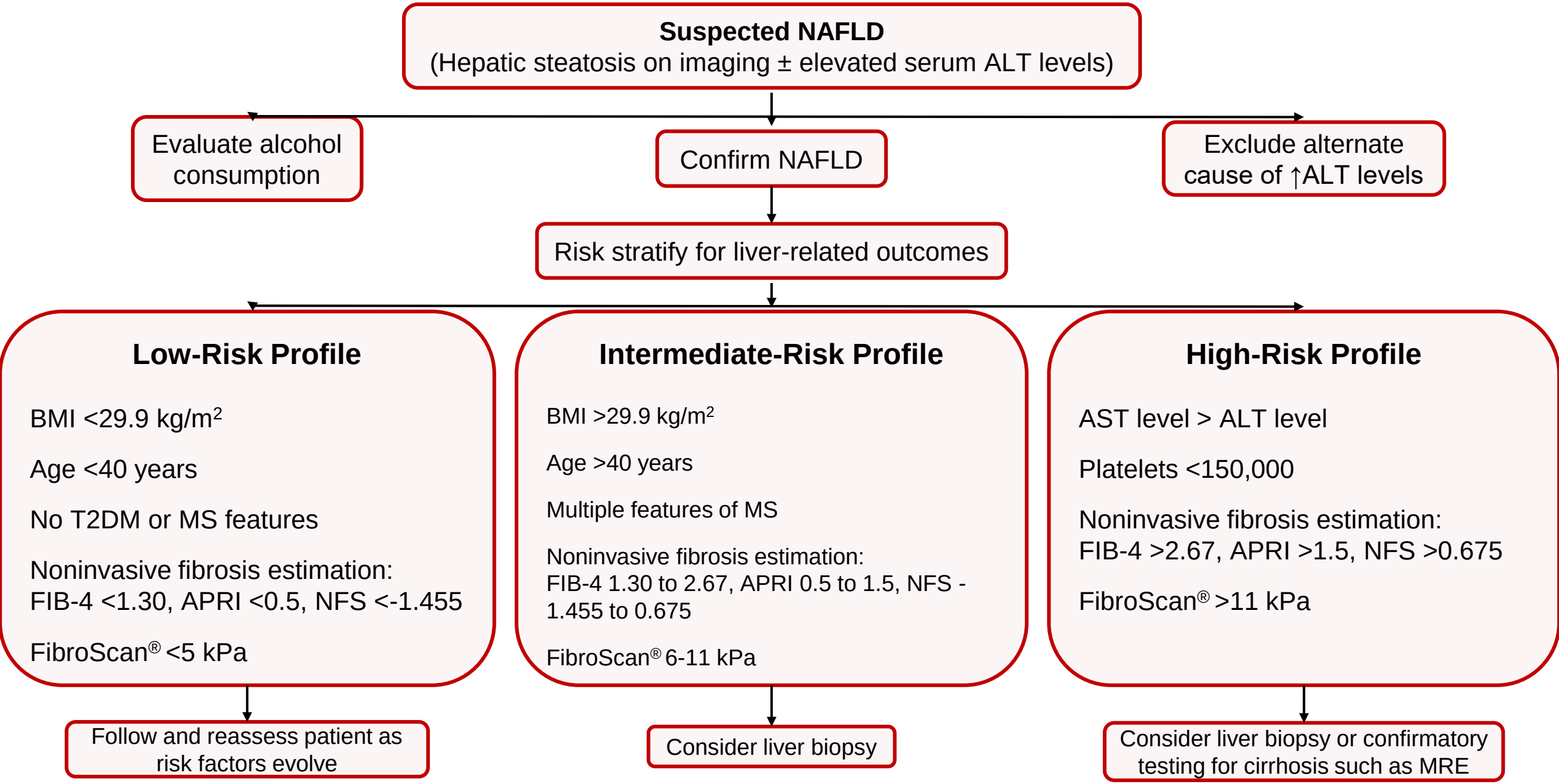
Kanwal et al. Gastroenterology, 2021.

Clinical Care Pathway for the Risk Stratification and Management of Patients with NAFLD

Kanwal F, Shubrook JH, Adams LA, Pfotenhauer K, Wong VWS, Wright E, Abdelmalek MF, Harrison SA, Loomba R, Mantzoros CS, Bugianesi E, Eckel RH, Kaplan LM, El-Serag HB and Cusi K.

	LOW RISK FIB-4 < 1.3 or LSM < 8 kPa or liver biopsy F0-F1	**INDETERMINATE RISK** FIB-4 1.3 - 2.67 and/or LSM 8 - 12 kPa and liver biopsy not available	**HIGH RISK**[1] FIB-4 > 2.67 or LSM > 12 kPa or liver biopsy F2-F4
	Management by PCP, dietician, endocrinologist, cardiologist, others	Management by hepatologist with multidisciplinary team (PCP, dietician, endocrinologist, cardiologist, others)	
Lifestyle intervention[2]	Yes	Yes	Yes
Weight loss recommended if overweight or obese[3]	Yes May benefit from structured weight loss programs, anti-obesity medications, bariatric surgery	Yes Greater need for structured weight loss programs, anti-obesity medications, bariatric surgery	Yes Strong need for structured weight loss programs, anti-obesity medications, bariatric surgery
Pharmacotherapy for NASH	Not recommended	Yes[4, 5]	Yes[4, 5]
CVD risk reduction[6]	Yes	Yes	Yes
Diabetes care	Standard of care	Prefer medications with efficacy in NASH (pioglitazone, GLP-1 RA)	Prefer medications with efficacy in NASH (pioglitazone, GLP-1 RA)

NASH Risk Assessment Algorithm



Rinella ME et al. Nat Rev Gastroenterol Hepatol, 2016.

Liver Biopsy

Pros

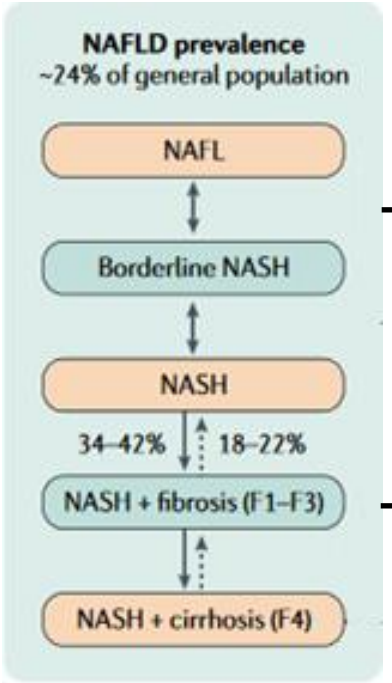
- The only one to differentiate between NAFLD and NASH
- Apart from fibrosis stage, it can also define disease activity in the form of lobular and portal inflammation and ballooning degeneration, using the NAFLD activity score (NAS) or the Steatosis Activity & Fibrosis score (SAF)
- Today it is used when there is diagnostic doubt and in clinical trials.

Cons

- Costly
- Invasive, associated with discomfort and occasional severe morbidity and even death
- Not available in primary care
- Limited by sampling and intra- and inter- observer variability

Seeff LB et al. Clin Gastroenterol Hepatol, 2010.
Ratziu V et al. Gastroenterology, 2005.
Younossi ZM et al. Mod Pathol, 1998.

NAFLD – Development of Novel Non-Invasive Diagnostic Algorithms



Liver Biopsy → ***Invasive, costly and not available in primary care***

US, MRI FLI, HSI → ***Too early to treat***

Fibroscan FIB-4 etc. → ***Too late to treat***

Diagnostic gap & **Unmet clinical need**

Non-invasive diagnosis of NASH, NAFL or healthy status

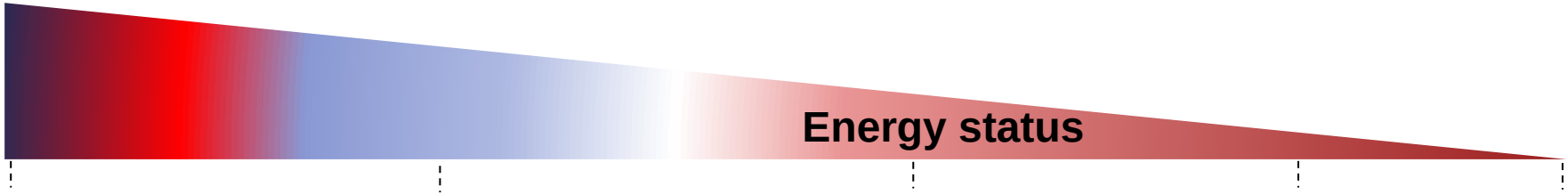
HARVARD MEDICAL SCHOOL

Prof. Karniadakis **Prof.**

BROWN

Perakakis N et al. Metabolism, 2019.

**submitted patent application (by BIDMC)*



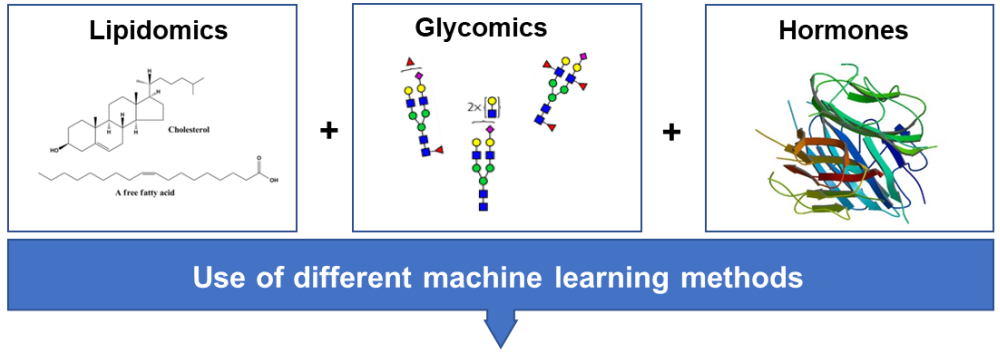
A. Metabolic role of hormones

1) Leptin

2) Follistatins/activins

3) Proglucagon peptides

B. Development of diagnostic tools



Non-invasive diagnosis of NASH, NAFL or healthy status with sensitivity and specificity > 90%

C. Evaluation of treatments

1) PPAR activators

2) Liraglutide

3) Empagliflozin

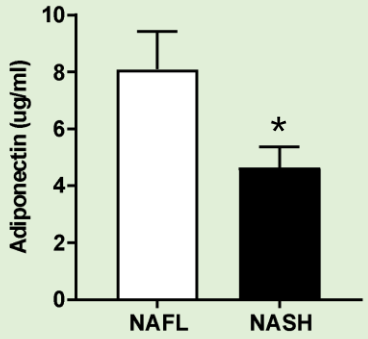
4) Lorcaserin

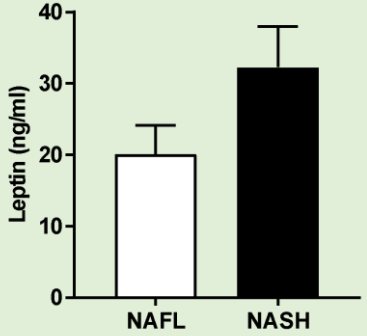
A1) Chrysafi P, Perakakis N ... Mantzoros C. Nature Commun, 2020.
A2) Perakakis N ... Mantzoros C. Diabetes Metab Res Rev, 2020; J Clin Endocrinol Metab, 2018
Perakakis N, Kokkinos A ... Mantzoros C. Diabetes Obes Metab, 2019.
Perakakis N, Upadhyay J ... Mantzoros C. Metabolism, 2018.
A3) Perakakis N, Kokkinos A ... Mantzoros C. Metabolism, 2019.

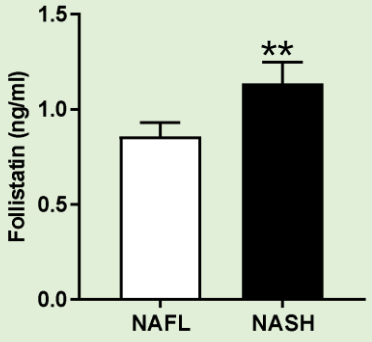
B) Perakakis N, Polyzos S, Yazdani A ... Mantzoros C. Metabolism, 2019.
Polyzos S, Perakakis N ... Mantzoros C. J Clin Endocrinol Metab, 2020.
C) Perakakis N, Stefanakis K ... Mantzoros C. Hepatol Commun, 2020.
Peradze N ... Mantzoros C. Cardiovascular Diabetol, 2019.
Tuccinardi D ... Mantzoros C. Diabetes Obes Metab, 2019.

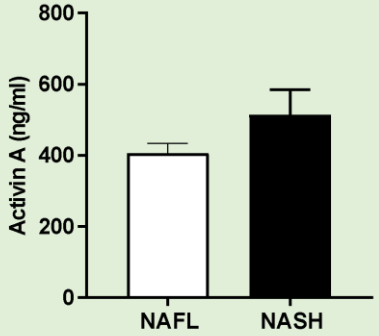
NAFLD – Development of Novel Non-Invasive Diagnostic Algorithms

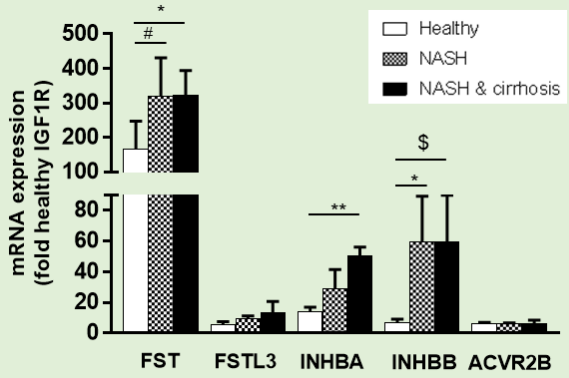
Hormones









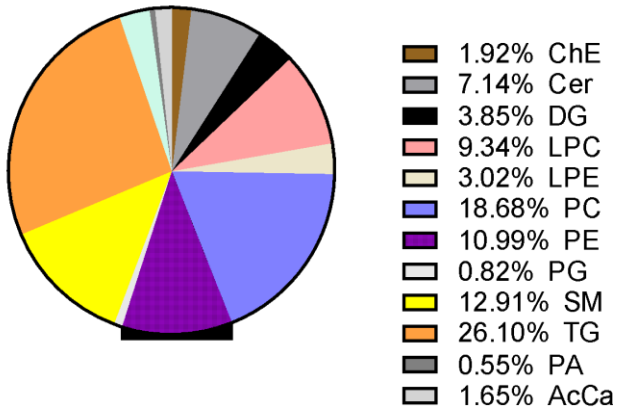


Polyzos A, Perakakis N* ... Mantzoros C. J Clin Endocrinol Metab, 2020. *equal contribution*

Mass spectrometry

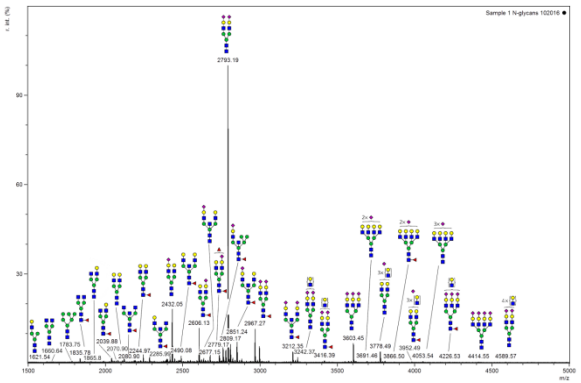
Lipidomics

365 lipid species



Glycomics

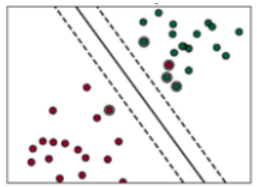
61 glycans



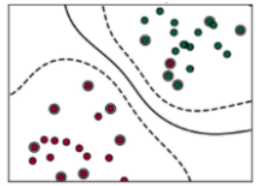
Machine Learning

Support Vector Machine

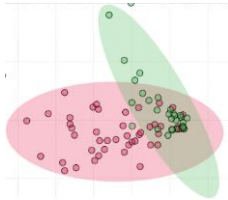
Linear



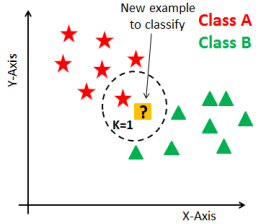
RBF



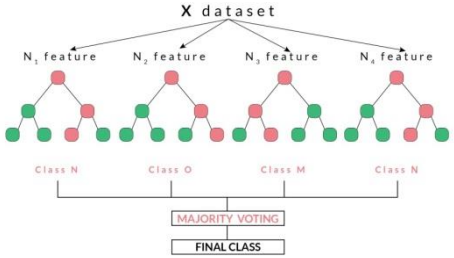
PLS-DA



K-nearest neighbour



Random Forest

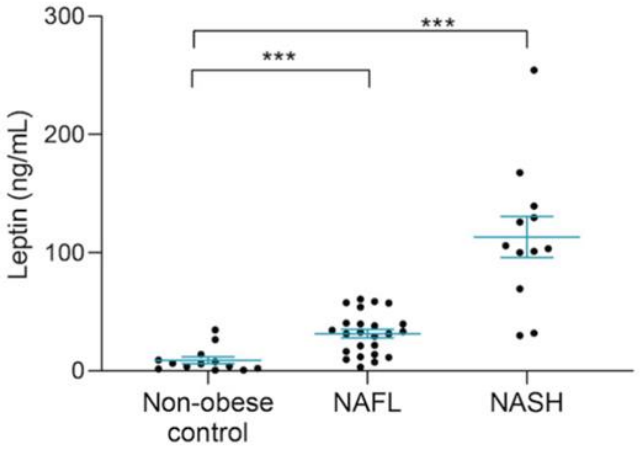


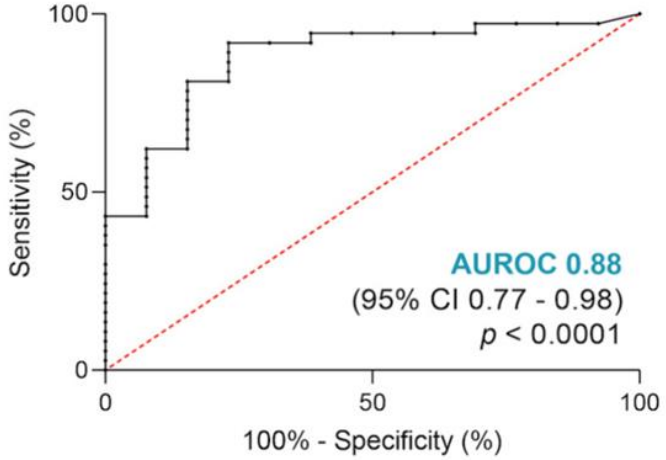
Perakakis N ... Mantzoros C. Metabolism, 2019 (BIDMC submitted patent application).

Adiponectin, Leptin and IGF-1 are Useful Diagnostic and Stratification Biomarkers of NAFL

- Leptin predicts NAFLD and correlates with serum content

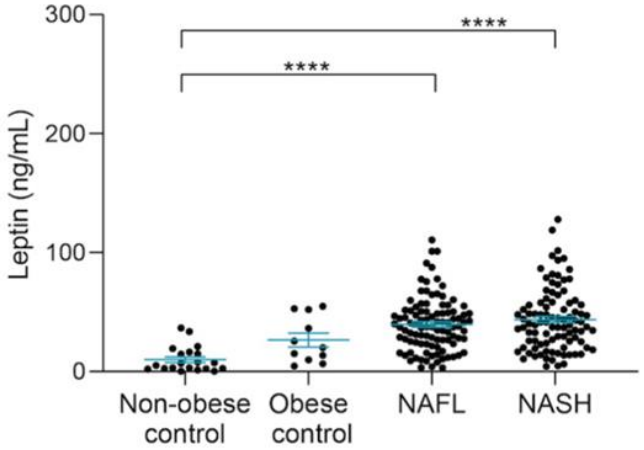
Discovery Cohort

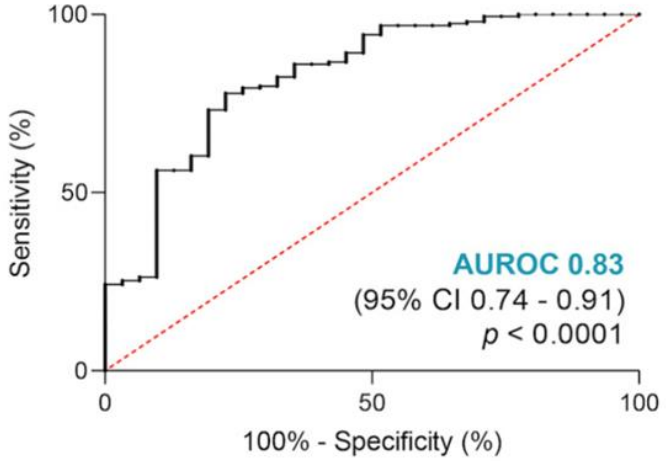




Statistics	Value (95% CI)
Cut-off	9.33 ng/mL (NA)
Sensitivity	0.94 (0.81 – 0.99)
Specificity	0.77 (0.46 – 0.95)
PPV	0.58 (0.34 – 0.79)
NPV	0.98 (0.91 – 0.99)
Accuracy	0.81 (0.68 – 0.91)

Validation Cohort





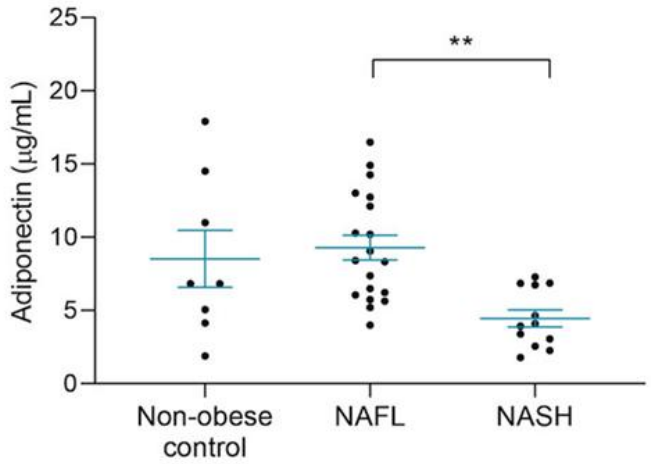
Statistics	Value (95% CI)
Cut-off	9.33 ng/mL (NA)
Sensitivity	0.95 (0.91 – 0.98)
Specificity	0.48 (0.30 – 0.67)
PPV	0.38 (0.30 – 0.46)
NPV	0.97 (0.94 – 0.98)
Accuracy	0.60 (0.53 – 0.67)

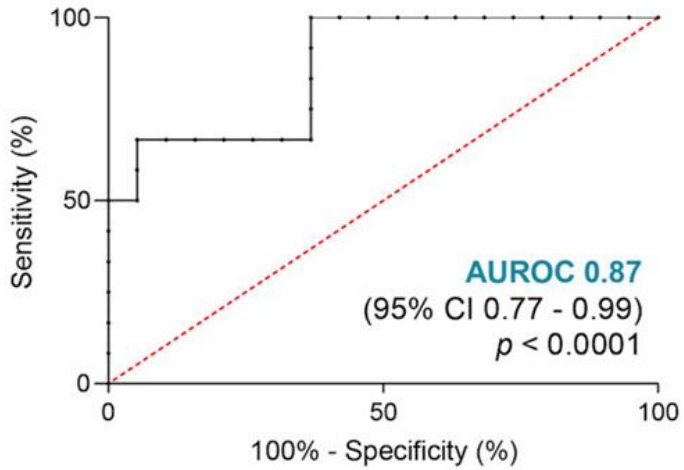
Marques V et al. Front Med, 2021.

Adiponectin, Leptin and IGF-1 are Useful Diagnostic and Stratification Biomarkers of NAFL

- Adiponectin distinguishes NASH

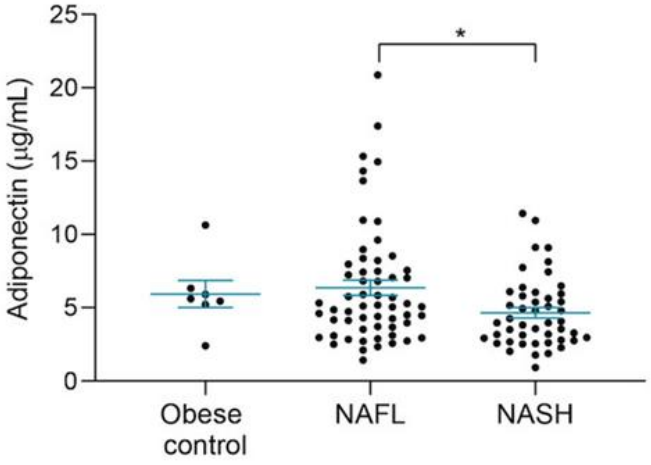
A. Discovery cohort

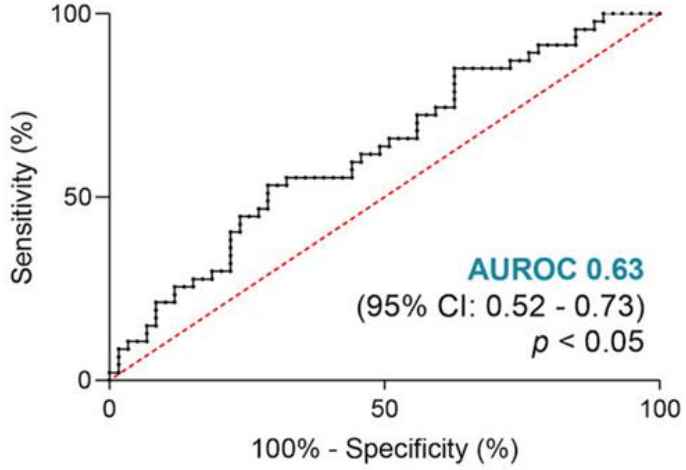




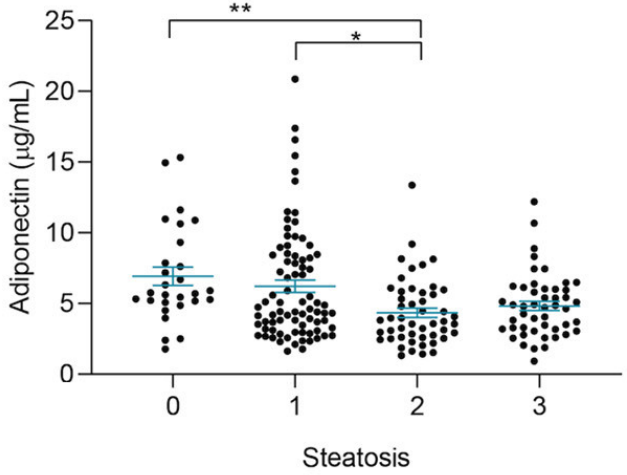
Statistics	Value (95% CI)
Cut-off	7.32 µg/mL (NA)
Sensitivity	1.00 (0.74 – 1.00)
Specificity	0.63 (0.39 – 0.84)
PPV	0.63 (0.49 – 0.58)
NPV	1.00 (NA)
Accuracy	0.77 (0.59 – 0.90)

B. Validation cohort





C. Adiponectin decreases with increasing steatosis

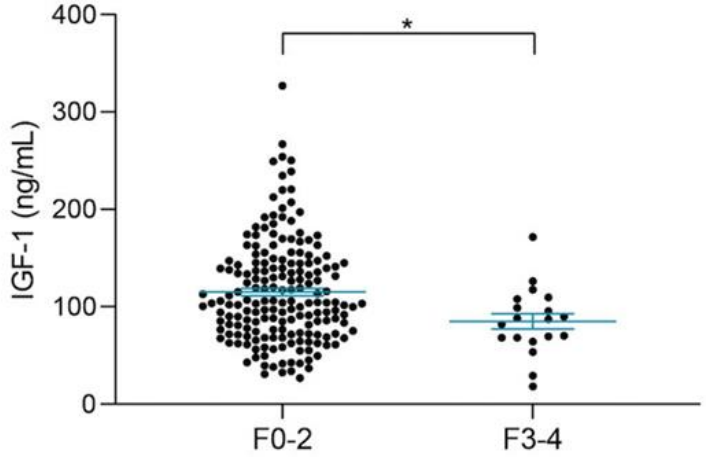


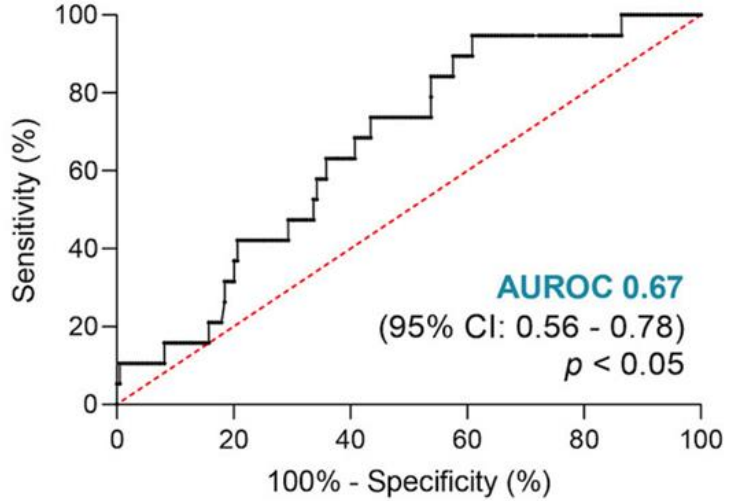
Marques V et al. Front Med, 2021.

Adiponectin, Leptin and IGF-1 are Useful Diagnostic and Stratification Biomarkers of NAFL

- No significant differences in IGF-1 relating to liver steatosis, lobular inflammation, or hepatocyte ballooning severity

- IGF-1 was significantly lower in NAFLD patients with advanced fibrosis (F3–4; $p < 0.05$)

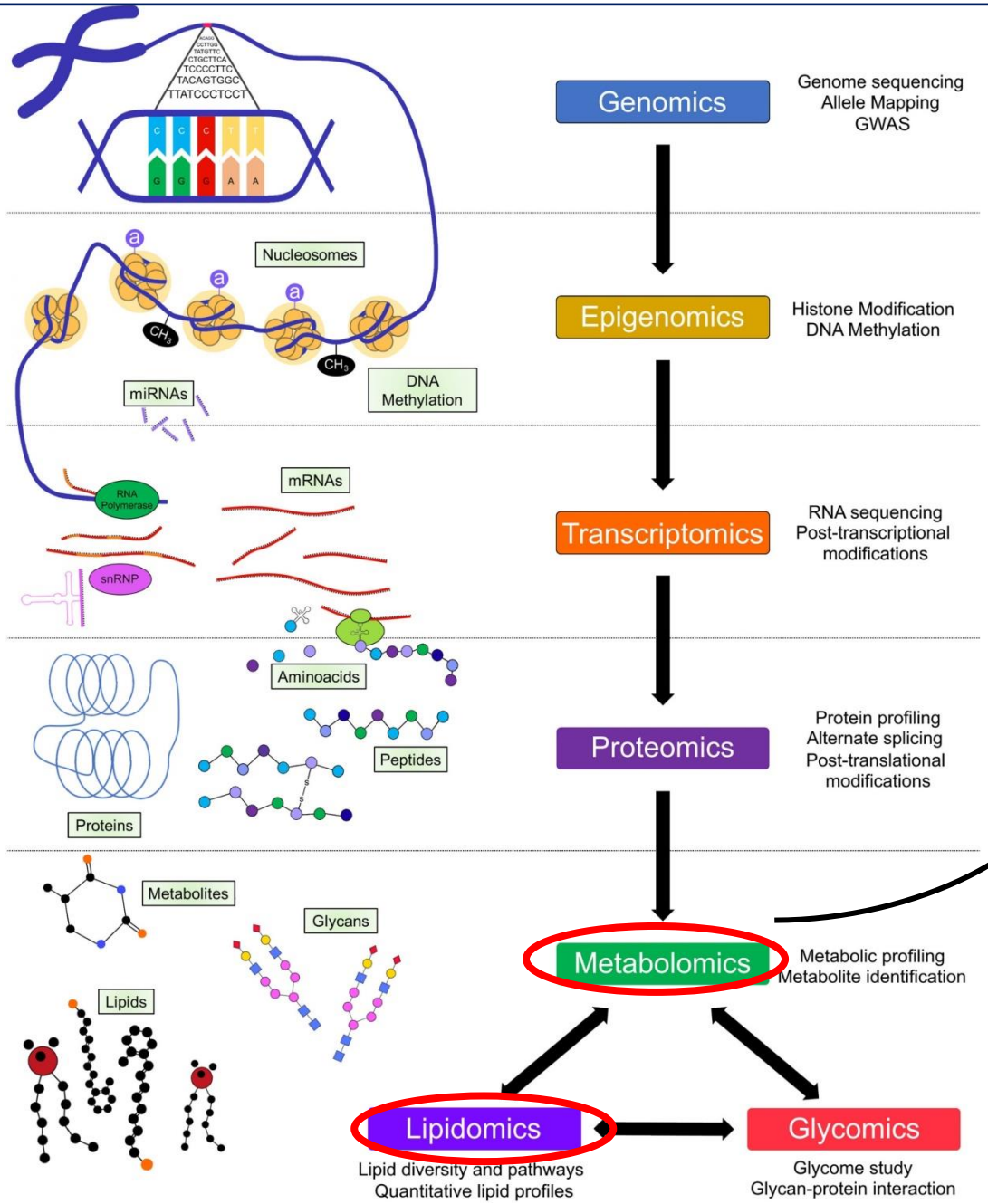


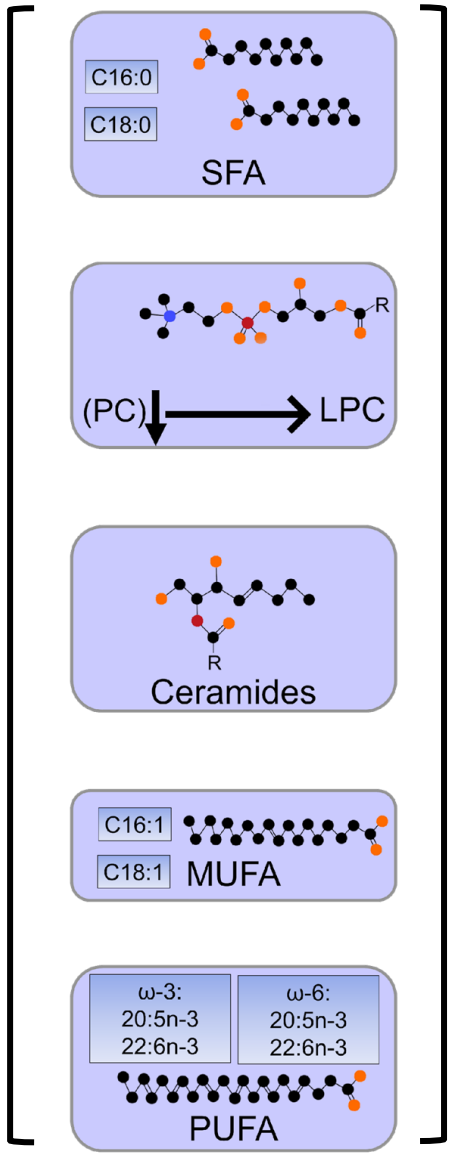


Statistics	Value (95% CI)
Cut-off	98.83 ng/mL (NA)
Sensitivity	0.70 (0.35 to 0.93)
Specificity	0.61 (0.13 to 0.72)
PPV	0.17 (0.11 to 0.25)
NPV	0.95 (0.88 to 0.98)
Accuracy	0.63 (0.53 to 0.72)

Marques V et al. Front Med, 2021.

OMICS and AI /ML Technologies in NAFLD





Modulation through existing metabolic drugs (for T2DM, obesity etc.)

Perakakis N ... Mantzoros C. Metabolism, 2020.

51 year-old-man at your office.
After identifying your patient's NAFLD Risk Factors and Past Medical History

How would you proceed with the diagnosis?

Which of the following is correct?

A. 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).
B. Liver fibrosis has been linked to morbidity and reduced overall patient survival
C. NAFLD and fibrosis are reversible with weight loss
D. To differentiate alcoholic vs nonalcoholic fatty liver, the AST/ALT can be used which is ≥ 2 in alcohol induced fatty liver.
E. All of the above.

What should be the next step after a FIB-4 or NFS calculation of intermediate risk?

A. Repeat it in 2-3 years

B. Repeat it in 1 year

C. Vibration-controlled transient elastography or blood tests measuring fibrosis markers

D. Refer to a gastroenterologist or hepatologist

Which of the following could most probably differentiate between NAFLD and NASH?

A. Ultrasonography
B. MRI
C. Vibration-controlled transient elastography
D. Biopsy

Thank you!

