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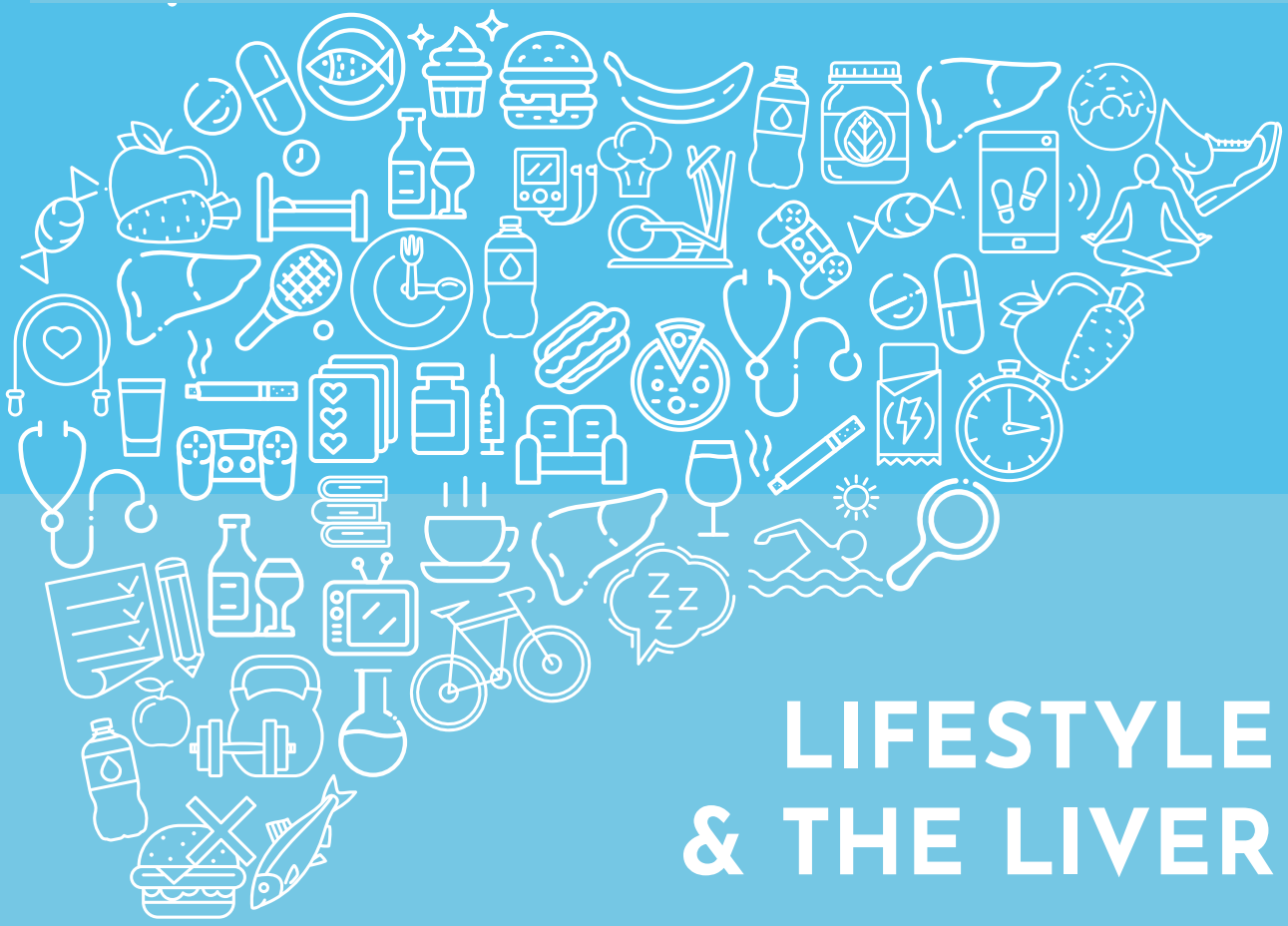

LIFESTYLE & THE LIVER

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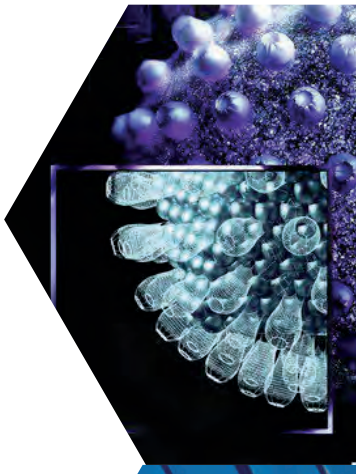

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Welcome message from the course organisers

On behalf of the European Association for the Study of the Liver (EASL), we are delighted to welcome you to ILC 2021 and especially to this Postgraduate Course (PGC) on "Lifestyle and the liver". Owing to the COVID-19 crisis, we've set this course up as a fully digital and highly interactive experience, drawing on experts on the field, to make it as enriching and educational as possible.

The liver is an organ particularly sensitive to lifestyles. The surge in non-alcoholic fatty liver disease (NAFLD) in the last decades is a paramount example of this sensitivity. This PGC will cover how unhealthy lifestyles affect the liver and how behavioural intervention, such as regular physical activity and nutritional changes, can improve liver diseases. This topic is very timely, given the effects of pandemic-related confinement and quarantine on people's lifestyles.

We will provide updates on NAFLD as well as on alcohol-related liver disease (ALD). Misuse of over-the-counter analgesics, and herbal and dietary supplement products, including illicit hormonal compounds, is an emerging cause of liver disease worldwide and thus a growing concern. This course will also address the potential harm of unsupervised use of drugs and other non-prescribed agents.

The PGC is divided into six sessions, including a closing panel discussion on a multidisciplinary, outpatient approach to NAFLD and ALD. It concludes with a State-of-the-Art lecture on lifestyle and public health. In each session, a case will be presented. As members of the audience, you will then be able to reply to questions from the chairs and live voting will be available. Take part and share your experience on Twitter, using the hashtags of #ILC2021, #LiverTwitter, and #NAFLD.

The organisers and the faculty wish you an enjoyable time at ILC 2021, and we hope you find the course stimulating and informative. We look forward to seeing you again in person at ILC 2022 taking place in London, UK.

Prof. Jean-François Dufour
Bern, Switzerland

Prof. María Isabel Lucena
Malaga, Spain

Prof. Mark Thursz
London, United Kingdom

Schedule

PGC: Lifestyle and the liver

Organisers

Jean-François DUFOUR, *Switzerland*

Maria Isabel LUCENA, *Spain*

Mark THURSZ, *United Kingdom*

WEDNESDAY 23 June 2021

Session 1. Non-alcoholic fatty liver disease (NAFLD)

Chairs:
Jean-François DUFOUR, *Switzerland*
Helen Louise REEVES, *United Kingdom*

10:00-10:05 **Case presentation: Non-alcoholic steatohepatitis**
Helen Louise REEVES, *United Kingdom*

10:05-10:15 **Non-drug management of NAFLD/NASH**
Elisabetta BUGIANESI, *Italy*

10:15-10:27 **Drug therapy in NASH in 2021**
Jean-François DUFOUR, *Switzerland*

10:27-10:32 **Is there a role for endoscopic strategies in NASH**
Guruprasad AITHAL, *United Kingdom*

10:32-10:37 **Bariatric surgery in NASH**
Helena CORTEZ-PINTO, *Portugal*

10:37-11:00 **Discussion and Q&A**

Session 2. Impact of physical activity and diet on liver disease

Chairs:
Elisabetta BUGIANESI, *Italy*
Manuel ROMERO GOMEZ, *Spain*

11:15-11:20 **Case presentation: NAFLD and CVD, I can't possibly do any exercise**
Manuel ROMERO GOMEZ, *Spain*

11:20-11:30 **Sodas and screens: threats for the liver**
Manal ABDELMALEK, *United States*

11:30-11:40 **What is wrong with my sleep?**
Jörn M SCHATTENBERG, *Germany*

11:40-11:50 **Patients with cirrhosis should exercise!**
Annalisa BERZIGOTTI, *Switzerland*

11:50-12:00 **How much exercise before liver transplantation on the transplant waiting list?**
Diethard MONBALIU, *Belgium*

12:00-12:15 **Discussion and Q&A**

Session 3. Nutritional and behavioural patterns affecting the liver

Chairs:
Guruprasad AITHAL, *United Kingdom*
Cyrielle CAUSSY, *France*

12:45-12:50 **Case presentation: Portion control**
Cyrielle CAUSSY, *France*

12:50-13:00 **Which diet should I be taking?**
Shira ZELBER-SAGI, *Israel*

13:00-13:10 **Effects of diet on the liver: Role of the microbiome**
Judith ARON-WISNEWSKY, *France*

13:10-13:20 **How much alcohol can I drink?**
Helmut SEITZ, *Germany*

13:20-13:30 **Smoking (cigarettes or cannabis), coffee and liver diseases**
Tracey G. SIMON, *United States*

13:30-13:45 **Discussion and Q&A**

Session 4. Alcohol-related liver disease

Chairs:
Maja THIELE, *Denmark*
Mark THURSZ, *United Kingdom*

14:00-14:05 **Case presentation: Severe alcoholic hepatitis**
Maja THIELE, *Denmark*

14:05-14:15 **Pathophysiology and treatment of alcoholic hepatitis**
Mark THURSZ, *United Kingdom*

14:15-14:25 **Impact of abstinence and diet on alcoholic liver disease progression**
Agustin ALBILLOS, *Spain*

14:25-14:35 **Alcohol Biomarkers in Clinical and Forensic Contexts**
Jessica MELLINGER, *United States*

14:35-14:45 **Interventions for Alcohol Use Disorders in patients with Alcohol Related Disease**
Giovanni ADDOLORATO, *Italy*

14:45-15:00 **Discussion and Q&A**

Session 5. Drugs and xenobiotic misuse and liver toxicity

Chairs:
Maria Isabel LUCENA, *Spain*
Victor NAVARRO, *United States*

15:30-15:35 **Case presentation: Noni juice and ALF**
Maria Isabel LUCENA, *Spain*

15:35-15:45 **Herbal remedies to improve well-being: healthy for the liver?**
Victor NAVARRO, *United States*

15:45-15:55 **Cathinone and derivatives: mechanisms of liver toxicity, clinical consequences and preventive interventions**
Karine LACOMBE, *France*

15:55-16:05 **The risk for the liver of bodybuilding and sport performance products**
Raul J. ANDRADE, *Spain*

16:05-16:15 **Over the counter pain killers and hepatoxicity**
Constantine J KARVELLAS, *Canada*

16:15-16:30 **Discussion and Q&A**

Session 6. Multidisciplinary outpatient approach to NAFLD and ALD

Chairs:
Jean-François DUFOUR, *Switzerland*
Maria Isabel LUCENA, *Spain*
Mark THURSZ, *United Kingdom*

16:45-17:45 **NAFLD – is this a job for the hepatologist on their own?**
Mary RINELLA, *United States*

ALD – it takes a team
Vijay SHAH, *United States*

Diet – what does it take?
Shira ZELBER-SAGI, *Israel*

Round Table discussion

State of the art lecture

18:00-18:30 **Obesity: the public health approach**
Harry RUTTER, *UK*

Abbreviations and Acronyms

AAS	Androgenic anabolic steroids
AASLD	American Association for the Study of Liver Diseases
ACC	Acetyl-CoA carboxylase
ADH	Alcohol dehydrogenase
AGB	Adjustable gastric banding
AGE	Advanced glycation end
AKI	Acute kidney injury
ALD	Alcoholic liver disease
ALF	Acute liver failure
ALFSG-PI	Acute liver failure study group prognostic index
ALT	Alanine aminotransferase
ALDH	Acetaldehyde dehydrogenase
AMP	Adenosine monophosphate
AP or AKP	Alkaline phosphatase
APAP	acetaminophen/paracetamol
AST	Aspartate aminotransferase
AUD	Alcohol use disorder
BA	Bile acids
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BP	Blood pressure
BPD	Biliopancreatic diversion
BS	Bariatric surgery
BSL	Blood sugar levels
CAR	Constitutive androstane receptor
CBT	Cognitive behavioural therapy
CCR	C-C motif chemokine receptor
CDT	Carbohydrate-deficient transferrin
CE	Cerebral edema
CP	Child-Pugh
CPET	Cardiopulmonary exercise testing
CRC	colorectal cancer
(C)RRT	(continuous) renal replacement therapy
CT	Computed tomography
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray absorptiometry
DILI	Drug-induced liver injury
DILIN	Drug-Induced Liver Injury Network
DM	Diabetes mellitus
DMR	Duodenal mucosal resurfacing
EASD	European Association for the Study of Diabetes
EASL	European Association for the Study of the Liver
EASO	European Association for the Study of Obesity
FBG	Fasting blood glucose
FITT	Frequency, intensity, time and type
FMT	Faecal microbiota transfer
FXR	Farnesoid X receptor
GGT	gamma-glutamyltransferase
GI	Gastrointestinal
GIP	Glucose-dependent insulinotropic peptide
GLP	Glucagon-like peptide
GSH	Glutathione
HbA1c	Glycosylated haemoglobin 1c
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HD	Hemodialysis
HDL	High-density lipoprotein
HDS	Herbal and dietary supplements
HE	Hepatic encephalopathy
HFCS	High-fructose corn syrup

HFD	High-fat diet
HR	Heart rate
HVP	High-volume plasma exchange
HVPG	Hepatic venous pressure gradient
ICH	intracranial hypertension
IMP	Inosine monophosphate
INR	International normalised ratio
IR	Insulin resistance
KCC	King's College criteria
LDL	low-density lipoprotein
LGB	Laparoscopic gastric banding
LPO	lipid peroxidation
LPS	Lipopolysaccharides
LRYGB	Laparoscopic Roux-en Y gastric bypass
LSG	Laparoscopic sleeve gastrectomy
LT	Liver transplantation
MAMC	Mid-arm muscle circumference
MARS	Molecular absorbent recirculating system
MCV	Mean cellular volume
MD	Mediterranean diet
MELD	Model for end-stage liver disease
MET	Motivational enhancement therapy
MetS	Metabolic syndrome
MPC	Mitochondrial pyruvate carrier
MRE	Magnetic resonance elastography
MRI	Magnetic resonance imaging
MUP	Minimum unit price
NAC	N-acetylcysteine
NAFLD	Non-alcoholic fatty liver disease
NPS	New psychoactive substances
PA	Phenolic acids
PAI-1	plasminogen activator inhibitor 1
PDFF	Proton-density fat fraction
PPAR	Peroxisone proliferator-activated receptors
PREP	Pre-exposure prophylaxis
PYY	peptide YY
RA	Retinoic acid
RCT	Randomised controlled trial
ROS	Reactive oxygen species
RPE	rate of perceived exertion
RYGB	Roux-en-Y gastric bypass
SBIRT	Screening, brief intervention and referral to treatment
SCN	Suprachiasmatic nucleus.
SF-36	Short-form 36 health survey
SG	Sleeve gastrectomy
SIRS	Severe inflammatory response syndrome
SNS	Sympathetic nervous system
SSB	Sugared-sweetened beverages
TFS	Transplant-free survival
TG	Triglycerides
TGF	Transforming growth factor
TLFB	Time-line Follow Back
TLR	Toll-like receptors
T2DM	Type 2 diabetes mellitus
(US)ALFSG	(United States) Acute Liver Failure Study Group
VLDL	Very low-density lipoproteins
WHO	World Health Organisation
YLL	Years of life lost
6MWT	6 minutes walking test

This syllabus was edited and compiled by Laura A. Kehoe, Medical Communications, Switzerland.

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SESSION 1

NON-ALCOHOLIC FATTY LIVER DISEASE

WEDNESDAY 23 JUNE
10H00 - 11H00

Non-drug management of NAFLD/NASH

Elisabetta Bugianesi

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Take-home messages

- The top three leading causes of death in patients with NAFLD in descending order are cardiovascular disease, cancer and liver disease.
- The increased risk of metabolic and macro/micro-vascular complications in NAFLD stems from the associated features of metabolic syndrome; nevertheless, NAFLD itself contributes to the spectrum of risk factors associated with insulin resistance.
- The incidence, prevalence and severity of metabolic and cardiovascular complications are proportional to the histological severity of liver damage, suggesting that NAFLD, but particularly NASH, can also contribute to a low-grade inflammatory state.
- The clinical implication of these findings is that patients with NAFLD require a multidisciplinary evaluation, with a major focus on type 2 diabetes mellitus and cardiovascular disease complications and may benefit from more intensive surveillance and early treatment interventions.

Introduction

The top three leading causes of death in patients with NAFLD in descending order are cardiovascular disease (CVD), cancer and liver disease. NAFLD has been proposed as the hepatic manifestation of the metabolic syndrome (MetS), having insulin resistance (IR) as a pathophysiologic mechanism [1]. Haemodynamic or metabolic imbalances shared by NAFLD and MetS may predispose individuals to common complications. It is now clear that the presence of NAFLD is a strong predictor of MetS, is a future risk of type 2 diabetes mellitus (T2DM) and CVD and is also associated to an increased risk of cancers in specific sites.

NAFLD and cardiovascular disease

The leading cause of death in patients with NAFLD is CVD [2]. While this may be partially explained by the association with the MetS, recent evidence suggests that NAFLD predicts CVD independently of MetS [3]. Hepatic IR leads to an overproduction of large triglyceride-rich very low-density lipoproteins (VLDL) particles in patients with NAFLD, which undergo additional modification to form small, dense LDL particles that are highly atherogenic [3]. NAFLD, but particularly NASH, can also contribute to a low-grade inflammatory state through the systemic release of several inflammatory mediators and pro-coagulant factors (e.g., plasminogen activator inhibitor 1, fibrinogen, and factor VII) [3]. The first important clinical implication is the increased risk of subclinical and overt atheromatous plaque formation in several vascular districts. A recent meta-analysis of 27 cross-sectional studies [4] reported a strong association between NAFLD, detected by imaging or biopsy, and markers of subclinical atherosclerosis (i.e. impaired flow-mediated vasodilatation and increased carotid-artery intimal medial thickness) independent of classical CVD risk factors. Overall, the risk of CVD events in NAFLD is roughly two-fold higher both in the general population and in high-risk groups [3]. In an Italian study on diabetic patients, the prevalence of coronary, cerebrovascular, and peripheral vascular disease was remarkably higher among patients with NAFLD (diagnosed by ultrasound) than among

those without, independent of traditional risk factors [5]. Two recent studies of over 11,000 adults from the United States have reported that NAFLD was significantly associated with increased CVD prevalence over 14 years of follow-up, but only NAFLD with advanced hepatic fibrosis (assessed by non-invasive scores) was independently associated with a ~70% increased risk of all-cause and CVD mortality [6,7].

NAFLD and type 2 diabetes mellitus

Hepatic lipid accumulation in NAFLD impairs hepatic glucose and lipid metabolism, increasing the risk of T2DM and CVD, independent of established risk factors [1]. It is well-documented that NAFLD increases the risk of prevalent and incident diabetes and precedes and predisposes patients to T2DM, independent of established risk factors. In a systematic review and meta-analysis [8] of 21 prospective, population-based studies in different ethnic groups, 1 log (x10) higher alanine aminotransferase (ALT) values (in U/L) conferred a hazard ratio of 1.85 (95% confidence interval [CI] 1.57-2.18, after adjustment for known risk factors. Notably, the incidence rate of T2DM increased progressively according to the ultrasonographic severity of NAFLD at baseline (normal: 7.0%, mild: 9.8%, moderate-to-severe: 17.8%, $p < 0.001$), after adjusting for multiple confounders [9]. Only one study evaluated the incidence of T2DM in adults with biopsy-proven NAFLD [2]. After a mean period of 13.7 years, 78% of these patients developed either T2DM (58%) or impaired glucose tolerance (20%). Of note, patients with NASH had an approximately threefold higher risk of developing T2DM than those with simple steatosis. As patients are diagnosed as diabetic, the amount of liver fat influences the severity of hepatic and peripheral IR. This leads to worse glycaemic control, demonstrated by increased levels of glycosylated haemoglobin 1c (HbA1c) in patients with T2DM and NAFLD, and can predict the amount of daily insulin needed to maintain glycaemic control [10].

NAFLD and extrahepatic cancers

The second most common cause of death among NAFLD patients is attributed to malignancies at either gastrointestinal (liver, colon, oesophagus, stomach and pancreas) or extra-intestinal sites (kidney in men and breast in women) [2,11]. In a community cohort of adults from the United States [12], after 21 years of longitudinal follow-up, NAFLD was associated with a nearly twofold increase in the risk of developing cancers. The highest risk for extrahepatic cancers was noted in uterine internal rate of return (IRR) = 2.3 (95% CI 1.4–4.1), stomach IRR = 2.3 (95% CI 1.3–4.1), pancreas IRR = 2.0 (95% CI 1.2–3.3) and colon IRR = 1.8 (95% CI 1.1–2.8). In this cohort, the obesity-related risk was largely driven by NAFLD, while obesity in the absence of NAFLD had a minimal association with malignancy risk [12]. Overall, the colon is the main extrahepatic site where a link between NAFLD and cancer seems to be most consistent [11]. Huang *et al.* reported that NAFLD was an independent risk factor for adenoma development (odds ratio [OR] 1.45) in 1522 asymptomatic subjects who underwent paired colonoscopies, after a negative index colonoscopy [13]. In a large European study (n= 1382), Stadlmayr *et al.* observed that male patients with ultrasound-diagnosed NAFLD had a higher prevalence of colorectal adenomas and early colorectal cancer compared to those without NAFLD, and the increased risk (OR 1.47) was independent of other known factors [14]. A generic increased risk of cancer in NAFLD is common to all the components of MetS and is due to increased insulin and insulin growth factors levels, which exert their normal activity as growth factors and stimulate cell proliferation, apoptosis and the production of vascular endothelial growth factor. The increased proinflammatory state characteristic of NASH may further influence apoptosis and tumour cell proliferation.

Screening strategy for extrahepatic diseases in NAFLD

All patients with NAFLD should be carefully evaluated for family and personal history of T2DM, myocardial infarction, angina, heart failure, stroke or other clinical CVD manifestations, and the presence of modifiable risk factors such as cigarette smoking and lifestyle habits. Regular assessments of body weight, waist circumference and blood pressure are mandatory. According to EASL Clinical Practice guidelines [15], screening procedures for T2DM prevention should be based on serial fasting blood glucose (FBG) or HbA1c measurements, while the two-hour oral glucose tolerance test could be limited to cases of impaired fasting glucose (i.e. FBG 100-126 mg/dl). A complete lipid profile, including total LDL- and HDL-cholesterol, should be regularly obtained and a yearly assessment of carotid intima-media thickness by artery ultrasonography is highly recommended [15].

Treatment

An ideal effective treatment for NAFLD might be expected to not only reduce the risk of chronic liver disease-related complications but also to decrease the risk of T2DM and CVD. Weight loss is the most effective way to promote liver fat removal, and several controlled studies have confirmed that an intense approach to lifestyle changes is able to attain the desired 7-10% weight loss, is associated with reduced liver fat, NASH remission, and also a reduction of fibrosis [15]. In most cases, any form of a healthy diet, which leads to caloric reduction and is acceptable to the patient, should be encouraged for patients with NAFLD. The American Heart Association's presidential advisory on dietary fats states that replacing saturated fat with polyunsaturated vegetable oil reduces the incidence of CVD by ~30% [16]. This shift towards more unsaturated fats occurs when a Westernised diet containing processed foods is replaced by the Mediterranean diet (MD). A MD contains lower amounts of dietary components that are thought to be potentially harmful for NAFLD and CVD, such as fructose, refined carbohydrates, trans fatty acids and red meat. A MD diet complies with current guidelines to reduce the risk of CVD and ameliorate liver damage in NAFLD patients [17].

Exercise is one of the cornerstones of NAFLD and NASH management [15]. A recent systematic review reveals that exercise, without weight loss, produced a 20–30% relative reduction in intrahepatic lipid [18]. Different forms of exercise (aerobic, resistance/strength training, or high-intensity intermittent training) appear to have similar effects on liver fat; if patients continue to exercise for 12 months, the benefits remain [18]. Exercise alone, in the absence of any change in body weight or composition, may enhance insulin sensitivity and glucose homeostasis. Thus, in people who are IR or have T2DM, exercise provides a way of improving glycaemic control.

Conclusions

NAFLD is a multisystem disease that affects many extrahepatic organ systems by disrupting the regulation of multiple metabolic and inflammatory pathways.

Patients with NAFLD require a multidisciplinary evaluation, with a major focus on T2DM and CVD complications. Their morbidity and mortality are more greatly affected by the occurrence of these extrahepatic complications rather than by liver disease itself. Accordingly, all NAFLD patients should be informed about the increased risk of T2DM and CVD in order to reinforce their compliance with lifestyle changes. Further research is needed to understand the pathways through which NAFLD influences these extrahepatic diseases to help decrease the global burden of morbidity and mortality caused by an inappropriate environment.

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Drug therapy for NASH: what should we be using in 2021?

Jean-François Dufour

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Take home messages

- The definition of NASH is based on liver histology and excludes other liver diseases, but the terminology is changing.
- Efficacy of drugs is measured on histological changes.
- Many drugs with different mechanisms of action are in phase 2 and phase 3 clinical trials.
- Efficacy of pharmacotherapy to treat NASH could be improved with better patient selection and use of drugs in combination.

Definition and endpoints

Non-alcoholic steatohepatitis (NASH) is defined by an alcohol intake too modest to harm the liver with accumulation of triglycerides in hepatocytes (steatosis) in the presence of lobular inflammation and ballooned cells. This is a suboptimal definition [1]. Alcohol consumption is based on anamnesis which is notoriously inaccurate in this regards and hepatic susceptibility to alcohol is not the same for all patients. In addition, the definition of NASH requires a liver biopsy, which 1) is an invasive procedure, 2) has some risks for the patients, 3) is costly, and 4) also inaccurate. Moreover, this definition is not associated with outcome. Fibrosis which is not required for the diagnosis of NASH dictates the outcome of patients with NASH. Overall mortality and liver-related mortality of patients with NASH correlate with the degree of fibrosis. Since the histology is required for the definition of NASH and since the biopsy is also providing the degree of liver fibrosis, surrogate endpoints of clinical trials are for phase 3 interim analyses histological changes. The regulatory approval pathway for pharmacological therapies for NASH requires therapies to show clinical benefit in improving liver-related outcomes for full regulatory approval, which may take several years due to low event rates. To accelerate drug development, liver histological improvements have been accepted as a surrogate for clinical improvements with either one-stage improvement in liver fibrosis or resolution of NASH. This approval is contingent upon showing clinical benefits over long-term follow-up for full approval.

Mode of action of drugs in clinical trials to treat NASH

FXR agonists – Farnesoid X receptor (FXR) is a transcription factor activated by bile acids. As such FXR regulates bile-acid metabolism, but since bile-acid biology is paced by food intake, FXR also controls hepatic metabolism. Drugs activating FXR have demonstrated effects in cholestatic liver disease.

PPAR agonists – Peroxisone proliferator-activated receptors (PPARs) comprise a family of three transcription factors – PPAR-α, PPAR-δ and PPAR-γ – which are involved in lipid and glucose metabolism and have anti-inflammatory effects.

Metabolic enzyme inhibitors – Steroyl-CoA desaturase-1 (SCD-1) converts saturated fatty acids to monounsaturated fatty acids. SCD-1 down-regulation reduces hepatic lipogenesis, enhances insulin sensitivity and promotes lipid oxidation.

Acetyl-CoA carboxylase (ACC) converts Acetyl-CoA to malonyl-CoA. Inhibition of ACC reduces hepatocellular malonyl-CoA levels, which in turn increases mitochondria beta-oxidation and decreases polyunsaturated fatty-acid synthesis; the net effect is improvement in hepatic steatosis.

Thyroid hormone receptor beta agonists – Selective thyroid hormone receptor beta (TRβ) agonist can modulate lipid metabolism without the side effects which are mediated by thyroid hormone receptor α.

Mitochondria pyruvate carrier inhibitors – Pyruvate fuels the tricarboxylic acid cycle to produce citrate and oxaloacetate, which supports lipogenesis and neoglucogenesis, respectively. The mitochondrial pyruvate carrier (MPC) transports pyruvate across the mitochondria so that it can interact with the enzymes of the cycle.

FGF21 agonists – Fibroblast growth factor 21 (FGF21) is produced by the liver, adipose tissue and pancreas, and has pleiotropic metabolic effects including increasing energy expenditure, improving insulin sensitivity, reducing sugar intake and browning adipose tissue.

GLP-1 agonists – Glucagon like peptide-1 (GLP-1) is an incretin hormone secreted by intestinal L-cells at the post-prandial phase. GLP-1 agonists target GLP-1 receptors expressed in various organs including the pancreas, intestine, adipose tissue and brain. GLP-1 regulates plasma glucose levels by stimulating glucose-dependent insulin secretion and inhibiting glucagon secretion. In addition, GLP-1 induces weight loss.

SGLT2 inhibitors – Sodium/glucose transport protein 2 (SGLT2) inhibitors are a class of anti-diabetic agents that exert their glucose-lowering effects by inhibition of SGLT2, which accounts for ~90% of the glucose reabsorbed by the kidney. SGLT2 inhibitors induce moderate weight loss.

Hepatoprotectant – Norursodeoxycholic acid is a side chain-shortened homologue of ursodeoxycholic acid that undergoes hepatic enrichment with hepatoprotective, anti-inflammatory, and antifibrotic activity.

Chemokine inhibitors – C-C motif chemokine receptor (CCR) type 2 plays a role in the recruitment, migration, and infiltration of proinflammatory monocytes and macrophages at the site of liver injury, and CCR5 in the activation and proliferation of collagen-producing activated hepatic stellate cells/ myofibroblasts.

As per February 2020 there were 159 clinical trials for NASH listed as active or enrolling on clinicaltrials.gov, 8 of these studies are phase 3 clinical trials. As per May 2021 there are 160 clinical trials for NASH listed as active or enrolling on clinicaltrials.gov, 11 of these studies are phase 3 clinical trials. They are listed in the table 1.

Table 1. Phase 3 randomized controlled trials for NASH

Drug (in alphabetical order)	Mechanism of action	Type and number of patients	Status	Duration of therapy
Aramchol	SCD-1 inhibitor	2000 NASH F2-3	Recruiting	52 weeks for histological analysis 5 years for clinical outcomes
Dapagliflozin	SGLT2 inhibitor	100 NASH with T2DM	Recruiting	12 months histological analysis
Obeticholic acid	FXR agonist	2480 NASH F1-3	Active, not recruiting	18 months for histological analysis 7 years for clinical outcomes
Obeticholic acid	FXR agonist	919 NASH F4	Recruiting	18 months histological analysis
Resmetirom	Thyroid hormone receptor β agonist	2000 NASH F1-3	Recruiting	12 months for histological interim analysis 54 months for clinical outcomes
Semaglutide	GLP-1 agonist	1200 NASH F2-F3	Not yet recruiting	18 months for histological analysis 5 years for clinical outcomes
Saroglitazar	Saroglitazar Vitamin E Combination	250 NAFLD		NAFL fibrosis score 6 months
Belapectin	Galectin-3 inhibitor	1010 NASH cirrhosis		Esophageal varices At 18 months

Rational for combination

There are several reasons to combine drugs to treat patients with NASH:

1. Improve the response rate in a population of patients with NASH. So far drugs tested in monotherapy trials have demonstrated a resolution of NASH below 60% with important differences of improvement in their placebo group (figure 1) [2], [3], [4], [5], [6], [7], [8]. Using combination of drugs the proportion of patients likely to respond will increase. This proportion can be further increased in patients are selected based on pathophysiological mechanisms. [9]

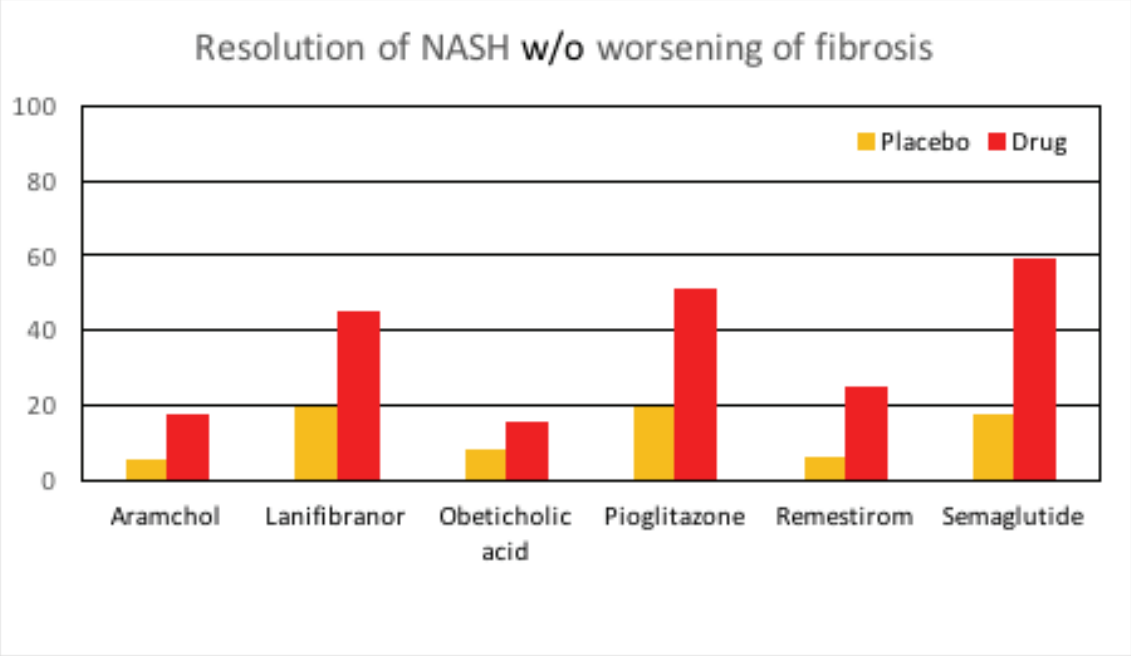

Figure 1. Percentages of resolution of NASH without worsening of fibrosis in different trials. These trials had different designs; this is not a head-to-head comparison of the drug effects.

2. Increase the degree of response in patients. Here the goal is to obtain in patients with NASH a more profound effect like resolution of NASH and improvement of fibrosis. Fibrosis is not part of the definition of NASH but it is a consequence of the chronic metabolic overload and inflammation. Fibrosis is a relevant therapeutic endpoint since it dictates the prognosis of the disease. It is logical from a clinical perspective to combine drugs to improve the fibrosis and to decrease the metabolic stress and inflammation that drives the fibrotic process. The phase 2 TANDEM trial assesses the combination of cenicriviroc with two doses of the FXR agonist tropifexor over 48 weeks (NCT03517540). Actually, approaches 1. and 2., if conceptually different are practically addressed together.
3. Allow intermittent treatment. On can envisage treating patients for a certain period of time, stop the combination therapy, follow the evolution, and eventually restart a treatment if indicated.
4. Avoid escape mechanisms. Targeting different pathways at the same time may prevent pathophysiological mechanism to escape the effect of a drug used in monotherapy.
5. Mitigate the side effect of a drug. There are currently two examples of this strategy. FXR agonists, including obeticholic acid, increase LDL cholesterol; combination with a statin may decrease this side effect. This was tested in the randomized, placebo-controlled, double-blind CONTROL phase 2 study. After 4 weeks of obeticholic acid, LDL cholesterol increased; addition of atorvastatin subsequently decreased LDL cholesterol below baseline values (NCT02633956) [10]. In the second example, ACC inhibition may be associated with hypertriglyceridaemia; combination with fenofibrate may decrease this side effect. In a phase 2 randomized trial, fenofibrate was prescribed 2 weeks before the addition of firsocostat in patients with advanced fibrosis due to NASH. Not only did the combination prevent increase in triglycerides, but it also improved hepatic fat and liver biochemistry (NCT02781584) [11].

Conclusion

With a rich pipeline of diverse drugs and many ongoing trials we are at a pivotal time in the field of NASH. The field needs to have non-invasive pathophysiologically meaningful biomarkers to diagnose NASH and to better select patients for specific treatments.

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Is there a role for endoscopic strategies in non-alcoholic steatohepatitis?

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Take-home messages

- Specialised enteroendocrine cells distributed in the gastrointestinal tract can sense the nutrients in the lumen and signal to tissues/organs that are involved in metabolism and the deposition of nutrients.
- Adaptive duodenal mucosal changes attributed to chronic exposure to fat and sugar-rich nutrients are associated with hyperinsulinemia, insulin resistance and diabetes.
- Duodenal mucosal resurfacing is a minimally invasive endoscopic procedure that results in a significant reduction in liver fat and improves glycaemic control in patients with type 2 diabetes.

Introduction

Flexible endoscopy provides access to gastrointestinal (GI) tract; minimally invasive endoscopic interventions are potentially reversible, repeatable and often cost-effective compared to surgical procedures. Therefore, a number of endoscopic procedures of varying degree of complexity including, intra-gastric balloon insertion, deployment of duodenojejunal sleeve and endoscopic sleeve gastroplasty have been designed. Structural alterations of the GI tract are associated with physiological alterations. This brief review will focus on the role of the duodenum as an endocrine organ, and the current evidence on the effect of duodenal mucosal resurfacing (DMR) on metabolic consequences of obesity.

Importance of duodenum as an endocrine organ

The GI tract constitutes the largest endocrine organ with over 40 hormones originating from specialised enteroendocrine cells scattered throughout the GI tract. Expression of these GI hormones exhibits a characteristic pattern with ghrelin, somatostatin and gastrin primarily produced in the stomach; glucose-dependent insulinotropic peptide (GIP) in the duodenum; cholecystokinin in the duodenum and jejunum, glucagon-like peptide (GLP)-1 in the jejunum, ileum and colon; and peptide YY (PYY) in the distal ileum and colon, respectively. This diverse group of cells senses the quantity and quality of nutrients in the intestinal lumen and signals to tissues/organs involved in metabolism and nutrients deposition. Incretins such as GLP-1 secreted by L cells and GIP secreted by K cells are among the products of enteroendocrine cells. These are key modulators of insulin secretion, glucose homeostasis, food intake, gastric emptying, energy expenditure, and hormonal regulation. There is an abundance of L cells in the hindgut. However, both L and K cells have been demonstrated in the first and second part of the human duodenum in equal numbers; their number is significantly increased in those with newly diagnosed type 2 diabetes [1]. The early phase in the post-prandial rise in both GIP and GLP-1 levels are linked to the exposure of duodenal K and L cells to luminal nutrients.

Within days of surgery, hyperglycaemia improves rapidly after Roux-en-Y gastric bypass (RYGB), a common bariatric procedure, suggesting weight loss independent mechanisms. With RYGB, nutrients

pass from a small gastric pouch through the 'alimentary limb' (about 125 cm of the jejunum) before meeting gastric, pancreatic juice and bile coming from the 'secretary limb'. Undigested food can directly stimulate nutrient-sensing endocrine cells in the ilium (hindgut hypothesis) stimulating incretins, GLP-1, anorexic hormones PYY and oxyntomodulin from L cells [2] and explains the rapid resolution of hyperglycaemia. Alternatively, the 'duodenal exclusion' (foregut hypothesis) from nutrient transit resulting from RYGB (Figure 1), could be the dominant mechanism in improving glucose homeostasis [3].

