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Mitochondrial alterations in fatty liver diseases

Bernard Fromenty¹, Michael Roden^{2,3,4,*}

Summary

Fatty liver diseases can result from common metabolic diseases, as well as from xenobiotic exposure and excessive alcohol use, all of which have been shown to exert toxic effects on hepatic mitochondrial functionality and dynamics. Invasive or complex methodology limits large-scale investigations of mitochondria in human livers. Nevertheless, abnormal mitochondrial function, such as impaired fatty acid oxidation and oxidative phosphorylation, drives oxidative stress and has been identified as an important feature of human steatohepatitis. On the other hand, hepatic mitochondria can be flexible and adapt to the ambient metabolic condition to prevent triglyceride and lipotoxin accumulation in obesity. Experience from studies on xenobiotics has provided important insights into the regulation of hepatic mitochondria. Increasing awareness of the joint presence of metabolic disease-related (lipotoxic) and alcohol-related liver diseases further highlights the need to better understand their mutual interaction and potentiation in disease progression. Recent clinical studies have assessed the effects of diets or bariatric surgery on hepatic mitochondria, which are also evolving as an interesting therapeutic target in non-alcoholic fatty liver disease. This review summarises the current knowledge on hepatic mitochondria with a focus on fatty liver diseases linked to obesity, type 2 diabetes and xenobiotics.

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Introduction

Fatty liver diseases are a burgeoning health problem and – upon the cure and control of viral hepatitis – their main causes have shifted towards non-communicable factors, such as excess alcohol consumption, common metabolic diseases, xenobiotic exposure and drug-induced liver injury.¹ The rising worldwide prevalence of obesity and type 2 diabetes mellitus (T2DM) has created the basis for a new syndemic, which may drive adverse liver disease outcomes in Europe.¹ Common metabolic diseases like obesity and T2DM share epidemiological and pathophysiologic features with non-alcoholic fatty liver disease (NAFLD)^{2,3} and their main characteristics, ectopic fat deposition, altered metabolic fluxes and insulin resistance are linked to abnormal energy metabolism.⁴ In adipose tissue, altered mitochondrial functionality contributes to adipose tissue dysfunction, with impaired insulin-mediated triglyceride storage and subsequent lipid overflow to other tissues.⁵ This may explain how hepatic lipid accumulation initiates dynamic changes in mitochondrial function and promotes the development and progression of steatosis (non-alcoholic fatty liver, NAFL) to non-alcoholic steatohepatitis (NASH) and hepatic fibrosis/cirrhosis.⁶

Detailed knowledge of the effects of xenobiotics on hepatic mitochondria and liver function^{7–9} has improved our understanding of the impact of mitochondria on metabolic disease-

related (lipotoxic) liver diseases. This has led to growing interest in the therapeutic potential of targeting mitochondria in fatty liver diseases.^{3,10}

In this review, we analyse the role of alterations in hepatic mitochondrial function across the spectrum of fatty liver diseases (of different aetiologies) with a focus on studies in humans.

Physiological role of hepatic mitochondria

A major role of hepatic mitochondria is energy production, via oxidation of substrates including amino acids, pyruvate, and fatty acids. The tight coupling between substrate oxidation and ATP synthesis, *i.e.* oxidative phosphorylation (OXPHOS), is finely tuned by many circulating and intrahepatocellular factors.^{11,12} Experimentally, OXPHOS coupling is reflected by a high respiratory control ratio (RCR), defined as the ratio of ADP-stimulated respiration (coupled respiration) to resting respiration. Pyruvate provided by glycolysis is oxidised by the tricarboxylic acid (TCA) cycle after its transformation into acetyl-coenzyme A (acetyl-CoA) by pyruvate dehydrogenase. Fatty acids are oxidised by the β -oxidation process (Fig. 1), which requires CoA and L-carnitine and involves several enzymes with specific activities according to their chain length.¹³ During fasting, hepatic mitochondrial fatty acid oxidation (FAO) generates ketone bodies that are oxidised in extrahepatic

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* Corresponding author. Address: Department of Endocrinology and Diabetology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany.

E-mail address: michael.roden@ddz.de (M. Roden).

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Keypoints

- Mitochondria are essential for multiple features of hepatic function, ranging from substrate metabolism and energy production through cellular signaling to biotransformation of xenobiotics.
- Assessment of mitochondrial functionality and quality control has largely been limited to preclinical studies, but recent developments have enabled us to gain more insight into these processes in human livers.
- Due to their role in fatty acid oxidation, lipogenesis and gluconeogenesis, mitochondria are involved in the pathogenesis of non-alcoholic fatty liver diseases.
- During common metabolic diseases, hepatic mitochondrial oxidative capacity may adapt to greater lipid availability and thereby help to prevent excessive lipid accumulation.
- With higher-grade obesity and type 2 diabetes, mitochondrial capacity can decline, while subsequent oxidative stress favors the progression of non-alcoholic fatty liver diseases from steatosis to steatohepatitis and fibrosis/cirrhosis.
- Microvesicular steatosis induced by xenobiotics such as amiodarone or valproic acid results from severe inhibition of mitochondrial fatty acid oxidation and bears the risk of lethal liver failure.
- Xenobiotic-induced macrovacuolar steatosis involves milder but chronic abnormalities of mitochondrial function, thereby favoring oxidative stress and progression to steatohepatitis and cirrhosis.
- Hepatic mitochondrial alterations play an important role in the mutual interaction of metabolic disorders with some drugs and alcohol abuse.
- Several interventions directly (e.g. thyroid hormone receptor agonists) or indirectly (e.g. weight loss) affect hepatic mitochondria and have beneficial effects on fatty liver diseases, suggesting that mitochondrial targets should be further evaluated for the treatment of non-alcoholic fatty liver diseases.

tissues via the TCA cycle to provide energy.⁷ Mitochondrial FAO is regulated by different transcription factors including peroxisome proliferator-activated receptor (PPAR) α , PPAR β/δ and forkhead box A2.⁷ Several mitochondrial NAD⁺-dependent protein deacetylases (sirtuins) also regulate mitochondrial FAO and other mitochondrial metabolic pathways.¹⁴

Oxidation of mitochondrial substrates generates NADH and FADH₂, whose electrons and protons feed the electron transport chain (ETC) in order to create a large electrochemical potential ($\Delta\psi$), which is mandatory for ATP production (Fig. 1). This process regenerates the NAD⁺ and FAD necessary for other cycles of fuel oxidation.⁷ Of note, 13 polypeptides of the ETC are encoded by mitochondrial DNA (mtDNA), while the remaining polypeptides are encoded by nuclear DNA (nDNA). Liver mitochondria contain all the components required for mtDNA replication, transcription and translation as well as enzymes involved in DNA repair.⁸ Permanent mtDNA replication is a major event during mitochondrial biogenesis, a complex programme orchestrated by several key transcription factors and coactivators including nuclear respiratory factors 1 and 2 (NRF1, NRF2) and peroxisome proliferator-activated receptor- γ coactivator 1 α and 1 β (PGC1 α , PGC1 β).¹⁵ AMP-activated protein kinase (AMPK) also plays a major role in mitochondrial biogenesis, in particular by activating PGC1 α .¹⁵ Beyond mitochondrial biogenesis, other processes regulate mitochondrial number and quality including mitochondrial dynamics (i.e. fusion and fission) as well as mitophagy, a selective autophagic pathway that specifically eliminates damaged mitochondria.^{16,17} Liver mitochondria are also involved in anabolic processes. During fasting, hepatic mitochondria play a pivotal role in gluconeogenesis, which transforms different carbon precursors (alanine, pyruvate,

lactate) into glucose. Indeed, the first steps of gluconeogenesis are catalysed by the mitochondrial enzymes, pyruvate carboxylase and malate dehydrogenase. After feeding, the TCA cycle metabolite citrate leaves the mitochondria to serve as a carbon source for hepatic *de novo* lipogenesis. Liver mitochondria also play a role in bile acid synthesis from cholesterol via cytochrome P450 27A1 (CYP27A1).¹⁸

Mitochondria further contribute to cell signalling by generating reactive oxygen species (ROS), via ETC complexes I, III and some enzymes of the mitochondrial FAO pathway.^{8,19} ROS activate different transcription factors, e.g. nuclear factor erythroid 2-like 2 (NFE2L2 or Nrf2), which contribute to antioxidant responses and mitochondrial biogenesis.^{8,20} When the mitochondrial unfolded protein response is activated, other mitochondria-derived signalling molecules (namely, small peptides) induce nuclear translocation of activating transcription factor 5.²¹

Finally, hepatic mitochondria also contain other CYPs like CYP1A2 and CYP2E1,^{22,23} the latter metabolises acetaminophen (paracetamol), 1,3-butadiene, ethanol, fatty acids, and ketone bodies.²²

Mitochondrial alterations in metabolic disease-related liver diseases

Metabolic disease-related liver diseases occur mainly in individuals with obesity and T2DM but are also present in those with rare diseases (lipodystrophies, inborn errors of metabolism), which are beyond the scope of this review. Herein, we focus on the comparison of hepatic mitochondrial function across the histopathological spectrum of NAFLD in obesity and the impact of diabetes. The methods used to assess various

