NIHR | Southampton Biomedical Research Centre

UNIVERSITY OF Southampton

‘Diabetes and nonalcoholic fatty liver disease: ‘screening’ and emerging therapies

Chris Byrne

Professor Endocrinology & Metabolism,

University of Southampton
& Southampton NIHR BRC, University Hospital Southampton

PCDS Masterclass
2023

Wednesday 13th September 2023. 11.20 - 12.05 & 14.10 - 14.55
T2D & NAFLD: Screening and emerging therapies.

Disclosures

- September 2023. Independent Research Grant Echosens France.

Background……beginning in 1980. Nonalcoholic steatohepatitis:

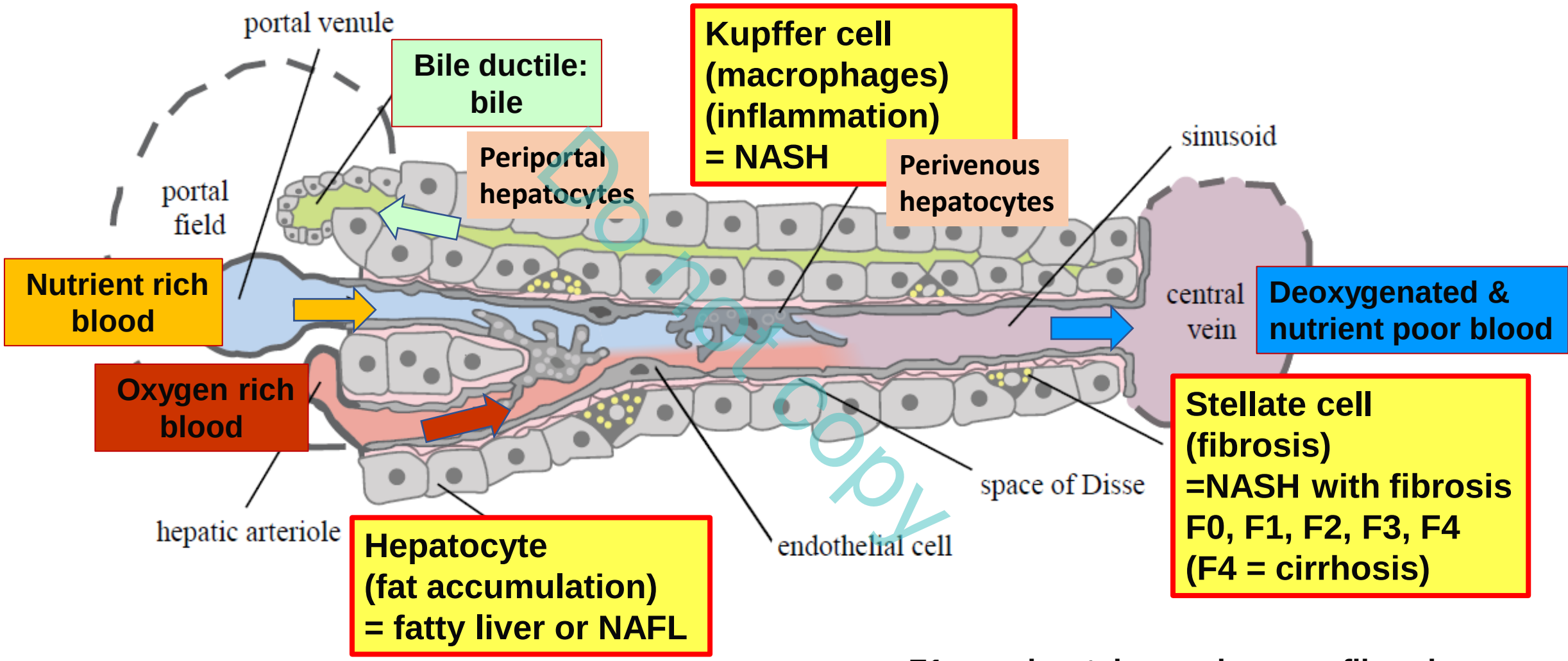
Mayo Clinic experiences with a hitherto unnamed disease

- Described here are findings in 20 patients with **nonalcoholic steatohepatitis of unknown cause**. The biopsy specimens were characterized by the presence of striking fatty changes with evidence of lobular hepatitis, focal necroses with mixed inflammatory infiltrates, and, in most instances, Mallory bodies;
- **Evidence of fibrosis was found in most specimens, and cirrhosis was diagnosed in biopsy tissue from three patients.**
- Most patients were moderately obese, and many had obesity-associated diseases, **such as diabetes mellitus** and cholelithiasis.
- Mild abnormalities of liver function were common clinical findings.
- Currently, we know of no effective therapy

Ludwig et al. Mayo Clin Proc 1980 Jul;55(7):434-8.

N.B. Diabetes + NAFLD = MAFLD (2020); Diabetes + NAFLD = MASLD (2023)

Structure of the liver lobule & flow of blood and bile

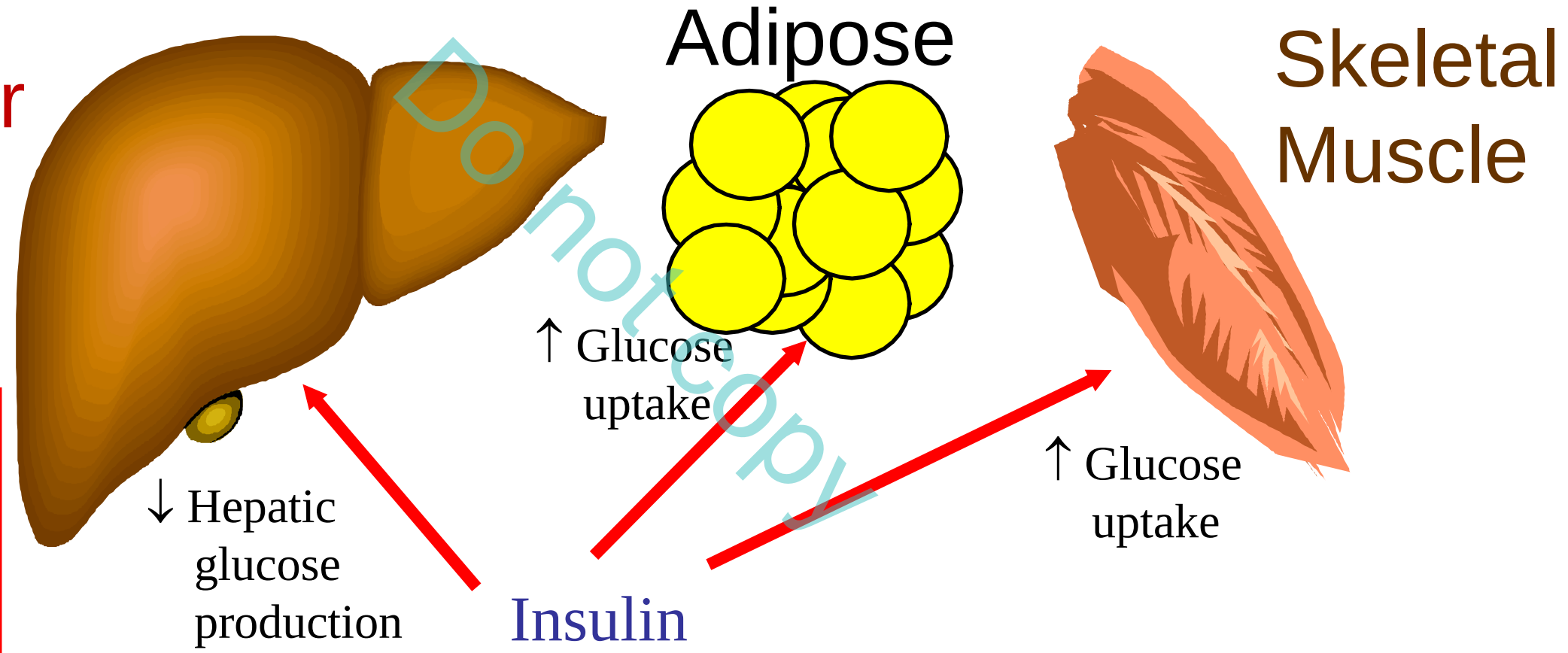


Modified from: Frevert U, Engelmann S, Zougbédé S, Stange J, Ng B, et al. (2005) PLOS Biology 3(6): e192.

F1 = periportal or perivenous fibrosis
F2 = periportal & perivenous fibrosis
F3 = bridging fibrosis
F4 = nodules of regenerating cells + fibrosis

NAFLD: insulin resistance

Insulin resistance occurs in each insulin-sensitive tissue with fatty liver disease



Dr Helen Holt

2006/7

Holt H et al. Diabetologia 2006 49:141-148

Holt H et al. *Diabetologia* 2007; 50: 1698–1706;

Holt H et al. *Diabetologia* 2007; 50: 1024–1032.

CASE REPORT (with 20 years of follow up from 2003 to 2023)

2003.

- Thank you for referring X.. He is a 40 year old man who has a history extending back over about five years of increasing thirst but his diagnosis of diabetes was made in February at a routine medical. His history is otherwise unremarkable. He has no other family history of type 2 diabetes or heart disease.

- X is a non-smoker but has noticed insidious weight gain over the last few years. His weight is now 83 kg (BMI 30kg/m2), whereas he weighed 70kg (BMI 25kg/m2) at the age of 24 years.

- I am concerned about his high carbohydrate consumption. I have encouraged him therefore to eat a more normal diet and encouraged him to eat unsaturated fat which will have very little effect on his cholesterol anyway. We discussed the various measures that he might adopt to reduce carbohydrate intake to precipitate weight loss and I have encouraged him to increase his levels of physical activity in an attempt to lose weight.