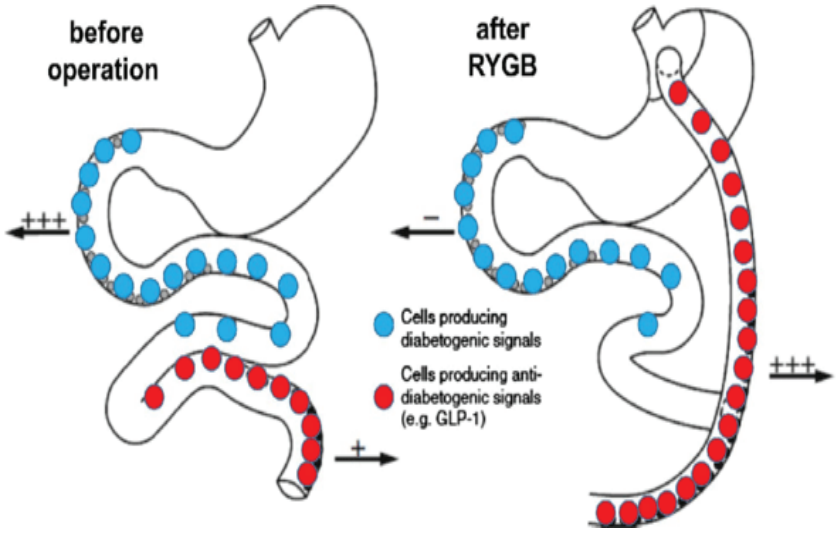

Figure 1. Foregut hypothesis: Putative mechanisms underlying the effectiveness of bariatric surgery in reversing type 2 diabetes (taken from [13]). RYGB, Roux-en-Y gastric bypass, GLP-1, glucagon-like peptide.

Pathophysiology of duodenal mucosal changes in obesity and type 2 diabetes

In the adult *Drosophila* midgut, intestinal stem cells interpret a nutrient cue to 'break homeostasis' and go through 'adaptive resizing' to drive reversible organ growth when food is abundant [4]. A discrete, local (not systemic) source of insulin appears to be important to adaptive midgut growth. High-fat feeding in Wistar rats stimulates progenitor cells to proliferate and differentiate, leading to GIP-expressing cell hyperplasia in the duodenum. On oral glucose tolerance test, circulating levels of glucose, insulin and GIP increased logarithmically, whereas GLP-1 decreased exponentially (despite a relative increase in the number of L cells). It is likely that chronic hyperinsulinemia determined by a high-fat diet, and which is also a characteristic of early diabetes and insulin resistance, may exert damaging effects upon L cell function via 'feed-forward loops' that regulates the cell cycle. In mice and pig models on a high-fat diet, GIP mediates the development of insulin resistance and diabetes. Blockade of GIP action using a GIP antagonist in mice results in a large weight loss, and a net improvement of both insulin resistance and diabetes.

In individuals with morbid obesity, both small intestinal enterocyte mass as estimated by plasma citrulline levels and enterocyte loss as assessed by intestinal fatty acid binding protein (I-FABP) levels, a marker of enterocyte loss, were significantly higher. Overall, I-FABP to citrulline ratio was higher in those with elevated glycated haemoglobin (HbA1c) levels when compared with those with normal HbA1c [5], indicating augmented epithelial proliferation. In another study involving obese individuals

with and without type 2 diabetes, delivery of nutrients to mid-jejunum (compared to the duodenum) through a balloon catheter, resulted in increased insulin sensitivity and inhibition of lipolysis [6]. Furthermore, the exclusion of the duodenum from nutrient transit after gastric bypass results in a net improvement of glucose metabolism in patients with type 2 diabetes, probably as a consequence of the lowering of circulating GIP levels [7].

Duodenal mucosal resurfacing as a treatment

The easy accessibility of the duodenum to flexible endoscopy makes it a potential target for intervention. Endoscopic DMR was first explored through proof-of-concept studies in preclinical rodent and porcine models, followed by the first human study testing the safety and efficacy in patients with type 2 diabetes [8].

In a human study of DMR [9], hydrothermal ablation of the duodenal mucosa from 1 cm distal to the ampulla of Vater to the proximal ligament of Treitz was performed in 39 patients with type 2 diabetes (screening HbA1c: 9.5% [80 mmol/mol]; body mass index; BMI: 31 kg/m^2). Mean HbA1c was reduced by 1.2% at 6 months in the full cohort (p <0.001). Those who had a long duodenal segment ablated (n = 28; 9.3 cm treated) had a mean HbA1c reduction of 2.5% at 3 months post-procedure compared with 1.2% in those who had a short segment ablated (n = 11; 3.4 cm treated). Three patients experienced duodenal stenosis and were treated successfully by balloon dilation.

DMR is a minimally invasive, upper GI endoscopic, catheter-based procedure (Figure 2) that uses an integrated dual-function catheter system (Fractyl Laboratories, Inc, Lexington, MA), which is passed over a guidewire alongside the endoscope. The duodenal mucosa is first lifted and then ablated by a pressure-based hydrothermal balloon at the tip of the catheter. Circumferential hydrothermal ablations last approximately 10 seconds at temperatures of approximately 90°C. These cycles are repeated to treat 10 cm of the post-papillary duodenum in a single endoscopic session. Ablation remains limited to the superficial intestinal mucosa and does not damage the underlying muscularis mucosa or deeper structures. DMR is followed by a re-epithelialisation that seems to initiate within days following the procedure, achieving a reset of the duodenal mucosa.

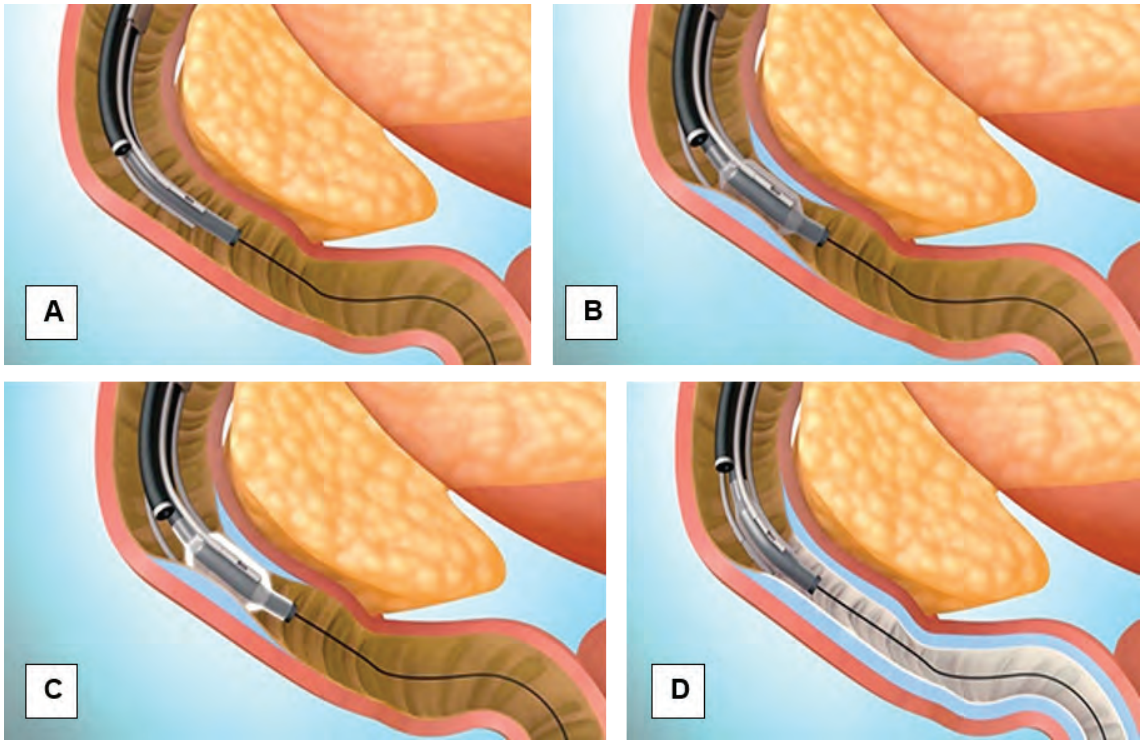

Figure 2. Duodenal mucosal resurfacing procedure. (A) Integrated dual-function catheter system is passed over a guidewire alongside the endoscope. (B) Duodenal mucosa is first lifted with submucosal saline injection. (C) The mucosa is ablated by the hydrothermal balloon. (D) These cycles are repeated to treat 10 cm of the post-papillary duodenum. Courtesy Fractyl Laboratories, Inc, Lexington, MA and used in [14].

In the first international multicentre, open-label study [9], 46 patients were enrolled (BMI of 24–40 kg/m^2) with type 2 diabetes (HbA1c 59–86 mmol/mol [7.5%–10.0%]) on stable oral glucose-lowering medication. DMR was completed in 37 patients (80%); in the remaining patients, technical issues were encountered during the procedure. At 24 weeks following the procedure (n = 36), patients showed an improvement in HbA1c (–10±2 mmol/mol (–0.9%±0.2%), p <0.001), fasting plasma glucose (–1.7±0.5 mmol/L, p <0.001), homeostatic model assessment for insulin resistance (–2.9±1.1, p <0.001), and weight reduced (–2.5±0.6 kg, p<0.001); these effects were seen 4 weeks after the procedure and sustained at 12 months. Changes in HbA1c did not correlate with the modest weight loss. 'Diabetes treatment satisfaction scores' improved significantly. In addition, alanine aminotransferase (ALT) levels decreased from 40±4 U/L at baseline to 31±2 U/L at 24 weeks (p = 0.016) and to 30±3 U/L at 12 months follow-up (p <0.001). The authors suggested that these findings are an indication of the beneficial effect of DMR on non-alcoholic fatty liver disease and the effect were sustainability.

In this study, general anaesthesia was used in 35 patients, and 11 patients were sedated with propofol. One procedure led to a serious adverse event, pyrexia (38°C), and elevated C-reactive protein on the first day after DMR; this resolved within 3 days. No patients experienced severe hypoglycaemia.

In a multicentre, randomised, double-blind, sham-controlled feasibility trial including patients with type 2 diabetes (REVITA-2) [10], before and 12 weeks following DMR, liver fat content was estimated using local magnetic resonance imaging (MRI) proton-density fat fraction (PDFF) acquisition protocols. In the modified intent-to-treat analysis (DMR n=56; sham n=52), 24weeks post DMR, median (IQR) HbA1c change was –10.4 (18.6) mmol/mol in DMR group versus –7.1 (16.4) mmol/mol in sham group

(p=0.147). In patients with baseline liver MRI-PDFF >5% (DMR n=48; sham n=43), 12-week post-DMR liver-fat change was –5.4 (5.6)% in DMR group versus –2.9 (6.2)% in sham group (p=0.096).

When results were stratified by region, among Europeans, post DMR, median (IQR) HbA1c change was –6.6mmol/mol (17.5mmol/mol) versus –3.3mmol/mol (10.9mmol/mol) post-sham (p=0.033); 12-week post-DMR liver-fat change was –5.4% (6.1%) versus –2.2% (4.3%) post-sham (p=0.035). Among Brazilians results trended towards DMR benefit in HbA1c, but not liver fat, in context of a large sham effect. Overall, on per protocol analysis, patients with high baseline fasting plasma glucose ((FPG)≥10mmol/L) had significantly greater reductions in HbA1c post-DMR versus sham (p=0.002).

No serious or unanticipated adverse device effects were reported throughout the 24 weeks study in the European population while 11.8% (n=2) in the Brazilian population including jejunal perforation requiring surgical repair.

In conclusion, DMR shows promise as an intervention with an ability to improve glycaemic control and reduce liver fat. Its impact on clinical practice will be determined by the sustainability of the beneficial effects as well as its cost-effectiveness.

Outlook

Research continues to discover new pathways in the gut-brain-liver axis. Recent publications have highlighted the role of duodenal nicotinamide adenine dinucleotide (NAD+)–dependent deacetylase sirtuin 1 (Sirt1) and Ampk–dependent pathways in reversing insulin resistance, reducing hepatic glucose production in obesity and type 2 diabetes [11, 12]. Increased understanding of the full range of physiological functions of the duodenum has the potential to expand our options to treat and prevent metabolic disorders and their consequences.

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Bariatric surgery in NASH

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Take-home messages

- Bariatric surgery results in weight loss and improvement of diabetes; prospective data have shown an improvement in all histological lesions of NASH, including fibrosis.
- Most of the bariatric surgeries performed currently are either the laparoscopic sleeve gastrectomy or the laparoscopic Roux-en-Y gastric bypass. No clear advantage of either procedure has been shown in general or for NAFLD/NASH patients.
- Bariatric surgery procedures should be considered only if non-surgical measures to lose weight cannot be achieved or are not lasting.
- Careful follow-up is needed after bariatric surgery to evaluate the evolution of liver disease; liver biopsy or non-invasive methods can be used.

Introduction

According to WHO, in 2016, more than 1.9 billion adults, 18 years and older, were overweight; of these, over 650 million were obese (WHO Obesity and overweight, 2020). In the context of this impressive number of obese individuals worldwide, the use of bariatric surgery (BS), has dramatically increased in recent years. Given the close association of obesity and in particular morbid obesity, with non-alcoholic fatty liver (NAFLD) and non-alcoholic steatohepatitis (NASH), there is now extensive experience in using these procedures in NAFLD/NASH patients.

There is strong evidence that weight loss may stabilize or reverse NASH. Consequently, management of NAFLD/NASH is very much focused in achieving weight loss, mostly in obese patients, by offering advice for lifestyle changes to promote a healthier dietary pattern and a regular practice of exercise. Nonetheless, although these measures are extremely effective when they result in significant weight loss, only 19% of individuals enrolled in an intensive program were able to achieve a reduction of more than 7% [1]. Furthermore, several studies have shown that after completion of a weight loss program, most people regain 30–50% of the weight that had been lost within the first year and that within 2–5 years most return to a weight very close to their original baseline. Consequently, more effective, and long-lasting measures to reduce weight, such as BS need to be considered [2]. One of the main advantages of BS is the durability of weight loss; a recent systematic review showed that all current bariatric procedures are associated with substantial and durable weight loss after 10 or more years follow-up [3].

Types of bariatric surgery

Currently, most of the bariatric surgeries performed are either laparoscopic sleeve gastrectomy (LSG) or laparoscopic Roux-en-Y gastric bypass (LRYGB) (Figure 1). These procedures were found to be superior to diet and exercise alone in achieving long-term weight control [4].

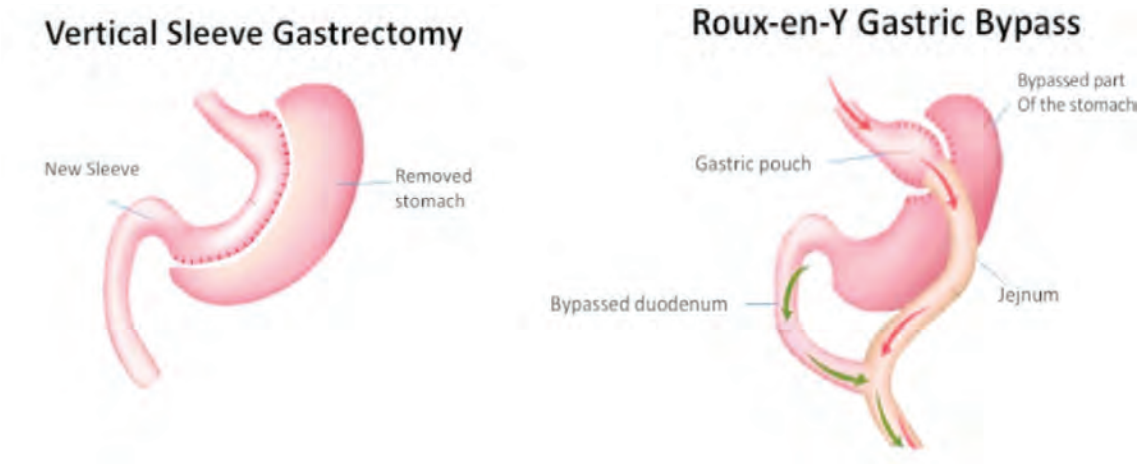

Figure 1. The two most common bariatric procedures, sleeve gastrectomy (SG) or Roux-en-Y gastric bypass (RYGB) (adapted from [2]).

LSG is a restrictive procedure, easy to be performed, but with less metabolic effects than LRYGB. This rapid surge of popularity of LSG may be due to its effectiveness in achieving weight loss and remission of comorbidities, being a less technically intensive procedure [5].

Regarding the combined restrictive and malabsorptive procedures, LRYGB is currently the most used procedure. It involves dividing the stomach into a small upper gastric pouch, connected to the lower part of the bowel, therefore, bypassing a major portion of the stomach and the upper part of the intestine. LRYGB is very effective in reducing weight and controlling comorbidities; the procedure achieves its benefits through bile flow alteration, reduction in gastric size, vagal manipulation, and subsequent enteric gut manipulation. It has a high-risk of complications, including a high early complication rate (like dumping syndrome). Furthermore, patients often require supplements due to the development of nutritional deficits.

In 2016, LSG was the most performed BS worldwide, making up 53.6% of operations compared to the 30.1% of LRYGB [6]. A recent meta-analysis was performed, comparing both techniques, including 33 studies with a total of 2475 patients, and found that LRYGB resulted in greater loss of body mass index (BMI) compared to LSG at 1 year, which persisted at 3 years, but there was insufficient evidence at 5 years; rate of complications was similar [5].

In the setting of NAFLD/NASH, a study including 310 patients matched for both procedures with biopsy-proven NAFLD/NASH and pre-operative abnormal liver function tests, found that both bariatric procedures were similarly effective in improving liver function tests [7]. Another recent systematic review and meta-analysis including 9940 individuals, comparing the impact of Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) on NAFLD/NASH, showed no difference regarding the histopathological outcomes [8]. In summary, no clear advantage of one of the procedures was demonstrated regarding NAFLD/NASH.

Metabolic effects of BS

Although initially used mostly for cosmetic reasons, soon it was demonstrated as very effective in treating the metabolic syndrome, type 2 diabetes mellitus (T2DM), hypertension, and hyperlipidaemia, thus leading to the concept of metabolic surgery.

In fact, one randomised controlled trial that assessed T2DM two years after gastric bypass surgery reported a remission rate of 75%, with a mean HbA1c decreasing from 8.6% at baseline to 6.4%

in the absence of pharmacologic therapy [9]. Also, a meta-analysis including a total of 16 studies (n = 6131) and a mean 17.3-month follow-up, showed that BS was significantly more effective than conventional medical therapy in achieving weight loss, HbA1c and fasting plasma glucose reduction, as well as diabetes remission. BS patients reaching T2DM remission ranged from 9.8 to 15.8-times the odds of conventional therapy [10].

Also, it was shown that BS improves long-term survival and death from cardiovascular disease and malignancy, the two most common causes of death in NAFLD.

Endocrine implications of bariatric surgery

A large part of the metabolic improvements from BS is related to the anatomical changes that are the result of limiting food consumption, reduced appetite, and the change in gut hormone secretion.

Table 1 shows the major differences and similarities between the effects of RYGB, SG and diet-induced weight loss on incretins and hormones.

Marked changes in gut hormone secretion occurs after gastric bypass surgery and these hormonal changes are largely influenced by the type of BS. These include a significant increase in glucagon-like peptide 1 (GLP-1) secretion just days after BS in both RYGB and SG, in a degree not seen in patients achieving a similar weight loss by caloric restriction. This may be due to the increased delivery of large nutrient loads to the distal gut containing a high number of incretins GLP-1 and peptide YY (PYY) producing L cells (hindgut hypothesis). Multiple studies have shown that GLP-1 signalling is a crucial player in the improvement of insulin sensitivity after BS. Indeed, changes in the secretion of gut hormones after gastric bypass surgery are in part responsible for the mechanism of weight loss and resolution of T2DM.

Ghrelin is secreted by gastric and duodenal enteroendocrine cells and is the only orexigenic hormone produced in the gastrointestinal tract that stimulates appetite and increases food intake. While caloric restriction results in a rise in the levels of ghrelin, they decrease after RYGB and SG [11].

Table 1. Effects of RYGB, SG and diet-induced weight loss on incretins and hormones taken from [11].

	RYGB	SG	Diet-Induced Weight Loss
Ghrelin	↓	↔ ↓	↑
PYY	↑	↑	↓
GLP-1	↑	↑	↔
GIP	↑ ↔	↑ ↔	↑ ↔
CCK	↑	↑	↓
Insulin	↑	↑	↓
Leptin	↓	↓	↓
Adiponectin	↑	↑	↑
Estrogen	↓	↓	—

Levels of GLP-1 and PYY rise after both RYGB and SG and not in diet-induced weight loss. Ghrelin levels decrease markedly after SG and increase after diet-induced weight loss. There are conflicting data regarding the change of GIP after surgical and diet-induced weight loss and ghrelin levels after RYGB. While estrogen reduction is associated with surgical weight loss and exercise-induced weight loss it does not significantly change in diet-induced weight loss.
↓ Decreased, ↑ Increased, ↔ Conflicting data, – No change.

RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; PYY, peptide YY; GLP-1, glucagon-like peptide 1; GIP, glucose-dependent insulinotropic peptide; CCK, cholecystokinin. Arrow up, increased; arrow down, decreased; horizontal arrow, conflicting data; en dash, no change.

Cost efficacy of bariatric surgery in NASH

A recent modelling study analysed the clinical and cost-effectiveness of surgery in patients with NASH [12]. It was found to be both effective and cost-effective for obese patients with NASH, regardless of fibrosis stage; in overweight patients, surgery increased QALYs for all patients regardless of fibrosis stage but was cost-effective only for patients with F3 fibrosis; the authors conclude that there is great promise of BS for treating NASH, but clinical trials in this area are required. More recently, the same group evaluated BS in patients with NASH compensated cirrhosis and found it could be highly cost-effective in patients with NASH and compensated cirrhosis and obesity or overweight patients [13].

Indication for bariatric surgery

Indication for BS in the context of NASH is somewhat unclear as there are no randomized controlled trials of BS in NASH, although there are several retrospective and prospective cohort studies. In fact, a single centre study with follow-up liver biopsies, from Lassailly *et al.* prospectively correlated clinical and metabolic data with liver histology at the time of surgery and 1 and 5 years after BS in 381 adult patients with severe obesity [14]. More recently, the same group published data, evaluated a group of 180 patients with biopsy-proven NASH and found that NASH resolved in liver biopsies from 84% of patients, 5 years after BS. The reduction of fibrosis began during the first year and continued through 5 years [15] (Figure 2).

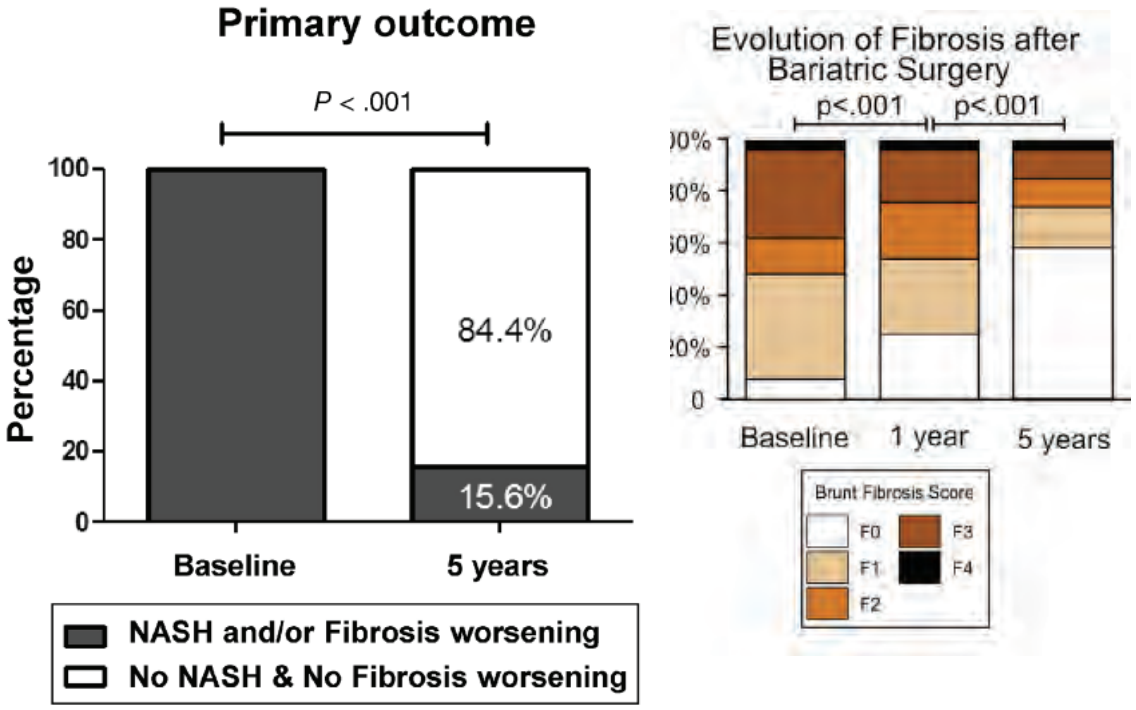

Figure 2. Bariatric surgery induced the disappearance of NASH in 85% and was maintained at 5 years [15].

A recent meta-analysis of 32 cohort studies comprising 3093 biopsy specimens, showed that BS induced a biopsy-confirmed resolution of steatosis in 66% of patients, inflammation in 50%, ballooning degeneration in 76% and fibrosis in 40%; with a patient's mean NAFLD activity score significantly reduced after BS (mean difference, 2.39). Nonetheless, BS resulted in new or worsening features of NAFLD, such as fibrosis, in 12% of patients [16].

Guidance from AASLD [17], recommends that BS can be considered in otherwise eligible obese individuals with NAFLD or NASH, but not yet as an established treatment for NASH. This guidance also considers that patients with compensated NASH or cryptogenic cirrhosis, that are otherwise eligible patients can be considered for BS, in experienced BS programs. In fact, there is a trend for further considering these patients, since although they may be at increased risk, they may greatly benefit from the procedure.

The EASL guidelines [18] state that BS is an option for reducing weight and metabolic complications in patients unresponsive to lifestyle changes and pharmacotherapy, with stable results in the long-term. It also states that there is no definite data on what the best surgical procedure is in this situation.

BS is indicated in obese patients with a BMI above 40, or those with a BMI between 35 and 39.9 and severe obesity-related comorbidity such as diabetes. In patients with a lower BMI, the indication as a treatment for NAFLD/NASH is still controversial.

Regarding specifically BS in patients with cirrhosis, data from case-series suggests that that bariatric surgery can be performed safely in patients with well compensated cirrhosis. Data regarding patients with decompensated disease and/or portal hypertension do not allow us to draw conclusions regarding safety and must be decided individually. Consideration should be given to cirrhosis severity, liver synthetic function, portal hypertension as well as the impact of surgical factors, and should be performed only in centres with large experience in BS [19].

Bariatric surgery after liver transplantation

Patients with morbid obesity and an indication for liver transplantation (LT), may benefit from bariatric surgery (BS) in the peri-transplant period. In fact, BS prior to LT could even prevent the progression of NASH and reverse the need for LT. Furthermore, BS during or after LT could also improve obesity-associated conditions such as diabetes, as well as reduce the incidence of NASH in the post-LT setting. However, there is still no consensus regarding the timing and use of BS in this setting [20].

Bariatric surgery and liver failure

The possibility of development of liver failure after BS, mostly after biliopancreatic diversion (BPD) has been reported in several case-reports and small case series. The causes are probably multi-factorial, relating to factors such as bacterial overgrowth, malnutrition, and rapid weight loss. Some of these cases have a fatal course. This procedure has been mostly abandoned, but if done, careful follow-up of these patients is mandatory.

Hepatologic follow-up after bariatric surgery

Since the prevalence of NAFLD is higher than 95% in morbidly obese patients, routine liver biopsies should be done at the time of BS, to diagnose and be able to evaluate the progression of the disease. More controversial is the issue of follow-up liver biopsies. In fact, with the availability of less invasive diagnostic tools, imaging studies such as magnetic resonance imaging (MRI-PDFF), magnetic

resonance elastography (MRE) and FibroScan® may be used either to supplement or even replace, repeated biopsies [21].

Regarding the recovery of liver function, a recent study found that after BS, especially sleeve gastrectomy, leads to an improvement of liver function (measured by LiMax), although a deterioration of liver function capacity may happen in patients with T2DM, higher preoperative weight and male sex [22].

Conclusions

BS seems to very effective in reducing and maintaining weight loss. Furthermore, it is now demonstrated to result in a histological improvement of NASH. Frequent NAFLD-metabolic associated conditions, such as diabetes, hypertension and dyslipidemia also tend to improve. Consequently, it is foreseeable that the role of BS in the treatment of NAFLD/NASH may become more prominent in the future, unless very effective drugs for obesity will be available.

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SESSION 2

IMPACT OF PHYSICAL ACTIVITY AND DIET ON LIVER DISEASE

WEDNESDAY 23 JUNE
11H15 - 12H15

Sodas and screens: threats for the liver

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Take-home messages

- Diets high in sugar (from sucrose and/or high-fructose corn syrup) and low in physical activity (i.e. increased screen time) not only increase the risk of NAFLD, but also non-alcoholic steatohepatitis.
- Fructose precipitates fatty liver acquisition and progression through various mechanisms, including increased *de novo* lipogenesis, impaired fatty acid oxidation, increased ATP consumption, and alterations in gut microbiota and gut permeability.
- Clinical studies demonstrate that increased consumption of sugar-sweetened beverages is associated with NAFLD acquisition and progression; however, current studies have not controlled well for potential confounding factors which may be associated with fructose consumption and metabolic syndrome (i.e. high-fat diet or sedentary lifestyle).
- Well-designed prospective clinical and epidemiological studies are necessary to clearly define the effect(s) of sugar-sweetened beverages and screen time at varying doses on human health and disease.

The threat of sugar-sweetened beverages and the liver

Sodas and screen time are an important public health issue. Americans consume more per-capita sugar-sweetened beverages containing high-fructose corn syrup (HFCS) than any other nation. Fructose consumption has doubled over the past three decades, and the consumption of excess fructose has been linked to hypertension, dyslipidemia, type 2 diabetes, obesity, gut and fatty liver disease. While some have argued that fructose is no different than sucrose and that HFCS-55 is roughly equivalent to or similar in composition to sucrose, a growing body of evidence suggests that fructose consumption plays a direct role in the risk for metabolic disease and may have adverse effects on central appetite regulation compared with glucose. Despite this evidence, current food-labelling practices do not provide information on fructose content in foods and beverages made with HFCS, fruit juice concentrate or crystalline fructose. Current FDA guidelines for the use of HFCS-55 as an ingredient only require it to be a “minimum” of 55% fructose and allow the unrestricted sales and use of HFCS-90.

Metabolism of fructose

Fructose metabolism is distinct from glucose metabolism [1]. Glucose is metabolised primarily by glucokinase or hexokinase, whereas fructose is principally metabolised by fructokinase. Fructokinase utilises ATP to phosphorylate fructose to fructose-1-phosphate, followed by the metabolism by aldolase B to generate D-glyceraldehyde and dihydroxyacetone phosphate. From this stage on, fructose metabolism is similar to glucose metabolism and results in the generation of glucose, glycogen, and triglycerides. Thus, the unique aspect of fructose metabolism lies in its first two enzymatic steps.

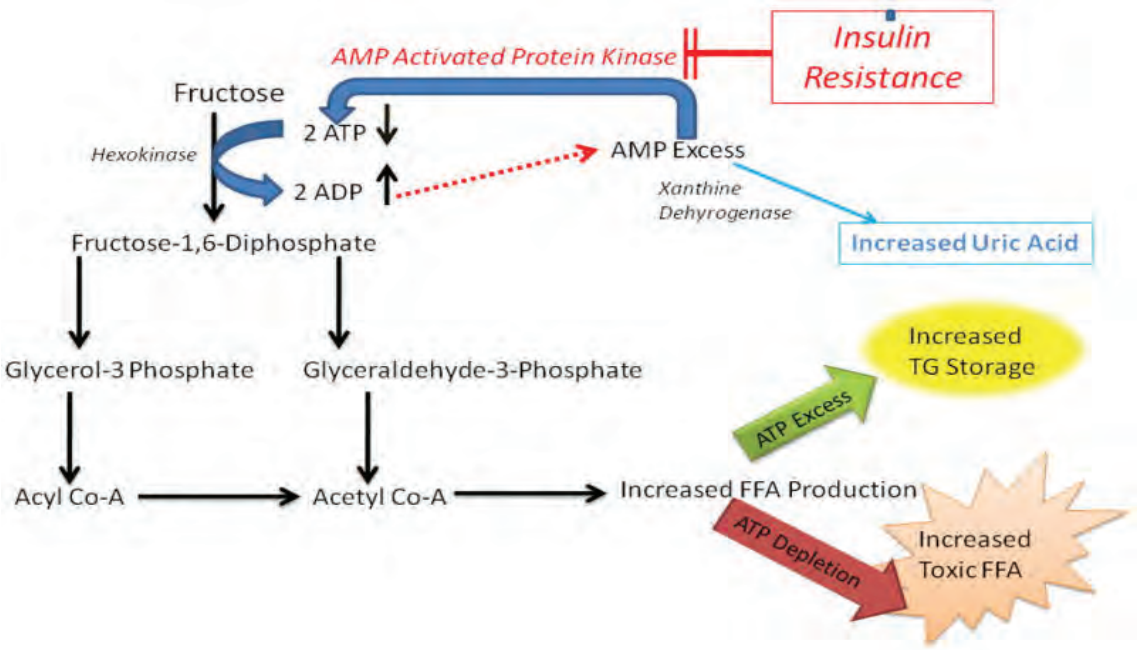

Figure 1. Fructose metabolism.

Fructokinase in the liver phosphorylates fructose rapidly and without any negative feedback control, resulting in a drop of ATP and intracellular phosphate. The fall in intracellular phosphate activates the enzyme, adenosine monophosphate (AMP) deaminase, that converts AMP to inosine monophosphate (IMP), resulting in purine nucleotide turnover that culminates in the formation of uric acid. In humans, intravenous and orally administered fructose induces an increase in uric acid and an ATP depletion. Unlike glucose, when fructose is metabolised there is a transient decrease in intracellular phosphate and ATP levels associated with nucleotide turnover and uric acid generation. This fall in ATP level induces a series of reactions, including a transient block in protein synthesis, an induction in oxidative stress, and mitochondrial dysfunction that turn out to have a key role in fructose-mediated effects in the pathogenesis of NASH [2].

Experimental studies

Dietary fructose, sucrose, or HFCS have been shown to have a special tendency to induce fatty liver in experimental animals as well as inflammation. To develop the fatty liver, it usually takes at least 8–24 weeks on a high-fructose diet with more progressive disease notable with longer exposure. Often the administration of fructose also induces other features of metabolic syndrome, including elevated blood pressure, elevated serum triglycerides, and insulin resistance. In part, the fatty liver may be due to increased energy intake, as high-fructose intake induces leptin resistance in rats. However, if diet is controlled so that the control group ingests the same amount of total energy, the fructose-fed rats will still develop features of metabolic syndrome, although weight gain does not differ between groups. Indeed, one can even induce fatty liver with a calorically restricted diet if the diet is high (40%) in sugar. Others have also reported that a high-fructose diet can induce fatty liver in the absence of weight gain. When administered to primates, fructose was shown to result in both an increase in liver fat and hepatic fibrosis after seven years, with the degree of fibrosis correlating with the time of fructose exposure.

Endogenously generated fructose may also have a role in fatty liver and NAFLD. High portal vein levels of glucose can induce the expression of aldose reductase in the liver, which can convert the glucose to sorbitol, which is then further metabolised to fructose by sorbitol dehydrogenase (the polyol pathway). Indeed, glucose-fed mice show increased fructose levels in their liver, and when fructose metabolism is blocked (by giving glucose to fructokinase knockout mice) the animals are almost completely protected from fatty liver and insulin resistance and are partially protected from obesity.

Clinical studies

Sugar-sweetened beverages are associated with NAFLD in humans. Patients with NAFLD were reported to have 2-3-fold higher intake of sugar-sweetened beverages compared to age, gender and BMI-matched controls [3]. Subsequently, the association of fructose from soft drinks has been associated with NAFLD in children, adolescents and adults, where it correlates in a dose-dependent manner with the severity of hepatic fibrosis [4].

Clinical studies also suggest a role for fructose in NAFLD. The administration of sugar-sweetened beverages for 6 months to humans resulted in increased liver fat confirmed by magnetic resonance spectroscopy [5]. Conversely, the restriction of fructose for 9 days in children with a high baseline fructose intake resulted in a reduction in liver fat compared to controls fed an isocaloric diet [6]. In a subset of the same study, there was also an improvement in other features of metabolic syndrome, including diastolic blood pressure, serum triglycerides and insulin resistance. While fruits contain fructose, they are less likely to induce metabolic syndrome due to the lower fructose content per fruit (compared to a soft drink). Reducing sugar or HFCS intake is a modifiable dietary risk factor for NAFLD/ NASH, which may improve the risk for disease acquisition and progression.

In a recent study of the Framingham Heart Study cohort (n = 2634 with CT imaging and n = 5908 with alanine aminotransferase (ALT)), participants were categorised as either non-consumers or consumers (3 categories: 1 serving/month to <1 serving/week, 1 serving/week to <1 serving/day, and ≥1 serving/day) of sugar-sweetened beverages or diet soda [7]. After adjustment for age, sex, smoking status, Framingham cohort, energy intake, alcohol, dietary fibre, fat (% energy), protein (% energy), diet soda intake, and body mass index, the odds ratios of fatty liver disease were 1, 1.16 (0.88, 1.54), 1.32 (0.93, 1.86), and 1.61 (1.04, 2.49) across sugar-sweetened beverage consumption categories, respectively (p trend = 0.04). Sugar-sweetened beverage consumption was also positively associated with ALT levels (p trend = 0.007). There was no association between diet, drinks and fatty liver.

In addition, frequent soda intake is more common in adolescents with visceral obesity. Soda intake was also more common in youth with elevated absolute visceral adipose tissue (>80 cm^2) than in youth without elevated absolute visceral adipose tissue (37% compared with 13%, respectively; p = 0.02). Two large randomised controlled trials have demonstrated that a reduction of sugar-sweetened beverages intake decreases adiposity in children and may benefit overweight and obese children with NAFLD [8,9]. Limiting screen time activities to <2 hours/day and increasing moderate to high-intensity physical activity is recommended for all children, including those with NAFLD [10].

The threat of increased screen time

Increases in usage time for computer/mobile devices (i.e. screen time) are inversely proportional to decreases in physical activity and risk for NAFLD. Adolescent boys with screen time of 2 or more hours per day on weekdays, have twice the risk of abnormal levels of insulin and HOMA-IR compared

with peers with screen time less than 2 hours per day on weekdays [11]. In a cross-sectional study conducted on 7516 adults in Tianjin, China for which screen time was calculated from questionnaires, findings showed that after adjustments for potential confounding factors, the odds ratios (95% confidence interval) of having overall NAFLD associated with increased screen time were 1.00 for <1 hour/day, 1.58 (1.22-2.05) for 1-3 hour/day, 1.58 (1.18-2.11) for 3-5 hour/day, 1.65 (1.21-2.27) for 5-10 hour/day, and 1.99 (1.29-3.05) for >10 hour/day (p trend = 0.02), respectively [12].

There are potential mechanisms that may underlie the inverse association between physical activity and risk for NAFLD. Firstly, physical activity might improve appetite control by enhancing satiety signalling. Secondly, physical activity not only reduces free fatty acids in circulation but also increases the uptake and utilisation of fatty acids in liver and skeletal muscle, leading to subsequently reduced hepatic fat accumulation. Thirdly, hepatic and muscle insulin resistance is considered the pathophysiological hallmark of NAFLD, which could be ameliorated directly by increased physical activity probably through a reduction in hepatic fat content or indirectly through an increase in muscle glucose transporter 4 expression and muscle glycogen synthase activity. A recent meta-analysis indicated that increased physical activity is dose-dependently associated with a lower risk of NAFLD. The current guidelines recommends that a minimum physical level (500 MET-minutes/week) is able to moderately reduce NAFLD risk [13]. Therefore, decreasing screen time and increasing physical activity are an essential foundation for the treatment of NAFLD/ NASH.

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What is wrong with my sleep?