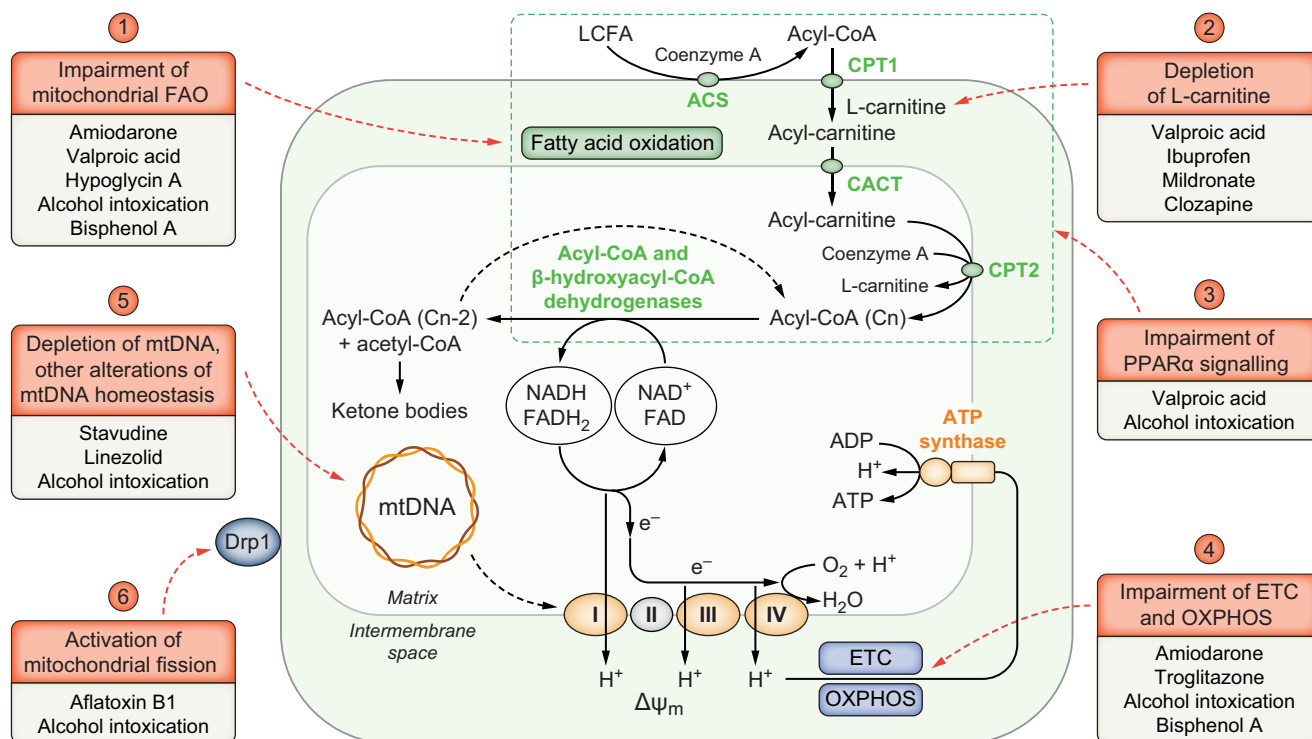


Fig. 1. Main mitochondrial functions and xenobiotic-induced alterations. Contrary to short/medium-chain fatty acids that freely enter mitochondria (not shown), LCFAs' entry requires a 4-step shuttle system. (1) LCFAs bind to CoA to form acyl-CoA thioesters in a process catalysed by long-chain ACS at the outer mitochondrial membrane. (2) Long-chain acyl-CoA thioesters are converted into acylcarnitines by CPT1 at the outer mitochondrial membrane. (3) Acylcarnitines are translocated across the inner mitochondrial membrane into the mitochondrial matrix by CACT. (4) CPT2 transfers the acyl moiety from carnitine back to CoA. Next, acyl-CoA thioesters (Cn) are oxidised via β -oxidation into fatty acyl-CoA thioesters shortened by 2 carbons (Cn-2) and an acetyl-CoA molecule. Each β -oxidation cycle is catalysed by 4 enzymes including different dehydrogenases requiring oxidised NAD^+ and FAD as cofactors. Whereas Cn-2 fatty acyl-CoA thioesters re-enter a new 4-step β -oxidation cycle, acetyl-CoA moieties can generate ketone bodies (mainly acetoacetate and β -hydroxybutyrate), which are released into the bloodstream and used by extrahepatic tissues for energy production during fasting. The β -oxidation pathway produces NADH and FADH_2 , which transfer electrons (e^-) to the ETC, thus regenerating NAD^+ and FAD used for further β -oxidation cycles. Within ETC, electrons are sequentially transferred to different inner mitochondrial membrane polypeptide complexes, I to IV. The final electron transfer to oxygen takes place at cytochrome c oxidase, also termed complex IV. mtDNA encodes 13 polypeptides that are components of complexes I, III, IV and V, also termed ATP synthase. The flow of electrons within the ETC is coupled to extrusion of H^+ from the mitochondrial matrix to the intermembrane space, thus creating the mitochondrial transmembrane potential, $\Delta\Psi_m$. When ATP is required, these protons re-enter the matrix through ATP synthase, thus liberating part of $\Delta\Psi_m$ energy, which is used to phosphorylate ADP into ATP. The process of tight coupling between substrate oxidation and ATP synthesis is referred to as OXPHOS. Xenobiotics can impair mitochondrial function through different mechanisms (number 1 to 6). Compounds mentioned in this figure are only examples illustrating these different mechanisms. ACS, acyl-CoA synthetase; CACT, carnitine-acylcarnitine translocase; CPT, carnitine palmitoyltransferase; CoA, coenzyme A; ETC, electron transport chain; FAO, fatty acid oxidation; LCFAs, long-chain fatty acids; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation; PPAR α , peroxisome proliferator-activated receptor- α .

mitochondrial features in human livers are summarised in Table 1.

Abnormalities in energy metabolism

Non-steatotic obesity

Assessing possible changes in mitochondrial functionality in metabolic diseases requires direct comparison with lean insulin-sensitive humans. Koliaki *et al.* employed *ex vivo* high-resolution respirometry with various substrates to measure oxygen fluxes (Table 1) in whole-liver tissue and isolated liver mitochondria from lean and obese individuals with different stages of biopsy-proven NAFLD.²⁴ These studies demonstrated that – contrary to skeletal muscle – maximal uncoupled respiration related to β -oxidation and TCA cycle activity was ~85% higher in livers from obese individuals without steatosis compared to lean controls (Fig. 2, top panel). The elevated OXPHOS capacity in the face of low intrahepatic triglyceride

levels supports the concept of an adaptation of hepatic mitochondria to rising lipid exposure, which – teleologically – should help to protect the liver from lipotoxicity and steatosis at the onset of obesity.²⁴ Accordingly, high-fat intake was shown to induce transient upregulation of 13 OXPHOS genes and mitochondrial respiration in steatosis-resistant A/J mice,²⁵ and increased hepatic ATP content by 16%, measured *in vivo* by ³¹P magnetic resonance spectroscopy (MRS; Table 1), in lean humans without steatosis.²⁶ Altogether, these data suggest that the absence of hepatic fat accumulation results from mitochondrial adaptation or plasticity,²⁷ which may characterise a moderately insulin-resistant obese phenotype²⁸ or an early state of obesity, which over time could lead to NAFLD.²⁹

Steatotic obesity

Despite upregulated hepatic OXPHOS capacity in non-steatotic obesity, studies in obese persons with steatosis (NAFL) revealed heterogeneous results, maybe owing to

Table 1. Methods used to assess features of mitochondria in humans or human liver tissue.

Parameter	Method	Readout	Pros	Cons
Mitochondrial content	Transmission electron microscopy	Mitochondrial area and number	Gold standard, morphologic assessment	Invasive, availability, time
	Protein expression, and activity ratios, proteomics	Maximal CSA, cardiolipin, mtDNA, ETC complexes I-IV, biomarkers of mitochondrial biogenesis	Availability, time	Invasive, no accepted marker, no validation in liver
Mitochondrial bioenergetic efficiency	High-resolution respirometry (HRR)	OXPHOS capacity	Quasi-gold standard, assessment of different OXPHOS states	<i>Ex vivo</i> , invasive, permeabilized tissue
	Near-infrared spectroscopy (NIRS)	Hb oxygenation, cytochrome oxidase aa3 (REDOX state)	Intact tissue	<i>Ex vivo</i> , invasive, indirect measure, limited use
	Liver ³¹ P MRS	[ATP], [Pi] or V _{ATP} (from saturation transfer or upon fructose challenge)	<i>In vivo</i> , intact tissue, suitable for repeated measures and clinical studies	Availability, multidisciplinary expertise, exclusion criteria, time
	Liver ¹³ C MRS + [1- ¹³ C]acetate and [3- ¹³ C]lactate	Mitochondrial oxidation and pyruvate cycling (from ¹³ C incorporation into hepatic glutamate and alanine)	<i>In vivo</i> , intact tissue	Availability, multidisciplinary expertise, exclusion criteria, time
	PET ¹¹ C- or ¹⁸ F-labeled fatty acids or analogues ¹³ C- or ² H-labeled metabolite dilution and positional isotopomer analysis Breath test + ¹³ C-labeled metabolites Genome-scale human metabolic model	Fatty acid β-oxidation Anaplerotic and TCA cycle fluxes (e.g. V _{PC} and V _{CS}), β-oxidation Mitochondrial oxidation Multiomics and splanchnic metabolite flux data	<i>In vivo</i> , intact tissue <i>In vivo</i> , intact tissue <i>In vivo</i> , intact tissue Integrated complex analysis	Radiation, availability, multidisciplinary expertise, indirect measure, time Availability, indirect measure, time Indirect measure, no validation Partly <i>in vitro</i> , partly invasive, multidisciplinary expertise
Mitochondrial quality control	Protein expression, activity, and ratios	Ubiquitin-proteasome system: USPs, ubiquitin Mitochondrial dynamics: Fusion: mitofusins (Mfn1, Mfn2), Opa1 Fission: Drp1 Mitophagy: PINK1/Parkin pathway, Tom20, COXII	Availability, Time	<i>In vitro</i> , invasive, indirect measure, no accepted single marker, no validation in human liver
	Fluorescence-activated cell sorting, confocal imaging	Mitophagy: cell analyses	Direct measure	<i>Ex vivo</i> , invasive, no validation in human liver

COXII, cytochrome c oxidase subunit II; CS, citrate synthase; CSA, CS activity; Drp, dynamin-related protein; ETC, electron transfer chain; Hb, hemoglobin; Mfn, mitofusin; MRS, magnetic resonance spectroscopy; mtDNA, mitochondrial DNA; PC, pyruvate carboxylase; PET, positron emission tomography; PINK1, PTEN-induced kinase 1; USPs, ubiquitin-specific proteases; Tom20, translocase of outer mitochondrial membrane 20.

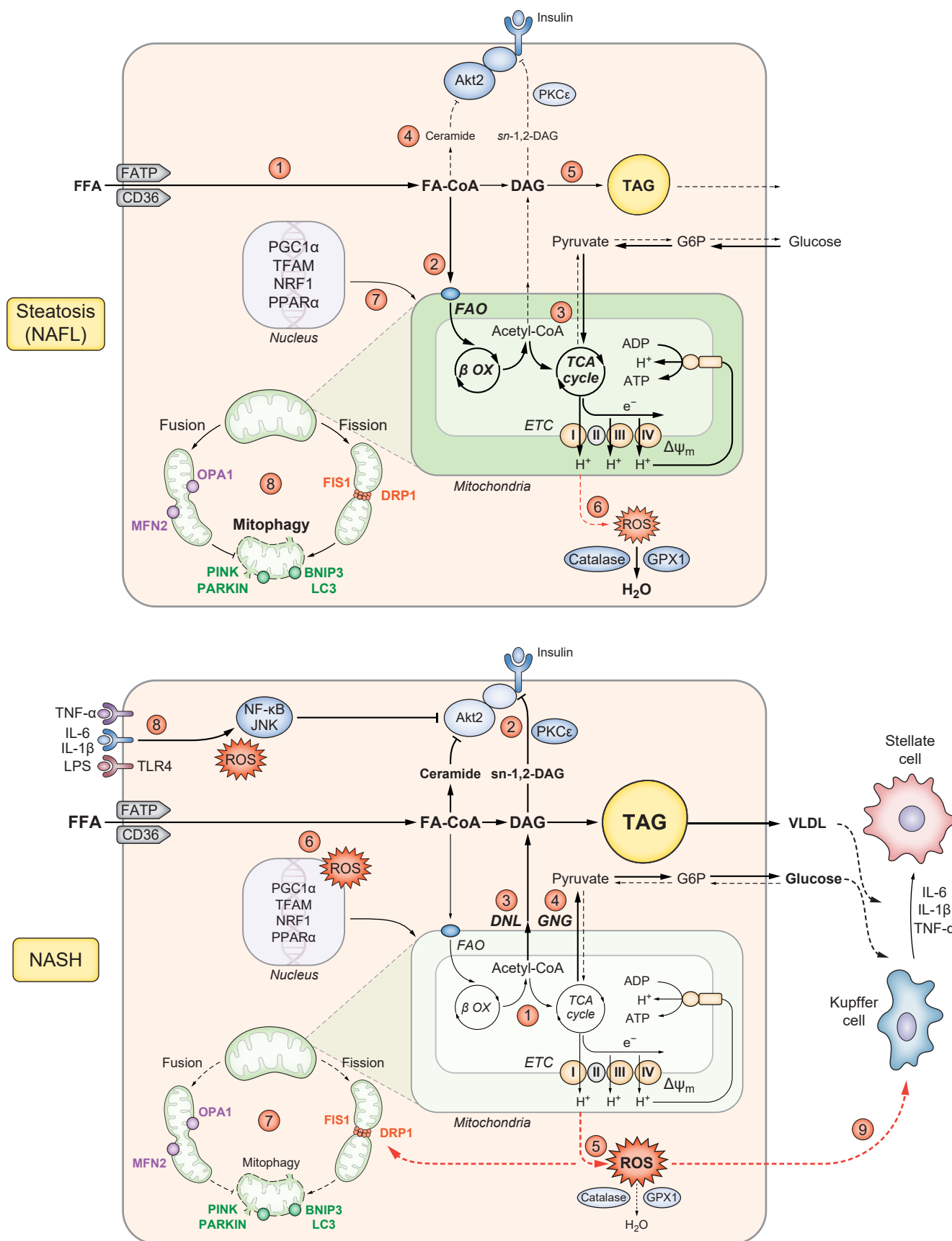


Fig. 2. Mitochondrial alterations in obesity, NAFL and NASH. Top panel: In obesity without or with NAFL (steatosis), greater availability of FFAs (1) increases the intracellular pool of FA-CoA, which (2) stimulates mitochondrial FAO and may also (3) increase TCA cycle and ETC activity. Upregulated mitochondrial oxidative capacity