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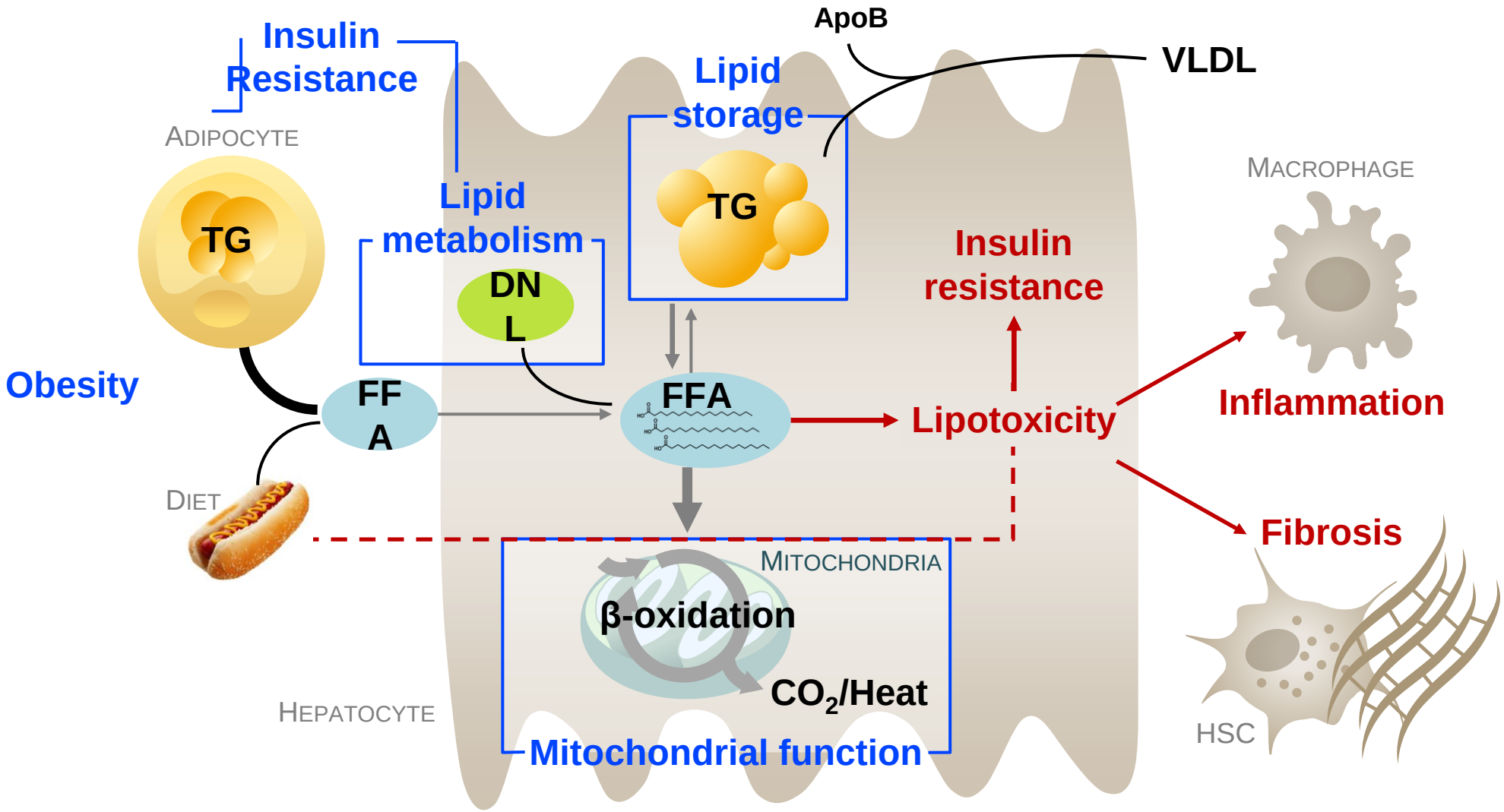
Certified Cardiometabolic Health Professional (CCHP)

Management of NASH: Lifestyle Modifications

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

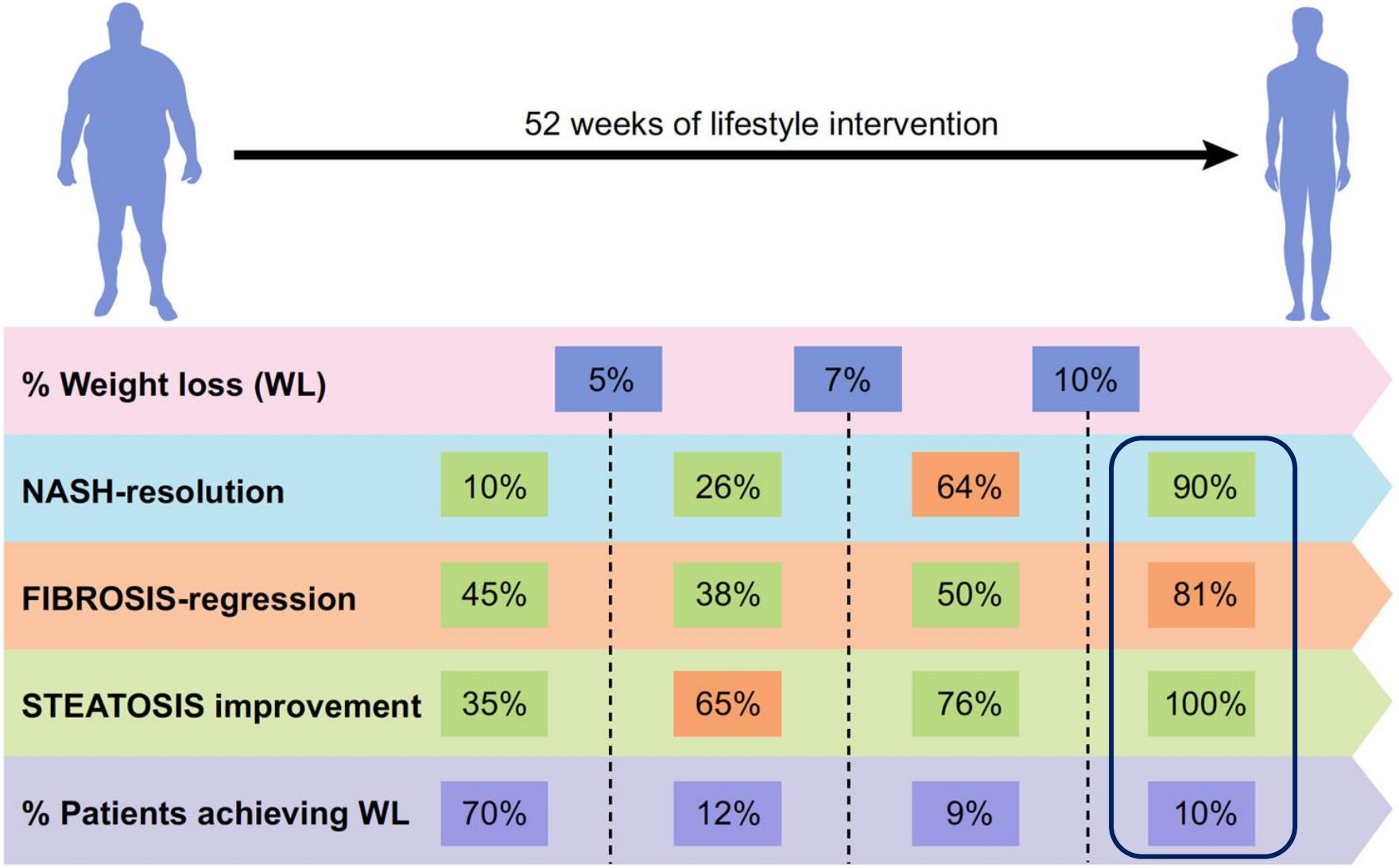


ApoB = apolipoprotein B; DNL = de novo lipogenesis; FFA = free fatty acid; VLDL = very low density lipoprotein.

1. Brown MD 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.

Management of NASH: Lifestyle Modification

- Weight loss through moderate exercise and dietary changes



Romero-Gomez M et al. J Hepatol, 2017.

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.

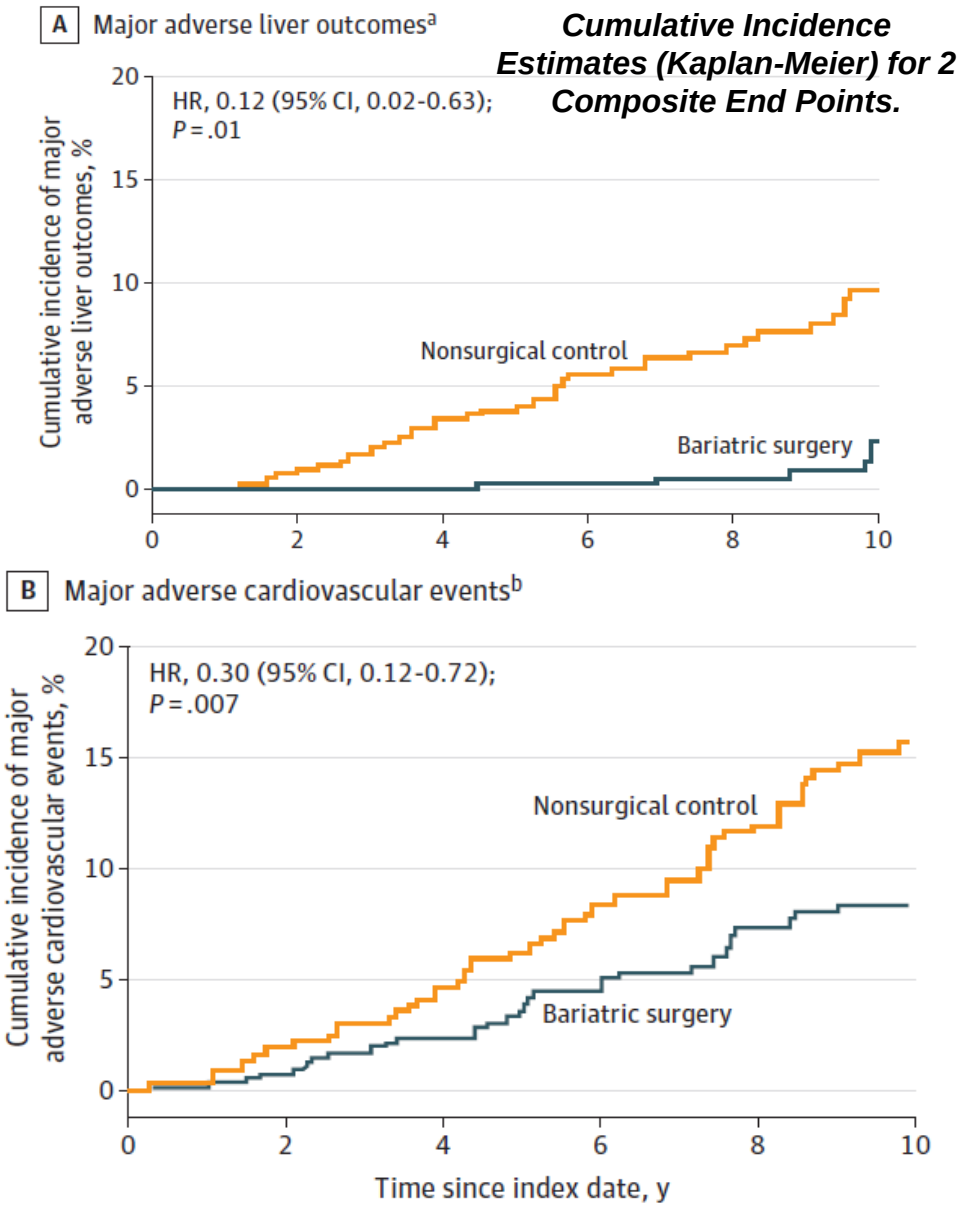
-Cumulative incidence at 10 years: 15.7%(95%CI, 11.3%-19.8%)

(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

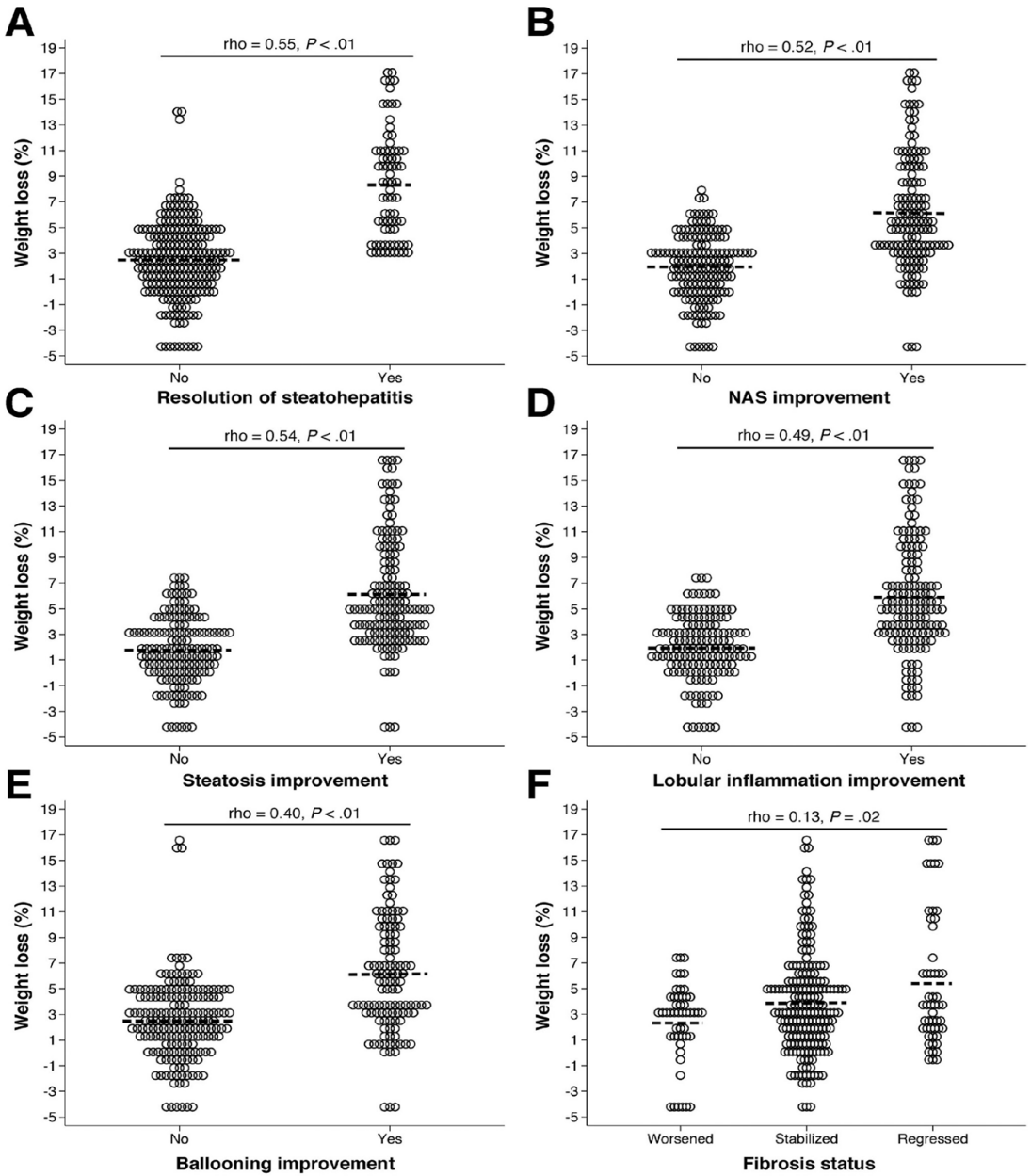
Cumulative Incidence Estimates (Kaplan-Meier) for 2 Composite End Points.



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

Weight Loss Through Lifestyle Modifications Significantly Reduces Features of Nonalcoholic Steatohepatitis



293 patients with histologically proven NASH

↓ Lifestyle changes for 52 weeks

Liver biopsies collected and compared to baseline

↓

The magnitude of weight loss correlated with decreases in intrahepatic triglyceride (IHTG) content, hepatocyte ballooning, and hepatic inflammation.

Vilar-Gomez E et al. Gastroenterology, 2015.

Management of NASH: Lifestyle Modification

- Mediterranean diet is inversely associated with liver steatosis and decreases ten-year cardiovascular risk and diabetes in NAFLD: evidence from the ATTICA prospective cohort study

Total sample			
	Total sample	**Men**	**Women**
N, cases	*2,020/317*	*1,006/198*	*1,014/119*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	3•01 (2•28, 3•95)*	2•70 (1•84, 3•95)*	2•83 (1•86, 4•30)*
Multi-adjusted model	1•37 (1•10, 2•10)**	1•61 (1•01, 2•57)*	1•11 (0•66, 1•88)
Multi-adjusted model plus MedDietScore	1•36 (0•96, 1•94)	1•62 (1•01, 2•63)**	1•08 (0•63, 1•85)
Sample stratified according to level of adherence to Mediterranean diet			
	Total sample	**Men**	**Women**
MedDietScore<27			
N, cases	*1,223/280*	*854/188*	*369/92*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	1•92 (1•41, 2•62)*	2•30 (1•54, 3•42)*	1•49 (0•89, 2•50)
Multi-adjusted model	1•40 (1•01, 2•03)**	1•65 (1•02, 2•69)**	1•09 (0•60, 1•98)
MedDietScore≥27			
N, cases	*797/37*	*152/10*	*645/27*
Liver steatosis (yes vs. no)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Crude model	2•05 (0•94, 4•50)	3•11 (0•66, 4•55)	1•72 (0•66, 4•48)
Multi-adjusted model	1•00 (0•38, 2•63)	1•26 (0•20, 5•64)	0•83 (0•24, 2•84)

HRs and their corresponding CIs were obtained through Cox regression analysis. Multi-adjusted model was adjusted for age, (gender), hypertension, hypercholesterolemia, current smoking, physical activity, body mass index, family history of cardiovascular disease. Abbreviations: Confidence Interval (CI); Hazard ratio (HR); Triglycerides-glucose (TyG). p<0•001, **p<0•05.

Kouvari M et al and the ATTICA study Investigators. Clin Nutr, 2021.

Management of NASH: Lifestyle Modification

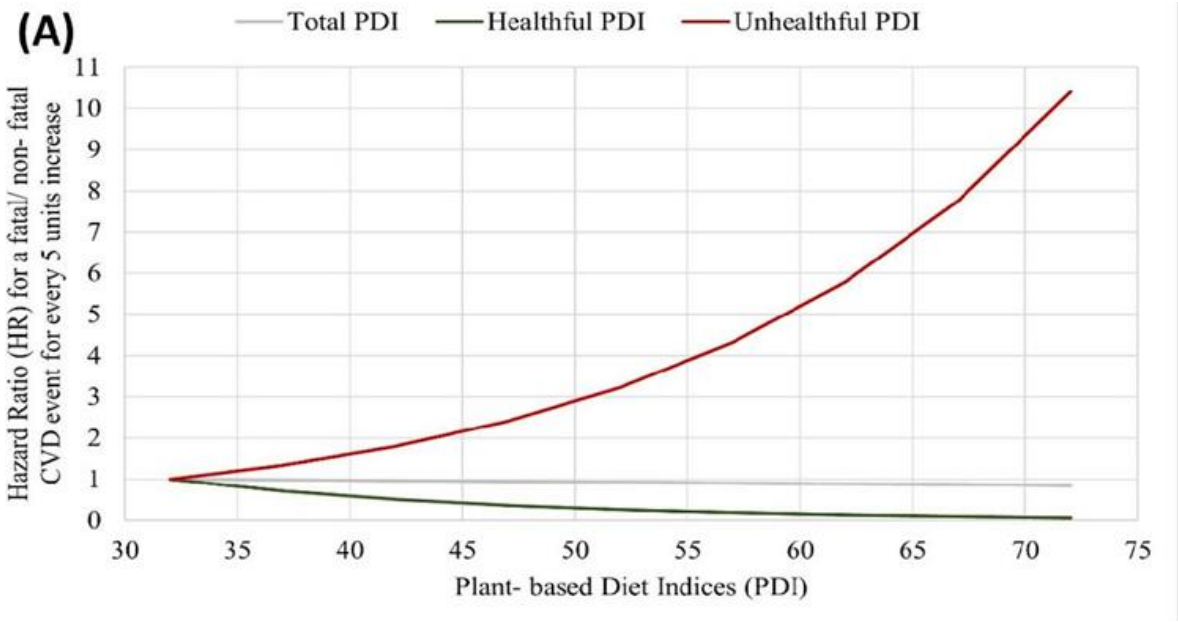
- Dietary changes

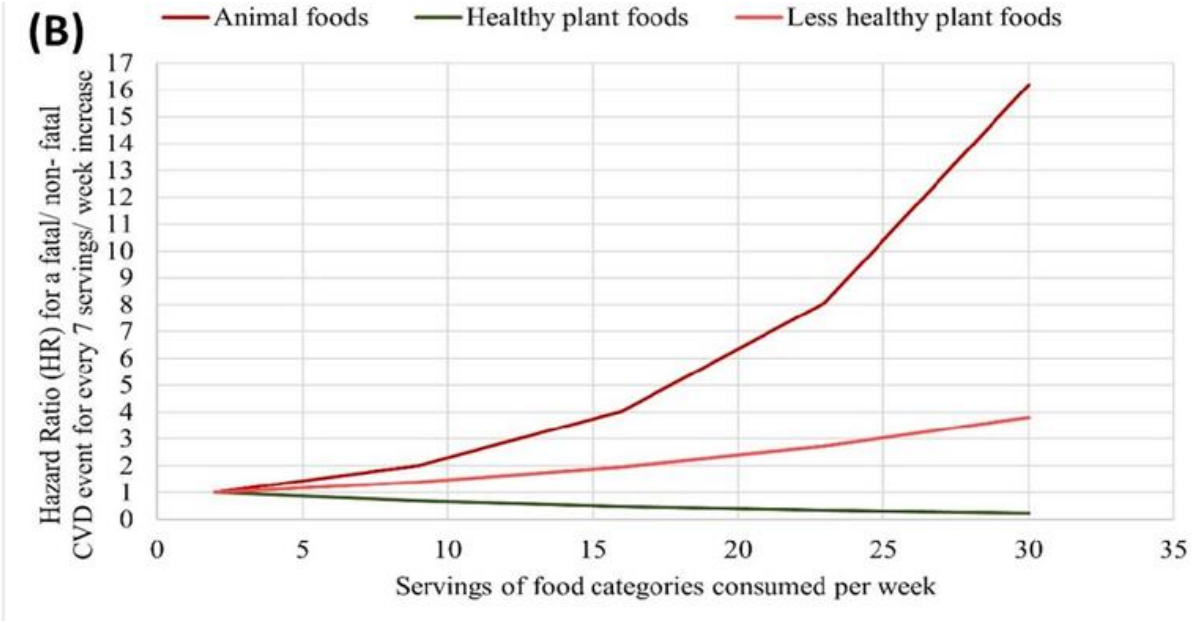
Dietary patterns	**Western Diet**	**Mediterranean Diet (MD)**	**Dietary Approach to Stop Hypertension (DASH)**
Foods intake	❖ *Processed foods* ❖ *Red meats* ❖ *Processed meats* ❖ *Sugary beverages* ❖ *Snacks* ❖ *cakes and biscuits* ❖ *eggs* ❖ *butter*	❖ *Extra virgin olive oil* ❖ *Vegetables and Fruits* ❖ *Cereals, legumes, nuts* ❖ *Moderate intakes of fish and other meat, dairy products and red wine* ❖ *Low intakes of eggs and sweets.*	❖ *Fruits and vegetables* ❖ *whole grains,* ❖ *fish, poultry, nuts,* ❖ *legumes* ❖ *low-fat dairy products* ❖ *reduced sodium* ❖ *Fresh food* ❖ *Minimally processed food*
Nutrients	↑**Energy intake** ↑SFA ↓PUFA ↑protein animal ↑sugar, fructose ↑cholesterol ↑Salt ↓fiber	↓SFA ↑**MUFA** ↑**PUFA** ↑protein vegetables ↓sugar fructose ↓cholesterol ↑**fiber** ↑**polyphenols,** ↑**carotenoids**	↓ **total fat** ↓**Salt** ↑protein vegetables ↓sugar fructose ↓cholesterol ↑fiber ↑polyphenols, ↑carotenoids

Berná G et al. Liver Int, 2020.

Quality of Plant-Based Diets in Relation to Cardiovascular Disease Risk in a Mediterranean Population: the ATTICA Cohort Study

➢ Dose-response relationship of plant-based diet indices, animal and plant foods with ten-year CVD incidence





The association with overall plant-based diet (PDI) was quite null.

A clear protective tend was evident increasing healthful plant-based dietary index (hPDI).

An aggravating effect of unhealthful plant-based dietary index (uPDI) was observed.

Kouvari M…Mantzoros C and the ATTICA study Investigators. Clin Nutr, 2021.

Food Items to Avoid

- Fruit juice
- Canned fruit

- Refreshments Cola type
- Other sugar refreshments
- Light refreshments

- Potatoes fried
- Potatoes boiled
- Potatoes baked
- Mashed potatoes

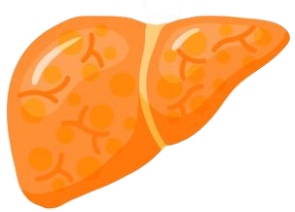
- Bread
- Rusks,
- French toast
- Cereals
- Pasta
- Rice

- Honey/jam
- Sugar
- Candies
- Chocolate
- Ice cream
- Cereal
- Cookies/biscuits
- Cake
- Greek traditional sweets

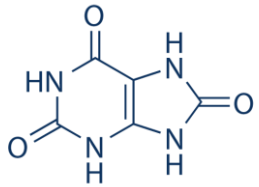
Kouvari M et al and the ATTICA study Investigators. In press.

Refined Sugars: Sucrose, Fructose and High Fructose Corn Syrup

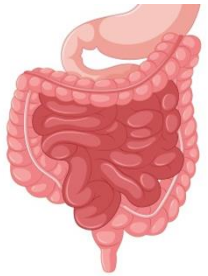
Refined sugars
Mainly found in sugar sweetened beverages (SSB)



Increase hepatic synthesis of triglycerides



Promote uric acid production which may cause oxidative stress and insulin resistance



Increase gut permeability and endotoxin

Framingham Heart Study
Three Generations of Dedication

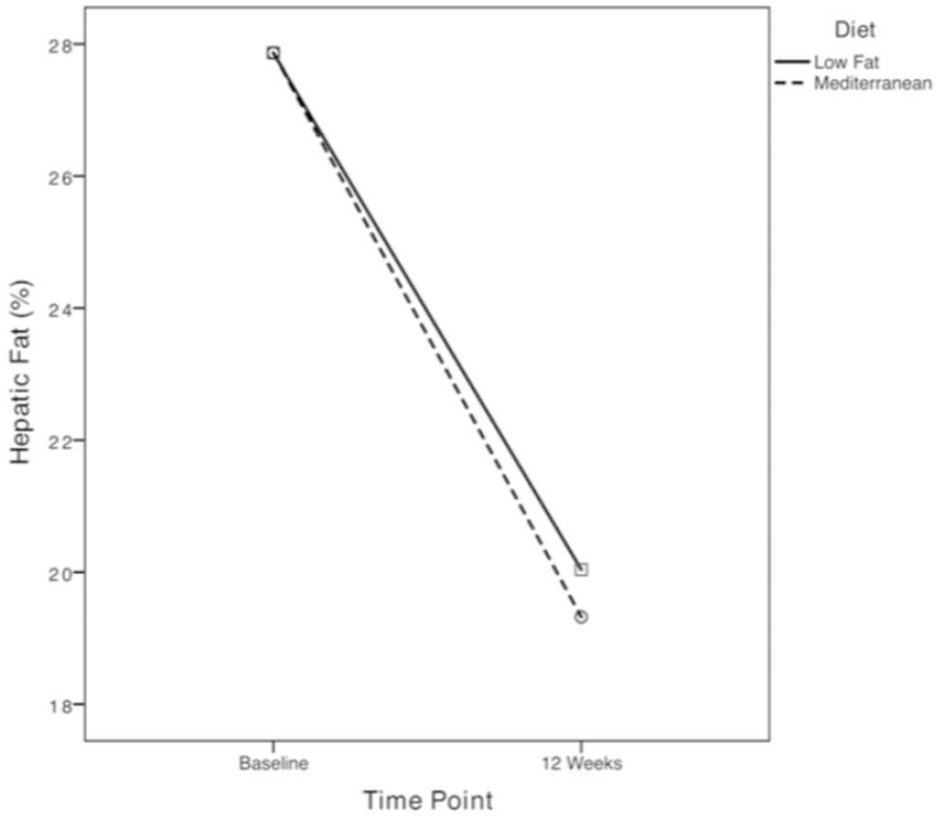
Dose-response association between soft drinks and fatty liver disease, with a 61% increased risk of fatty liver disease in daily consumers of SSB compared to non-consumers.

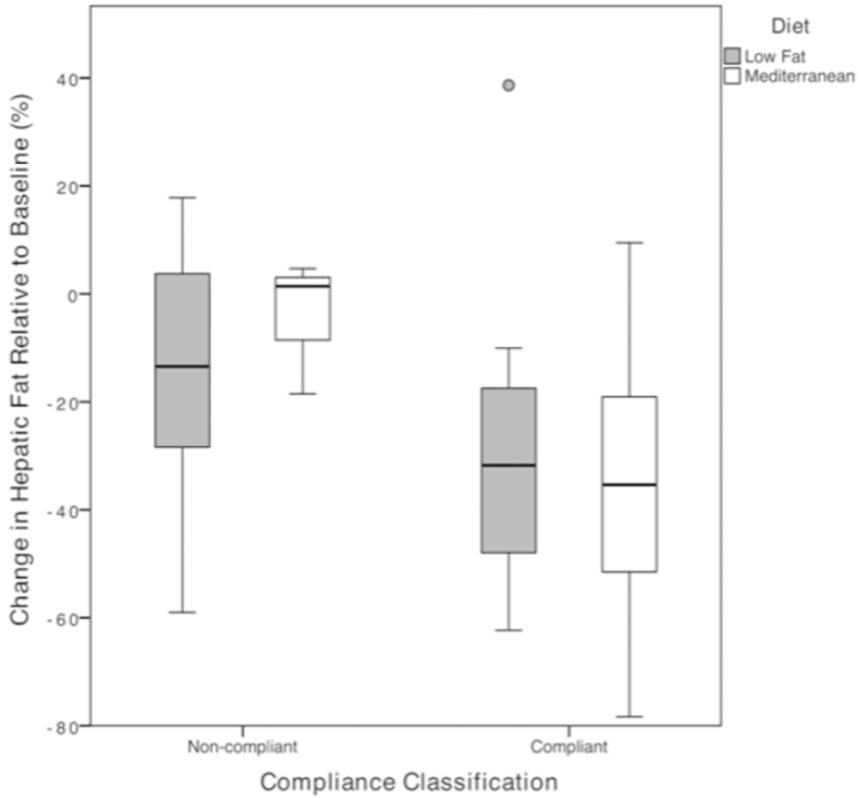
1) Howard BV et al. Circulation, 2002. 2) Poulsom R et al. Prog Biochem Pharmacol, 1986. 3) Herman RH et al. Fed Proc, 1970. 4) Vos MB et al. Hepatology, 2013. 5) Choi JW et al. Arthritis Rheum, 2008. 6) Afzali A et al. Hepatology, 2010. 7) Ma J et al. J Hepatol, 2015.

Ad Libitum Mediterranean and Low-Fat Diets Both Significantly Reduce Hepatic Steatosis: A Randomized Controlled Trial

- At week 12, hepatic steatosis had reduced significantly in both groups ($P < 0.01$), and there was no difference in liver fat reduction between *ad libitum* isocaloric Mediterranean vs Low-Fat Diet ($P = 0.32$)

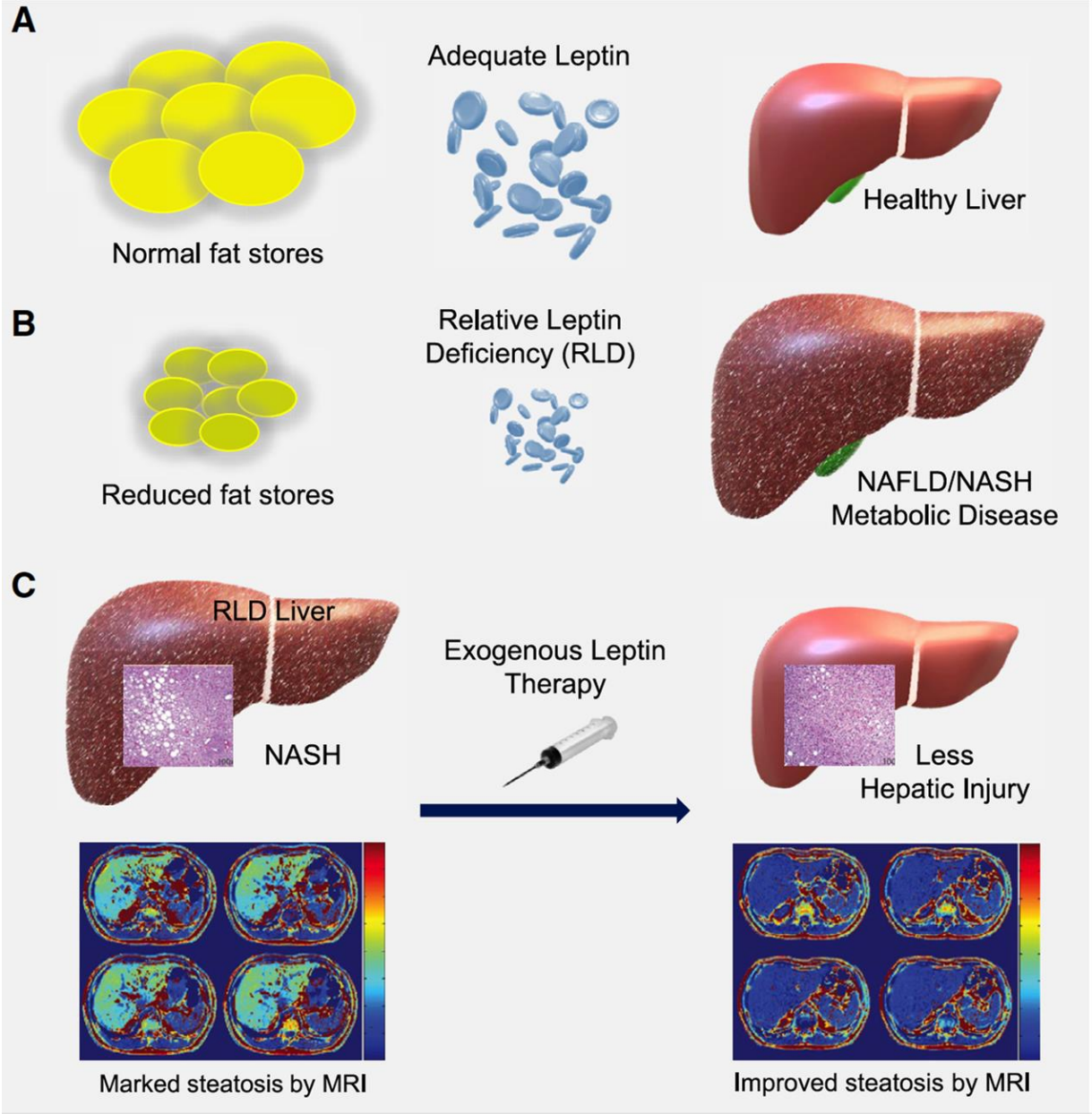
- The Mediterranean diet was easier to adhere to than a low-fat diet (88% vs. 64%) and improved CVD risk factors, including lipids and glycated haemoglobin, to a greater degree.





Properzi C et al. Hepatology, 2018.

Metreleptin Therapy for NASH: Open Label Interventions in Two Different Clinical Settings



Akinci B et al. Med, 2021.