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Take-home messages

- The circadian rhythm and sleep patterns are impacting health and disease.
- The hepatic circadian clock network regulates genes and proteins by post-transcriptional/post-translational mechanisms.
- Disorganisation of the central and hepatic circadian clock network promotes non-alcoholic fatty liver disease (NAFLD), fibrogenesis, and hepatocellular carcinoma (HCC) in animal models.
- Translational studies support an influence of chronobiology on liver disease.
- Disruption of sleep-wake patterns increase metabolic derangement in NAFLD and promotes steatohepatitis and HCC.
- Nutrition affects the circadian clock network and is a powerful tool to modulate the hepatic clock network to promote liver health.

Introduction

Chronobiology – a term that reflects the biological changes that underline the day and night cycles in mammals – has a strong impact on health and disease. Light and activity affect the ability to develop, learn and regulate well-being, but also subtler body processes including hormone signalling, body temperature, immune function, and digestive activity. Wake and sleeps phases have a major impact on the metabolism - most prominently reflected by periods of feeding and fasting - which by themselves have a major influence on health and disease. The complex interaction of behaviour and biological processes makes it difficult to dissect independent contribution to health and disease in humans. Animal models have produced evidence on the direct interaction of wake and sleep phases and the regulation of genes and proteins. In the central nervous system, the suprachiasmatic nucleus in the hypothalamus has been identified as the clock region. It constitutes the master oscillator of the circadian system in mammals which regulates the circadian rhythm and exerts neuronal (e.g. the sympathetic nervous system) and hormonal (e.g. glucocorticoid signalling) activities to regulate many body functions depending on a 24-hour cycle. Importantly, peripheral organs exhibit an additional circadian clock network that exerts organ specific effects and regulates gene transcription, but also affects post-translational modification to adjust to organ specific requirements (Figure 1).

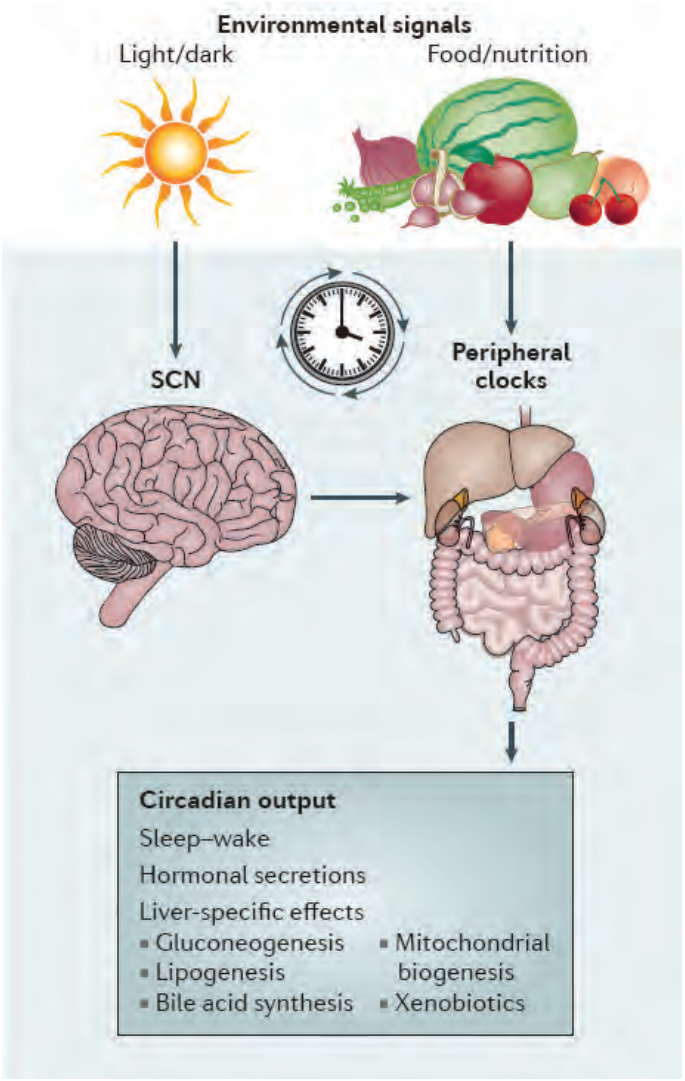

Figure 1. A central and peripheral clock network exist to sense environmental, external factors and conveys these to regulate gastrointestinal and liver-specific functions (adapted from [1]). SCN, suprachiasmatic nucleus.

Scientific evidence linking circadian rhythm to liver disease

The reciprocal influence of feeding and wake phases highlights the tight link of the clock network with metabolism - even across species despite differences in the involved genes and proteins. Animal models have been helpful to delineate the circadian network. Both global *Clock* gene knockout mice as well as liver-specific knockouts have been used to study clock dysfunction during health and disease states. These have given rise to the concept, that clock network dysfunction accelerates the development of liver disease. In particular, non-alcoholic fatty liver diseases (NAFLD) and hepatocellular carcinoma (HCC) have been implicated in the context of circadian clock network dysregulation. To recapitulate the tight link of nutrition and sleep-wake phases, the term chrononutrition has been coined. In particular, the timing and the composition of nutrients can synergise with the circadian clock network to affect liver disease. In mice, the hepatic clock network regulates cell homeostasis, including the energy metabolism and the expression of enzymes controlling the absorption and metabolism of xenobiotics. Alterations of either the circadian rhythm or the metabolic system (e.g. behaviour affecting wake-sleep

circle, interruption of sleeping phases or a high-fat diet) can result in the dysregulation of metabolic pathways and circadian clock function. In mice that are homozygous for dominant negative *Clock* alleles hyperphagia, obesity and hyperglycaemia develop (Figure 2; adapted from [2]). Likewise, disruption of *Bmal1* in adipocytes leads to obesity [3], while islet-specific *Bmal1* ablation promotes type 2 diabetes [4]. These animal models demonstrate the importance of the peripheral clock networks in addition to the central nervous system circadian clock network.

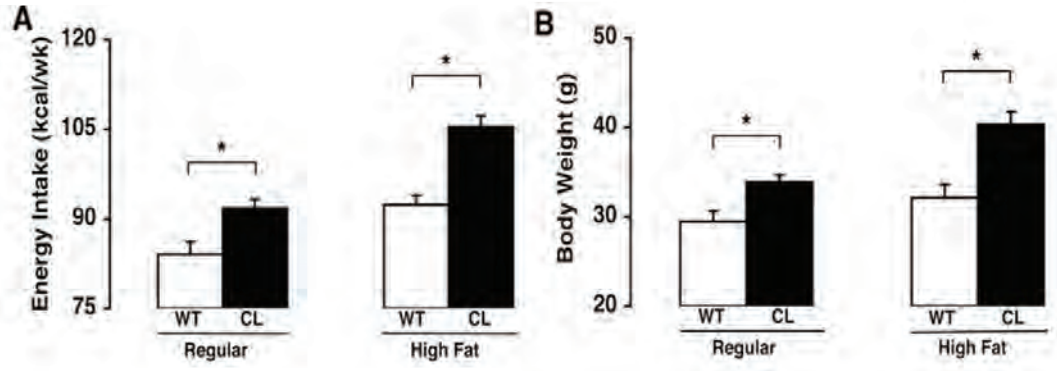

Figure 2. Disruption of the clock gene exacerbates weight gain in mice on a high-fat diet (taken from [2]). WT, wild-type; CL, *clock* knockout.

In these knockout models, several nuclear receptors have been identified that are crucially involved in liver tissue homeostasis and influenced by the circadian clock network, in particular, regulators of the energy metabolism. Interestingly, some of these nuclear receptors have also been identified as targets that are currently explored in clinical trials for NASH. The implication that the circadian rhythm could influence the effect strength of these emerging therapeutic approaches highlights the importance to address nutrition and behaviour on top of pharmacotherapy in NASH (Table 1).

Table 1. Circadian clock genes and controlled nuclear receptor genes that have been identified as regulators of liver function (adapted from [1]).

Circadian clock genes	Clock-gene controlled genes	Pharmacokinetic and hepatic function
Period circadian regulator 1 (Per1)	Peroxisome proliferator-activated receptor alpha (PPARalpha)	Adsorption (gut)
Period circadian regulator (Per2)	Proline and acidic amino acid rich basic leucine zipper (PAR bZIP)	Uptake (liver)
Rev-erb alpha	D-site binding protein (Dbp)	Metabolism (liver)
Rev-erb beta	Thyrotroph embryonic factor (Tef)	Elimination (liver/bile)
RAR-related orphan receptor alpha (Ror alpha)	Hlf	
Rot gamma	Nuclear factor interleukin-3-regulated protein (Nfil3)	
	Constitutive androstane receptor (CAR)	
	Pregnane X receptor (PXR)	
	Aryl hydrocarbon receptor (Ahr)	

Animal models have also highlighted that the hepatic clock network modulates the expression of cytochromes (e.g. Cyp2a4, Cyp2a5, Cyp2b10, Cyp2c22, Cyp2e1, Cyp3a11, Cyp4a3, Cyp4a14, Cyp7 and Cyp17), which are involved in the detoxification, bile synthesis and oxidative stress by hepatocytes and cholangiocytes.

In a recent study, the role of bile acids and the sympathetic nervous system was proposed in the context of hepatocarcinogenesis from NAFLD. Herein, mice with a disrupted sleep/wake cycle - a model of repeated jet lag - developed a number of abnormalities including neurodegeneration, ulcerative dermatitis, accelerated ageing, and NAFLD with HCC at the age of 90 weeks as a direct consequence of the disruption of the circadian clock. Interestingly, two nuclear hormone receptors, Farnesoid X receptor (FXR) and constitutive androstane receptor (CAR) were dysregulated leading to the accumulation of bile acids (BA). BA-induced over activation of CAR, and eventual CAR-dependent liver injury, fibrosis, and neoplasia occurred (Figure 3). The activation of CAR was dependent on beta-adrenergic receptors activation and could be augmented by blockade of the sympathetic nervous system. This study highlighted the fundamental role of the integrated networks of metabolic responses that are controlled by FXR and CAR during the disruption of the circadian rhythm [5].

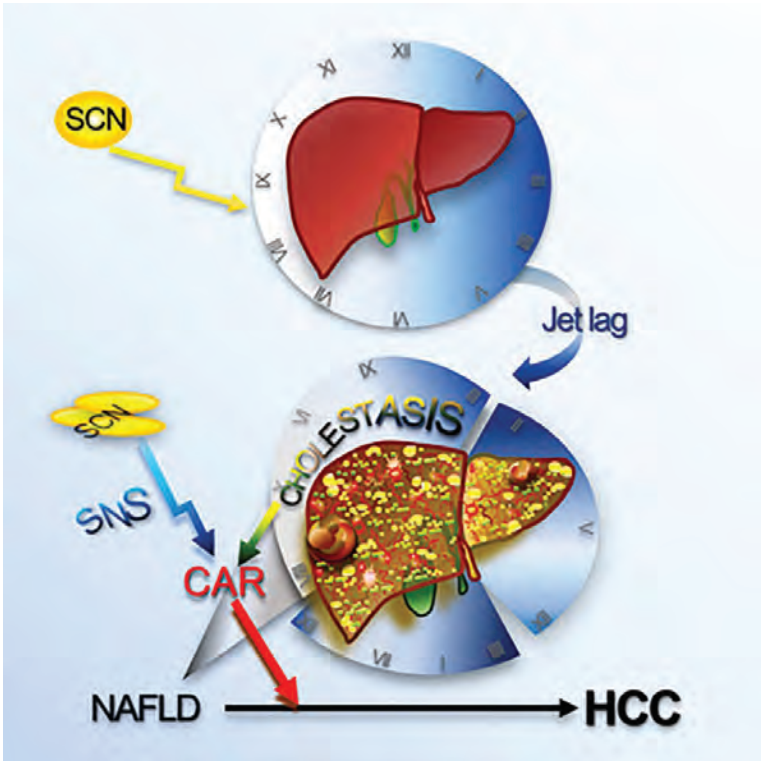

Figure 3. Clock network disruption - mimicking a model of jet lag - produces fatty liver (NAFLD) and hepatocellular carcinoma (HCC) from CAR activation through bile acids, which is dependent on the sympathetic nervous system (SNS), taken from [5].

Translational aspects of chronobiology on liver disease

In humans, the effect of a dysregulated sleep-wake cycle has been associated with metabolic abnormalities. Both external, environmental factors, as well as internal, genetic aspects in patients with metabolic syndrome and NAFLD have been identified. In a large, longitudinal study from the United States and Puerto Rico, the exposure to artificial light at night - e.g. from a TV in the bedroom -

predisposed women to the development of obesity [6]. Additional observational studies have provided evidence that desynchronicity of circadian rhythms from shift work promotes NAFLD. The alterations of the energy balance arising from a dysregulated clock network and the consequence on humoral factors and autonomic nerve fibres are thought to be the underlying mechanisms (Figure 4). These signals are integrated in the liver and here chronodisruption alters energy homeostasis promoting hepatic steatosis. In a second step, alterations of time-related oscillations of nutrients and BAs contribute to the progression of steatohepatitis, involving metabolic and inflammatory signalling pathways. In the end, it is a complex interplay of dietary signals, BAs, immune cell activation, autophagy, and gut microbiota that conveys an effect in humans through the clock network. As a consequence, socially driven circadian dysregulation should be ameliorated by reducing night-time exposure to blue light from electronic screens to prevent NAFLD. Additionally, planning of school and work schedules to synchronise with the circadian rhythm (e.g. forward rather than backward shift rotation) could have protective effects. In the context of treatment and dietary interventions, the clock network could be exploited using intermittent or periodic fasting or diets that mimic fasting schedules. The concept of chrono-recovery to improve metabolic derangements in NAFLD remains to be established [7]. On the other hand, therapeutic interventions that restore a deranged glucose metabolism, e.g. by using SGL-T2 inhibitors [8] or alternating BA metabolism through FXR agonists [5], could be beneficial in patients with NAFLD-related to an altered sleep-wake cycle. The restoration of diurnal metabolic rhythms and flexibility by SGL-T2 may have therapeutic implications beyond those demonstrated in diabetes [8].

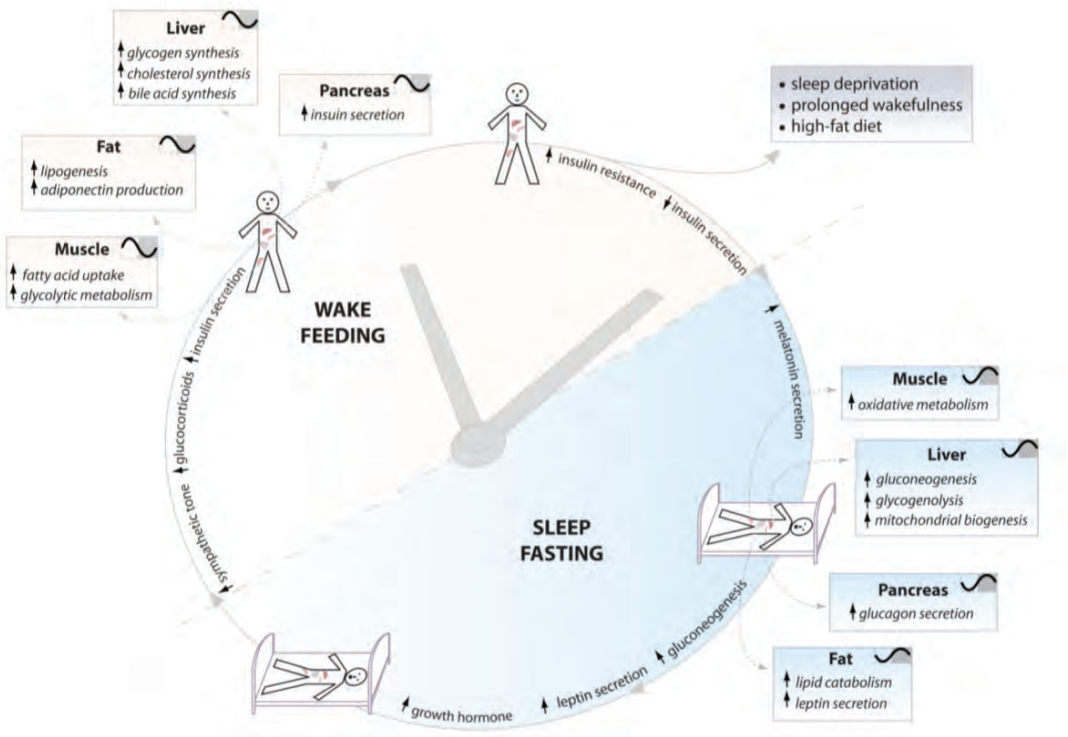

Figure 4. Integration of behaviour, feeding and metabolism (taken from [9]).

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Patients with cirrhosis should exercise!

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Take-home messages

- Sarcopenia is a common finding in cirrhosis, particularly in decompensated patients, and is associated with complications and higher mortality irrespective of MELD score.
- Among the existing tools available for testing sarcopenia, skeletal muscle mass area on CT scan at L3 for muscle mass, and handgrip test for muscle function have been extensively validated and correlate with prognosis.
- Exercise training, and particularly resistance training, improve muscle mass and function in cirrhosis, and exercise improves health outcomes (portal hypertension; cardiovascular fitness; quality of life) beyond skeletal muscle health in cirrhosis.
- Exercise should be then seen as a therapy, and as such, it should be properly prescribed following the frequency, intensity, time and type principles.
- Patients with cirrhosis should be screened for conditions potentially contraindicating exercise and should receive nutritional counselling before starting to exercise.
- Motivational interviews can help to implement lifestyle changes, including change in sedentary behaviour, in patients with cirrhosis.

Sarcopenia and risk of complications in cirrhosis

Sarcopenia is defined as the loss of skeletal muscle mass two standard deviations below the healthy young adult average (1). This condition is very frequent in patients with cirrhosis, with a prevalence ranging between 20-65%, depending on the method used for the assessment and on the severity of the underlying liver disease (2). Together with worsening of nutritional status (which in turn is one of the causes of sarcopenia in cirrhosis), sarcopenia is the major factor leading to frailty (3). In addition to an insufficient protein/calorie intake, systemic inflammation, increased muscle myostatin levels, increased susceptibility to the effect of starvation and sedentary behaviour accelerate skeletal muscle breakdown, leading commonly to overt sarcopenia in decompensated patients. Sarcopenia is a strong negative prognostic factor in this population, irrespective of the method used for its assessment (4). Few data are available in compensated patients, but existing evidence suggests a higher risk of clinical decompensation and bacterial infections in this stage of the disease. In decompensated patients awaiting liver transplantation (LT), sarcopenia is clearly associated with higher mortality on the waiting list, independent of the model for end-stage liver disease (MELD) score. Models based on the quantification of muscle mass/presence of sarcopenia added to MELD score have shown an improved prognostic value, and higher ability to identify patients with low MELD score and a higher risk of death. Similar results have been shown in patients undergoing transjugular intrahepatic portosystemic shunt due to the complications of portal hypertension. In addition, hospital costs for patients with sarcopenia in the United States have been estimated to double the costs for non-sarcopenic patients. Using a meta-analytic approach, pretransplant sarcopenia is also associated with longer intensive care unit stay, infection risk and almost doubles the risk of death after LT as compared to patients without this condition, independent of pre-LT MELD score. Importantly, sarcopenia is a frequent condition in patients with NASH cirrhosis and patients with obesity, irrespective of the cause of cirrhosis, and

sarcopenic obesity seems to be associated with further worsening of outcomes. There is, therefore, a strong rational to treat patients with sarcopenia in cirrhosis to attempt to improve outcomes. The mainstay of treatment remains protein and calories supplementation (2), but additional strategies are required to try to revert sarcopenia.

How to assess sarcopenia in cirrhosis

Assessment of muscle mass

Skeletal muscle mass quantification requires cross-sectional imaging. The most accepted and validated is the analysis of images of the psoas muscle alone or combined to paraspinal and abdominal wall muscles at the level of the third lumbar vertebra (L3) on computed tomography (CT) (2). Images are analysed using software packages to obtain the cross-sectional area and density (reflecting among myosteatosis) of the abdominal skeletal muscle; measurements are normalised to the height of subjects to obtain the skeletal muscle index (cm^2/m^2). Normal values for male and female subjects, age and ethnicity are available and radiation exposure to obtain a single slice CT at L3 is low. In addition, patients with cirrhosis often require a CT scan for other purposes, and images at L3 can be analysed on the existing scans. Consequently, the EASL guidelines on clinical nutrition in chronic liver disease (2) recommend assessing muscle mass on CT whenever this is available. Suggested cut-offs for the diagnosis of sarcopenia in Western countries are 50 cm^2/m^2 for men and 39 cm^2/m^2 for women (5).

Although measurements can also be performed on images on magnetic resonance, normal values to compare to are not yet available, and this method is not recommended.

Alternatives include simple anthropometric methods (measurement of mid-arm muscle circumference [MAMC], mid-arm muscular area [MAMA], and triceps skinfold [TSF]), ultrasound methods (assessment of thigh muscle thickness; psoas muscle diameter), whole-body dual-energy X-ray absorptiometry (DEXA) and tetrapolar bioelectrical impedance analysis (BIA) (2). All have shown prognostic value for mortality in patients with cirrhosis, but each has limitations. Anthropometric methods and ultrasound assessment of skeletal muscles have low cost, can be performed quickly and at the bedside and are not affected by fluid retention, but should be performed by trained personnel to reduce intra- and interobserver variability. DEXA measures fat mass and fat-free mass in addition to bone mineral density; its main limitations include radiation exposure and water retention influences the calculation of fat-free mass (which is not only skeletal muscle mass). BIA uses a two-compartment model to assess fat and fat-free mass, has a low cost and is portable and easy to use. However, result variability according to the amount of water retention is a major limitation in cirrhosis, in particular in decompensated patients (2).

Assessment of muscle function and of frailty

The concept of sarcopenia goes beyond the reduction of skeletal muscle mass and should also include muscle function; although skeletal muscle contractile function is not directly measuring muscle mass, it can be considered another measure related to sarcopenia. Handgrip strength measured by dynamometry is a cheap, simple, bedside, validated and effective method that correlates with complications and mortality in cirrhosis (2).

More global tests include the short physical performance battery and the Liver Frailty Index (https://liverfrailtyindex.ucsf.edu/). Both include timed repeated chair stands and balance testing; the first also uses a short walk test, and the second uses a handgrip test in combination. Both are quick to use (2-3 minutes) and correlate with mortality on the waiting list for LT (6). As such, they can be used as additional tools in cirrhosis (2).

As for the best test to be used, data comparing these methods head-to-head are very scanty. A recent single centre study performed in patients on the waiting list for LT compared models based on MELD plus CT-based muscle mass measurements or DEXA or handgrip test; results showed the MELD–handgrip strength was superior compared to a MELD-CT muscle model to predict mortality (7).

Rationale for exercise and effects of exercise in patients with cirrhosis

Among the several factors driving sarcopenia in cirrhosis, sedentary behaviour plays an important role. It has been shown that patients with cirrhosis tend to be very sedentary, with over 75% of their waking time spent not physically active (6, 8). The cause of sedentary behaviour in cirrhosis is multifactorial, including fatigue, reduced hepatocellular function and protein-energy malnutrition among others, but sedentary behaviour further worsens the aerobic capacity in this population, leading to a vicious circle and a further loss of muscle mass. In decompensated patients with cirrhosis, decreased aerobic capacity (VO_2) is correlated with MELD score and mortality, and not surprisingly, patients with cirrhosis show a reduced tolerance to exercise and usually stop exercise testing due to symptoms before reaching their predicted maximal cardiac frequency (6). Alcohol consumption and pulmonary complications of cirrhosis and portal hypertension are associated with worse aerobic capacity irrespective of the severity of liver dysfunction.

The benefits of exercise in the general population have been validated in several large studies. Thanks to its anabolic properties, exercise can improve muscle mass in patients with sarcopenia, providing improvement in the overall cardiopulmonary function as well as improvement in the quality of life (6).

For all the above-mentioned reasons, exercise is an attractive intervention to potentially improve outcomes in cirrhosis (6). Despite this, studies are limited, and in particular, randomised controlled trial (RCT) studies that compare exercise programmes with usual care in this setting (Table 1).

Table 1. Summary of the study findings for exercise programs in randomised controlled trials.

Publication	Number and type of participants	Design and duration	Exercise intervention	Results
Roman E et al. 2014	N = 17 MELD 7-13, 82% CP-A	RCT 12 W	Supervised moderate exercise at the study site or usual care (control) 3 x 1 hour/week Intensity: 60-70% of max HR	Six Minutes Walking Test (6MWT), 2 min step test and thigh muscle mass improved only in the exercise group. General health, vitality & social function on SF-36 significantly improved only in the exercise group
Zenith L et al. 2014	N = 20 MELD 10, 84% CP-A	RCT 8 W	Supervised aerobic exercise (cycle ergometry) at the study site+ 250–300 kcal on exercise days or usual care (control). 3 x 1 hour/week 60-80% of VO_2 peak	Peak VO_2, thigh muscle, thigh circumference and thickness on ultrasound, fatigue and self-perceived health better in the exercise group vs. control
Debette-Gratien et al. 2015	N = 13 (6 dropouts) MELD 13–21, 63% CP-A	Cohort 12 W	Supervised exercise Aerobic and resistance 2 x 1 hour/week 70-80% repetition max.	Peak VO_2, maximum power, ventilatory power, 6MWT and knee muscle improved
Macias-Rodriguez RU et al. 2016	N = 22 MELD 7–14, 64% CP-A	RCT 14 W	Supervised aerobic (cycle ergonomy) exercise + 30% calories on exercise days or usual care. All received nutritional therapy 3 x 40–70 min/week	Reduction of portal pressure and improved ventilator efficiency only in exercise group; improved phase angle on BIA.
Berzigotti A et al. 2016	N = 60 (10 dropouts) MELD 9 ± 3, 92% CP-A BMI $\geq$ 26 kg/m^2	Cohort 16 W	Supervised/gym exercise, aerobic and resistance, at the study site + 500–1000 kcal/day reduction in calories Min 1 x 1 hour/week	Reduction in body weight; reduction in HVPG, improve VO_2, improved quality of life No measure of muscle mass available

Publication	Number and type of participants	Design and duration	Exercise intervention	Results
Roman E et al. 2016	N = 23 (2 dropouts) MELD 8, All CP A	RCT 12 W	Supervised aerobic, resistance, balance, coordination, stretching and relaxation exercises at the study site or usual care (control) 3 x 1 hour/week 60-70% or patient tolerance	Exercise group showed muscle mass and lean body mass increase (DEXA), fat body mass decrease and fall risk decrease
Hiraoka A et al. 2017	N = 33 (2 dropouts) 91% CP A	Cohort 12 W	Home-based exercise training	Average daily steps; muscle volume, leg and handgrip strength improved
Kruger C et al. 2018	N = 60 (3 dropouts) 70% CP A	RCT 8 W	Home-based aerobic exercise (cycle ergometry) + 250–350 kcal on exercise days or usual care (control) 3 x 30mins/week, increasing to 3x 1 hour/week 60-80% of max HR	6MWT increase in the exercise group. Patients adherent to exercise (≥80% sessions): 6MWT increase and peak VO_2 increase better than controls No change in thigh thickness on ultrasound
Chen HW et al. 2019	N = 17 65% CP-B and 35% CP-C	RCT 12 W	Home-based exercise + Leucine or usual care (control)	6MWT improved in the exercise group; improved psoas muscle index (by CT) in the exercise group
Aamann L et al. 2019	N = 39 (3 dropouts) 95% CP-A	RCT 12 W	Supervised resistance exercise training at the study site or usual care (control) 3 x 1 hour/week Gradual increase in intensity	Improved muscle strength, increased thigh cross-sectional area by CT and increased body-cell mass by BIA in the resistance training group
Lai JC et al. 2020	N=58 active arm N=25 SOC 54% CP B or C	RCT 12 W Multicentric	Home-based strength training intervention (STRIVE): 30-minute strength training video plus a health coach	Only 14% of STRIVE participants adhered to the strength training video for 10-12 weeks. LFI improved both in the STRIVE arm and in the SOC arm. CLDQ scores improved from 4.6 to 5.2 in STRIVE and did not change in SOC (P = 0.09 for ΔCLDQ difference). One patient died (SOC arm) of bleeding. No adverse events were reported by STRIVE participants.

MELD, model for end-stage liver disease; CP, Child-Pugh; RPE, rate of perceived exertion; W, week; N, number; HR, heart rate; 6MWT, 6 minutes walking test; SF-36, Short-form 36 health survey; BIA, bioelectrical impedance analysis; HVPG, hepatic venous pressure gradient; CT, computer tomography; DEXA, dual-energy X-ray absorptiometry; SOC, Standard of Care.

As shown in Table 1, frequency, intensity, time and type (FITT) of exercise programs used in patients with cirrhosis vary among the different studies, but the results invariably show positive effects of exercise on muscle strength or mass; muscle mass seems particularly influenced by resistance training, as shown in a recent well-conducted RCT (9). Results of home-based exercise are encouraging, and this approach removes one of the major obstacles of exercising (i.e. displacement to a gym), however, adherence was poor in the largest RCT published so far (10). The small series/number of randomised patients and the inclusion of mostly compensated, Child-Pugh A patients are a clear limitation of the existing data.

Nonetheless, it is important to note that the benefits of exercise in cirrhosis go beyond a positive effect on skeletal muscle. As for liver-specific effects, two studies reported a significant reduction in portal pressure in patients undergoing either exercise and nutrition supplementation (malnourished patients (11) or exercise and moderate hypocaloric diet (overweight/obese patients (8)). In the latter study, the amount of physical activity over the 16 weeks of the intervention correlated with the reduction of portal pressure. In addition, exercise reduces anxiety and depression and improves the quality of life in this specific population. Even if specific studies are lacking, it is plausible that the well-known cardiovascular and metabolic effects of exercise also take place in patients with cirrhosis.

Best strategies to implement an intervention in this special population

A pre-exercise safety screening should be conducted to screen for cardiopulmonary and other comorbidities (6); in addition, the presence of decompensation of cirrhosis means that the exercise program needs to be carefully personalised and optimised for caloric and protein intake to avoid worsening of ascites (2). Hence, a nutritional assessment should be performed, and in malnourished patients, nutritional therapy should be started before or in parallel to exercise. A late evening snack intake should be encouraged in all patients, and a carbohydrate snack of 250-300 kcal pre- or post-exercise on exercise days can be recommended for patients starting on exercise programs.

It should be underlined that there is no absolute contraindication to low-intensity exercise and to standard physical activity, but patients with severe ascites and encephalopathy have limited tolerance and usually a poor adherence to exercise that need to be addressed. The presence of a caregiver for patients with hepatic encephalopathy improves adherence and reduces the risk of a fall.

A short motivational interview to elicit behaviour change is important and takes only a few minutes (6). It helps to understand how ready the patient is to modify his/her behaviour, to clearly formulate reasons that should help patients to understand why more physical activity and exercise is needed in their specific case, to agree on achievable targets, and to identify potential barriers of exercising. Patients should be asked to maintain activity at a rate of perceived exertion not over 5-6 out of 10 on the Borg 0-10 scale, meaning that they should feel some degree of exertion, but still allow themselves to talk during exercise training (6). Exercises with a high-risk of injury or falling should be avoided, especially in patients with severe thrombocytopenia (<20 G/l). In patients with severe portal hypertension, abdominal press/crunch has to be avoided since it acutely increases abdominal pressure.

A baseline physical performance assessment should be completed by using simple tools such as the short physical performance battery and gait speed, this helps to objectively compare the progress over time and is needed to identify patients who are severely deconditioned.

In all cases, non-exercise activity thermogenesis should be encouraged, meaning avoiding sedentary behaviours by taking all the opportunities to move more in the daily routine.

As for proper exercise, training prescription should follow the FITT of exercise principles (6). Exercise should contain aerobic, resistance and flexibility components. Since specific studies in cirrhosis are scarce, the existing guidelines for adults belonging to the general population have been used and adapted. As for the aerobic component, patients should start exercising 4 times per week, with the aim to increase to everyday exercising. Intensity should be moderate (see above). Time should be minimal at the beginning in very deconditioned patients (aiming at even just 1 minute walking/1 minute rest for 5 times), and increase progressively, with the aim of achieving sessions of 40 minutes, for a total of 150 minutes/week. The type of activity can include walking, cycling, or any other activity that the patient can tolerate. Resistance (e.g. progressive weight; stair climbing) and flexibility (stretching and balance exercises for the large muscle groups of the upper and lower body) sessions should be taken at least twice/week.

Conclusion

In conclusion, exercise should be routinely recommended to patients with cirrhosis in order to prevent and treat sarcopenia, improve liver-related outcomes, and improve quality of life.

Studies carefully addressing prehabilitation strategies of patients with decompensated cirrhosis on the waiting list for LT, and rehabilitation of patients surviving episodes of decompensation, with strong clinical endpoints are among the several unmet needs in this field.

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How much exercise before liver transplantation on the transplant waiting list?

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Take-home messages

- In patients with cirrhosis and end-stage liver disease, physical inactivity, sarcopenia, and frailty are common and independent predictors of morbidity and mortality.
- As for many chronic diseases, physical exercise training in patients with cirrhosis is feasible, safe, and effective (improving physical fitness, muscle mass and health-related quality of life).
- Prehabilitation programs should be routinely prescribed for all eligible (mental and physically capable) patients with cirrhosis and/or awaiting liver transplantation. Such programs should include endurance and strength training, promote physical activity, offer nutritional counselling/interventions, and tackle all risk factors associated with worse outcome and postoperative complications (cessation of smoking and alcohol consumption, correction of iron-deficiency anaemia, etc.).
- The impact of exercise training programs on outcomes after liver transplantation is an area to be evaluated further. Such exercise programs should include a personalised postoperative holistic intervention with exercise training and promotion of physical activity as key actionable factors to prevent/reduce early post-liver transplantation complications and long-term cardiovascular disease and mortality.
- Currently, existing barriers hampering the widespread implementation of physical activity for patients with cirrhosis and before/after liver transplantation should be knocked down. For both the physician and patient: it is TIME TO MOVE!

Summary of the literature and practical recommendations

Background

With his statement *"eating alone will not keep a man well, he must also take exercise",* Hippocrates recognised the importance of physical exercise more than 2400 years ago. Anno 2020, health benefits of a physically active lifestyle are extensive and robust; physical activity not only fosters normal growth and development, but also enables people to feel, function, and sleep better. Physical inactivity is regarded a primary cause of chronic diseases and an important predictor of cardiovascular disease, type 2 diabetes mellitus, obesity, some cancers, poor skeletal health, some mental health aspects, overall mortality, and poor quality of life [1]. Public health recommendations currently recommend at least 150 minutes of moderate-intensity aerobic physical activity per week (or ≥75 minutes/week at vigorous intensity), with additional muscle strengthening (2-3 sessions/week), flexibility (≥2-3 sessions/week), and balance exercises (≥2-3 sessions/week) [2,3]. Such recommendations are also relevant for patients with chronic diseases, including liver disease. However, in those not able reach

these targets, it is important to note that any increase in physical activity generates clinical benefits [4]. Lack of physical activity may contribute to the development of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). In parallel with the pandemic of physical inactivity and obesity, NASH is becoming the primary indication for liver transplantation (LT). While exercise at least partially reverses hepatic steatosis in NAFLD, even in the absence of reductions in body weight, accumulating evidence also indicates beneficial effects of exercise in the later stages of chronic liver disease as discussed below.

Physical fitness and frailty in chronic liver disease

Advanced chronic liver disease is associated with a deterioration in patients' physical fitness. Physical fitness encompasses cardiorespiratory fitness (i.e., peak oxygen uptake [VO_2peak]), musculoskeletal fitness (i.e., muscle strength, endurance, flexibility, etc.), and motor fitness (i.e., speed, agility, and balance). In a systematic review, the mean VO_2peak of 1107 patients with cirrhosis evaluated for LT was 17.4 $ml \cdot kg^{-1} \cdot min^{-1}$ [5]. This level of cardiorespiratory fitness is hardly as much as the minimum level required for fully independent living (≥18 $ml \cdot kg^{-1} \cdot min^{-1}$ in men, ≥15 $ml \cdot kg^{-1} \cdot min^{-1}$ in women). There are multiple causes for this reduced physical fitness. End-stage liver disease results in dysfunction of (i) the respiratory system (hepatopulmonary syndrome, portopulmonary hypertension, and ascites/hydrothorax), (ii) the heart (cirrhotic cardiomyopathy and chronotropic incompetence), (iii) the cerebrum (hepatic encephalopathy), (iv) blood (anaemia), and (v) muscle (sarcopenia, mitochondrial dysfunction, impaired contractility). Furthermore, hepatic dysfunction is associated with impaired glucose output and hence impaired oxygen-efficient substrate delivery to the contracting muscle during (high-intensity) exercise. Deterioration in musculoskeletal fitness and muscle mass is common in liver cirrhosis, particularly in men. Likewise, muscle quality (typically assessed as the ratio of muscle strength over muscle mass and amongst others determined by the presence of intra- and intermuscular fat deposits, which can be measured as radiation attenuation index on CT scans) is commonly impaired in end-stage liver disease. A muscular catabolic state prevails in chronic liver disease due to increased dependence on muscle breakdown for hepatic glucose production, low-grade systemic inflammation, poor dietary protein intake, hypermetabolism, androgen deficiency, and muscle hyperammonemia stimulating myostatin-associated autophagy and mTOR pathway inhibition.

Furthermore, patients with end-stage liver disease and/or awaiting LT are highly sedentary, which attenuates the anabolic muscle response to protein intake. Sarcopenia (depletion of muscle mass) and progressive inactivity are major contributors to physical frailty in patients with liver cirrhosis [6]. Frailty is a status characterised by poor physiological reserve and high vulnerability for health stressors. It is a multidimensional concept representing the end-manifestation of physiological derangements in solid organs, skeletal muscle, and the regulation of inflammatory status and endocrine function. In its broader conceptualisation, frailty also includes social and emotional aspects.

End-stage liver disease affects all three key components of physical frailty: functional capacity, cardiorespiratory fitness (exercise capacity and cardiorespiratory reserve) and sarcopenia (muscle mass, muscle strength).

Physical activity, physical fitness, muscle mass, muscle quality, and frailty have all been identified as predictors of pre-, peri-, and/or post-LT hospitalisation, complications (i.e., infections), waiting list and postoperative mortality and/or short- and long-term survival.

Assessment of physical fitness and frailty in chronic liver disease

In clinical practice, the assessment of physical fitness should be incorporated into baseline and longitudinal assessments of liver transplant candidates and recipients. The type and number of measurements depend on time and resources available.

The Liver Frailty Index is a composite measure of handgrip strength, chair stands, and postural balance which takes about 5 minutes to execute and has the advantage of being low cost, objective, performance-based, and suitable for longitudinal assessments of frailty.

Ideally, a comprehensive intake procedure is led by a physiotherapist or exercise physiologist as part of a multidisciplinary team. Besides an extensive anamnesis and sports medical screening excluding contraindications to exercise (i.e., untreated varices and high-risk cardiovascular diseases), such assessment should ideally aim to evaluate cardiorespiratory fitness (VO_2peak and/or 6 minutes walking test [6MWT]), muscle strength (handgrip, quadriceps, and inspiratory muscle strength), balance, body composition (muscle mass, muscle quality, fat mass, bone mineral density), physical activity (questionnaires or accelerometry), barriers and facilitators for physical activity implementation, and quality of life.

Baseline and longitudinal assessments of frailty, physical fitness, and body composition serve multiple aims: (i) to complement standard evaluation criteria for LT candidacy, (ii) to tailor physical activity interventions, nutrition, and possibly pharmacological therapy before and after LT to improve patient outcomes, and (iii) to advance the research field of physical rehabilitation in liver disease and transplantation.