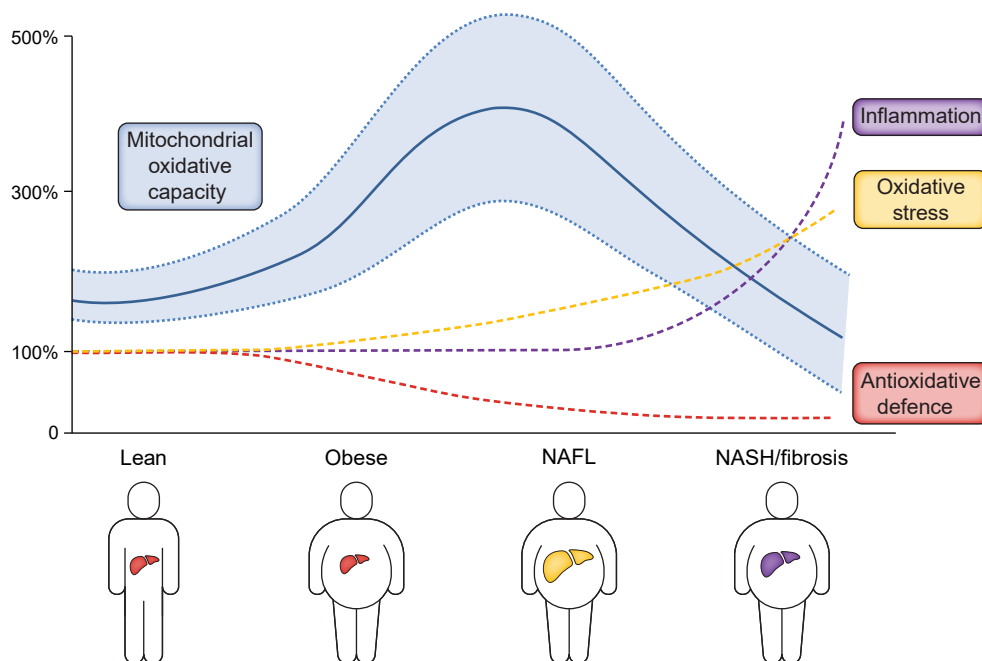


Fig. 3. Concept of hepatic mitochondrial adaption/plasticity during NAFLD development. Mitochondrial oxidative capacity varies broadly across the spectrum of obesity and NAFLD, mainly due to body mass, but also age, insulin sensitivity, concomitant type 2 diabetes, chronic alcohol abuse and possibly genetic variants. Nevertheless, oxidative capacity can transiently increase with longer duration of obesity, stimulating fatty acid oxidation and thereby limiting triglyceride deposition. This favours the generation of oxidative stress, which gradually exhausts antioxidative capacity and contributes to the progressive mitochondrial abnormalities observed in NASH and fibrosis. Chronic oxidative stress stimulates intracellular inflammatory pathways followed by local -intrahepatic- and later systemic inflammation during the course of NASH and fibrosis. NAFL, non-alcoholic fatty liver (steatosis); NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

differences in obesity grade/duration, age, liver fat content and/or the absence of liver histology. The following studies reported on *in vivo* investigations of hepatic energy metabolism in obese people with steatosis but without progressive NAFLD. Non-invasive ^{31}P MRS detected no difference in hepatic ATP content³⁰ and ATP synthase flux rates (V_{ATP})³¹ between elderly obese people with NAFL and young lean volunteers. A study using $[1-^{13}\text{C}]$ acetate infusion to label hepatic $[5-^{13}\text{C}]$ glutamate and $[1-^{13}\text{C}]$ glutamate for *in vivo* ^{13}C MRS (Table 1) also revealed no difference in hepatic citrate synthase (CS) flux (V_{CS}) between young lean/overweight persons without or with NAFL.³² In contrast, $[U-^{13}\text{C}]$ propionate administration to label plasma glucose revealed increased hepatic TCA cycle flux rates (V_{TCA}) as well as anaplerotic flux in middle-aged obese people with steatosis.³³ Greater hepatic V_{TCA} has been repeatedly found in other cohorts^{34–36} and is associated with a switch from lactate to glycerol as the substrate for gluconeogenesis.³⁴ High-resolution respirometry measurements originally revealed a comparable 4–5-fold increase in malate-

glutamate-, malate-octanoylcarnitine-stimulated and maximal uncoupled respiration in hepatic mitochondria isolated from either steatotic or non-steatotic livers of obese individuals vs. non-steatotic livers of lean individuals²⁴ (Fig. 2, top panel). Recent high-resolution respirometry studies also reported increased maximal coupled (significant) and uncoupled ($p = 0.054$) mtDNA/ndDNA-adjusted respiration from malate-glutamate-octanoylcarnitine in livers of obese individuals with NAFL vs. lean individuals without steatosis.³⁷ But no differences were found with this substrate³⁷ or with malate-palmitoylcarnitine between steatotic or non-steatotic livers in people with obesity.³⁸ Interestingly, hepatic oxidative capacity correlated with hepatic triglycerides, plasma free fatty acids and insulin resistance in isolated mitochondria,²⁴ but only with body mass index in liver tissue.³⁷ While these data would suggest steatosis-independent upregulation of oxidative capacity in obesity, some mitochondrial abnormalities could occur early in NAFL, including lower hepatic RCR, along with reduced expression of genes involved in mitochondrial

temporarily (4) protects against lipotoxic-insulin resistance and (5) TAG accumulation, while (6) ROS are scavenged by increased catalase and GPX1 activities. With the onset of steatosis, (7) mitochondrial biogenesis and (8) quality control start to decline. Bottom panel: In NASH, continuous excess FFA overload progressively (1) impairs the efficiency of mitochondrial oxidative capacity, which leads to (2) accumulation of lipotoxic metabolites (e.g. ceramides and DAGs), which induce insulin resistance with (3) augmented GNG and (4) DNL. In the face of decreasing antioxidant activity, (5) increasing ROS production favours oxidation of membrane lipids, proteins and DNA, which (6) impairs mitochondrial biogenesis and (7) quality control, and (8) activates JNK and NF- κ B. Ongoing oxidative stress, hyperglycaemia and dyslipidaemia (9) activate Kupffer cells and stellate cells, which via cytokines (e.g. TNF- α , IL-1 β and IL-6) drive inflammation, fibrosis and disease progression. BNIP3, BCL2 interacting protein 3; DAG, diacylglycerol; DRP1, dynamin-related protein 1; ETC, electron transport chain; FATP, fatty acid transport protein; FA-CoA, fatty acyl coenzyme A; FFA, free fatty acid; FIS1, mitochondrial fission 1 protein; GNG, gluconeogenesis; GPX1, glutathione peroxidase 1; IL-, interleukin; JNK, c-Jun N-terminal kinase; LC3, autophagy-related protein 8 (ATG8); MFN2, mitofusin 2; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; NRF1, nuclear respiratory factor 1; PINK, PTEN-induced kinase; PGC1 α , peroxisome proliferator-activated receptor- γ coactivator 1 α ; PKC ϵ , protein kinase C ϵ ; PPAR α , peroxisome proliferator-activated receptor- α ; ROS, reactive oxygen species; TAG, triacylglycerol; TCA, tricarboxylic acid; TFAM, transcription factor A mitochondrial; TLR4, Toll-like receptor 4; TNF α , tumour necrosis factor- α ; VLDL, very low-density lipoprotein.

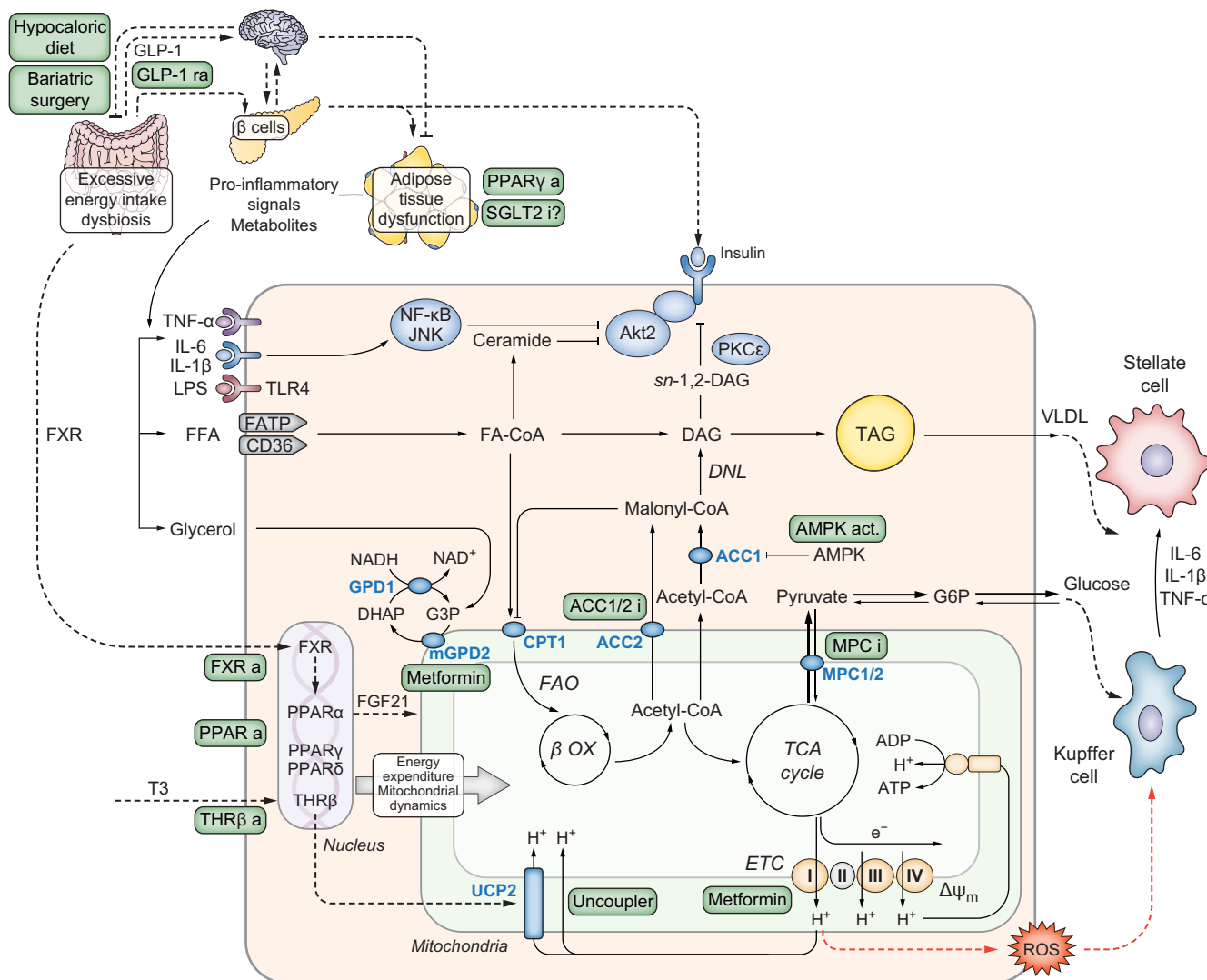


Fig. 4. Interventions (in)directly targeting liver mitochondria in obesity, type 2 diabetes and NAFLD. Aside from lifestyle (hypocaloric diet)- and surgically induced (bariatric surgery) weight loss, several drugs that are being assessed for the treatment of NAFLD target liver mitochondria directly or indirectly (green boxes) respiration. Metformin can inhibit ETC complex I and subsequently activate AMPK, but at low doses metformin inhibits mitochondrial GPD2 and increase cytosolic redox. AMP activators inhibit DNL via cytosolic ACC1, which is also targeted by ACC i. Inhibition of mitochondrial ACC2 and of the MPC complex favours FAO. Other pharmacological agents, e.g. agonists of PPAR $\alpha/\delta/\gamma$, FXR, THR β , and the T3 receptor, act via transcriptional control to augment energy expenditure but also mitochondrial quality control (dynamics). Of note, FXR a target DNL. GLP-1 ra, SGLT2 i and PPAR γ a exert indirect effects on hepatic mitochondrial functionality mainly via weight loss. a, agonist; ACC i, acetyl-coenzyme A carboxylase inhibitors; AMPK, adenosine monophosphate kinase; β OX, β oxidation; CER, ceramides; CPT1, carnitine palmitoyltransferase 1; DAG, diacylglycerols; DNL, *de novo* lipogenesis; DHAP, dihydroxy acetone phosphate; E, energy; ETC, electron transport chain; FA-CoA, fatty acyl-coenzyme A; FAO, fatty acid oxidation; FATP, fatty acid transporters; FGF, fibroblast growth factor; FFA, free fatty acid; FXR, farnesoid X receptor; G3P, glycerol-3-phosphate; G6P, glucose-6-phosphate; GLP-1 r, glucagon-like peptide-1 receptor; GPD, glycerol-3-phosphate dehydrogenase; i, inhibitor; IL-, interleukin-; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; mtDNA, mitochondrial DNA; MPC, mitochondrial pyruvate carrier; PPAR, peroxisome proliferator-activated receptor; ra, receptor agonist; ROS, reactive oxygen species; SGLT2, sodium glucose cotransporter-2; T3, triiodothyronine; TAG, triacylglycerol; TCA, tricarboxylic acid; THR, thyroid hormone receptor; TLR-4, toll-like receptor-4; TNF- α , tumour necrosis factor- α ; UCP2, uncoupling protein 2; VLDL, very low-density lipoprotein.

biogenesis²⁴ or activities of rate-controlling enzymes such as β -hydroxyacyl-CoA dehydrogenase.³⁸ Such mitochondrial alterations might then contribute to oxidative stress and lipid peroxidation²⁴ (Fig. 3).