The Effect of Dietary Patterns on NAFLD and its Related Hepatic and Extra-Hepatic Complications in Adults: a Systematic Review of Randomized Controlled Trials

Angeliki M. Angelidi,*Angeliki Papadaki,* Eric Nolen-Doerr, Chrysoula Boutari, & Christos S. Mantzoros (under Review)

Methods

3 trials included

Overall cohort
N=128

Effect of dietary patterns on hepatic and extra-hepatic complications related to NAFLD

Results

Hepatic fat content

Low-carbohydrate
-27%, P=0.008
one study, n=18

vs Low-calorie

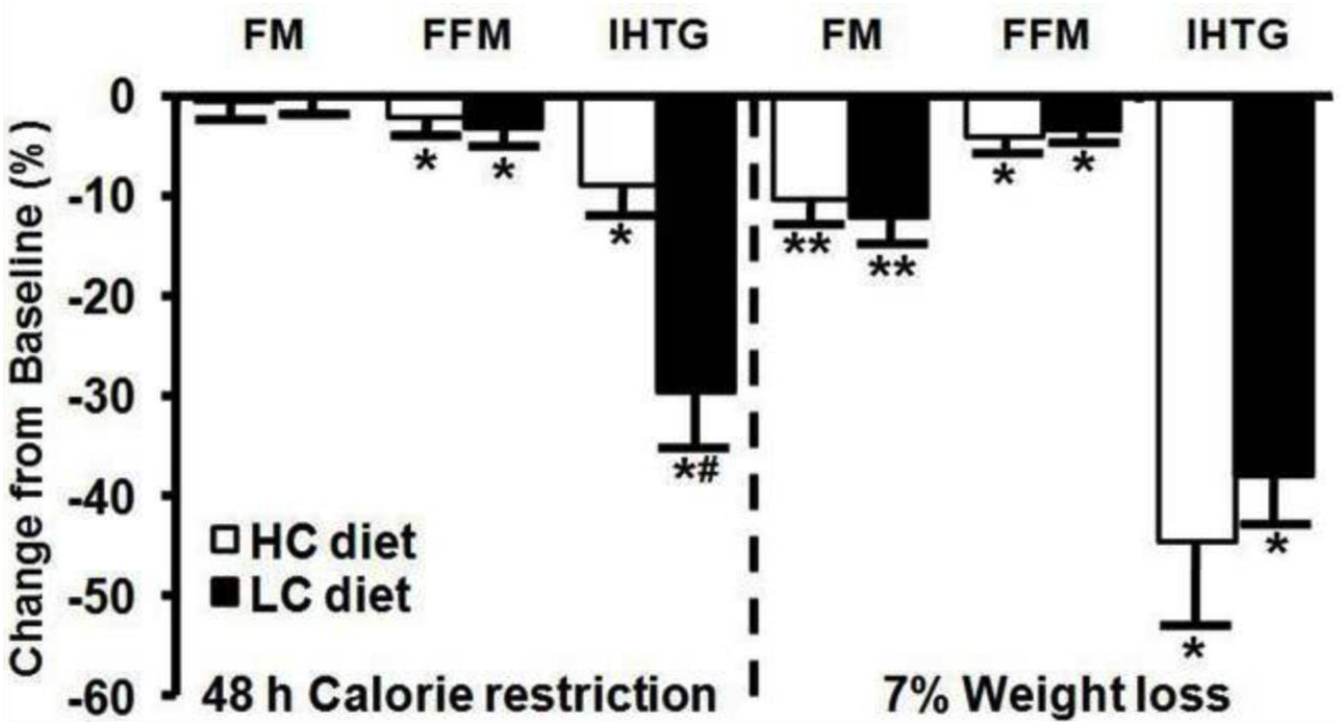
Mediterranean
-4.4%, P=0.030
one study, n=12

vs low-fat, high-carbohydrate

Intrahepatic Triglyceride Content (IHTG) is extremely sensitive to changes in energy balance

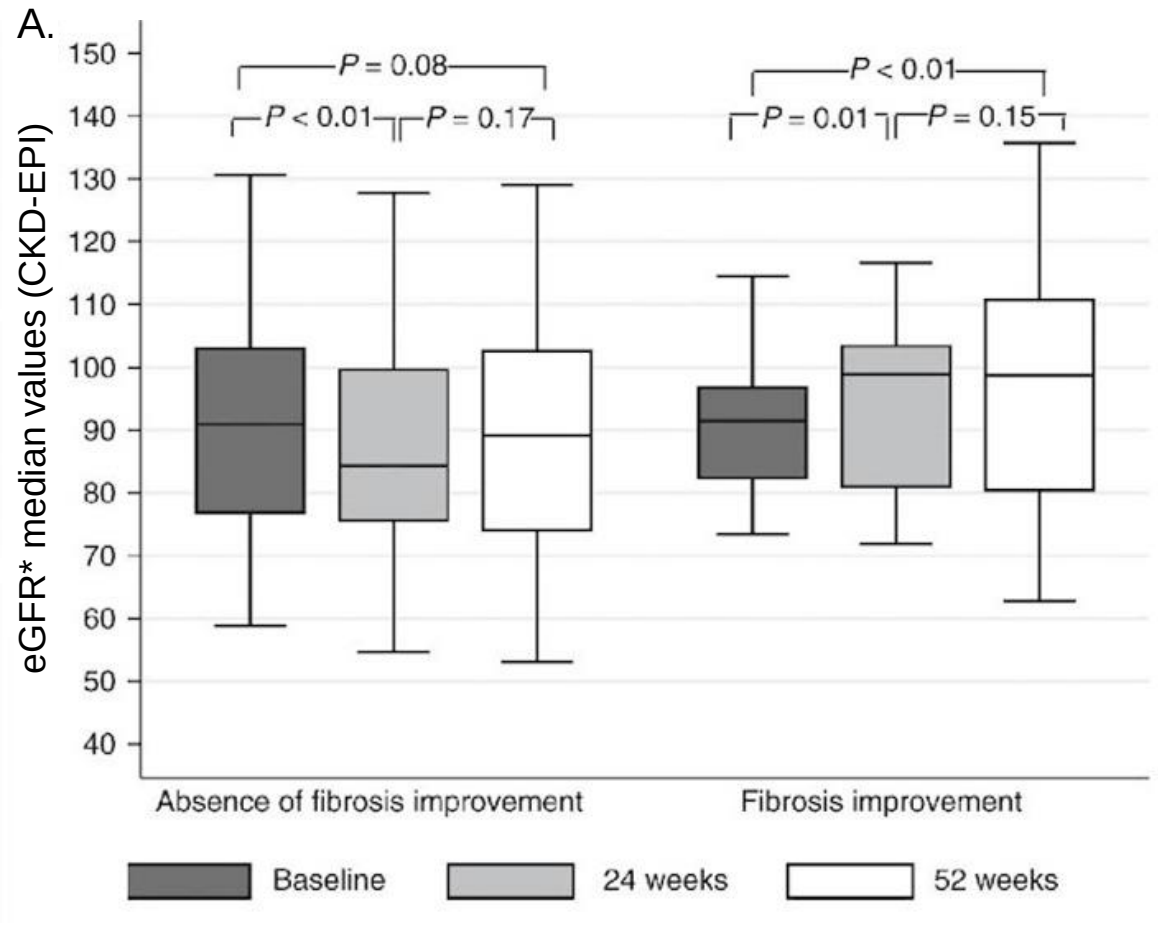
- 22 obese subjects (BMI=36.5±0.8kg/m^2) were randomized to a high-carbohydrate (>180g/d) or low-carbohydrate (<60g/d) energy-deficit diet and were assessed after 48 h, and after ~11 wks (7% weight loss) of diet therapy.

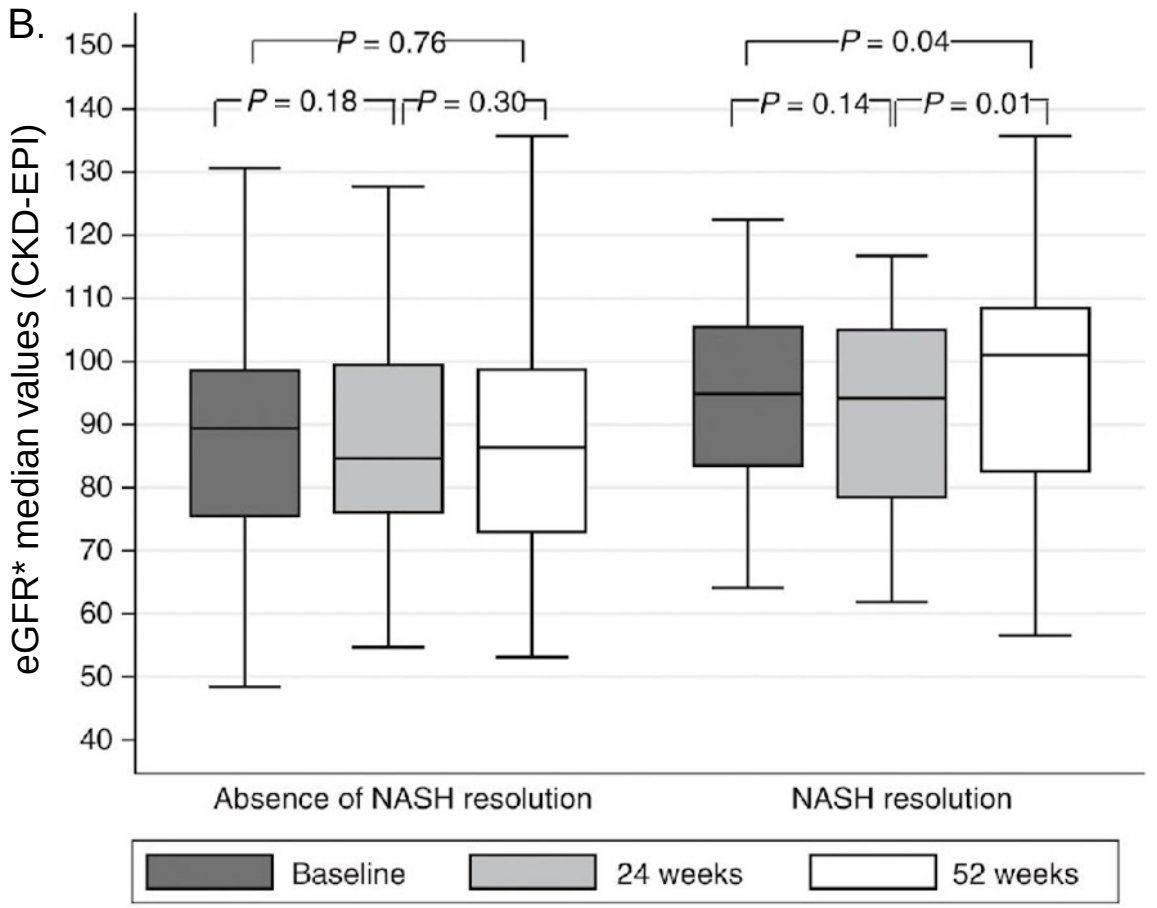
- Even 48 h of a low-calorie diet can decrease IHTG by about 20%, and 7% weight reduction decreases IHTG by approximately 40%.



Kirk E et al. Gastroenterology, 2009.

Improvement in liver histology due to lifestyle modification is independently associated with improved kidney function in patients with non-alcoholic steatohepatitis

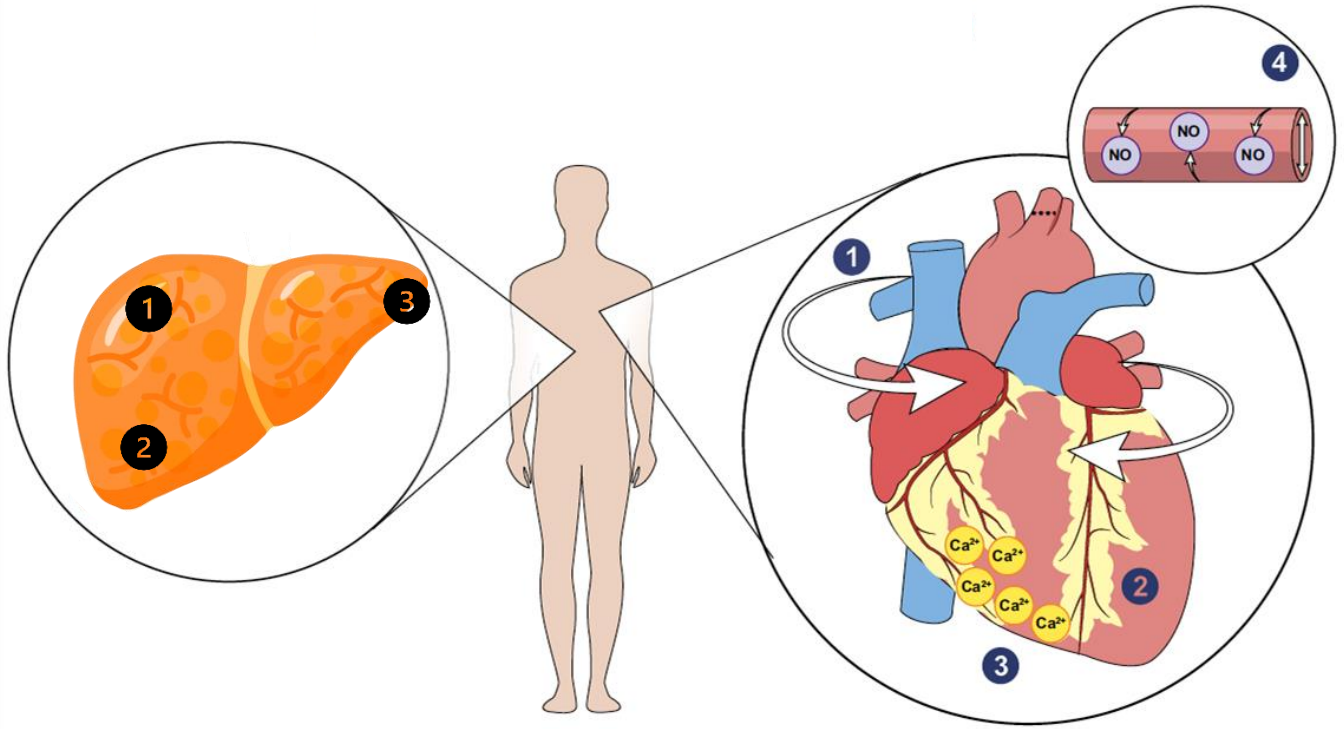




Vilar-Gomez E et al. Aliment Pharmacol Ther, 2017.

Benefits of Physical Activity and Exercise in NAFLD

- Regular endurance or resistance exercise in the absence of weight loss decreases IHTG content only slightly but improves metabolic health.

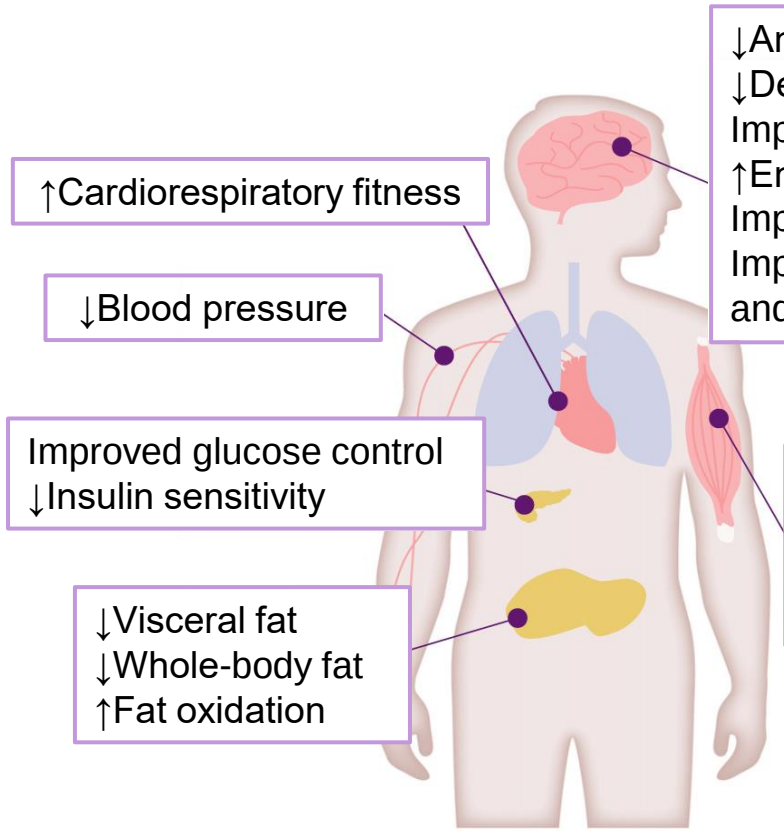


Changes in the liver

1. Peripheral insulin sensitivity ↑ = *de novo* lipogenesis ↓
2. Visceral fat ↓ = lipid supply to liver ↓
3. VLDL clearance ↑ = lipid storage ↓

Changes to cardiovascular system

1. Torsion ↓ = myocardial damage ↓
2. EDV ↑ = preload ↑
3. Ca^{2+} handing ↑ = SV ↑ + EF ↑
4. FMD ↑ = O^2 supply ↑



↑Cardiorespiratory fitness

↓Blood pressure

Improved glucose control
↓Insulin sensitivity

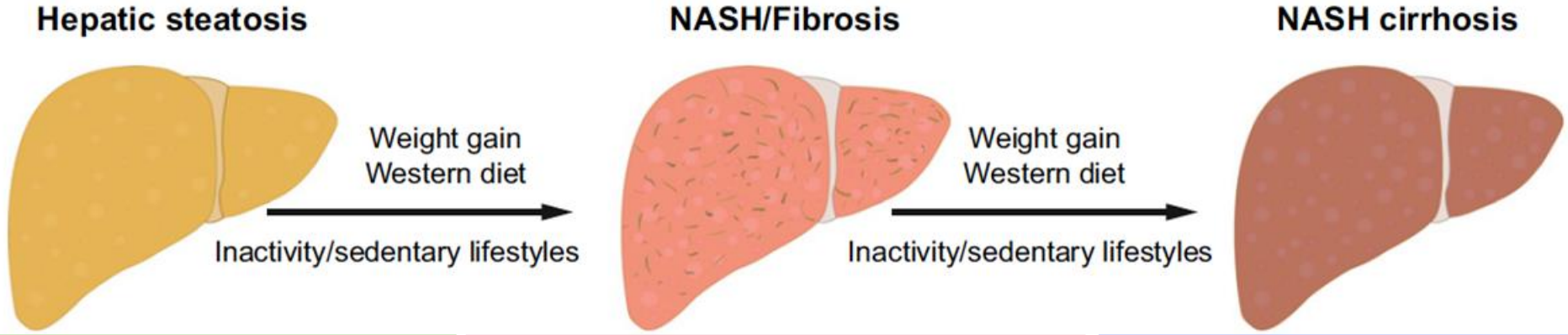
↓Visceral fat
↓Whole-body fat
↑Fat oxidation

↓Anxiety
↓Depression
Improved mood
↑Energy levels
Improved sleep patterns
Improved self-esteem
and self-confidence

↑Muscle strength
↑Muscle bulk
↑Bone density
↑Flexibility

1) Hashida R et al. J Hepatol, 2017. 2) Sabag A et al. Diabetes Care, 2020. 3) Hallsworth K et al. Gut, 2011. 4) *Romero-Gomez M et al. J Hepatol, 2017. 5) Hallsworth K et al. JHEP Rep, 2019.*

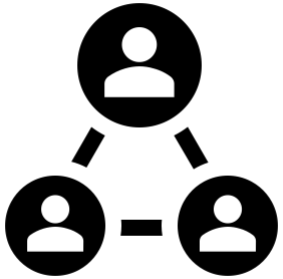
Summary of Lifestyle Treatment Options Through the Course of NAFLD



	Hepatic steatosis	NASH/Fibrosis	NASH cirrhosis
Weight loss	Weight loss ≥ 5%	Minimum 7-10% weight loss	Modest caloric restriction 500-800 kcal/day (overweight/obese patients) Increase weight loss targets in well-compensated obese patients
Dietary options	• Mediterranean diet • Encourage a regular meal pattern • Avoid snacking • Reduce alcohol intake (if relevant) • Reduction in fructose/glucose intake (especially sugar-containing drinks) and processed foods	• Mediterranean diet • Encourage a regular meal pattern • Avoid snacking • Reduce alcohol intake (if relevant) • Reduction in fructose/glucose intake (especially sugar-containing drinks) and processed foods	• Protein intake 1.2-1.5 g/kg/day • Small frequent meals • Encourage abstinence from alcohol • Reduction in fructose/glucose intake (especially sugar-containing drinks and processed foods)
Exercise options	Increase daily physical activity levels (aim for 10,000 steps/day if applicable) Decrease total sedentary time and break p sedentary time Aerobic or resistance exercise (aiming for 150 min/week of moderate intensity exercise)		

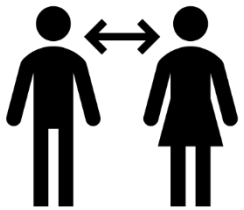
Hallsworth K et al. JHEP Rep, 2019.

The Role of the Multidisciplinary Approach and Behavioral Therapy in the lifestyle Treatment of NASH



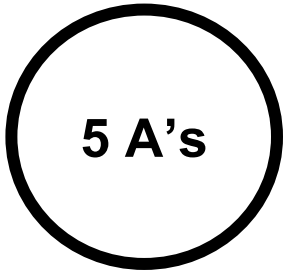
Multidisciplinary team

- Physicians, dieticians, psychologists and physical activity supervisors
- Not provided in most clinical setups due to limited resources



Address a patient's lack of understanding and comprehension of diagnosis

- Provide information and refer the patients to appropriate resources about NAFLD implications and treatment
- Obtain training in behavioral therapy.



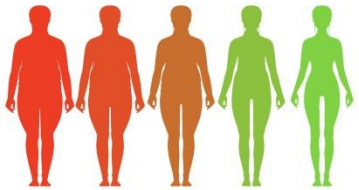
Behavioral Therapy and Motivational Interviewing

- Ask
- Advise
- Assess
- Assist
- Arrange

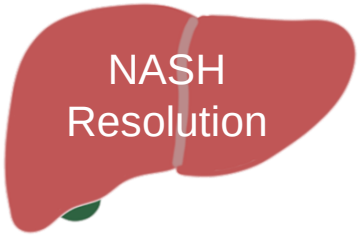
Romero-Gomez M et al. J Hepatol, 2017.
Hallsworth K et al. JHEP Rep, 2019.

Barriers and Facilitators to Implementing Lifestyle Changes

- Genetic Predisposition: The PNPLA3 rs738409 gene polymorphism: associated with a 3-fold greater reduction in hepatic triglyceride in response to lifestyle intervention.



- Baseline BMI: 3–5% body weight reduction sufficient for NAFLD resolution in 50% of non-obese individuals, compared to the 7–10% body weight reduction required to achieve a similar outcome in obese individuals.



- Number of metabolic components and duration: Less likely if individuals are morbidly obese (BMI ≥35 kg/m2), have type 2 diabetes (T2DM) or severe NASH demonstrated by significant hepatocyte ballooning

Long-term weight loss maintenance

- Neurohormonal defense mechanisms: The key factors influencing long-term weight loss are intensity of physical activity and percentage of weight loss during the first year.

Romero-Gomez M et al. J Hepatol, 2017.
Hallsworth K et al. JHEP Rep, 2019.

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Foundations of Cardiometabolic Health Certification Course

Certified Cardiometabolic Health Professional (CCHP)

Management of NASH: Overview of Guidelines, Current and Select Emerging Treatment Options

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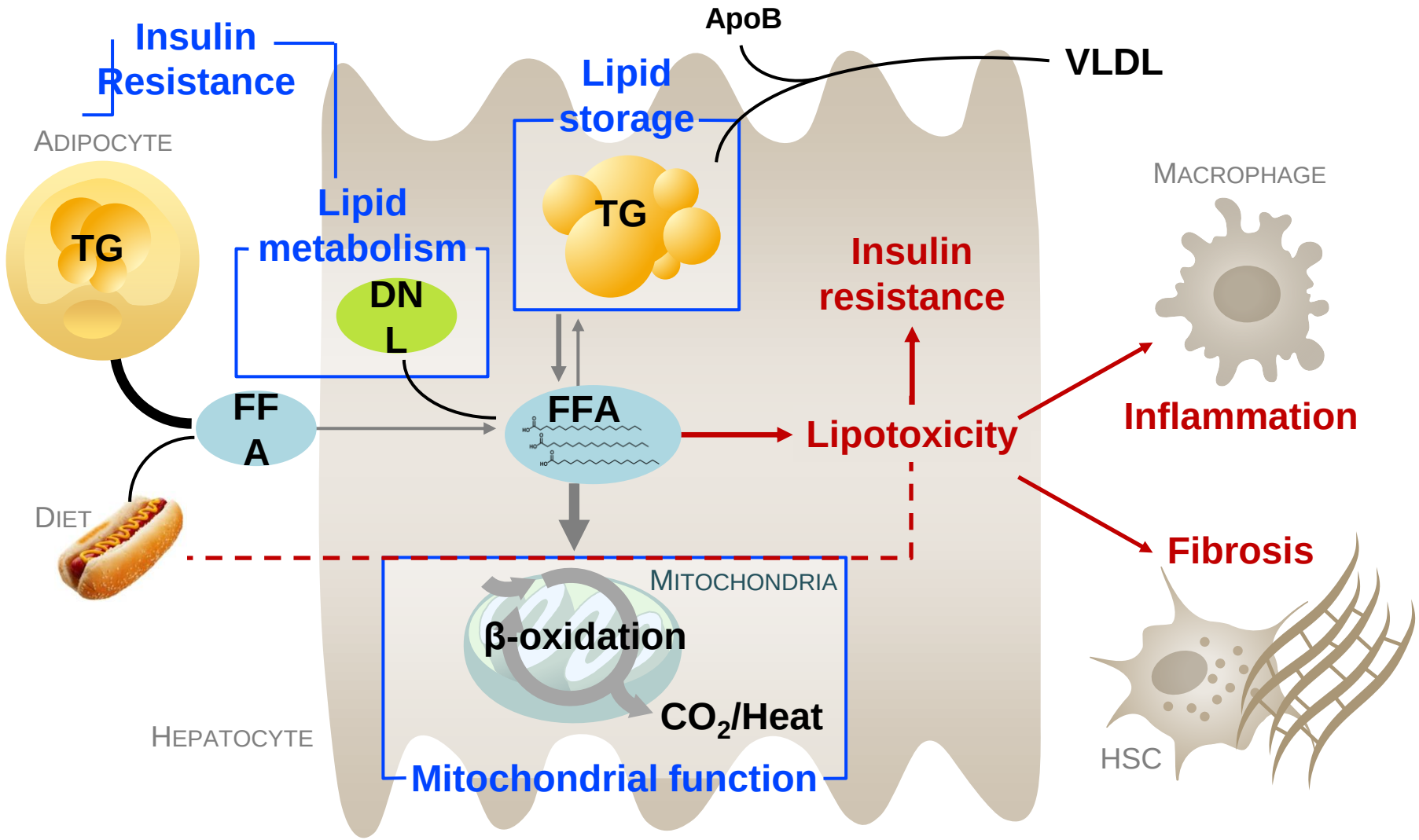
Proposed Pathophysiologic Mechanisms for NAFLD/NASH

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.*

Activins/Follistatins in Response to Exercise and their Associations with Metabolic and Anthropometric Variables

Exercise

1. Intervention study in healthy normal weight population (n=80)

- n=20/group [young or old], [fit vs sedentary]
- Aerobic exercise up to exhaustion

2. Intervention study in patients with metabolic syndrome (n=9) vs. without (n=13)

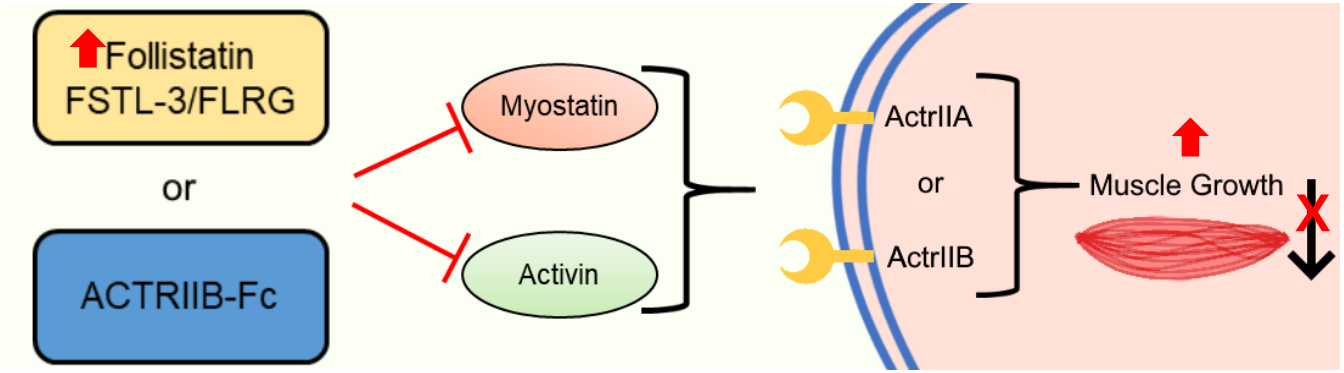
- High-intensity, Moderate-Intensity, Resistance Exercise
- Metabolic parameters in both studies

Conclusions

A. ↑ 10-21% during exercise independent of type of exercise and MetS

B. Follistatin and FSTL3 correlate with BMI, %body fat and lipid profile

Obesity



Perakakis et al. J Clin Endocrinol Metab, 2018.

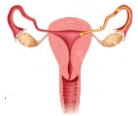
ARTICLES
https://doi.org/10.1038/s41591-018-0048-0
nature medicine
Corrected: Publisher Correction

Inactivating hepatic follistatin alleviates hyperglycemia

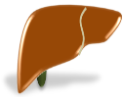
KO of Follistatin
- ↑ WAT insulin sensitivity
- ↑ Suppression of HGP by insulin

Tao et al. Nat Med, 2018.

Activins / Follistatins



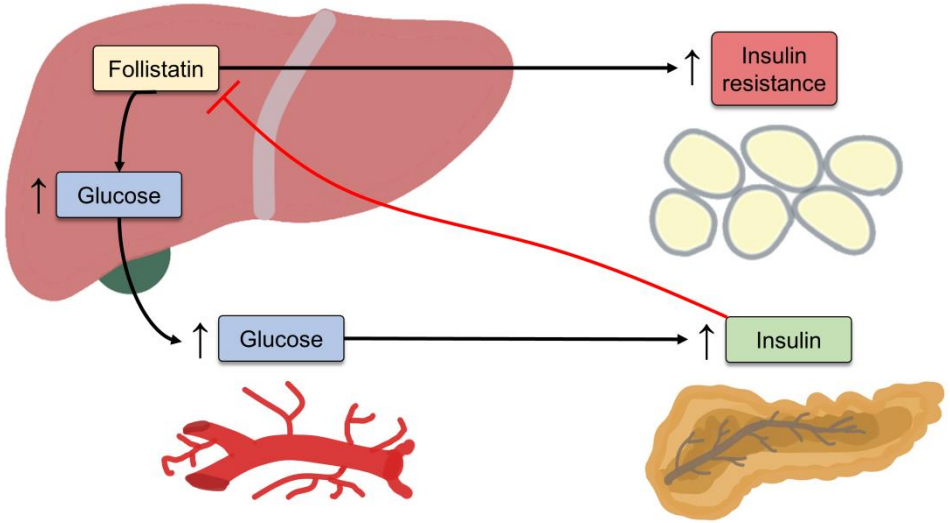

Activin A, Activin B, Activin AB → **A. Activate Reproductive function growth** **B. Inhibit muscle**




Follistatin & Follistatin-like 3 → **Antagonize Activins**

Clinical studies with activins/follistatins

1. Glucose or lipid intake p.o. or i.v. for 6 hours (Cross-sectional intervention) — Perakakis…Mantzoros Diabetes Obes Metab. 03/2019; Perakakis…Mantzoros Diabetes Res Rev 02/2020
2. Before vs after bariatric surgery in morbid obesity
3. Exercise in patients with Metabolic Syndrome (Cross-sectional intervention) — Perakakis…Mantzoros J Clin Endocrinol Metab 08/2018
4. Exercise in healthy population (Intervention)
5. Complete fasting for three days vs isocaloric state (Cross-sectional) — Perakakis…Mantzoros Metabolism 05/2018
6. Athletes with hypothalamic amenorrhea vs eumenorrheic women (Case-Control)



Early reduction in follistatin predicts long-term impovement in insulin sensitivity after bariatric surgery

Management of Patients with NAFLD and NASH

Variable	Lifestyle intervention[a]	Liver-directed pharmacotherapy	Diabetes care (in individuals with diabetes)	Cardiovascular risk reduction
NAFL	Yes	No	Standard of care	Yes
NASH with fibrosis stage 0 or 1 (F0, F1)	Yes	No	Standard of care	Yes
NASH with fibrosis stage 2 or 3 (F2, F3)	Yes	Yes	Pioglitazone, GLP-1 receptor agonists[b]	Yes
NASH cirrhosis (F4)	Yes	Yes	Individualize[c]	Yes

[a] All patients require regular physical activity and healthy diet and to avoid excess alcohol intake; weight loss recommended.
[b] Among GLP-1 receptor agonists, semaglutide has the best evidence of benefit in patients with NASH and fibrosis.
[c] Evidence for efficacy of pharmacotherapy in patients with NASH cirrhosis is very limited and should be individualized and used with caution.

aga American Gastroenterological Association

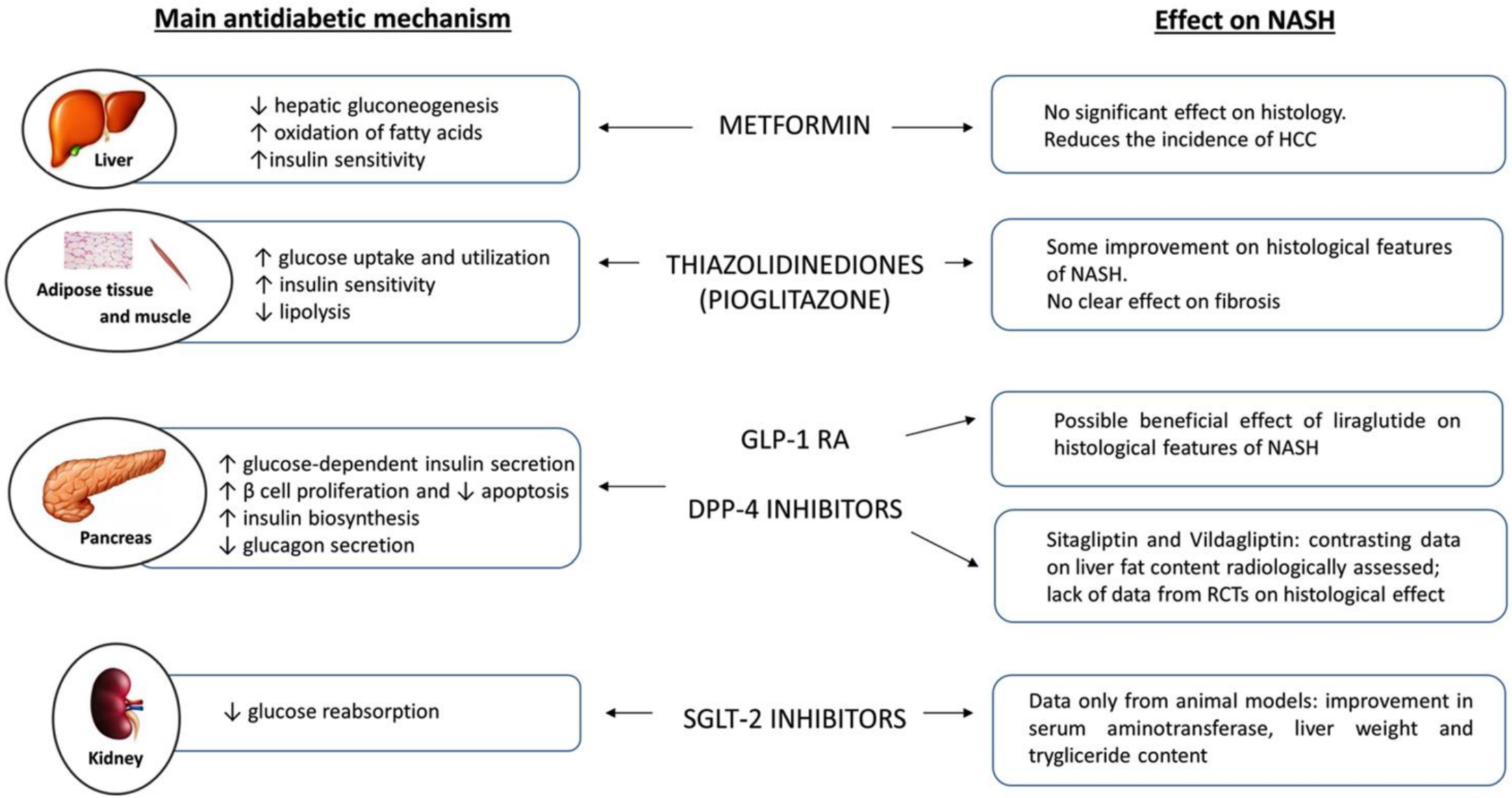
Metabolism Clinical and Experimental

Obesity A Research Journal

Fasiha K et al. Metabolism, 2021.