Efficacy of physical training in chronic liver disease and liver transplant candidates

Since 2014, an increasing number of randomised controlled trials (RCT) has reported on the feasibility, safety, and effectiveness of physical activity interventions to counteract the pretransplant catabolic status and improve the physiological reserve in patients either or not listed for LT. With a few exceptions, physical activity interventions implementing moderate-intensity aerobic exercise have been shown effective to improve VO_2peak and/or 6MWT, with greater improvements observed in well-adherent patients. In a recent review of 11 studies addressing physical exercise in patients with cirrhosis [7], there was only 1 home-based exercise program. Supervised (partly) home-based programs could increase patient adherence in frail patients. No studies evaluated the effects of strength training alone, but added to an aerobic training intervention, significant improvements in lean body mass and muscular strength have been reported [8,9]. In fact, in this patient population, aerobic training by itself may already stimulate muscle anabolism, potentially through decreased muscle myostatin protein concentrations and associated decrements in intermuscular fat infiltration. In patients listed for transplantation, supervised tailored physical training was shown to be feasible and safe in a small cohort of 8 patients, including patients graded Child-Pugh C and with MELD-scores as high as 21 [9]. Training included moderate-intensity endurance training (≥20 minutes at ventilatory threshold) and moderate-load strength training (3 sets of 8 repetitions at 70-80% of their 1-repetition maximum) which considerably improved patients' VO_2peak and quadriceps strength. No cardiovascular events or complications related to portal hypertension were observed [9]. Another recent pilot RCT supports the safety and good adherence to training in LT candidates, though caution remains warranted given the small sample size of this study (exercise group: n = 4; usual care group: n = 4) [10]. To date, no major exercise-induced adverse events have been reported. However, one training study reported a patient experiencing transient and mild bronchospasm during exercise, while another study reported

a knee injury not precluding continued training. At present, there is still a lack of solid data on the effects of physical training on pre- and peri-operative complications. Only one pilot study with four patients in the exercise and control groups reports on intra-, peri-, and postoperative outcomes, yet as expected with an underpowered study, the findings did not show any differences between groups [8]. In contrast to LT, one RCT in patients considered at high-risk for postoperative complications (i.e., patients >70 years and/or with the American Society of Anesthesiologists score III/IV and low Duke activity status index) following major abdominal surgery showed a 51% reduction in the number of patients with complications in those allocated to a pre-operative aerobic training intervention [9]. Given that exercise training improves physical fitness, muscle mass, and thus physiological reserve in liver transplant candidates, it is indeed very reasonable to expect similar training effects in this patient population.

This can be underpinned knowing that the optimal level of the ventilatory anaerobic threshold for favourable short-term survival after LT is >9.0 ml·kg·min^{-1} [12], and that the ventilatory anaerobic threshold can be improved with a mean of 1.5 ml·kg·min^{-1} in a 4-week training period prior to liver surgery [13].

Safety, potential benefits and practical recommendations for physical training in chronic liver disease and liver transplant candidates

Safety concerns related to consequences of exercise-induced elevations in portal hypertension may be - partially - responsible for the slow progress in the field of physical rehabilitation in liver disease. Cycling exercise at 30 and 50% of the maximal workload increased HVPG in patients with cirrhosis from 16.7 mmHg at rest to 19.2 and 19.9 mmHg during exercise, respectively [14]. Contrary, exercise-induced changes in portal pressure were also found to depend on the use of beta-blockers: a decrease from rest to exercise in those patients administered propranolol (16.3 to 12.9 mmHg) versus an increase from rest to exercise in those receiving placebo (16.7 mmHg at rest to 19.0 mmHg). Interestingly, two trials reported a reduced portal pressure at rest following approximately four months of physical training, irrespective of the use of beta-blockers [15,16]. These findings may indicate that portal pressure *per se* should not be considered a contraindication for physical/exercise training when prophylactic interventions for variceal bleeding are in place. On the contrary, and perhaps provokingly, physical training could be considered a non-pharmaceutical treatment option to improve portal hypertension.

Concerns exist with regards to exercise-induced ammonia production and hence subsequent encephalopathy. Indeed, during strenuous exercise muscle fibres may become a substantial source of ammonia production, with the increase in ammonia levels relating to the imposed relative workload. However, at rest, muscle tissue aids the liver in detoxifying ammonia. It is, therefore, not surprising that training interventions attenuate the increase in ammonia in response to acute exercise in both cirrhotic and non-cirrhotic patients. Improving cardiorespiratory fitness and muscle mass may thus be expected to reduce the incidence and severity of future episodes of hepatic encephalopathy.

Based on the collective experience of six North American centres, a practical guide prescribing physical exercise in liver cirrhosis has recently been developed [17]. Pre-exercise safety screening on cirrhosis-related complications comes first. Patients with liver cirrhosis should be screened and treated for high-risk varices. Complications of liver cirrhosis such as ascites, peripheral oedema, hepatic encephalopathy, or low platelet count may warrant modifications to the exercise program such as (para-) medical supervision and supported physical activity to reduce the risk of falling. Cardiovascular assessment is required in those with symptoms/history suggestive for cardiovascular disease, metabolic disease, or renal insufficiency, but nonetheless willing to engage in moderate-

intensity exercise. Cardiopulmonary exercise testing (CPET), measuring ventilatory anaerobic threshold and VO_2peak, could help exclude unstable cardiovascular disease because a continuous electrocardiogram is used throughout the exercise test. Therefore, using CPET, the gold standard to assess cardiorespiratory fitness should be encouraged. Activities not surpassing the intensity of brisk walking do not necessitate medical clearance ***per se***. As a general advice: "start low, progress slowly, be alert for symptoms", and adapt the training content to present the physical status of the patient. Poor cardiorespiratory fitness may necessitate the initiation of aerobic training with 1-minute exercise intervals interspersed by 1-minute recovery bouts for a total of 5 minutes of exercise. Whenever possible, one should aim to gradually increase training duration and intensity to 40 minutes per session and ≥150 minutes of moderate-intensity exercise per week. Weight training (1-3 sets, 10-15 repetitions, intensity set so to induce muscle fatigue by the end of the exercise) or functional strengthening exercises targeting the major muscle groups should be performed two to three non-consecutive days per week. Flexibility and balance exercises can be incorporated in the warming up and cooling down phases of the exercise sessions.

Besides training of the cardiovascular system and peripheral muscles, one should also consider pretransplant training of the inspiratory muscles. Impaired respiratory muscle strength is a common finding in liver cirrhosis and a risk factor of mortality. Inspiratory muscle strength further weakens shortly after transplantation. It is well-known that improved inspiratory muscle strength reduces postoperative length of hospital stay and pulmonary complication rates after major upper abdominal or cardiothoracic surgery. It is likely, but yet to be proven, that similar effects could benefit abdominal transplant recipients.

Based on the recent review of 11 pretransplant exercise studies, the optimal duration of the exercise program has been suggested to be 12 weeks or more (i.e., till transplantation) [7].

Exercise training after liver transplantation

LT is the treatment of choice for end-stage liver failure. Despite excellent short-term outcomes (80-90% one-year patient survival) muscle wasting and impaired physical fitness associated with chronic liver failure only partially recover following LT. Due to continued physical inactivity and immunosuppressive therapy, LT recipients are at higher risk to become chronically fatigued, while obesity and the development of metabolic syndrome are increasingly diagnosed after LT. About one in five of previously normal weight individuals become obese within 2 years. Increased BMI at one-year post-LT accurately predicts the development of metabolic syndrome while one-third will have cardiovascular disease within 8 years.

Cardiovascular disease is one of the leading causes of death after LT, and its prevention should be a priority to improve longer-term outcomes. Addressing weight gain and implementing regular exercise, early in the postoperative period and continued thereafter, should be seen as a priority to reduce cardiovascular disease in the future. Regular exercise has positive effects on cardiovascular disease and mortality for the normal ('healthy') population; even a small increase in physical activity related to a significantly lower risk of death [15].

Although exercise training is likely to at least partially restore physical fitness (muscle strength and cardiorespiratory fitness) and improve cardiometabolic health in LT recipients, evidence on the effects of exercise training on VO2peak and quadriceps muscle strength in LT recipients is limited. We systematically reviewed its safety and effectiveness in this population, including only RCTs that reported the effect of exercise training on VO_2peak and muscle strength after LT (unpublished data). Meta-analysis of six studies (n=275) showed a trend for favourable effects of exercise training on

cardiorespiratory fitness assessed or estimated by VO2peak and 6MWT, respectively (SMD: 0.23, 95% CI: -0.01 to 0.48, P=0.06; Chi²=2.57, P=0.77; I²=0%). Meta-analysis restricted to three studies (n=114) implementing muscle strengthening exercises showed a trend towards improved lower body muscle strength, assessed by either dynamometry or 30-s sit-to-stand test, in favour of exercise training (SMD: 0.34, 95% CI: -0.03 to 0.72, P=0.07; Chi²=1.77, P=0.41; I²=0%).

Exercise training has the potential to affect long-term outcomes following LT, including physical function, health-related quality of life and cardiovascular mortality. However, research on the optimal timing, type, dose of exercise, mode of delivery (home-based, community-based, or hospital-based) and relevant outcomes is limited and become top research priorities. There is an urgent need for multicentre, larger scale, intervention studies, and to study various modes of exercise training as part of a holistic approach. Additionally, the impact of exercise training studies on immunity, infection, cognition and economic outcomes should not be neglected.

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SESSION 3

NUTRITIONAL AND BEHAVIOURAL PATTERNS AFFECTING THE LIVER

WEDNESDAY 23 JUNE
12H45 - 13H45

Which diet should I be taking?

Shira Zelber-Sagi

School of Public Health, Faculty of Social Welfare and Health Sciences, University of Haifa, Haifa, and Department Gastroenterology, Tel-Aviv Medical Center, Tel-Aviv, *Israel*

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Take-home messages

- Clinical evidence strongly supports the role of lifestyle modification and weight reduction as the primary therapy for the management of NAFLD and NASH. This must be a lifelong treatment to avoid relapse and weight regain.
- The Mediterranean diet can reduce liver fat even without weight loss and is the most recommended dietary pattern in NAFLD. Other similar healthy dietary patterns are also helpful, and the choice of diet should be negotiated with the patients and tailored to their cultural and personal preferences.
- Changing dietary composition is important for both NAFLD and liver cancer prevention, especially reduction in the intake of sugar/fructose, saturated fat, ultra-processed food in general and processed meat specifically, and better adherence to the components of the Mediterranean diet.
- Normal weight NAFLD patients can also benefit from a modest weight reduction, physical activity and reduced consumption of dietary fructose, sugared-sweetened beverages and cholesterol.
- The global growing consumption of ultra-processed foods which is calorie-dense and rich in fructose, saturated fats and other unhealthy compounds, poses a great challenge in the treatment of NAFLD.
- Avoidance from sugar sweetened beverages should be encouraged starting from early childhood. Like alcohol, questions regarding sugar sweetened beverages consumption should be part of the NAFLD patient medical history.

Introduction

There is no doubt that lifestyle modification, such as diet and exercise is the key factor in preventing and treating NAFLD. Data are still evolving mainly from observational studies but also recently from clinical trials with regard to the exact type of diet and nutrients that are needed. In parallel, there is an increased understanding that lifestyle modification and weight loss pose a great challenge on both the patient and caregiver and much needs to be overcome in relation to patient-related and environmental barriers. The efforts required to overcome these barriers include policy measures to create a healthier environment to support a healthy lifestyle as acknowledged in a recent EASL policy statement on NAFLD and obesity (Figure 1). The current review will cover the most updated evidence for lifestyle treatment in NAFLD and provide practical tools.

Figure 1. EASL policy statement aimed to inform politicians, policy-makers and the general population across Europe about NAFLD and the measures required for prevention and treatment. Accessed from https://easl.eu/wp-content/uploads/2019/04/EASL-POLICY-Obesity-and-NAFLD-FINAL.pdf

What weight reduction, for who and how?

There is a consensus that gradual weight reduction achieved by caloric restriction, with or without increased physical activity, leads to improved serum liver enzymes, liver fat, degree of hepatic inflammation and fibrosis (1, 2). A meta-analysis of randomised clinical trials (RCTs) compared any intervention aiming to reduce weight (behavioural weight loss programs, pharmacotherapy, and surgical procedures) with no or lower-intensity weight loss (intervention duration 3-8 months). Weight loss interventions were associated with improvements in alanine aminotransferase (ALT), histologically or radiologically-measured liver steatosis, histologic NAFLD activity score, and the presence of non-alcoholic steatohepatitis (NASH). No significant change in histologic liver fibrosis was found (3), but perhaps longer interventions and follow-up are needed (Figure 2). Longer-term non-randomised studies demonstrated a reduction in the level of fibrosis following lifestyle modification (1). In patients with NASH, those who lost 10% or more of total body weight exhibited significantly higher rates of fibrosis regression (63% *vs.* 9%)(4). Importantly, lifestyle changes that produce even modest results such as sustained weight loss of about 5% of initial body weight can reduce steatosis, liver enzymes, NASH (1) and induce health benefits as clinically meaningful reductions in triglycerides and blood glucose and other cardiovascular risk factors (5, 6). The European Association for the Study of the Liver (EASL)/ diabetes (EASD)/ obesity (EASO) Clinical Practice Guidelines recommends that in overweight/obese NAFLD patients, a 7–10% weight loss is the target of most lifestyle interventions (2). Generally, for the treatment of obesity, many diets have been shown to produce and sustain weight loss, if they are followed. Clinicians can choose a diet that the patient will follow, and which has health benefits.

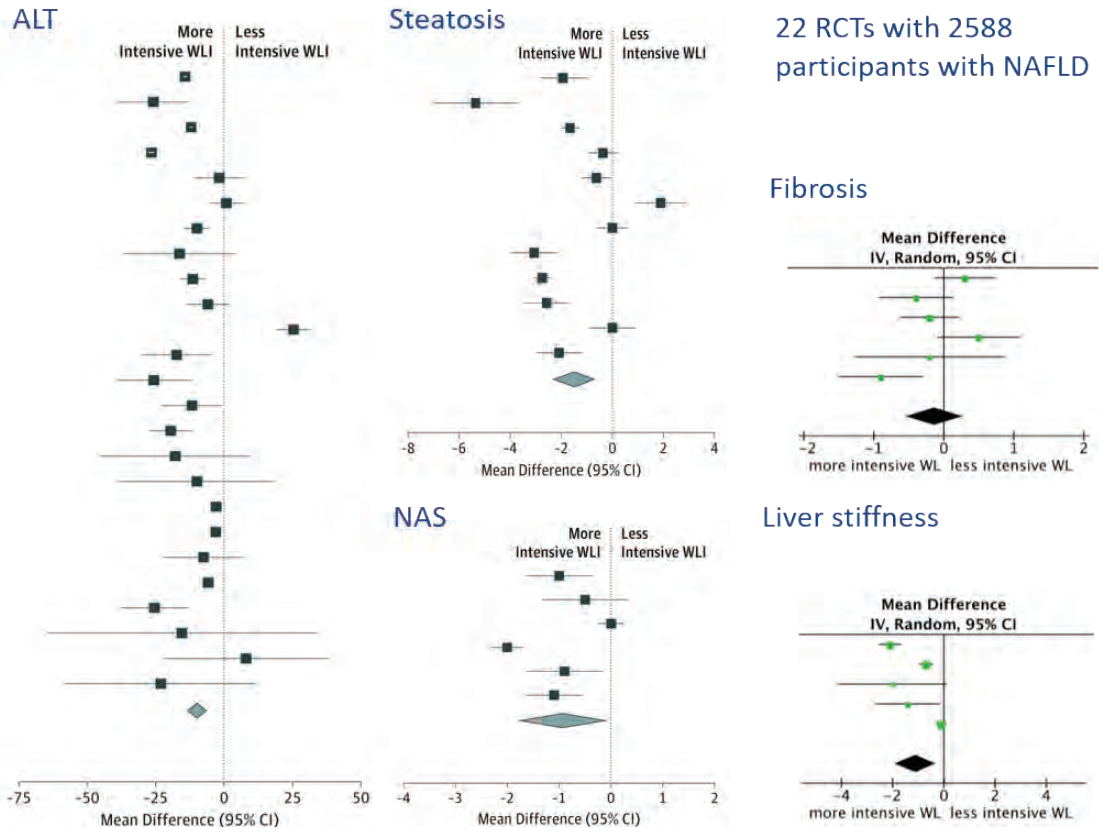

Figure 2. Effect of weight loss on NAFLD, Systematic Review and Meta-analysis of RCTs. Taken from [3]. ALT, alanine aminotransferase; NAS, NAFLD activity score; RCTs, randomised controlled trials.

What can we do for the "Normal-weight/Non-obese" NAFLD patients?

NAFLD can also develop in subjects with BMI within the ethnic-specific cut-off (25 kg/m^2 BMI in Caucasian, and 23 kg/m^2 in Asian subjects) (7). NAFLD subjects with normal weight have milder features of the metabolic syndrome when compared with patients with obesity, but have a higher prevalence of dyslipidemia, arterial hypertension, insulin resistance, and diabetes compared with healthy controls (8). NAFLD subjects with normal weight also have a greater visceral obesity and decreased muscle mass (8), and therefore, are recommended to perform physical activity which decreases visceral fat , increases muscle mass and improves insulin resistance (9).

Non-obese patients can achieve remission of NAFLD with 3-5% weight reduction following a lifestyle intervention program. Non-obese patients also showed to be more likely than obese patients to maintain weight reduction and normal liver enzymes in the long-term (10). In observational studies, normal weight NAFLD patients had a higher consumption of dietary fructose, sugared-sweetened beverages (SSBs) and cholesterol and thus would especially benefit from reducing their intake. It has been suggested that the lean NAFLD phenotype might be consistent with obesity resistance, where individuals are still prone to develop steatosis in response to an obesogenic environment (and perhaps a diet enriched in cholesterol), driven by genetic and gut-driven mechanisms (11).

It's not all about weight – what is the independent role of dietary composition?

Overfeeding with polyunsaturated (PUFA) and saturated fat (SFA) has distinct effects on liver and visceral fat accumulation as shown in short-term RCTs. In a double-blind RCT, adding to the habitual diet muffins that are high in either palm (SFA)- or sunflower oil (PUFA) led to a similar body weight gain of about 2 kg, but only SFA markedly induced liver fat content along with liver enzymes and atherogenic serum lipids (12). In another RCT, 1000 extra kcal/day of SFA or PUFA or simple sugars for 3 weeks demonstrated similar results, where SFA induced the greatest increase in liver fat and insulin resistance (13). In summary, different types of fat have different effects in NAFLD and NASH. Therefore, a simple recommendation for a reduction in total fat intake is inappropriate.

While there are many causes of NAFLD, the intake of fructose-containing sugars plays a major role. Added sugars refer to refined sugars (sucrose, fructose and high fructose corn syrup) added to SSBs and incorporated into food. Evidence from epidemiological studies and clinical trials show an association between added sugars and NAFLD, which is more prominent with SSBs. Fructose intake has been shown to stimulate *de novo* lipogenesis as well as to block fatty acid oxidation in the liver, and to alter gut permeability and microbiome resulting in associated endotoxemia. The unique aspect of fructose compared to glucose is that when fructose is metabolised there is a transient decrease in intracellular phosphate and ATP levels associated with nucleotide turnover and uric acid generation, leading to oxidative stress, inflammation and fibrosis (14).

Among children and adolescents, fructose consumption was independently associated with NASH (15). Importantly, reduced sugar consumption among children led to a regression of steatosis within a short time (weeks) (16, 17). Similarly, among overweight adults, in a double-blind RCT, six weeks of fructose restriction per se led to a small, but statistically significant, decrease in liver fat content in comparison with an isocaloric control group; both groups were asked to follow a 6-wk fructose-restricted diet, while in addition to this diet, the control group was supplemented with fructose powder aimed at achieving a fructose intake similar to baseline, whereas the intervention group remained fructose-restricted and received glucose powder to allow an isocaloric comparison (18).

Even infants at the age of 1 year who consumed >2 sugar-containing beverage servings per day were three times more likely to develop NAFLD at 10 years of age compared to those with <1.0 serving/day, regardless of BMI (19). Taken together, these findings imply that, like alcohol, questions regarding SSB consumption should be part of the NAFLD patient medical history, and avoidance from SSBs should be encouraged starting from early childhood.

One of the most studied dietary patterns is the traditional Mediterranean diet, characterised by a high intake of olive oil, vegetables, fruits and nuts, legumes, whole grains, fish and seafood, and a low intake of red meat and especially processed meat. The Mediterranean diet has a well-established protective role against non-communicable diseases and large prospective observational studies support also the inverse association of NAFLD with the Mediterranean diet (20, 21), reinforced by clinical trials comparing it to a regular low-fat diet (22). It should be mentioned that other similar healthy eating patterns have also been beneficial (23). For example, a prospective study of elderly population implied that adherence to the World Health Organization (WHO) recommended healthy dietary pattern was related to regression of NAFLD on repeated ultrasonography (24). Similarly, in the Multi-ethnic Cohort study, keeping a Healthy Eating Index pattern, was related with lower NAFLD risk (25). The Mediterranean diet has been recommended for the treatment of NAFLD by the EASL–EASD–EASO Clinical Practice Guidelines (2)] and recently by the European Society of Clinical Nutrition and Metabolism (ESPEN) guidelines (9). In contrast, the effect of specific hypocaloric diets, such as low-carbohydrate/high-protein diets, and intermittent fasting, on NAFLD/NASH have not been adequately studied (26).

Interestingly, one of the principles of the Mediterranean diet is to minimise processed and high sugar food and to have more home-cooked meals. The global growing consumption of ultra-processed foods which is calorie-dense and rich in fructose, saturated fats and other unhealthy compounds, poses a great challenge in the treatment of NAFLD.

A reduction in processed food in general and specifically processed meat and high fructose food (27, 28) can also lead to reduced intake of advanced glycation end products (AGEs). AGEs dietary intake has shown to be related to insulin resistance among the general population and also specifically among subjects with NAFLD. Furthermore, the soluble receptor of AGEs (sRAGE), which prevents the binding of extracellular AGEs to the cell-surface RAGE, thus exerting protective effects, showed an inverse correlation with the level of liver fat, and more interestingly, sRAGE levels were increased by lifestyle changes (29). In support, a cross-sectional analysis of 743 subjects, showed exercise was independently protective from low sRAGE levels while pack-years, working and sedentary time, intake of red and/or processed meat were associated with increased odds for low sRAGE levels. In turn, low sRAGE levels were independently associated with elevated ALT and NAFLD with elevated ALT (30).

Several studies have shown the harmful association between high meat intake and NAFLD (31-33). In a large population-based study of ethnically diverse populations, higher intakes of red meat, processed red meat, poultry, and cholesterol were risk factors for NAFLD/ NAFLD-related cirrhosis, while dietary fibre was a protective factor. Importantly, the associations were generally similar across a wide spectrum of racial/ethnic groups, supporting the external validity of the observed associations (34). Moreover, a recent prospective cohort of the general population from six states in the United States and 16-year follow-up data, indicated that high intake of total meat, processed and unprocessed red meat (beef, lamb, and pork), heme iron, and nitrite from processed meat were associated with liver disease-related mortality (35).

Interestingly, a recent project published by the “EAT-Lancet Commission on healthy diets from sustainable food systems” recommended a diet similar to the Mediterranean diet; consisting of a diversity of plant-based foods, low amounts of animal source foods, unsaturated rather than saturated fats, and small amounts of refined grains, highly processed foods and added sugars (36). Indeed, a diet rich in fruits and vegetables suggests a lower risk of cardiometabolic disorders and NAFLD (37, 38) attributed to fibres, vitamins and non-vitamin antioxidants they contain. A cross-sectional study of patients undergoing abdominal ultrasonography and non-invasive evaluation for the level of steatosis using SteatoTest, NASH NashTest and fibrosis using FibroTest, indicated that vitamin E and C dietary intake might be protective from NAFLD-related liver damage; NAFLD, NASH and level of steatosis, but not fibrosis(38). Furtheremore, non-vitamin antioxidants, phenolic acids (PA) abundantly present in foods such as berries, nuts, coffee, tea and whole grains were demonstrated to be inversely associated with the presence of NAFLD and insulin resistance (39). Nut consumption has been associated with reduced inflammation, insulin resistance, and oxidative stress and recently with the prevalence and severity of (NAFLD) in a study among 4655 subjects undergoing abdominal ultrasound. Nut consumption on a daily basis compared to less than once a week, was inversely associated with NAFLD and advanced fibrosis assessed by markers (adjusted for: sex, age, BMI, metabolic syndrome, hepatic steatosis, alcohol consumption, intake of fast-food, vegetables, fruits, sweets, red and processed meat, white meat, fish, coffee and consumption of SSB) (40).

The association with NAFLD of many of the above mentioned harmful and protective foods and nutrients, demonstrated mostly in observational studies, is reinforced by a recent 18-month RCT including 294 people with abdominal obesity or dyslipidemia, aiming to examine the effectiveness of green-Mediterranean (MED) diet, further restricted in red/processed meat, and enriched with green plants and polyphenols on

NAFLD, compared to healthy dietary guidelines (HDG), all accompanied by physical activity. There were two isocaloric MED groups, both consumed 28 g/day walnuts, while the green-MED group further consumed green tea (3–4 cups/day), Mankai (a Wolffia globosa aquatic plant strain) and green shake (+1240 mg/day total polyphenols). Despite similar moderate weight-loss in both MED groups, green-MED group achieved almost double liver fat loss (–38.9% proportionally), as compared with MED (–19.6% proportionally; p=0.035 weight loss adjusted) and HDG (–12.2% proportionally; p<0.001). Liver fat loss was independently associated with increased Mankai and walnuts intake, decreased red/processed meat consumption and changes in microbiome Composition (41).

Is there a role for lifestyle in prevention of hepatocellular carcinoma (Figure 3)

Evidence for a potential association between dietary composition and hepatocellular carcinoma (HCC) in humans is mostly driven from large observational prospective studies and meta-analyses, in the general population and is not specific to NAFLD patients. But in fact, the diet that is good for the treatment of NAFLD is also the diet which may help prevent HCC including; higher intake of monounsaturated fatty acids (MUFA) or PUFA, n-3 PUFA-rich fish (42), fiber (43) and vegetables (44), with lower intake of red and processed meat (45, 46), high-fat dairy products and butter (47), SFA (48), cholesterol (49), and sugar. Interestingly, the Mediterranean diet pattern, which captures all the above-mentioned foods and nutrients, has been shown to be associated with lower odds for liver cancer, in a large case-control study (50). Furthermore, a recent prospective study with 32 years of follow-up, demonstrated that a better adherence to the Alternative Healthy Eating Index-2010 (AHEI-2010), which is similar in many ways to the Mediterranean diet, may decrease the risk of developing HCC (51).

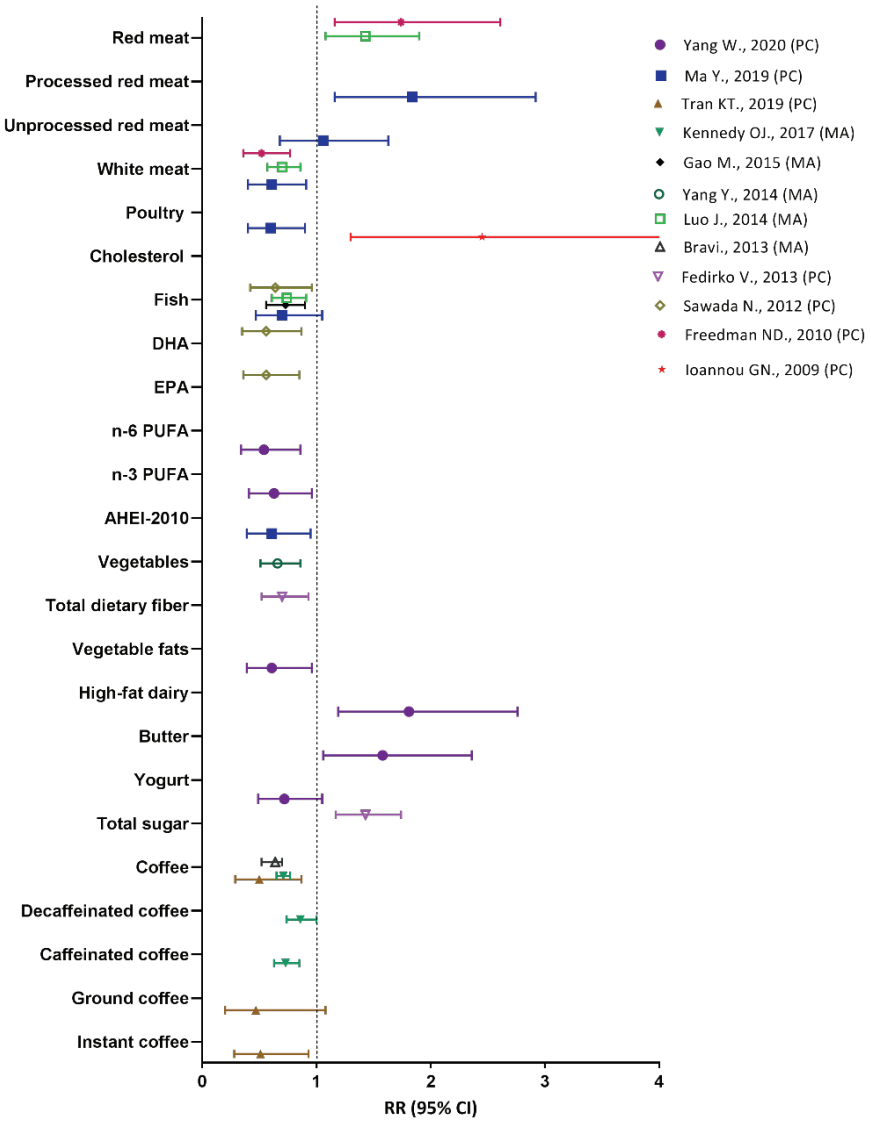

Figure 3. Lifestyle parameters related with increased or reduced risk for HCC, demonstrated in large prospective cohort studies and meta-analyses of cohort studies. Figure obtained from Zelber-Sagi Shira, **Seminars in Liver Disease 2021** ahead of print.

Relative risks (RR) (or Hazards Ratios) are presented with confidence interval (CI). The multivariate adjusted associations are presented. The categories compared were the highest dietary intake category vs. the reference category (lowest intake).

Abbreviations: eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA; polyunsaturated fatty acid, PUFA; Alternative Healthy Eating Index-2010, AHEI-2010; prospective cohort, PC; meta-analysis, MA.

AHEI-2010 consists of high intake of fruit, vegetables, whole grains, nuts and legumes, n-3 fats, and low intake of sugar-sweetened beverages and fruit juice, red and processed meat, trans fat, sodium, and a moderate alcohol consumption.

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Effects of diet on the liver: role of the microbiome

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Take-home messages

- The gut microbiome is involved in the development of NAFLD through altered gut-liver axis (i.e. altered intestinal permeability leading to increased endotoxemia and subsequent inflammatory signalling within the liver).
- Human and rodent experiments have shown that gut microbiota differs between healthy controls, patients with NAFLD/NASH and cirrhosis.
- Several gut bacterial signatures of NASH or NASH-related fibrosis are concordant across the literature.
- Nevertheless, some discrepant signatures are also observed potentially linked to several confounding factors (among which corpulence, metabolic disease and their related treatments, ethnicity, food intake, sequencing methods).
- Several means to modulate the gut microbiome (probiotics, prebiotics, diet intervention, polyphenols, physical activity) have shown positive effects on both the gut microbiota dysbiosis and NAFLD outcomes mostly in animal studies but some were also replicated in humans.

NAFLD and the microbiome (composition and function)

The role of the gut microbiome in the pathophysiology of NAFLD originates from faecal microbiota transfer (FMT) experiments from mice or humans to germ-free mice (mice without any microbiome). Indeed, the sole FMT from mice or individuals with NAFLD replicated liver alterations in the receiver. Such results were also confirmed using antibiotic-treated mice, which were prevented from high-fat diet–induced NASH development. The mechanistic pathways linking the gut microbiota to NAFLD development and its progression is outside the topic of this syllabus but is reviewed in details in (1–3). In brief, it includes altered intestinal permeability leading to increased endotoxemia and subsequent inflammation, enterohepatic circulation of bile acids, altered immunity and the role of microbiome-related metabolite (4).

Therefore, bacterial signatures of the disease have been researched by comparing patients with NAFLD or NASH or NAFLD-related fibrosis with healthy controls, using different microbiota sequencing methods. These signatures have been extensively reviewed (1,2,5)which often also includes obesity, diabetes, and dyslipidemia. It is rapidly becoming the most prevalent liver disease worldwide. A sizable minority of NAFLD patients develop nonalcoholic steatohepatitis (NASH. In brief, NAFLD and NASH are associated with an increase in Proteobacteria at the phylum level, *Gammaproteobacteria* at the class levels, while there is an increase in *Escherichia* and *Dorea* and a decrease in *Anaerosporobacter, Coprococcus, Eubacterium, Faecalibacterium* and *Prevotella* at the genera level. Likewise, some signatures are associated with advanced fibrosis such as an increase in *Bacteroides* and *Escherichia* (2), signatures which are similarly detected during cirrhosis. Differential microbiota composition within the increasing severity of NAFLD spectrum is important since it could be useful as a diagnostic marker.

Indeed, in advanced liver fibrosis, 37 species were differentially expressed between patients and healthy controls and were further included in a model together with clinical patient's characteristics. This model enabled very high accuracy detection for the most severe case of fibrosis (6) we characterized the gut microbiome compositions using whole-genome shotgun sequencing of DNA extracted from stool samples. This study included 86 uniquely well-characterized patients with biopsy-proven NAFLD, of which 72 had mild/moderate (stage 0-2 fibrosis and could potentially be used as a non-invasive diagnostic tool in the future.

Nevertheless, although being distinct, bacteria from different genera or class are able to perform similar functions. Thus, looking for differential functionality between patients with NAFLD and healthy controls seem more relevant than their sole different composition. To this end, omics tools have been used such as metagenomic sequencing, which not only assesses composition but also their functional potential. Furthermore, it can be coupled with serum metabolomic analysis to evaluate microbiota-related metabolite production. Thus, in NAFLD-related gut microbiota dysbiosis, several metabolites or pathways are increased in NAFLD patients, such as branched-chain amino acids (also associated with insulin resistance (7)) or lipopolysaccharides (LPS) synthesis, which participate in the pathophysiology. Likewise, during NAFLD-related fibrosis, metagenomic signature highlight the importance of amino acid dysregulation, which also translates within the systemic circulation with the increase of 3-(4-hydroxyphenyl) lactate, a microbial-derived metabolite involved in amino acid metabolism (2).

Interestingly, while the gut microbiota composition is highly dependent of an individual's usual dietary habits, a rapid change in the diet can induce some bacterial changes. Furthermore, composition changes after a diet modification will display inter-individual variability, which depends upon the initial microbiota composition. Amongst its numerous physiological functions, the gut microbiota is able to process food items leading to metabolite production that acts on the host physiology (8). According to the initial gut microbiota composition and the diet regimen, different metabolites will be produced, some of which are involved in NAFLD pathophysiology through the gut-liver axis. Hence, the question that arises is, how can diet modification potentially improve gut microbiota dysbiosis, metabolite production and subsequently beneficially impact liver outcomes?

Effects of lifestyle recommendations on NASH-associated gut microbiota dysbiosis or related altered gut-liver axis

EASL (European Association for the Study of Liver) (9) have listed several lifestyle propositions to improve NAFLD, however, to date their recommendations are based on moderate to low quality of evidence (B and C, nevertheless graded 1 or 2). Several of these recommendations are also concordant with the AASLD (American Association for the Study of Liver Diseases) guidelines (10). Whereas the specificity of the diet intervention remains vague in terms of macro and micronutrient content, the Mediterranean diet is advised. Most importantly, it is proposed that some specific food items should be excluded such as processed food or high-fructose food or beverages (B2). Patients with NAFLD/NASH should follow a healthy diet (B2/C2) (9). Overweight or obese patients should undergo weight loss through a hypocaloric diet and achieve a 7-10% body weight loss (B1). Finally, weight maintenance should be targeted to prevent recurrence of NAFLD. Anyhow, EASL recommends that lifestyle intervention should be personally tailored.

How do those lifestyle guidelines relate to microbiome changes and whether these changes affect NAFLD is not present in these recommendations but will be discussed and addressed thereafter.

Mediterranean diet and gut microbiota health

The Mediterranean diet (MD) is composed of a high intake of mono- and polyunsaturated fatty acids, increased consumption of fruits and vegetables thus enriched in fibre and polyphenols as well as decreased processed food and refined sugars. MD can reduces weight and improves metabolic diseases (11)we randomly assigned 322 moderately obese subjects (mean age, 52 years; mean body-mass index [the weight in kilograms divided by the square of the height in meters], 31; male sex, 86% as well as reduce liver fat, when associated with polyphenol supplementation as shown in several randomized controlled trials, and reviewed in (12). Thus, this led EASL to recommend good compliance with this specific diet (9). Data shows that diet components within the Mediterranean diet modify the gut microbiota towards a healthier state, thus potentially explaining its beneficial effect on NAFLD.

For example, a recent 3-weeks overnutrition randomized controlled trial evaluated the comparative effects of saturated fat, unsaturated fat or carbohydrate intake both on the gut microbiota and the liver. Saturated fat induced the most important hepatic fat accumulation, insulin resistance, and intestinal permeability, evaluated by the indirect measure of serum LBP/CD14 as compared to the two other diet interventions (13). More interestingly, solely saturated fat induced a significant shift in gut microbiota composition towards increased proteobacteria [(13)], a signature often observed during NAFLD and NASH (5)two metabolic diseases strongly intertwined with non-alcoholic fatty liver disease (NAFLD. Therefore, guidelines that propose a reduction of saturated fat towards an increase in unsaturated fat seems healthier both for the liver, intestinal permeability and microbiome. Nevertheless, more studies evaluating the impact of saturated fat reduction within the diet and its effects on the microbiome and NAFLD are needed to draw firm conclusions.

The literature on the effects of polyphenol (contained in the MD) is growing, mainly in rodent models. Polyphenol seems to improve both microbiota dysbiosis as well as the altered gut-liver axis observed during NAFLD. Furthermore, it is associated with improved histological features of NAFLD as extensively reviewed in (3). However, to date, studies translating those positive effects also in humans are still lacking and are thus warranted. For example, a recent meta-analysis of human studies evaluating the effect of resveratrol (one of the many available polyphenols) on human NAFLD and gut microbiome dysbiosis failed to find a beneficial effect (14)a dietary phytochemical, is capable of attenuating NAFLD development and progression; however, results from clinical studies are inconsistent and inconclusive. Here, we conducted a meta-analysis to evaluate the efficacy of resveratrol on NAFLD, using several parameters to provide new insights for clinical application. We systematically searched EMBASE, PubMed, Science Citation Index, Elsevier, and Cochrane Library databases for studies published up to date (July 2016.

Nevertheless, a recent study evaluating the effect of MD diet with or without polyphenol supplementation (this time using Wolffia globose Mankai strain) showed a significant and clinically relevant reduction of intra-hepatic fat, evaluated by proton magnetic resonance spectroscopy (15). Furthermore, the same trial demonstrated that MD associated with Wolffia globose Mankai strain supplementation induced gut microbiota composition modification, weight loss and improved metabolic alterations (16)abdominally obese or dyslipidemic participants in Israel were randomly assigned to (1. Most interestingly, autologus fecal microbiota transfer, collected during the weight loss phase of this study, from individuals submitted to this MD diet supplemented with polyphenols is able to preserve weight loss and metabolic improvement (16)abdominally obese or dyslipidemic participants in Israel were randomly assigned to (1, thus again suggesting the role of the MD-induced microbiome changes

in the improvement of metabolic complications (one of the many culprits of NAFLD physiopathology). The effect of MD on the gut microbiota composition continues to be studied and suggests that it at least partly reverses gut bacterial signatures associated with NASH (3). More humans studies are still needed to evaluate the effects of MD on gut microbiota dysbiosis as well as on the different NAFLD histologic alterations, specifically liver fibrosis which represent the prognostic lesions of the disease.