Diabetes mellitus

Studies in T2DM, which frequently co-exists with NAFLD,^{1–3,39} also revealed varying results. Elderly persons with long-standing T2DM have lower hepatic ATP content and V_{ATP} than age- and body mass-matched glucose-tolerant

persons.^{30,31} Hepatic Pi, but not ATP content, decreased in parallel with rising liver fat content during the 5 years following a diabetes diagnosis.⁴⁰ A ¹³C-ketoisocaproate approach showed an inverse association of reduced mitochondrial function with age, adiposity and diabetes status in people with NAFLD.⁴¹ Conversely, upregulation of hepatic mitochondrial gene expression was reported in overweight individuals with T2DM vs. lean controls in a Japanese cohort,⁴² but data on liver fat content were not provided. One high-resolution respirometry study detected no difference in hepatic OXPHOS capacity

and CS activity between lean individuals, and those with obesity or T2DM.⁴³ The observation that liver lipid droplet area and density only tended to be higher in individuals with T2DM or non-diabetic obesity points to a specific phenotype, as most people with overt T2DM will have steatosis.^{1,2} In this context, recent cluster analyses identified T2DM endotypes (subtypes), discriminating between severely insulin deficient and severely insulin-resistant (from moderate obesity- and age-related diabetes) cases. The severe insulin-resistant diabetes endotype was not only associated with the highest liver fat content at diagnosis, but also with the greater risk of liver fibrosis²⁹ and a higher frequency of the rs738409(G) single nucleotide polymorphism in the *PNPLA3* (patatin-like phospholipase domain-containing 3) gene.⁴⁴ Nevertheless, despite growing evidence, there is currently no proof of a causal interaction between mitochondrial function and genes that increase the risk of NAFLD development and progression.^{45,46}

Also, individuals with autoimmune or type 1 diabetes mellitus may display altered mitochondrial features in different tissues, which mainly relate to glycaemic control and insulin resistance.⁴⁷ Recent analyses of the German Diabetes Study showed that hepatic γ ATP decreases and negatively correlates with glycaemic control, despite unchanged liver fat content, 5 years after the diagnosis of type 1 diabetes mellitus.⁴⁰ It is conceivable that the decreased ATP content resulted from unphysiological insulin supply, leading to impaired suppression of gluconeogenesis and glucose production.

NASH and fibrosis

It has long been known that progressive NAFLD is associated not only with complex metabolic derangements including severe insulin resistance,^{1,2,27} but also with ultrastructural mitochondrial defects, e.g. larger size, loss of mitochondrial cristae, paracrystalline inclusions or linear crystalline inclusions in swollen mitochondria.^{48,49} This might suggest a progressive decline in hepatic respiratory capacity during the transition from steatosis to NASH and cirrhosis.^{50,51} Indeed, several human studies reported evidence for a decline in hepatic mitochondrial functionality, i.e. impaired hepatic ATP repletion after fructose-induced ATP depletion (Table 1),⁵² decreased ETC complex activity⁵³ and increased hepatic expression of uncoupling protein-2.⁵⁴ Uncoupling protein-2 upregulation leads to dissociation of OXPHOS from ATP production and reduction of the redox pressure on the ETC, thereby protecting against liver damage at the expense of impaired capacity to respond to metabolic demands, as present in a dyslipidaemic and inflammatory milieu.^{55,56} Nonetheless, the excess availability of free fatty acids will overload the ETC, increase ROS production and oxidative stress, as well as disrupt redox homeostasis^{27,57,58} (Figs 2 and 3).

To clarify whether hepatic mitochondrial oxidative capacity is indeed reduced in human biopsy-proven NASH (NAFLD activity score [NAS] ≥ 5), Koliaki *et al.* compared hepatic oxygen fluxes by high-resolution respirometry between lean and obese people without NASH and obese people with NASH²⁴ (Fig. 2, bottom panel). In NASH, hepatic OXPHOS capacity was ~ 40 – 51% lower than in obese individuals with/without steatosis and $\sim 10\%$ lower than in lean non-steatotic humans.²⁴ RCR remained low, as in obese people without NASH, whereas leaking control ratio (reflecting abnormal proton leak

across the inner membrane), H_2O_2 production and oxidative DNA damage increased, while antioxidative defence declined. Another study expanded on these observations by stratifying people with NAFLD into borderline and definite NASH groups based on the presence of histopathological ballooning and fibrosis (by NAS).³⁸ The definite NASH group featured lower complete [1 - ^{14}C]palmitate oxidation and lower incomplete FAO (i.e. oxidation to ^{14}C -acid-soluble metabolites) in whole-liver tissue. This study further confirmed higher mitochondrial H_2O_2 emission in the presence of palmitoyl-CoA, which rises with increasing inflammation and ballooning stages.³⁸ Further high-resolution respirometry studies reported a similar pattern of respiratory capacity, being numerically highest in NAFL, intermediate in NASH and non-steatotic obesity, and lowest in non-steatotic lean livers, but it was concluded that hepatic respiration is preserved in NAFLD.³⁷

Despite some evidence for sexual dimorphism in obesity and NAFLD, the available studies on hepatic oxygen fluxes in humans were too small to fully address this question.^{24,37,38} One study showed no association between sex and hepatic maximal OXPHOS capacity,³⁷ whereas another study found higher liver pyruvate kinase in males, which related to NAFLD severity, and demonstrated improved mitochondrial function in male mice following pyruvate kinase-silencing.⁵⁹

Notably, *in vivo* ^{13}C -octanoate breath testing (Table 1) revealed increased hepatic β -oxidation in NASH.⁶⁰ This discrepancy with palmitate oxidation hints at specific impairment of the mitochondrial entry of long-chain fatty acids via the carnitine palmitoyltransferase (CPT) shuttle, in line with investigations in rodent NASH models.^{61–63} The lower efficiency of mitochondrial β -oxidation of long-chain fatty acids could explain the cellular accumulation of lipotoxins, i.e. free fatty acids, diacylglycerols (DAGs) and ceramides (Fig. 4). Hepatic DAG concentrations correlate with steatosis grade, NAS and insulin resistance in people with obesity,⁶⁴ maybe due to specific membrane-bound DAG species.⁶⁵ Of note, certain hepatic ceramides and sphingolipids do not necessarily correlate with hepatic insulin resistance, but rather with hepatic oxidative capacity, oxidative stress and inflammation, and are higher in obese people with NASH than in those with or without NAFL.^{66,67} Further studies are needed to evaluate whether lipotoxins mediate mitochondrial effects on NAFLD progression and can serve as biomarkers in NASH.⁶⁸ As T2DM is not only associated with greater insulin resistance and lipotoxicity, but also with accelerated NAFLD progression, one might hypothesise that T2DM associates with more severe hepatic mitochondrial abnormalities. Gancheva *et al.* compared hepatic oxygen fluxes in obese individuals with histologically proven NASH, with/without T2DM, vs. lean individuals without steatosis.⁶⁹ Despite comparable histopathology, individuals with T2DM and NASH had $\sim 33\%$ lower complex II-linked oxidative capacity from TCA cycle substrates than those with non-diabetic NASH and had higher H_2O_2 production than lean individuals.⁶⁹ Although hyperglycaemia and reactive dicarbonyls may cause oxidative stress, advanced glycation end products largely failed to explain the altered hepatic mitochondrial capacity.⁶⁹ Further analysis demonstrated lower hepatic OXPHOS capacity and antioxidant defences in obese

people with NASH and hepatic fibrosis score ≥ 1 (F1+) vs. those without fibrosis (F0).⁶⁹

Concept of mitochondrial adaptation/plasticity

Collectively, these data support the contention that hepatic OXPHOS capacity is upregulated in obesity and early obesity-related NAFL in response to adipose tissue-derived lipid flux to the liver, but continuously declines with rising lipotoxic and oxidative stress during progression to NASH and fibrosis (Fig. 3). Indeed, rodent models demonstrated transient upregulation of mitochondrial function during development of metabolic disease-related fatty liver,^{70–72} but impairment in NASH.^{51,73–75} Moreover, integrating multiomics and splanchnic metabolite flux measurements in humans (Table 1) provided independent support for the concept of increasing metabolic stress and loss of metabolic adaptability in NAFLD.³⁴ This study showed increased mitochondrial metabolism, glyceroneogenesis and a switch from lactate to glycerol as the substrate for gluconeogenesis, which may contribute to a vicious cycle in the pathogenesis of NAFLD and T2DM.³⁴ Of note, these cross-sectional studies do not allow us to draw firm conclusions on causality and the sequence of events but provide support for prospective studies monitoring hepatic mitochondrial functionality over time in humans, specifically in advanced NAFLD, to prove the concept of gradually declining mitochondrial adaptation/plasticity.

Changes in mitochondrial dynamics

In humans, assessment of mitochondrial content and quality control, *i.e.* mitophagy and mitochondrial dynamics,⁷⁶ bears some limitations, because it is based on *ex vivo* measurements⁷⁷ rather than on direct real-time monitoring of the processes (Table 1). As for mitochondrial content, CS activity was found to be increased in NASH,²⁴ gradually higher in T2DM with NASH,⁶⁹ but not different in borderline or definite NASH in another cohort.³⁸ Protein expression of ETC complexes was unchanged,³⁸ slightly to moderately^{24,69} or markedly lower in NASH⁵³ and decreased in F1+⁶⁹ and F2-F4.³⁸ Likewise, mtDNA copy number was lower in obese individuals with NASH than in lean controls.⁶⁹ The mRNA expression of biomarkers of mitochondrial biogenesis was already slightly lower in obese individuals with or without NAFL (*PGC1A*, *NRF1*, *TFAM*)²⁴ and was markedly diminished in those with NASH (*PGC1A*, *NRF1*, *TFAM*, sirtuin 1, *AMPK*).^{24,38} Despite certain variability, these studies demonstrate defects in mitochondrial biogenesis and point to differences in mitochondrial quality control across the NAFLD spectrum. While mitochondrial dynamics were clearly impaired in many murine models, *e.g.* diet-induced hepatic insulin resistance,⁷⁸ *PGC1 α* overexpression,⁷⁹ liver-specific deletion of dynamin-related protein GTPases for division/fission (dynamin-related protein 1 [Drp1]) and fusion (*Opa1*),⁸⁰ or of mitochondrial fission factor,⁸¹ few clinical studies examined mitochondrial quality control. The higher mitochondrial content despite lower oxidative capacity observed in some studies would suggest impaired removal of dysfunctional mitochondria, which is supported by the loss of the mitophagy-

associated adaptor protein BNIP3 (BCL2-interacting protein 3) with progression of NAFLD and reduction in *OPA1*, mitofusin 2, and pDRP^{S616} in NAFL and definite NASH.³⁸ Liver-specific pDRP^{S616} deletion leads to impaired FAO and mitochondrial enlargement, resulting in megamitochondria,⁸⁰ a typical sign of human NASH. Another study reported a reduction only in the fusion marker *MFN2*, in a NASH cohort with and without T2DM, despite impressive mitochondrial ultrastructural alterations, *e.g.* swelling, autophagosome activity and matrix degeneration.⁶⁹ Among other factors, excessive ROS production could impede the physiological fission/fusion cycles, resulting in mitophagy arrest and leaving hepatocytes with high amounts of damaged mitochondria that cannot be recycled, but inducing the mitochondrial unfolded protein response and cytochrome *c*-related apoptosis.⁸²

Mitochondrial alterations in xenobiotic-induced fatty liver diseases

Fatty liver diseases can also be induced by some xenobiotics, including pharmaceuticals and environmental toxicants. However, in contrast to metabolic disease-related liver diseases, there are only a few clinical studies reporting mitochondrial alterations in xenobiotic-induced fatty liver diseases, likely due to their relatively low incidence and the challenge of diagnosis.⁸³ Hence, the following sections are mainly based on experimental studies (Fig. 1).