Management of NASH

Currently Available Pharmacotherapies – mechanisms of action



Iogna Prat L et al. Hormones (Athens), 2018.

Clinical Care Pathway for the Risk Stratification and Management Of Patients with NAFLD

Kanwal F…Mantzoros C…Cusi K, Gastroenterology 2021 in press.

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

- The AGA assembled a congress of experts to develop a white paper providing guidance on the screening, diagnosis, and treatment of NAFLD.
- A second goal was to develop a clinical care pathway to be widely implemented in primary care practices.
- This paper introduces the latter care pathway and provides a rationale supporting proposed steps to assist clinicians in diagnosing and managing clinically significant fibrosis based on the best available evidence.

There are Currently No Approved Therapies Specifically for NASH

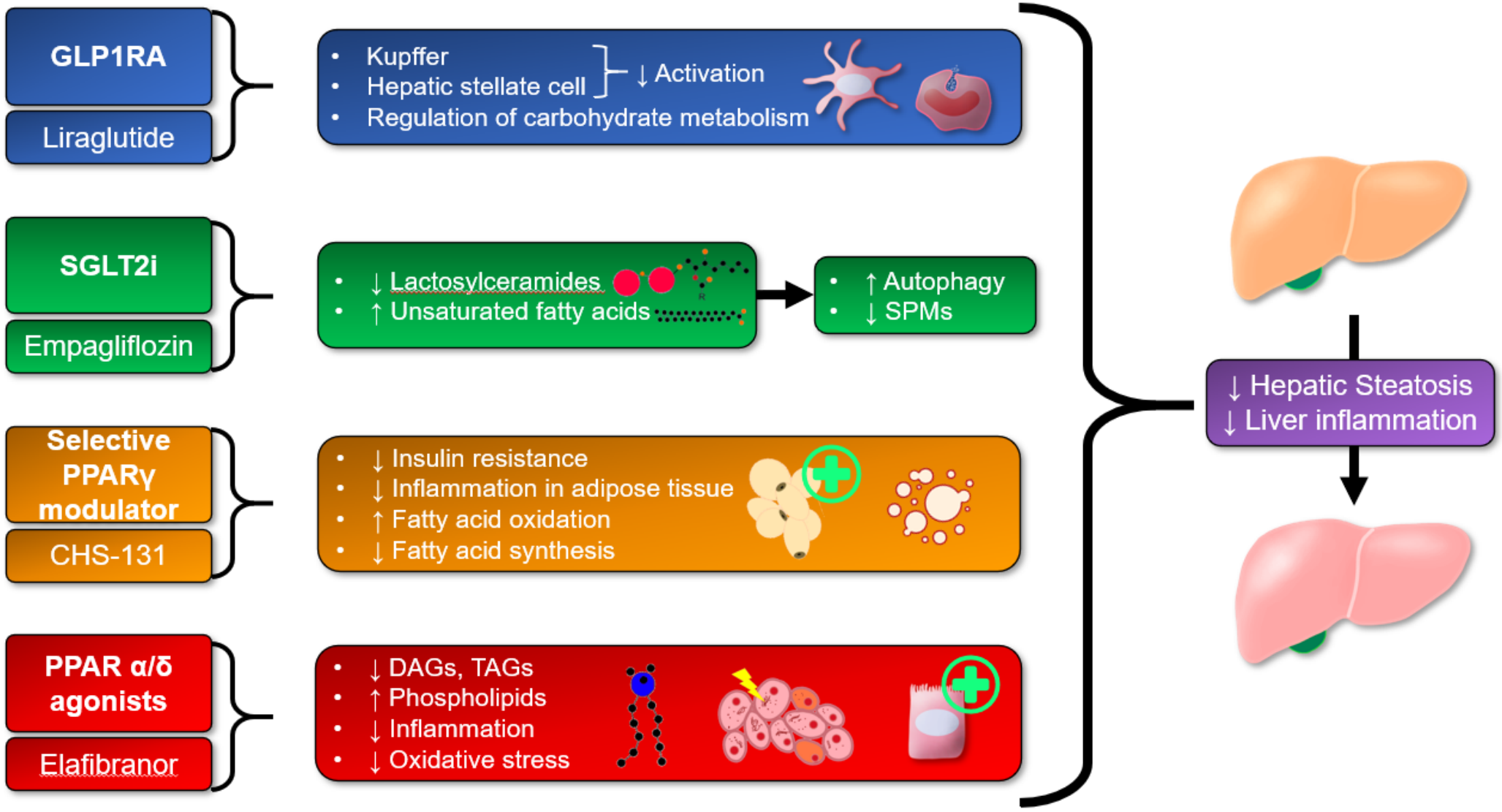
Lifestyle Modification	While weight loss is associated with mild/moderate improvement in NASH, maintaining the weight loss is very challenging.
Weight Loss Surgery	Bariatric surgery and endoscopic devices have demonstrated improvement in NASH and metabolic syndrome, but evidence for fibrosis improvement is limited.
Commonly Prescribed Medications	▪ Vitamin E (RRR-a-tocopherol) administered at a daily dose of 800 IU/day improves liver histology in nondiabetic adults with biopsy-proven NASH and therefore may be considered for this patient population. Risks and benefits should be discussed with each patient before starting therapy ▪ Until further data supporting its effectiveness become available, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis

Chalasani N et al. Hepatol, 2018.

Klein EA et al. JAMA, 2011.

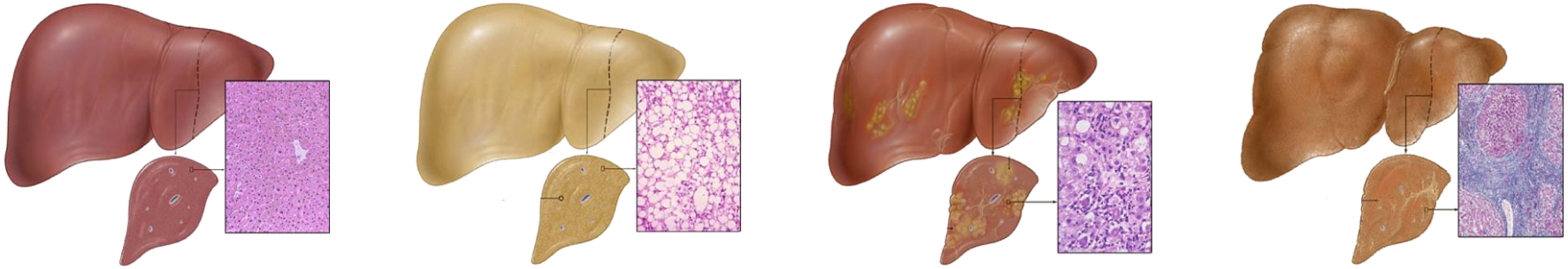
Miller ER 3rd et al. Ann Intern Med, 2005.

NAFLD– Evaluation of Treatments in Preclinical Models



1) Perakakis N ... Mantzoros C. Hepatol Commun, 2020 2) Perakakis N ... Mantzoros C. Liver Int, 2021 3) Nasiri-Ansari et al. Int J Mol Sci, 2021 4) Perakakis N ... Mantzoros C. Int J Mol Sci, 2021.

Medications Targeting Pathophysiological Processes



Targets related to insulin resistance and/or lipid metabolism

PPARγ:	Pioglitazone
GLP-1:	Liraglutide, Semaglutide
GLP-1/GR:	MEDI0382
ACC:	GS-0976, PF-05221304
SCD1:	Aramchol
SGLT1/2:	LIK066
FGF21:	BMS-986036, AKR-001,BIO89-100
THR-β:	MGL-3196, VK2809
FGFR1/KLB	BFKB8488A
MPC	MSDC-0602K, PXL065
Mixed ag-antagonist GR and antagonist MR	miricorilant
GLP-1/GIP	Tirzepatide

Targets related to lipotoxicity & oxidative stress

PPARα/∂:	Elafibranor
PPARα/∂/γ:	Lanifibranor
PPARα/γ:	Saroglitazar
MPC	MSDC-0602K, PXL065
FXR:	OCA, GS-9674, tropifexor, LMB-763, EYP001, MET409
TGR5:	INT-767, INT-777
ASBT:	Volixibat
FGF19:	NGM282
Vitamin E	

Targets related to inflammation and immune activation

CCR2/5:	Cenicriviroc
AOC3:	BI 1467335
TLR4:	JKB-121
Anti-LPS:	IMM-124E
CRV431	

Targets related to cell death (apoptosis and necrosis)

ASK1	Selonsertib
Caspase	Emricasan
CRV431	

Targets related to fibrogenesis & collagen turnover

LOXL2:	Simtuzumab
Galectin	GR-MD-02
CRV431	

Phase II trials (planned or ongoing): more than 60

Phase III trials: Cenicrivoric, elafibranor, obeticholic acid, and selonsertib

AASLD Guidelines Recommendations on Use of Diabetes Treatments

Metformin	▪ Metformin is not recommended for treating NASH in adult patients.
Statins & Thiazolidinediones	▪ Pioglitazone improves liver histology in patients with and without T2DM with biopsy-proven NASH. Therefore, it may be used to treat these patients. Risks and benefits should be discussed with each patient before starting therapy. ▪ Until further data support its safety and efficacy, pioglitazone should not be used to treat NAFLD in patients without T2DM and biopsy-proven NASH.
GLP-1 analogues & SGLT-2s	▪ It is premature to consider GLP-1 agonists or SGLT-2is to specifically treat liver disease in patients with NAFLD or NASH
Vitamin E	▪ Vitamin E (RRR-a-tocopherol) administered at a daily dose of 800 IU/day improves liver histology in nondiabetic adults with biopsy-proven NASH and therefore may be considered for this patient population. Risks and benefits should be discussed with each patient before starting therapy ▪ Until further data supporting its effectiveness become available, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis

Chalasani N et al. Hepatology, 2018.

The Effect of Metformin on Weight and other Metabolic Parameters in Obese Non-Diabetic Patients: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

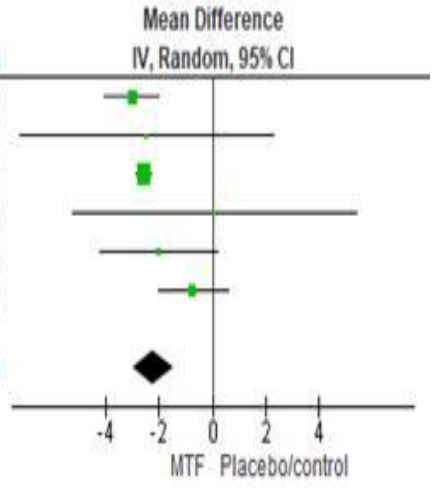
Zarzour F, Saadeh N, Haber R, Basha D, Jebali L, Ghezzawi M, Chakhtoura M, Mantzoros CS

The effect of metformin compared to placebo/control on % weight loss

Study or Subgroup	MTF Mean	MTF SD	MTF Total	Placebo/control Mean	Placebo/control SD	Placebo/control Total	Weight	Mean Difference IV, Random, 95% CI
Goodwin 2015	-2.3	4.64	237	0.7	6.76	255	24.7%	-3.00 [-4.02, -1.98]
Guimaraes 2007	-3.77	4.17	8	-1.3	6.08	10	2.4%	-2.47 [-7.22, 2.28]
Meyerhardt 2019	-1	0.59	35	1.55	0.58	34	42.4%	-2.55 [-2.83, -2.27]
Rodriguez 2004	-5.97	4.23	10	-6.06	7.81	11	1.9%	0.09 [-5.22, 5.40]
Torres 2009	-3.3	4.17	49	-1.3	6.08	41	9.3%	-2.00 [-4.20, 0.20]
Walton 2019	-2.36	3.17	54	-1.63	3.75	55	19.2%	-0.73 [-2.03, 0.57]
Total (95% CI)			393			406	100.0%	-2.21 [-2.96, -1.45]

Heterogeneity: $Tau^2 = 0.33$; $Chi^2 = 9.25$, df = 5 (P = 0.10); $I^2 = 46\%$

Test for overall effect: Z = 5.74 (P < 0.00001)

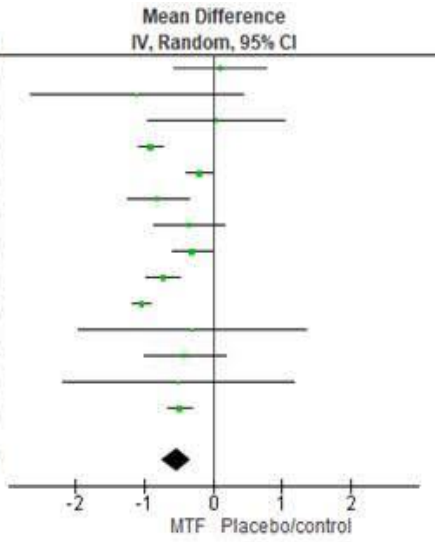


The effect of metformin compared to placebo/control on change in BMI (kg/m2)

Study or Subgroup	MTF Mean	MTF SD	MTF Total	Placebo/control Mean	Placebo/control SD	Placebo/control Total	Weight	Mean Difference IV, Random, 95% CI
Anushiravani 2019	-0.6	1.35	30	-0.7	1.29	30	5.6%	0.10 [-0.57, 0.77]
Davis 2018	-0.58	3.21	36	0.52	3.84	45	1.7%	-1.10 [-2.64, 0.44]
Dos santos 2019	0.05	1.4	10	0	0.86	13	3.5%	0.05 [-0.94, 1.04]
Goodwin 2015	-0.7	1.04	237	0.2	0.96	255	11.0%	-0.90 [-1.08, -0.72]
He 2012	-0.3	1	180	-0.1	0.9	180	10.9%	-0.20 [-0.40, -0.00]
Kim 2015	-1.2	2.1	122	-0.4	1.4	127	8.0%	-0.80 [-1.25, -0.35]
Kulkarni 2018	-0.76	1.07	30	-0.42	0.91	28	7.2%	-0.34 [-0.85, 0.17]
Lim 2009	-0.4	0.81	65	-0.1	0.98	79	9.8%	-0.30 [-0.59, -0.01]
Meyerhardt 2019	-0.29	0.71	35	0.43	0.12	34	10.4%	-0.72 [-0.96, -0.48]
Schuster 2004	-0.53	0.4	45	0.5	0.34	81	11.4%	-1.03 [-1.17, -0.89]
Snogaard 1997	-0.4	0.81	10	-0.1	1.78	5	1.5%	-0.30 [-1.94, 1.34]
Torres 2009	-0.1	1.22	49	0.3	1.6	41	6.3%	-0.40 [-1.00, 0.20]
Uygun 2004	-2.4	1.9	13	-1.9	2.1	10	1.5%	-0.50 [-2.16, 1.16]
Zhang 2009	-0.18	0.46	49	0.3	0.41	45	11.1%	-0.48 [-0.66, -0.30]
Total (95% CI)			911			973	100.0%	-0.53 [-0.75, -0.31]

Heterogeneity: $Tau^2 = 0.10$; $Chi^2 = 75.65$, df = 13 (P < 0.00001); $I^2 = 83\%$

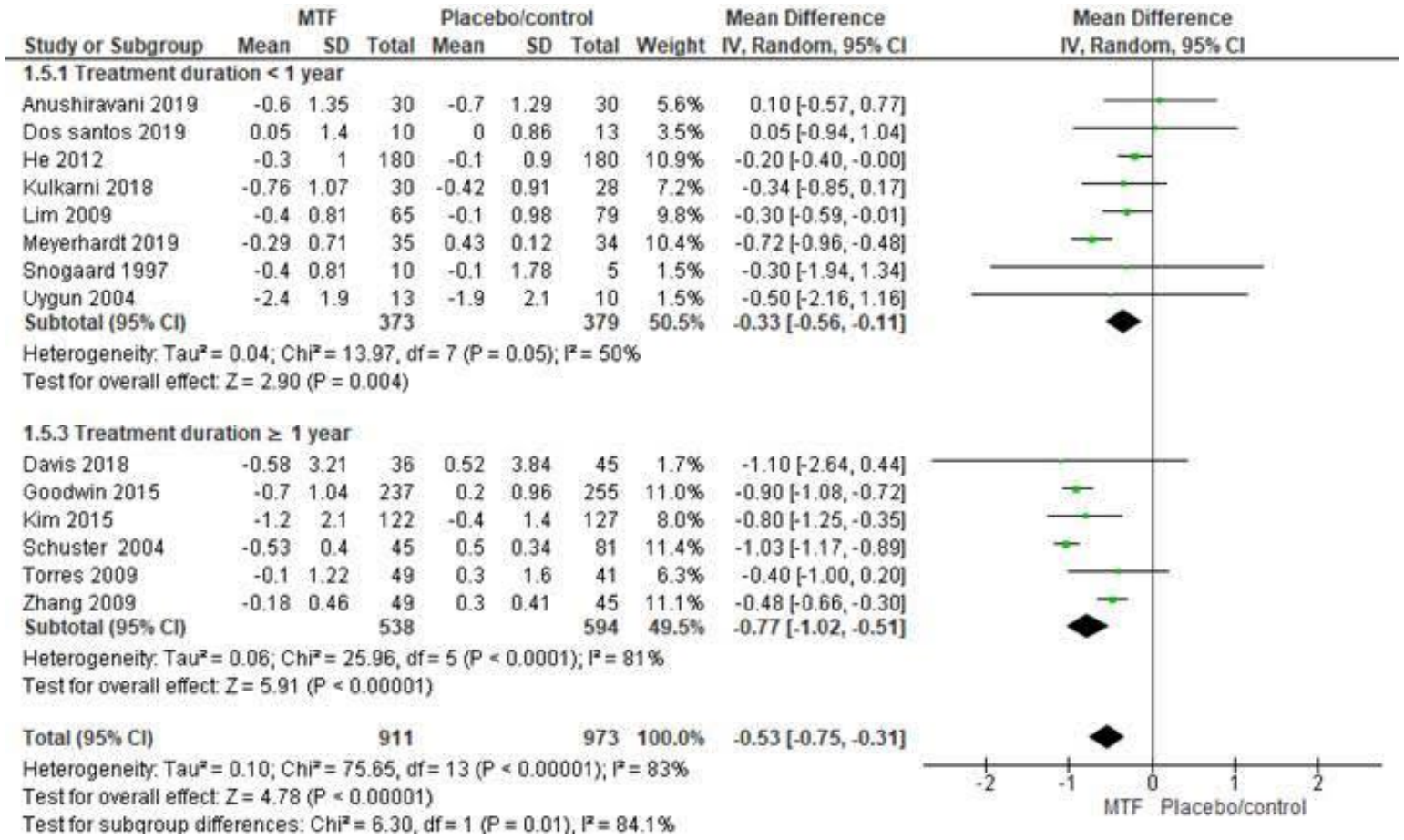
Test for overall effect: Z = 4.78 (P < 0.00001)



The Effect of Metformin on Weight and other Metabolic Parameters in Obese Non-Diabetic Patients: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

Zarzour F, Saadeh N, Haber R, Basha D, Jebali L, Ghezzawi M, Chakhtoura M, Mantzoros CS

The effect of metformin compared to placebo/control on change in BMI (kg/m2) according to treatment duration



Study or Subgroup	MTF Mean	MTF SD	MTF Total	Placebo/control Mean	Placebo/control SD	Placebo/control Total	Weight	Mean Difference IV, Random, 95% CI
1.5.1 Treatment duration < 1 year								
Anushiravani 2019	-0.6	1.35	30	-0.7	1.29	30	5.6%	0.10 [-0.57, 0.77]
Dos santos 2019	0.05	1.4	10	0	0.86	13	3.5%	0.05 [-0.94, 1.04]
He 2012	-0.3	1	180	-0.1	0.9	180	10.9%	-0.20 [-0.40, -0.00]
Kulkarni 2018	-0.76	1.07	30	-0.42	0.91	28	7.2%	-0.34 [-0.85, 0.17]
Lim 2009	-0.4	0.81	65	-0.1	0.98	79	9.8%	-0.30 [-0.59, -0.01]
Meyerhardt 2019	-0.29	0.71	35	0.43	0.12	34	10.4%	-0.72 [-0.96, -0.48]
Snogaard 1997	-0.4	0.81	10	-0.1	1.78	5	1.5%	-0.30 [-1.94, 1.34]
Uygun 2004	-2.4	1.9	13	-1.9	2.1	10	1.5%	-0.50 [-2.16, 1.16]
Subtotal (95% CI)			373			379	50.5%	**-0.33 [-0.56, -0.11]**
Heterogeneity: Tau² = 0.04; Chi² = 13.97, df = 7 (P = 0.05); I² = 50%								
Test for overall effect: Z = 2.90 (P = 0.004)								
1.5.3 Treatment duration ≥ 1 year								
Davis 2018	-0.58	3.21	36	0.52	3.84	45	1.7%	-1.10 [-2.64, 0.44]
Goodwin 2015	-0.7	1.04	237	0.2	0.96	255	11.0%	-0.90 [-1.08, -0.72]
Kim 2015	-1.2	2.1	122	-0.4	1.4	127	8.0%	-0.80 [-1.25, -0.35]
Schuster 2004	-0.53	0.4	45	0.5	0.34	81	11.4%	-1.03 [-1.17, -0.89]
Torres 2009	-0.1	1.22	49	0.3	1.6	41	6.3%	-0.40 [-1.00, 0.20]
Zhang 2009	-0.18	0.46	49	0.3	0.41	45	11.1%	-0.48 [-0.66, -0.30]
Subtotal (95% CI)			538			594	49.5%	**-0.77 [-1.02, -0.51]**
Heterogeneity: Tau² = 0.06; Chi² = 25.96, df = 5 (P < 0.0001); I² = 81%								
Test for overall effect: Z = 5.91 (P < 0.00001)								
Total (95% CI)			911			973	100.0%	**-0.53 [-0.75, -0.31]**
Heterogeneity: Tau² = 0.10; Chi² = 75.65, df = 13 (P < 0.00001); I² = 83%								
Test for overall effect: Z = 4.78 (P < 0.00001)								
Test for subgroup differences: Chi² = 6.30, df = 1 (P = 0.01), I² = 84.1%								

AASLD Guidelines Recommendations on Use of Diabetes Treatments

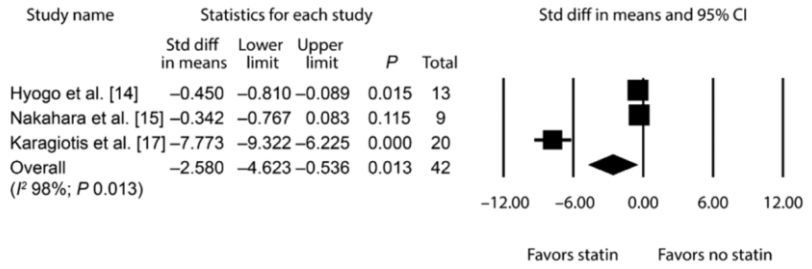
Metformin	▪ Metformin is not recommended for treating NASH in adult patients.
Statins & Thiazolidinediones	▪ Pioglitazone improves liver histology in patients with and without T2DM with biopsy-proven NASH. Therefore, it may be used to treat these patients. Risks and benefits should be discussed with each patient before starting therapy. ▪ Until further data support its safety and efficacy, pioglitazone should not be used to treat NAFLD in patients without T2DM and biopsy-proven NASH.
GLP-1 analogues & SGLT-2s	▪ It is premature to consider GLP-1 agonists or SGLT-2is to specifically treat liver disease in patients with NAFLD or NASH
Vitamin E	▪ Vitamin E (RRR-a-tocopherol) administered at a daily dose of 800 IU/day improves liver histology in nondiabetic adults with biopsy-proven NASH and therefore may be considered for this patient population. Risks and benefits should be discussed with each patient before starting therapy ▪ Until further data supporting its effectiveness become available, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis

Chalasani N et al. Hepatology, 2018.

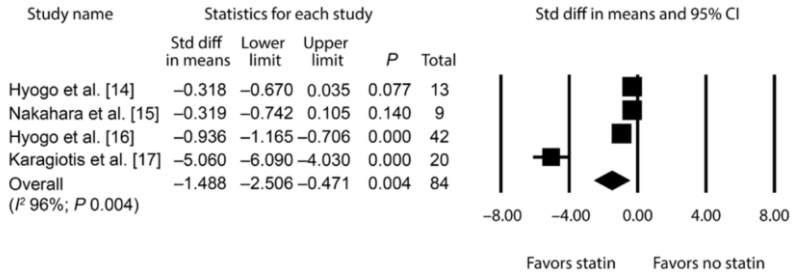
Statin Use in Patients with Non-Alcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis

Boutari C, Anastasilakis D, Pappas P, Mantzoros C

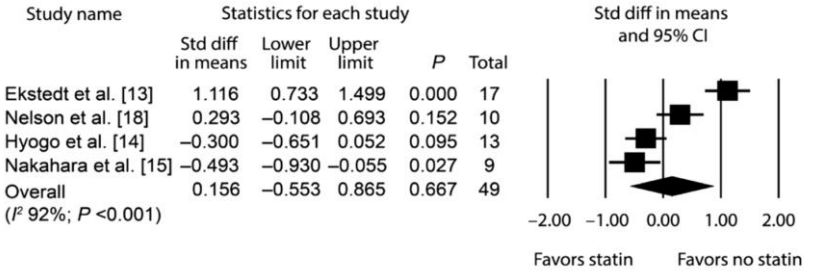
The effect of statin on Std diff of steatosis grading



The effect of statin on Std diff on NAFLD activity score



The effect of statin on Std diff on fibrosis stage



Other studies with statins on NAFLD

Author	Year	Country	Study design	N	Type of statin (mg)	Follow-up time (months)	Change from baseline
Maroni et al.	2011	Italy	Retrospective trial	43	Atorvastatin (n=19), simvastatin (n=11), rosuvastatin (n=10), fluvastatin (n=2) lovastatin (n=1)	13	AST (IU/L) 26.33 ± 8.15-->34.28 ± 31.58 (p=0.06) ALT (IU/L) 37.6 ± 14.21-->44.7 ± 30.43 (p=0.1) γ-GT (IU/L) 76.39 ± 59-->86.5 ± 93.33 (p=0.3)
Han et al.	2012	South Korea	RCT	189	Pitavastatin (2-4) vs Atorvastatin (10-20)	3	Changes in liver attenuation index (CTL-S) values PITA: -6.7 ± 12--> -3.4 ± 9.6 (p=0.008) ATOR: -7.1 ± 10.1--> -5.0 ± 9.2 (p=0.158)
Rana et al.	2016	India	RCT	98	Metformin (n=31) Rosuvastatin (n=34) Pioglitazone (n=33)	6	USG score, mean ± SD 2.35 ± 0.49-->2.42 ± 0.81 (p=0.593) 2.59 ± 0.50-->1.32 ± 0.47 (p<0.001) 2.45±0.51--> 1.76 ± 0.71 (p<0.001)
Rinella et al.	2019	USA	RCT	66	Rosuvastatin 40mg	3	MRI-PDFF (relative liver fat content), mean (SD) up to -66.6% (17.1)

Rinella ME et al. J Hepatol, 2019.
Han KH et al. J Clin Lipidol, 2012.
Maroni L et al. Am J Med Sci, 2011.
Rana H et al. J Clin Diagn Res, 2016.
Rattanachaisit P et al. Asian Biomedicine, 2018.

➢ EDITORIAL

Long-term statin treatment for hepatic fibrosis in patients with nonalcoholic fatty liver disease: Is it time to give the emperor a statin robe?

Christopher M. Tessier, Stergios A. Polyzos, Vasilios G. Athyros, Christos S. Mantzoros

Published: May 11, 2021

➢ CROSS-SECTIONAL STUDY

Statin use is associated with lower prevalence of advanced liver fibrosis in patients with type 2 diabetes

Stefano Ciardullo, Gianluca Perseghin

AASLD Guidelines Recommendations on Use of Diabetes Treatments

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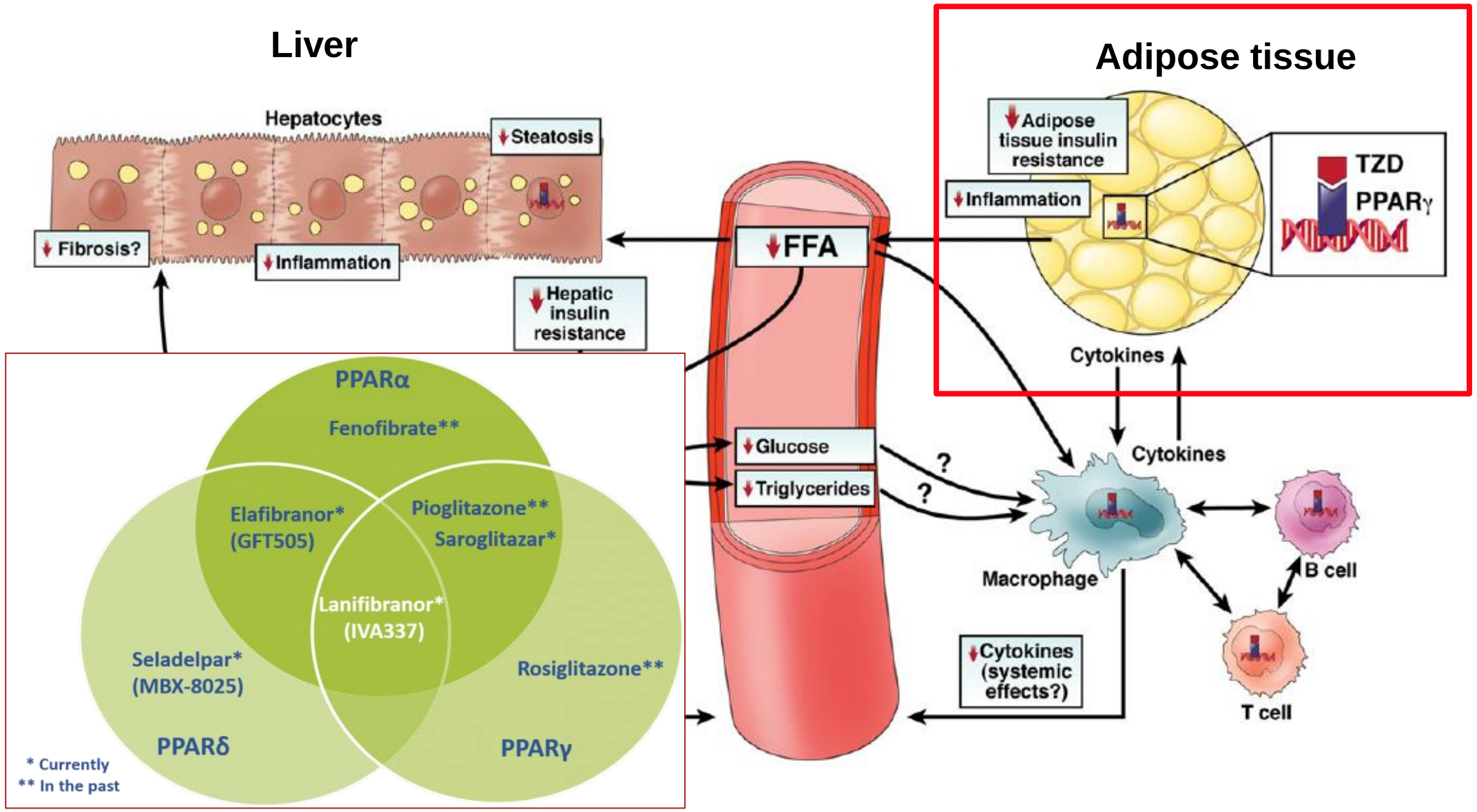
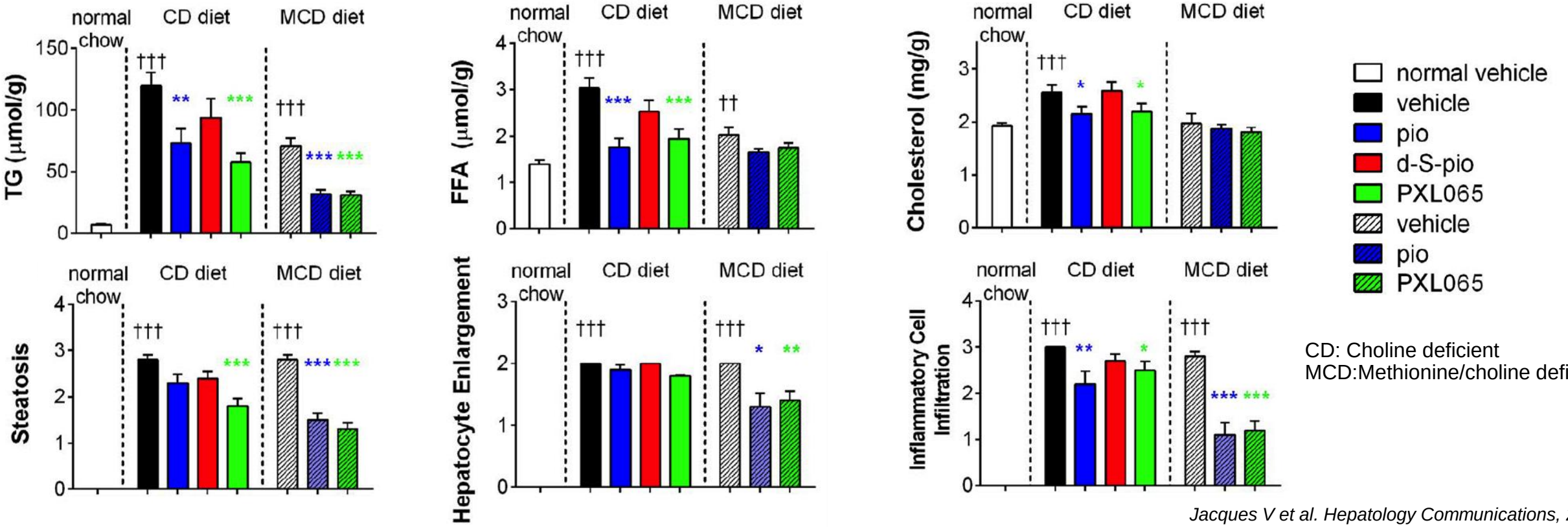
Chalasani N et al. Hepatology, 2018.

Rationale for PPARs in NASH



Deuterium-Stabilized (R)-Pioglitazone (PXL065) Is Responsible for Pioglitazone Efficacy in NASH yet Exhibits Little to No PPARγ Activity

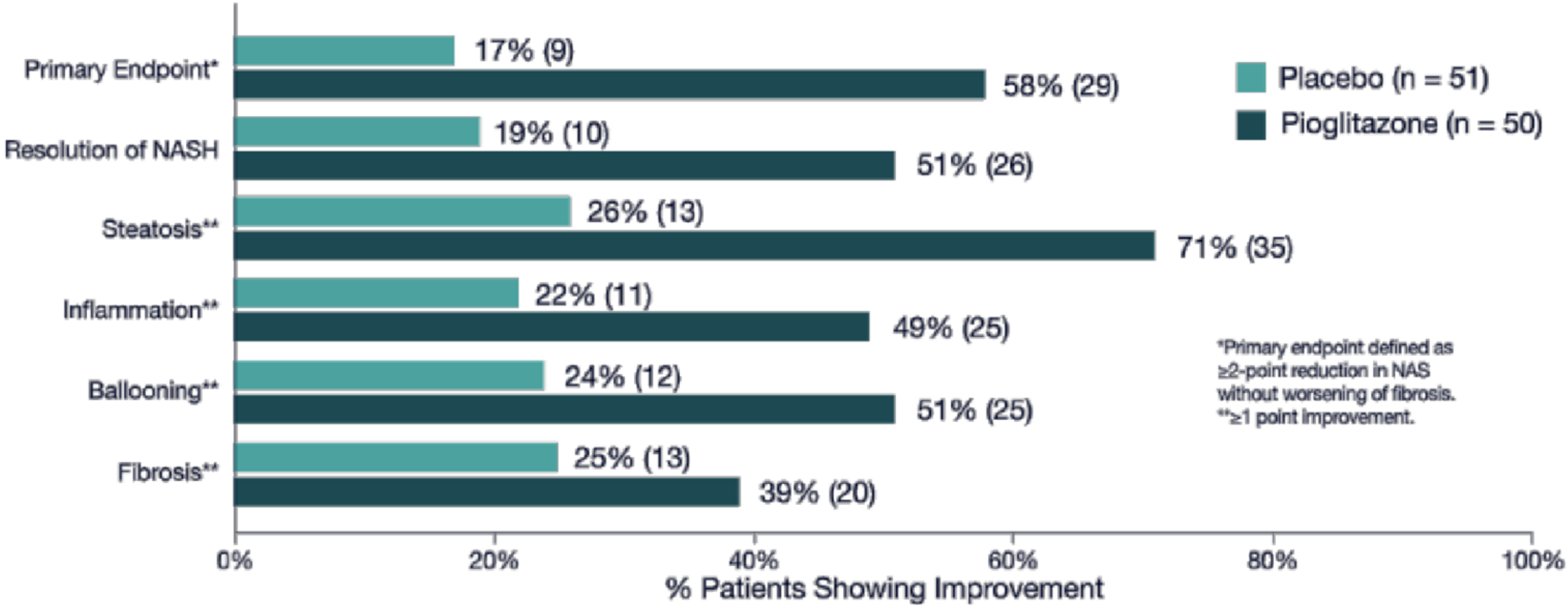


CD: Choline deficient
MCD:Methionine/choline deficient

Jacques V et al. Hepatology Communications, 2021.