Fructose and gut microbiota dysbiosis

Fructose has been associated with the development of several liver alterations leading to NAFLD, especially in rodent experiments as reviewed in detail in (17)including steatosis (fatty liver. Interestingly, data in rodents show how fructose-induced NAFLD lesions originate at least in part through the gut microbiota. Nevertheless, since most studies associated a fructose and lipid challenge, the latter being known to induce microbiota dysbiosis *per se*, it is complex to incriminate the sole effect on fructose. Focusing on fructose challenge only, most rodent studies demonstrate an increased intestinal permeability (as seen with reduced intestinal tight junction, increased circulating LPS, increased TLR induction leading to liver damage and inflammation) (17)including steatosis (fatty liver. Some studies have also observed fructose-induced microbiota composition changes. Yet, those results need to be validated and confirmed in humans. However, indirect proof has started to accumulate linking fructose consumption and gut microbiota dysbiosis during NAFLD. Indeed, subjects with NAFLD had higher endotoxemia (i.e. increased circulating concentration of the LPS, a membrane part of Gram-negative bacteria) than obese matched individuals without NAFLD. More interestingly, in adolescent with NAFLD, fructose beverage intake led to even higher post-prandial endotoxemia as compared to healthy subjects (18). Furthermore, this increased endotoxemia was not observed after glucose consumption. This study, although using indirect endpoints, suggests that fructose is associated with gut microbiota dysbiosis and leads to increased intestinal permeability, a pathophysiologic feature characterising NAFLD. Nevertheless, more studies are needed, such as diet intervention studies that aim to reduce fructose intake and further evaluate whether it improves both gut microbiota dysbiosis and NAFLD alterations.

Weight maintenance and gut microbiota

It is known that weight loss often results in subsequent increased weight regain in humans, a phenomenon called the “yoyo effect”. It has recently been shown in rodent studies that weight cycling modifies the gut microbiota (19) and is thus responsible for increasing weight regain after initial weight loss.

Indeed, Thaiss *et al.* have exposed mice to cycles of HFD interleaved with normal chow able to induce successful weight loss. These mice were compared to a control group of mice submitted to constant HFD during the whole experiment. They observed that weight regain after successful weight loss increased with the number of weight cycles. Furthermore, mice who underwent two or more weight cycling experiments (despite successful weight loss reduction during the chow diet periods) finally reached a similar weight than the group submitted to constant HFD. Furthermore, weight cycling altered gut microbiota composition, which remained very dysbiotic even after successful weight loss. More importantly, weight regain was prevented in the absence of microbiota, obtained by broad antibiotic treatment. Finally, they demonstrated the obligatory role of the gut microbiota in this increased weight regain since FMT from mice who underwent several weight cycling experiments replicates *per se* weight gain in germ-free mice upon HFD. There is a need to now confirm these findings in humans. Overall, these experiments suggest that achieving weight maintenance after initial weight gain might

prevent further gut microbiota dysbiosis exacerbation, thus leading to an increased weight regain in cases of recurrent positive energy balance.

Conclusions

The role of the gut microbiota in NAFLD pathophysiology is now well-identified, and microbiome signature of NAFLD or related fibrosis have been identified in humans. The links between diet and microbiota composition and function has also been demonstrated. Studies have started to show how diet modification could lead to improved gut microbiota dysbiosis, improved gut barrier function and mechanisms involved in the gut-liver axis, thus leading to improved NAFLD outcomes. Nevertheless, more research is still needed in this field, which particularly should consider individual microbiota composition variability in order to propose personalised nutrition programs that further improve liver outcomes. Furthermore, other types of intervention (probiotics, prebiotics, diet intervention, polyphenols, physical activity, FMT) aiming to modulate the gut microbiota, have also been studied and promising results have emerged showing their potential benefice in NAFLD reviewed in (3).

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(references in **BOLD** are required reading)

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How much alcohol can I drink?

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Take-home messages

- Any alcohol consumption, regardless of the doses, contains a health risk. The magnitude of health risks depends on the amount of alcohol consumed and differs with respect to the target organ, as well as individual genetic and non-genetic factors.
- Daily alcohol intake of approximately 24 g for men and half of that for women has a relatively low risk for alcohol-associated diseases. Prerequisite for this is that the person is healthy and has no disease which deteriorates with alcohol
- No alcohol during pregnancy.
- Children and adolescents below the age of 18 years should not drink at all.
- Older people above 70 years of age should limit their alcohol consumption.
- Risk factors for alcoholic liver disease are mutations of some genes *(PNPLA3; TM6SF2; MBOAT7, minor C allele in HNRNPUL1)*, female gender, obesity, and the presence of other types of liver disease.
- Even moderate alcohol intake raises arterial blood pressure.
- Light to moderate alcohol intake may slightly improve peripheral insulin resistance especially in women. Patients with diabetes mellitus may drink alcohol only when blood sugar levels are well controlled.
- Obese individuals with metabolic syndrome and pure fatty liver should try to limit their alcohol intake.
- Patients with non-alcoholic steatohepatitis should avoid alcohol.

Introduction

According to the World Health Organization, alcohol is responsible for over 200 diseases [1]. Recent epidemiologic data emphasise that any alcohol consumption, regardless of the doses, contains a health risk and that only abstinence is completely risk-free [2]. However, the magnitude of health risk depends on the amount of alcohol consumed and differs with respect to the target organ, as well as individual genetic and non-genetic factors, which may increase alcohol toxicity. According to various public health guidelines, daily alcohol intake of approximately 24 g for men and half of that for women is called moderate and has a relatively low risk for alcohol-associated diseases [3]. Prerequisite for this is that the person is healthy, metabolises alcohol adequately (no polymorphisms of alcohol dehydrogenase such as ADH1C1*1 or acetaldehyde dehydrogenase ALDH21*2, which leads to an accumulation of acetaldehyde [AA]) or possesses other risk genes e.g. *PNPLA3* or others with an increased risk for alcoholic liver disease (ALD). These recommendations consider the most sensitive organ towards alcohol toxicity, which seems to be the female breast where no threshold dose for alcohol toxicity exists and where alcohol even at low doses is a risk factor for breast cancer [4].

The most important diseases associated with chronic alcohol consumption and their alcohol-attributable fractions for selected causes of death in percentage are given in Table 1 [1].

Table 1. The most important diseases associated with chronic alcohol consumption and their alcohol-attributable fractions for selected causes of death in percentage.

Alcohol use disorders (100%)	Poisoning (18%)
Fetal alcohol syndrome (100%)	Traffic injury (15%)
Liver cirrhosis (50%)	Tuberculosis (12%)
Oral cavity cancer and pharynx cancer (30%)	Liver cancer (12%)
Pancreatitis (25%)	Epilepsy (12%)
Laryngeal cancer (23%)	Colorectal cancer (12%)
Oesophageal cancer (22%)	Hypertension (8%)
Interpersonal violence (22%)	Breast cancer (8%)
Self-harm (22%)	Ischemic heart disease (7%)

High-risk groups

High-risk groups consist of children and adolescents (in many countries, alcohol is already consumed regularly at the age of 12 years). Children and young adolescents, who drink chronically or binge, have an increased risk for addiction and cancer later in life. In addition, their brain development is disturbed, leading to behavioural abnormalities. Thus, under the age of 18, alcohol should not be consumed.

The elderly, over the age of 70 years, have also an increased risk for organ damage, because they metabolise alcohol slower since their metabolising systems also age, and their brains and livers become more susceptible to the toxicity of alcohol. Furthermore, many elderly people take drugs for various reasons and an interaction between alcohol and drugs may result in increased drug toxicity (central nervous system side effects with disorientation, fatigue and stumbling). Thus, alcohol should not be consumed regularly in the elderly.

In addition, some cardiovascular (e.g. cardiomyopathy, arrhythmia, hypertension), gastrointestinal (e.g. gastro-oesophageal reflux, Celiac disease, liver disease of other aetiologies) and metabolic (e.g. porphyria, lipid metabolism) diseases deteriorate with alcohol. Children of individuals with alcohol use disorders have an increased risk to develop alcohol dependency by themselves. Thus, in these situations, alcohol consumption should be completely avoided.

Smoking

Most important is simultaneous smoking since the carcinogens present in tobacco smoke are activated by Cytochrome P4502E1 (CYP2E1), which is increased in the mucosa of the oral cavity and the oesophagus by chronic alcohol consumption. Furthermore, smoke contains AA which is toxic and adds to the risk of cancer in these areas. Smoking may also change oral bacteria, which are capable of metabolising alcohol to acetaldehyde [5].

Genetics

AA is a major toxin which is generated by alcohol dehydrogenase (ADH) from ethanol, and ALDH detoxifies it to acetate. Out of seven ADHs, two show polymorphisms, namely ADH1B and ADH1C. The polymorphism of ADH1B may lead to a high active ADH, which creates a huge amount of AA that cannot be tolerated. Therefore, individuals who possess ADH1B2*2 homozygosity cannot drink alcohol at all because of the side effects. On the other hand, individuals with ADH1C1*1 homozygosity code for an enzyme, which is 2.5-times more active to produce AA. Various studies have reported that these individuals have a higher risk for cancer in the upper elementary tract and the large intestine [5].

When AA is not metabolised to acetate adequately, an increased risk for cancer occurs. ALDH2 mutation occurs in 50% of Asians, Japanese, Koreans and Chinese, 40% are heterozygotes and 10% are homozygotes. Homozygotes cannot detoxify AA at all; thus, they must abstain from alcohol completely. Heterozygotes are able to metabolise AA, but their activity is approximately 15% of that of Caucasians. Therefore, AA levels increase after alcohol consumption leading to a flush syndrome (red face, tachycardia, sweating, vomiting). Despite these symptoms, some individuals continue to drink with an increased risk for cancer of the upper alimentary tract [5]. Risk factors for ALD are summarised in Table 2.

Table 2. Risk factors for alcoholic liver disease

Genetics
Female gender
Overweight or obese
Liver disease of other aetiologies (Hepatitis B and C, hereditary hemochromatosis, a1-Anti-Trypsin deficiency, NASH).
Simultaneous intake of certain drugs such as paracetamol, methotrexate, isoniazid
Exposure to toxins (vinyl chloride, solvents, nitrosamines, aflatoxins)
Simultaneous intake of ß-carotene or vitamin A
Smoking

Several large genome-wide association studies revealed that patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) and, to a lesser extent, transmembrane 6 superfamily member 2 (*TM6SF2*) and membrane-bound O- acetyltransferase domain-containing protein 7 (*MBOAT7*) are important genetic determinants for risk and severity of ALD [3]. *PNPLA3* is closely involved with lipid metabolism and is a risk factor for NAFLD and hepatocellular carcinoma (HCC). By contrast, a mutation in *TM6SF2* can result in hepatic fat accumulation owing to a defect in the secretion of very-low-density lipoproteins, and a mutation in *MBOAT7* can cause a disturbance in the acetylation of phosphatidylinositol, but it is not clear whether this results in hepatic fat accumulation [3]. More recently, *HNRNPUL1* and *MARC1* were identified as additional risk loci for alcohol-related cirrhosis.

Female gender

Women are more prone to alcohol compared to men, especially with respect to ALD [3]. The reason for this is not clear but may include a lower first-pass metabolism of alcohol in the stomach and a decrease in the water distribution space leading to higher alcohol levels as compared to men when the same amount of alcohol per kg body weight is ingested. Additionally, oestrogen metabolism is inhibited by alcohol and oestrogens may influence the development of fatty liver.

Overweight and obesity

How a low amount of alcohol consumption affects arterial hypertension and insulin resistance

Arterial blood pressure

Epidemiological, preclinical and clinical studies established the association between **high alcohol consumption** and hypertension. However, the mechanism through which alcohol raises BP remains elusive. Possible mechanisms include an impairment of the baroreceptors as well as various endocrinologic abnormalities such as an enhanced sympathetic activity, stimulation of the renin-angiotensin-aldosterone system, and increased cortisol levels. In addition, vascular reactivity may be affected by alcohol with a loss of relaxation due to inflammation and oxidative injury of the endothelium leading to inhibition of endothelium-dependent nitric oxide production. To prevent alcohol-induced hypertension, persons must reduce the amount of alcohol intake and partake in exercise.

For a long time, it was believed that **small amounts of alcohol** have a beneficial effect on blood pressure (BP). 150 ml of red wine among type 2 diabetes patients did not have a discernible effect on mean 24 hour BP. In fast ethanol metabolisers, even a BP-lowering effect was noted [6]. But a study with almost 4000 current drinkers without hypertension found that alcohol increased their BP by 1 mmHg for every 10 g consumed per day, and it was concluded that any amount of alcohol above 10 g raises the systolic BP. Furthermore, a most recent study of more than 17,000 United States adults demonstrated that even moderate alcohol consumption (7-13 drinks per week) increased BP significantly [7]. Ethnicity, age, and gender may be factors which could explain the contradictory results of the effect of small amounts of ethanol on BP.

Peripheral insulin resistance

Many reports have suggested that moderate consumption of alcohol improves peripheral insulin resistance (IR). However, this effect seems to be variable depending on the amount of alcohol consumed, the pattern of drinking, and on other factors. In a meta-analysis with 38 studies pooled [8], it was found that with one standard drink per day gave an 18% less likelihood to develop diabetes mellitus (DM). However, this was only seen in women with 2 drinks per day, with 5 or more drinks, no effect was noted. However, in men, alcohol increased the risk to develop DM. Furthermore, when Asians and Caucasians were compared an effect was only seen in Caucasians, possibly due to the difference in the genetics of alcohol metabolising enzymes in Asians.

Susceptibility to low amounts of alcohol in diabetic patients and obese patients

Alcohol affects type 2 DM primarily by influencing blood sugar levels (BSL). Patients who already suffer from type 2 DM may consume alcohol when their BSL are well controlled. They should not consume more than 1 drink per day, should drink slowly with meals and avoid sweet alcoholic beverages or alcoholic beverages high in carbohydrates such as beer. Alcohol should not be consumed chronically and/or in high quantities since BSL may be affected, hepatic fat deposition increases and side effects such as peripheral neuropathy will be enhanced.

According to most recent publications the data is still controversial with respect to the effect of alcohol in patients with type 2 DM. While a most recent study from Malaysia demonstrated that modest alcohol intake (< 21 units/week in men and < 14 units/week in women) is not associated with higher prevalence of hepatic steatosis or more severe liver disease among patients with type 2 DM, data from Sweden show a high risk of hepatic fibrosis in these patients when they drink even moderately. Furthermore, light-to-moderate alcohol consumption increases the risk of type 2 DM in Chinese individuals with NAFLD. Again, ethnicity and genetics of ethanol metabolizing enzymes may affect the results.

Unquestionably, obesity is a risk factor for ALD [3,9]. Obese individuals often suffer from metabolic syndrome (DM, hypertension and disturbances in cholesterol and triglyceride metabolism). As discussed, moderate alcohol consumption has only marginal effects on DM and BP, the caloric content of 1 drink (13 g of ethanol equalising approximately 90 kcal) is small and the effect on cholesterol metabolism shows mostly both an increase in LDL- and HDL-cholesterol. However, obese individuals frequently have NAFLD mostly in the form of pure fatty liver. Pure fatty liver may advance to NASH. Thus, obese individuals with pure fatty liver may consume alcohol, but not regularly and only moderately. The situation in patients with NASH will be discussed below.

Should patients with NASH completely abstain from alcohol?

The pathogenesis of ALD and NAFLD share similarities in hepatic morphology and pathogenesis, including fatty liver as a prerequisite (Figure 1). The histological features of alcoholic steatohepatitis (ASH) and NASH appear similar, which suggests similar pathogenetic mechanisms in the generation of hepatic inflammation. However, the mechanisms for non-alcoholic and alcoholic fatty liver are somehow different. As alcohol consumption and an excess of dietary caloric intake may occur together, the effect of chronic alcohol consumption on patients with obesity and patients with NAFLD is of special interest. Various epidemiological studies report that >40 g of alcohol per day and even moderate (20-40 g of alcohol per day) alcohol consumption can enhance hepatic steatosis, inflammation, fibrosis, and cirrhosis in patients who are overweight or obese [3]. A more recent cross-sectional study of 2475 participants of the Framingham Heart Study with hepatic steatosis demonstrated that alcohol even after excluding heavy alcohol users is a risk factors for NAFLD and a Japanese study demonstrated that even moderate drinking promote hepatic fibrosis in patients with NAFLD.

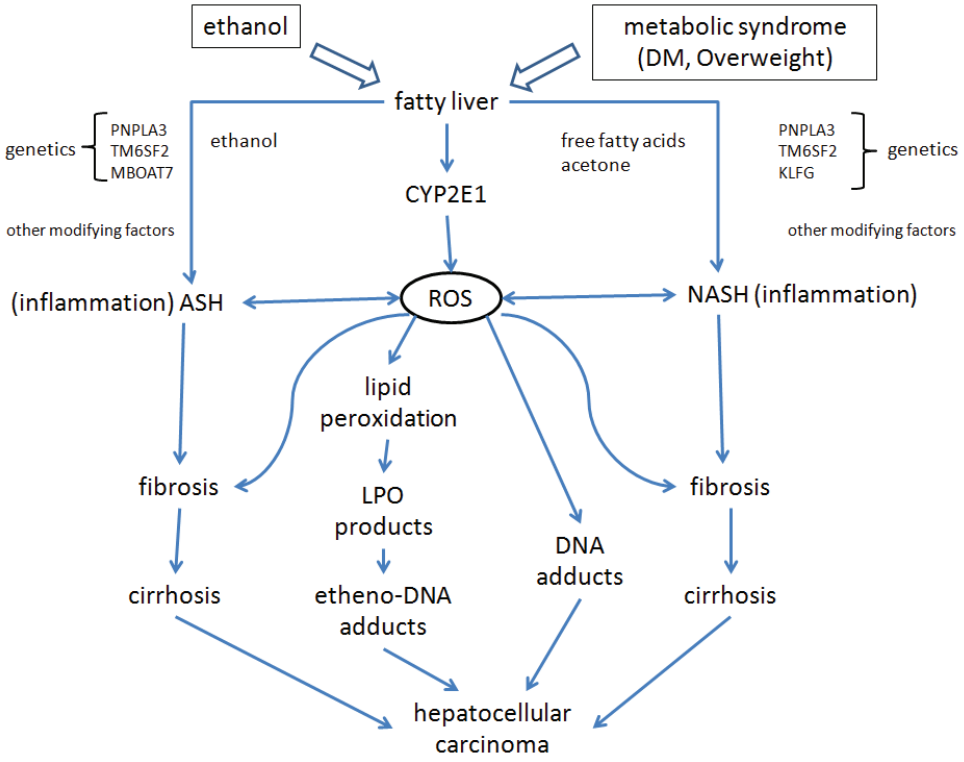

Figure 1. Oxidative stress as a factor in the pathogenesis of ASH and NASH. This results in the generation of varying amounts of reactive oxygen species (ROS), which might lead to inflammation but, at the same time, might also occur due to inflammation (ASH and NASH) via cytokines/

chemokines. The occurrence of ASH and NASH is further modified by genetics and other modifying factors. ROS lead to fibrosis and lipid peroxidation (LPO). LPO products, such as malondialdehyde and 4-hydroxy-nonenal can bind to DNA, which results in the generation of highly carcinogenic etheno-DNA adducts. Direct bonds of ROS to DNA, etheno-DNA adducts, and the cirrhotic milieu play a decisive role in hepatic carcinogenesis and finally can lead to hepatocellular carcinoma. PNPLA3, patatin-like phospholipase domain-containing 3; TM6SF2, transmembrane 6-superfamily member 2; KLF6, Kruppel-like factor 6; MBOAT7, O-acetyltransferase domain-containing 7. Adapted from [11].

By contrast, epidemiological studies from Japan and Europe suggest that moderate alcohol consumption improves hepatic steatosis compared to non-drinkers due to an improvement of peripheral IR. Furthermore, various cross-sectional studies on NAFLD report a beneficial effect of alcohol consumption (>40 g per day) on hepatic fat. In addition, some studies examining the effect of alcohol on histopathologically diagnosed NAFLD had controversial findings. Although in some studies moderate alcohol intake in patients with NAFLD resulted in an accelerated progression of fibrosis and an elevation of serum transaminase activities, other studies (some in morbidly obese patients) did not confirm this finding. However, these studies are small, and most of them do no account for various confounding factors. Thus, based on currently available data, it may be difficult to determine the role of moderate alcohol consumption on NAFLD progression. Moreover, results may also vary depending on whether alcohol is consumed in patients with pure fatty liver or in patients with NASH [3,9].

In contrast, the data on alcohol and the development of HCC in patients who are overweighed or obese and in patients with NAFLD patients are clearer. Almost all retrospective studies report an increased risk with alcohol consumption at any level for the development of HCC in patients with NASH [3,9].

In conclusion, in clinical practice, it seems wise to recommend that at least patients with NASH should refrain from any amount of alcohol consumption.

Drugs, xenobiotics and alcohol

Many drugs interact with alcohol even when alcohol is consumed in low quantities. This is predominantly relevant for central acting drugs, but also for paracetamol, methotrexat, and isoniazid. In this context, it is referred to in more detailed literature [10].

ß-carotene and vitamin A

Chronic alcohol consumption induces CYP2E1, which is responsible for the degradation of retinol and retinoic acid (RA) to polar apoptotic metabolites. Although the loss of RA has deleterious consequences in cell differentiation and cancer development, the substitution of RA, retinol or ß-carotene further enhances the generation of these apoptotic metabolites resulting in liver damage [10].

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Smoking [cigarettes or cannabis], coffee and liver diseases

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Take Home Messages

- Preclinical and epidemiological studies suggest that cigarette smoking (CS) contributes to the development and progression of numerous types of liver disease, including primary biliary cholangitis, alcohol-related liver disease and chronic viral hepatitis; in contrast, data are still limited and conflicting regarding the link between CS and progressive non-alcoholic fatty liver disease (NAFLD).
- While preliminary data suggest that cannabis and endocannabinoids may impact the development and progression of chronic liver disease, published clinical evidence is limited, and further research is needed before clinical recommendations can be made.
- Coffee consumption currently is recommended by the most recent EASL guidelines for protecting against the progression of chronic liver diseases, including NAFLD, but little is currently known about the optimal dose or preparation of coffee to consume, to achieve maximal benefit.

Introduction

Cirrhosis is responsible for nearly 1.3 million deaths, worldwide[1,2], including over 40,000 deaths per year in the United States[3], where cirrhosis-related mortality is projected to triple by the year 2030[4]. For patients with cirrhosis the prognosis remains poor, and there are currently no approved therapies to reverse cirrhosis and prevent liver decompensation events. Thus, there remains a need to develop effective strategies to prevent the progression of chronic liver disease, at earlier stages.

A growing body of literature demonstrates that dietary and lifestyle factors play a key role in the liver disease progression to cirrhosis and HCC, including diet, alcohol intake, coffee consumption, smoking, physical activity and use of certain medications[5-9] (Figure 1). Accordingly, it is increasingly recognized that lifestyle modifications may provide impactful benefits for preventing the development of cirrhosis and its sequelae[10,11]. Herein, we will review current understanding of three specific modifiable lifestyle factors associated with chronic liver disease: cigarette smoking, cannabis use, and coffee consumption.

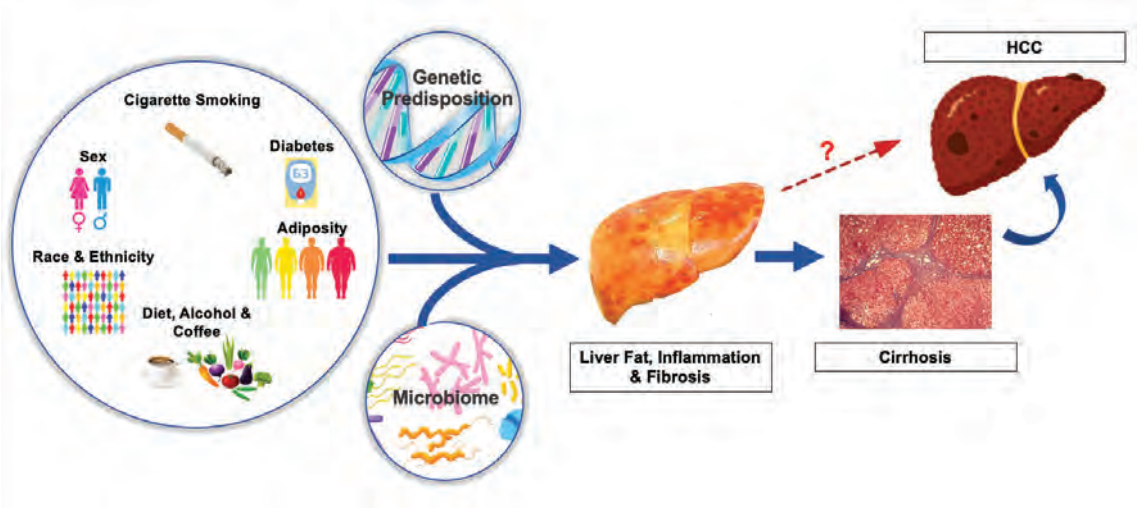

Figure 1. Emerging Risk Factors for Cirrhosis and Hepatocellular Carcinoma. Taken from Simon, TG, "Lifestyle and Environmental Approaches for the Primary Prevention of Hepatocellular Carcinoma". Clinics in Liver Disease, 2020.

Cigarette Smoking (CS) and Chronic Liver Disease

Worldwide, CS represents the leading cause of preventable morbidity and mortality[12]. It is estimated that the worldwide prevalence of daily CS among men and women are 25.0% and 5.4%, respectively, and in many countries the prevalence of CS has increased over the past decade[12]. [13]Although the principal complications of CS include chronic respiratory disease, cardiovascular disease and certain cancers, a body of preclinical and clinical data now link CS to an increased risk of some liver diseases, as well as a 51-70% higher risk of developing primary liver cancers, including hepatocellular carcinoma (HCC)[13 14].

Several potential mechanisms have been proposed to explain the association between CS and the development and progression of certain forms of chronic liver disease. First, CS may induce insulin resistance and hyperglycemia[15], which in turn can promote hepatic inflammation and fibrogenesis. Second, CS contributes to fat redistribution and to the development of central obesity, even in those who lose body weight while smoking[16 17]. Moreover, weight gain after smoking cessation may also promote the development of obesity and diabetes[18], and thus promote the development and progression of non-alcoholic fatty liver disease (NAFLD). Third, CS results in chronic hypoxia and oxidative stress[19-21], which has been shown in Zucker rats to compound hepatocyte injury and induce the expression of pro-inflammatory and pro-fibrotic genes, resulting in progressive liver injury and fibrosis[22]. Finally, in high-fat diet-fed mice, CS accentuates hepatic steatosis through diverse pathways, including by inhibiting phosphorylation of adenosine-5-monophosphate (AMP)-activated protein kinase, which increases hepatic lipogenesis via upregulation of sterol response-element-binding protein 1-c and activation of fatty acid synthase and acetyl-coenzyme-A-carboxylase[23 24].

The earliest clinical evidence linking CS to progressive liver disease emerged in the 1990s[25 26]; since that time, CS has been shown to accelerate the progression of primary biliary cholangitis, alcohol-related liver disease as well as chronic viral hepatitis[27 28-31]. Numerous studies also have demonstrated that CS is associated with significantly higher risk of developing incident HCC. In support of this, exome sequencing of HCC tumours recently revealed specific mutational signatures (MSig1 and MSig3) that are associated with tobacco exposure, suggesting smoking contributed to direct, genotoxic effects in the liver[32].

Currently, robust clinical data linking CS specifically to non-alcoholic fatty liver disease (NAFLD) are still limited. Recently, a large cohort study of 199,468 Korean adults followed by serial abdominal ultrasonography found that current CS, total pack-years of smoking and urinary cotinine levels were each significantly and positively associated with an increased risk of developing incident NAFLD[33], consistent with findings from a previous, smaller retrospective study from Japan[34]. However, among the few published studies to date that have included detailed NAFLD histology, all have been limited by cross-sectional designs and the results have been conflicting, with some reporting positive associations between long-term CS and the prevalence of advanced fibrosis[35 36], while others have found null associations[37]. Given these limited and conflicting data, additional well-designed, prospective studies are needed to more precisely quantify the magnitude of risk associated with CS in patients with NAFLD.

Cannabis and Liver Disease

Worldwide, approximately 147 million people consume cannabis on an annual basis[38]. Cannabis contains over 400 components, among which two of the most-studied ingredients are Cannabidiol (CBD) and tetrahydrocannabinol (THC). CBD and THC both exert their effects through G-protein coupled receptors, the cannabinoid receptor-1 and -2 (CB-1 and CB-2), which interact with endocannabinoid ligands. While THC is a partial agonist that avidly binds to both receptors, CBD is a CB-1 antagonist and a full agonist at CB-2, but it has low affinity for both receptors[39 40].

Under normal physiologic conditions in the liver, the CB-1 and CB-2 receptors are expressed relatively weakly within hepatic endothelial cells and hepatocytes (CB-1), and in Kupffer cells (CB-2); however, both receptors are upregulated in the setting of chronic liver disease[39 41]. Within the liver, cellular activation of CB-1 and CB-2 receptors induce opposing, pro- and anti-fibrotic effects, respectively[39 42-44] (Figure 2). Specifically, in preclinical models, CB-2 deficiency promotes fibrogenesis, while CB-2 agonism accelerates hepatic regeneration, and improves liver fibrosis, while decreasing inflammatory infiltration in the liver[39 42-44]. CB-2 agonism has also been shown to reduce obesity in rats[45], and to reduce rates of developing incident diabetes, in both mice[46] and humans[47], and it may also attenuate alcohol-related liver injury[40 48]. Accordingly, liver samples from patients with ALD demonstrate increased expression of pro-inflammatory and pro-fibrotic CB-1 receptors[49], while CB-1 receptor knockout mice are resistant to the development of alcohol-related liver disease[49 50]. Finally, Dai and colleagues recently demonstrated that CB-1 and CB-2 receptors were both over-expressed in patients with chronic HBV infection, and the degree of this over-expression correlated with increasing liver fibrosis severity[51].

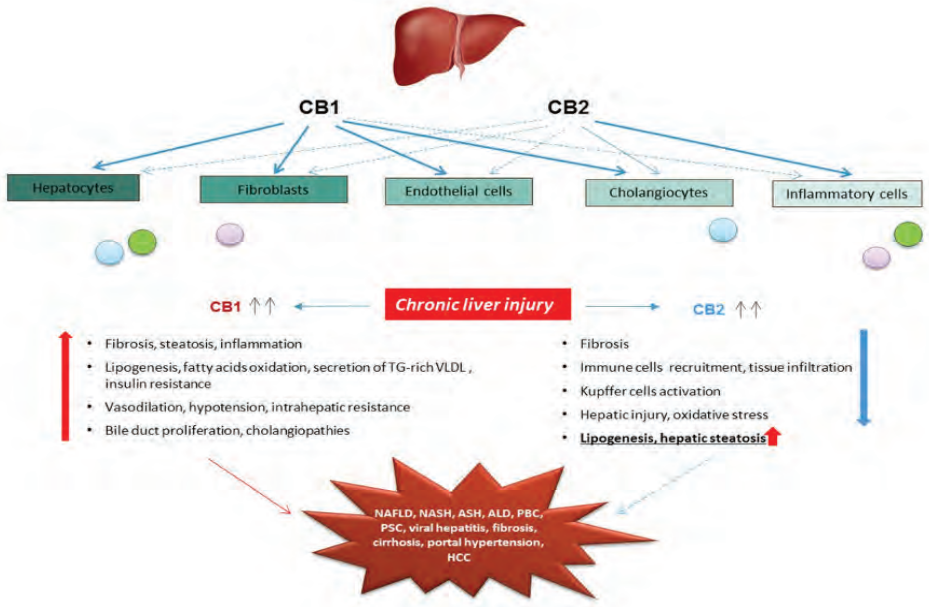

Figure 2. Actions of the CB-1 and CB-2 receptors in the liver. Taken from [40].

Despite these promising preliminary evidence, more clinical data are still needed regarding the effects of cannabis on the prevalence and severity of liver disease. To date, several cross-sectional studies have found significant, inverse associations between marijuana use and decreased odds of having prevalent steatosis in patients with chronic HCV infection, or HIV/HCV co-infection[52] or NAFLD[53], however others have found null associations. A recent meta-analysis of 3 cross-sectional studies with 5,973,595 patients found that marijuana use was associated with a significantly lower odds of having prevalent steatosis (pooled OR=0.80, 95%CI=0.75-0.85)[54]; however, in subgroup analyses, no significant association was found for either prevalent fibrosis (pooled OR=1.96, 95% CI 0.78-4.92), or for fibrosis progression. Recently, a cross-sectional analysis of an administrative dataset of 319,514 U.S. adults with a past or current history of alcohol abuse, found that individuals with concurrent cannabis use had significantly lower odds of developing alcoholic hepatitis, cirrhosis and HCC (adjusted OR: 0.57 [0.53-0.61], 0.45 [0.43-0.48] and 0.62 [0.51-0.76], respectively)[55]. Given the limitations of existing evidence, high-quality, prospective data are still needed from large study populations with well-phenotyped liver disease, before clinical recommendations can be made.

Coffee

Coffee contains well-described anti-inflammatory, antioxidant and antifibrotic properties, and it has been observed that coffee drinkers tend to have lower risk of developing advanced liver disease, including liver fibrosis[56], cirrhosis and incident HCC[57 58]. Both the World Cancer Research Fund and the International Agency for Research on Cancer have also published reports supporting the beneficial effects of coffee for the prevention of HCC[59 60]. A recent meta-analysis of 26 studies and 1,825 incident HCC cases demonstrated that consumption of at least 2 cups/day of coffee was associated with significantly reduced risk of incident HCC compared to no coffee consumption, with a pooled relative risk of 0.71[61] (Figure 3). Per each additional 2 cups of coffee consumed per day, the magnitude of observed benefit was significantly greater with caffeinated coffee (27% relative risk reduction) than with decaffeinated coffee (14% relative risk reduction)[61]. Overall, the strength and consistency of the epidemiological associations for coffee has led to recommendations for moderate coffee consumption for HCC prevention in the 2018 guidelines from the European Association for the Study of the Liver (EASL)[62].

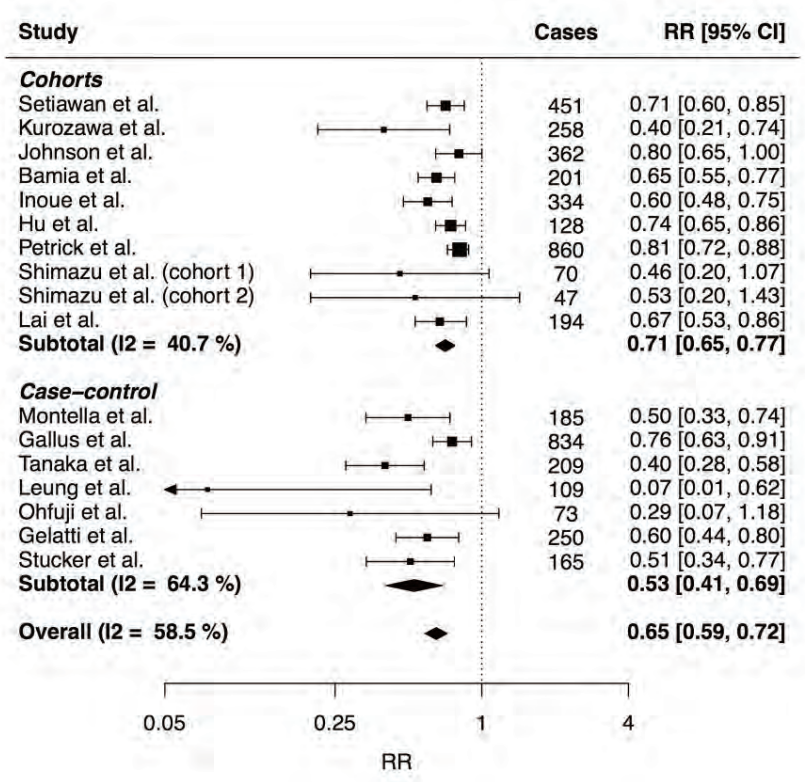

Figure 3. Association between coffee consumption and the development of incident hepatocellular carcinoma, systematic review and meta-analysis of observational studies. Taken from [61].

Observational studies have also examined the relationship between coffee consumption and the development and progression of chronic liver disease. Increasing coffee intake has been associated with lower alanine aminotransferase (ALT) levels, in population-based cohorts[63 64], and both case-control and cross-sectional studies have found that coffee consumption is inversely associated with the odds of having prevalent fibrosis, defined both histologically[65], or by the FibroTest[66], and also with a lower odds of having cirrhosis, particularly alcoholic cirrhosis[67]. In 2014, a systematic review comprised primarily of cross-sectional and case-control studies reported consistent, inverse

associations between coffee consumption and the prevalence and severity of NASH, cirrhosis and the odds of liver-related mortality[57]. More recently, a large cohort study in 63,275 Chinese adults found a dose-dependent, inverse association between coffee consumption and reduced risk of non-viral cirrhosis-related mortality (P-value for linear trend=0.014)[68].

On the basis of these epidemiological findings, the most recent EASL guidelines currently recommend coffee consumption as being protective in NAFLD and other etiologies of liver disease, for reducing histological severity and liver-related outcomes[69]. However, several very important questions remain unanswered, including the optimal "dose" and preparation of coffee (i.e. espresso vs. drip-coffee, type of coffee bean or roasting process), the optimal timing to initiate coffee intake and the necessary duration of consumption during the natural history of liver disease, to achieve meaningful risk reduction. Thus, in order to provide patients with precise, meaningful clinical recommendations regarding coffee consumption, additional more detailed prospective studies are still needed.

Conclusions

Given the diversity of chronic liver diseases, their underlying risk factors and the lack of effective therapies to reverse cirrhosis and prevent its sequelae, strategies for primary prevention are likely to have broad clinical applicability. Lifestyle modification including potentially the avoidance of CS and modest consumption of coffee, could be readily combined with etiology-specific prevention strategies to offer synergistic benefits for patients. Ultimately, combining lifestyle modification strategies with targeted biomarkers for predicting the development of cirrhosis and HCC could provide a robust and cost-effective prevention strategy for patients with chronic liver disease.

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SESSION 4

ALCOHOL-RELATED LIVER DISEASE

WEDNESDAY 23 JUNE
14H00 - 15H00

Pathophysiology and treatment of alcoholic hepatitis

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Take-home messages

- There are no safe thresholds for alcohol consumption. Quantity and patterns of drinking influence the risk of cirrhosis.
- In the majority of cases, the diagnosis of alcohol-related liver disease and alcoholic hepatitis can be made non-invasively. Biopsies should be reserved for cases of diagnostic uncertainty or research studies.
- Stratification of patients using disease severity scores (DF, MELD, ABIC or GAHS) is important to guide patient management.
- In alcoholic hepatitis, steroids do not confer any benefit beyond 28 days and should be reserved for selected cases.
- Infection is the most common complication of alcoholic hepatitis necessitating clinical vigilance and aggressive treatment.
- Long-term survival is dependent on achieving abstinence. Clinical teams should look to integrate addiction services for inpatients and outpatients.

Introduction

Alcoholic hepatitis is a clinical syndrome characterised by jaundice and liver failure occurring in people who are still actively drinking or who have become abstinent within the last 4 weeks [1]. Alcoholic hepatitis invariably develops in people who already have the histological lesion of steatohepatitis, but it is not necessary to have cirrhosis. Alcoholic hepatitis is a distinct clinical entity and is associated with a high-risk of short-term mortality [2]. Scoring systems, which assess residual liver function, are used to classify disease severity and conventionally a discriminant function ≥32 or MELD ≥20 identify a population who have a 20% chance of dying within 30 days of presentation and 30% chance of dying within 90 days [3].