- The mainstay of his therapy at least in the short term is weight loss and alteration of his diet to control his hyperglycaemia.

- Today in clinic there was no evidence of proteinuria but he had + glucose in his urine. His blood pressure was 124/77 mmHg, his waist measurement 96 cm and his weight 83 kgs at a height of 1.67m. BMI 30kg/m2.

CASE REPORT 2003 to 2022

- DOB 06/11/1962
- **2003 to 2022** . HbA1c 10.9% (98mmol/mol (2003) to 70mmol/mol (2022)
- **2003.** TC 7.0mmol/L, TG 3.1mmol/L (2003).
- **2003 to 2022.** ALT 69IU to 160IU (2017) but often within the normal range.
- 66iu/L in 2022

CASE REPORT 2003 to 2022-treatment

- **2014 (age 51 years) Southampton NIHR BRC Participant in INSYTE RCT. Insulin 57.6 pmol/L, HOMA-IR = 3.4. HbA1c 63mmol/mol. ALT 62IU/L. AST 37 IU/L. GGT 57 IU/L. ACR 2.4. GLP-1 204..**
- **10 year CVD risk prediction 10.2%. BMI 28.8kg/m2. ELF 6.8. Mean MRS Liver fat % =39. Liver stiffness 5.9kPa. V02 max 24mls/kg/min**
- **Metformin 1g bd. and rosuvastatin 10mg/day.**
- **2017.** ELF =9.5. Dapagliflozin 10mg/day added.
- **11/2021** – prescribed semaglutide 1mg/week.
- **05/2022** -prescribed pioglitazone 30mg/day

CASE REPORT 2023 results & treatment

- **May 2023** – HbA1c 44mmol/mol, ALT 25IU/L.
- HbA1c now 46 mmol/mol; NASH. FibroScan in 2020: 8.6 kPa (equivalent to F2); Primary hypothyroidism
- **Recent blood tests: 2nd May 2023.** ALT 25 international units/litre. LDL cholesterol 1.83 millimoles/litre. Triglycerides 1.7 mmols per litre. Free T4 13.5 picomoles per litre. TSH 1.08 mU/L.
- **15 May 2023:** Weight 74.4 kg. BMI 26.2kg/m2. BP 124/71mmHg.
- **Current medication:** Dapagliflozin 10 milligrams daily. L-thyroxine 100 micrograms daily. Metformin 1 gram BD. Pioglitazone 30 milligrams once daily. Rosuvastatin 10 milligrams daily. Semaglutide 1 milligram by injection per week.

Diabetes is the most important risk factor for cirrhosis and HCC

18 million patients four European cohorts

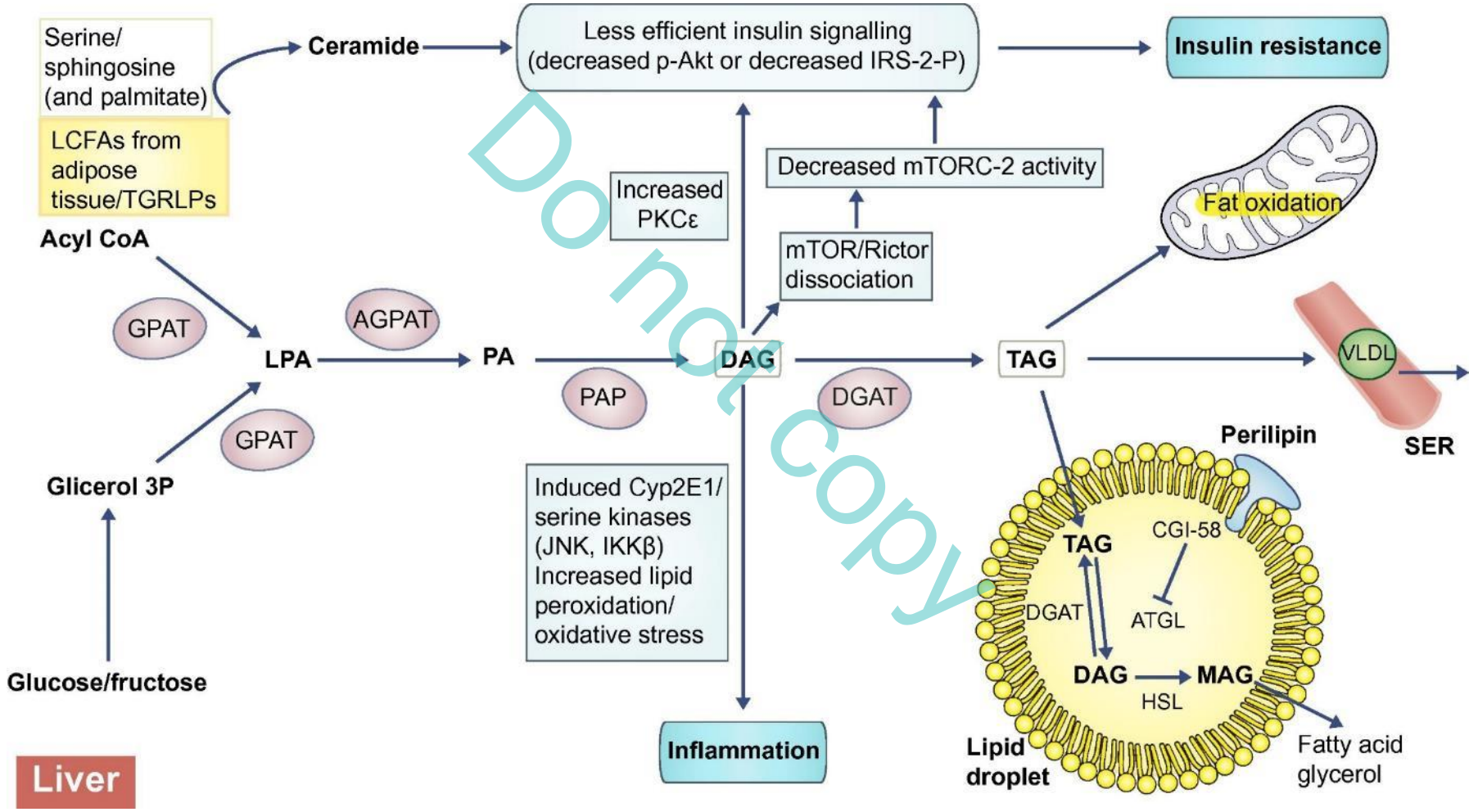
- Four databases, the median duration of follow-up was 3.3 years (IQR 1.8–5.3) totalling 531,452 person-years for patients with coded NAFLD/NASH and 43,385,495 person-years for controls (no NAFLD/NASH).

- Coded NAFLD/NASH: more likely to have diabetes/hypertension/obesity
- Apart from a diagnosis of NAFLD/NASH, diabetes was the strongest independent risk factor for acquiring a diagnosis of cirrhosis or HCC.
- HR for cirrhosis in patients compared to controls was 4.73 (95% CI 2.43–9.19) and for HCC, 3.51 (95% CI 1.72–7.16).

- N.B. In the matched control population, the HR for diabetes was even higher than the coded NAFLD/NASH cohort, which may reflect a significant number of individuals with undiagnosed NAFLD/NASH among the controls

Alexander et al. BMC Medicine (2019) 17:95

Liver lipids induce hepatic IR and inflammation

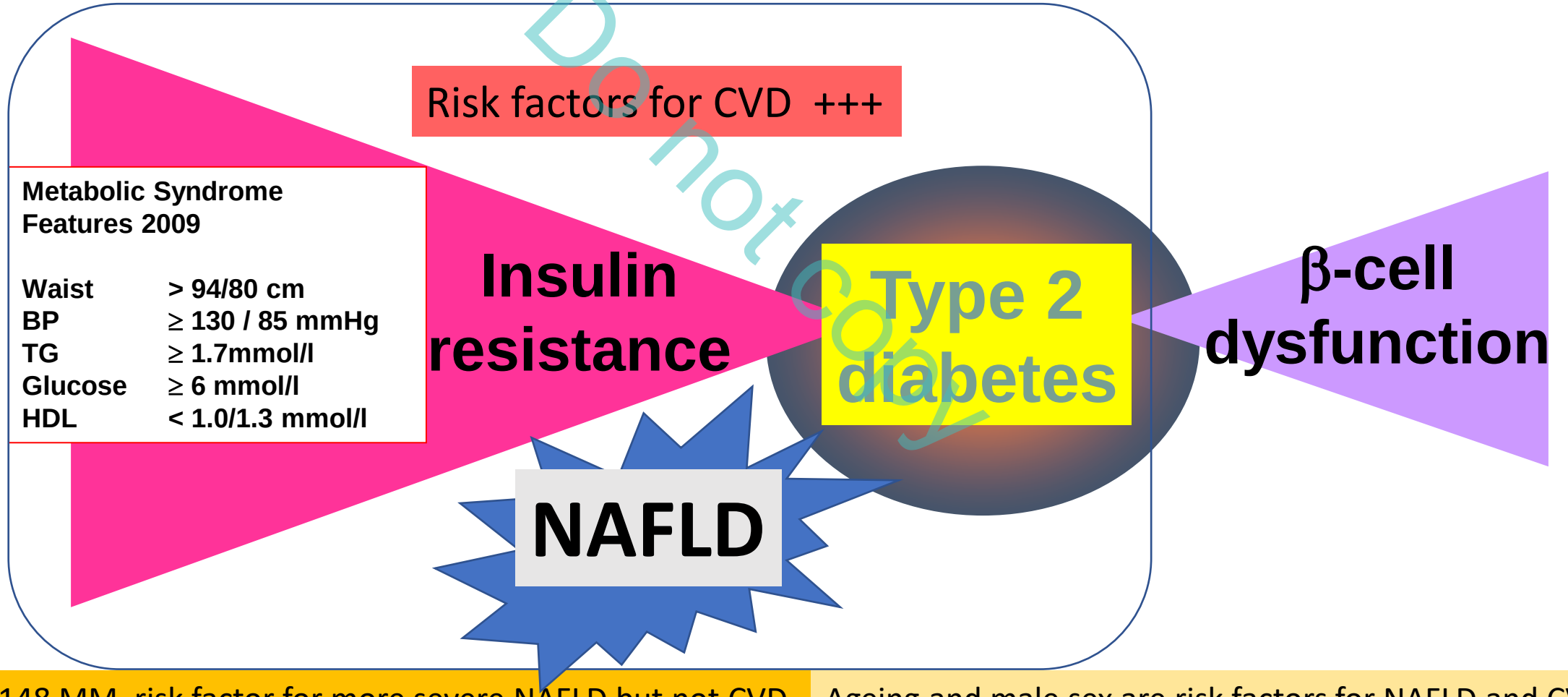


Byrne CD, Targher G. J Hepatol 2015;62:S47–64

MetS and type 2 diabetes are important CVD risk factors:

& MetS features are easily recognised and occur frequently with NAFLD

N.B. fatty liver disease + MetS features (or T2DM, or obesity) = MAFLD (2020)/MASLD (2023)

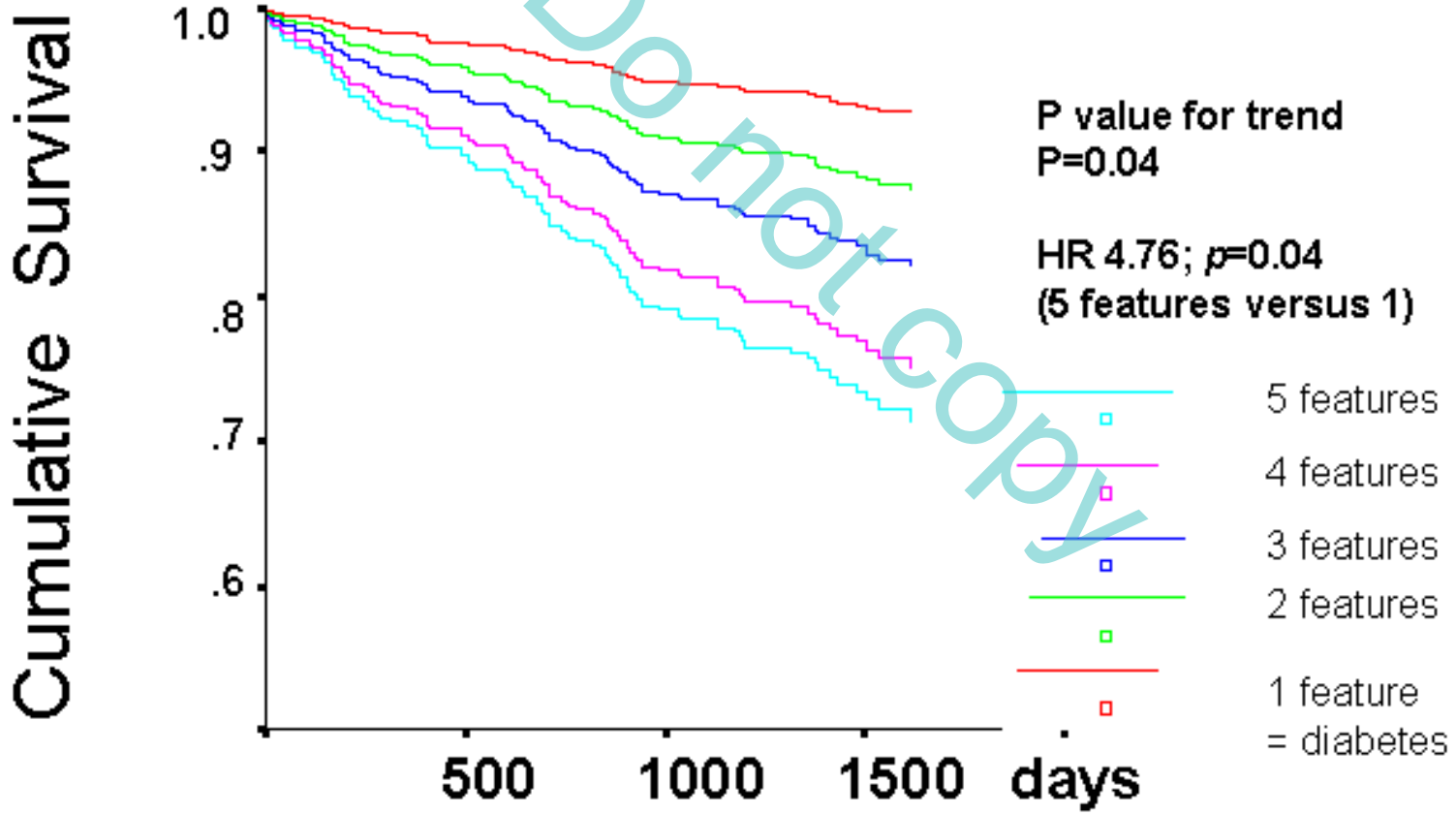


PNPLA3 148 MM, risk factor for more severe NAFLD but not CVD

Ageing and male sex are risk factors for NAFLD and CVD

In type 2 diabetes:

Survival is worsened with increasing number of features of Metabolic syndrome



& NAFLD occurs frequently with MetS features

Metabolic Syndrome Features

Waist	**> 94/80 cm**
BP	**≥ 130 / 85**
TG	**≥ 1.7mmol/l**
Glucose	**≥ 6 mmol/l**
HDL	**< 1.0/1.3 mmol/l**

Gudzer, Gatling, Mullee, Byrne Diabetologia 2006 Jan;49(1):49-55

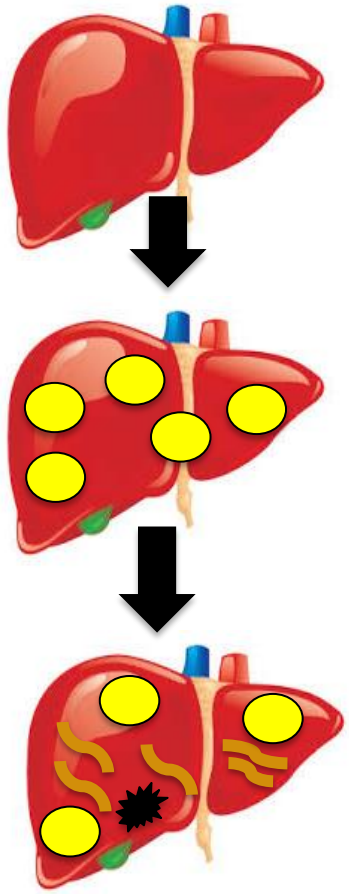
2) NAFLD: a multisystem disease

J Hepatology. 2015 & Lancet Gastroenterol Hepatol. 2021

Normal liver

Steatosis

NASH ± fibrosis



Insulin resistance/hyperinsulinaemia and related disorders
(e.g. atherogenic dyslipidaemia, hypertension, dysglycaemia, high non-esterified fatty acids)

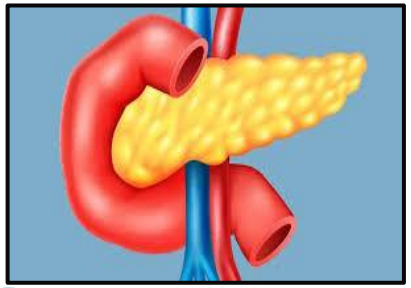
Vasoactive and thrombogenic molecules
(e.g. fibrinogen, transforming growth factor-beta, plasminogen activator inhibitor-1, reactive oxygen species)

Proinflammatory factors
(e.g. interleukin-1 beta, interleukin-6, tumour necrosis factor-alpha, C-reactive protein)

CVD, Arrhythmias & HF

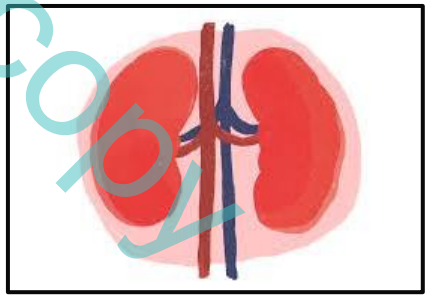
Lancet Gastroenterol Hepatol. 2021 6(11):903-913

Gut. 2022 25:gutjnl-2022-327672



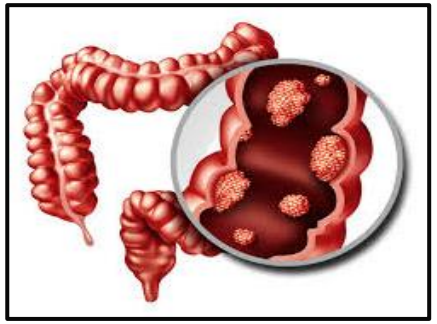
Type 2 diabetes

Gut. 2021 May;70(5):962-969



Chronic kidney disease

Gut. 2022 71:156-162.