Management of NASH

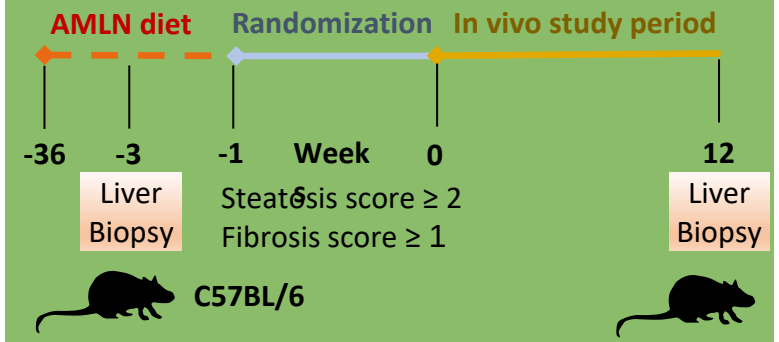
Efficacy of Pioglitazone and Vitamin E in Biopsy-Confirmed NASH



Cusi K et al. Ann Intern Med, 2016.

The Selective PPARγ Modulator CHS-131 Improves Liver Histopathology and Metabolism in a Mouse Model of Obesity and NASH

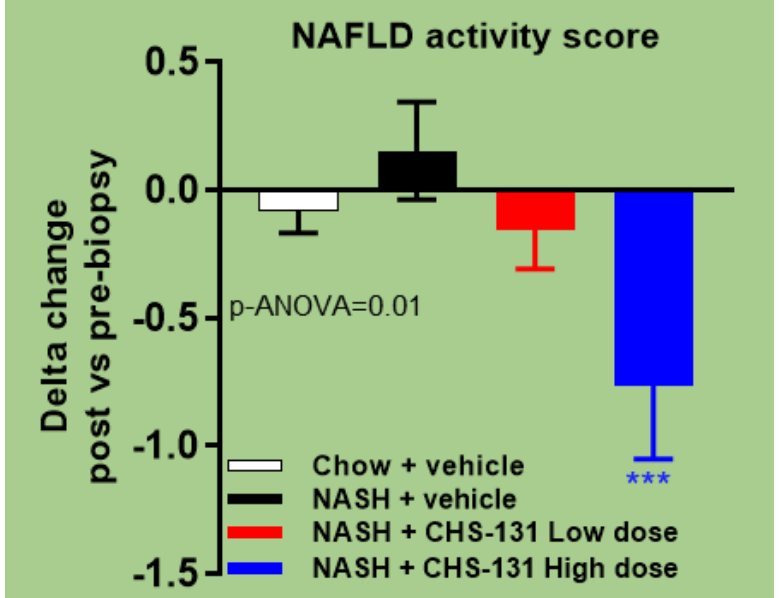
Experimental model



Aim: Effects of CHS-131 (SPPARM) in NAFLD

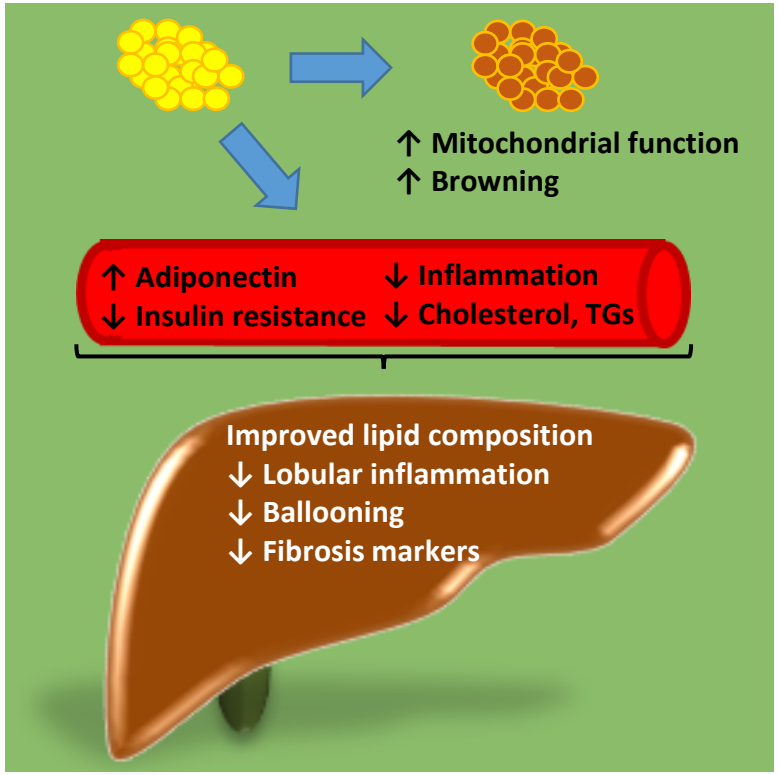
CHS-131 Low dose: 10 mg/kg
CHS-131 High dose: 30 mg/kg
AMLN: 40% fat with trans-fat
20% fructose, 2% cholesterol

CHS-131 improves liver histology



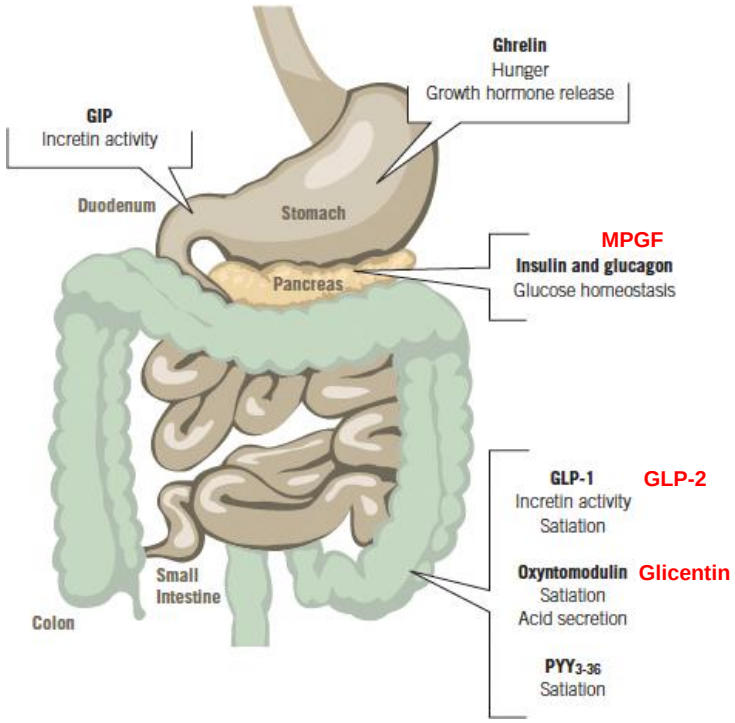
***, p<0.001 NASH+CHS-131 High dose vs NASH + Vehicle

Mechanisms



Perakakis N ... Mantzoros C. Hepatol Commun, 2020.

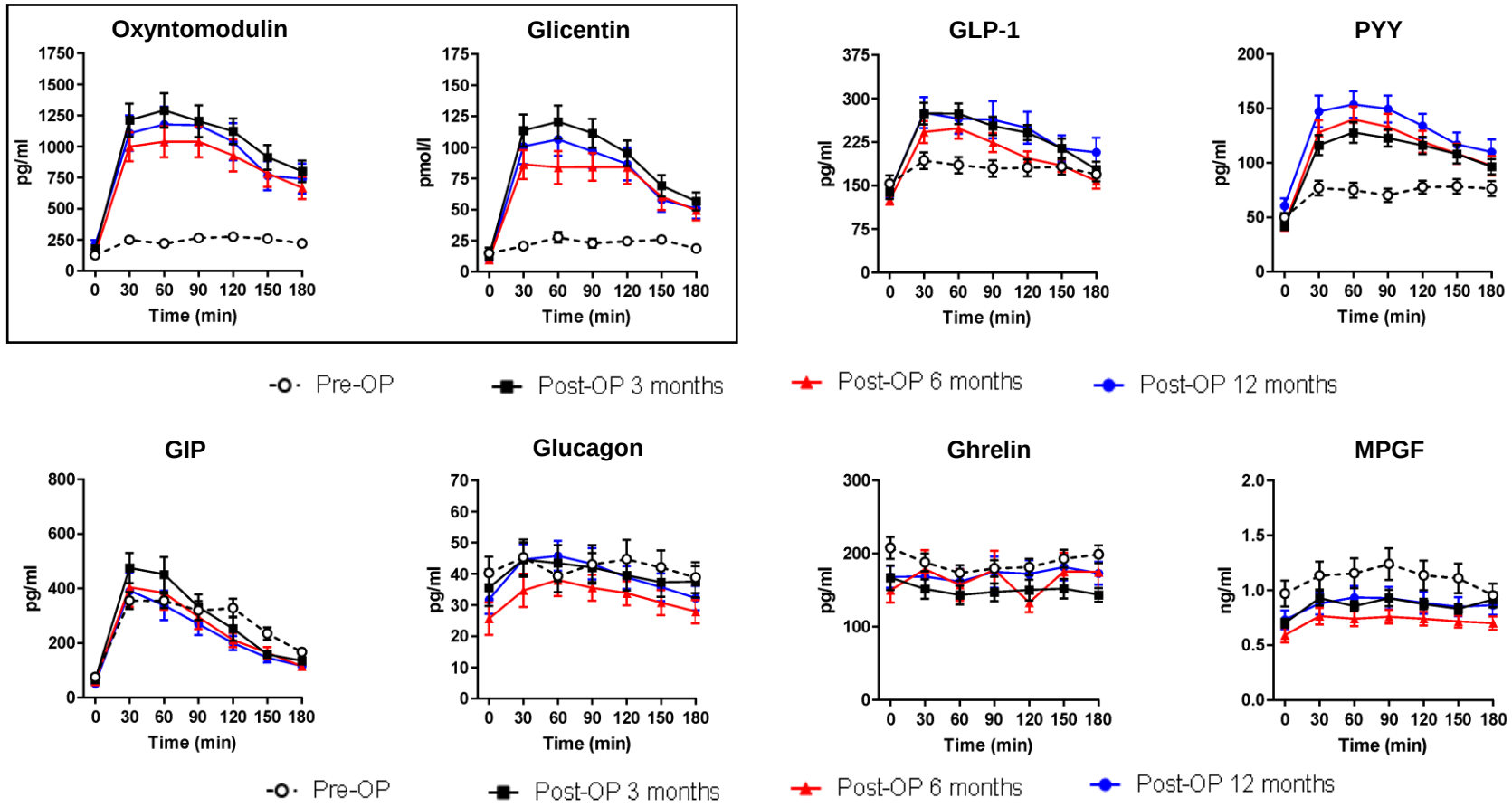
Gastrointestinal Hormones



Treatments for T2DM, Obesity and NAFLD

- GLP-1 receptor agonists (e.g. liraglutide)
- GLP-1/GIP
- GLP-1/Glucagon
- GLP-1/GIP/Glucagon
- Oxyntomodulin
- GLP-1/Oxyntomodulin/PYY

A. Comparison of profiles of 8 Gastrointestinal hormones with mixed meal after Bariatric surgery (n=36)



- Changes in Oxyntomodulin and Glicentin correlate strongly with satiety scores
- Changes in Oxyntomodulin and Glicentin 3 months after Op predict weight loss at 12 months

Perakakis N et al. J Clin Endocrinol Metab, 2020.
Pilitsi E et al. Metabolism, 2019.
Alford S. Obes Ver, 2018.
Upadhyay J, Polyzos S*, Perakakis N* et al. Metabolism, 2018.*equal contribution*

Perakakis N ... Mantzoros C. Metabolism, 2019.
** confirmed by Nielsen et al. J Clin Endocrinol Metab, 2020.*

Gastrointestinal Hormones

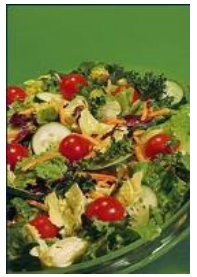
Cross-sectional study – 36 subjects with obesity undergoing fMRI

HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

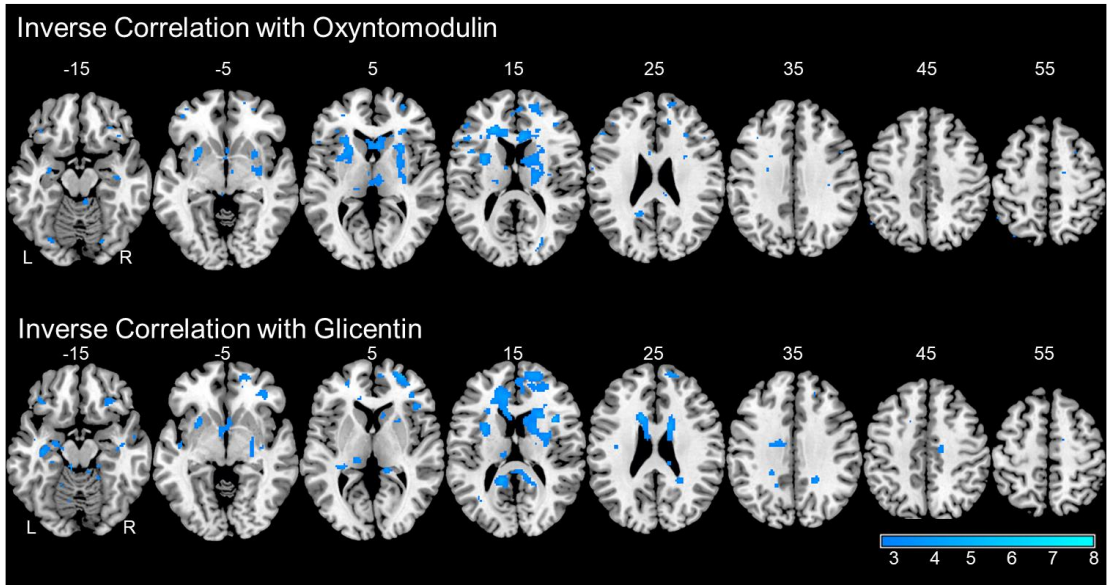
Dr. Olivia Farr

Highly desirable

Less desirable



Non-food



↑Oxm & Glicentin levels □ ↓ Activation of reward centers (Insula, putamen, caudate, OFC)

Farr O ... Mantzoros C. Diabetologia, 2016.
Perakakis N ... Mantzoros C. Diabetes Obes Metab, 2021.

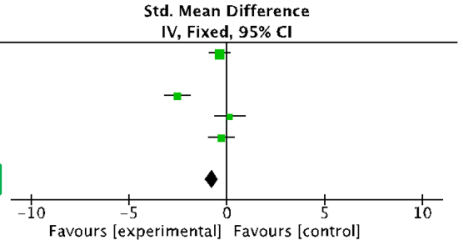
GLP-1 analogues

- Effects of liraglutide on hepatic fat change and AST levels

Hepatic fat change

Study or Subgroup	Liraglutide Mean	SD	Total	Placebo Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
Armstrong MJ 2016	−0.7	0.8	26	−0.4	0.8	26	36.1%	−0.37 [−0.92, 0.18]
Bajaj HS 2018	0	0	0	0	0	0		Not estimable
Frossing S 2018	−1.57	0.41	44	−0.23	0.71	21	23.1%	−2.53 [−3.21, −1.84]
Khoo J 2017	−7.2	7.1	12	−8.9	13.4	12	16.9%	0.15 [−0.65, 0.95]
Smits MM 2016	−2.1	1.2	17	−1.8	0.9	17	23.8%	−0.28 [−0.95, 0.40]
Total (95% CI)			**99**			**76**	**100.0%**	**−0.76 [−1.09, −0.43]**

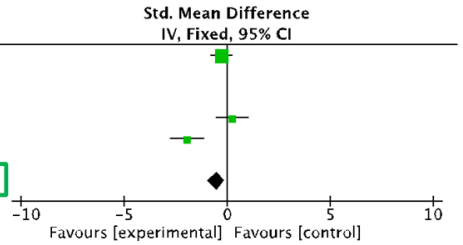
Heterogeneity: Chi² = 34.34, df = 3 (P < 0.00001); I² = 91%
Test for overall effect: Z = 4.50 (P < 0.00001)



AST change

Study or Subgroup	Liraglutide Mean	SD	Total	Placebo Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
Armstrong MJ 2016	−15.8	21.8	26	−8.6	28.3	26	52.9%	−0.28 [−0.83, 0.27]
Bajaj HS 2018	0	0	0	0	0	0		Not estimable
Frossing S 2018	0	0	0	0	0	0		Not estimable
Khoo J 2017	−18	15	12	−23	24	12	24.4%	0.24 [−0.56, 1.04]
Smits MM 2016	−1.8	0.6	17	−0.6	0.6	17	22.7%	−1.95 [−2.79, −1.12]
Total (95% CI)			**55**			**55**	**100.0%**	**−0.53 [−0.93, −0.14]**

Heterogeneity: Chi² = 15.52, df = 2 (P = 0.0004); I² = 87%
Test for overall effect: Z = 2.63 (P = 0.009)



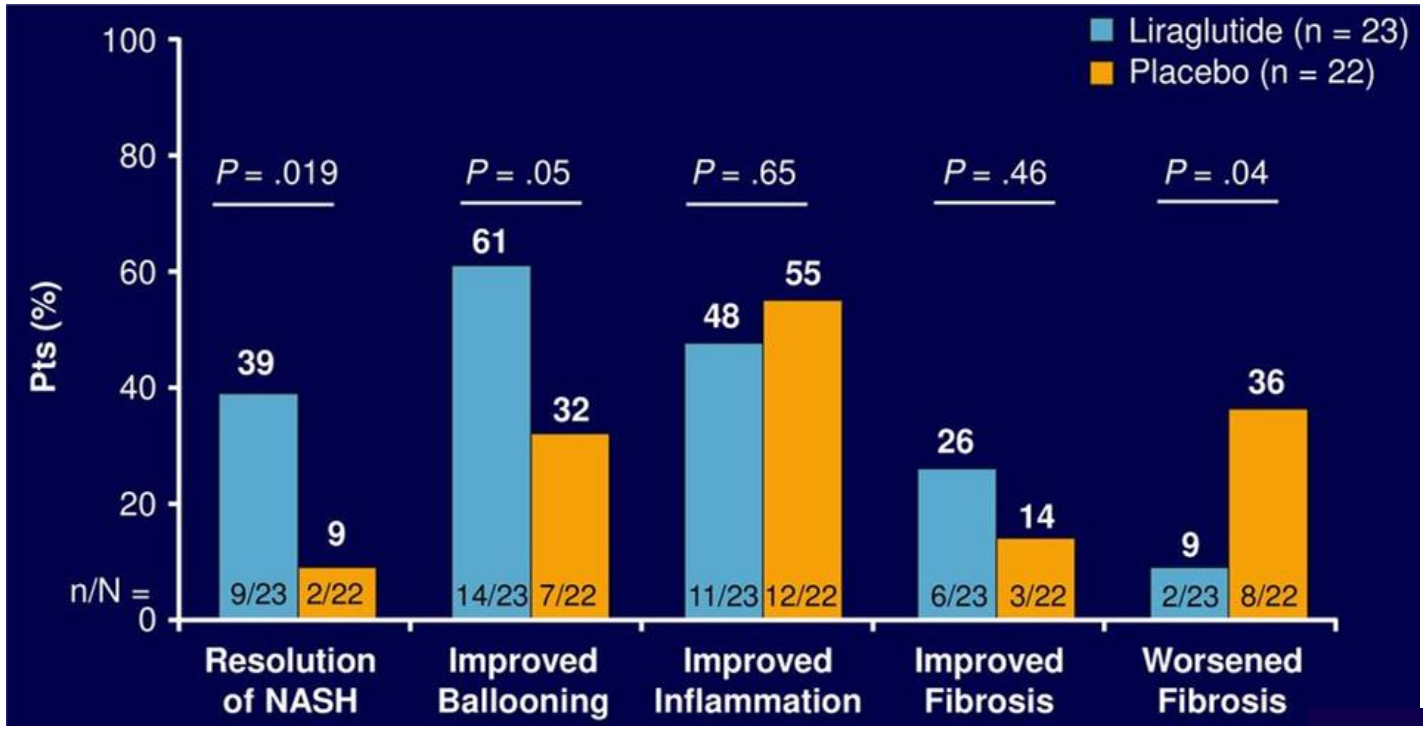
- Changes in hepatocyte ballooning, steatosis, and lobular inflammation with liraglutide vs placebo

	Liraglutide	Placebo	Relative risks or mean changes (95% CI) from baseline to 48 weeks (liraglutide *vs* placebo)	p value*
Primary outcome				
Number of patients with paired liver biopsies	23	22	..	..
Patients with resolution of non-alcoholic steatohepatitis	9 (39%)	2 (9%)	4·3 (1·0 to 17·7)	0·019
Changes from baseline in histopathological parameters				
Total NAFLD activity score				
Change in score	−1·3 (1·6)	−0·8 (1·2)	−0·5 (−1·3 to 0·3)	0·24
Patients with improvement	17 (74%)	14 (64%)	1·2 (0·8 to 1·7)	0·46
Hepatocyte ballooning score				
Mean change	−0·5 (0·7)	−0·2 (0·6)	−0·3 (−0·7 to 0·1)	0·15
Patients with improvement	14 (61%)	7 (32%)	1·9 (1·0 to 3·8)	0·05
Steatosis				
Change in score	−0·7 (0·8)	−0·4 (0·8)	−0·2 (−0·6 to 0·2)	0·32
Patients with improvement	19 (83%)	10 (45%)	1·8 (1·1 to 3·0)	0·009
Lobular inflammation				
Change in score	−0·2 (0·6)	−0·2 (0·5)	−0·01 (−0·3 to 0·3)	0·97
Patients with improvement	11 (48%)	12 (55%)	0·9 (0·5 to 1·6)	0·65
Kleiner fibrosis stage				
Change in score	−0·2 (0·8)	0·2 (1·0)	−0·4 (−0·8 to 0·1)	0·11
Patients with improvement	6 (26%)	3 (14%)	1·9 (0·5 to 6·7)	0·46†
Patients with worsening	2 (9%)	8 (36%)	0·2 (0·1 to 1·0)	0·04†

Armstrong MJ et al. Lancet, 2016.

GLP-1 analogues

- The liraglutide efficacy and action in non-alcoholic steatohepatitis (LEAN) study showed that liraglutide contributed to liver biopsy resolution of definite NASH, which occurred in 9/23 patients compared with 2/22 such patients on placebo.



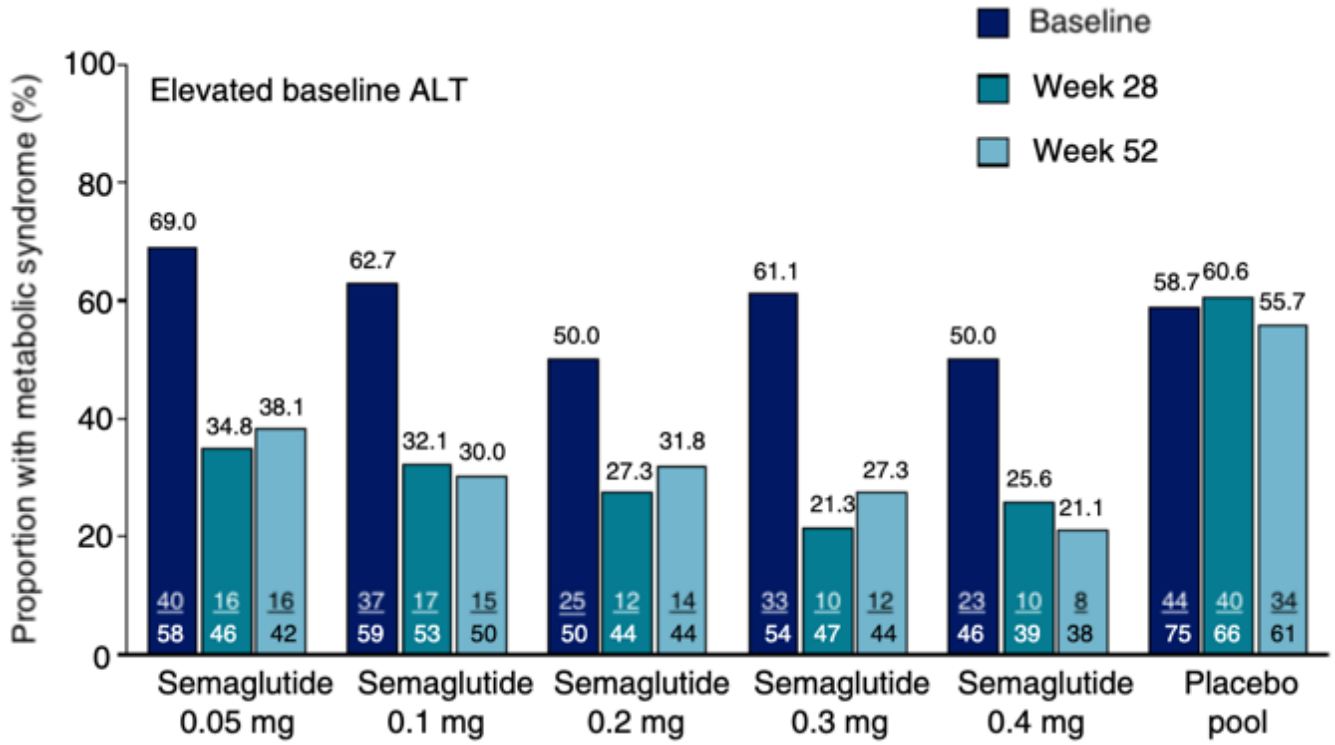
Armstrong MJ et al. Lancet, 2016.

- Dulaglutide, another GLP-1 RA, seems to be able to improve NAFLD in patients with T2DM, due to its potential to reduce body weight with a weekly injection.

Seko Y et al. Hepatol Res, 2017.

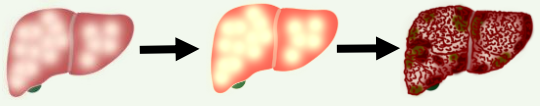
GLP-1 analogues - Semaglutide

- Data from a 104-week cardiovascular outcomes trial in type 2 diabetes (semaglutide 0.5 or 1.0 mg/week) and a 52-week weight management trial (semaglutide 0.05-0.4 mg/day) were analysed.
- Semaglutide significantly reduced ALT and hsCRP in clinical trials in subjects with obesity and/or type 2 diabetes.
- Ongoing phase 2 clinical trial (***NCT03884075***) - Non-Alcoholic Fatty Liver Disease, the Hepatic Response to Oral Glucose, and the Effect of Semaglutide (***NAFLD HEROES)***



Newsome P et al. Aliment Pharmacol Ther, 2019.

Glucagon-Like Peptide-1 Receptor Agonists for Treatment of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis: An Updated Meta-Analysis of Randomized Controlled Trials



11
studies

Patients receiving GLP-1 receptor agonists

Assessment of liver fat:

Biopsy | US | MRI-PDFF MRS

Effects of different GLP-1RAs on liver fat content% as assessed by magnetic resonance-based techniques vs placebo or reference

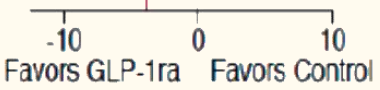
Study	Year	Molecules	Treatment N	Treatment Mean	Treatment SD	Control N	Control Mean	Control SD	Liver fat (%) WMD with 95% CI	Weight (%)
Dutour	2016	Exenatide (10 ug)	22	-8.00	2.00	22	1.00	2.00	-9.00 [-10.18, -7.82]	18.09
Frossing	2017	Liraglutide (1.8 mg)	48	-1.57	0.41	24	-0.23	0.71	-1.34 [-1.65, -1.03]	18.89
Khoo	2018	Liraglutide (1.8 mg)	15	-7.00	7.10	15	-8.10	13.20	1.10 [-6.49, 8.69]	6.39
Yan	2019	Liraglutide (1.8 mg)	24	-4.00	4.50	24	-0.80	5.30	-3.20 [-5.98, -0.42]	14.98
Liu	2020	Exenatide (10 ug)	38	-17.55	12.93	38	-10.49	11.38	-7.06 [-12.54, -1.58]	9.35
Kuchay	2020	Dulaglutide (1.5 mg)	27	-5.80	1.00	25	-2.30	1.20	-3.50 [-4.10, -2.90]	18.72
Bizino	2020	Liraglutide (1.8 mg)	23	-6.30	7.10	26	-4.00	4.60	-2.30 [-5.70, 1.10]	13.58
Overall									-3.92 [-6.27, -1.56]	

Heterogeneity: $\tau^2 = 7.61$, $I^2 = 96.66\%$, $H^2 = 29.90$

Test of $\theta_i = \theta_j$: $Q(6) = 179.40$, $p = 0.00$

Test of $\theta = 0$: $z = -3.26$, $p = 0.00$

Random-effects DerSimonian-Laird model

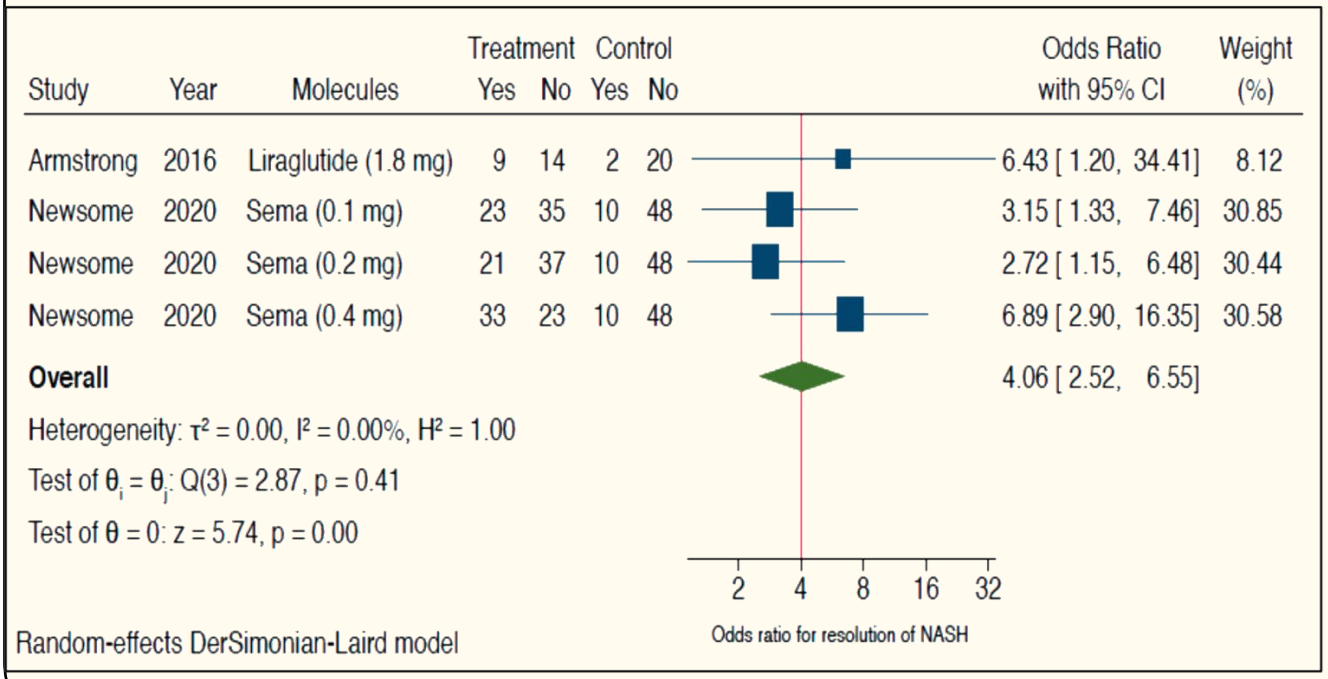


Mantovani A et al. Metabolites, 2021.

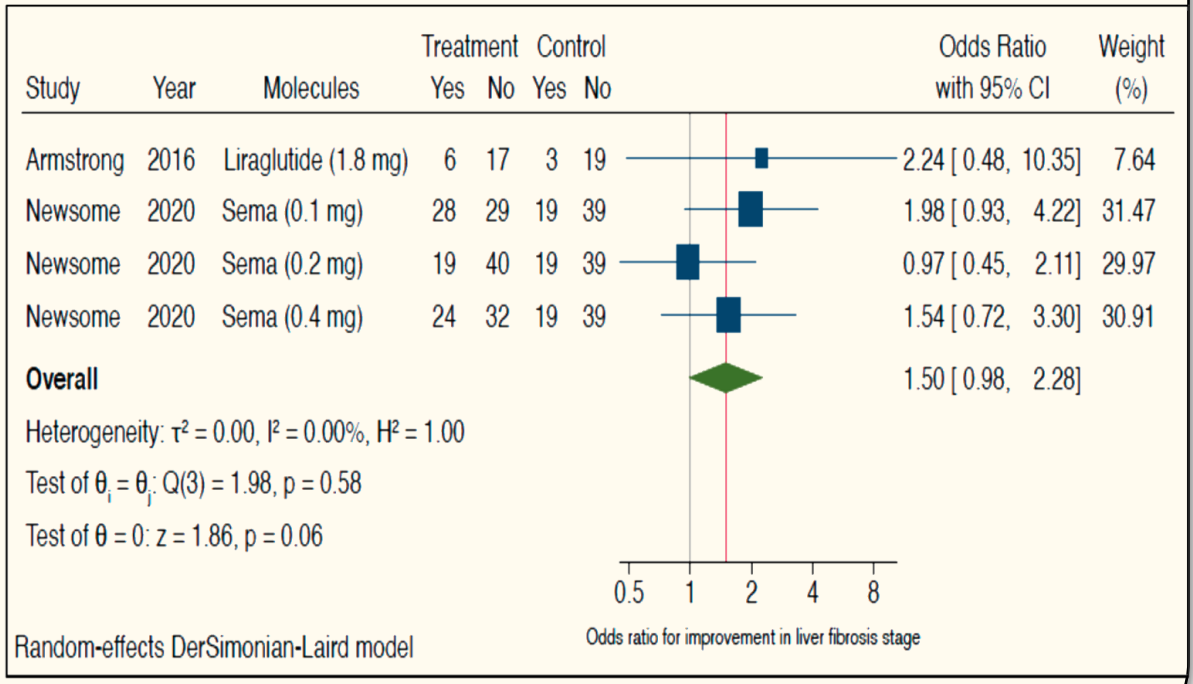
Glucagon-Like Peptide-1 Receptor Agonists for Treatment of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis: An Updated Meta-Analysis of Randomized Controlled Trials

RCTs using liraglutide 1.8 mg/day or semaglutide at a dose of 0.1 mg, 0.2 mg or 0.4 mg/day subcutaneously vs placebo

Resolution of NASH, no worsening of fibrosis



Improvement of fibrosis, no worsening of NASH



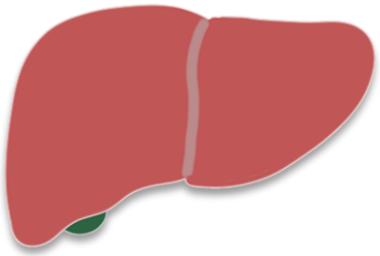
Mantovani A et al. Metabolites, 2021.