Abnormalities in energy metabolism

Xenobiotic-induced fatty liver diseases can present as two main entities, associated with distinct mitochondrial alterations. Microvesicular steatosis, a rare but potentially fatal liver lesion, is caused by severe inhibition of mitochondrial FAO,^{7,83} whereas macrovesicular steatosis is not a pure mitochondrial disease and is often asymptomatic in the short term.^{7,83} Lipid accumulation in macrovesicular steatosis results partly from moderate impairment of mitochondrial FAO, but may also result from other mechanisms *e.g.* activated *de novo* lipogenesis and reduced very low-density lipoprotein secretion.^{84–86}

There is a number of mechanisms by which xenobiotics can induce inhibition of mitochondrial FAO (Fig. 1), which are not mutually exclusive: (i) direct FAO impairment, *e.g.* through inhibition of CPT1 and acyl-CoA dehydrogenases,^{87,88} (ii) reduced PPAR α expression and activity;⁸⁹ (iii) inhibition of ETC complexes, limiting NAD⁺ and FAD availability for FAO;^{7,87,90} (iv) mtDNA depletion, which impairs ETC activity and FAO.^{7,8} Of note, alcohol-related microvesicular steatosis might be secondary to these four mechanisms.^{86,91,92} Interestingly, alcohol-related alterations in both mitochondrial FAO and ETC activity could result from impaired mitochondrial targeting of methionine adenosyltransferase α -1 (*MAT α 1*).⁹³ Reduced mtDNA after repeated or chronic alcohol intoxication seems to be linked, at least partly, to the blockage of mtDNA replication caused by the accumulation of unrepaired oxidative DNA lesions.^{91,94} Alcohol-related mtDNA depletion could also result from impairment of the *NRF1* signalling pathway.⁹⁵

Another mechanism of xenobiotic-induced impairment of mitochondrial FAO and steatosis is intrahepatic L-carnitine depletion (Fig. 1), which can result from the formation and renal excretion of xenobiotic-carnitine derivatives.^{87,96,97}

Like NAFLD, xenobiotic-induced macrovacuolar steatosis can progress in the long-term to steatohepatitis and cirrhosis.^{7,83,98} Regarding drugs, progression to steatohepatitis has been described in people treated with anti-anginal agents, amiodarone and perhexiline, and the antifolate drug methotrexate.^{7,99} Carbon tetrachloride, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and alcohol abuse can also induce steatohepatitis.^{98,100,101}

Although the mechanisms underlying the xenobiotic-induced transition of steatosis to steatohepatitis may be complex, altered mitochondrial function likely plays a significant role. For instance, amiodarone- and perhexiline-induced impairment of the ETC favours ROS overproduction and lipid peroxidation, which in turn could promote necroinflammation and fibrosis,^{102–104} as suggested for ethanol-induced steatohepatitis.^{101,105} Ethanol-induced mitochondrial ROS overproduction may result from impaired ETC, but also higher CYP2E1 levels in these organelles.^{22,105}

It remains unclear why some xenobiotics induce severe mitochondrial alterations and microvesicular steatosis in a few people, but only moderate alterations and macrovacuolar steatosis in a larger number of individuals. An attractive hypothesis is the presence of genetic predisposition affecting basal mitochondrial function, e.g. congenital defects in enzymes involved in mitochondrial FAO, OXPHOS and antioxidant defence.^{103,106,107} For instance, polymorphisms (or mutations) in the gene encoding DNA polymerase γ may favour mitochondrial failure and microvesicular steatosis induced by valproic acid and some antiretroviral nucleoside analogues.^{103,106}

Changes in mitochondrial dynamics

The altered mitochondrial dynamics induced by some xenobiotics have mostly been investigated in the context of alcohol-related fatty liver disease. Recent experimental studies suggested that alcohol-related steatosis could result from abnormal activation of mitochondrial fission via increased expression of Drp1.^{108,109} Interestingly, ethanol-stimulated Drp1 expression resulted from upregulation of orphan NR4A1 (nuclear receptor subfamily 4 group A member 1) signalling and p53 activation.¹⁰⁸ Drp1 upregulation might also be involved in aflatoxin B1-induced exacerbation of steatosis in the liver of HBV-transgenic mice and HBV-X protein-expressing human hepatocytes.¹¹⁰ Finally, several investigations suggested that acetaminophen promotes mitochondrial fission directly via increased Drp1 expression and indirectly by suppressing fusion proteins such as mitofusin 1 and OPA1.¹¹¹ However, it remains to be confirmed whether acetaminophen-induced mitochondrial fission is pathogenic in the few reported cases of hepatic steatosis caused by this painkiller.^{7,112}

Hepatic mitochondrial alterations in the mutual interaction of xenobiotics and metabolic disorders

Some xenobiotics can aggravate metabolic disease-related liver diseases either by worsening preexisting hepatic lipid

deposition and/or by hastening the transition from fatty liver to NASH.^{23,113} Although most knowledge is derived from rodents and cultured cells, several clinical studies also support the interaction between some xenobiotics and metabolic diseases.²³ From a clinical perspective, compelling evidence has been gathered for drugs, e.g. methotrexate, stavudine, corticosteroids, irinotecan and tamoxifen.^{23,114–116} Alcohol abuse also increases the risk of aggravating fatty liver disease in people with obesity.^{23,117} Experimental investigations further demonstrated worsening of obesity-associated fatty liver disease by environmental toxicants, e.g. bisphenol A, TCDD, perchloroethylene and nonylphenol.²³

Some studies pointed to abnormal mitochondrial function as a mechanism underlying xenobiotic-induced aggravation of metabolic disease-related fatty liver diseases.²³ Obese ethanol-intoxicated mice showed reduced hepatic ATP content and mRNA levels of *Nrf1*, *Ppargc1a* (encoding PGC1 α) and *Ppara* (encoding PPAR α).^{118,119} Reduced CPT1 expression might explain why exposing pregnant rats to bisphenol A exacerbates hepatic lipid accumulation in male offspring fed a high-fat diet after weaning.¹²⁰ Of note, methotrexate, stavudine, corticosteroids and tamoxifen can impair mitochondrial function through different mechanisms.^{114,121} However, no studies have shown an additive/synergistic effect of obesity and these drugs on mitochondrial function or quality control in the liver.

The zebrafish model has been useful to examine NAFLD and toxicant-induced liver injury.^{122,123} A recent study showed that ethanol and benzo[a]pyrene exposure triggers the progression of NAFL to NASH in obese zebrafish larvae.¹²⁴ This was most probably due to aryl hydrocarbon receptor-dependent mitochondrial accumulation of labile iron, thereby impairing mitochondrial respiration.¹²⁵ Interestingly, combined ethanol- and benzo[a]pyrene-induced NAFLD progression was also reproduced in human HepaRG cells incubated with a mixture of stearic and oleic acids.¹²⁶ Moreover, the transition of steatosis to a NASH-like state in HepaRG cells was associated with mitochondrial ROS overproduction, lower mtDNA levels and reduced mitochondrial respiration.¹²⁶

Therapeutic implications

Caloric restriction

Low-energy nutrition and bariatric (metabolic) surgery currently represent the most efficient strategies to treat or even reverse common metabolic disorders (Fig. 4). While some studies have addressed the acute effects of hypercaloric nutrition on hepatic energy metabolism in lean humans,^{26,127} Luukkonen *et al.* recently described the effects of a 6-day ketogenic diet in overweight/obese volunteers using co-infusion of [D₇]glucose, [¹³C₄]β-hydroxybutyrate and [3-¹³C]lactate.¹²⁸ In the face of a 3% weight loss, liver fat content decreased by 31% despite elevated plasma free fatty acid concentrations, which was associated with multiple adaptations including lower hepatic CS flux – possibly due to 1.67-fold higher mitochondrial NADH levels.¹²⁸

Bariatric (metabolic) surgery

Despite the impressive effect of surgery on body weight and NAFLD in humans, evidence of its effects on hepatic mitochondrial function, *i.e.* increased OXPHOS capacity, CS

activity, ETC gene expression and reduced oxidative stress, has mainly come from studies in rodents.^{129,130} One clinical study performed liver biopsies during Roux-en-Y gastric bypass surgery or sleeve gastrectomy and Tru-Cut percutaneous liver biopsies 12 months later in people with obesity. Along with metabolic improvements and a 2-point reduction in NAS, hepatic respiration (specifically from ETC complexes II and IV) increased along with a higher mtDNA/nDNA ratio independent of the type of surgery.³⁷ This mirrors the gradual increase in skeletal muscle respiration from ETC complex II and expression of genes involved in mitochondrial function 12 months after metabolic surgery.¹³¹

Physical exercise training

Exercising improves mitochondrial features in skeletal muscle and clinical features of NAFLD.^{3,132} There is some evidence for exercise-mediated mitochondrial adaptation in rodent livers.¹³³ In humans, a 3-month combined aerobic and resistance training programme led to a decrease in hepatic fat content in people with or without NAFL, but no changes in the hepatic ATP/Pi ratio,¹³⁴ which of course does not exclude other effects on liver mitochondrial functionality.

Pharmacological treatments

Metformin has been the first-line antihyperglycaemic drug for T2DM, which among a plethora of systemic effects also targets mitochondrial functionality¹³⁵ (Fig. 4), by inhibiting ETC complex I (with subsequent AMPK activation at high concentrations) or AMPK-independent inhibition of mitochondrial glycerol-3-phosphate dehydrogenase and the subsequent rise in cytosolic redox state and fall of lactate-mediated gluconeogenesis.^{136,137} However, no reports from prospective placebo-controlled studies have addressed the effects of metformin on hepatic mitochondrial function in humans. Interestingly, a phase IIa trial with a direct AMPK activator (PXL770) failed to show an improvement in liver fat content in people with MRI-diagnosed steatosis,¹³⁸ which suggests that AMPK activation alone may not be sufficient to alleviate NAFLD.

Because of their potential to stimulate hepatic β -oxidation and restore mitochondrial function, thyroid hormone-related strategies have recently attracted interest as treatments for NAFLD. They include thyroxin and its analogues, iodothyronines and more recently thyromimetics.¹³⁹ Liver-selective thyroid hormone receptor agonists should improve NAFLD independently of weight loss and without (extra-)hepatic side effects. In a phase II placebo-controlled trial, the liver-directed, orally active, selective THR β agonist, resmetirom (MGL-3196), resulted in lower liver fat content by MRI-proton density fat fraction, in a >2-point NAS reduction, but – despite decreased serum fibrosis markers – did not affect fibrosis as assessed by histology.¹⁴⁰ Resmetirom is currently being studied in larger clinical trials in NASH cohorts (MAESTRO-NASH/NCT03900429).

Finally, mitochondrial uncouplers that use an ATP synthase-independent pathway to generate heat from dissipation of the

proton gradient cause effective weight loss, but were banned from clinical use, mainly because of the risk of life-threatening hyperthermia.¹⁴¹ Currently, several compounds with mitochondrial protonophore properties are being developed and showed some beneficial effects on dyslipidaemia and steatosis in animal models¹⁴² (Fig. 4).

Given the dynamic mitochondrial adaptation that occurs during the development and progression of NAFLD, upregulation of intrahepatic mitochondrial pathways will not necessarily be beneficial. Consequently, stimulating oxidative metabolism might accelerate oxidative stress, lipid peroxidation and inflammation in NASH.⁷³ In this context, current pharmacological concepts mainly act indirectly on the liver via improvement of adipose tissue function either due to weight loss (liraglutide, semaglutide, empagliflozin, dapagliflozin) or due to adipose tissue remodelling (pioglitazone)³ (Fig. 4). Novel strategies, such as dual agonists addressing glucagon-like peptide-1 and glucagon receptors, could combine both the weight losing effect with direct effects on hepatic mitochondrial functionality to more efficiently treat NAFLD, as demonstrated for cotadutide in murine NASH models.¹⁴³

Regardless of the potential benefit of the aforementioned pharmacological treatments in NAFLD, physicians should be aware that the fatty liver is more susceptible to drug-induced injury. Hence, people with NAFLD who are prescribed new drugs should be closely monitored for drug-induced liver injury,^{144,145} which is particularly relevant in the context of possible mitochondrial toxicity.¹⁴⁶

Conclusion

Fatty liver diseases due to common metabolic diseases or xenobiotics share several similarities, but also substantial differences regarding toxic effects on mitochondrial functionality. Nevertheless, metabolic disorders and alcohol abuse and/or pharmaceutical drugs frequently go hand-in-hand and can potentiate each other's toxic effects on energy metabolism,^{23,117} which makes it difficult to delineate their specific action in fatty liver disease. Additionally, the assessment of the various features of hepatic mitochondrial functionality and quality control remains challenging in humans (Table 1), for whom only expensive and time-consuming techniques or invasive tests are currently available.¹⁴⁷ Thus, detailed examination of hepatic mitochondria calls for non-invasive methods, which should also enable more time-course studies to follow disease progression,^{37,40} and monitor the effects of drugs affecting hepatic energy metabolism in clinical trials. In this context, novel biomarkers of abnormal mitochondrial function, e.g. extracellular vesicles¹⁴⁸ or circulating cell-free mitochondria,¹⁴⁹ are currently being evaluated. Such developments might also help to identify drugs and other xenobiotics that can aggravate mitochondrial alterations in NAFLD. Finally, such biomarkers will not only increase our knowledge on the pathophysiological role of mitochondria, but will further enable validation of targets aimed at restoring defective mitochondrial function in fatty liver diseases, particularly by

concomitantly improving FAO and ETC activity to prevent oxidative stress.^{3,10,73} Aside from the aforementioned therapeutic concepts, further innovative strategies could aim to

increase PGC1 α activity^{73,79} and mitochondrial MAT α 1 levels⁹³ or reduce the expression of methylation-controlled J protein¹⁵⁰ and OPA1.^{80,151}

Affiliations

¹INSERM, Univ Rennes, INRAE, Institut NUMECAN (Nutrition Metabolisms and Cancer) UMR_A 1341, UMR_S 1241, F-35000, Rennes, France; ²Department of Endocrinology and Diabetology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany; ³Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich-Heine University Düsseldorf, Düsseldorf, Germany; ⁴German Center for Diabetes Research, Partner Düsseldorf, München-Neuherberg, Germany

Abbreviations

Acetyl-CoA, acetyl-coenzyme A; AMPK, AMP-activated protein kinase; CPT, carnitine palmitoyl transferase-I; CS, citrate synthase; CYP, cytochrome P450; ETC, electron transport chain; FAO, fatty acid oxidation; MAT α 1, methionine adenosyltransferase α -1; MRS, magnetic resonance spectroscopy; mtDNA, mitochondrial DNA; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; nDNA, nuclear DNA; NRF, nuclear respiratory factor; OXPHOS, oxidative phosphorylation; PGC1, peroxisome proliferator-activated receptor- γ coactivator 1; PPAR, peroxisome proliferator-activated receptor; RCR, respiratory control ratio; ROS, reactive oxygen species; T2DM, type 2 diabetes mellitus; TCA, tricarboxylic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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Conflict of interest

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Please refer to the accompanying ICMJE disclosure forms for further details.