The risk of developing a significant liver disease is closely related to the volume of alcohol that is consumed [4]. Using epidemiological data, it is not possible to define a safe threshold for alcohol consumption; the risk of cirrhosis appears even with an average 1-unit alcohol consumption per day. Certain patterns of alcohol consumption are associated with a higher risk of liver disease. Specifically, people who only drink with meals are less at risk than those who drink at any time. People who drink every day without a break are at a higher risk than people who have at least two days abstinence each week. Amongst patients with severe alcoholic hepatitis, alcohol consumption is frequently very high; in the STOPAH trial, the average consumption was 150 g/day in women and 200 g/day in men [5].

Virtually all patients who drink heavily will develop hepatic steatosis. There are two explanations for this. Firstly, alcohol is a source of calories, which in the majority of people is excess to requirements putting them into a positive calorie balance. Secondly, oxidative metabolism of alcohol modulates the ratio of reduction to oxidised nicotinamide adenine dinucleotide (NADH: NAD+) ratio, which inhibits fatty acid oxidation and promotes triglyceride synthesis. Inhibition of peroxisome proliferator-activated receptor a (PPAR-a) and AMP kinase along with inhibition of sterol regulatory element binding protein 1 (SREBP-1) also favours lipogenesis [1].

In approximately 20% of heavy drinkers, hepatic steatosis will be accompanied by inflammation, characterised by ballooned hepatocytes and inflammatory cell infiltrates which constitute steatohepatitis [6]. A number of processes are thought to contribute to the evolution of steatohepatitis. It is recognised that lipogenesis does not always result in inert triglycerides but sometimes generates cytotoxic or immunoactive lipids [7]. Ceramides may cause cell damage through endoplasmic reticulum stress, whereas arachidonic acid derivatives appear to modulate inflammatory responses.

High levels of alcohol ingestion cause damage to the small intestinal mucosa resulting in translocation of microbial products across the epithelium into the mesenteric veins [8]. Bacterial lipopolysaccharide and CpG rich DNA reach the liver through the portal venous system where they interact with Toll-like receptors (TLRs) in hepatic sinusoids. TLR ligation stimulates the release of proinflammatory cytokines, which stimulates the recruitment of inflammatory cells across the sinusoidal epithelium.

Metabolism of alcohol via alcohol dehydrogenase generates acetaldehyde, a reactive intermediate which may form neoantigens stimulating an adaptive immune response. This may be exacerbated in people who have genetically determined acetaldehyde dehydrogenase deficiency [9]. In excess concentrations, alcohol metabolism overwhelms the alcohol dehydrogenase enzymes and is metabolised through the cytochrome P450 enzyme system and specifically P450-2E1. This generates oxygen-derived free radicals, which damage cytosolic proteins and mitochondrial membranes leading to endoplasmic reticulum stress and cellular apoptosis through the release of cytochrome C [10].

Long-term alcohol excess and steatohepatitis lead to epigenetic changes in hepatocytes. Modification of the hepatocyte nuclear factor 4a (*HNF4a*) gene leads to an overexpression of the fetal HNF4a-P2 isoform rather than the adult HNF-4a-P1 isoform in response to transforming growth factor b (TGF-b) [11]. TNF4a-P2 suppresses a number of genes involved in normal hepatocyte functions such as bile acid transport, gluconeogenesis and the cytochrome P450 system. This probably explains the markedly diminished liver function observed in alcoholic hepatitis even in patients without cirrhosis.

Diagnosis

The diagnosis of alcoholic hepatitis is usually straightforward [12]. A clinical presentation with recent onset jaundice on a background of heavy alcohol consumption is typical. Features of liver failure, including encephalopathy, ascites and coagulopathy are common. Aspartate aminotransferase (AST) is invariably raised, whereas alanine aminotransferase (ALT) may be within normal limits and the AST: ALT ratio should be >1.5. Other causes of jaundice and transaminitis should be excluded with viral serology, autoantibody screens and liver ultrasound. A drug history to exclude possible drug-induced liver injury is important. Patients who have a large variceal haemorrhage at the time of presentation should be assessed carefully as the liver dysfunction may arise through a period of hypotension – shock liver - rather than alcoholic hepatitis.

In clinical practice, a liver biopsy is rarely required but may aid diagnosis when viral serology or autoantibody tests cause diagnostic uncertainty. Liver histology should show typical features of steatohepatitis including steatosis, hepatocyte ballooning, inflammatory infiltrates and peri-cellular, peri-venular fibrosis [6]. Cirrhosis is present in around 80% of cases but is not necessary to make the diagnosis. Additional histological features in alcoholic hepatitis are bile plugging and mega mitochondria.

Assessment of severity

Disease severity scores are important to determine the prognosis and have been widely used to select patients for treatment or for inclusion in clinical trials [3]. The most established scoring system is Maddrey's discriminant function (mDF) based on bilirubin level and prothrombin time. An mDF ≥32 indicates severe disease with an associated mortality of 20% at 28 days and 30% at 90 days. The mDF has been widely used for the inclusion of patients into clinical trials. The Model for End-Stage Liver Disease (MELD), based on creatinine, bilirubin and international normalised ratio is now more commonly used, and a value of ≥20 is considered to be a threshold for severe disease. The Glasgow Alcoholic Hepatitis Score, based on age, white blood cell count, urea, prothrombin time and bilirubin, identifies a group of patients with a GAHS score >9 who may potentially benefit from corticosteroid treatment [13].

Treatment

Currently, EASL guidelines suggest that corticosteroids, usually prednisolone 40 mg daily, may be considered for patients with severe alcoholic hepatitis [12]. Large scale trials and meta-analysis show that prednisolone improves survival by a small margin at 28 days but does not improve survival at 90 days due to the increased risk of infection [5,14]. It is probable that a small proportion of patients do benefit from prednisolone treatment; patients with a baseline neutrophil: lymphocyte ratio between 5 and 8 appear to have improved survival if treated with steroids [15]. If steroids are used, then it is essential that the response is assessed at 7 days using the Lille score. If the Lille score is >0.45, then steroids should be stopped to reduce the risk of infection and because further treatment is futile [16]. Corticosteroids are usually stopped abruptly as there is little risk of an Addisonian crisis after such a short period of treatment.

Previously, pentoxifylline was used to treat severe alcoholic hepatitis. However, a therapeutic benefit was only seen in one trial and recent studies have failed to show a therapeutic effect.

Survival is significantly better in patients who have a good intake of calories and protein. Unfortunately, many patients with alcoholic hepatitis have profound anorexia and it is difficult to persuade them to eat adequately. Intensive nutritional interventions, such as placement of naso-gastric tubes can result in aspiration and other consequences which undermine the potential benefit [17]. Naso-gastric tubes should probably be avoided in patients with encephalopathy. Intravenous feeding is challenging as the risk of line sepsis is high.

In the absence of effective therapeutic interventions for patients with severe alcoholic hepatitis, transplantation may be considered in a minority of patients, even though abstinence has not been durably demonstrated [18]. Outcomes from liver transplantation are as good or better than for other indications [19]. At present, a set of consistent criteria to select patients have not been agreed.

Novel treatments

A number of novel interventions have recently been tested in phase II clinical trials [20]. Two of the main causes of death in patients with alcoholic hepatitis are susceptibility to infection and failure of hepatitis regeneration. Granulocyte-colony stimulating factor (G-CSF) has been shown to stimulate hepatocyte proliferation in human experimental studies and is also thought to improve immune function through mobilising bone marrow CD34+ cells. In one European trial, G-CSF was shown not to benefit patients with alcoholic hepatitis, but in an Indian study in patients with acute-on-chronic liver failure (mostly due to alcoholic hepatitis), G-CSF appeared to improve survival [21,22]. A recent trial presented at the Liver Meeting in November 2019 was unable to confirm any benefit.

The gut microbiome has been shown to be abnormal in patients with alcoholic liver disease [8]. This, so-called, dysbiosis may result in increased translocation of bacterial products, production of toxic metabolites or translocation of more toxic bacterial products. Following the success of faecal microbial transfer (FMT) in *C. difficile* infection, it is widely accepted that FMT is reasonably safe although the criteria for selecting donors and the route of delivery are not well-established. A trial of FMT in patients with alcoholic hepatitis who were not eligible for steroid treatment was conducted in India and showed a significant benefit in terms of survival [23]. However, the mortality in the control arm of this study was unusually high and further trials are required to evaluate this treatment.

An intriguing study, recently published in Nature revealed that the microbiome of many patients with alcoholic hepatitis contains *E. faecalis* strains, which encode a cytolytic toxin [24]. In mouse models of alcoholic hepatitis, the toxin producing *E. faecalis* can be targeted with highly specific bacterial phages, resulting in marked improvement in the disease. This will potentially lead to a novel therapeutic option in the future.

Alcoholic hepatitis is associated with raised serum levels of a large number of inflammatory cytokines. It is challenging to decode which of these cytokines is causal in disease pathogenesis and which are paraphenomena. IL-1 has been shown to exert a number of effects in animal models replicating some of the pathogenesis of alcoholic hepatitis, suggesting that it plays a key role in disease aetiology [25]. IL-1 signalling can be inhibited by receptor blockade using anakinra and IL-1b can be inhibited using the monoclonal antibody canakinumab. A trial of anakinra in combination with zinc and pentoxifylline, compared to corticosteroids has recently been presented. The results were encouraging but not definitive, and a further trial is currently being set up. A phase II trial of canakinumab is currently in progress.

DUR-928 is a sulphated oxysterol that appears to influence gene transcription through epigenetic regulation, but the precise mode of action is unknown. A small phase II trial, presented at the Liver Meeting in November 2019 demonstrated a high rate of Lille responses in patients with severe alcoholic hepatitis compared to controls, treated with corticosteroids [26]. A phase IIb study is now planned.

IL-22 is a pleotropic cytokine produced mainly by T cell populations under the influence of IL-23. It is involved in wound healing and epithelial barrier function and has been shown to induce proliferation of hepatocytes. A phase IIa trial with F-652, a recombinant fusion protein of IL-22, demonstrated higher proportions of Lille responses compared to controls [27]. A phase IIb study is now in preparation.

Infection and sepsis

The rates of infection and sepsis are extremely high amongst patients with alcoholic hepatitis. Approximately 25% of patients will have an infection at the time of admission (known as baseline infection), and a further 25-30% will develop an infection while in hospital (known as incident infection) [28]. If patients presenting with infections are treated aggressively with antibiotics and fluid management their prognosis is not adversely affected. However, patients presenting with severe inflammatory response syndrome (SIRS) have a significantly worse prognosis [29]. Incident infections, particularly when occurring in patients treated with corticosteroids, also have a poor prognosis as they may lead to haemodynamic instability, acute kidney injury and multi-organ failure [30].

There are multiple explanations for the increased susceptibility to infection. Firstly, there is increased translocation of bacteria across the gut epithelial barrier, and secondly, there is impaired innate immunity. Not all causes of immunoparesis have been described, but failure of phagocytic cell oxidative burst has been associated with a high-risk of infection in this group of patients [31].

Vigilant clinical observation coupled with aggressive antibiotic treatment is required to increase the chances of survival. In addition, it is crucial to be aware of the risk of fungal infection.

Management of alcohol use disorder

By definition, all patients with alcoholic hepatitis suffer with an alcohol use disorder and the majority will have a high level of alcohol dependence. It is therefore mandatory that clinicians with experience in alcohol addiction thoroughly assess all patients [12]. The majority of patients will require psychosocial interventions such as motivational enhancement therapy or cognitive behavioural therapy to manage alcohol dependence. In addition, pharmacological therapy with baclofen should be considered [32]. It is not appropriate to delay interventions for alcohol use disorder until the patient has been discharged, so units that manage alcoholic hepatitis should have direct access to addiction services.

Summary

Alcoholic hepatitis is a life-threatening event, which is superimposed on alcohol-related steatohepatitis. Mortality risk varies considerably with the severity of disease and can be assessed using DF or MELD. The best supportive care, including nutrition, careful fluid management, vigilance for infection and acute kidney injury, make a significant impact on the chances of survival. A small proportion of patients may benefit from corticosteroids, but treatment increases the risk of severe infection. Management of alcohol use disorder should be integrated into the medical management in order to optimise longer-term clinical outcomes

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Impact of alcohol abstinence and diet on alcoholic liver disease progression

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Take-home messages

- Alcohol abstinence remains the cornerstone of alcoholic liver disease management as it improves long-term survival in patients with both compensated and decompensated alcoholic cirrhosis and after an episode of severe acute alcoholic hepatitis.
- Obesity, metabolic syndrome and alcohol consumption have supra-additive impacts on liver-related death. Active management of metabolic syndrome is mandatory in patients with alcoholic liver disease.
- Amounts of alcohol considered permissive could be harmful in patients with metabolic syndrome.
- Lifestyle interventions, including a hypocaloric normoproteic diet and moderate-intensity physical activity to control excessive body weight, improve outcomes in patients with alcoholic and non-alcoholic cirrhosis.

Lifestyle modifications are crucial for the management of alcoholic liver disease (ALD). This strategy is particularly important in early disease stages when most patients are asymptomatic and liver damage is potentially reversible. Besides alcohol abstinence and intense counselling, adequate diet avoiding excessive body weight and cigarette smoking cessation are also essential.

Alcohol abstinence

Sustained alcohol abstinence has been shown to improve outcomes in all stages of ALD and remains the cornerstone of ALD management. Abstinence improves long-term survival in patients with the compensated or decompensated stage of alcoholic cirrhosis [1-7], as well as in patients discharged after severe acute alcoholic hepatitis [8,9]. Improvement after abstinence in Child-Pugh C to Child-Pugh B or A patients was observed within 3 months in 66% of patients [6]. The benefit extends to the patients in the liver transplantation waiting list in which 9% can be delisted due to improvement after abstinence [8]. Furthermore, a reduction in the amount of alcohol ingested improves outcomes and survival compared with persistent excessive drinking, although studies are limited by the difficult nature of quantifying alcohol consumption [3]. It is conceivable that similarly to other forms of chronic liver disease in which the aetiology is treated if patients stop drinking, those with alcoholic cirrhosis can revert to compensated disease after an initial episode of decompensation. Effectively, in patients with alcoholic cirrhosis and oesophageal varices but not bleeding varices, abstinence was found to reduce portal pressure, promote the regression of varices and lower the risk of a first episode of variceal bleeding [11]. Interestingly, after a median follow-up of 45 months no patients showed a HVPG fall below 10 mmHg. Thus, as in other forms of cirrhosis, after treatment of its aetiology, clinically significant portal hypertension may persist after prolonged abstinence leaving patients at an increased risk of decompensation and malignancy. The effects of abstinence are influenced by sex and the presence of other causes of hepatic damage and comorbidities. In fact, the benefits of abstinence are less likely in women with alcoholic hepatitis with or without cirrhosis, and despite abstinence, the disease progresses in roughly 50% [1,12-14].

Diet and body weight reduction

Obesity and metabolic syndrome have synergistic effects when added to excessive alcohol consumption causing liver-related death. In a Scottish prospective cohort study, the excess risk of liver-related death of an increased body mass index was small (1.29 95% CI, 0.70-2.80) compared with heavy alcohol consumption (3.66, 95% CI, 1.74-7.71), but the relative excessive risk due to the interaction was elevated to 9.53 (95% CI, 4.98-18.2) [15]. Excess weight and hyperglycaemia, along with age and female sex, are independent risk factors for fibrosis in ALD [14]. Moreover, metabolic syndrome and its individual components, specifically diabetes mellitus, insulin resistance, and obesity, are independent predictors of both liver-related and overall mortality in ALD [16-18]. The synergistic effect of metabolic syndrome and alcohol on liver damage was further shown in a longitudinal population-based cohort study in which alcohol use within the limits used to define NAFLD emerged as a risk factor for incident liver disease in patients with single metabolic syndrome components [18]. This evidence suggests that metabolic syndrome treatment should improve liver-related outcomes in patients with ALD. Additionally, patients with metabolic syndrome, and specifically with NAFLD, should be warned that drinking even a small amount of alcohol could trigger liver disease progression.

Obesity negatively impacts the course of compensated cirrhosis. Indeed, obesity and metabolic syndrome are as frequent in alcoholic or viral compensated cirrhosis as in the general population and increases the risk of decompensation by 3-fold [19, 20]. These observations support lifestyle interventions, including a personalised hypocaloric normal-protein diet and supervised moderate-intensity physical activity to reduce weight in patients with compensated cirrhosis. A 16-week program of such characteristics was able to reduce portal pressure in patients with compensated cirrhosis, provided that the reduction in body weight was of at least 10% [20]. This evidence supports EASL recommendations for Nutrition in Liver Disease that indicate “a tailored, moderately hypocaloric (-500–800 kcal/d) diet, including adequate protein intake (>1.5 g proteins/kg.ideal BW/d) can be adopted to achieve weight loss without compromising protein stores in obese patients with cirrhosis” [21]. It is important to avoid protein depletion to minimise the risk of exacerbating sarcopenia.

With regards to diet in patients with cirrhosis, a further aspect to consider is the recent observation that a diet rich in fermented milk, vegetables, cereals, coffee, and tea is associated with a greater microbial diversity and an independently lower risk of 90-day hospitalisations in patients with compensated and decompensated cirrhosis of various aetiologies [22].

Other lifestyle modifications

ALD is commonly associated with addictions other than alcohol abuse such as tobacco. Smoking exacerbates the effect of ALD by 3-fold and has a synergistic effect with alcohol in causing, cardiovascular disease and malignancy, including hepatocellular carcinoma [18,23-25]. Hence, efforts should be made to help patients with ALD to give up smoking. In chronic liver disease of any aetiology, including alcohol, increasing daily coffee consumption by 2 cups has been found to nearly half the risk of cirrhosis, liver-related death and hepatocellular carcinoma [26].

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Alcohol Biomarkers in Clinical and Forensic Contexts

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Take-home messages

- Alcohol biomarkers are an important aid in detecting undisclosed alcohol use and identify patients struggling with alcohol use.
- Alcohol biomarkers can be direct or indirect, but direct are preferred for use where clinical decision-making is taken.
- Biomarkers should not be used on their own to make decisions regarding listing or other clinical care, but, where positive, should prompt discussion with the patient.
- Urinary ethyl glucuronide and phosphatidylethanol are validated in advanced ALD and have good sensitivity and specificity.

Alcohol Biomarkers: What are they?

Alcohol biomarkers are moieties found in urine, blood, or hair which indicate, to varying degrees of accuracy, the presence of alcohol consumption over varying lengths of time. Biomarkers fall into two broad categories: indirect and direct. Indirect biomarkers are those that do not directly measure an alcohol metabolite, but rather a metabolic consequence of alcohol use. Macrocytosis, AST and ALT elevations, elevated GGT levels, and % carbohydrate deficient transferrin are examples of indirect biomarkers. Direct biomarkers are breakdown products of alcohol that are found in urine, blood, and hair and provide direct, more specific, evidence of alcohol consumption with greater specificity than indirect biomarkers. Blood alcohol levels, urinary ethyl glucuronide and ethyl sulftate, and phosphatidylethanol are examples of direct alcohol biomarkers.

How good are the different biomarkers?

Indirect Biomarkers. Overall, indirect biomarkers are widely available (with the exception of % carbohydrate deficient transferrin), generally easy to order, but have low specificity for alcohol use.

Gamma glutamyl transferase (GGT) is a widely known and common indirect biomarker of alcohol use. GGT is not found only in the liver but also in kidney, brain, pancreas, spleen, heart and prostate. It is elevated in the presence of heavy alcohol use due to damage to hepatocytes from alcohol or due to increased production from cells under oxidative stress. Like other indirect biomarkers, it is poorly specific for alcohol use and has only moderate sensitivity. False positives are abundant and include non-alcohol related liver disease, smoking, obesity, and diabetes. Medications which induce microsomal enzymes such as anticonvulsants and even NSAIDs can produce elevations of GGT.

The **aminotransferases**, AST and ALT, are enzymes involved in amino acid metabolism and are often elevated in many types of liver cell injury, though the degree of elevation in aminotransferases does not always correlate with the degree of liver injury. Liver enzymes elevations are frequently seen in heavily drinking patients, but lower levels of alcohol use may not produce measurable increases in aminotransferases. A 2:1 AST to ALT ratio is frequently used to signal alcohol-related liver disease, especially in diagnostic criteria for acute alcoholic hepatitis but overall sensitivity of AST and ALT are lower than that of GGT.

Mean corpuscular volume (MCV). MCV is frequently elevated, producing macrocytosis in those with heavy alcohol use as a consequence of direct marrow toxicity of alcohol, inadequate folic acid intake or absorption. While macrocytosis has been linked with folate deficiency, this is relatively rare in alcohol-related cirrhosis and most cases of macrocytosis occur with normal folate levels. Like other indirect biomarkers, macrocytosis is non-specific as MCV can be elevated in many other conditions including other liver disease, bleeding, vitamin deficiencies (B12 or folate), hypothyroidism, and hematologic conditions such as hemolysis, hemoglobinopathies, and bone marrow disorders.

Carbohydrate Deficient Transferrin (%CDT). %CDT is produced as a result of alcohol inhibition of transferrin glycosylation. It is reported as the percentage of CDT per total transferrin and has a half-life of approximately 2-3 weeks. It has lower sensitivity (25-50%) and is inaccurate in more advanced liver disease where false positives even in the absence of drinking can occur. As a result, %CDT is less useful a biomarker of alcohol use in liver disease, but may have more accuracy in the post-transplant period where liver function normalizes.

Direct Biomarkers

Blood alcohol levels (BAL). Blood alcohol levels are widely used, typically in acute settings such as emergency departments or in forensic settings, to determine if alcohol has been ingested. Legal limits of blood alcohol content vary depending upon country. Because of its short half-life, BAL typically can only determine alcohol use in the past 12-24 hours. As such, its utility in clinical care settings outside the emergency department is more limited.

Urinary ethyl glucuronide and **ethyl sulfate**. Urinary ethyl glucuronide (Ueg) and urinary ethyl sulfate (Ues) are direct breakdown products of alcohol produced by direct metabolism from UDP-gluruonyltransferase and UDP-sulfotransferase, respectively. They are excreted in the urine and also found in blood and hair. Ueg/Ues detect alcohol use approximately 3-5 days prior to testing, while hair ethyl glucuronide can detect alcohol use months before and is used predominantly in forensic contexts. False positives and false negatives can occur, but urinary ethyl sulfate does not have these drawback and is thus frequently used as a confirmatory test for Ueg. See Table 1 for test performance. Ueg has been validated in studies including liver disease patients both pre and post-liver transplant. Several studies have shown high specificity (81-90%) in patients with chronic liver disease due to alcohol with reasonable sensitivity (76-89%) depending upon cut-off values (1, 2). Most studies have used a cut-off of 0.5 mg/L. Ueg and Ues are not effected by liver disease but are renally cleared. Therefore, in patients with decreased glomerular filtration rates, these biomarkers can be prolonged. Hair ethyl glucuronide can measure alcohol use months in the past, and is most accurate at detecting chronic heavy alcohol use(3). A rather large chunk of hair is needed for testing, which makes it less practical for the type of frequent monitoring required in clinic settings. As such, it is most commonly used in forensic settings.

Phosphatidylethanol. Phosphatidylethanol (PETH) is a direct biomarker of alcohol use produced when phospholipase D (PLD1 and PLD2) catalyzes the formation of PETH on red blood cells. There are several phospholipids species, but 2 (PETH 16:0/18:1 and PETH 16:0/18:2) make up the majority of those produced and are the most common species targeted in detection assays. The window of detection is 2-3 weeks, though there is potential individual variation in metabolism that may lengthen this detection window . PETH has been tested in ALD patients and at cut-off levels of 20 ng/mL or higher, sensitivity was 73% and specificity was 96% for any alcohol use(4). In a similar study of pre- and post-liver transplant patients using a 20 ng/mL cut-off, sensitivity was 100% and specificity was 96% for any alcohol use. False positives can occur. Recent data has also shown that sensitivity may be higher in those with more advanced liver fibrosis(5).

Wearable Alcohol Sensors

Newer technologies that measure alcohol use via transdermal alcohol vapor detection have been developed. These include wearable devices that detect transdermal alcohol content and are worn either on the ankle or wrist. Only one device (SCRAM®) is commercially available in the US. Others are in laboratory testing. Currently, wearable alcohol sensors are largely used in research populations or in criminal justice systems and have not, to date, been validated in liver disease populations though such work is under way.

Table 1. Biomarker Performance (6, 7).

Test	Source	Detection Time	Sensitivity	Specificity	PPV	NPV
%CDT	Blood	2-3 weeks	21-50%	50-100%	64-100%	86-93%
EtG	Urine	3-5 days	76-89%	93-99%	81-90%	91-99%
EtG	Hair	Months	81-100%	83-98%	68-95%	86-100%
EtS	Urine	3-5 days	82%	86%	70%	93%
PETH	Blood	2-3 weeks	98-100%	66-96%	85%	100%

Why should we use alcohol biomarkers?

Alcohol biomarkers are necessary to aid in detecting underlying alcohol use that patients, very often and very understandably, may be reluctant to disclose. Discordance between patient reports of alcohol use and biomarker results is common. In studies with Ueg and PETH, for example, discordance rates of 20-26% have been reported, including in transplant clinics(8, 9). While biomarkers should never be used to punish patients, they are very useful in helping to detect alcohol use so that a fuller discussion can be had with the patient and clinician about what struggles the patient might be having with alcohol that they did not want to disclose. Maintaining an open, trusting, and nonjudgmental relationship with one's patient is critical to maximal candor and to being able to help a patient through a disclosure of alcohol use, whether during the clinical interview or through discovery by alcohol biomarkers.

How should we use these in clinical contexts?

First, understand that alcohol biomarkers should be used. Alcohol biomarkers are *recommended* for use by major addiction societies, including the American Society of Addiction Medicine and the American Psychiatric Association as well as by the American Association for the Study of Liver Disease and the European Association for the Study of the Liver in their guidance documents regarding ALD. However, they should not be used in order "catch" and punish patients using substances. Biomarkers should be used as an aid to detect alcohol use so that slips or relapses in patients can be found early and patients can be directed to alcohol treatment where needed. In transplant clinics, biomarkers are invaluable in helping teams uncover undisclosed alcohol use, enabling patients struggling with sobriety to be identified and given proper care for their alcohol use disorder. As more and more patients with advanced ALD are being considered for transplant, determining abstinence prior to transplant and ensuring abstinence after transplant are critical goals that alcohol biomarker use facilitates.

Interpretation of biomarkers performance depends on the prevalence of alcohol use in the population under study. For example, a biomarker with 90% sensitivity and 90% specificity will have different positive predictive values in different prevalence settings. In a primary care clinic, with an estimated 10% prevalence of heavy alcohol use, PPV would be 50%, while in an addiction clinic with a higher population prevalence of heavy drinkers, PPV would be 99%. Thus, once would expect more false positives in the primary care clinic and more false negatives in the addiction clinic(10). Knowing the rough prevalence of heavy alcohol use in your clinical population will aid in the interpretation of biomarker results.

In general, interpretation of direct alcohol biomarkers should fall into a "yes/no" rather than "how much" line of interpretation. While longer periods of heavier drinking tend to produce higher levels of all direct alcohol biomarkers, sufficient variation exists in individual metabolism, renal function, and other test characteristics to warrant caution in interpreting biomarkers as direct measures of the amount of drinking that has occurred. In addition, some aspects of biomarkers remain unknown in liver disease patients and are an active area of research (11).

Be aware of false positives and false negatives. While deception and lack of candor in reporting drinking is quite common in both general hepatology and transplant practices, false positives can and do occur with these tests. In addition, alcohol content in common commercial items can also result in positive tests. For example, the alcohol content in some common cough and cold medications is 20-25% and for mouthwashes can be 15-25%. Hand sanitizer does contain high alcohol levels but intermittent topical application in the absence of ingestion does not produce measurable levels of alcohol biomarkers.

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Assessment, psychosocial intervention and pharmacological treatment of alcohol use disorder in patients with alcohol-associated liver disease

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Take-home message

- The most effective recommendation for patients with alcohol-associated liver disease (ALD) is total alcohol abstinence.
- ALD should be considered as a dual pathology, including both a liver and an addiction disease. For this reason, these patients should be treated by a team of specialists, including hepatologists and providers (i.e. psychiatrist and psychologist) with mandatory expertise in addiction medicine and integrated into the Liver Unit.
- Biological markers can be used in clinical practice to monitor alcohol consumption during follow-up.
- The most effective management strategy to increase abstinence and to prevent relapse is the combination of psychosocial interventions and pharmacological therapy.
- Among psychosocial intervention, in patients with ALD, an integrated combination of psychotherapy with cognitive behavioural therapy, motivational enhancement therapy and comprehensive medical care is effective. Among medication useful to promote total alcohol abstinence and to prevent relapse, baclofen seems to be safe and effective in patients with ALD.

How would you follow-up this type of patient for the management of alcohol use disorder?

The primary effective strategy for patients affected by alcohol use disorder (AUD) and alcohol-associated liver disease (ALD) is represented by total alcohol abstinence, because medical and surgical (i.e. liver transplantation) interventions for ALD and its complications have limited success when drinking continues [1]. Medical recommendations and motivational advice offered by physicians may not be sufficient to induce total alcohol abstinence and/or to prevent relapse. Specific medications combined with psychosocial interventions seems to be the most effective treatment to achieve these outcomes [1]. However, since AUD is a chronic and relapsing disease, AUD patients need a regular and strict follow-up, particularly those with ALD.

Follow-up of AUD patients consist of the following points, which would be assessed at each outpatient/inpatient control:

- Evaluation of alcohol craving through interview and specific scales (i.e. visual analogue scale, obsessive-compulsive drinking scale, Penn Alcohol Craving Scale, etc.).

- Monitoring of pharmacological treatment used to prevent relapse (anti-craving drugs) and/or drugs used to reduce alcohol withdrawal syndrome, to evaluate the most appropriate medication/dose and to assess both the efficacy and the possible onset of side effects. The use of Brief Behavioural Compliance Enhancement Treatment (BBCET), a manual-driven, low-intensity supportive program, could represent an effective instrument to enhance a patient's compliance with medications.
- Assessment of risks factors for relapse.
- Monitoring of alcohol abstinence through biological markers of alcohol abuse.
- Monitoring of liver disease (for example, abdominal ultrasound and liver function test) and extrahepatic alcohol-related disease.

To date, AUD patients are mainly evaluated and managed by a psychiatrist, social workers and psychologist. However, at present, it is still not well-established the most appropriate specialist who should follow-up AUD patients with ALD (i.e. internist, hepatologist, psychiatrist). AUD patients affected by ALD represent a special population. In particular, ALD should be considered as a dual pathology, including both a liver and an addiction disease. For this reason, these patients should be treated and followed by a team of specialists, including hepatologist and providers (i.e. psychiatrist and psychologist) with mandatory expertise in addiction medicine and integrated into the Liver Unit. In particular, integrating alcohol interventions with medical care, ALD patients who would not accept an external consultant for alcoholism treatment could be engaged in the Liver Unit as they are usually willing to return for medical appointments. The usefulness of this integrated model was first reported in AUD patients with end-stage alcoholic liver disease who underwent liver transplantation [2-4]. A lower rate of relapse and mortality after liver transplantation have been reported in patients following this integrated model [2-4].

How sure can we be about abstinence? What is the scientific evidence for different methods?

Physicians should evaluate abstinence during an interview with an AUD patient and its family members at each outpatient control. Quantity-frequency questionnaires and retrospective diaries, such as Time-line Follow Back (TLFB) can be used to estimate an individuals' alcohol consumption.

Biological markers can be also used in clinical practice to monitor alcohol consumption during follow-up (for review see [5-6]). The most routinely used biological markers are gamma-glutamyltransferase (GGT), mean cellular volume (MCV), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and AST/ALT ratio (DeRitis ratio). ALT and AST are less sensitive than GGT in detecting excessive alcohol consumption. When aminotransferases are elevated, if the AST/ALT ratio is greater than 2.0, 90% of cases are due to alcohol abuse. An increase of 40% or more in AST level and 20% or more in ALT value have been reported to be suggestive of relapse to drinking in AUD patients. This was true even if the marker remained within the reference range.

However, these biological markers are likely to lose their utility in patients with liver disease. Carbohydrate-deficient transferrin (CDT) is less affected by false-positive results due to liver disease. Moreover, CDT is more specific for heavy alcohol consumption (about 4-5 drinks per days) and it remains elevated for about two weeks after drinking. Its main limitation is its relatively low sensitivity. However, a combination of CDT, GGT, and MCV will further improve the diagnostic value. In particular, the combination of CDT and GGT increases the sensitivity for identifying alcohol consumption during follow-up.

Recently, several studies suggested a role of ethylglucuronide as a biomarker for alcohol use detection. Ethylglucuronide is a direct metabolite of alcohol, that can be measurable in tissue, blood, hair, and,

most commonly, urine. The detection time ranges from hours to 4 or 5 days. However, traditional biomarkers for alcohol use in blood and urine allow only limited detection windows (hours to days), while ethylglucuronide in the hair can detect long-term alcohol consumption.

What is the most effective treatment for AUD patients with ALD?

The most effective management strategy to increase abstinence and to prevent relapse in AUD is the combination of psychosocial interventions and pharmacological therapy.

The most frequently used psychosocial interventions for AUD treatment include twelve-step facilitation therapy, motivational enhancement therapy (MET) and cognitive behavioural therapy (CBT). They represent the backbone of AUD treatment. However, a recent meta-analysis showed that a single psychosocial intervention is useful only for AUD patients without ALD, while in AUD patients affected by ALD only, an integrated combination of psychotherapy with CBT, MET and comprehensive medical care is effective [7]. These data underline that AUD patients with ALD are a special population, which need intensive behavioural approaches integrated within medical care.

Despite progress in pharmacological treatments of AUD, pharmacotherapy is still underutilised in clinical practice. A few drugs are approved for AUD (Table 1), such as disulfiram, naltrexone, nalmefene and acamprosate, although the exact panel of approved drugs may vary across countries [1,8]. In some EU countries, sodium oxybate (Italy and Austria) and baclofen (France) have been approved. Other drugs have been proposed and tested in this population of patients, based on the growing knowledge of the neurobiology of AUD [8]. All these medications are effective to reduce alcohol craving, to improve abstinence rate and to reduce relapse. However, their use is limited to AUD patients without advanced ALD disease and/or with early stage of liver disease (and in this case liver function must be monitored) because most of these medications have not been tested in AUD patients with advanced ALD. In particular, to date, only baclofen has been formally evaluated in randomised clinical trials in AUD patients affected by liver cirrhosis [9]. The safety and efficacy of baclofen in prospective cohort studies are discussed in Mosoni *et al.* review [10].

Table 1. Anti-craving drugs approved for the treatment of alcohol use disorder adapted from [11].

Anti-craving drug	Approved Country	Mechanism of action	Dose	Available data on efficacy and safety in AUD patients with ALD
ACAMPROSATE	U.S. and EU	Glutamate receptor modulation	1.3 g/day (weight <60 kg) and 2 g/day (weight >60 kg) in three daily administrations	Only one-day administration study in Child-Pugh A-B liver cirrhosis*
BACLOFEN	France	GABA-B agonist	10 mg t.i.d. in patients with liver disease	In Child-Pugh A-C liver cirrhosis and in AAH (see ref [10])

Anti-craving drug	Approved Country	Mechanism of action	Dose	Available data on efficacy and safety in AUD patients with ALD
DISULFIRAM	U.S. and EU	Inhibitor of aldehyde dehydrogenase	800-1200 mg/ day for 3-4 days, then 400 mg/day until the 7th day, after 200 mg/day	NO
NALMEFENE	EU	selective opioid receptor ligand with antagonist activity at the μ and δ receptors and partial agonist activity at the κ receptor	18 mg/day "on demand"	NO
NALTREXONE	U.S. and EU	Opiate antagonist with the highest affintiy for the μ receptor	50-100 mg/day	NO
SODIUM OXIBATE (GHB)	Italy and Austria	GABA-B/ GHB receptor agonist	50 mg/kg divided into three or six daily administrations	Only one case report**

*Delgrange, T., *et al.* Effect of acute administration of acamprosate on the risk of encephalopathy and on arterial pressure in patients with alcoholic cirrhosis. Gastroenterol Clin Biol 1992; 16: 687–91

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SESSION 5

DRUGS AND XENOBIOTIC MISUSE AND LIVER TOXICITY

WEDNESDAY 23 JUNE
15H30 - 16H30

Herbal remedies to improve well-being: Healthy for the liver?

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Take-home messages

- Herbal and dietary supplements (HDS) are used commonly and for many purposes worldwide.
- Regulations vary among countries; consumer safety is not assured.
- Little data exist to support a therapeutic benefit from HDS.
- The diagnosis of HDS-associated liver injury requires fastidious exclusion of other causes using a careful medical history and exclusionary lab testing.
- Some HDS can cause liver injury that is more serious than injury caused by prescribed medications.
- In the future, chemical analysis of ingredients and patient genetic testing may play a role in establishing a diagnosis.

Background & introduction

The acronym "HDS", derived from Herbal and Dietary Supplements, encompasses many products that arise from traditional medicine, naturopathic beliefs, and long-standing safe use of naturally occurring ingredients with suspected health benefits. In the United States (U.S.), the more accurate designation of such compounds is simply Dietary Supplements (DS), which includes vitamins, minerals, herbs, and amino acids (https://ods.od.nih.gov/About/DSHEA_Wording.aspx: accessed 31/12/2019). Although regulation in the U.S. exists to control the sale and distribution of DS, the focus of regulation is to guide manufacturers on purity, strength, and composition; not safety. Some countries have regulatory guidelines that place greater emphasis on safety, building upon many years of safe use of ingredients. Over time, commercialisation of DS has led to the development and marketing of complex mixtures of naturally occurring compounds or chemically synthesised products. In most situations, DS ingredients, either alone or in various combinations, have not been tested for safety in humans.

In general, drug-induced liver injury, or DILI, is recognised by elevated liver chemistries: cholestatic injury is reflected in a predominant elevation of alkaline phosphatase; hepatocellular injury by a predominant elevation of the alanine aminotransferase and/or the aspartate aminotransferase; and mixed pattern with both hepatocellular and cholestatic enzymes being elevated simultaneously. Severe DILI should be regarded as that which is accompanied by symptoms and signs of hepatic insufficiency (cognitive dysfunction, severe constitutional symptoms, coagulopathy).

It remains useful to consider the types of DILI as direct (intrinsic, predictable) or idiosyncratic (unpredictable). In the former, cell death occurs from direct injury leading to hepatocyte necrosis or apoptosis. Most idiosyncratic liver injury occurs through complex interplays between an agent's metabolites and the host immune system or metabolic processes.

There are several purported risk factors for developing DILI. These factors include age, gender, race, pregnancy, alcohol use, underlying chronic liver disease, dose and metabolic pathway. However, a person's genetic constitution is emerging as one of the most important potential risks for the development of hepatotoxicity; the strong association of flucloxacillin injury with HLA-B*5701 genotype

drew attention to this fact [1]. Subsequent work by Lucena *et al.* expanded on this issue to include the association of multiple HLA class I and II alleles with amoxicillin-clavulanate injury [2]. More recently, the association of the HLA-B*35:01 allele with liver injury due to *Polygonum multiflorum* raises our awareness even further that genetics play a pivotal role in predisposing people to injury from exogenous agents, even natural compounds [3].

Frequency of HDS use

Because of the free-market proliferation of DS manufacturers, and the lack of costly safety testing requirements, DS use in most countries is very common. In the U.S. alone, it is estimated that over half of all households use DS [4]. Exact statistics are unknown, as the sale of products from all sources is not monitored. The most common users of DS are adult females in their middle years. The best surrogate marker indicating rising herbal dietary supplement use in the USA is the increasing retail sales which, in 2018, exceeded $8.8 billion (http://cms.herbalgram.org/press/2019/2018HerbMarketReport.html). Globally, the market is projected by at least one source to exceed $140 billion by 2024 (https://www.strategyr.com/market-report-herbal-supplements-and-remedies-forecasts-global-industry-analysts-inc.asp: accessed 31/12/2019).