First Liver-Specific Metabolomics and Lipidomics

Liraglutide

GLP-1 receptor agonist

- ↓BW
- ↑Insulin secretion
- ↑Insulin sensitivity



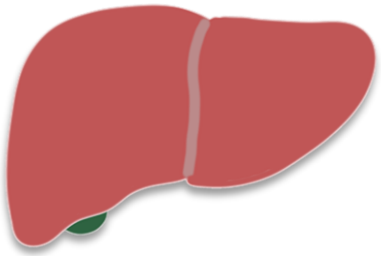
- ↓Hepatic glucose production

- Histological resolution of NASH (to a lesser extent in humans)
- **Phase 3 trials ongoing**

Elafibranor

Dual PPARα PPARδ agonist

- Peroxisome proliferation & gene expression activation



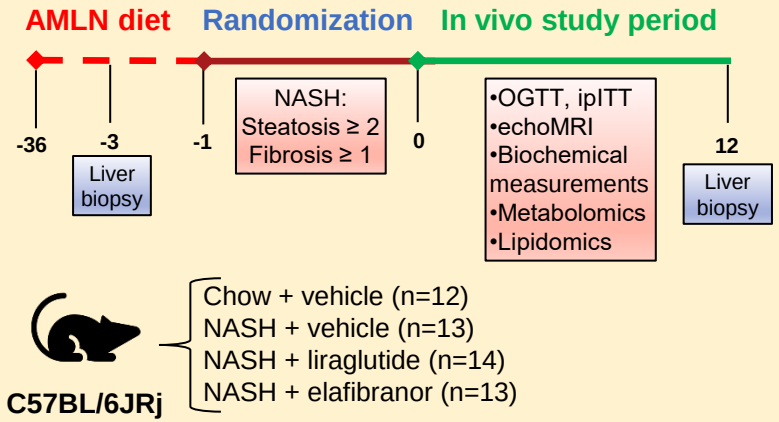
- ↑Fatty acid uptake, binding, transportation and oxidation
- ↓Liver injury

- Histological resolution of NASH and fibrosis
- **Phase 3 trial unsuccessful**

Perakakis N ... Mantzoros C. Liver International, 2021

Elafibranor and Liraglutide Differentially Improve the Hepatic Lipidome and Metabolome in a Biopsy-Proven Mouse Model of NASH

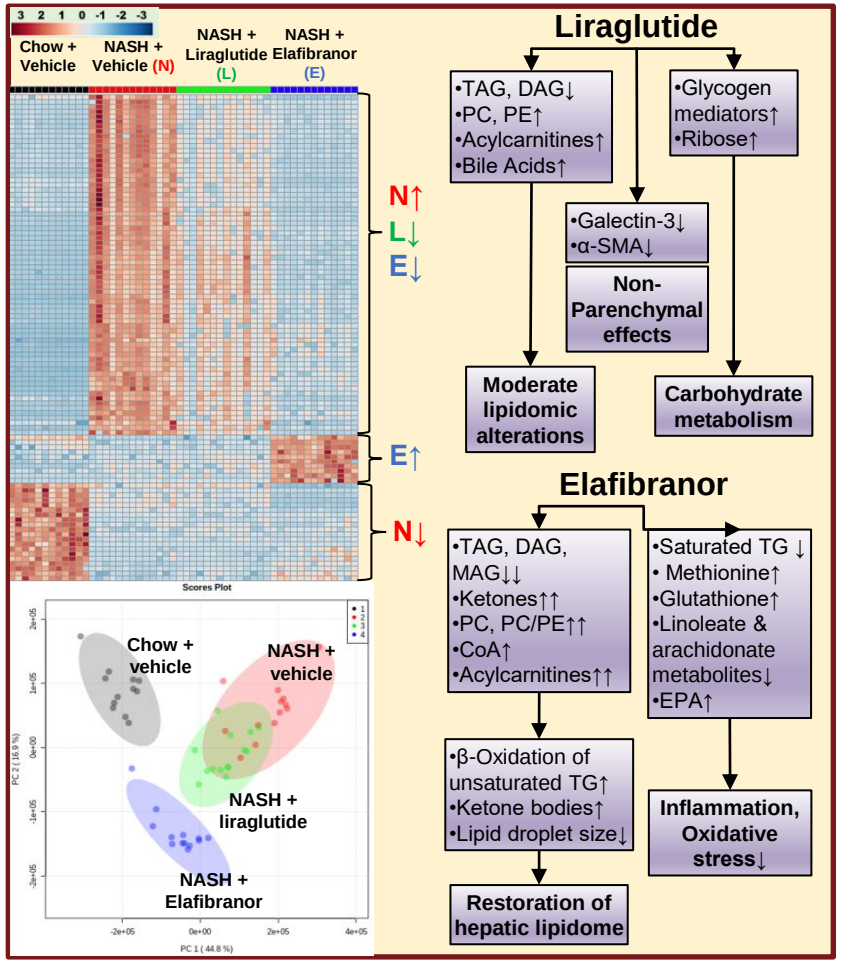
Experimental model



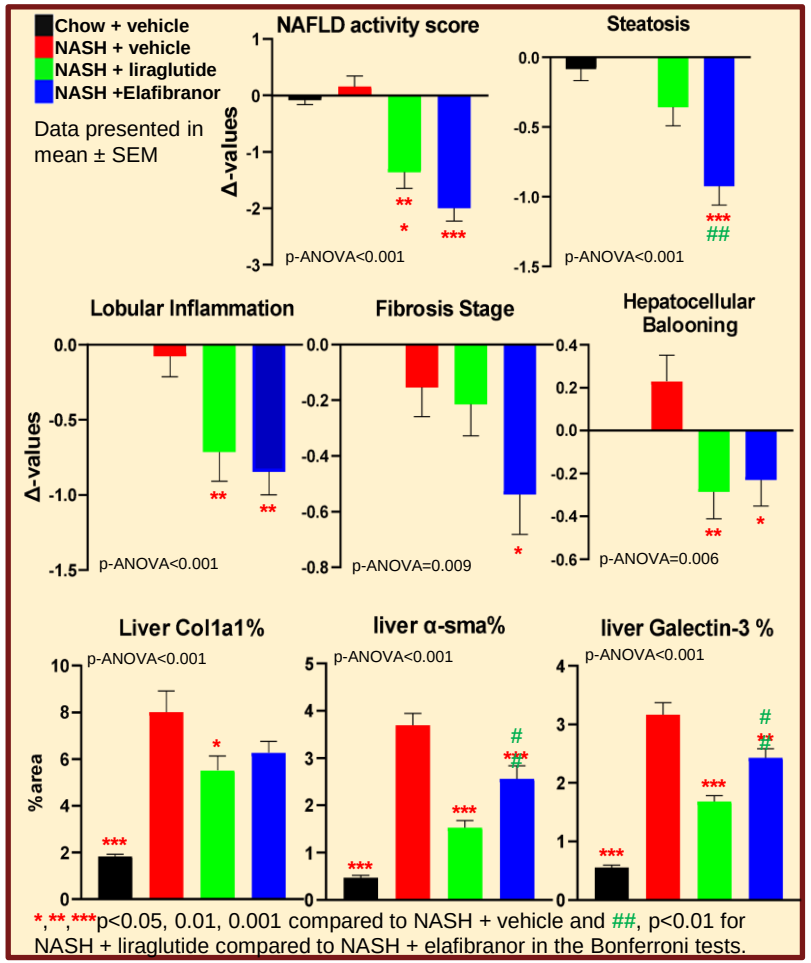
Aim: Effects of Liraglutide (GLP1Ra) and Elafibranor (dual PPARa) on the NASH metabolome and lipidome

Liraglutide: 0.4 mg/kg/d s.c.
Elafibranor: 30 mg/kg/d p.o.
AMLN: 40% fat with 15% trans-fat
20% fructose, 2% cholesterol

PCA, heatmap and pathways



Liver histology changes & markers



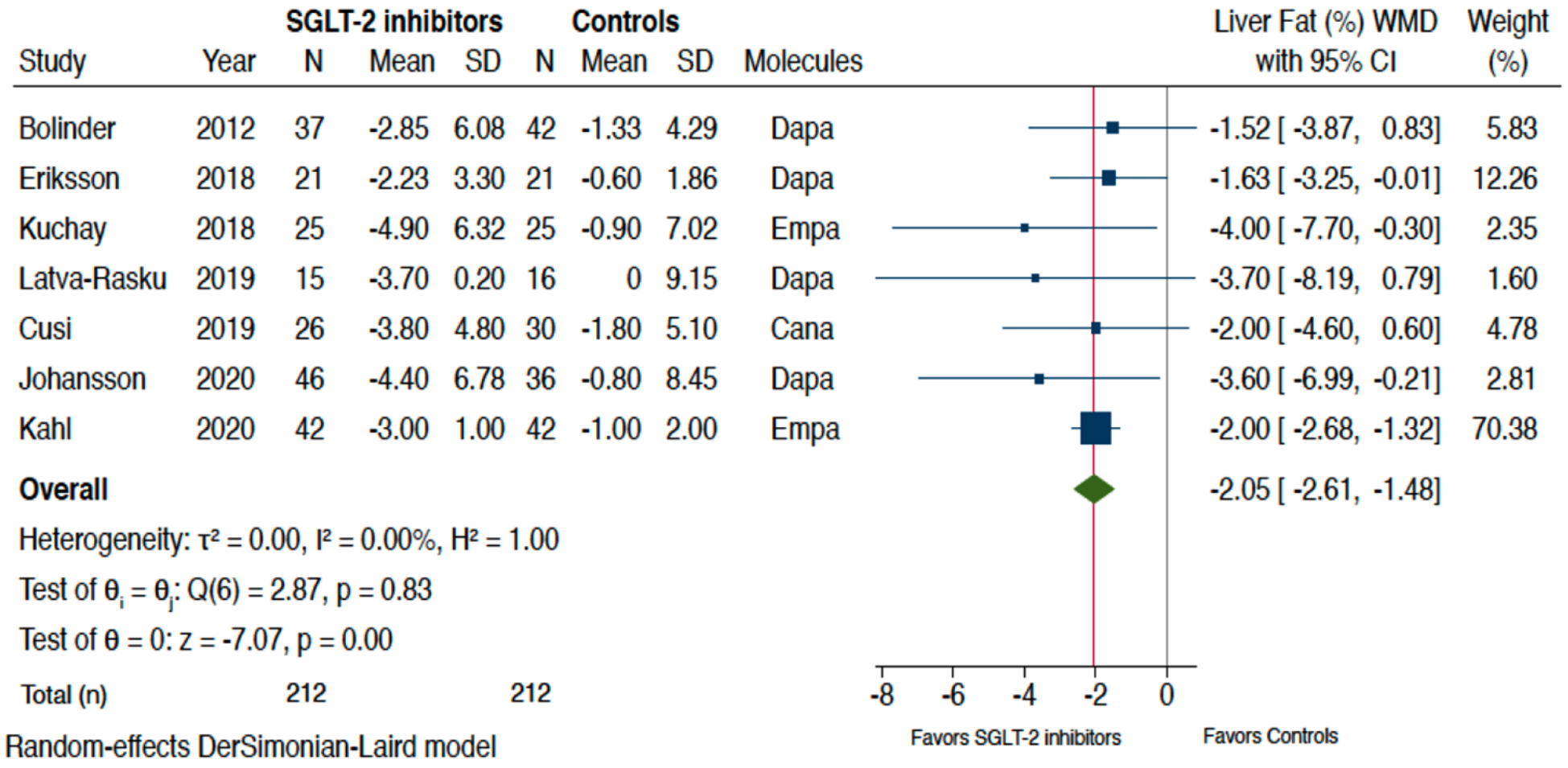
Perakakis N ... Mantzoros C. Liver International, 2021.

AASLD Guidelines Recommendations on Use of Diabetes Treatments

Metformin	▪ Metformin is not recommended for treating NASH in adult patients.
Statins & Thiazolidinediones	▪ Pioglitazone improves liver histology in patients with and without T2DM with biopsy-proven NASH. Therefore, it may be used to treat these patients. Risks and benefits should be discussed with each patient before starting therapy. ▪ Until further data support its safety and efficacy, pioglitazone should not be used to treat NAFLD in patients without T2DM and biopsy-proven NASH.
GLP-1 analogues & SGLT-2s	▪ It is premature to consider GLP-1 agonists or SGLT-2is to specifically treat liver disease in patients with NAFLD or NASH
Vitamin E	▪ Vitamin E (RRR-a-tocopherol) administered at a daily dose of 800 IU/day improves liver histology in nondiabetic adults with biopsy-proven NASH and therefore may be considered for this patient population. Risks and benefits should be discussed with each patient before starting therapy ▪ Until further data supporting its effectiveness become available, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis

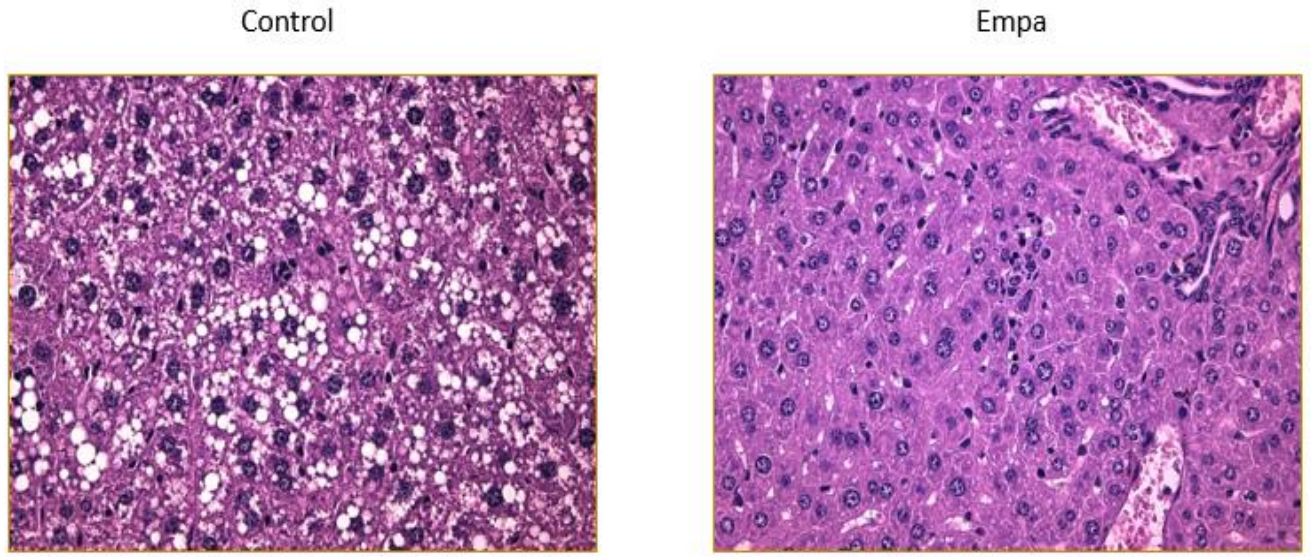
Chalasani N et al. Hepatology, 2018.

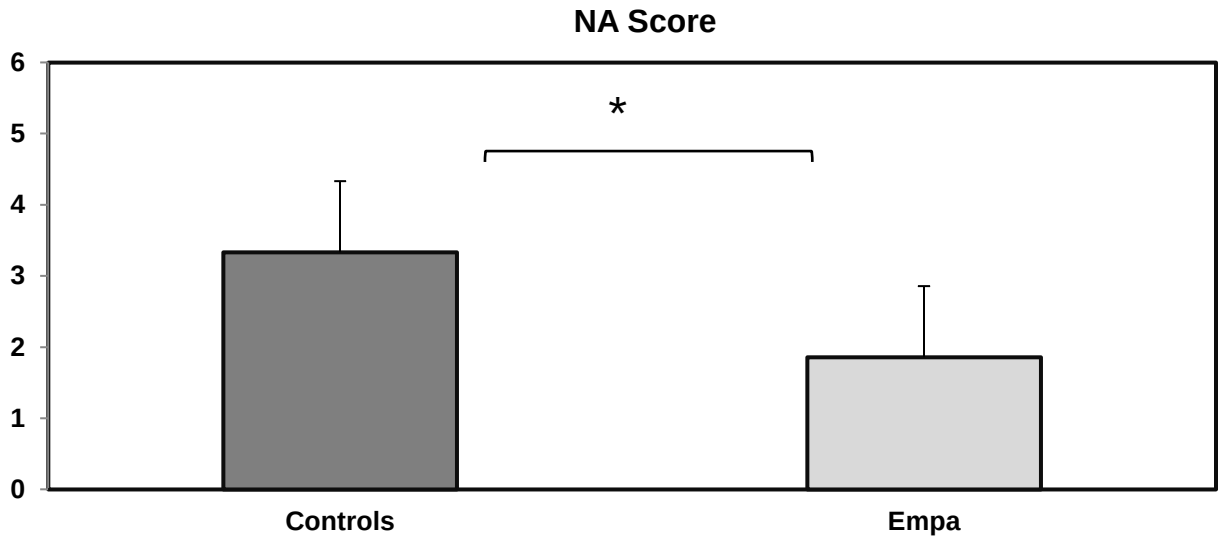
Sodium-Glucose Contrasporter-2 Inhibitors for Treatment of Nonalcoholic Fatty Liver Disease: A Meta-Analysis of Randomized Controlled Trials



Mantovani A et al. Metabolites, 2021.

Sodium-Glucose Co-Transporter-2 Inhibitors (SGLT2i)

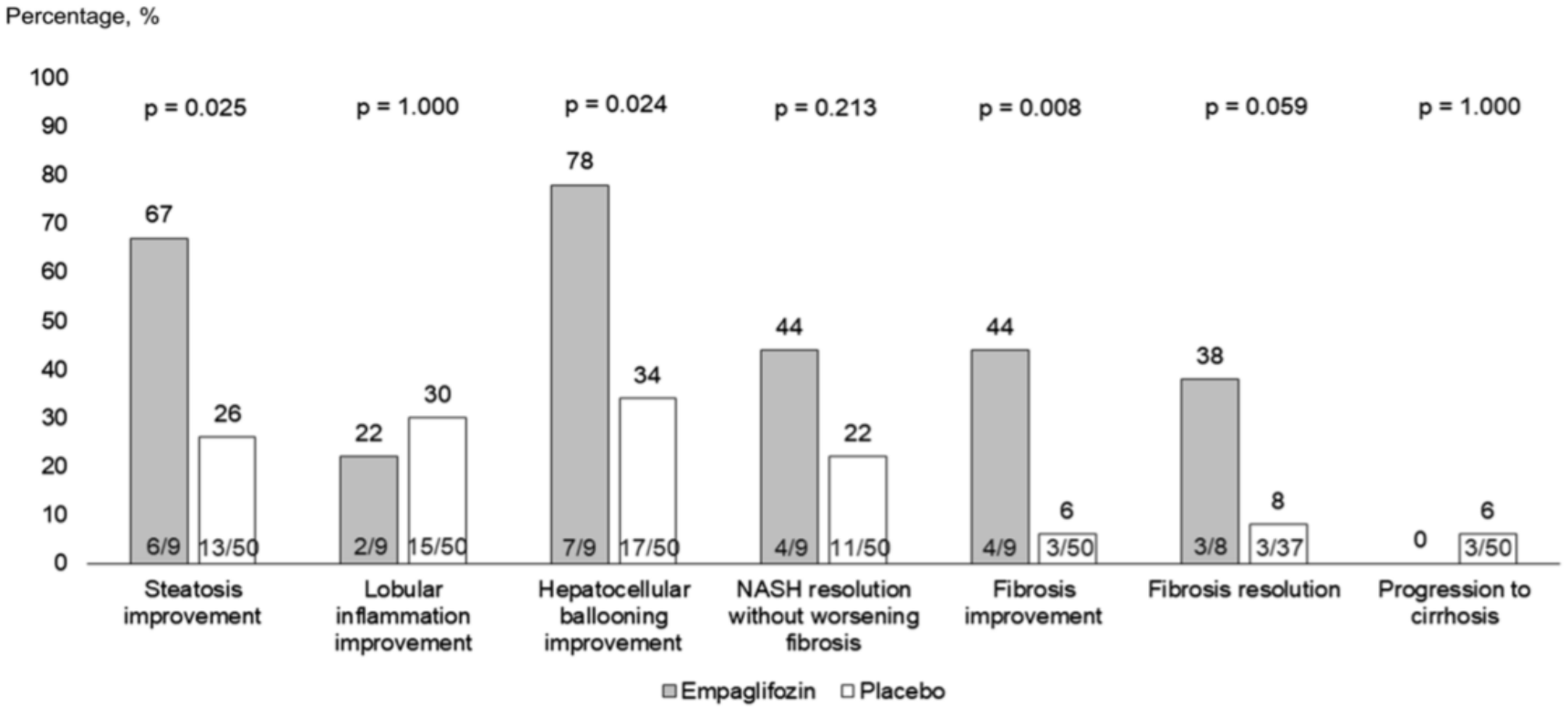




Kassi… Mantzoros, 2020

Sodium-Glucose Co-Transporter-2 Inhibitors (SGLT2i)

- There is primary histological evidence that empagliflozin, a SGLT2i, can significantly decrease the liver fat fraction, steatosis, ballooning and fibrosis.
- Moreover, SGLT2i treatment improved glycaemic control but also reduced liver fat mass in patients with NAFLD and T2DM in another study. Body weight loss was mainly attributable to the reduction in fat mass, particularly in visceral fat.



Lai LL et al. Dig Dis Sci, 2019.
Arase Y et al. Clin Drug Investig, 2019.

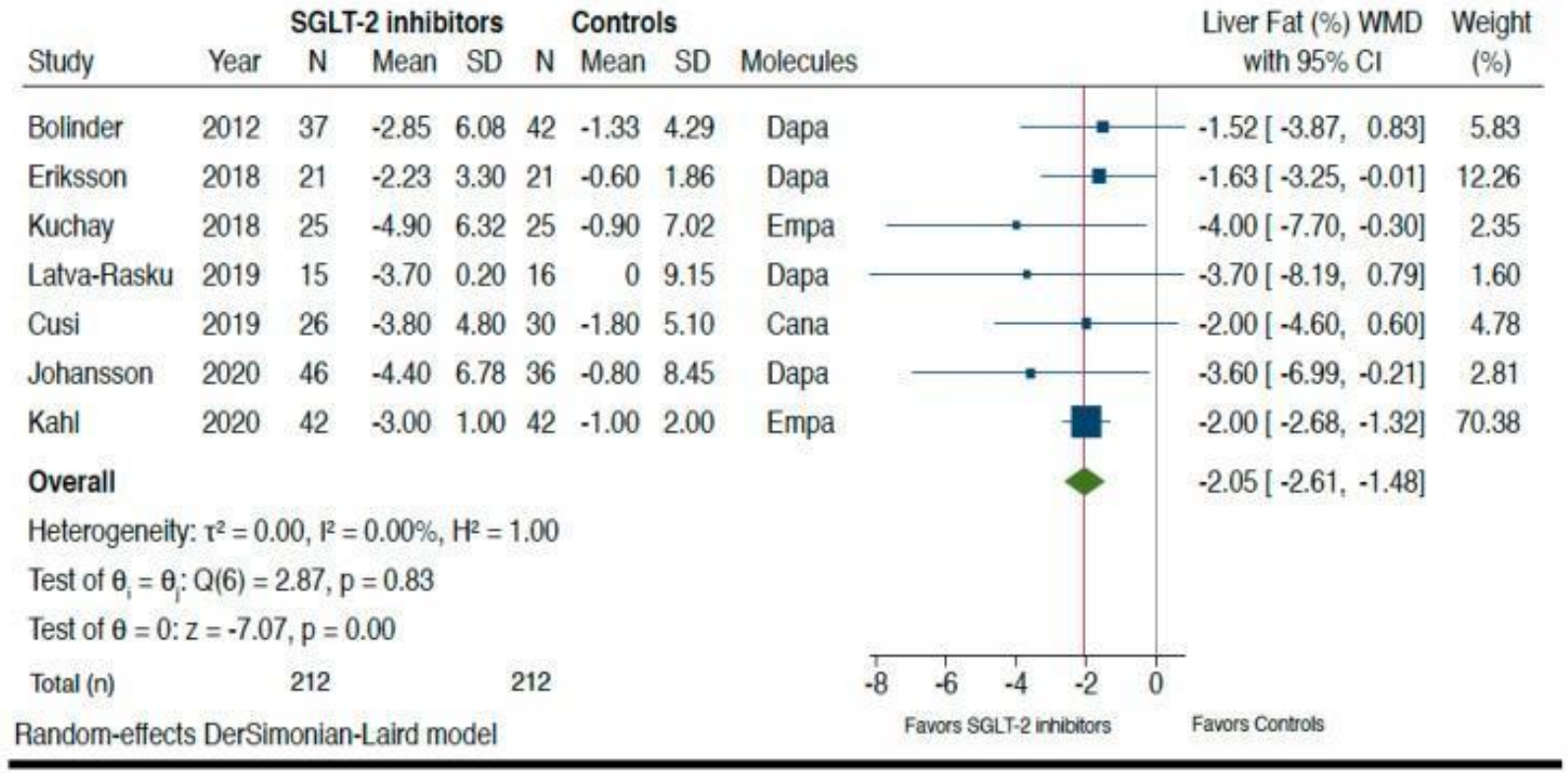
metabolites

MDPI

Review

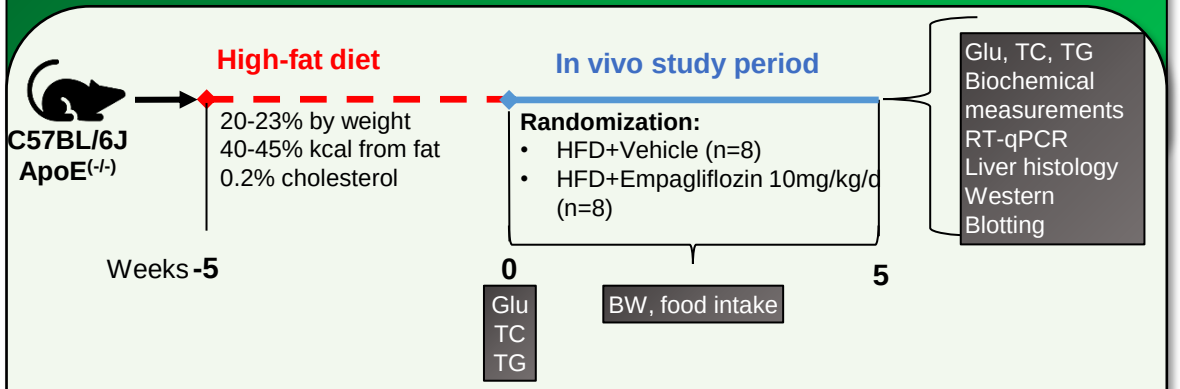
Sodium-Glucose Cotransporter-2 Inhibitors for Treatment of Nonalcoholic Fatty Liver Disease: A Meta-Analysis of Randomized Controlled Trials

Alessandro Mantovani, Graziana Petracca, Alessandro Csermely, Giorgia Beatrice and Giovanni Targher *



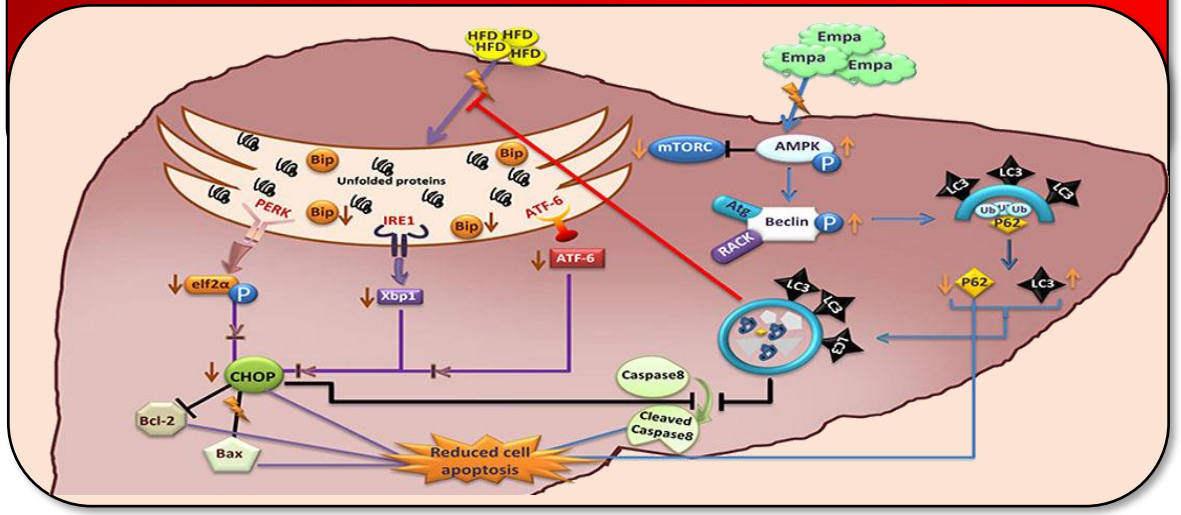
Empagliflozin Attenuates Non-Alcoholic Fatty Liver Disease (NAFLD) in High Fat Diet Fed ApoE(-/-) Mice by Activating Autophagy and Reducing ER Stress and Apoptosis

Experimental model



Aim: Investigate the effects of empagliflozin (SGLT2i) on NAFLD progression, particularly ER stress, hepatocellular autophagy and apoptosis.

Graphical Abstract



Results

Empagliflozin vs vehicle

- **Pre-to-post** → Glu, TG, TC, TG/HDL ↓
- **Post-intervention** → ALT ↓

Liver histology
- Liver weight ↓
- Steatosis↓
- Lobular inflammation ↓
- NAS score↓

Lipid metabolism & Inflammation genes
- Fasn↓
- Srebp-1↓
- PCK-1 ↓ — Lipogenic
- MCP-1↓
- F4/80↓ — Inflamm.

ER Stress core genes
- EIF2a, GRP78 Chop, IRE1, Xbp1, ATF-6, GRP94 ↓

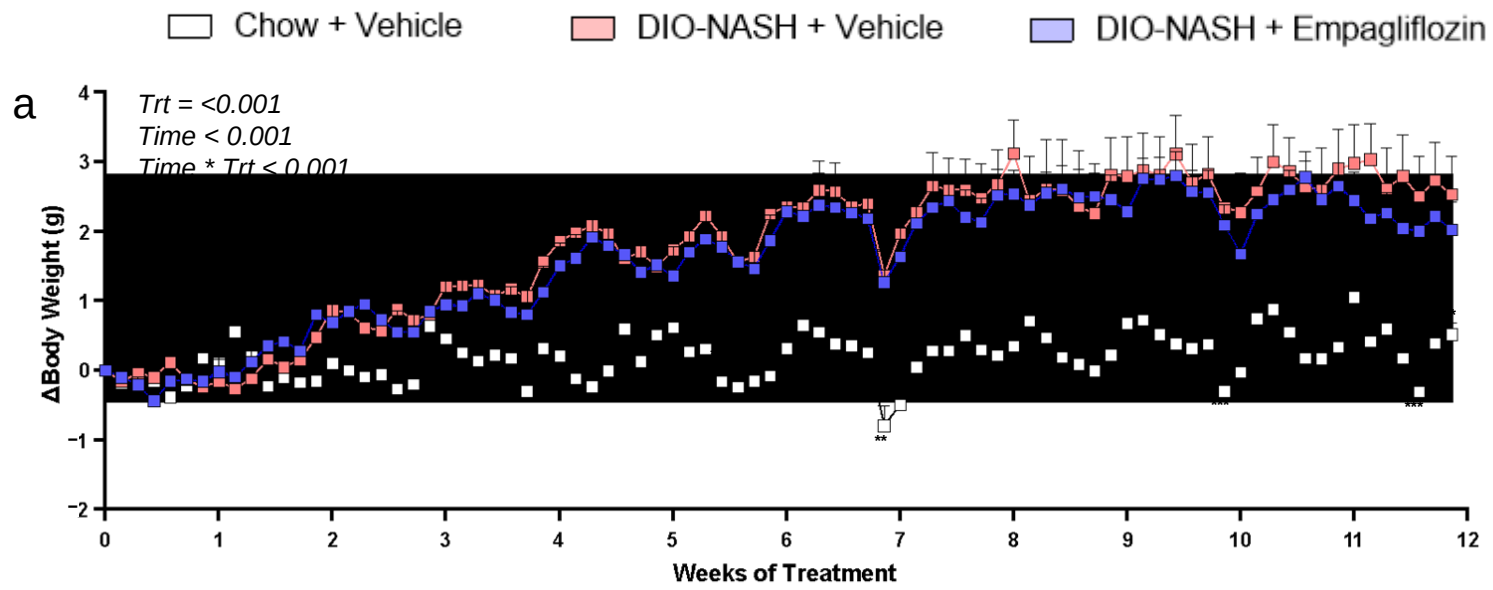
Hepatic autophagy genes & markers
- mTOR↓
- p62↓, pAMPK / AMPK ↑, Beclin-1 ↑

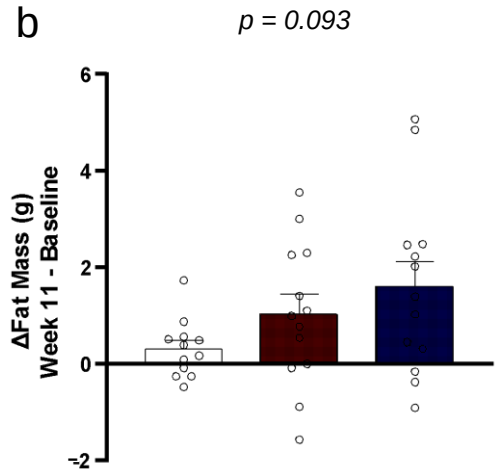
ER Stress markers
- CHOP↓
- P-eIF2a / eIF2a↓

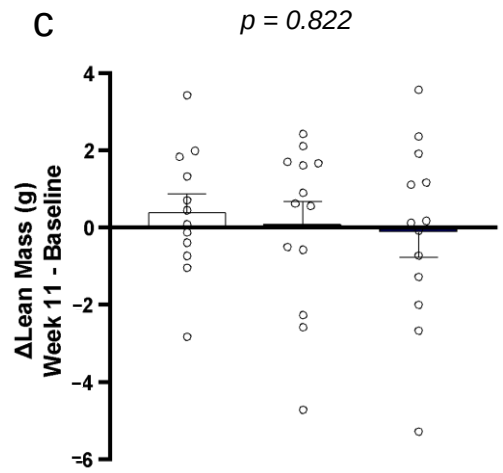
Hepatic apoptosis markers
- Bcl2/Bax↑, Cleaved Caspase 8 ↓

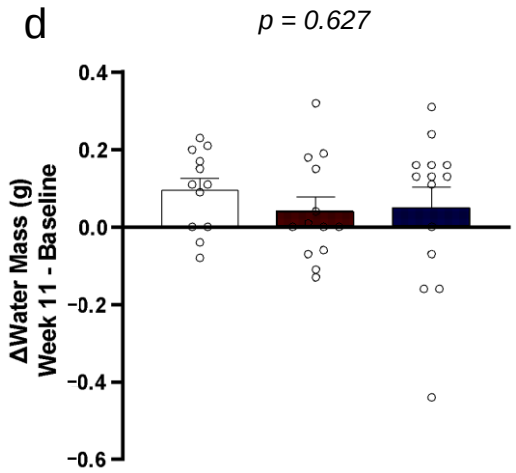
Nasiri-Ansari et al. Int J Mol Sci, 2021.

Empagliflozin Improves Metabolic and Hepatic Outcomes in a Non-Diabetic Obese Biopsy-Proven Mouse Model of Advanced NASH



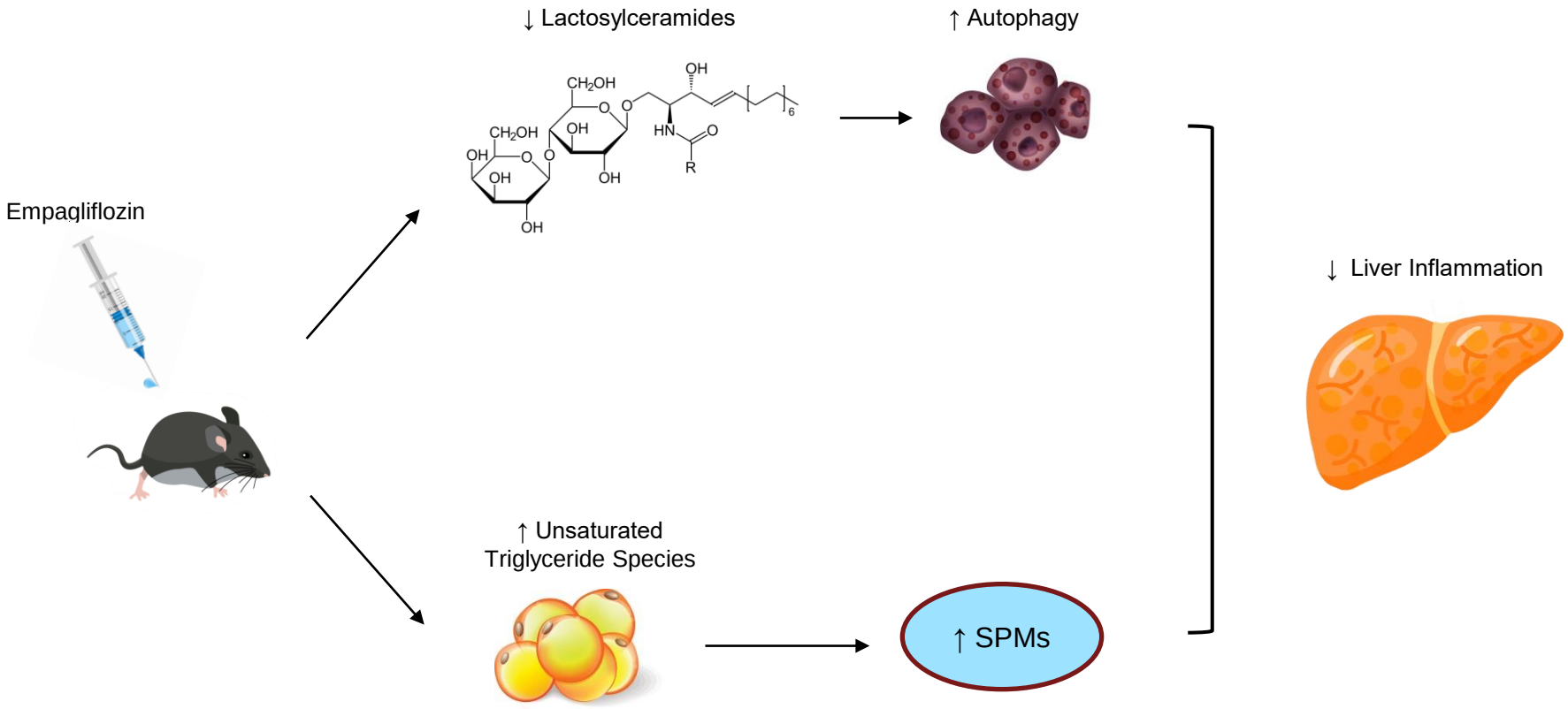






Perakakis N ... Mantzoros C. Int J Mol Sci, 2021.