Extra-hepatic cancers

Gut. 2022;71:778-788

Ale Mantovani
Gio Targher

Ki-Chul Sung
Yoosoo Chang
Seungho Ryu

Sarah Wild

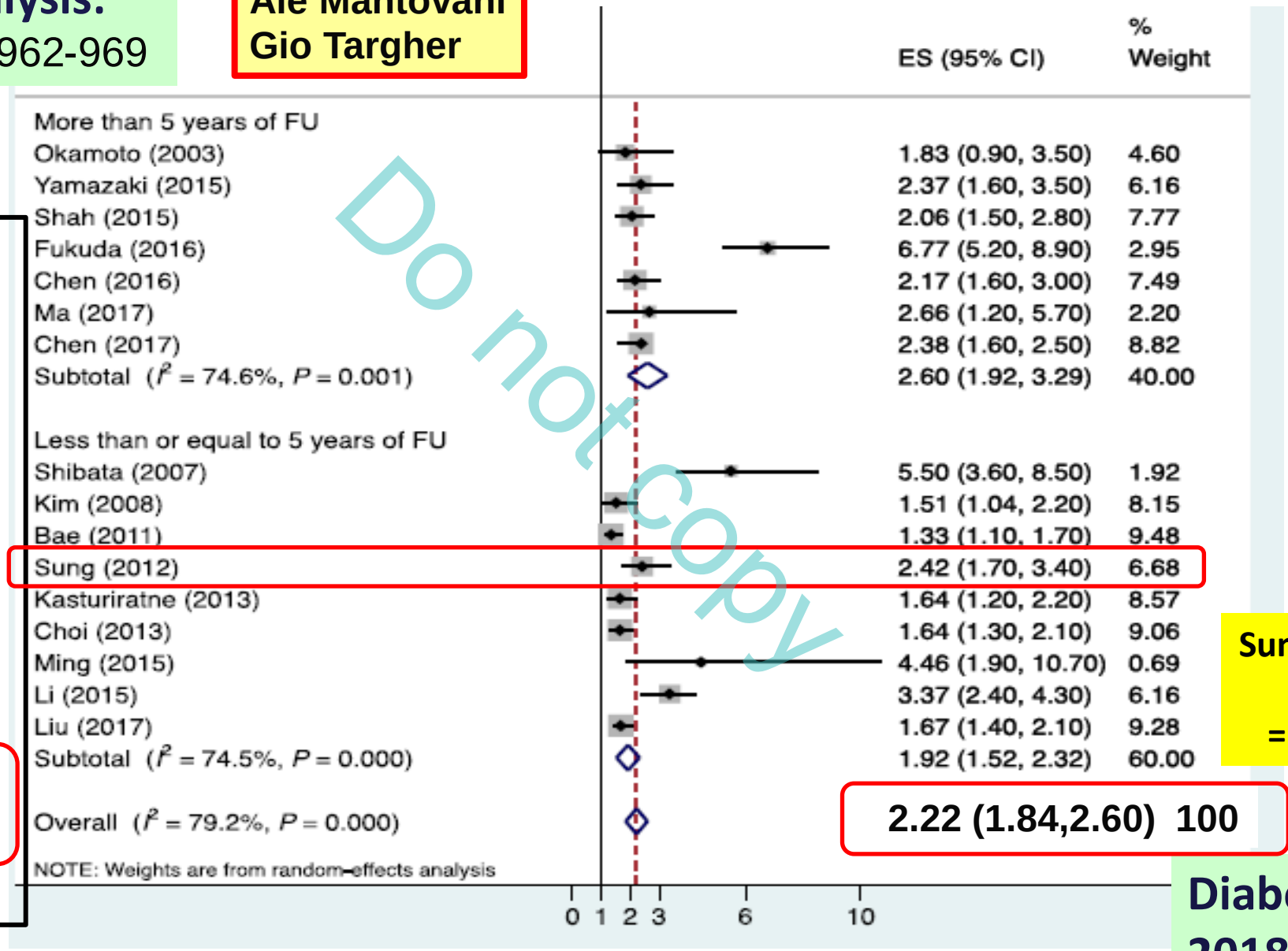
NAFLD increases risk of incident diabetes

Updated meta-analysis:
Gut. 2021 May;70(5):962-969

Ale Mantovani
Gio Targher

- **33 studies (501 022 individuals) (30.8% with NAFLD)**
- **27 953 cases of incident diabetes over a median of 5 years (IQR: 4.0-19 years).**
- **Patients with NAFLD had a higher risk of incident diabetes than those without NAFLD**

random-effects HR 2.19, 95% CI 1.93 to 2.48; I^2 =91.2%)

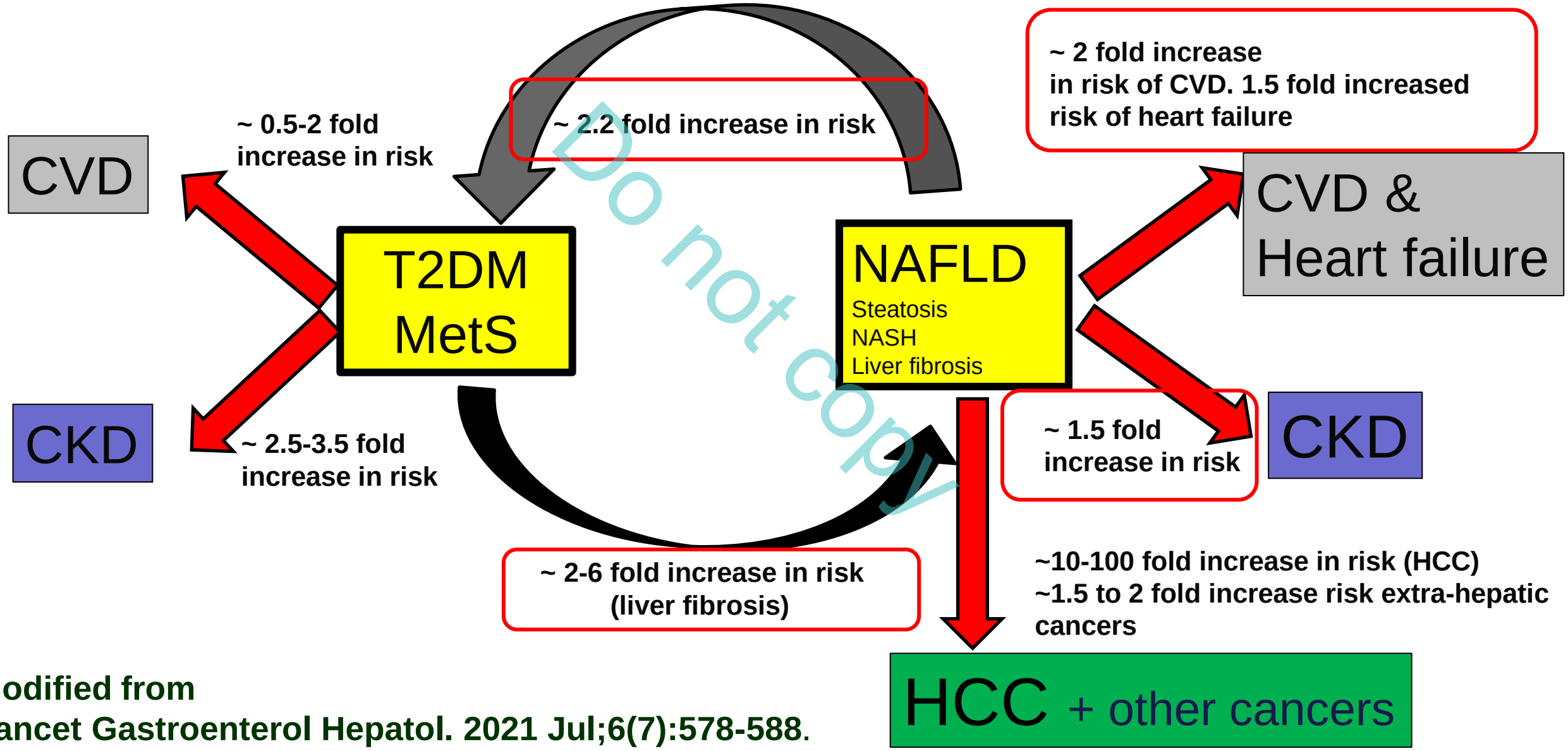


Summary HR (95%CIs)

= 2.22 (1.84, 2.60)

Diabetes Care 2018; 41: 372-382

……..and Type 2 diabetes and NAFLD represent a vicious spiral of adverse outcomes



Modified from
Lancet Gastroenterol Hepatol. 2021 Jul;6(7):578-588.

NAFLD improves risk prediction for type 2 diabetes and effect modification by sex and age (Hepatology. 2022 Dec;76(6):1755-1765)

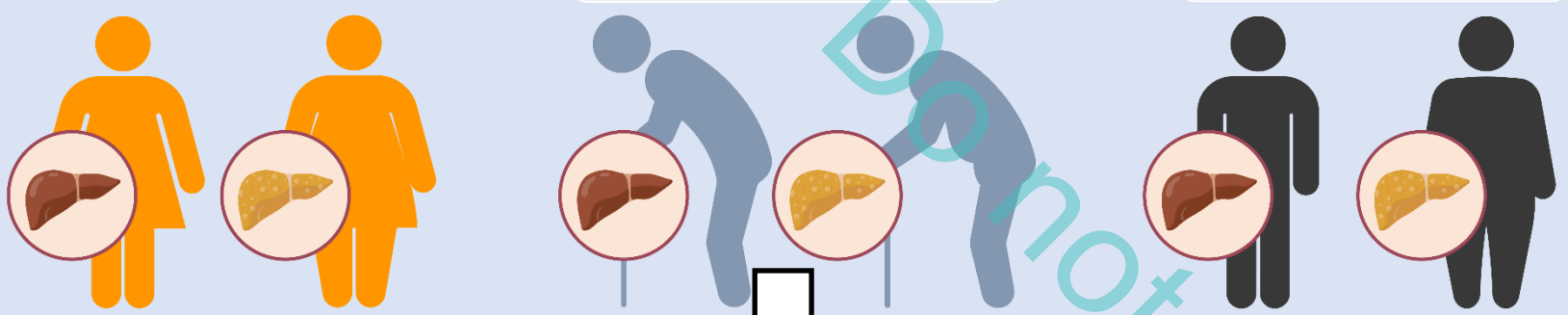
Yoosoo Chang
Seungho Ryu
Sarah Wild

A total of 245,054 adults without diabetes were included.