Author contribution

Both authors jointly contributed to concept, review of the available literature, preparation of illustrations and interpretation of the evidence.

Supplementary data

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References

Author names in bold designate shared co-first authorship

- [1] Karlens TH, Sheron N, Zelber-Sagi S, Carrieri P, Dusheiko G, Bugianesi E, et al. The EASL-Lancet Liver Commission: protecting the next generation of Europeans against liver disease complications and premature mortality. *Lancet* 2022;399(10319):61–116.
- [2] Targher G, Corey KE, Byrne CD, Roden M. The complex link between NAFLD and type 2 diabetes mellitus - mechanisms and treatments. *Nat Rev Gastroenterol Hepatol* 2021;18:599–612.
- [3] Pafili K, Roden M. Nonalcoholic fatty liver disease (NAFLD) from pathogenesis to treatment concepts in humans. *Mol Metab* 2021;50:101122.
- [4] Gancheva S, Jelenik T, Álvarez-Hernández E, Roden M. Interorgan metabolic crosstalk in human insulin resistance. *Physiol Rev* 2018;98:1371–1415.
- [5] **Roden M, Shulman GI.** The integrative biology of type 2 diabetes. *Nature* 2019;576(7785):51–60.

- [6] Azzu V, Vacca M, Virtue S, Allison M, Vidal-Puig A. Adipose tissue-liver cross talk in the control of whole-body metabolism: implications in non-alcoholic fatty liver disease. *Gastroenterology* 2020;158:1899–1912.
- [7] Fromenty B. Inhibition of mitochondrial fatty acid oxidation in drug-induced hepatic steatosis. *Liver Res* 2019;3:157–169.
- [8] Fromenty B. Alteration of mitochondrial DNA homeostasis in drug-induced liver injury. *Food Chem Toxicol* 2020;135:110916.
- [9] Ramachandran A, Visschers RGJ, Duan L, Akakpo JY, Jaeschke H. Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives. *J Clin Transl Res* 2018;4:75–100.
- [10] Dewidar B, Kahl S, Pafili K, Roden M. Metabolic liver disease in diabetes – from mechanisms to clinical trials. *Metabolism* 2020;111S:154299.
- [11] Rui L. Energy metabolism in the liver. *Compr Physiol* 2014;4:177–197.
- [12] Schirmacher V. Mitochondria at work: new insights into regulation and dysregulation of cellular energy supply and metabolism. *Bio-medicines* 2020;8:526.
- [13] Houten SM, Violante S, Ventura FV, Wanders RJ. The biochemistry and physiology of mitochondrial fatty acid β -oxidation and its genetic disorders. *Annu Rev Physiol* 2016;78:23–44.
- [14] Wang CH, Wei YH. Roles of mitochondrial sirtuins in mitochondrial function, redox homeostasis, insulin resistance and type 2 diabetes. *Int J Mol Sci* 2020;21:5266.
- [15] Ploumi C, Daskalaki I, Tavernarakis N. Mitochondrial biogenesis and clearance: a balancing act. *FEBS J* 2017;284:183–195.
- [16] Ni HM, Williams JA, Ding WX. Mitochondrial dynamics and mitochondrial quality control. *Redox Biol* 2015;4:6–13.
- [17] Xian H, Liou YC. Functions of outer mitochondrial membrane proteins: mediating the crosstalk between mitochondrial dynamics and mitophagy. *Cell Death Differ* 2021;28:827–842.
- [18] Conde de la Rosa L, Garcia-Ruiz C, Vallejo C, Baulies A, Nuñez S, Monte MJ, et al. STARD1 promotes NASH-driven HCC by sustaining the generation of bile acids through the alternative mitochondrial pathway. *J Hepatol* 2021;74:1429–1441.
- [19] Kakimoto PA, Tamaki FK, Cardoso AR, Marana SR, Kowaltowski AJ. H₂O₂ release from the very long chain acyl-CoA dehydrogenase. *Redox Biol* 2015;4:375–380.
- [20] Galicia-Moreno M, Lucano-Landeros S, Monroy-Ramirez HC, Silva-Gomez J, Gutierrez-Cuevas J, Santos A, et al. Roles of Nrf2 in liver diseases: molecular, pharmacological, and epigenetic aspects. *Antioxidants (Basel)* 2020;9:980.
- [21] Callegari S, Dennerlein S. Sensing the stress: a role for the UPR^{mt} and UPRsm in the quality control of mitochondria. *Front Cell Dev Biol* 2018;6:31.
- [22] Massart J, Begriche K, Hartman JH, Fromenty B. Role of mitochondrial cytochrome P450 2E1 in healthy and diseased liver. *Cells* 2022;11:288.
- [23] Massart J, Begriche K, Corlu A, Fromenty B. Xenobiotic-induced aggravation of metabolic-associated fatty liver disease. *Int J Mol Sci* 2022;23:1062.
- [24] Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metab* 2015;21:739–746.
- [25] Poussin C, Ibberson M, Hall D, Ding J, Soto J, Abel ED, et al. Oxidative phosphorylation flexibility in the liver of mice resistant to high-fat diet-induced hepatic steatosis. *Diabetes* 2011;60:2216–2224.
- [26] **Hernández EÁ, Kahl S, Seelig A, Begovatz P, Irmiler M, Kupriyanova Y, et al.** Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. *J Clin Invest* 2017;127:695–708.
- [27] Koliaki C, Roden M. Alterations of mitochondrial function and insulin sensitivity in human obesity and diabetes mellitus. *Annu Rev Nutr* 2016;36:337–637.

- [28] Blüher M. Metabolically healthy obesity. *Endocr Rev* 2020;41:bnaa004.
- [29] Zaharia OP, Strassburger K, Strom A, Böhnhof GJ, Karusheva Y, Antoniou S, et al. Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. *Lancet Diabetes Endocrinol* 2019;7:684–694.
- [30] Szendroedi J, Chmelik M, Schmid AI, Nowotny P, Brehm A, Krssak M, et al. Abnormal hepatic energy homeostasis in type 2 diabetes. *Hepatology* 2009;50:1079–1086.
- [31] Schmid AI, Szendroedi J, Chmelik M, Krssak M, Moser E, Roden M. Liver ATP synthesis is lower and relates to insulin sensitivity in patients with type 2 diabetes. *Diabetes Care* 2011;34:448–453.
- [32] Petersen KF, Befroy DE, Dufour S, Rothman DL, Shulman GI. Assessment of hepatic mitochondrial oxidation and pyruvate cycling in NAFLD by ¹³C magnetic resonance spectroscopy. *Cell Metab* 2016;24:167–24171.
- [33] Sunny NE, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metab* 2011;14:804–810.
- [34] **Hyötyläinen T, Jerby L, Petäjä EM, Mattila I, Jääntti S, Auvinen P, et al.** Genome-scale study reveals reduced metabolic adaptability in patients with non-alcoholic fatty liver disease. *Nat Commun* 2016;7:8994.
- [35] Satapati S, Kucejova B, Duarte JAG, Fletcher JA, Reynolds L, Sunny NE, et al. Mitochondrial metabolism mediates oxidative stress and inflammation in fatty liver. *J Clin Invest* 2015;125:4447–4462.
- [36] **Fletcher JA, Deja S, Satapati S, Fu X, Burgess SC, Browning JD.** Impaired ketogenesis and increased acetyl-CoA oxidation promote hyperglycemia in human fatty liver. *JCI Insight* 2019;4:e127737.
- [37] Pedersen JS, Rygg MO, Chrøis K, Sustarsic EG, Gerhart-Hines Z, Wever Albrechtsen NJ, et al. Influence of NAFLD and bariatric surgery on hepatic and adipose tissue mitochondrial biogenesis and respiration. *Nat Commun* 2022;13:2931.
- [38] Moore MP, Cunningham RP, Meers GM, Johnson SA, Wheeler AA, Ganga RR, et al. Compromised hepatic mitochondrial fatty acid oxidation and reduced markers of mitochondrial turnover in human NAFLD. *Hepatology* 2022 Jan 9. <https://doi.org/10.1002/hep.32324>. Online ahead of print.
- [39] Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. *J Hepatol* 2019;71:793–801.
- [40] Kupriyanova Y, Zaharia OP, Bobrov P, Karusheva Y, Burkart V, Szendroedi J, et al. Early changes in hepatic energy metabolism and lipid content in recent-onset type 1 and 2 diabetes mellitus. *J Hepatol* 2021;74:1028–1037.
- [41] Afolabi PR, Scorletti E, Smith DE, Almeshmadi AA, Calder PC, Byrne CD. The characterisation of hepatic mitochondrial function in patients with non-alcoholic fatty liver disease (NAFLD) using the ¹³C-ketocaproate breath test. *J Breath Res* 2018;12:046002.
- [42] Misu H, Takamura T, Matsuzawa N, Shimizu A, Ota T, Sakurai M, et al. Genes involved in oxidative phosphorylation are coordinately upregulated with fasting hyperglycaemia in livers of patients with type 2 diabetes. *Diabetologia* 2007;50:268–277.
- [43] Lund MT, Kristensen M, Hansen M, Tveskov L, Floyd AK, Stockel M, et al. Hepatic mitochondrial oxidative phosphorylation is normal in obese patients with and without type 2 diabetes. *J Physiol* 2016;594:4351–4358.
- [44] Zaharia OP, Strassburger K, Knebel B, Kupriyanova Y, Karusheva Y, Wolkersdorfer M, et al. Role of patatin-like phospholipase domain-containing 3 gene for hepatic lipid content and insulin resistance in diabetes. *Diabetes Care* 2020;43:2161–2168.
- [45] Mehta R, Jeiran K, Koenig AB, Otagonsuren M, Goodman Z, Baranova A, et al. The role of mitochondrial genomics in patients with non-alcoholic steatohepatitis (NASH). *BMC Med Genet* 2016;17:63.
- [46] Longo M, Meroni M, Paolini E, Erconi V, Carli F, Fortunato F, et al. TM6SF2/PNPLA3/MBOAT7 loss-of-function genetic variants impact on NAFLD development and progression both in patients and in in vitro models. *Cell Mol Gastroenterol Hepatol* 2022;13:759–788.
- [47] **Kaul K, Apostolopoulou M, Roden M.** Insulin resistance in type 1 diabetes mellitus. *Metabolism* 2015;64:1629–1639.
- [48] Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001;120:1183–1192.
- [49] Shami GJ, Cheng D, Verhaegh P, Koek G, Wisse E, Braet F. Three-dimensional ultrastructure of giant mitochondria in human non-alcoholic fatty liver disease. *Sci Rep* 2021;11:3319.
- [50] Begriche K, Igoudjil A, Pessayre D, Fromenty B. Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. *Mitochondrion* 2006;6:1–28.
- [51] Serviddio G, Bellanti F, Vendemiale G, Altomare E. Mitochondrial dysfunction in nonalcoholic steatohepatitis. *Expert Rev Gastroenterol Hepatol* 2011;5:233–244.
- [52] Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA* 1999;282:1659–1664.
- [53] Pérez-Carreras M, Del Hoyo P, Martín MA, Rubio JC, Martín A, Castellano G, et al. Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. *Hepatology* 2003;38:999–1007.
- [54] Serviddio G, Bellanti F, Tamborra R, Rollo T, Capitano N, Romao AD, et al. Uncoupling protein-2 (UCP2) induces mitochondrial proton leak and increases susceptibility of non-alcoholic steatohepatitis (NASH) liver to ischaemia-reperfusion injury. *Gut* 2008;57:957–965.
- [55] Chavin KD, Yang S, Lin HZ, Chatham J, Chacko VP, Hoek JB, et al. Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. *J Biol Chem* 1999;274:5692–5700.
- [56] Baffy G. Uncoupling protein-2 and non-alcoholic fatty liver disease. *Front Biosci* 2005;10:2082–2096.
- [57] Rostaing C, Piquet MA, Leterrier D, Rey B, Mourier A, Servais S, et al. Mitochondrial adaptations to steatohepatitis induced by a methionine- and choline-deficient diet. *Am J Physiol Endocrinol Metab* 2008;294:E110–E119.
- [58] Morris EM, Rector RS, Thyfault JP, Ibdah JA. Mitochondria and redox signaling in steatohepatitis. *Antioxid Redox Signal* 2011;15:485–504.
- [59] Chella Krishnan KC, Floyd RR, Sabir S, Jayasekera DW, Leon-Mimila PV, Jones AE, et al. Liver pyruvate kinase promotes NAFLD/NASH in both mice and humans in a sex-specific manner. *Cell Mol Gastroenterol Hepatol* 2021;11:389–406.
- [60] Miele L, Grieco A, Armuzzi A, Candelli M, Forgione A, Gasbarrini A, et al. Hepatic mitochondrial beta-oxidation in patients with nonalcoholic steatohepatitis assessed by ¹³C-octanoate breath test. *Am J Gastroenterol* 2003;98:2335–2336.
- [61] **Serviddio G, Giudetti AM, Bellanti F, Priore P, Rollo T, Tamborra R, et al.** Oxidation of hepatic carnitine palmitoyl transferase-I (CPT-I) impairs fatty acid beta-oxidation in rats fed a methionine-choline deficient diet. *PLoS One* 2011;6:e24084.
- [62] Ji C, Dai Y, Jiang W, Liu J, Hou M, Wang J, et al. Postnatal overfeeding promotes early onset and exaggeration of high-fat diet-induced nonalcoholic fatty liver disease through disordered hepatic lipid metabolism in rats. *J Nutr Biochem* 2014;25:1108–1116.
- [63] Zamani-Garmsiri F, Hashemnia SMR, Shabani M, Bagherieh M, Emamgholipour S, Meshkani R. Combination of metformin and genistein alleviates non-alcoholic fatty liver disease in high-fat diet-fed mice. *J Nutr Biochem* 2021;87:108505.
- [64] Magkos F, Su X, Bradley D, Fabbrini E, Conte C, Eagon JC, et al. Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. *Gastroenterology* 2012;142:1444–1446.
- [65] Lyu K, Zhang Y, Zhang D, Kahn M, Ter Horst KW, Rodrigues MRS, et al. A membrane-bound diacylglycerol species induces PKC ϵ -mediated hepatic insulin resistance. *Cell Metab* 2020;32:654–664.
- [66] Apostolopoulou M, Gordillo R, Koliaki C, Gancheva S, Jelenik T, De Filippo E, et al. Specific hepatic sphingolipids relate to insulin resistance, oxidative stress, and inflammation in nonalcoholic steatohepatitis. *Diabetes Care* 2018;41:1235–1243.
- [67] Apostolopoulou M, Gordillo R, Gancheva S, Strassburger K, Herder C, Esposito I, et al. Role of ceramide-to-dihydroceramide ratios for insulin resistance and non-alcoholic fatty liver disease in humans. *BMJ Open Diabetes Res Care* 2020;8:e001860.
- [68] Ooi GJ, Meikle PJ, Huynh K, Earnest A, Roberts SK, Kemp W, et al. Hepatic lipidomic remodeling in severe obesity manifests with steatosis and does not evolve with non-alcoholic steatohepatitis. *J Hepatol* 2021;75:524–535.
- [69] Gancheva S, Kahl S, Pesta D, Mastroianni L, Dewidar B, Strassburger K, et al. Impaired hepatic mitochondrial capacity in nonalcoholic steatohepatitis associated with type 2 diabetes. *Diabetes Care* 2022;45:928–937.
- [70] Crescenzo R, Bianco F, Falcone I, Coppola P, Liverini G, Iossa S. Increased hepatic de novo lipogenesis and mitochondrial efficiency in a model of obesity induced by diets rich in fructose. *Eur J Nutr* 2013;52:537–545.
- [71] **Franko A, von Kleist-Retzow JC, Neschen S, Wu M, Schommers P, Böse M, et al.** Liver adapts mitochondrial function to insulin resistant and diabetic states in mice. *J Hepatol* 2014;60:816–823.