Empagliflozin Improves Metabolic and Hepatic Outcomes in a Non-Diabetic Obese Biopsy-Proven Mouse Model of Advanced NASH



Perakakis N ... Mantzoros C. Int J Mol Sci, 2021.

Management of NASH

Investigational Pharmacotherapies

- More than 60 phase 2 trials are planned or ongoing
- Cenicrivoric, elafibranor, obeticholic acid, and selonsertib are in phase 3 trials
- Selective PPRgamma Modulators (SPARMs) that may provide same or better efficacy than pioglitazone but with fewer side effects are also in development.
- Specific thyroid receptor activators are also in development.

Improvement in insulin sensitivity and prevention of high fat diet-induced liver pathology using AZD5069, a CXCR2 antagonist

Phillips BE, Lantier L, Engman C, Garciafigueroa Y, Singhi A, Trucco M, Mantzoros C, Wasserman D, and Giannoukakis N.

Experimental model

6-week old C57BL/6J male mice

- Control diet (**CN**) (n=
- High-fat (**HF**) diet
- **HF + AZD5069**

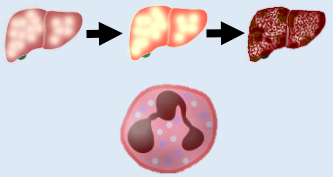
N=5-9 from each group

Randomization — **In vivo study period**

Weeks 0 — **16**

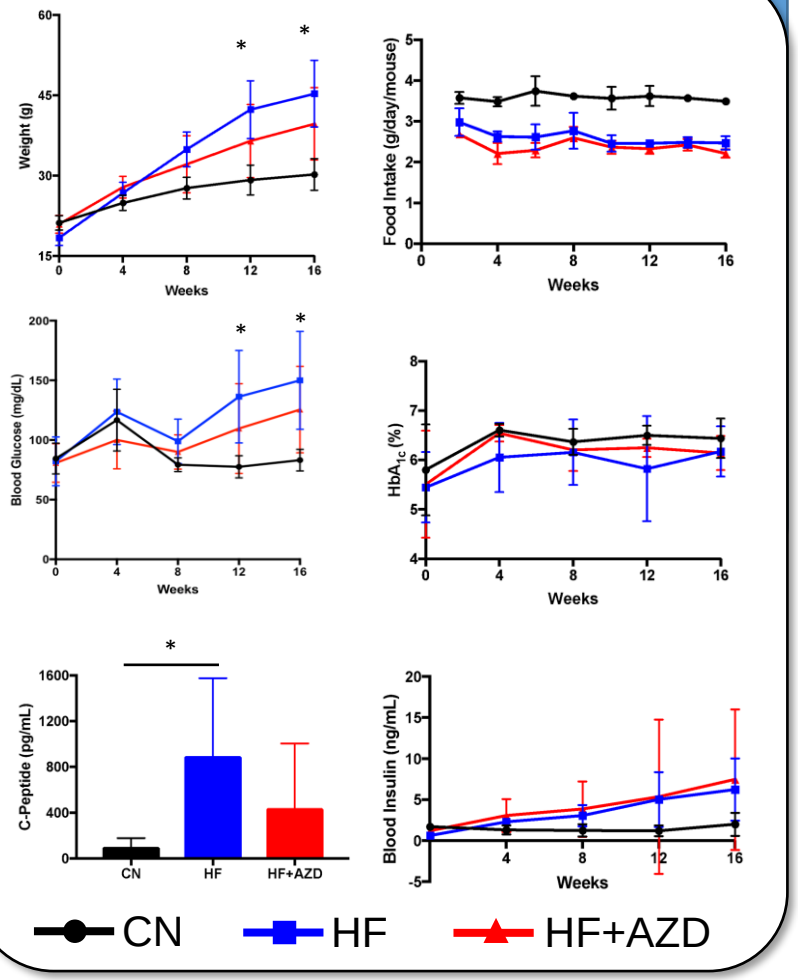
Every 4 weeks:
- ipITT, hyperinsulinemic clamp
- Biochemical and hormonal measurements
- **At euthanasia:** Organ histology and immunofluorescence

Aim: To test the capabilities of CXCR2 antagonist AZD5069 (modulator of neutrophil accumulation) in improving glycemia, insulin sensitivity and preventing the progression towards liver pathology reminiscent of NAFLD/NASH in a murine model.

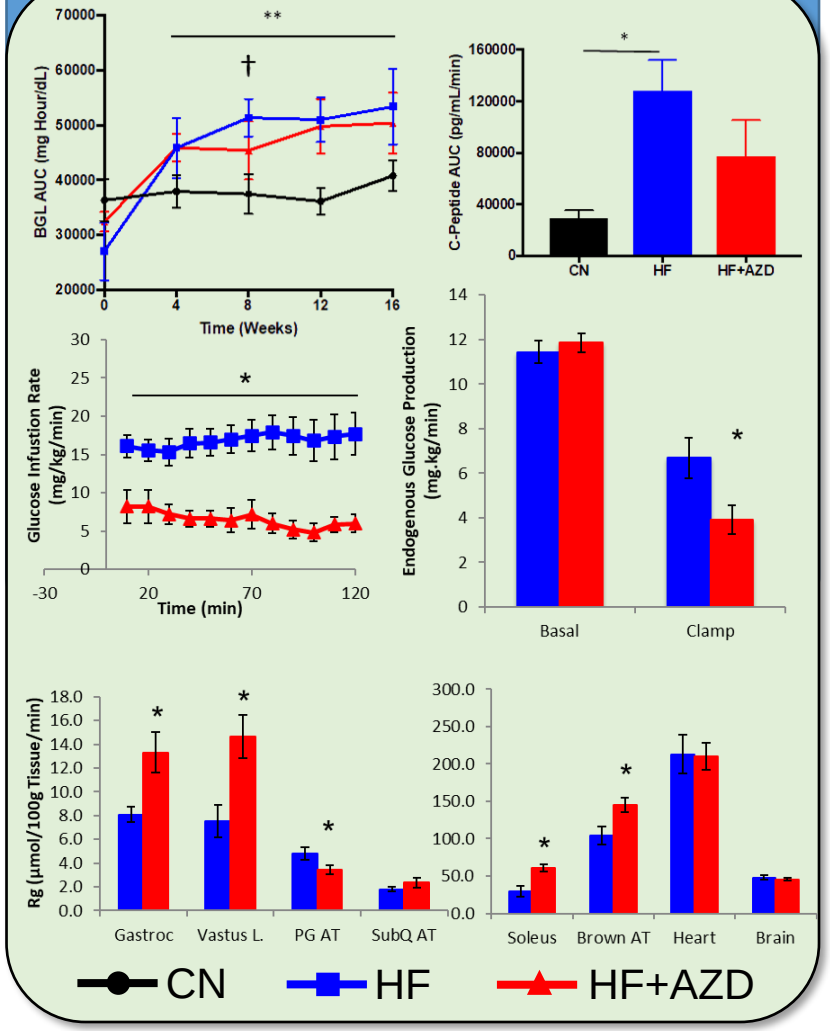


CN: 13% kcal fat
HF: 60% kcal fat
AZD5069: 593.8 mg/4057 kcal

Body weight, food intake, and fasting glycemic indices



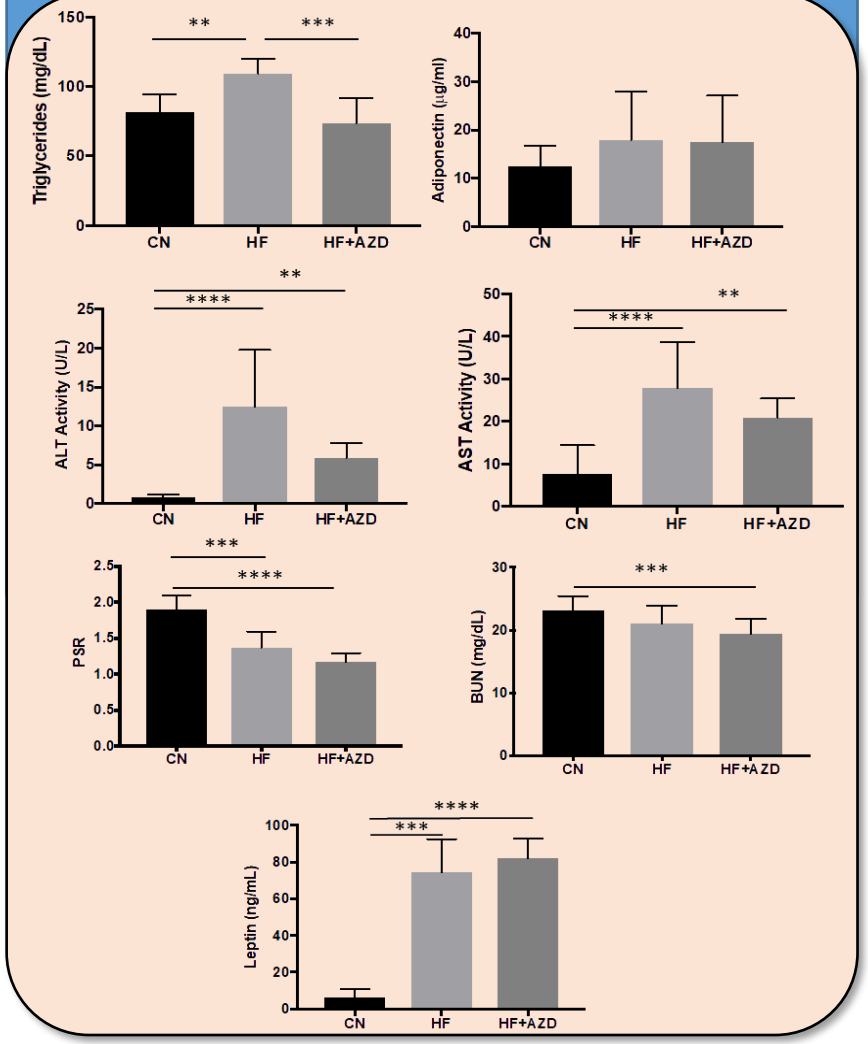
ipITT and hyperinsulinemic clamp data



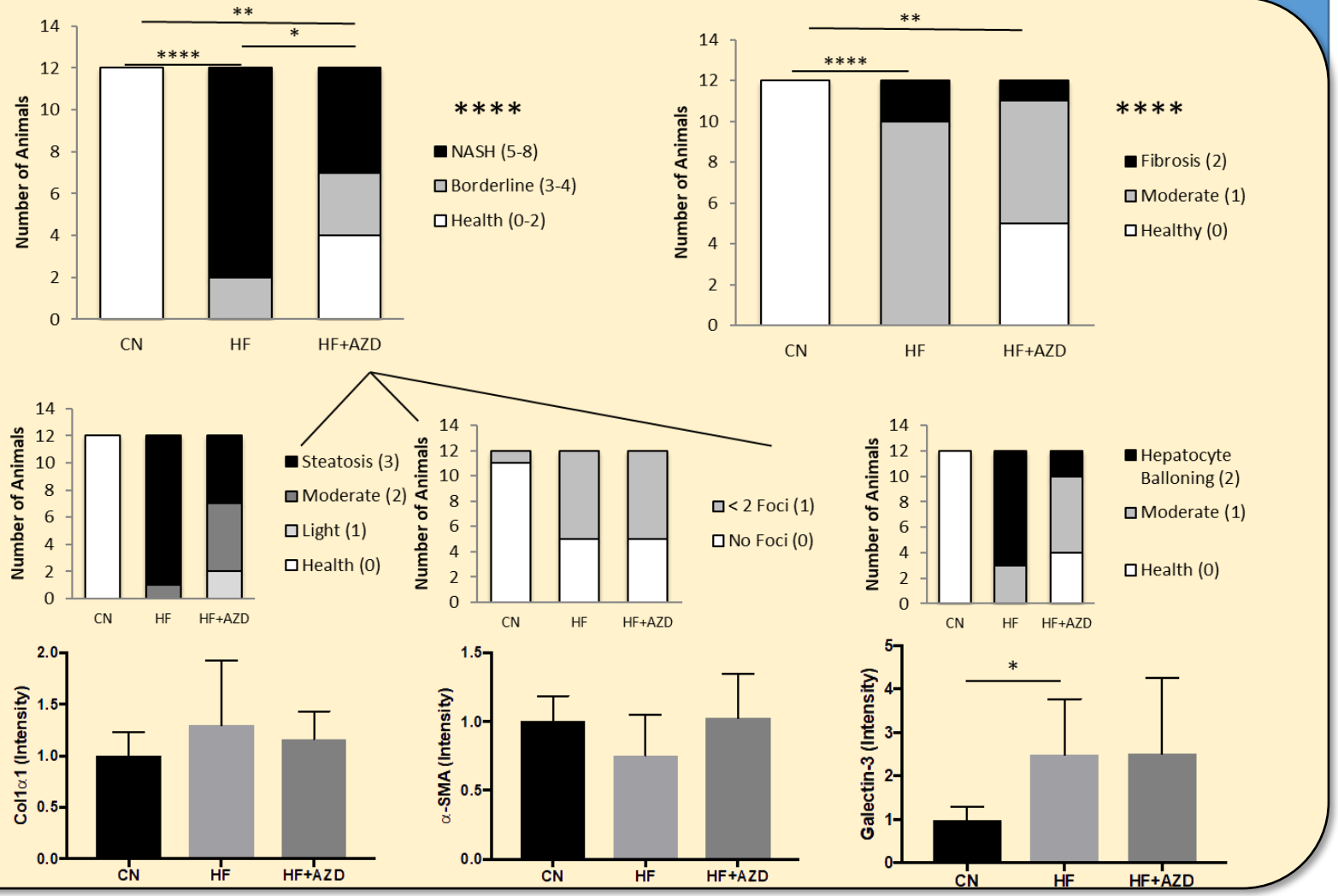
Improvement in insulin sensitivity and prevention of high fat diet-induced liver pathology using AZD5069, a CXCR2 antagonist

Phillips BE, Lantier L, Engman C, Garciafigueroa Y, Singhi A, Trucco M, Mantzoros C, Wasserman D, and Giannoukakis N.

Circulating liver metabolites and leptin



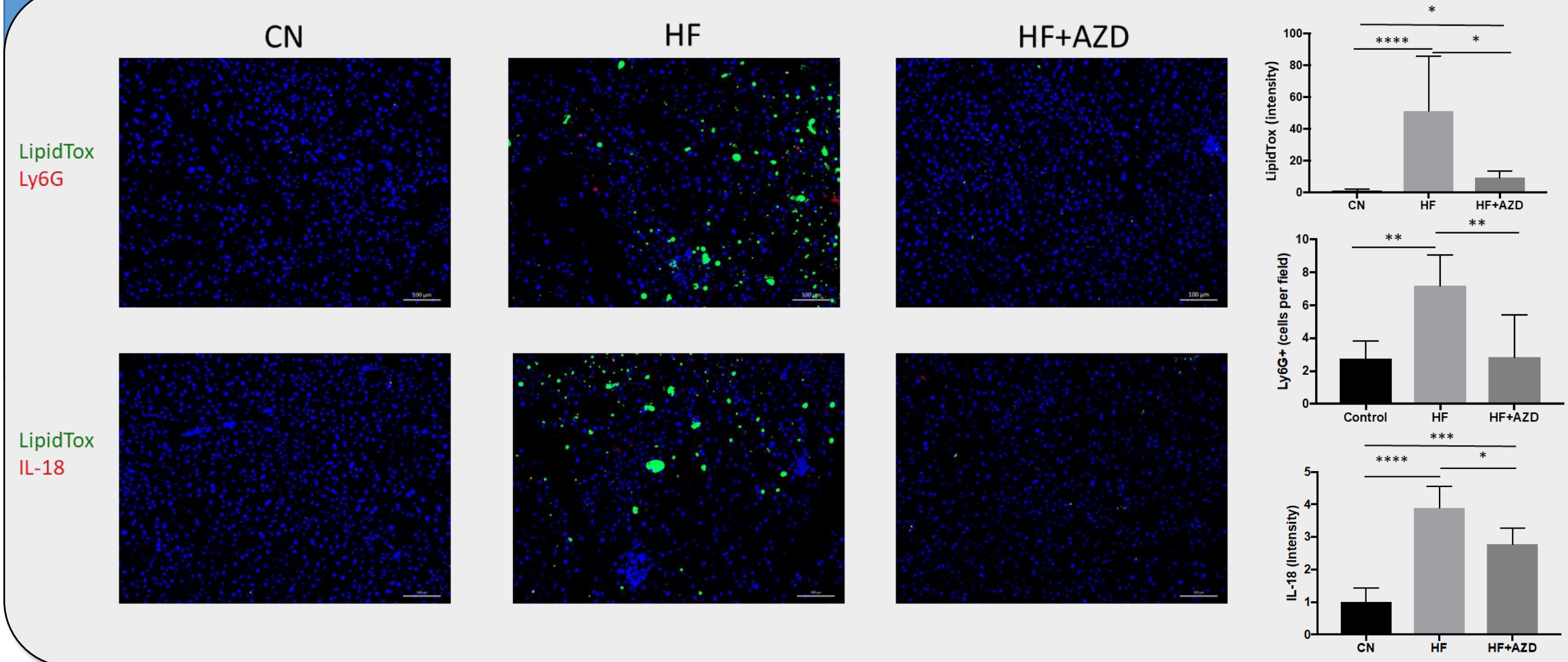
NAFLD activity score and liver biopsy data at sacrifice



Improvement in insulin sensitivity and prevention of high fat diet-induced liver pathology using AZD5069, a CXCR2 antagonist

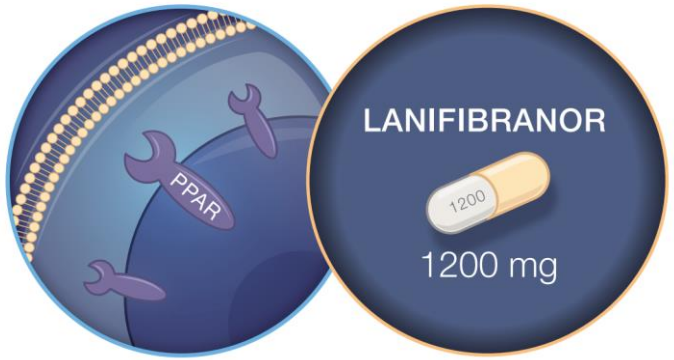
Phillips BE, Lantier L, Engman C, Garciafigueroa Y, Singhi A, Trucco M, Mantzoros C, Wasserman D, and Giannoukakis N.

Hepatic lipid content, accumulated neutrophils and IL-18 density



A Randomized, Controlled Trial of the Pan-PPAR Agonist Lanifibranor in NASH

The NEW ENGLAND JOURNAL of MEDICINE

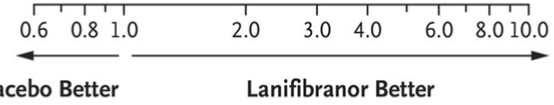


Adverse Events

	Lanifibranor 1200 mg	Lanifibranor 800 mg	Placebo
	number (percent)		
Severe adverse events	3 (4)	3 (4)	3 (4)
Most frequent adverse events			
Diarrhea	10 (12)	8 (10)	1 (1)
Nausea	7 (8)	8 (10)	3 (4)
Weight gain	7 (8)	8 (10)	0

The pan-PPAR agonist lanifibranor, at a dose of 1200 mg daily, improved histologic outcomes in patients with non-cirrhotic, highly active NASH.

End Point	Placebo	Lanifibranor	Risk Ratio (95% CI)	P Value
	percent of patients with response			
Primary end point: reduction of ≥2 points in SAF-A score and no worsening of fibrosis				
Lanifibranor, 800 mg	33	48	1.45 (1.00–2.10)	0.07
Lanifibranor, 1200 mg	33	55	1.69 (1.22–2.34)	0.007
Secondary end point: resolution of NASH without worsening of fibrosis				
Lanifibranor, 800 mg	22	39	1.70 (1.07–2.71)	
Lanifibranor, 1200 mg	22	49	2.20 (1.49–3.26)	
Secondary end point: improvement in fibrosis stage of ≥1 without worsening of NASH				
Lanifibranor, 800 mg	29	34	1.15 (0.72–1.85)	
Lanifibranor, 1200 mg	29	48	1.68 (1.15–2.46)	
Composite secondary end point: resolution of NASH and improvement in fibrosis stage of ≥1				
Lanifibranor, 800 mg	9	25	2.57 (1.20–5.51)	
Lanifibranor, 1200 mg	9	35	3.95 (2.03–7.66)	



Decrease of ≥2 Points in SAF-A Score and No Worsening of Fibrosis

	Lanifibranor	Placebo	Risk Ratio (95%CI)	P Value
Lanifibranor, 800 mg	48%	33%	1.45 (1.00–2.10)	P=0.07
Lanifibranor, 1200 mg	55%	33%	1.69 (1.22–2.34)	P=0.007

Sanyal AJ et al. NEJM 2021

Leptin

Study 1: RCT cross-over study of lean subjects in fed state and during 72h- fasting treated with placebo or leptin

Fed		Weight-maintaining diet				
	Day 0	Day 1	Day 2	Day 3	Day 4	
Fasting		Placebo or Leptin	Placebo or Leptin	Placebo or Leptin	Placebo or Leptin	
	Day 0	Day 1	Day 2	Day 3	Day 4	

Study 2: Pharmacokinetic study (3 leptin doses) in lean and obese during fasting and fed state

Fed		Leptin Dose A / B / C				
	Day 0	Day 1				
Fasting		Leptin Dose A / B / C	Leptin Dose A / B / C	Leptin Dose A / B / C	Leptin Dose A / B / C	
	Day 0	Day 1	Day 2	Day 3	Day 4	

Study 3: Open-label leptin replacement study in females with chronic mild hypoleptinemia over 3 months

Baseline
Duration: 30 days → Month 1 & 2
Metreleptin
0.08mg/kg/day → Month 3
Metreleptin
0.2mg/kg/day

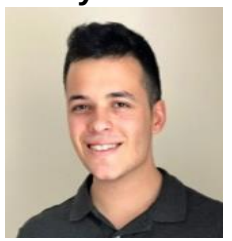
Study 4: RCT with leptin replacement in females with chronic mild hypoleptinemia over 12 months

12 months
Metreleptin 0.08mg/kg/day vs placebo → 4 months follow-up
(no treatment)

Dr. Perakakis

P. Chrysafi

Dr. Farr



K. Stefanakis

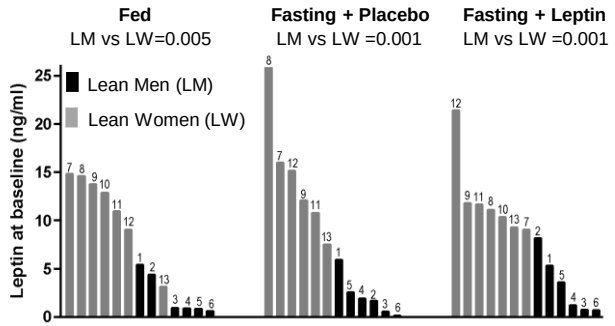
Dr. Peradze

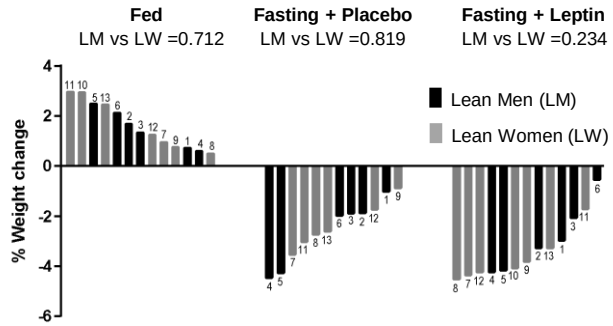
Dr. Sala-Vila

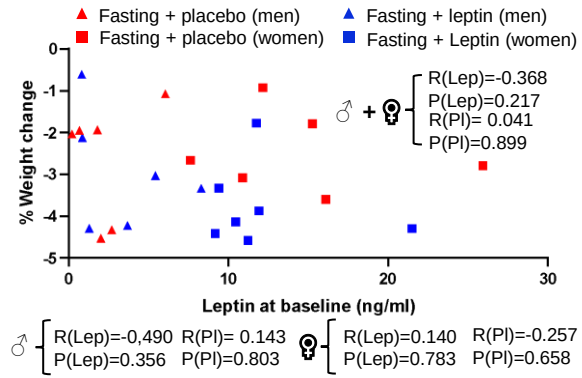
Dr. Chan

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

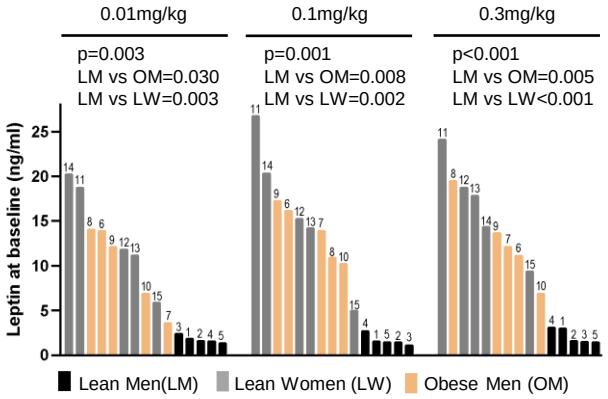
a . Correlation of % weight change with leptin at baseline in study 1 (72h-fed untreated or fasting treated with leptin or placebo)

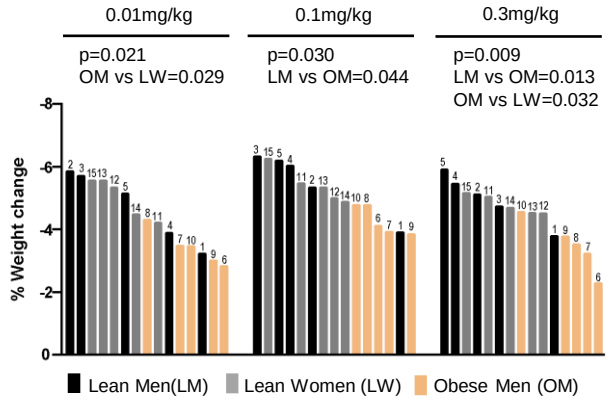


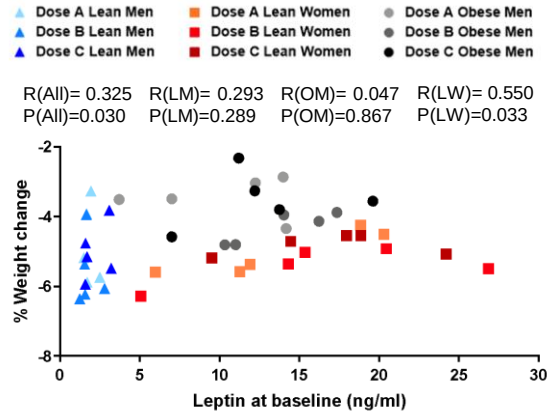




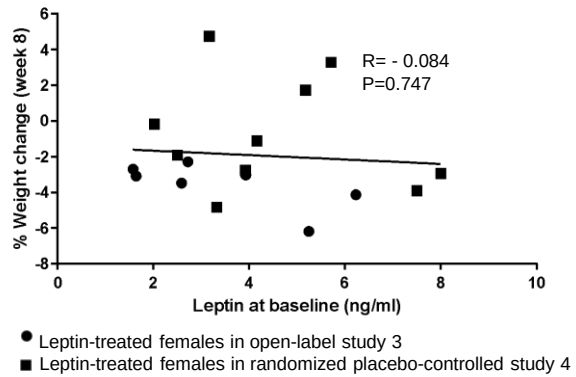
b. Correlation of % weight change with leptin at baseline in study 2 (72h-fasting treated with escalating leptin doses)

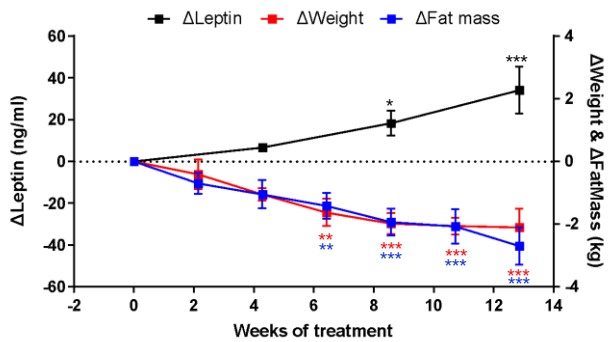




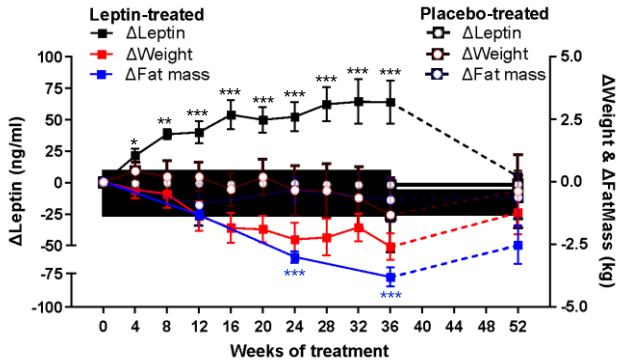


c. Correlation of % weight change with leptin at baseline and weight and fat mass changes in relation to leptin in studies 3 and 4 (Long-term leptin treatment)





Leptin(ng/ml)	Body weight (kg)	Fat mass (kg)
p<0.001	p<0.001	p<0.001



Leptin(ng/ml)	Body Weight(kg)	Fat Mass(kg)
G<0.001	G=0.093	G=0.010
T<0.001	T<0.001	T<0.001
G*T<0.001	G*T=0.059	G*T<0.001

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

Leptin

Food intake

- **Hypothalamus**

Energy Expenditure

- **SNS activity**
- **Adrenal hormones**
- **Body Temperature**
- **BP, Pulse**

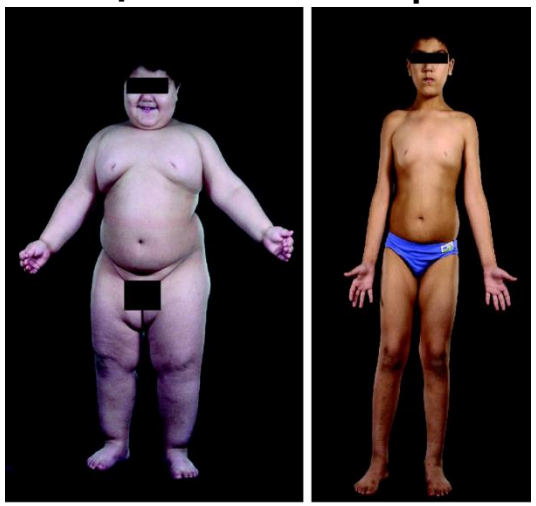
Lipid metabolism

- **Lipolysis**
- **Lipid utilization**

Perakakis, Farr, Mantzoros
JACC State-of-the art review (02/2021)

Congenital Leptin Deficiency

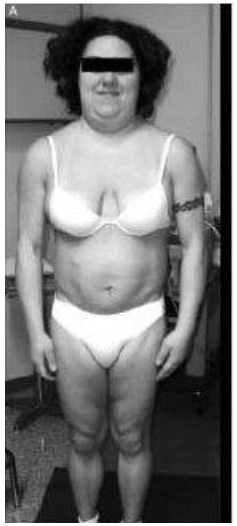
- Leptin **+ Leptin**



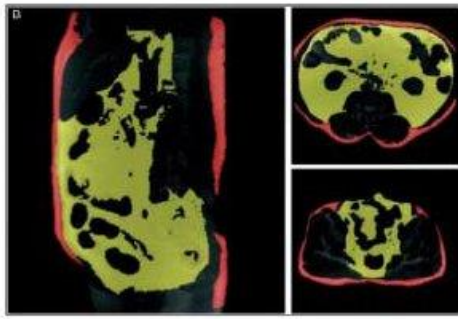
3yr old weighing 42 kg 7yr old weighing 32 kg

Farooqi S et al. J Endocrinol, 2014.