Premenopausal women (n=109,810)

Postmenopausal women (n=4,958)

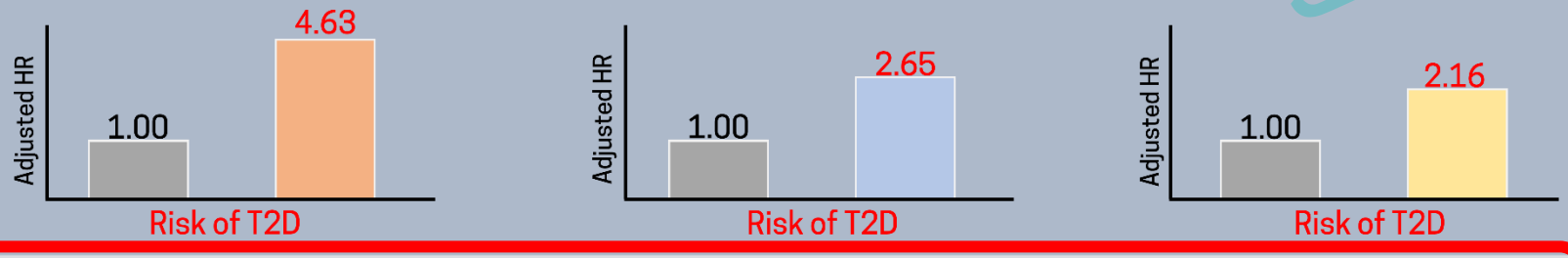
Men (n=130,286)



Median follow-up: 5.3 years

Incidence density : per 10^3 person-years

1.5 vs. 18.7	6.2 vs. 27.5	4.5 vs. 16.6



NAFLD is a much stronger risk factor for incident diabetes in premenopausal women than in post-menopausal women or men.

Conventional risk factors + NAFLD

Age, Family history of diabetes, Hypertension, BMI, waist circumference

Combined risk prediction model for T2D (AUROC)

	Base	+NAFLD
Premenopausal	0.823	0.838 ↑
Postmenopausal	0.708	0.743 ↑
Men	0.744	0.758 ↑

The addition of NAFLD to conventional risk factors improved risk prediction for incident T2D in both sexes, with a highest discriminatory power in pre-menopausal women than men or post-menopausal women.

NAFLD increases risk of incident CVD events (fatal, non-fatal or both)

J. Hepatology 2016; 65: 589-600 → Updated systematic review and meta-analysis → Lancet Gastroenterol Hepatol. 2021 Nov;6(11):903-913

SUMMARY:

36 longitudinal studies aggregate data on 5.8 million middle-aged adults

Mean (SD) age 53 (7) years

99668 incident fatal and non-fatal CVD events

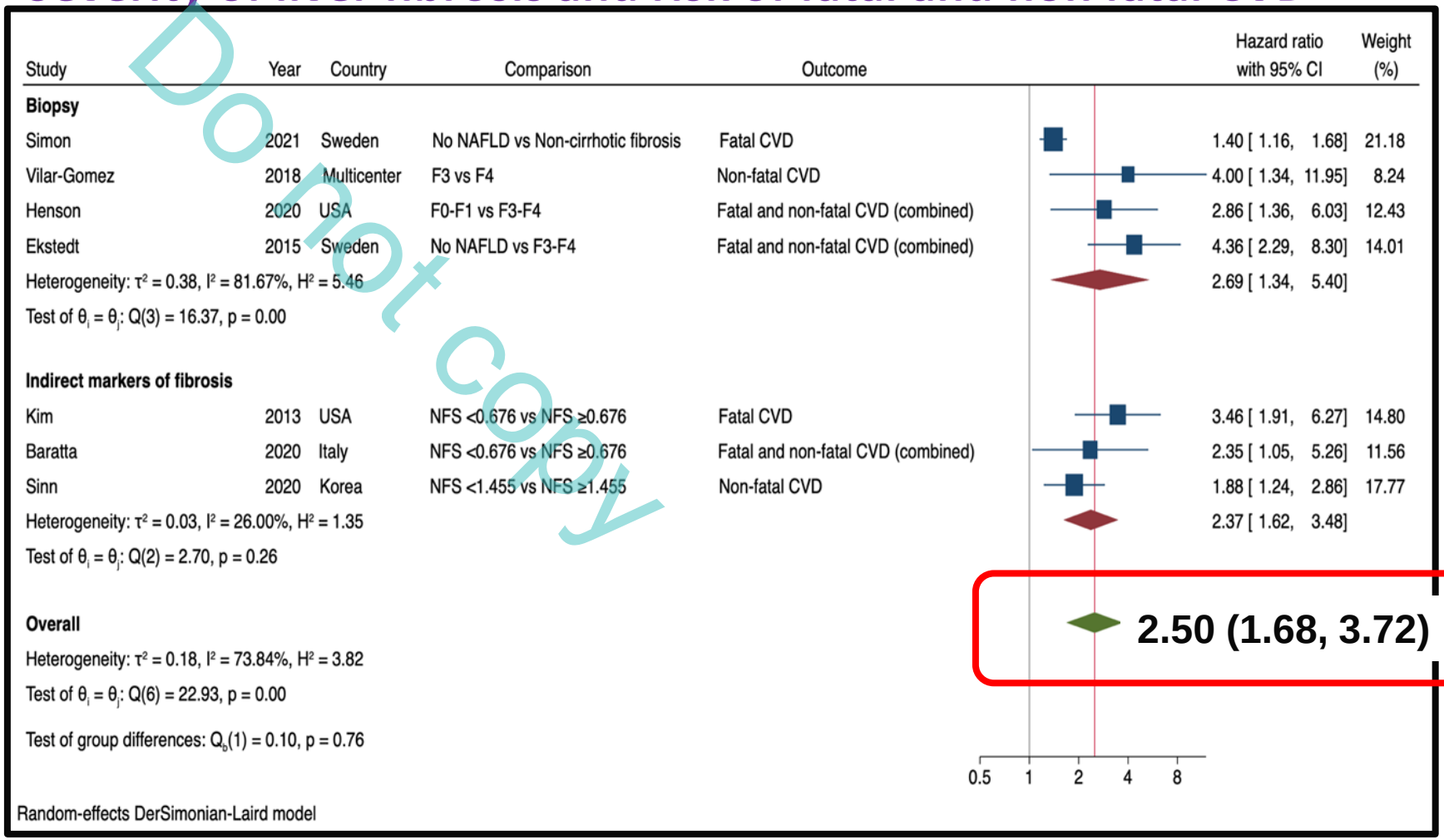
Median (IQR) follow up 6.5 (5.0-10.2) years

NAFLD associated with increased risk of incident CVD events pooled random-effects **HR 1.45 (95%CI 1.31, 1.61)**

(independent of age, sex, diabetes, adiposity measures, common CVD risk factors.

Ale Mantovani
Gio Targher

Severity of liver fibrosis and risk of fatal and non fatal CVD



NAFLD and risk of CVD: modified by T2DM, genotype and maybe LDL-C

- Meta-regression analyses to examine the effect of potential moderator variables, showed a significant positive association between the proportion of patients with pre-existing type 2 diabetes (p=0·001) and LDL-C (p=0.04) **Lancet Gastroenterol Hepatol. 2021 Nov;6(11):903-913**

Ale Mantovani
Gio Targher

- NAFLD increases risk of CVD in patients with T2DM

Wild et al. Diabetes Care 2018

Sarah Wild

- Risk of CVD increases with liver fibrosis **Lancet Gastroenterol Hepatol. 2021 Nov;6(11):903-913**
- Risk of CVD attenuated with PNPLA3 I148M & TM6SF2 E167K

Non-alcoholic fatty liver disease and increased risk of: a) incident CKD and b) extrahepatic cancers: meta-analyses of observational cohort studies.

a) 13 studies with 1 222 032 individuals (28.1% with NAFLD) and 33 840 cases of incident CKD stage ≥3

b) 10 cohort studies with 182 202 middle-aged individuals (24.8% with NAFLD) and 8485 incident cases of extrahepatic cancers at different sites over a median follow-up of 5.8 years.