Purposes for supplement use

Consumers use DS for many reasons. These include self-empowerment, disease self-management, perceived benefits of HDS that may complement or replace conventional medical therapies, and disillusionment with conventional treatments. Whatever the motivations for use, consumers do not uniformly inform their providers of HDS use, and providers may not uniformly ask.

HDS studied for liver health

Plant products and other naturally occurring compounds have biological activity. In *in vitro* systems, some ingredients have been shown to reduce liver enzymes, and diminish hepatic inflammation or fibrosis. As regards to human studies, a search of ClinicanTrials.gov for the terms “liver” and “herbal” as of November 2019 yielded 77 active or completed studies. These involved naturally occurring/herbal ingredients being tested in humans with or at risk for liver disease, for either safety or effectiveness.

Many clinical studies have been done to test the effectiveness of HDS for various disorders, but fewer in patients with liver disease. Such trials for liver patients are challenging to conduct, given the concern for safety, and the lack of efficacy markers to prove benefit. Table 1 below illustrates ingredients with purported health benefits for liver disease.

Table 1. Supplements studied for the benefits in liver diseases.

	Proposed mechanism	Target disease	Outcomes	Best evidence	References
Betaine	Methyl donor	NASH	No improvement in LFTs, histology	RCT, fair quality	Abdelmalek 2009
Carniture	Fatty acid transport/ oxidation	NASH	Improved LFTs Reduced NAS	RCT, fair quality	Malaguamera 2010 Somi 2014
Glycyrrhizin	Stimulates INF-α production	HCV, HCC	Improved LFTs No effect on HCV RNA	RCT, good quality	Van Rossum 1999 Kumada 2002
Omega-3	Fatty acid oxidation	NASH	Improved LFTs	Meta-analysis	Parker 2012
Resveratrol	Improved insulin sensitivity	NASH	Improved LFTs Improved steatosis Conflicting data	RCT, fair quality	Poulsen 2013 Chachay 2014 Fagihzadeh 2015
Vitamin E	Antioxidant	NASH in non-diabetics	Improved NAS Conflicting data	Improved NAS Conflicting data	Sanyal 2010 Lavine 2011 Sato 2015
Silymarin	Antioxidant	HCV, NASH	No effect on HCV RNA No effect on NASH histology	No effect on HCV RNA No effect on NASH histology	Fried 2012 Navarro 2019

NASH, non-alcoholic steatohepatitis; NAS, NALFD activity score; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; LFTs, liver function tests; RCT, randomised controlled trials.

Frequency of herbal dietary supplement-induced liver injury

In Western countries, liver injury from all agents is uncommon, ranging from 3 cases per 100,000 residents in the U.S. State of Delaware [5] to 19 per 100,000 in Iceland [6]. In China, where investigators have access to huge databases of patients with liver injury, an incidence of 24 per 100,000 residents was found in a retrospective study [7]. With regards to DILI attributed to HDS, approximately 20% of DILI cases were attributed to supplements in the U.S. Drug-Induced Liver Injury Network (DILIN) [8]. The proportion of such cases attributed to HDS in other countries has been reported to range from as low as 1.3% up to 73%, as nicely summarised by Andrade *et al.* [9] (see required reading).

HDS linked to harm

The U.S. DILIN is just one among several robust networks focused on understanding, treating, and mitigating liver injury from drugs and HDS. A detailed look at the DILIN data shows that about 20% of all case of DILI within the network were due to DS, divided into bodybuilding or performance-enhancing products and other, non-building or non-performance-enhancing products (such as weight loss products) [8]. Typically, HDS implicated in liver injury today tend to be multi-ingredient products, rather than single herbal agents; 63% of HDS-induced liver injury cases in the Spanish DILI network, for example, were due to products with multiple ingredients [10]. Descriptions from both the DILIN and the Spanish Networks reveal that most liver injury, particularly when due to non-bodybuilding products, presents with a predominantly hepatocellular pattern of injury, and more commonly occurs in women.

Many different dietary supplement ingredients have been linked to liver injury. However, there are a few, which have emerged more frequently in the literature, and selected agents, ingredients and associated commercial products are listed in Table 2 below; the reader is directed to the suggested readings for more details and source references.

One of the most compelling studies linking liver injury to a natural product comes from Yu and colleagues [11]; in the context of a randomised trial to study green tea extract as a cancer preventative agent in women, it was found to cause enzyme elevations in exposed participants that improved upon withdrawal and recurred upon re-exposure.

Table 2. Selected agents, ingredients and associated commercial products of dietary supplements linked to liver injury.

Agent/ingredient	Example HDS and commercial products	Reported patterns of injury & presentations
Alkaloids/alkylating agents	Aloe vera Comfrey Greater celandine Groundsel Heliotropium Ma Huang Skull cap/MOVE FREE Valerian	Sinusoidal obstruction syndrome Hepatocellular Cholestatic Severe acute hepatitis, necrosis Fibrosis/cirrhosis
Anabolic steroids	Appearance and performance enhancement products	Early hepatocellular, prolonged cholestasis, renal dysfunction
Anthraquinones	Noni Juice Senna	Hepatocellular Cholestatic
Catechins	Green Tea Extract HYDROXYCUT HERBALIFE SLIMQUICK	Hepatocellular

Agent/ingredient	Example HDS and commercial products	Reported patterns of injury & presentations
Creosote	Chaparral	Cholestatic, hepatocellular
Garcinia cambogia		Hepatocellular Severe acute hepatitis
Kavalactones	Kava Kava	Cholestatic, hepatocellular Severe acute hepatitis
Mitragyna speciosa	KRATOM	Cholestatic, mixed

Diagnosis & causality assessment

The lack of a diagnostic test for liver injury due to HDS means that a careful medical history forms the foundation for a confident diagnosis. Most important is the elicitation of a complete history of supplement use, and documentation of which supplement(s) was used prior to the onset of injury.

Liver injury from HDS can appear to be no different than that due to other insults such as viruses and immune injury. Thus, exclusionary testing is important. Specifically, this approach must exclude viral hepatitis (A through to E), as well as immune injury, alcohol, anatomical/vascular insult, and flare of metabolic and inherited disease, as illustrated in the Figure 1. But even with this careful approach, there are special considerations, unique to HDS-associated liver injury, as discussed below.

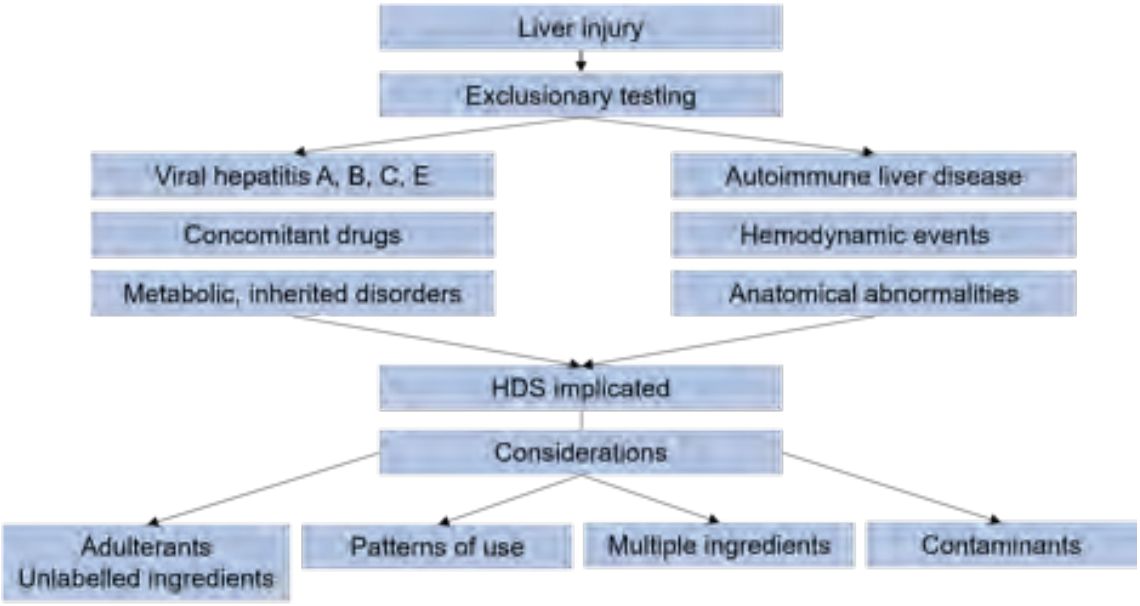

Figure 1. Diagnostic approach to liver injury from HDS.

Combining an accurate medical history with exclusionary lab testing is facilitated by structured causality assessment. There are several such approaches that have been applied to liver injury from HDS; most commonly applied are the Roussel Uclaf Causality Assessment Method (RUCAM), DILIN Expert opinion method, and Maria Victorino Scale. Each scale offers the user a degree of confidence in attributing injury to a specific agent; drug or dietary supplement. It is important to note that all approaches to causality assessment are limited by some of the nuances unique to HDS-associated

injury. First, many supplements on the market are complex mixtures and rarely have their ingredients been tested either alone or in combination with other ingredients for safety. Thus, attributing injury to a specific supplement may be facilitated by a causality assessment scale, but pinpointing the precise ingredient(s) responsible for injury is extraordinarily difficult. Second, given rather permissive regulation of HDS as regards to safety, it is not uncommon for HDS to contain unlabelled ingredients or contaminants. Finally, the conditions of use may have some impact on the development of liver injury; for example, overuse, use with alcohol, or under extreme fasting conditions. Absent any conventional approach to recommend the use of HDS, the best and safest way to consume supplements is unknown, but this information is not captured by current scales.

Ultimately, it is left to the judgement of the clinician to attribute injury to a specific supplement based on a complete medical history and application of diagnostic testing in the context of a structured approach. The degree of confidence when multiple supplements or multi-ingredient supplements are consumed is highly dependent upon the clinician's experience and what is reported in the medical literature. Although speculated upon, there remains no clinically useful biomarkers for DILI other than conventional lab parameters [12].

Analysis of HDS can be performed for chemical components and DNA composition. The former is applied commonly to HDS using High-Performance Liquid Chromatography-Mass Spectrometry. This approach is particularly valuable to validate the composition of HDS for regulatory and research purposes. Testing of supplements collected from patients enrolled into the DILIN led to the striking observation that about 50% of all supplements linked to harm had a chemical composition that was not accurately represented on the label [13].

The use of chemical (or DNA) analysis for something more than chemical verification is a complex endeavour. Linking injury to a specific ingredient would require isolation of the ingredient from the parent compound (supplement) and performing either toxicity testing or re-exposing the patient to observe the effect. In either case, a negative test would not exclude the agent as being responsible for injury as interactions of a suspected ingredient with other components of the supplement could not be excluded.

Prognosis, management, & outcome

Simply recognising that a supplement or other non-prescribed natural product may be the cause of injury and stopping the agent are the most important initial interventions. Conventional management entails a keen eye to identify the patient with severe liver dysfunction (jaundice, rising INR, encephalopathy) and referral to a transplant centre when suspected. Outcomes of liver injury from HDS are different and in some cases more severe than injury due to drugs. For example, liver injury from non-bodybuilding products (such as weight loss supplements) is more likely to lead to transplantation than injury from drugs [8]. Similarly, Hillman *et al.* [14] found that when due to supplements, acute liver injury and acute liver failure is associated with higher transplant rates and lower transplant-free survival than DILI due to prescription medication.

There are no specific antidotes for liver injury from HDS. The use of steroids remains at the discretion of the clinician when there is a suspicion of immune activation. Additionally, for patients with non-acetaminophen acute liver failure, the use of N-acetylcysteine is prudent and evidence-based.

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Additional reading

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Zheng E, Sandhu N, Navarro V. Drug-induced liver injury secondary to herbal and dietary supplements. Clin Liver Dis 2020;24:141-155.

Cathinone and derivatives: mechanisms of liver toxicity, clinical consequences and preventive interventions

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Take-home messages

- The use of cathinone and derivatives is increasing with the wide access to illicit drugs on the web.
- The spectrum of clinical consequences is very large, driven by the mitochondrial toxicity of the drugs, as well as their serotonin-like effects. It also includes indirect effects due to their route of administration (drivers of HIV and hepatitis C epidemics).
- Because there is no efficient substitution therapy, the management of cathinone addiction relies on prevention, screening and offer of psychosocial support and behavioural psychotherapy.

Introduction

Cathinone is a phenylalkylamine belonging to the family of amphetamines. It is derived from the leaves of the psychostimulant plant called Khat (*Catha edulis*) used for centuries in the Arabic Peninsula and Eastern Africa for its reported properties against asthenia and for improving concentration and libido. The use of Khat is culturally accepted in the communities living in these areas, as is the use of cocaine-derived Coca leaves (*Catha erythroxylaceae*), shewed in the Andes Mountains.

The first synthetic production of a cathinone-derived component, ephedrone, took place in the 1960s and this drug has been commercialised as an antidepressant in the Soviet Union and a neurostimulant in the United States. Very quickly, misuse and abuse of ephedrome have been reported [1] and clandestine laboratories have started to produce derivates of cathinone classified as “new psychoactive substances” or NPS, which possess a close resemblance to the empathogenic amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA). The aggressive marketing of cathinone-derived drugs has started with the wide expansion of dark business on the web in the 2010s. Because the synthesis of derivative agents is very easy from a chemical point of view, new derivatives are constantly hitting the (dark) market with ever-changing names, which makes it difficult to implement a surveillance system of those drugs.

Alongside their own properties of inducing organ damages, which pathophysiological pathways will be described hereunder, the cathinone derivatives have been shown to be associated with numerous cases of hepatitis C, HIV and acute psychiatric disorders (with fatal cases regularly reported) acquired through the practice of slam (injection or intra-anal absorption of drugs in the context of sexual activities with multiple partners) [2,3]. That is why it can be considered as an emerging public health threat.

Physiopathology of drug-induced liver injury due to cathinones

All cathinones alter behaviour by increasing monoamine transmission in the central nervous system sites of action (i.e., dopamine, noradrenaline, serotonin) through amphetamine-like release facilitation

and cocaine-like reuptake inhibition mechanisms. Because of the euphoria and hallucination effects induced by synthetic cathinone drugs, they are dangerously addictive. However, those effects are fading with time and cause a compulsory demand for more and more consumption.

Apart from those neurological effects, drug-induced liver injuries have been experienced first artificially in rats and reported *in vivo* in individuals, although this is quite a rare event.

To better understand the mechanism underlying the risk of hepatotoxicity associated with cathinones derivatives use, an *in vitro* model based on HepG2 cells has been built to study the effect of 5 drugs (cathinone designer drugs 3,4-methylenedioxypyrovalerone [MDPV], 4-methylmethcathinone [4-MMC; mephedrone], 4-methoxymethcathinone [4-MeOMC; methedrone], 3,4- methylenedioxymethcathinone [βk-MDMA; methylone], and naphthylpyrovalerone [naphyrone] and bupropion, used as an antidepressant and smoking cessation agent) [4]. Globally, those agents are acting as mitochondrial toxicants impairing the function of the electron transport chain and depleting cellular ATP. But given the relatively low number of cases of severe liver damages reported, other factors such as genetic susceptibility to liver injury may render individuals more sensitive to the drug's effects.

Clinical consequences of the use of cathinones

The clinical spectrum of morbidities associated with the use of cathinone and its synthetic derivatives are twofold; either due to a direct effect of the drug on organs such as the brain, liver, kidney or heart; or due to an indirect effect of the addiction induced by the drugs and its correlated infectious and psychosocial consequences.

Clinical manifestations may be related to the sympathomimetic toxicity of the drugs: hypertension, tachycardia, cardiac, kidney and liver failure, rhabdomyolysis, electrolyte imbalance, metabolic toxicity, paradoxical hypoglycaemia and cerebral oedema. The classic serotonin syndrome may also be experienced with hyperthermia and psychotic disorders. A high number of suicidal ideations and suicide attempts (hanging) are also reported [5]. However, alongside those acute clinical manifestations, sub-acute effects may also be experienced, particularly affecting sleep, mood and general physical condition (insomnia, depression, physical pain and fatigue) [6].

Because of the route of administration, individuals are also exposed to the risk of acquiring blood-transmitted infections such as HIV and hepatitis C. Recently, two epidemics of HIV infection related to the injection of cathinones have been observed, one in men having sex with men in Israel [7] and one in homeless people in Ireland [2]. Moreover, most epidemics of acute hepatitis C reported throughout Europe since the 2010s have been proved to be associated with the practice of slam and chemsex. Furthermore, suboptimal adherence to HIV pre-exposure prophylaxis (PREP) may be an indirect consequence of binge use of cathinones and its subsequent mental impact, consequently increasing the risk of acquiring HIV (as shown in the IPREX trial that first demonstrated the effect of PREP in males) [3].

Finally, a large number of individuals taking cathinone derivatives on a regular basis do report dependence and experience tolerance with withdrawing syndromes that lead to binge use with a compulsive behaviour, where there is no time left for activities other than seeking drugs and injecting. De-socialising, loss of job, progressive distancing with family and friends are strong markers of excessive use of new psychoactive substances.

Harm reduction-based interventions in the context of cathinones use

Because cathinone and their derivatives are often taken in combination with other traditional and new psychoactive substances (cocaine, cannabis, heroin, ecstasy, etc.), it is very difficult to design a unique harm reduction intervention.

Users may present to the emergency room with serotonin syndrome and not report the use of any drug. Most of the synthetic drugs are impossible to identify when using the regular drug screening battery. Therefore, educating physicians working in the emergency and intensive care units in detecting situations where individuals (often young and males, but not always) may have symptoms related to the consumption of cathinone derivatives is paramount.

In individuals susceptible to use drugs, screening for abuse of drugs is the first step in the management plan. Very few tools exist that efficiently detect the need for psychosocial and addiction support. The screening, brief intervention and referral to treatment (SBIRT) is an evidence-based practice used to identify, reduce and prevent problematic use and abuse of, and dependence on, tobacco, alcohol and psychoactive substances. A French team has applied this intervention with some success in a cohort of men having sex with men and infected with HIV [8].

Because there is no substitution therapy active in the context of cathinone dependence (unlike methadone or buprenorphine for heroine), behavioural psychotherapy and psychosocial support have to be provided in the setting of addiction centres when possible.

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The risk for the liver of bodybuilding and sport performance products

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Take-home messages

- Consumption of illegal bodybuilding and sport performance products containing androgenic anabolic steroids, which have several potential deleterious effects, including an array of liver lesions is becoming a serious health issue.
- Androgenic anabolic steroids consistently induce a phenotype of liver damage characterised by very high bilirubin with low transaminase and alkaline phosphatase levels.
- Liver biopsy typically shows cholestasis and mild lobular inflammation, not necessary for the diagnosis of this entity.
- Jaundice and pruritus can be protracted and severe, and despite the cases frequently meeting Hy's law, no fatalities have been reported.
- Acute kidney injury and renal failure can occur particularly in cholestatic damage with very high bilirubin levels, because of bile cast nephropathy, but androgenic anabolic steroids can also induce glomerular damage in the absence of cholestasis.
- To decrease the exposure to anabolic steroids the adoption of strict regulatory and educational measures is paramount.

Herbal and dietary supplements (HDS) used for "improving and maintaining health" represent a profitable market in Western countries. Among these, bodybuilding and sport performance products are widely used nowadays. They include dietetic supplements containing different, apparently non-hazardous for the liver, ingredients such as serum proteins (whey), branched-chain amino acids, and/or creatine. However, 12-58% of supplements taken by bodybuilders and athletes contain unlabelled substances such as androgenic anabolic steroids (AAS) [1]. It has been estimated that among Americans aged 13 to 50 years, 2.9–4.0 million have used AAS [2]. In United Kingdom it has been estimated that 0.2% of people 16 to 59-year-old uses AAS [3].

Although illegal in most countries and banned by elite sports bodies, these products can be easily purchased in different points of sale such as fitness shops, gyms, through personal trainers or via the internet. The fact that in some countries import/export of AAS is permitted if they are intended for personal use could favours the growing distribution of these products [3]. Analysis of 24 bodybuilding products sold in fitness shops in the United Kingdom showed that 23 had AAS, 12 of them were already withdrawn from the market, and 16 were mislabelled [4]. More recently, the Drug-Induced Liver Injury Network (DILIN) from the United States, analysed 272 HDS and detected mislabelling in more than half of these. Products for performance-enhancing and weight loss were the most frequently mislabelled [5].

AAS are synthetic testosterone derivatives with a greater anabolic and lower androgenic effect. The specific medical indications of these drugs are the treatment of hypogonadism, breast cancer, aplastic anaemia, wasting and hereditary angioneurotic oedema. In sport and bodybuilding settings,

the pursued effects are an increase in muscle size and strength and improved physical performance. Moreover, these drugs are also used for appearance enhancement and psychological purposes.

The chemical derivatives containing 17-alpha-alkylation, as methyltestosterone, methandrostenolone, oximetolone, oxandrolone, or stanozolol, enable oral intake, although with greater risk of liver toxicity, presumably at least in part because they are resistant to inactivation through first-pass hepatic metabolism. In addition, these products can be detrimental for other organs such as the kidney, heart, and central nervous and reproductive systems.

The DILIN and the Spanish DILI Registry have recently highlighted the increasing prevalence of DILI due to HDS, including bodybuilding and sport enhancing products [6,7]. Growing prevalence of DILI due to HDS has been described by the DILIN study group, where liver injury from HDS has been increasing over time from 7% to 20% in 10 years (p <0.001), with bodybuilding products related DILI cases accounting for the higher increase [6]. A similar trend has been observed in the Spanish DILI Registry from 1994 to 2016. The yearly proportion of HDS DILI was 1.5% in 1998 and increased steadily to 6% of the enrolled cases from 2010 to 2013 and from 2014 to 2016. The AAS-related DILI cases had a more dramatic increase in recent years, representing 15% of the cases identified from 2014 to 2016 [7].

Overall, more than 150 cases of bodybuilding-induced DILI have been described [8], which included a variety of AAS such as androstenedione, dehydroepiandrosterone, desoxymethyltestosterone, maasdrol, methasterone, methylepithiostanol, stanozolol or superdrol (Table 1).

Clinical and pathological phenotypes of liver disease associated with the use of bodybuilding products

Anabolic androgenic steroids are associated with an array of clinical and pathological types of liver injury. The most serious complication of AAS use, which typically occurs in patients on long-term therapy for aplastic anaemia or hypogonadism, but occasionally in athletes or bodybuilders, is the development of hepatic tumours, either adenoma or hepatocellular carcinoma. (LiverTox https://www.ncbi.nlm.nih.gov/books/NBK548931/).

Other rare hepatic complications of AAS seen in the same exposure setting are vascular lesions, such as peliosis hepatis and nodular regenerative hyperplasia. These vascular hepatic lesions can be asymptomatic, but occasionally peliosis hepatis can lead to liver rupture with haemorrhagic shock. (LiverTox https://www.ncbi.nlm.nih.gov/books/NBK548931/).

Nevertheless, the typical signature of 17-alpha-alkylated derivatives induced liver injury is acute protracted cholestasis with marked jaundice. The Spanish and Latin-American DILI Registries described such distinct phenotype in 25 cases of AAS hepatotoxicity. All patients included in this study were young men with a median age of 32 years old, 60% had a hepatocellular type of liver injury, 92% were jaundiced, the median latency period was of more than a month with a de-challenge median period of 151 days, and 68% required hospitalisation. No fatal or chronic cases were described. The AAS involved in these cases were stanozolol (68%), metilepitiostanol (28%) and mestaterone (4%). In comparison to DILI caused by other drug classes or to no bodybuilding herbal products, these patients had higher peaks of bilirubin, lower transaminase and alkaline phosphatase (AP) levels [9].

Table 1. Publications reporting cases of AAS-induced liver injury, clinical-pathological phenotypes and main identified constituents (modified from [8]).

Dietary supplement	Citations	Number of cases	Constituents	Marketed properties	Type of liver injury	Pathogenesis	Regulatory measures EMA or FDA
Androgenic Anabolic steroids	Kafrouni *et al.* 2007	2	Androstenedione	Bodybuilding, improving fitness and exercise performance	Hepatocellular hepatitis Cholestatic hepatitis	17 alpha alkylated AAS	Episdrol
Celtic	Sánchez Osorio M *et al.* 2008	1	Dehydroepiandrosterone		Hepatocellular adenoma Hepatocellular carcinoma	Inhibition of biliary transporter proteins	Epistane
Dragon	Shah *et al.* 2008	5	Desoxymethyltestosterone		Peliosis hepatitis	Lysosomal and mitochondrial degeneration, oxidative stress	Trim fast
Epidrol	Krishnan *et al.* 2009	3	Maasdrol		Focal nodular regenerative hyperplasia		Uprising 2.0 (Superdrol)
Epistane	Singh *et al.* 2009	3					
Trim fast	Wingert N*et al.* 2010	1	Methylepithiostanol				
No explode	Avelar-Escobar *et al.* 2012	1	Stanozolol				
Uprising 2.0	Timcheh-Hariri A *et al.* 2012	20					

Dietary supplement	Citations	Number of cases	Constituents	Marketed properties	Type of liver injury	Pathogenesis	Regulatory measures EMA or FDA
(Superdrol)	El Sherrif *et al.* 2013	2		Bodybuilding, improving fitness and exercise performance			
	Vilella AL *et al.* 2013	1					
	Martin DJ *et al.* 2013	12					
	Navarro *et al.* 2014	45					
	Luciano RL *et al.* 2014	1					
	Robles-Diaz *et al.* 2015	25					
	Brazeau MJ *et al.* 2015	1					
	Schwingel *et al.* 2015	23					
	El Rahi C *et al.* 2015	1					

More recently, the DILIN experience with bodybuilding supplements-related DILI comprising a series of 44 cases has been published [10]. All cases were also young men, with a median age of 31 years old. All patients had jaundice with median bilirubin levels of 9.8 mg/dl and 84% of cases with pruritus. The most frequent type of liver injury was hepatocellular (40%), followed by mixed (31%) and cholestatic (29%) damage. After withdrawal of the culprit product, an initial reduction of alanine aminotransferase (ALT) with an increase of bilirubin and AP was observed in these cases and bilirubin went down slowly (Figure 1). This study provided a compelling histological description of liver histological lesions induced by AAS as 22 patients underwent a liver biopsy and an experienced pathologist centrally revised histological specimens (Figure 1). The most common histopathological pattern was mixed hepatocellular and cholestatic injury (77%), and only four patients (18%) had acute cholestasis. Interestingly, the liver histology features noted close to the time of elevated serum aminotransferases were not significantly different from those seen when the R <5, although there was a trend of increased hepatocyte apoptosis in hepatocellular cases. Hepatocellular injury was typically spotty and mild, and no patient had bridging or confluent necrosis. Some degree of cholestasis was found in all biopsies, but bile duct injury without evidence of bile duct loss was found in only 14%. Steatosis was uncommon (10%), and no patient had more than mild portal fibrosis. One case showed microscopic evidence suggestive of peliosis hepatis and nodular regeneration. This consistent histological picture, in the setting of young males with a history of exposure, makes liver biopsy unnecessary for diagnostic reassurance.

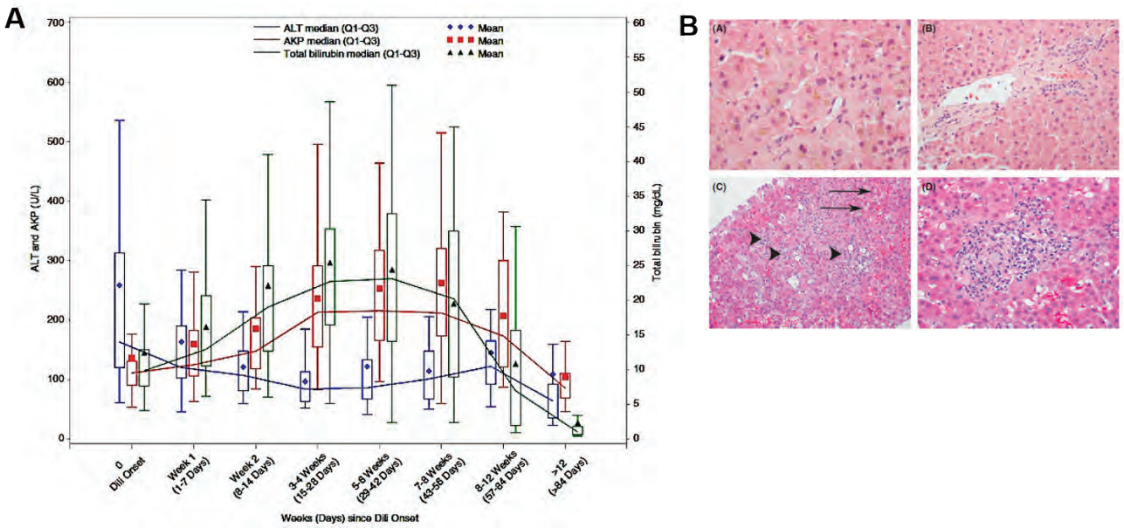

Figure 1. Biochemical and histological description of liver histological lesions induced by AAS. (A) Median total bilirubin (mg/dl) (µmol) or alkaline phosphatase (AKP) and ALT activity in U/L plotted over time in weeks after presentation. (B) Histological findings in anabolic steroid DILI. Taken from Stolz A *et al.* Aliment Pharmacol Ther. 2019;1–10.

Of note, in the largest case series and despite a high proportion of hepatocellular type of liver injury associated with high bilirubin levels, no fatalities occurred, showing a low prognostic value of Hy´s law (hepatocellular injury with high levels of bilirubin are associated with a higher mortality or need for liver transplantation) in this setting. Interestingly, the only woman diagnosed with AAS hepatotoxicity in the Latin-American DILI Registry went into acute liver failure and died [11], confirming that females are at higher risk of severe liver toxicity than males [3].

Renal dysfunction is common in AAS-induced liver injury. Twelve patients from the Spanish and American cases (24% and 14% respectively) developed renal dysfunction [9]. Six cases of AAS

hepatotoxicity included in the Spanish Registry developed acute kidney injury (AKI) along with another five previously published cases of AAS DILI and AKI were analysed. In a bivariate logistic regression model, the risk factors associated with this complication were a cholestatic type of liver injury with higher bilirubin levels (OR 1.26). The best bilirubin cut-off point for the prediction of AKI development was 21.5 mg/dl (AUROC 0.92) (Figure 2). AKI related to bile acids known as "cholemic nephrosis" was described in the 19th century, and nowadays it is called "bile cast nephropathy". The histological changes include a tubular epithelial injury in the distal nephron and luminal obstruction by bile casts. For many years the mechanism of renal impairment has been attributed to bile acid nephropathy [12] but direct drug toxicity probably also occurs as both AKI in the absence of cholestasis. In addition, focal segmental glomerulosclerosis with nephrotic syndrome and renal insufficiency have been reported in bodybuilders [13]; the latter probably an under-recognised complication because of the expected rise in serum creatinine as a result of increased muscle mass in these subjects [13]. Renal impairment generally resolves without therapeutic intervention, although cases requiring dialysis have been described [14].

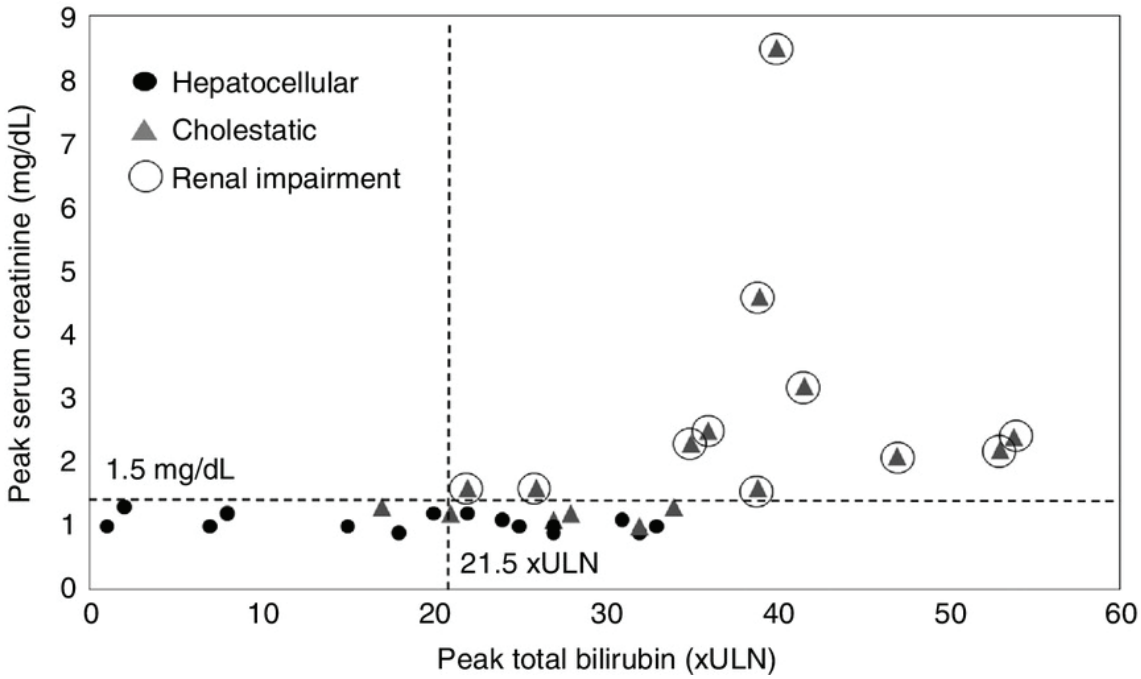

Figure 2. Peak values of total bilirubin and serum creatinine in anabolic androgenic steroid-induced liver injury (AAS DILI). Taken from Robles-Diaz M, *et al.* Aliment Pharmacol Ther 2015; 41: 116–125.

Diagnosis

No validated biomarkers are yet available for DILI diagnosis. Therefore, as in other types of DILI, diagnosis of AAS-related liver injury is based in a detailed pharmacological history, where the presence of a compatible temporal relationship is paramount, and exclusion of other causes of liver injury is a crucial step [15]. In addition to the usual diagnostic issues of DILI related to conventional medications, recognising hepatotoxicity from bodybuilding supplements is hampered by other difficulties such as no information of the supplements ingested by the patient, multi-ingredient supplements, mislabelling, adulteration, and the lack of notification of adverse events in this population. On the contrary, AAS hepatotoxicity has a recognisable signature that can help in detecting this type of liver injury.

Identification of AAS consumption in subjects who denied intake may be addressed by screening of AAS in urine or blood or an evaluation of the endocrine effect of AAS [16].

Pathogenesis and risk factors of acute cholestasis associated with AAS use

Pathogenic mechanisms of cholestasis with marked jaundice associated with C-17 substituted androgens are not completely understood. The damage might be somewhat dose related (direct-intrinsic) as it has been estimated to occur in ~1% of patients treated with methyltestosterone, danazol, stanozolol or oxymetholone and high doses also cause cholestasis in some animal models. As the syndrome is similar to cholestasis of pregnancy and the jaundice is associated with high doses of oestrogens or birth control pills, it might be due to genetic variants of bile salt transporter proteins. A candidate gene study targeting *ABCB11* (BSEP), *ABCB4* (MDR3) and *ATP8B1* (PFIC 1), which are genes whose mutations cause progressive familial intrahepatic cholestasis and was undertaken by the DILIN group in 41 AAS DILI cases. The majority of the patients did not have causal mutations in the three candidate genes but variants in *ABCB11* gene that encodes the BSEP occurred in 20% of the cases ***vs.*** 12% in controls. Hence, genetic variants responsible for rare genetic cholestatic disorders would account for a minority of AAS DILI cases. The authors speculated that injury may be due to unknown hepatic transporters either at the canalicular or sinusoidal domain of hepatocytes, or variants in other genes [10]. In *in vitro* experiments using human hepatocytes, epistane upregulated the expression of key bile acid synthesis genes (*CYP7A1*, by 65% and *CYP8B1*, by 67%) and bile acid transporters (NTCP, OSTA and BSEP) [17].

Treatment

Treatment is mainly based on withdrawal of the androgenic anabolic drug and supportive therapy. Steroids, cholestiramine and ursodesoxicholic acid have not shown a clinical benefit. Anecdotal report has shown the potential efficacy of N-Acetyl cysteine [18]. Liver support systems such as molecular absorbent recirculating system (MARS) or plasmapheresis have been tried in selected cases and have reduced concentrations of inflammatory cytokines and bilirubin [19] (Figure 3). Pruritus can be severe in these patients and should be managed according to guidelines. Rifampicin may improve pruritus and also decrease bilirubin levels, but again the evidence is anecdotal [20].

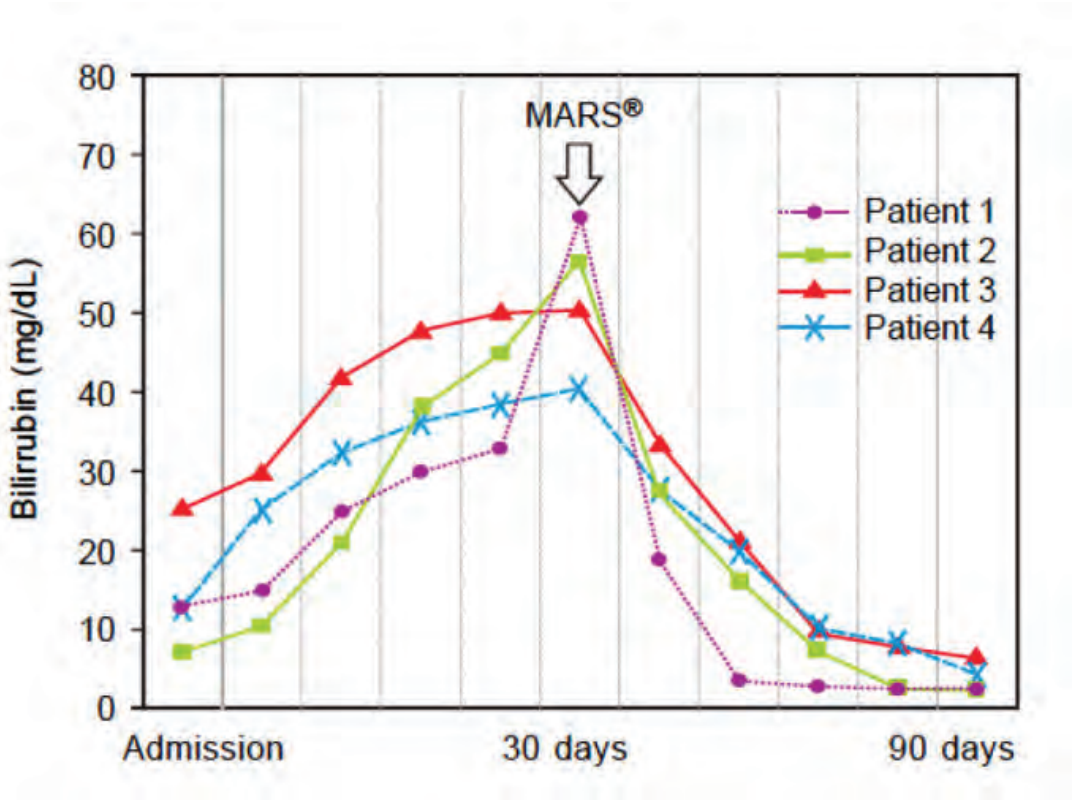

Figure 3. Bilirubin levels before and after MARS® therapy. Taken from Diaz FC, *et al.* Ann Hepatol 2016;15:939–43.