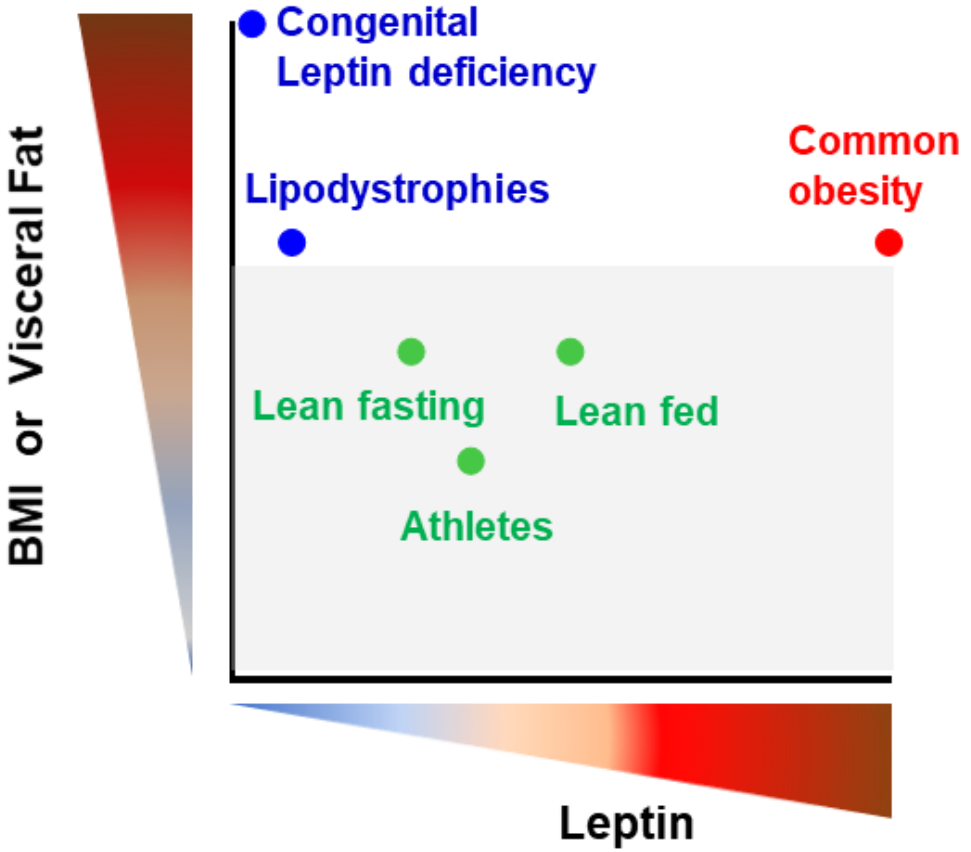
Lipodystrophy



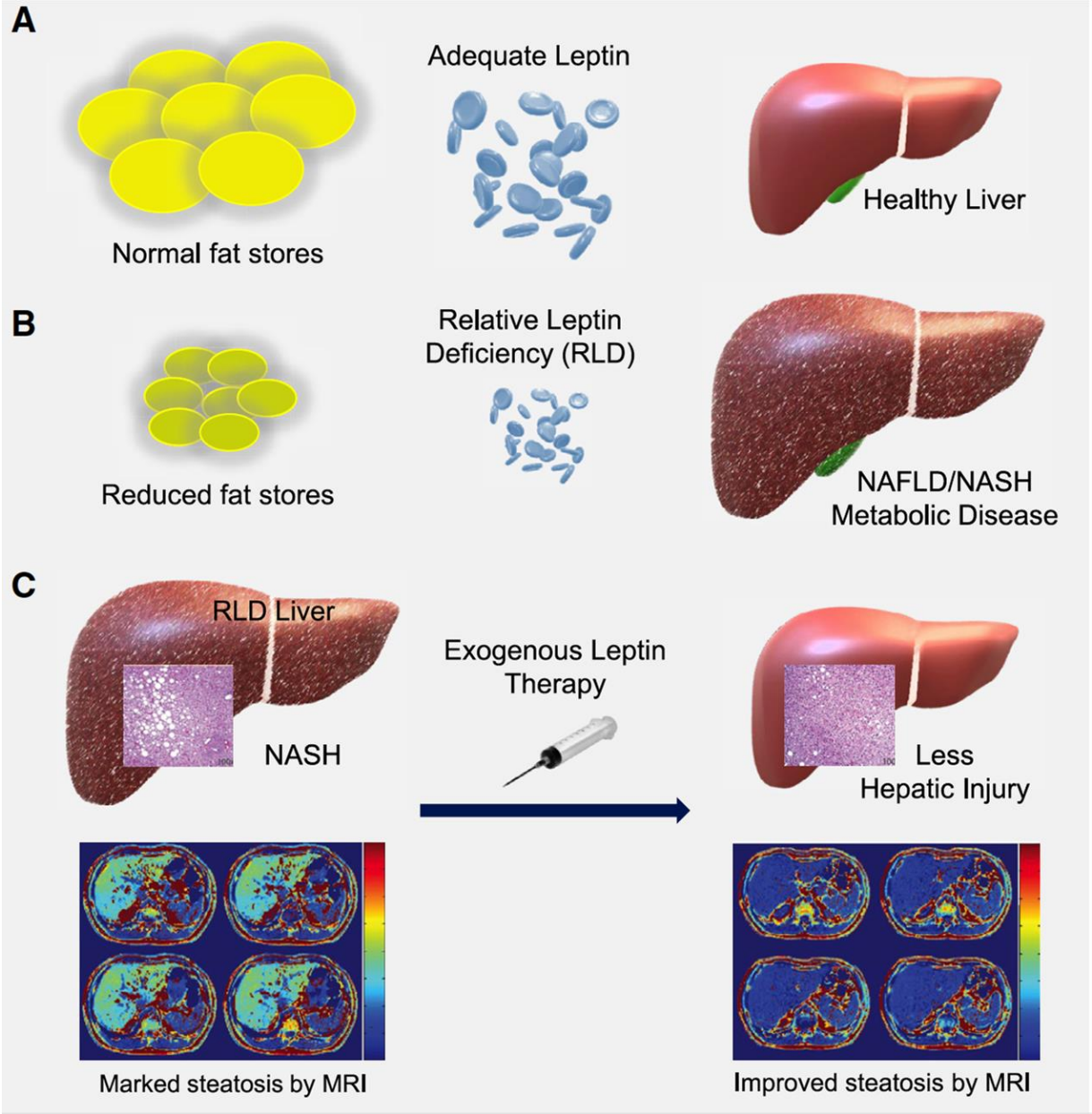
Reduced subcutaneous fat (red)
Increased visceral fat (yellow)



Parker et al. Eur J Endocrinol, 2013.



Metreleptin Therapy for NASH: Open Label Interventions in Two Different Clinical Settings

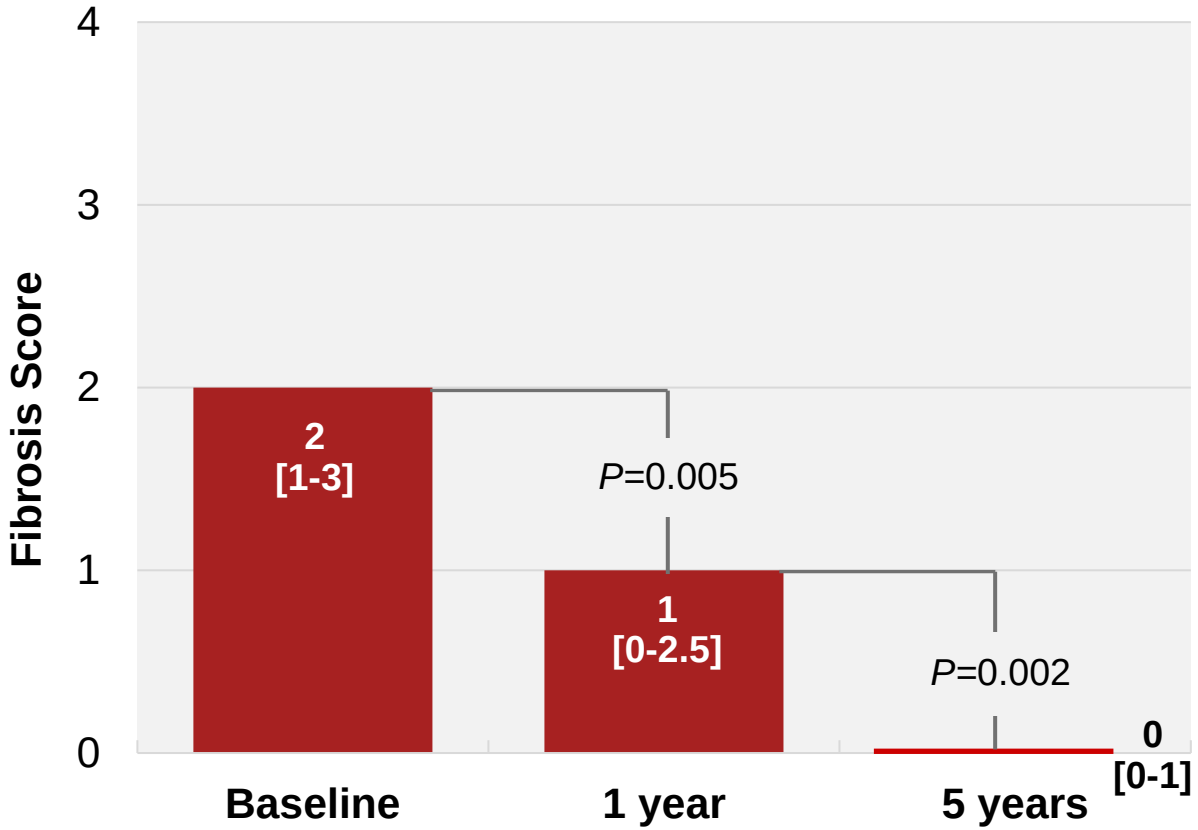


Akinci B et al. Med, 2021.

Bariatric Surgery Improved NASH and Fibrosis in one Long-Term Study

Proportion of Patients With NASH Resolution

Fibrosis Improvement



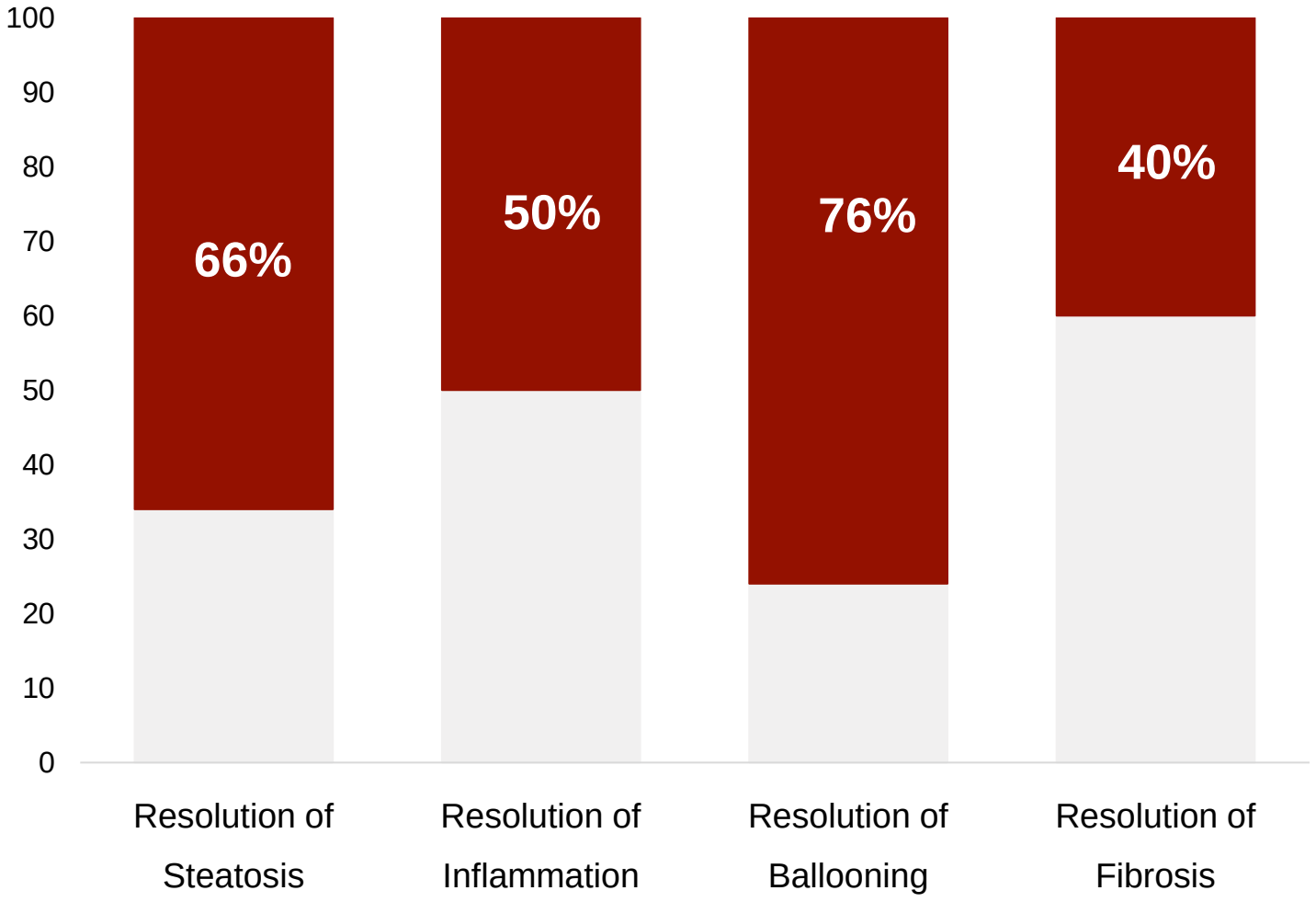
Total subjects N=190, results using paired biopsies

Lassailly G et al. AASLD, 2018. Abstract 70.

Complete Resolution of Nonalcoholic Fatty Liver Disease After Bariatric Surgery: A Systematic Review and Meta-analysis

- 32 cohort studies: 3093 liver biopsies at baseline and 2649 biopsies at follow-up evaluation (85.65% follow-up rate).
- Median follow-up period of 15 months (range, 3–55 mo)

Resolution of histopathologic features in % of patients

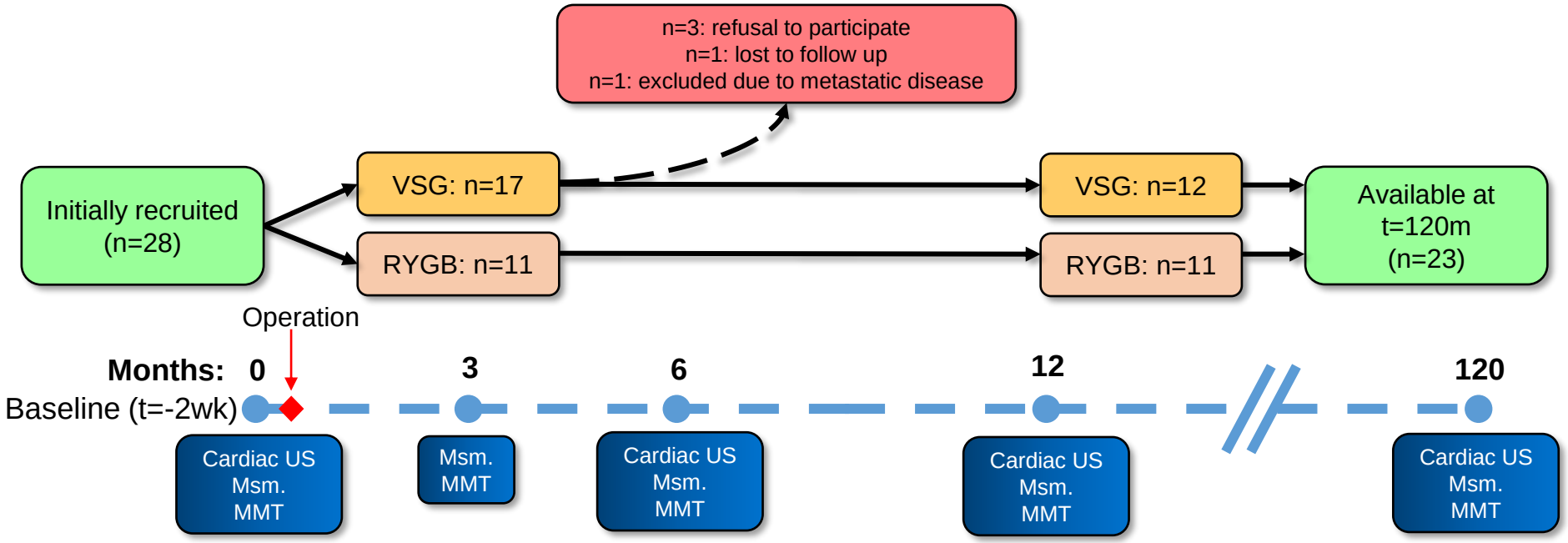


Bariatric surgery also resulted in a significant decrease in NAS compared with baseline (mean difference, 2.39; 95% CI, 1.58–3.20; P < .001; 11 studies)

Lee Y et al. Clin Gastroenterol Hepatol, 2019.

Long-Term Beneficial Effects of Bariatric Surgery on Cardiometabolic Risk and Exploration of Underlying Mechanisms

Kokkinos A., … Mantzoros C. (submitted to NEJM)



Measurements at each visit:

- Anthropometrics
- Body composition
- BP, RMR
- Basic glucose and lipid profile
- Insulin resistance indices
- CBC
- Liver function tests, non-invasive scores
- Renal function tets
- Markers of inflammation
- Adipokines
- Activins / Follistatins
- Metabolomics / lipidomics
- Metabolomics / lipidomics

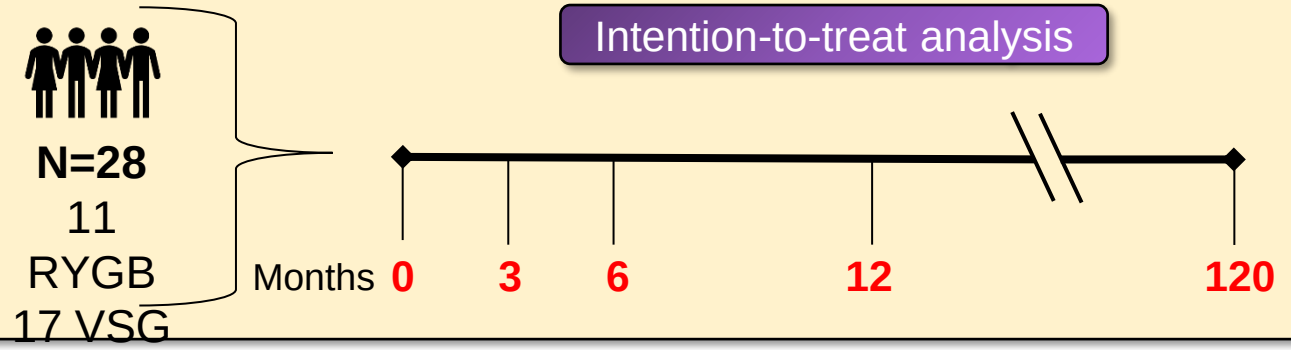
Measurements during MMT (0-180 min, 30-minute intervals)**:**

- Glucose
- Insulin
- Triglycerides
- Proglucagon-derived peptides:
  - GLP-1
  - GLP-2
  - Glucagon
  - Glicentin
  - Oxyntomodulin
  - MPGF
- Ghrelin
- PYY
- Hunger VAS
- Satiety VAS

Long-Term Beneficial Effects of Bariatric Surgery on Cardiometabolic Risk and Exploration of Underlying Mechanisms

Kokkinos A., … Mantzoros C. (submitted to NEJM)

Study design



Main endpoints (all patients)

	Baseline	3 months	6 months	12 months	10 years	p-ANOVA
Body weight (kg)	138.9 ± 21	114.5 ± 17.3***	100.9 ± 17***†††	90.6 ± 17.1***†††§§§	105.8 ± 25.4***‡‡‡	**Time<0.001**
BMI (kg/m^2)	49.6 ± 6.8	41 ± 6.2***	36.5 ± 5.7***†††	32.8 ± 6.2***†††§§§	37.6 ± 8.7***‡‡‡	**Time<0.001**
Fat mass %	49.8 ± 8.2	43.9 ± 7.2***	38.1 ± 9.4***†††	35.4 ± 8.9***†††	38.3 ± 11.5***	**Time<0.001**
Lean Mass %	50.2 ± 8.2	55.1 ± 6*	61.9 ± 9.4***†††	64.6 ± 8.9***†††	61.7 ± 11.5***	**Time<0.001**
Waist circumference (cm)	128.1 ± 12.7	112.9 ± 13.4***	104.6 ± 12.6***††	96.3 ± 13***†††§§	106.1 ± 17.4***‡‡‡	**Time<0.001**
Hip circumference (cm)	143 ± 13.3	127.7 ± 14.4***	121.1 ± 11.3***†	114 ± 12.3***†††§§	125 ± 16.5***‡‡	**Time<0.001**
Waist / hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1*	0.8 ± 0.1**	**Time=0.01**
RMR/energy expenditure (kcal/d)	2090.4 ± 330	1685.2 ± 284.1***	1594 ± 220.8***	1606.9 ± 302.3***	1683.4 ± 358.9**	**Time<0.001**

P-ANOVA for p of time in 2-way ANOVA. *, **, *** for p<0.05, 0.01, 0.001 vs Baseline; †, ††, ††† likewise for p-values vs 3 months, for timepoints after 3 months; §, §§, §§§ likewise for p-values vs 6 months, for timepoints after 6 months; ‡, ‡‡, ‡‡‡ likewise for p-values vs 12 months for the 10-year timepoint. Data presented as means ± SD.

Long-Term Beneficial Effects of Bariatric Surgery on Cardiometabolic Risk and Exploration of Underlying Mechanisms

Clinical outcomes (all patients)

	Baseline	3 months	6 months	12 months	10 years	p-ANOVA
Basic Metabolic panel						
Fasting plasma glucose (mg/dL)	109.7 ± 30.3	89.1 ± 14.6*	92.1 ± 8.5**	90.4 ± 7.1*	92.9 ± 7.2*	**Time<0.001**
Fasting plasma insulin (mIU/L)	21.1 ± 6.2	9.8 ± 3.4***	9.3 ± 4.3***	7.5 ± 4.8***	8.7 ± 5.1***	**Time<0.001**
Fasting plasma triglycerides (mg/dL)	107.6 ± 39.4	104.1 ± 20	89.1 ± 25.9†	82.5 ± 20†††	61.3 ± 30.9***†††§§‡	**Time<0.001**
Total Cholesterol (mg/dL)	186.1 ± 27.3	178.8 ± 35	176 ± 39.2	170.3 ± 25.1	161.5 ± 25.4*	**Time<0.001**
HDL cholesterol (mg/dL)	41.6 ± 5.9	41.3 ± 6.1	41.8 ± 5.8	41.2 ± 4.2	51.3 ± 11.9**†§‡‡	**Time=0.04**
LDL cholesterol (mg/dL)	126.1 ± 28	116.6 ± 33.8	116.4 ± 39.3	113.5 ± 23.1	96.8 ± 23.1**	**Time<0.001**
HOMA-IR	4.8 ± 1.5	2.3 ± 0.9***	2.1 ± 1***	1.6 ± 1.1***	2 ± 1.2***	**Time=0.01**
Liver Function tests						
AST (IU/L)	19.4 ± 5.5	18.2 ± 4.3	14.9 ± 5*†	17.7 ± 8.1	17.2 ± 4	Time=0.07
ALT (IU/L)	25.3 ± 10.7	18 ± 7.1*	15.5 ± 11*	18.3 ± 9.5	14.9 ± 4.5**‡‡	**Time=0.004**
ALP (IU/L)	170.9 ± 67.4	150.8 ± 60.1	162.4 ± 52.9	150.8 ± 68.5	70.4 ± 18.9***††§§§‡	**Time<0.001**
γGT (IU/L)	24.1 ± 10	13.5 ± 5.6**	13.4 ± 6.6**	16.9 ± 11.4	10.7 ± 4.3***	**Time<0.001**
Renal, inflammation and gut microbiota markers						
eGFR (CKD-Epi)	103.1 ± 11.9	110.1 ± 7.2	108.3 ± 8.9	112.3 ± 4.6	101.3 ± 8.6†‡‡	Time=0.11
CRP (mg/dL)	10 ± 9.8	8.4 ± 10.1	6.3 ± 7.7	3.2 ± 3.3***††	6.5 ± 3.2	**Time<0.001**
GlycA (µmol/L)	468.2 ± 59.1	441.5 ± 68.7	418 ± 72.8**	392.3 ± 67.6**	395.7 ± 56.9**	**Time<0.001**
TMAO (µmol/L)	1.99 ± 1.23	2.55 ± 2.55	2.91 ± 3.98	2.8 ± 2.7	4.27 ± 4.32	**Time=0.005**
Data from CBC						
Neutrophils (10^3)	5.3 ± 1.7	3.6 ± 1.2***	3.6 ± 1.4***	3.5 ± 1.5*	3.5 ± 1.3***	**Time<0.001**
Lymphocytes (10^3)	2.2 ± 0.6	1.9 ± 0.4	1.8 ± 0.5	1.9 ± 0.3	1.6 ± 0.4**†‡	**Time<0.01**

	Baseline	6 months	12 months	10 years	p-ANOVA
Cardiac US					
Left ventricular end systolic diameter (mm)	35.4 ± 2.8	33.4 ± 2.4***	32.4 ± 2.4***§§§	30.3 ± 3.1**§§‡	**Time<0.001**
Left ventricular end diastolic diameter (mm)	54 ± 2.9	52.8 ± 2.4*	51.9 ± 2.4***§§§	48.4 ± 2.7**§§§‡‡	**Time<0.001**
Epicardial fat thickness (cm)	1.61 ± 0.12	1.42 ± 0.09***	1.42 ± 0.12***	1.21 ± 0.19***§§‡‡‡	**Time<0.001**
Ejection Fraction (%)	58.7 ± 3.5	62.6 ± 4.3**	67.1 ± 14.2*	62 ± 2.9	**Time<0.01**

P-ANOVA for p of time in 2-way ANOVA. *, **, *** for p<0.05, 0.01, 0.001 vs Baseline; †, ††, ††† likewise for p-values vs 3 months, for timepoints after 3 months; §, §§, §§§ likewise for p-values vs 6 months, for timepoints after 6 months; ‡, ‡‡, ‡‡‡ likewise for p-values vs 12 months for the 10-year timepoint. Data presented as means ± SD.

Long-Term Beneficial Effects of Bariatric Surgery on Cardiometabolic Risk; Exploration of Underlying Mechanisms

Peptide responses to mixed meal test and hormonal tests (all patients)

	Baseline	3 months	6 months	12 months	10 years	p-ANOVA
Basic Indices (AUC)						
Glucose (mg/dL)xmin	109.7 ± 30.3	89.1 ± 14.6*	92.1 ± 8.5**	90.4 ± 7.1*	92.9 ± 7.2*	**Time<0.001**
Insulin (mIU/L)xmin	21.1 ± 6.2	9.8 ± 3.4***	9.3 ± 4.3***	7.5 ± 4.8***	8.7 ± 5.1***	**Time<0.001**
Triglycerides (mg/dL)xmin	107.6 ± 39.4	104.1 ± 20	89.1 ± 25.9†	82.5 ± 20†††	61.3 ± 30.9***†††§§‡	**Time<0.001**
Gut Peptides (AUC)						
GLP-1 (pg/mL)xmin	32485.9 ± 12179.5	42528.4 ± 11898.3	37099.2 ± 10312.2	43253.5 ± 18712.8*	52847.6 ± 12101.8***†§§§	**Time<0.001**
GLP-2 (ng/mL)xmin	297.8 ± 148.1	414.3 ± 134.3**	355.9 ± 133.9	344.8 ± 101.1	406.1 ± 148.5*	**Time<0.001**
Glucagon (pg/mL)xmin	7621.6 ± 4895.4	7391.5 ± 4096.6	6075.5 ± 3012.3	7201.8 ± 3352	10631.8 ± 5104.8†§§‡	**Time<0.001**
Oxyntomodulin(pg/mL)xmin	43854.3 ± 26505.7	188159.9 ± 89174.5***	153461 ± 82790.1***	173905.8 ± 95938.7***	191910.9 ± 112041.6***	**Time<0.001**
Glicentin (pg/ml)xmin	4215.5 ± 2439.2	16651.3 ± 8123.3***	12790.3 ± 7659.6***	15012.6 ± 8867.1***	12799.8 ± 6662.6***	**Time<0.001**
MPGF (ng/mL)xmin	207.5 ± 125.1	160 ± 54.4	131 ± 52.4*	157.8 ± 76.8	141.1 ± 63.2*	**Time<0.002**
Ghrelin (pmol/L)xmin	32252.8 ± 11087.6	16673.2 ± 13292.6***	18426.9 ± 16879.5*	23770.6 ± 15269.8	28446.9 ± 25403.8	**Time<0.001**
PYY (pmol/L)xmin	13624.7 ± 5598.6	19355.5 ± 5617.5**	20976.5 ± 7849.7**	23470.8 ± 9014.2***	22619.9 ± 9528.6***	**Time<0.001**
Hunger and satiety iAUC						
Hunger iAUC (VASxmin)	-81.4 ± 3597	-1596.6 ± 4900.2	-3227.4 ± 4560.8	-2069.3 ± 4059.6	-2172.4 ± 3612.7	**Time=0.047**
Satiety iAUC (VASxmin)	1917.9 ± 4199.6	1636.3 ± 5466	3518.1 ± 4999.5	3605.5 ± 4616.9	3390.7 ± 3742.4	**Time=0.33**
Adipokines and activins/follistatins						
Leptin (ng/mL)	89.6 ± 28.9	42.8 ± 24.7***	33.9 ± 23.2***	26.4 ± 18.9***†††	58.3 ± 42.4*§‡	**Time<0.001**
Adiponectin (ug/mL)	9.6 ± 3.6	10.8 ± 2.9	12 ± 2.6**	13.9 ± 5**†	15.8 ± 4.4***†††§§	**Time<0.001**
Adiponectin/leptin ratio	0.1 ± 0.1	0.3 ± 0.2***	0.5 ± 0.3**	0.5 ± 0.3**†††	0.3 ± 0.4§‡	**Time<0.001**
Activin A (pg/mL)	432.8 ± 149.7	377 ± 98.3	319.3 ± 83*†	316.7 ± 86.5*†	455.7 ± 107.3§§§‡‡‡	**Time<0.001**
Activin B (pg/mL)	120.7 ± 42.6	124.1 ± 49.8	122.7 ± 54.3	123.2 ± 49.6	152.2 ± 42.1**§	**Time=0.02**
Activin AB (pg/mL)	5.7 ± 2.6	8.8 ± 5.2**	7.3 ± 3.8	8.9 ± 5.3	12.3 ± 4.3***§§§‡	**Time<0.001**
Follistatin (ng/mL)	4.5 ± 1.3	4 ± 1.1	3.5 ± 1*	3 ± 0.9***†§	5.6 ± 1.5††§§§‡‡‡	**Time<0.001**
Follistatin-like 3 (ng/mL)	16.2 ± 4	15.1 ± 3.6	13.3 ± 2.8***†††	14.2 ± 4.3	17.5 ± 6.8	**Time=0.02**

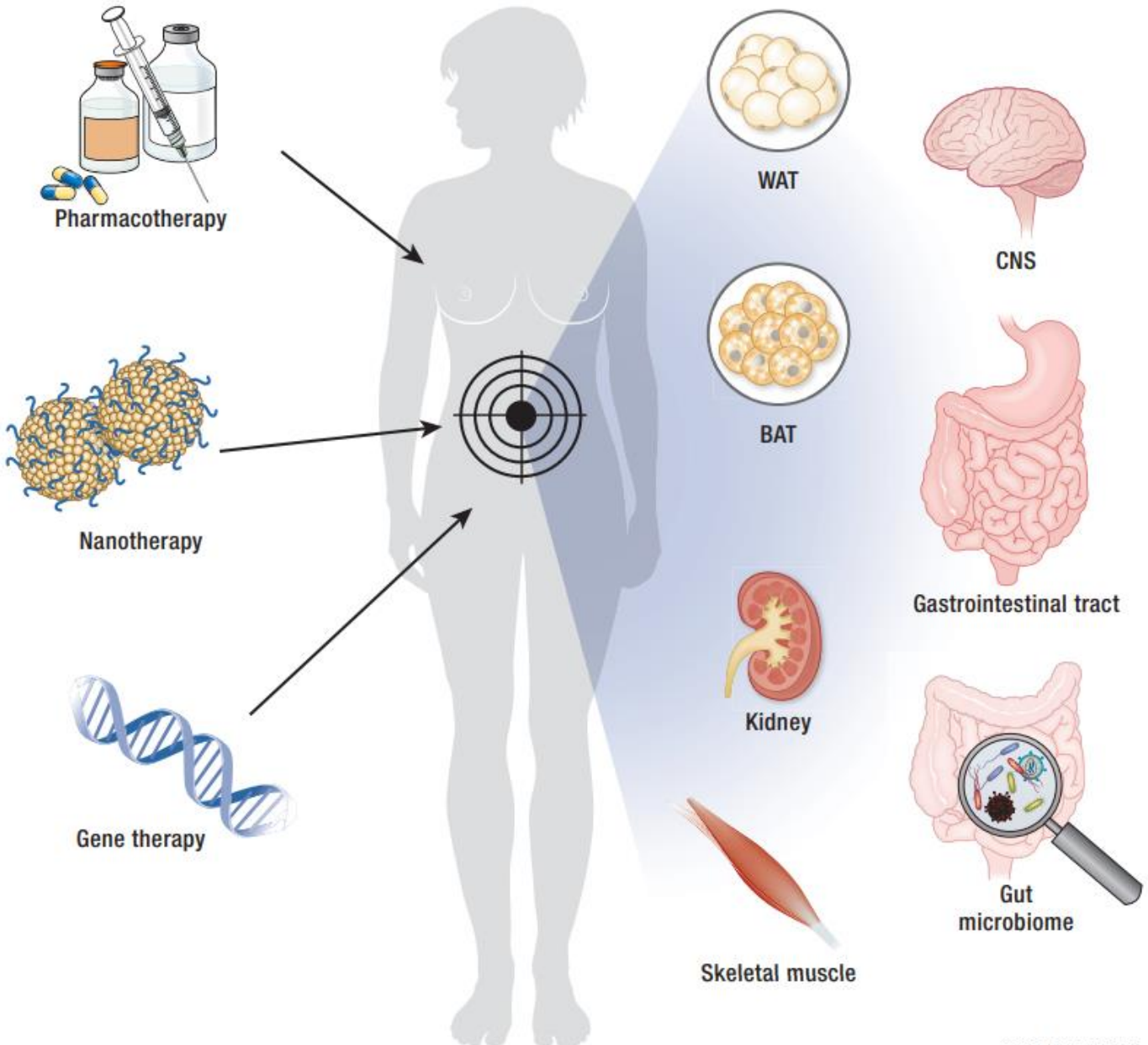
P-ANOVA for p of time in 2-way ANOVA. *, **, *** for p<0.05, 0.01, 0.001 vs Baseline; †, ††, ††† likewise for p-values vs 3 months, for timepoints after 3 months; §, §§, §§§ likewise for p-values vs 6 months, for timepoints after 6 months; ‡, ‡‡, ‡‡‡ likewise for p-values vs 12 months for the 10-year timepoint. Data presented as means ± SD.

Next Steps

- **BRAVES** study focusing on the effects of surgery on NASH outcomes:
  - Trial just completed enrollment and we started assessing laboratory parameters

- Make medications using these hormones alone or in combination and administer them instead of having surgery **- "surgery in a pill"**
  - (We have applied for funding to study the latter)

The future: Developing Potential Therapies for Metabolic Diseases



Angelidi A, et al. Endocrine Society, 2021.

Novel Non-invasive Approaches to the Treatment of Obesity: From Pharmacotherapy to Gene Therapy

Angelidi A, Belanger M, Koliaki C, Kokkinos A, Mantzoros C.

Endocrine Reviews 2021 (in press)

Central Nervous System

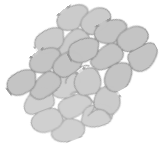
CNC Secreted Neuropeptides and Antagonists

- Tesofesine
- Oxytocin
- NPY antagonists
- Methylphenidate
- GDF-15

Endocannabinoid System Agents

- CB1R Antagonists *(rimonabant, AM251, AM6545, JD5037)*
- Agents targeting GPR *(GPR55, GPR18, GPR119)*

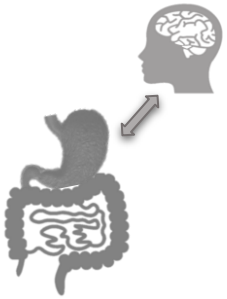
Adipose tissue



- Leptin
- B3-adrenoreceptor agonists (*mirabegron*)
- Brown fat transplantation
- PPAR-γ agonists
- Other Adipokines

GI System

Gut-derived hormones and gut-brain axis



- CKK
- PYY analogues
- OXM
- Glicentin
- Ghrelin
- Secretin
- GLP-1 agonists *(exenatide, liraglutide, lixisenatide, dulaglutide, albiglutide, semaglutide)*

Combinations

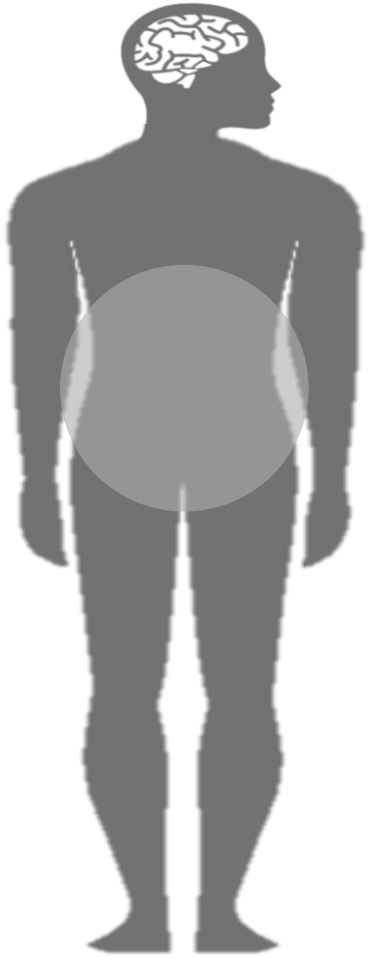
- Dual agonists *(GLP-1/glucagon, GLP-1/GIP)*
- Triple agonists (GLP-1/GIP/glucagon, GLP-1/OXM/PYY)
- Synthetic GLP-1/glucagon coagonists *(Cotadutide, SAR425899)*
- Synthetic GLP-1/GIP coagonists (*tirzepatide*)
- DACRAs

Other gut peptide-related approaches

- FGF21 analogues
- Bile acids

Pancreas

- Insulin
- Amylin analogues



Modulation of gut microbiome

- Prebiotics
- Probiotics
- Fecal microbiota transplantation

Kidney

- SGLT2i *(dapagliflozin, canagliflozin, empagliflozin, ertugliflozin)*
- SGTL1/2i *(sotagliflozin, licogliflozin)*

Skeletal muscle

- Bimagrumab

Novel Drug Delivery Systems

- Oral peptide engineering *(oral semaglutide)*
- PHB-targeted nanotherapy
- Nanotechnology-based photothermal lipolysis
- WAT browning

Gene therapy

Antiobesity Vaccines

- Oral immunization
- VLP vaccines
- Ghrelin-targeted antiobesity vaccines