- NAFLD was associated with a moderately increased risk of incident CKD (n=10 studies; random-effects **HR 1.43, (95% CI 1.33 to 1.54)**; I2=60.7%). **Gut. 2022 71:156-162.**

- NAFLD was significantly associated with a nearly **1.5-fold to 2 fold increased risk of developing GI cancers (oesophagus/stomach/pancreas/colorectal cancers). GUT. 2022**

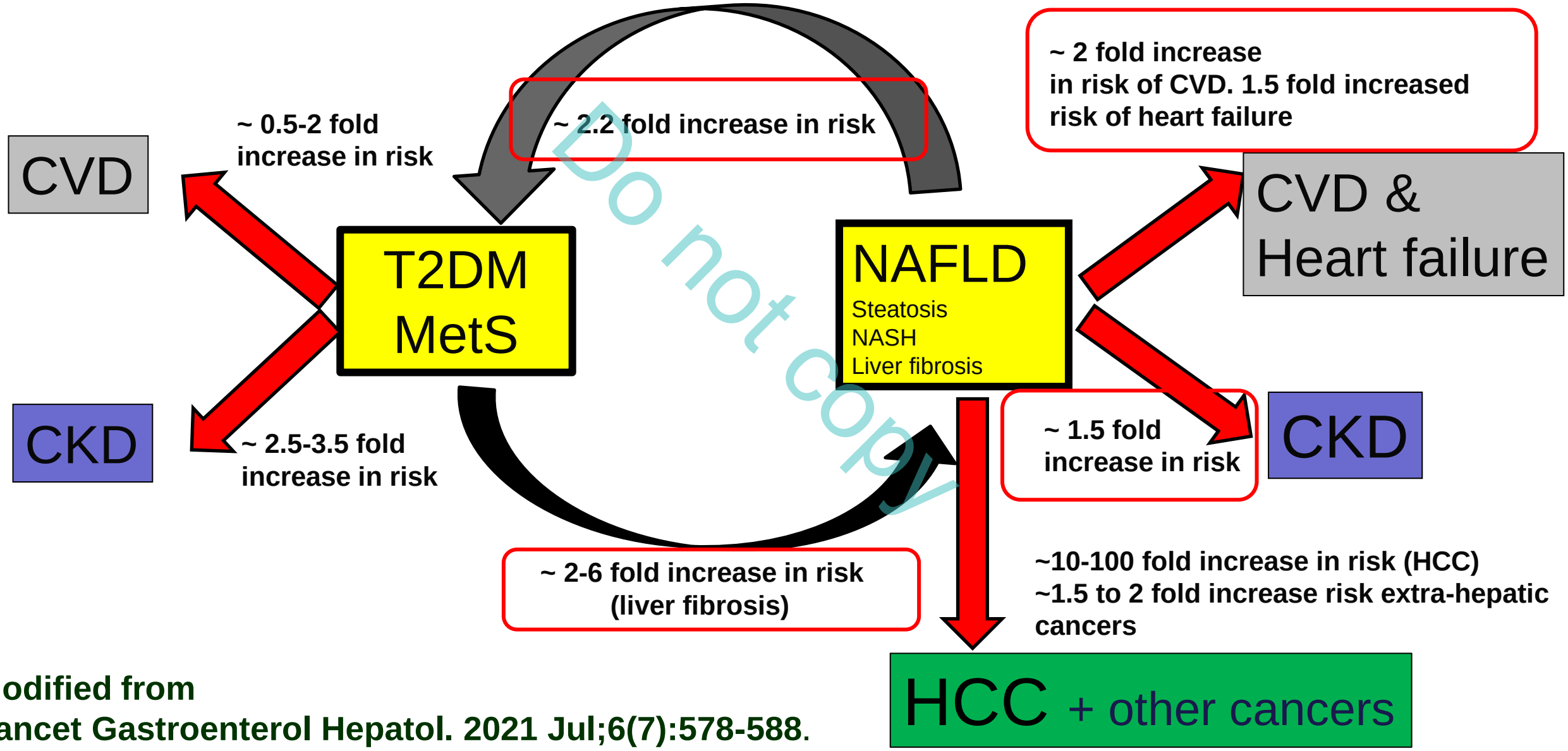
- NAFLD was associated with an approximately **1.2-fold to 1.5-fold**
- **increased risk of developing lung, breast, gynaecological**
- **or urinary system cancers. Gut. 2022;71:778-788**

(all risks were independent of age, sex, smoking, obesity, diabetes)

a) Gut. 2022 71:156-162.

b) Gut. 2022;71:778-788

Summary. Type 2 diabetes and NAFLD: a vicious spiral of adverse outcomes



Modified from
Lancet Gastroenterol Hepatol. 2021 Jul;6(7):578-588.

The liver plays a key role in glucose homeostasis and that role changes with increased severity of liver disease

- in the fed state the liver stores glycogen and glycogen synthesis is stimulated by insulin, whereas during fasting the liver produces glucose through glycogenolysis and gluconeogenesis
- it is important to bear in mind the severity of the liver disease and the relative health of the liver.
- it is also important to consider the health of other organs that influence glucose metabolism such as the adrenal glands because for example, the adrenal gland produces counter regulatory hormones to insulin, such as glucocorticoid hormones, and these hormones can have a profound effect to stimulate hepatic gluconeogenesis and increase hepatic glucose output

Glucose metabolism in patients with insulin resistance and cirrhosis

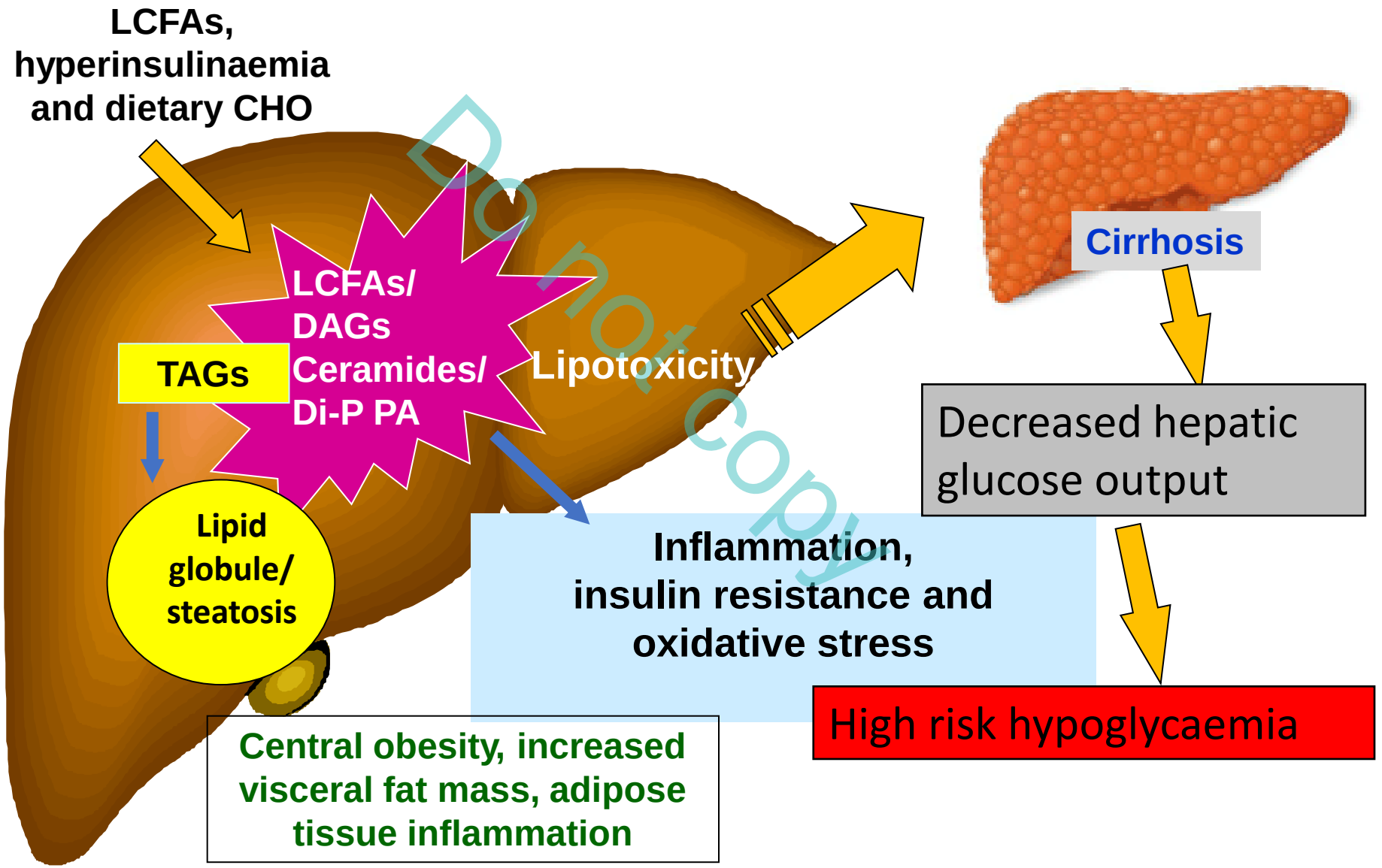
- INSULIN RESISTANCE: With hepatic lipid accumulation, there is accumulation of lipid synthesis intermediaries, such as hepatic diacylglycerol (DAG), which has the potential to activate protein kinase C-ε (PKC-ε), impairing insulin receptor activation (**causing hepatic insulin resistance) and impairing insulin-stimulated glycogen synthesis**

- In addition to hepatic insulin resistance, peripheral insulin resistance (usually due to obesity) indirectly influences hepatic glucose and lipid metabolism by **increasing flux of substrates that promote lipogenesis (fatty acids) and gluconeogenesis (glycerol and fatty acid-derived acetyl-CoA**, an allosteric activator of pyruvate carboxylase in the gluconeogenic pathway)

- Insulin stimulates glycogen synthesis in the liver, but in patients with insulin resistance and T2DM there is a decreased capacity to synthesise glycogen.

- **CIRRHOSIS: Liver glycogen levels are decreased in cirrhosis**

NASH and decompensating cirrhosis: decreased hepatic gluconeogenesis



How can NAFLD be diagnosed in 2023?

(N.B. Liver biopsy is invasive, costly, impractical and associated with increased morbidity)

- Biomarkers (liver fat, inflammation and fibrosis)
  -Liver fibrosis biomarkers (**ELF**, FIB-4, NFS, APRI)

- Imaging (liver fat, inflammation and fibrosis)
  -MRI/MRS, ultrasound, **elastography (Fibroscanning) & ultrasound elastography**

How should patients with NAFLD be diagnosed and monitored in routine clinical practice?

Rational Testing: building on NICE NAFLD Guideline ng49 (2016)
Tests for diagnosing and monitoring non-alcoholic fatty liver disease in adults

Byrne C, Patel J, Scorletti E, Targher G.
BMJ. 2018 Jul 12;362:k2734.