- [72] Jelenik T, Kaul K, Séquaris G, Flögel U, Phielix E, Kotzka J, et al. Mechanisms of insulin resistance in primary and secondary nonalcoholic fatty liver. *Diabetes* 2017;66:2241–2253.
- [73] **Begrliche K, Massart J**, Robin MA, Bonnet F, Fromenty B. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. *Hepatology* 2013;58:1497–1507.
- [74] Ramirez-Tortosa MC, Ramirez-Tortosa CL, Mesa MD, Granados S, Gil A, Quiles JL. Curcumin ameliorates rabbits's steatohepatitis via respiratory chain, oxidative stress, and TNF-alpha. *Free Radic Biol Med* 2009;47:924–931.
- [75] **Li S, Li X**, Chen F, Liu M, Ning L, Yan Y, et al. Nobiletin mitigates hepatocytes death, liver inflammation, and fibrosis in a murine model of NASH through modulating hepatic oxidative stress and mitochondrial dysfunction. *J Nutr Biochem* 2022;100:108888.
- [76] **Chiu YH, Amy Lin SC**, Kuo CH, Li CJ. Molecular machinery and pathophysiology of mitochondrial dynamics. *Front Cell Dev Biol* 2021;9:743892.
- [77] **Hoppins S, Lackner LL, Lee JE, Mears JA**. In vitro and in vivo assays for mitochondrial fission and fusion. *Methods Cell Biol* 2020;155:491–518.
- [78] **Liu HY, Han J**, Cao SY, Hong T, Zhuo D, Shi J, et al. Hepatic autophagy is suppressed in the presence of insulin resistance and hyperinsulinemia inhibition of FoxO1-dependent expression of key autophagy genes by insulin. *J Biol Chem* 2009;284:31484–31492.
- [79] Morris EM, Meers GM, Booth FW, Fritsche KL, Hardin CD, Thyfault JP, et al. PGC-1 α overexpression results in increased hepatic fatty acid oxidation with reduced triacylglycerol accumulation and secretion. *Am J Physiol Gastrointest Liver Physiol* 2012;303:G979–G992.
- [80] Yamada T, Murata D, Adachi Y, Itoh K, Kameoka S, Igarashi A, et al. Mitochondrial stasis reveals p62-mediated ubiquitination in Parkin-independent mitophagy and mitigates nonalcoholic fatty liver disease. *Cell Metab* 2018;28:588–604.
- [81] Takeichi Y, Miyazawa T, Sakamoto S, Hanada Y, Wang L, Gotoh K, et al. Non-alcoholic fatty liver disease in mice with hepatocyte-specific deletion of mitochondrial fission factor. *Diabetologia* 2021;64:2092–2107.
- [82] Longo M, Meroni M, Paolini E, Macchi C, Dongiovanni P. Mitochondrial dynamics and nonalcoholic fatty liver disease (NAFLD): new perspectives for a fairy-tale ending? *Metabolism* 2021;117:154708.
- [83] Satapathy SK, Kuwajima V, Nadelson J, Atiq O, Sanyal AJ. Drug-induced fatty liver disease: an overview of pathogenesis and management. *Ann Hepatol* 2015;14:789–806.
- [84] Klauing JE, Li X, Wang Z. Role of xenobiotics in the induction and progression of fatty liver disease. *Toxicol Res (Camb)* 2018;7:664–680.
- [85] Allard J, Bucher S, Massart J, Ferron PJ, Le Guillou D, Loyant R, et al. Drug-induced hepatic steatosis in absence of severe mitochondrial dysfunction in HepaRG cells: proof of multiple mechanism-based toxicity. *Cell Biol Toxicol* 2021;37:151–175.
- [86] Cano R, Pérez JL, Dávila LA, Ortega Á, Gómez Y, Valero-Cedeño NJ, et al. Role of endocrine-disrupting chemicals in the pathogenesis of non-alcoholic fatty liver disease: a comprehensive review. *Int J Mol Sci* 2021;22:4807.
- [87] Fromenty B, Pessayre D. Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. *Pharmacol Ther* 1995;67:101–154.
- [88] Kennedy JA, Unger SA, Horowitz JD. Inhibition of carnitine palmitoyl-transferase-1 in rat heart and liver by perhexiline and amiodarone. *Biochem Pharmacol* 1996;52:273–280.
- [89] Wang W, Lin R, Zhan Wang W, Lin R, Zhang J, Mao Y, et al. Involvement of fatty acid metabolism in the hepatotoxicity induced by divalproex sodium. *Hum Exp Toxicol* 2012;31:1092–1101.
- [90] Nadanaciva S, Dykens JA, Bernal A, Capaldi RA, Will Y. Mitochondrial impairment by PPAR agonists and statins identified via immunocaptured OXPHOS complex activities and respiration. *Toxicol Appl Pharmacol* 2007;223:277–287.
- [91] Demeilliers C, Maisonneuve C, Grodet A, Mansouri A, Nguyen R, Tinel M, et al. Impaired adaptive resynthesis and prolonged depletion of hepatic mitochondrial DNA after repeated alcohol binges in mice. *Gastroenterology* 2002;123:1278–1290.
- [92] Xu L, Zhang X, Xin Y, Ma J, Yang C, Zhang X, et al. Depdc5 deficiency exacerbates alcohol-induced hepatic steatosis via suppression of PPAR α pathway. *Cell Death Dis* 2021;12:710.
- [93] Barbier-Torres L, Murray B, Yang JW, Wang J, Matsuda M, Robinson A, et al. Depletion of mitochondrial methionine adenosyltransferase α 1 triggers mitochondrial dysfunction in alcohol-associated liver disease. *Nat Commun* 2022;13:557.
- [94] Cahill A, Stabley GJ, Wang X, Hoek JB. Chronic ethanol consumption causes alterations in the structural integrity of mitochondrial DNA in aged rats. *Hepatology* 1999;30:881–888.
- [95] Hao L, Zhong W, Dong H, Guo W, Sun X, Zhang W, et al. ATF4 activation promotes hepatic mitochondrial dysfunction by repressing NRF1-TFAM signalling in alcoholic steatohepatitis. *Gut* 2021;70:1933–1945.
- [96] Spaniol M, Kaufmann P, Beier K, Wüthrich J, Török M, Scharnagl H, et al. Mechanisms of liver steatosis in rats with systemic carnitine deficiency due to treatment with trimethylhydraziniumpropionate. *J Lipid Res* 2003;44:144–153.
- [97] Wang W, Bai M, Jiang T, Li C, Li P, Zhou H, et al. Clozapine-induced reduction of l-carnitine reabsorption via inhibition/down-regulation of renal carnitine/organic cation transporter 2 contributes to liver lipid metabolic disorder in mice. *Toxicol Appl Pharmacol* 2019;363:47–56.
- [98] Wahlang B, Beier JI, Clair HB, Bellis-Jones HJ, Falkner KC, McClain CJ, et al. Toxicant-associated steatohepatitis. *Toxicol Pathol* 2013;41:343–360.
- [99] Schumacher JD, Guo GL. Mechanistic review of drug-induced steatohepatitis. *Toxicol Appl Pharmacol* 2015;289:40–47.
- [100] Lu H, Cui W, Klaassen CD. Nrf2 protects against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced oxidative injury and steatohepatitis. *Toxicol Appl Pharmacol* 2011;256:122–135.
- [101] Seitz HK, Bataller R, Cortez-Pinto H, Gao B, Gual A, Lackner C, et al. Alcoholic liver disease. *Nat Rev Dis Primers* 2018;4:16.
- [102] Berson A, De Beco V, Lettéron P, Robin MA, Moreau C, El Kahwaji J, et al. Steatohepatitis-inducing drugs cause mitochondrial dysfunction and lipid peroxidation in rat hepatocytes. *Gastroenterology* 1998;114:764–774.
- [103] Begrliche K, Massart J, Robin MA, Borgne-Sanchez A, Fromenty B. Drug-induced toxicity on mitochondria and lipid metabolism: mechanistic diversity and deleterious consequences for the liver. *J Hepatol* 2011;54:773–794.
- [104] Serviddio G, Bellanti F, Giudetti AM, Gnoni GV, Capitanio N, Tamborra R, et al. Mitochondrial oxidative stress and respiratory chain dysfunction account for liver toxicity during amiodarone but not dronedarone administration. *Free Radic Biol Med* 2011;51:2234–2242.
- [105] Zhang P, Qiang X, Zhang M, Ma D, Zhao Z, Zhou C, et al. Demethyleneberberine, a natural mitochondria-targeted antioxidant, inhibits mitochondrial dysfunction, oxidative stress, and steatosis in alcoholic liver disease mouse model. *J Pharmacol Exp Ther* 2015;352:139–1347.
- [106] Bassett JT, Rodriguez B, Mulligan L, Fontana RJ. Acute liver failure in a military recruit treated with valproic acid and harboring a previously unrecognized POLG-1 mutation. *Epilepsy Behav Rep* 2019;12:100342.
- [107] Degoul F, Sutton A, Mansouri A, Cepanec C, Degott C, Fromenty B, et al. Homozygosity for alanine in the mitochondrial targeting sequence of superoxide dismutase and risk for severe alcoholic liver disease. *Gastroenterology* 2001;120:1468–1474.
- [108] Zhou H, Zhu P, Wang J, Toan S, Ren J. DNA-PKcs promotes alcohol-related liver disease by activating Drp1-related mitochondrial fission and repressing FUNDC1-required mitophagy. *Signal Transduct Target Ther* 2019;4:56.
- [109] Choi Y, Seo H, Cho M, Kim J, Chung HS, Lee I, et al. Rutin inhibits DRP1-mediated mitochondrial fission and prevents ethanol-induced hepatotoxicity in HepG2 cells and zebrafish. *Anim Cells Syst (Seoul)* 2021;25:74–81.
- [110] Chen YY, Lin Y, Han PY, Jiang S, Che L, He CY, et al. HBx combined with AFB1 triggers hepatic steatosis via COX-2-mediated necrosome formation and mitochondrial dynamics disorder. *J Cell Mol Med* 2019;23:5920–5933.
- [111] Ramachandran A, Umbaugh DS, Jaeschke H. Mitochondrial dynamics in drug-induced liver injury. *Livers* 2021;1:102–115.
- [112] Bouvet R, Cauchois A, Baert A, Fromenty B, Morel I, Turlin B, et al. Fatal acetaminophen poisoning with hepatic microvesicular steatosis in a child after repeated administration of therapeutic doses. *Forensic Sci Int* 2020;310:110258.
- [113] Liebe R, Esposito I, Bock HH, Vom Dahl S, Stindt J, Baumann U, et al. Diagnosis and management of secondary causes of steatohepatitis. *J Hepatol* 2021;74:1455–1471.
- [114] Massart J, Begrliche K, Moreau C, Fromenty B. Role of nonalcoholic fatty liver disease as risk factor for drug-induced hepatotoxicity. *J Clin Transl Res* 2017;3(Suppl 1):212–232.
- [115] Mori S, Arima N, Ito M, Ueki Y, Abe Y, Aoyagi K, et al. Incidence, predictive factors and severity of methotrexate-related liver injury in rheumatoid arthritis: a longitudinal cohort study. *Rheumatol Adv Pract* 2020;4:rkaa020.
- [116] **Lee B, Jung EA**, Yoo JJ, Kim SG, Lee CB, Kim YS, et al. Prevalence, incidence and risk factors of tamoxifen-related non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Liver Int* 2020;40:1344–1355.