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(references in **BOLD** are required reading)

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Over the counter pain killers and hepatotoxicity: Paracetamol (acetaminophen)

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Take home messages

- Paracetamol (acetaminophen, APAP) toxicity is the most common cause of drug-induced liver injury leading to acute liver failure (ALF) in the Western world.
- APAP toxicity may be 'intentional' (i.e. single point overdose) or 'unintentional' (therapeutic misadventure) where multi-day dosing leading to toxicity often in the setting of alcohol, lack of awareness of possible self-harm and delaying access to rescue treatments.
- APAP-ALF follows a hyper-acute pattern that may lead to multisystem organ failure with intracranial hypertension responsible for up to 25% of ALF deaths.
- Advances in the critical care management (neuroprotective strategies, including continuous renal replacement therapy) have been instrumental in reducing mortality in APAP-ALF and, in some cases, prevent the need for liver transplantation.

Introduction

Paracetamol (acetaminophen, APAP) toxicity is an ongoing public health problem in Europe and North America. Despite the presence of a highly effective antidote, N-acetylcysteine, APAP toxicity continues to dwarf all other forms of drug-induced liver injury leading to acute liver failure in the Western world. APAP-ALF represents 65.4% and 45.7% of cases in the United Kingdom (UK) and North America, respectively.[1] Overall, outcomes from APAP overdoses that reach the threshold of acute liver failure (encephalopathy and INR $\geq$ 1.5), are better than observed with most other aetiologies of ALF, but still result in nearly 30% dying and 8% requiring transplantation.

Mechanism of Liver Injury

Progression to ALF following excessive APAP ingestion is uncommon and occurs in < 1% of patients presenting to emergency medicine departments. Following a single time point APAP overdose, hepatic transaminases and INR rise within 24 hours and peak within 72-96 hours. Despite the severity of illness associated with APAP-ALF, there remains significant potential for hepatic recovery. With safe doses, APAP is predominantly bound to glucuronides or sulfates and renally excreted. At toxic doses, this metabolic pathway becomes saturated, with excess APAP oxidized by cytochrome P-450 enzymes to the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI). Toxic NAPQI may be bound to hepatic glutathione (GSH), rendering a benign molecule. Enhanced production of NAPQI may be driven by ethanol or other medications (secondary to promotion of cytochrome P-450 activity). Availability

of hepatic GSH is also reduced with chronic ethanol abuse/malnutrition; thus, decreasing NAPQI detoxification capability.[2] As such, varying APAP dosages may lead to ALF, with excess NAPQI disrupting cellular integrity and rapidly inducing hepatocyte necrosis. Administration of NAC in APAP-ALF replenishes hepatic GSH and decreases NAPQI. N-acetylcysteine (NAC) administration within 12 hours of APAP ingestion can lessen APAP-related hepatotoxicity.

Intentional toxicity vs. therapeutic misadventure

APAP toxicity may be classified as 'intentional if the overdose occurred at single time point. In contrast, 'unintentional' APAP toxicity (or 'therapeutic misadventure') often reflects a staggered overdose with multi-day dosing leading to toxicity, typically in the setting of alcohol, lack of awareness of possible self-harm and therefore late presentation for rescue treatments. The majority of APAP-ALF cases in the UK are the result of intentional overdose, which has driven legislation to restrict over-the-counter access to APAP. In contrast, half of North American cases are reported to be the result of therapeutic misadventure. In a recent study from the ALFSG registry, MacDonald ***et al.*** demonstrated that 1190 patients presenting with APAP-ALF, intentional APAP overdoses were more likely to be associated with a history of depression or another psychiatric comorbidity. In contrast, patients with unintentional toxicity were more likely to have a history of narcotic use either as separate medications or combination preparations with acetaminophen (Table 1) [3]. Furthermore, in a single-centre French study, 25% of all patients admitted with severe acute liver injury due to APAP admitted to ICU over a 20-year period were patients who took doses within the therapeutic range (< 6g/day) [4].

Management of complications of APAP-ALF

APAP-ALF follows a hyper-acute pattern, in which maximum hepatocyte destruction is complete by 72 hours following ingestion. Resulting cerebral edema (CE) and multisystem organ failure are associated with substantial morbidity and mortality, with intracranial hypertension (ICH) responsible for up to 25% of ALF deaths. Management aims to control or prevent ICH, correct metabolic derangements, and maintain hemodynamic stability. Advances in the critical care management of APAP-ALF have been instrumental in reducing mortality and, in some cases, prevent the need for LT [5].

Neurologic Management

In APAP-ALF, astrocyte swelling result in cytotoxic CE, which may culminate in tonsillar herniation and death. The incidence of patients developing ICH has decreased, from 76% in the 1980s to 20% more recently [1, 3]. The basis for decreasing rates of ICH is likely due to in neurocritical care/neuroprotective strategies. These include intubation/airway protection for high grade coma, propofol-based sedation (avoiding benzodiazepines), avoiding hypercapnia, hypernatremia (maintaining serum sodium between 145 and 150 mmol/l to counteract astrocyte swelling) and continuous renal replacement therapy (see below). Moderate hypothermia (targeting body temperature of 34°C) has not been demonstrated to improve outcomes prophylactically but main be considered in refractory cases [6]. Intracranial pressure monitoring remains the gold standard for real-time detection of ICH; however, no associated mortality benefit has been demonstrated and use has declined over time [7]. Non-invasive ultrasonography techniques, such as optic nerve sheath diameter measurement and transcranial Doppler (middle cerebral artery pulsatility index) have promise, although neither technique have been validated in a clinical context.

Hyperammonemia and extracorporeal therapies in APAP-ALF

Hyperammonemia has been implicated in the development of HE, ICH and neurological death with serum ammonia levels > 200 μmol/l correlating with development of ICH [8]. Thus, ammonia lowering remains a therapeutic goal. Lactulose and Rifaximin, used in chronic liver patients, have no proven value in ALF patients. Continuous renal replacement therapy (CRRT) has been demonstrated to achieving significant ammonia clearance has been associated with improved transplant-free survival in ALF [9]. In a study of over 1000 ALF patients from the US ALFSG registry, Cardoso ***et al.*** demonstrated an improvement in 21-day TFS with CRRT, while worsening 21-day TFS was associated with intermittent hemodialysis [9]. Most recently, Warrillow *et al.* demonstrated in 54 ALF patients in Australia who underwent CRRT (continuous venovenous hemofiltration, median time to initiation ~ 4 hours) that CRRT was associated with significant reduced ammonia concentrations in ALF patients with its effect proportionate to cumulative dose [10]. In addition to traditional indications for renal replacement therapy, CRRT should be considered in all ALF patients with hyperammonemia (> 200) or those deemed at high risk for intracranial hypertension.

Albumin dialysis (MARS ~ molecular adsorbent recirculating system) in ALF was evaluated FULMAR Trial [11]. Comparing MARS (n=53) versus SMT (n=49) patients, 6-month survival did not differ between groups (85% vs. 76%; p=0.3) although significant confounding factor in this study was the short median listing-to-LT time (16.2 hours). High volume plasma exchange has also been evaluated in a prospective randomized trial in ALF (n=182 ALF patients) [12]. Non-transplanted HVP-treated patients (largely APAP patients) displayed significantly increased survival (hazard ratio: 0.56; p=0.0083).

Prognosis in APAP-ALF

The most commonly used prognostic index remains the King's College Criteria (KCC). These criteria have a high specificity, but low sensitivity and negative predictive value [13]. Recently, the US ALFSG prognostic index (ALFSG-PI) was developed to predict likelihood of TFS using data from 1974 ALF patients [13]. Admission values of HE coma grade, etiology, vasopressor use, INR, and bilirubin were significantly associated with TFS. While ALFSG-PI was found to outperform both KCC and MELD in predicting TFS in ALF; however, external validation remains necessary [13].

Are we getting better?

Outcomes in APAP-ALF are consistently improving over time as demonstrated recently in US ALFSG study by MacDonald and colleagues of 1190 patients with APAP-ALF enrolled between January 1998 and December 2018 (Table 1) [3]. Twenty-one-day transplant-free survival (TFS) significantly increased from 61.7% during 1998-2007 to 69.8% during 2008-2018. Similarly, incidence of intracranial hypertension and 21-day mortality secondary to cerebral edema significantly decreased from 51.5% to 29.9% and from 11.6% to 4.5%, respectively, over the same time intervals. Notably, these findings occurred in association with increased use of early continuous renal replacement therapy (CRRT; from 7.6% to 22.2% during first 7 days), with use of CRRT found to be significantly associated with improved 21-day TFS (Odds Ratio 1.62; p=0.023) (Figure 1). Finally, overdose intentionality and presence of psychiatric comorbidities were not found to be independently associated with 21-day TFS. Similar results were demonstrated in the UK in a large single centre cohort (Kings College Hospital's) 33-year experience with 3300 ALF patients. Improvements in TFS likely reflects evolving intensive care/ neurocritical care strategies [1, 14].

Liver Transplantation

Comparable survival rates have been reported beyond one year following LT in transplanted APAP-ALF patients and cirrhotic. Recurrent self-harm and poor compliance and follow-up represent potential problems in post-LT APAP-ALF patients; however, long-term outcomes post-LT are similar to those of non-APAP ALF patients.

Conclusions

Paracetamol (acetaminophen) toxicity is the most common cause of ALF in North America and Europe, of which half are intentional and the other half unintentional/therapeutic misadventure. Psychiatric comorbidity is more prevalent in intentional paracetamol overdose while therapeutic misadventures are more commonly associated with alcohol co-ingestion, combination preparations and narcotic use/co-preparation. APAP-ALF follows a hyper-acute pattern that may lead to multisystem organ failure with cerebral edema (CE) responsible for up to 25% of ALF deaths. Transplant-free survival has significantly increased in the last two decades (now > 70%), with rates of intracranial hypertension and death rates from cerebral edema have significantly decreased in the past two decades. Advances in the critical care management (neuroprotective strategies, including continuous renal replacement therapy) have been instrumental in reducing mortality in APAP-ALF and, in some cases, prevent the need for LT.

Table 1. Characteristics of 1162 APAP-ALF enrolled in the US-ALFSG Registry between 1998-2018 stratified by intentionality of overdose.

	Intentional Overdose (N=445)		Unintentional Overdose (N=617)		P-value
	N		N		
Age (years)	445	33 (25-44)	617	38 (30-48)	<0.0001
Sex (male)	445	122 (24.4%)	617	145 (23.5%)	0.147
Acetaminophen Level (µg/ml)	378	54.5 (16.8-137.0)	473	21.0 (10.0-55.0)	<0.0001
Highest MELD (days 1-7)	437	28 (18-34)	612	27 (18-34)	0.70
Coma Grade 3/4 (days 1-7)	433	265 (61.2%)	598	376 (62.9%)	0.58
King's College Criteria met (days 1-7)	445	72 (16.2%)	617	117 (19.0%)	0.24
ALFSG Prognostic Index (admission)					
– Survival Predicted Probability 80%	409	161 (39.4%)	571	226 (39.6%)	0.95
Psychological Comorbidities	445	322 (72.4%)	617	255 (41.3%)	<0.001

	Intentional Overdose (N=445)		Unintentional Overdose (N=617)		P-value
	N		N		
Depression	339	216 (63.7%)	541	179 (33.1%)	<0.001
Schizophrenia	133	10 (7.5%)	367	5 (1.4%)	<0.001
Chronic Pain	123	0 (0.0%)	365	3 (0.8%)	0.31
Bipolar Disorder	191	68 (35.6%)	392	30 (7.7%)	<0.001
Anxiety	162	39 (24.1%)	442	80 (18.1%)	0.10
Alcohol Use (7 drinks/week)	133	40 (30.1%)	231	87 (37.7%)	0.14
Intravenous Drug Use	440	48 (10.7%)	614	35 (5.7%)	0.002
Opioid Use	440	127 (28.9%)	608	362 (59.5%)	<0.001
Intracranial Hypertension (days 1-21)	220	85 (38.6%)	289	100 (34.6%)	0.35
Death (days 1-21)	391	98 (25.1%)	549	139 (25.3%)	0.93
– Cerebral Edema Death	391	37 (9.5%)	547	35 (6.4%)	0.08
Listed for Liver Transplantation	445	95 (21.3%)	616	151 (24.5%)	0.23
Received Liver Transplant (days 1-21)	444	30 (6.8%)	614	55 (9.0%)	0.19
Transplant-free Survival (day 21)	392	266 (67.9%)	564	373 (66.1%)	0.58

*In 38/1190 patients, intentionality of overdose could not be determined

Data from MacDonald *et al.*, Clin Gastroenterol Hepatol 2020 [3]

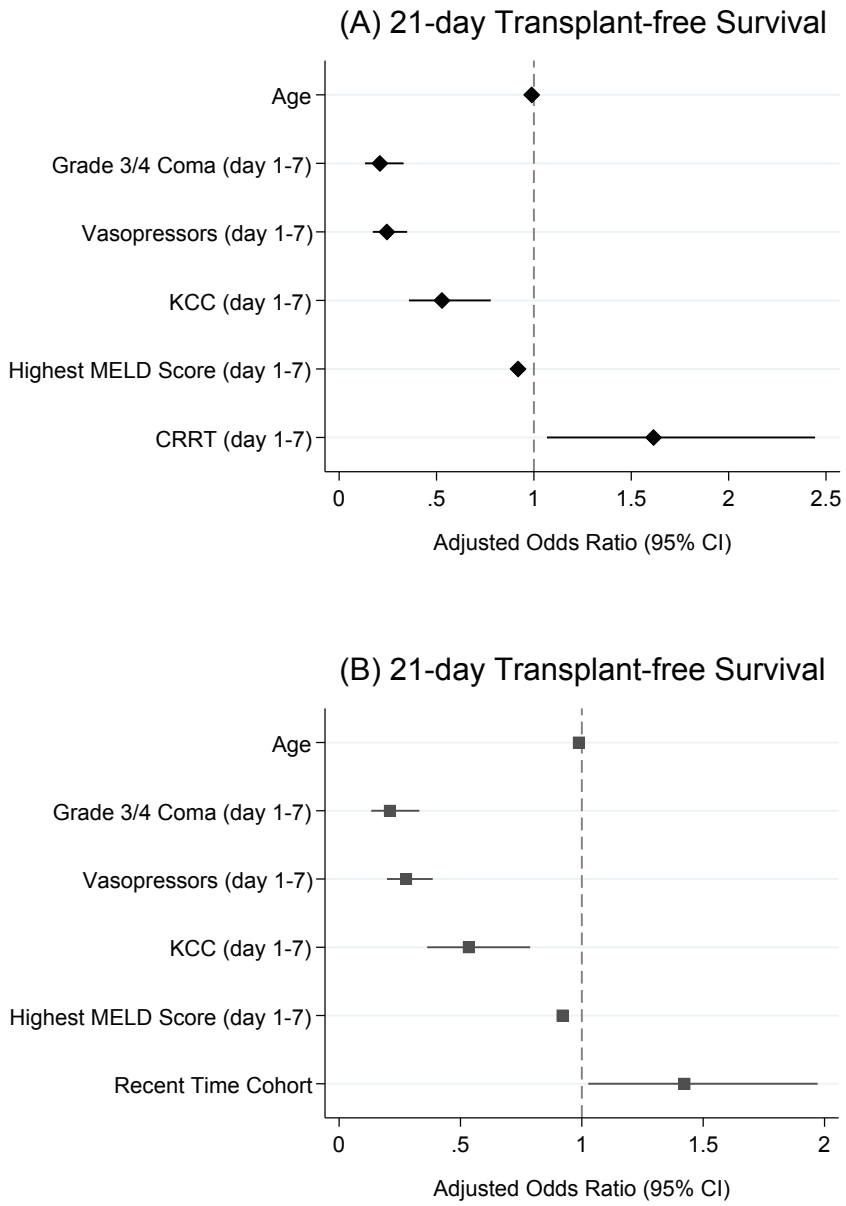

Figure 1. Adjusted associations with 21-day transplant-free survival in 1190 APAP-ALF patients. (A) Model 1 adjusting for CRRT and (B) Model 2 (adjusting for era (2008-2018 vs. 1998-2007).

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SESSION 6

MULTIDISCIPLINARY OUTPATIENT APPROACH TO NAFLD AND ALD

WEDNESDAY 23 JUNE
16H45 - 17H45

NAFLD – is this a job for the hepatologist on their own?

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Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of disease that requires careful risk stratification to identify those at highest risk for liver related outcomes. However, overall, patients with NAFLD are at highest risk of death from cardiovascular disease and malignancy. Therefore, modification of these risks needs to be central to the approach to the patient with NAFLD. Between individuals, the dominant driver of disease in may differ, underscoring the importance of an individualized approach to the patient. Optimal management of patients with NAFLD, thus requires a thoughtful multidisciplinary approach to proactively intervene on modifiable risk factors and provide long term supportive care for this chronic disease. A true multidisciplinary clinic requires substantial organization and institutional commitment. However, comprehensive care can be provided by a Hepatologist with external support as needed.

NAFLD is largely a disease of overnutrition and addressing this needs to be central to the management plan. Weight loss and modification of dietary macronutrient content are not only critical to the treatment of NAFLD, but they also improve metabolic comorbidities, which will reduce cardiovascular and potentially cancer related morbidity and mortality.

At the initial visit with a Hepatologist, the patient should be risk stratified for severity of liver disease, other causes or contributors to liver disease elucidated, and the burden of comorbid illness assessed. Nearly all patients with NAFLD will benefit from nutritional counselling and the vast majority will benefit from weight loss. This is best achieved by a structured nutritional plan with frequent follow up visits. Another critical component to a successful weight loss program is the assessment of psychological factors driving eating patterns and barriers to weight loss. For many patients, behavioural intervention with a health psychologist can be instrumental to breaking unhealthy habits and serves as a compliment to dietary counselling (Figure 1).

The Endocrinologist plays a pivotal role, not only in the management of Diabetes, but also in the identification and management of other Endocrine disorders associated with NAFLD (Figure 1). Diabetes management incorporating the use of drugs that may be beneficial in NASH such as the GLP-1 agonists or SGLT2 inhibitors is best done with an Endocrinologist, however depending on the comfort level of the Hepatologist, can be prescribed without referral. If an Endocrinologist is not formally a part of a multidisciplinary NAFLD clinic, a relationship should be established with one on a consultatory basis, or if the patient has already established care with an Endocrinologist, this can be done on an individual basis. Quality multidisciplinary care can be accomplished in a variety of settings with a thoughtful approach tailored to the resources available.

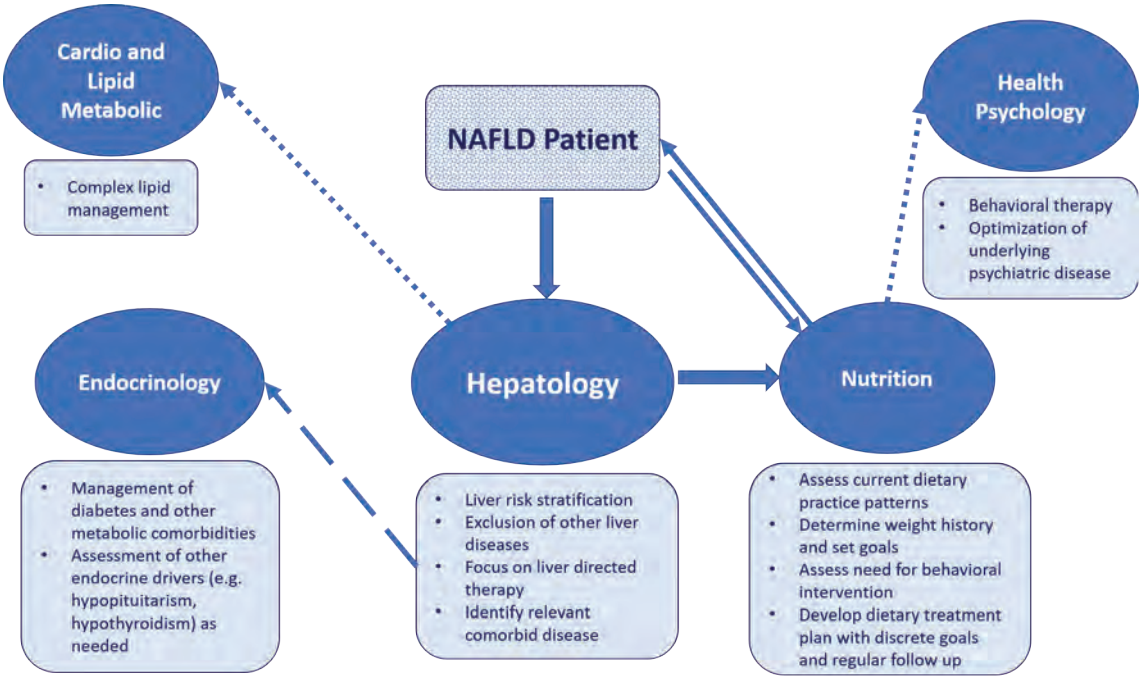

Figure 1: A multidisciplinary approach to the management of NAFLD. Once referred, the patient is evaluated by Hepatology and risk stratified. The evaluation should include a careful assessment of relevant endocrine co-factors and referral to an Endocrinologist embedded in the clinic or in consultation as this will be applicable to the majority of patients with NAFLD (semi-solid arrow). All patients should undergo nutritional assessment and a plan established for regular follow up independent of Hepatology visits. The need for Health Psychology and additional Cardiology or Lipid metabolic support should be assessed on an individual basis (dotted arrows).

ALD – it takes a team

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Take-home message

- ALD patients have complexities outside of the liver that are difficult for hepatologists alone to manage.
- Multidisciplinary management includes team-based care at the medical setting as well as at the home setting and includes psychologists, social workers, and others.
- Initial descriptions of such models indicate improvements in quality of care in this patient population.
- There are a number of benefits to the multidisciplinary care model, although barriers exist for implementation outside of the transplant setting.

Why do we need multidisciplinary outpatient approaches to ALD?

ALD is a dual disease that requires liver attention and AUD attention. Many hepatologists at the current time do not have the capabilities for managing AUD[1]. Many ALD patients also have psychosocial challenges that present complications for their management[2]. Models for integrated care include gastroenterologists or hepatologists, primary care physician, patient's home and a multidisciplinary clinic that may involve a social worker and a psychologist. There are algorithms that suggest which patients might benefit from this integrated care model[3]. These are based on presence of AUD based on AUDIT score and presence of ALD based on laboratory testing, history, and imaging.

What are the outcomes of multidisciplinary care units?

An early description in 2013 by Addolorato[4] stated that the percentage of patients who showed recidivism after liver transplant with an alcohol addiction unit was lower, suggesting benefit of this model. Indeed, transplant centres are the most common setting for this type of multidisciplinary model. A more recent study by Mellinger[5] shows, even in the non-transplant setting, that rates of hospital admission and ER utilization/person/month are reduced in a multidisciplinary model.

What are the recommendations and barriers to this type of model[2]?

Recommendations include colocation of the team, multidisciplinary approach, a focus on interpersonal team relations, novel approaches to patient encounters, and engagement with unaffiliated community and outreach locations. Barriers include financial sustainability of the model, logistical complexity, disparities of the patient population, and the patient's cognitive status.

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Dietary treatment as part of a multidisciplinary outpatient approach to NAFLD

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Take-home messages

- NAFLD is mostly a nutritional and lifestyle driven disease, and in addition, it is a multisystem disease accompanied by dietary-related comorbidities. Therefore, these patients will often benefit from multidisciplinary care which incorporates comprehensive dietary treatment.
- Critical evidence shows that a comprehensive lifestyle intervention can induce clinically significant weight loss (i.e., ≥5%). However, the majority of NAFLD patients do not utilize structured, comprehensive lifestyle treatment.
- Without investment in comprehensive accessible and affordable nutritional care, no sustainable dietary modifications can be expected. Like medications, a diet can work only as long as it is maintained.
- The active support of the physicians whose advice has a motivational value and is a catalyst for lifestyle change, is very much needed.
- The role of healthcare providers includes: teaching healthy eating skills, enhancing confidence in the benefits of diet, engaging family members, discussing potential barriers and finding shared solutions.

Why is a multidisciplinary team needed?

Establishment of multidisciplinary teams is an effective way to manage the diverse clinical needs of patients with liver disease. This approach improves NAFLD patient's self-management, since it provides a comprehensive follow up by physicians, dieticians, psychologists and physical activity supervisors (1). The advantages of multidisciplinary team are better management of complex cases, promotion of research, teaching and exchange of knowledge between specialists, and perhaps reduction of professional wear out. From the patient's perspective, a multidisciplinary team enables getting all aspects of treatment under a single roof, improved communication and agreement between the various specialists on the route of treatment, which may enhance confidence and motivation. The composition and structure of the multidisciplinary team and the services that are provided will determine the quality and comprehensive nature of the care provided to the NAFLD patient in this healthcare setting.

NAFLD is mostly a nutritional and lifestyle driven disease, and in addition, it is a multisystem disease accompanied by dietary-related comorbidities. Therefore, these patients will often benefit from multidisciplinary care, incorporating comprehensive dietary treatment by nutrition experts with experience in treating liver disease patients with and without advanced fibrosis. Currently, diet is the cornerstone and the only established treatment of NAFLD, but even when pharmacologic treatment becomes available, it will still need to be accompanied by lifestyle treatment and is not intended to replace it, similarly to the case with type-2 diabetes and obesity.

There are a few published examples of multidisciplinary secondary care clinics for NAFLD (2-6). The scientific evidence to support justification of multidisciplinary approach is insufficient, due to a gap between evidence-based practice and active design of relevant studies, and structured data collection needed to gather high-level evidence-based medicine. For example, establishment of a multidisciplinary metabolic hepatology clinic offering lifestyle advice, weight loss services and pharmacological treatment of diabetes and cardiovascular risk factors led to reduced alanine aminotransferase (ALT), weight, HbA1c, total cholesterol and liver stiffness (5).

In contrast, it is demonstrated that adults with NAFLD receiving merely care in usual clinical practice do not get to see a clinical dietitian in the vast majority of cases; 3.5% in the United States cohort of patients (n=2,019). Diet was recommended only to 53% of patients and exercise was recommended only to 15% of them. In this study, only 32% overweight or obese adults with NAFLD receiving usual care achieved >5% weight reduction during a median follow-up of 39 months, and weight regain back to baseline was common (21.2%) (7).

Barriers for establishment of a multidisciplinary team and suggested compromises

Unfortunately, in many cases a full multidisciplinary team is not available for the patient due to limited resources. At the minimum, availability of a comprehensive and long-term nutritional treatment should be provided to NAFLD patients. Availability should be ensured at the economic level (e.g., insurance coverage), geographic/practical accessibility (e.g. convenient distance, ability to set appointments in a reasonable time interval) and clearly defined referral pathways.

Within or outside of a multidisciplinary setting, one of the important triggers for successful nutritional treatment is the active support of physicians, since the physician's advice is a catalyst for lifestyle change (8). Several studies indicate that a physician's advice to lose weight has positive effects on the likelihood of adhering to diet and exercise recommendations (9) and on the patients' motivation for weight loss (10). The importance of the physician to increase awareness to the need for weight loss and referral to dietary interventions is emphasized by a large study showing that weight loss programs are under-utilized. Among 3,822 persons with NAFLD (Fatty Liver Index ≥ 30) from the US National Health and Nutrition Examination Survey (2001-2014) only 53.9% of people with NAFLD intended to lose weight even though over 95% were overweight or obese. Notably, amongst those who tried to lose weight ≤10% (lower rates among men) attended weight loss programs (11).

Furthermore, general practitioners and hepatologists treating NAFLD patients should provide information and refer the patients to appropriate resources regarding NAFLD implications and treatment options and have training in providing behavioural therapy. Similarly to the treatment approach in other chronic diseases, healthcare providers need to discuss the broader picture of complications with their NAFLD patients; the message should be that risk reduction of liver cancer, diabetes and cardiovascular disease, is possible (12). In a cross-sectional study among 146 NAFLD patients, a better nutritional behaviour was associated with higher patient's perceptions of understanding what NAFLD is, believing in treatment effectiveness and a higher self-efficacy (13).

The 5 *A*'s model (ask, assess, advise, agree, and assist) (Figure 1) may be useful as a tool to assist clinicians advising NAFLD patients on how to modify their behaviour, assessing their interest in doing so, assisting in their efforts to change, and arranging appropriate follow-up (14, 15). Most physicians do not have the skills and time to deal with long-term comprehensive nutritional treatment, and thus the referral to nutrition specialist is imperative for effective nutritional treatment.

Even use of a simple tool such as filling a structured form (a "care bundle", Figure 2), intended to checklist all aspect of care for NAFLD patients, can be helpful as a first step. In a study describing the current management of patients with NAFLD attending hospital clinics in North East England, among 147 patients attending gastroenterology, hepatology and a specialist NAFLD clinic, there was significant variability in the lifestyle advice given and management of metabolic risk factors. Weight loss advice was infrequently documented prior to implementation of a care bundle. Use of the bundle was associated with significantly better documentation and implementation of most aspects of patient management, including management of metabolic risk factors, documented lifestyle advice and provision of NAFLD-specific patient advice booklets. Patients attending a NAFLD clinic were more likely to achieve >10% body weight loss and have metabolic risk factors addressed compared to non-specialist clinics (general gastroenterology and hepatology clinics) (16).

Recently, it has been suggested that a web-based intervention can be helpful in reaching out to patients who are unable to attend face-to-face treatment or access to care. It has been shown that a web-based interactive intervention provided to NAFLD patients was not inferior to a standard group-based intervention with respect to weight loss, adherence to healthy diet and habitual physical activity, normalization of liver enzymes, and stable surrogate markers of fibrosis (17). However, attrition rate in the web-based intervention was higher, and web-based tools need further confirmation and may be more suitable for young patients.

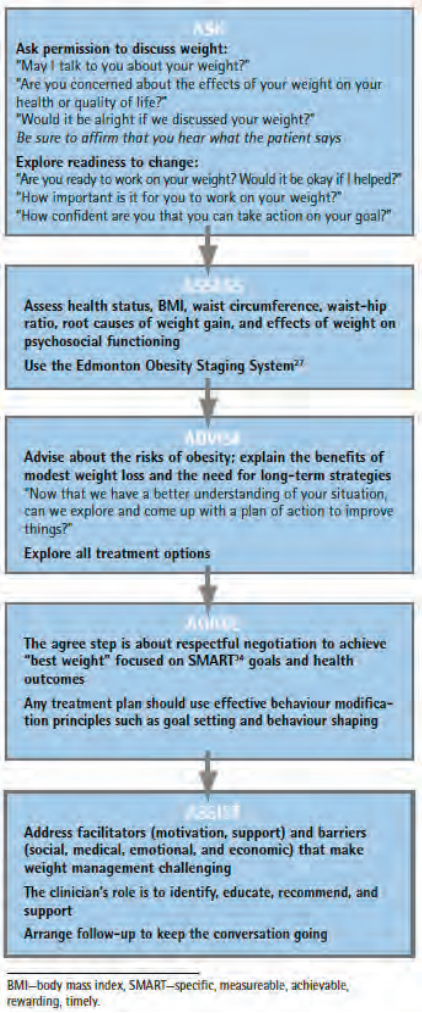

Figure 1. The 5 As for obesity and lifestyle counseling (obtained from Vallis M., Can Fam Physician 2013(15))

Care bundle: Management of patients with non-alcoholic fatty liver disease

Date seen.....

Weight (kg)	Height (m)	BMI	BP (mmHg)
Overweight/ Obesity □	Type 2 diabetes □ Year diagnosed:	Hypertension □	Dyslipidaemia □
Current alcohol consumption units/week If alcohol consumption consistently greater than 14/21 units per week for females/males, this is not NAFLD			
Current stage of NAFLD **Last staging date**: ..... Stage at diagnosis and then re-stage every 3 years or more frequently, non-invasively where possible.			
Liver biopsy: NAS Grade..... Stage..... Date..... N/A			
FIB-4 score =			
Fibroscan = kPa Date..... N/A			
Lifestyle changes			
Ensure information leaflets on NAFLD given	Y N		
Change in weight since last clinic appointment (+ or -)	kg %		
Target weight (aim >5% weight loss if overweight and >10% if obese)	kg		
Discuss/reinforce dietary advice	Y N		
If not losing weight offer referral to dietician	Y N N/A decline		
Current activity levels & discuss increasing activity/exercise			
Managing metabolic risk factors			
Review BP (further monitoring or treatment if BP>140/90 via GP)	Y N		
Review diabetic control/ screen for diabetes (If suboptimal control, then advise GP/diabetologist to review regimen)	Y N		
Ensure on statin - If no, why not? Not tolerated □ Low risk □ (statins are recommended for patients with T2DM or a QRISK2 >10%)	Y N		
Smoking cessation advice	Y N N/A		
Specific NAFLD treatment: If patients have NASH and/or ≥F2 on biopsy (or Fibroscan > 8 and FIB-4 >1.3) consider referring to specialist clinic for a trial or specialist treatment	Y N N/A		
Routine investigations: FBC, U/E, LFT, AST, GGT, HbA1c, glucose, lipids (fasting preferred). If Cirrhotic: AFP, vitamin D. Check liver screen completed.			

Figure 2 Care bundle for management of patients with non-alcoholic fatty liver disease. (Obtained from Neilson LJ, Macdougall L, Lee PS, et al. Frontline Gastroenterology ahead of print) (16).

What does it take to achieve successful lifestyle modifications?

Lifestyle modification should be presented to patients as a lifetime treatment, with varying intensity according to their needs (i.e., ≥14 sessions in 6 months comprehensive weight-loss interventions, and for weight loss maintenance; a long-term (≥1 year) comprehensive program) (18). In the Look AHEAD (Action for Health in Diabetes) study, 5145 overweight/obese men and women with type-2 diabetes were randomly assigned to an intensive lifestyle intervention (ILI) or a usual care group, referred to as Diabetes Support and Education (DSE). At year 4, ILI participants lost an average of 4.7% of initial weight, compared with 1.1% for DSE. More ILI than DSE participants lost ≥5% (46% vs 25%) and ≥10% (23% vs 10%) of initial weight. Importantly, participants in the ILI who maintained the weight loss, compared with those who did not, attended more treatment sessions. These results provide critical evidence that a comprehensive lifestyle intervention can induce clinically significant weight loss (i.e., ≥5%) and maintain this loss in more than 45% of patients at the fourth year of follow-up (19).

A qualitative study (20) highlights the important role of healthcare providers as educators on the significance of NAFLD (in itself and in the broader context of the metabolic syndrome) and its potential to regress; teaching healthy eating skills, enhancing confidence in the benefits of diet, engaging family members in the treatment to gain support and avoid conflicts, discussing potential barriers (e.g. life stressors and 'obesogenic environment') and finding shared solutions. Although this personalised intervention approach will “cost” a few more minutes of the provider’s time, this may be a reasonable price to pay for a measure that could potentially make a difference between adherence and non-adherence. Otherwise, even the most effective diet will end-up falling into the gap between clinical trials and clinical practice (21).

Combined NAFLD & Alcohol-related Fatty Liver Disease (AFLD)

The separation of both aetiologies is arbitrary as many people with obesity can also have alcohol-induced liver damage and vice versa. Behavioural risk factors for AFLD and NAFLD frequently co-exist, particularly among populations of a lower socioeconomic status. Moreover, the presence of both metabolic and alcohol-related liver disease synergistically accelerates liver damage. There is therefore a pressing need to simultaneously prevent and treat these two leading causes for liver disease. The establishment of holistic referral pathways and structured treatment programs able to deal with patients with joint alcohol-related and metabolic liver disease should be favoured. Clinical networks between general practitioners, endocrinologists, cardiologists, nutritionists and hepatologists should ideally be able to provide a comprehensive management (Policy statement on the coexistence of alcohol-related liver disease and non-alcoholic fatty liver disease, 2020 https://easl.eu/wp-content/uploads/2020/08/Full-version-Policy-Statement-on-the-coexistence-of-NAFLD-and-ARLD_26Aug2020.pdf).

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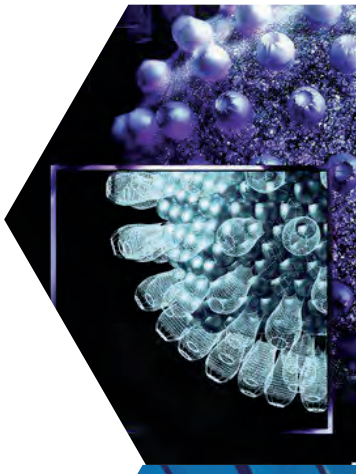

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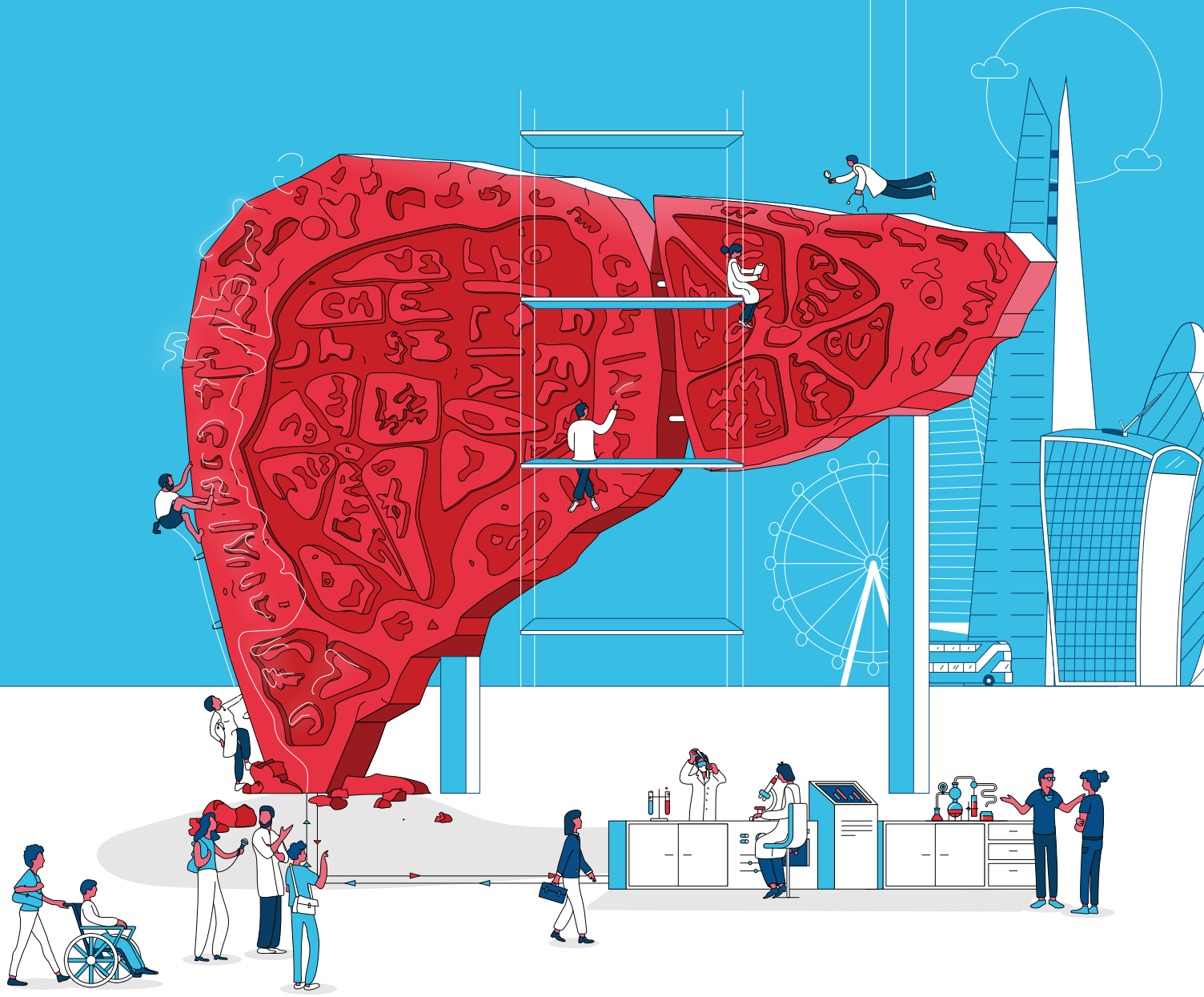

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