Diagnostic performance of common simple liver fibrosis biomarkers for identifying ≥F2 fibrosis

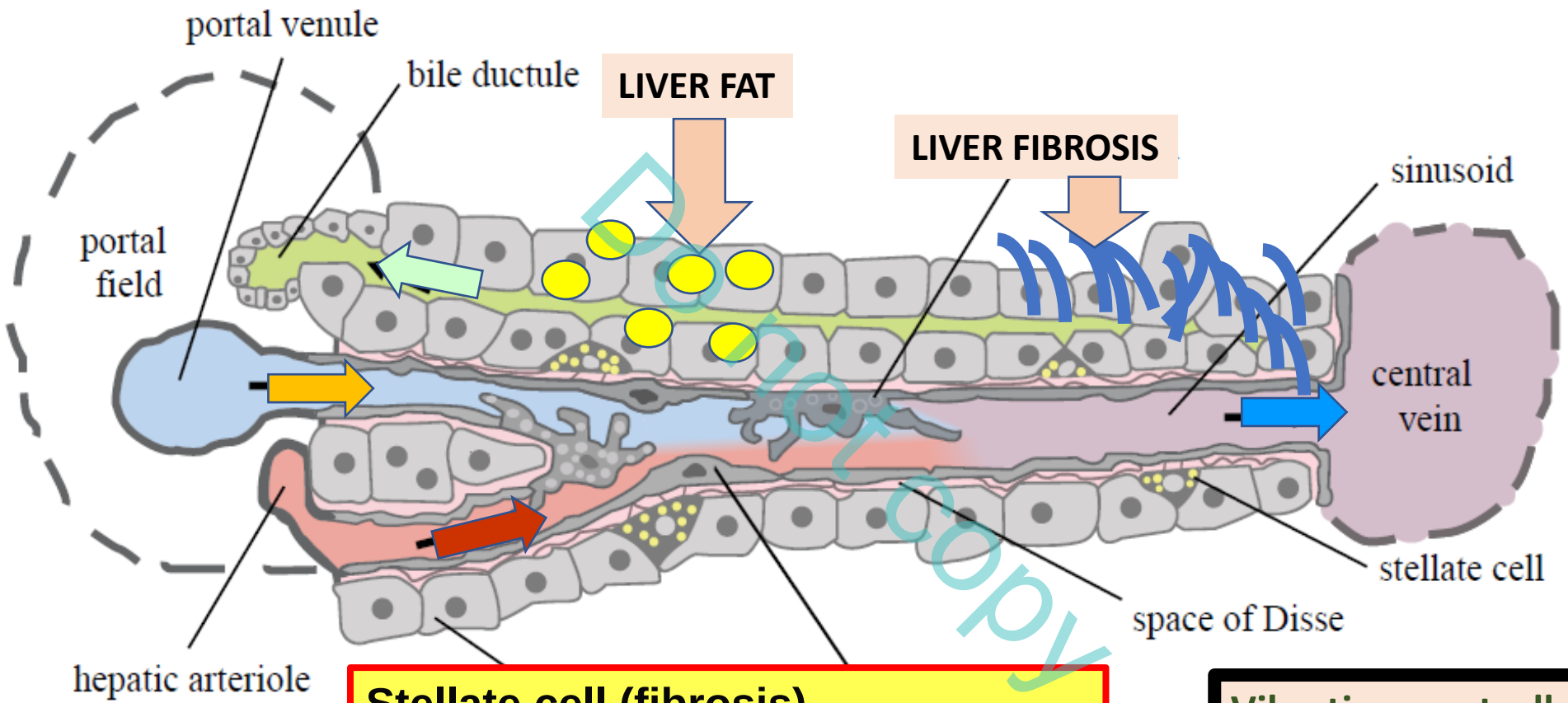
Table 2. Comparison of the performance of ELF™, FIB-4, APRI, FibroTest®, and NFS for identifying ≥F2 fibrosis

Biomarkers	Cut-off values	AUC	Summary sensitivity (%)	Summary specificity (%)	Summary PPV (%)	Summary NPV (%)
APRI[32]	0.43–1.50	0.70	59.3 (33.3–71.1)	77.1 (66.2–90.6)	67.5 (61.1–74.3)	70.6 (57.6–87.5)
FIB-4[32]	0.37–3.25	0.75	64.4 (54.4–77.8)	70.0 (60.0–87.5)	73.3 (66.2–77.8)	60.6 (40.5–74.2)
FibroTest®[33]	0.30–0.75	0.77	56.0 (45.0–66.0)	77.0 (74.0–80.0)	NR	NR
NFS[32,*]	–1.1	0.72	66.5 (60.9–70.1)	82.5 (68.7–96.3)	81.7 (76.6–86.7)	73.6 (61.1–86.0)
ELF™[35]	7.7[†]	0.81	Sensitivity=0.96	Specificity=0.12	PPV=0.42	NPV=0.83

Values are presented as mean (range).

Clin Mol Hepatol. 2023 Feb;29(Suppl):S157-S170

Structure of the liver lobule & flow of blood and bile



Stellate cell (fibrosis)
F0, F1, F2, F3, F4 (F4 = cirrhosis)

Vibration-controlled transient elastography (kPa thresholds)

F1 = periportal or perivenous fibrosis
F2 = periportal & perivenous fibrosis……..≥ 8.2kPa
F3 = bridging fibrosis……..≥ 9.7kPa
F4 = nodules of regenerating cells + fibrosis……≥ 13.6kPa

Eddowes et al. Gastroenterology 2019 156:1717-1730.

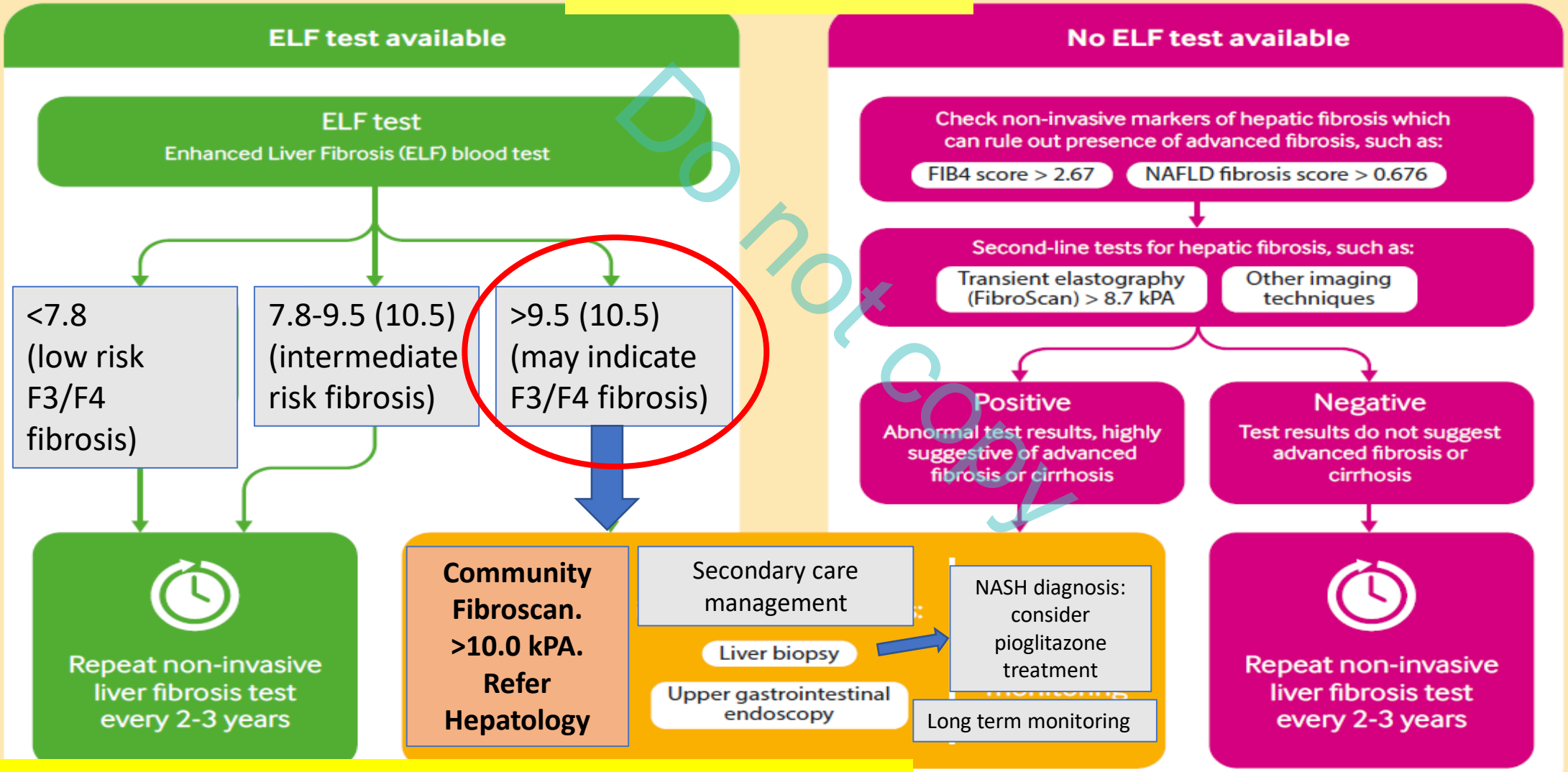
Modified from: Frevert U, Engelmann S, Zougbédé S, Stange J, Ng B, et al. (2005) PLOS Biology 3(6): e192.