- [117] Åberg F, Färkkilä M. Drinking and obesity: alcoholic liver disease/nonalcoholic fatty liver disease interactions. *Semin Liver Dis* 2020;40:154–162.
- [118] Xu J, Lai KKY, Verlinsky A, Lugea A, French SW, Cooper MP, et al. Synergistic steatohepatitis by moderate obesity and alcohol in mice despite increased adiponectin and p-AMPK. *J Hepatol* 2011;55:673–682.
- [119] Everitt H, Hu M, Ajmo JM, Rogers CQ, Liang X, Zhang R, et al. Ethanol administration exacerbates the abnormalities in hepatic lipid oxidation in genetically obese mice. *Am J Physiol Gastrointest Liver Physiol* 2013;304:G38–G47.
- [120] Strakovsky RS, Wang H, Engeseth NJ, Flaws JA, Helferich WG, Pan YX, et al. Developmental bisphenol A (BPA) exposure leads to sex-specific modification of hepatic gene expression and epigenome at birth that may exacerbate high-fat diet-induced hepatic steatosis. *Toxicol Appl Pharmacol* 2015;284:101–112.
- [121] Pessayre D, Fromenty B, Berson A, Robin MA, Lettéron P, Moreau R, et al. Central role of mitochondria in drug-induced liver injury. *Drug Metab Rev* 2012;44:34–87.
- [122] Faillaci F, Milosa F, Critelli RM, Turola E, Schepis F, Villa E. Obese zebrafish: a small fish for a major human health condition. *Animal Model Exp Med* 2018;1:255–265.
- [123] Katoch S, Patial V. Zebrafish: an emerging model system to study liver diseases and related drug discovery. *J Appl Toxicol* 2021;41:33–51.
- [124] Bucher S, Tête A, Podechard N, Liamin M, Le Guillou D, Chevanne M, et al. Co-exposure to benzo[a]pyrene and ethanol induces a pathological progression of liver steatosis in vitro and in vivo. *Sci Rep* 2018;13(8):5963.
- [125] Imran M, Chalmel F, Sergent O, Evrard B, Le Mentec H, Legrand A, et al. Transcriptomic analysis in zebrafish larvae identifies iron-dependent mitochondrial dysfunction as a possible key event of NAFLD progression induced by benzo[a]pyrene/ethanol co-exposure. *Cell Biol Toxicol* 2022 Apr 12. <https://doi.org/10.1007/s10565-022-09706-4>. online ahead of print.
- [126] Bucher S, Le Guillou D, Allard J, Pinon G, Begriche K, Tête A, et al. Possible involvement of mitochondrial dysfunction and oxidative stress in a cellular model of NAFLD progression induced by benzo[a]pyrene/ethanol co-exposure. *Oxid Med Cell Longev* 2018;4396403.
- [127] Sarabhai T, Kahl S, Szendroedi J, Markgraf DF, Zaharia OP, Barosa C, et al. Monounsaturated fat rapidly induces hepatic gluconeogenesis and whole-body insulin resistance. *JCI Insight* 2020;5:e134520.
- [128] Luukkonen PK, Dufour S, Lyu K, Zhang XM, Hakkarainen A, Lehtimäki TE, et al. Effect of a ketogenic diet on hepatic steatosis and hepatic mitochondrial metabolism in nonalcoholic fatty liver disease. *Proc Natl Acad Sci U S A* 2020;117:7347–7354.
- [129] Verbeek J, Lannoo M, Pirinen E, Ryu D, Spincemaille P, Vander Elst I, et al. Roux-en-y gastric bypass attenuates hepatic mitochondrial dysfunction in mice with non-alcoholic steatohepatitis. *Gut* 2015;64:673–683.
- [130] Sacks J, Mulya A, Fealy CE, Huang H, Mosinski JD, Pagadala MR, et al. Effect of Roux-en-Y gastric bypass on liver mitochondrial dynamics in a rat model of obesity. *Physiol Rep* 2018;6:e13600.
- [131] Gancheva S, Ouni M, Jelenik T, Koliaki C, Szendroedi J, Toledo FGS, et al. Dynamic changes of muscle insulin sensitivity after metabolic surgery. *Nat Commun* 2019;10:4179.
- [132] El-Agroudy NN, Kurzbach A, Rodionov RN, O'Sullivan J, Roden M, Birkenfeld AL, et al. Are lifestyle therapies effective for NAFLD treatment? *Trends Endocrinol Metab* 2019;30:701–709.
- [133] Stevanović J, Beleza J, Coxito P, Ascensão A, Magalhães J. Physical exercise and liver “fitness”: role of mitochondrial function and epigenetics-related mechanisms in non-alcoholic fatty liver disease. *Mol Metab* 2020;32:1–14.
- [134] Brouwers B, Schrauwen-Hinderling VB, Jelenik T, Gemmink A, Sparks LM, Havekes B, et al. Exercise training reduces intrahepatic lipid content in people with and people without nonalcoholic fatty liver. *Am J Physiol Endocrinol Metab* 2018;314:E165–E173.
- [135] Mastrototaro L, Roden M. Insulin resistance and insulin sensitizing agents. *Metabolism* 2021;125:154892.
- [136] LaMoia TE, Shulman GI. Cellular and molecular mechanisms of metformin action. *Endocr Rev* 2021;42:77–96.
- [137] Feng J, Wang X, Ye X, Ares I, Lopez-Torres B, Martínez M, et al. Mitochondria as an important target of metformin: the mechanism of action, toxic and side effects, and new therapeutic applications. *Pharmacol Res* 2022;177:106114.
- [138] Fouquerey P, Bolze S, Dubourg J, Hallakou-Bozec S, Theurey P, Grouin JM, et al. Pharmacodynamic effects of direct AMP kinase activation in humans with insulin resistance and non-alcoholic fatty liver disease: a phase 1b study. *Cell Rep Med* 2021;2:100474.
- [139] Zucchi R. Thyroid hormone analogues: an update. *Thyroid* 2020;30:1099–1105.
- [140] Harrison SA, Bashir MR, Guy CD, Zhou R, Moylan CA, Frias JP, et al. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2019;394(10213):2012–2024.
- [141] Grundlingh J, Dargan PI, El-Zanfaly M, Wood DM. 2,4-Dinitrophenol (DNP): a weight loss agent with significant acute toxicity and risk of death. *J Med Toxicol* 2011;7:205–2012.
- [142] Goedeke L, Shulman GI. Therapeutic potential of mitochondrial uncouplers for the treatment of metabolic associated fatty liver disease and NASH. *Mol Metab* 2021;46:101178.
- [143] Boland ML, Laker RC, Mather K, Nawrocki A, Oldham S, Boland BB, et al. Resolution of NASH and hepatic fibrosis by the GLP-1R/GcgR dual-agonist Cotadutide via modulating mitochondrial function and lipogenesis. *Nat Metab* 2020;2:413–431.
- [144] Lammert C, Imler T, Teal E, Chalasani N. Patients with chronic liver disease suggestive of nonalcoholic fatty liver disease may be at higher risk for drug-induced liver injury. *Clin Gastroenterol Hepatol* 2019;17:2814–2815.
- [145] Regev A, Palmer M, Avigan MI, Dimick-Santos L, Treem WR, Marcink JF, et al. Consensus: guidelines: best practices for detection, assessment and management of suspected acute drug-induced liver injury during clinical trials in patients with nonalcoholic steatohepatitis. *Aliment Pharmacol Ther* 2019;49:702–713.
- [146] Rana P, Aleo MD, Wen X, Kogut S. Hepatotoxicity reports in the FDA adverse event reporting system database: a comparison of drugs that cause injury via mitochondrial or other mechanisms. *Acta Pharm Sin B* 2021;11:3857–3868.
- [147] Georgiev A, Granata C, Roden M. The role of mitochondria in the pathophysiology and treatment of common metabolic diseases. *Am J Physiol Cell Physiol* 2022;322:C1248–C1259.
- [148] Thietart S, Rautou PE. Extracellular vesicles as biomarkers in liver diseases: a clinician's point of view. *J Hepatol* 2020;73:1507–1525.
- [149] Al Amir Dache Z, Otandault A, Tanos R, Pastor B, Meddeb R, Sanchez C, et al. Blood contains circulating cell-free respiratory competent mitochondria. *FASEB J* 2020;34:3616–3630.
- [150] Barbier-Torres L, Fortner KA, Iruzubieta P, Delgado TC, Giddings E, Chen Y, et al. Silencing hepatic MCJ attenuates non-alcoholic fatty liver disease (NAFLD) by increasing mitochondrial fatty acid oxidation. *Nat Commun* 2020;11:3360.
- [151] Yamada T, Murata D, Kleiner DE, Anders R, Rosenberg AZ, Kaplan J, et al. Prevention and regression of megamitochondria and steatosis by blocking mitochondrial fusion in the liver. *iScience* 2022;25:103996.