Investigation of the severity of liver fibrosis

Southampton clinical pathway for primary care (Tina Reinson & Janisha Patel)

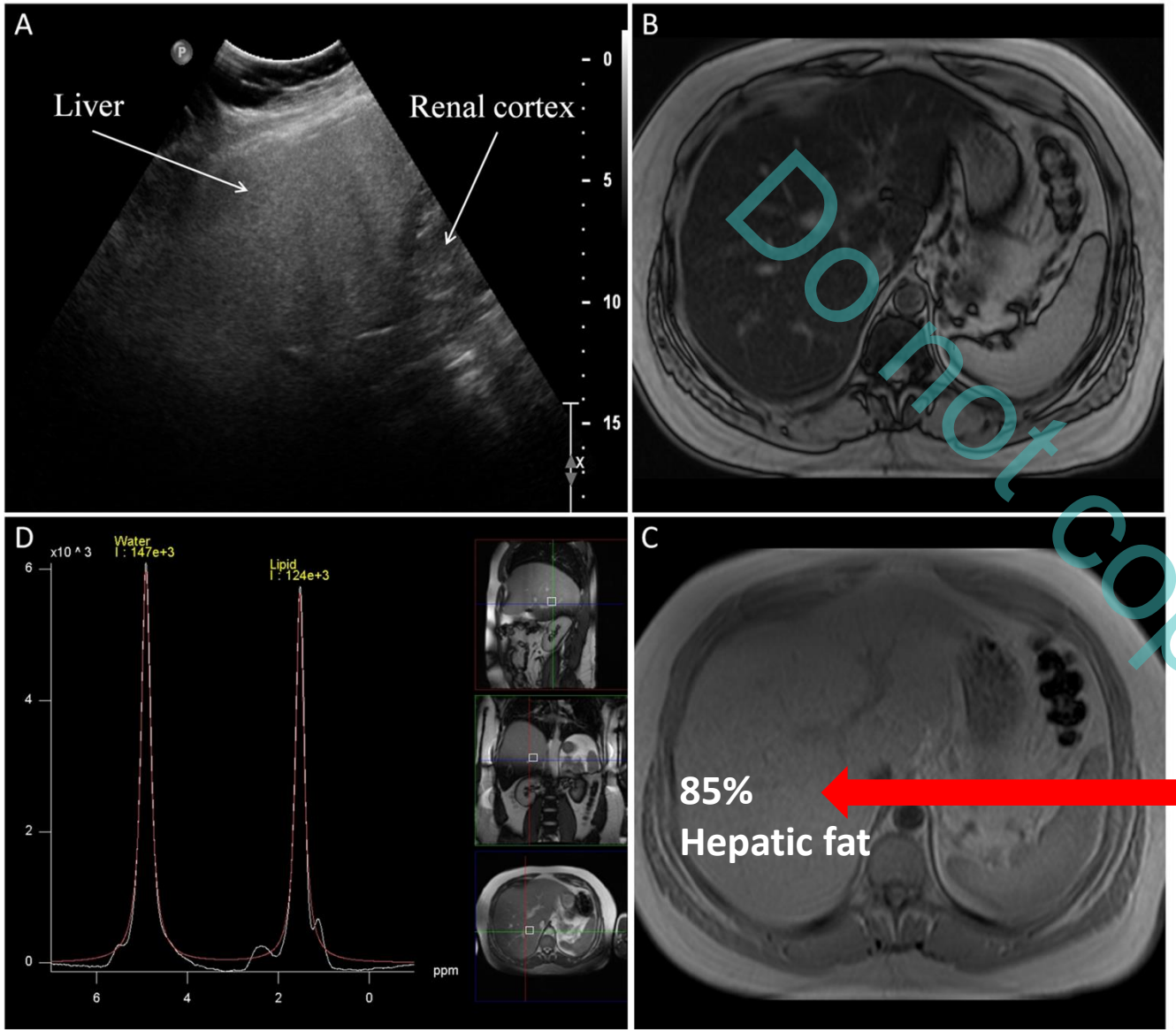
BJGP Open. 2021 Dec 14;5(6): BJGPO.2021.0145

2019: ELF score pathway modified and Community Fibroscanning added



Modified from BMJ. 2018 Jul 12;362:k2734. doi: 10.1136/bmj.k2734.

MR Spectroscopy and MRI are very sensitive techniques for measuring small changes in liver fat in clinical trials



(A) Ultrasound scan showing diffuse increased echogenicity of liver parenchyma compared to renal cortex.

(B) Chemical shift **MR Imaging** showing marked hepatic signal drop-off during out-of-phase image compared to in-phase

(C) image, suggesting significant diffuse fatty liver.

(D) Single-voxel **MR Spectroscopy** measuring area under lipid spectrum (2nd peak) relative to water spectrum (1st peak), allowing accurate **quantification of hepatic fat of 85% in the same patient.**

1. Scorletti et al. Hepatology. 2014 Oct;60(4):1211-21

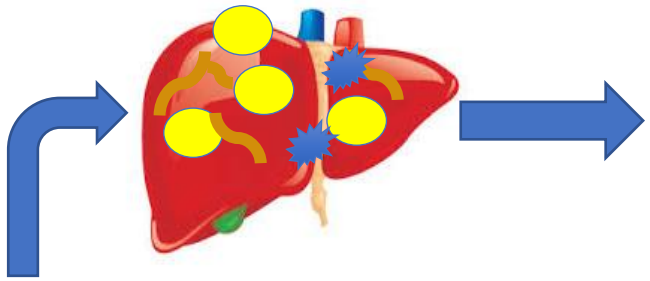
2. Scorletti et al. Gastroenterology 2020 May;158(6): 1597-1610.e7

Angela Darekar
David Breen
Charles Peebles

Treatment of NAFLD as a multisystem disease:

Potential benefits of incretin receptor agonists and combination therapy with glucagon receptor agonists on processes relevant to both liver disease and the extra-hepatic consequences of liver disease

NAFLD and NASH



Treatment with incretin receptor agonists (GLP-1, GIP) & glucagon receptor agonists

Weight loss with reduction in visceral and subcutaneous fat depots (decreased appetite)

Improved metabolic syndrome features (improved blood pressure, atherogenic dyslipidaemia, dysglycaemia and peripheral and hepatic insulin resistance)

Improved liver lipid metabolism and decreased liver fat content (decreased lipotoxicity)

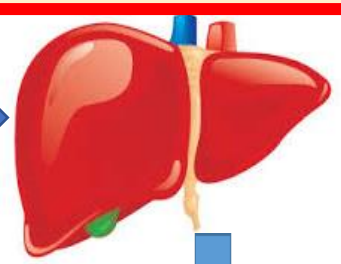
Decreased low-grade inflammation (e.g. C-reactive protein, interleukin-6, TNF-alpha, and other acute-phase proteins)

Decreased prothrombotic state (e.g. factor VII, fibrinogen, tissue factor, plasminogen activator inhibitor-1, and other fibrinolytic factors)

Decreased reactive oxygen species

Others (e.g. improved adiponectin, change in appetite, increased energy expenditure and heart rate)

NAFLD and NASH attenuation/amelioration

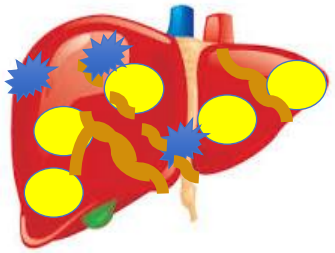


Improvements in risk of extrahepatic complications:
T2D
CVD
CKD
Obesity-related cancers

Lancet Gastroenterol Hepatol. 2023 Aug 16:S2468-1253(23)00159-0

Treatment with SGLT2is, GLP-1 & GIP receptor agonists, PPAR gamma agonists (pioglitazone), AT-II receptor blockers, RAS inhibitors,

Effects of treatments on risk factors for liver and kidney disease



MASLD

RISK FACTORS

Excess body fat
(weight loss with reduction in visceral and subcutaneous fat depots

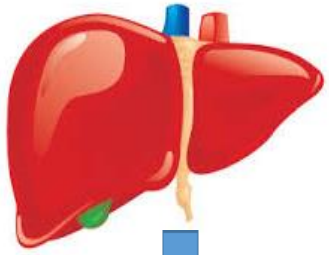
Cardio-metabolic risk factors
(improvement in: blood pressure, atherogenic dyslipidaemia, dysglycaemia and peripheral and hepatic insulin resistance)

Liver lipid metabolism
(decreased liver lipid content & lipotoxicity)

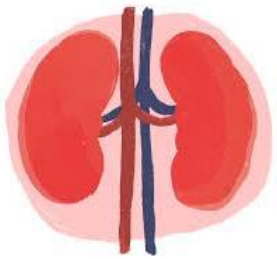
Low-grade inflammation
(decreased C-reactive protein, interleukin-6, TNF-alpha, and other acute-phase proteins)

Prothrombotic state
(decreased factor VII, fibrinogen, tissue factor, plasminogen activator inhibitor-1, and other fibrinolytic factors)

Others (e.g. increased adiponectin, decreased reactive oxygen species, decrease in proteinuria



MASLD attenuation or amelioration



Improvements in CVD risk factors and CKD

Figure

Conclusions:

There currently not sufficient data to advocate screening for NAFLD in T2DM- 'REFLEX Study' aiming to assess cost effectiveness.

Tina Reinson, Ryan Buchanan, Josh Bilson

- However, consider whether NAFLD maybe present in T2DM (patient centrally obesity, hypertensive, hyperTG, low HDL-C, very insulin resistant)
- ALT ≥ 20IU/L in women and ≥ 30IU/L in men is probably abnormal
- Assessment of liver fibrosis is key (NILS/fibrosis markers/imaging)

And allows estimation of liver disease severity and need for HCC surveillance/referral to Hepatology

- Ultrasound elastography/Fibroscanning is useful but requires training and has some limitations
- Weight loss and increased phys activity – important in management
- Consideration of: pioglitazone/GLP-1R agonists/SGLT2is/statins/ACEI/AII receptor blockers
- Bariatric surgery helps but potentially introduces new problems!