Mitochondrial alterations in fatty liver diseases

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Review

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Bernard Fromenty[1], 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.

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 chracteristics, 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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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, PPARβ/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 $FADH_2$, 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 1α and 1β (PGC1α, PGC1β).[15] AMP-activated protein kinase (AMPK) also plays a major role in mitochondrial biogenesis, in particular by activating PGC1α.[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

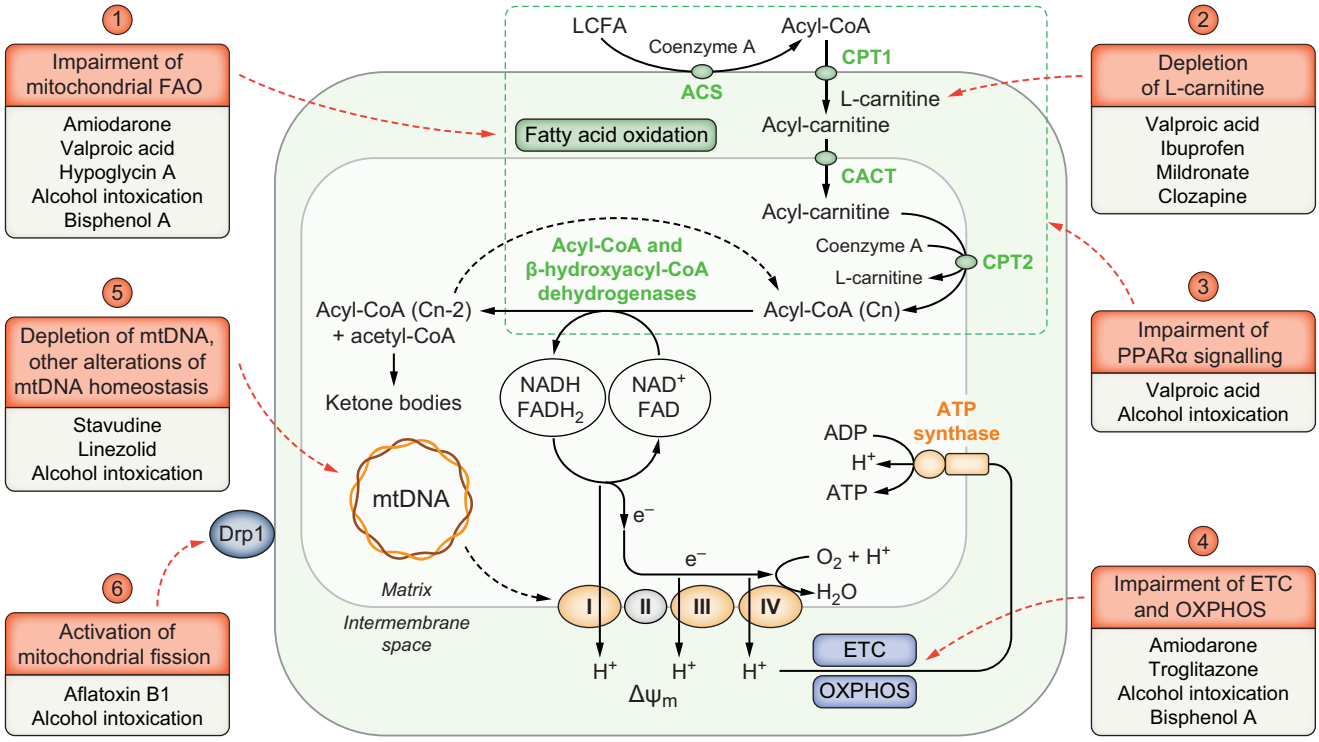

Fig. 1. Main mitochondrial functions and xenobiotic-induced alterations. Contrary to short/medium-chain fatty acids that freely enter mitochondria (not shown), LCFAs' entry requires a 4-step shuttle system. (1) LCFAs bind to CoA to form acyl-CoA thioesters in a process catalysed by long-chain ACS at 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; PPARα, 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 ^{31}P 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 phenotype[28] 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	*Ex vivo*, invasive, permeabilized tissue
	Near-infrared spectroscopy (NIRS)	Hb oxygenation, cytochrome oxidase aa3 (REDOX state)	Intact tissue	*Ex vivo*, invasive, indirect measure, limited use
	Liver ^{31}P MRS	[ATP], [Pi] or V_{ATP} (from saturation transfer or upon fructose challenge)	*In vivo*, 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)	*In vivo*, intact tissue	Availability, multidisciplinary expertise, exclusion criteria, time
	PET +^{11}C- or^{18}F-labeled fatty acids or analogues	Fatty acid ß-oxidation	*In vivo*, intact tissue	Radiation, availability, multidisciplinary expertise, indirect measure, time
	^{13}C- or ^{2}H-labeled metabolite dilution and positional isotopomer analysis	Anaplerotic and TCA cycle fluxes (*e.g.* V_{PC} and V_{CS}), β-oxidation	*In vivo*, intact tissue	Availability, indirect measure, time
	Breath test +^{13}C-labeled metabolites	Mitochondrial oxidation	*In vivo*, intact tissue	Indirect measure, no validation
	Genome-scale human metabolic model	Multiomics and splanchnic metabolite flux data	Integrated complex analysis	Partly *in vitro*, 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	*In vitro,* invasive, indirect measure, no accepted single marker, no validation in human liver
	Fluorescence-activated cell sorting, confocal imaging	Mitophagy: cell analyses	Direct measure	*Ex vivo,* 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.

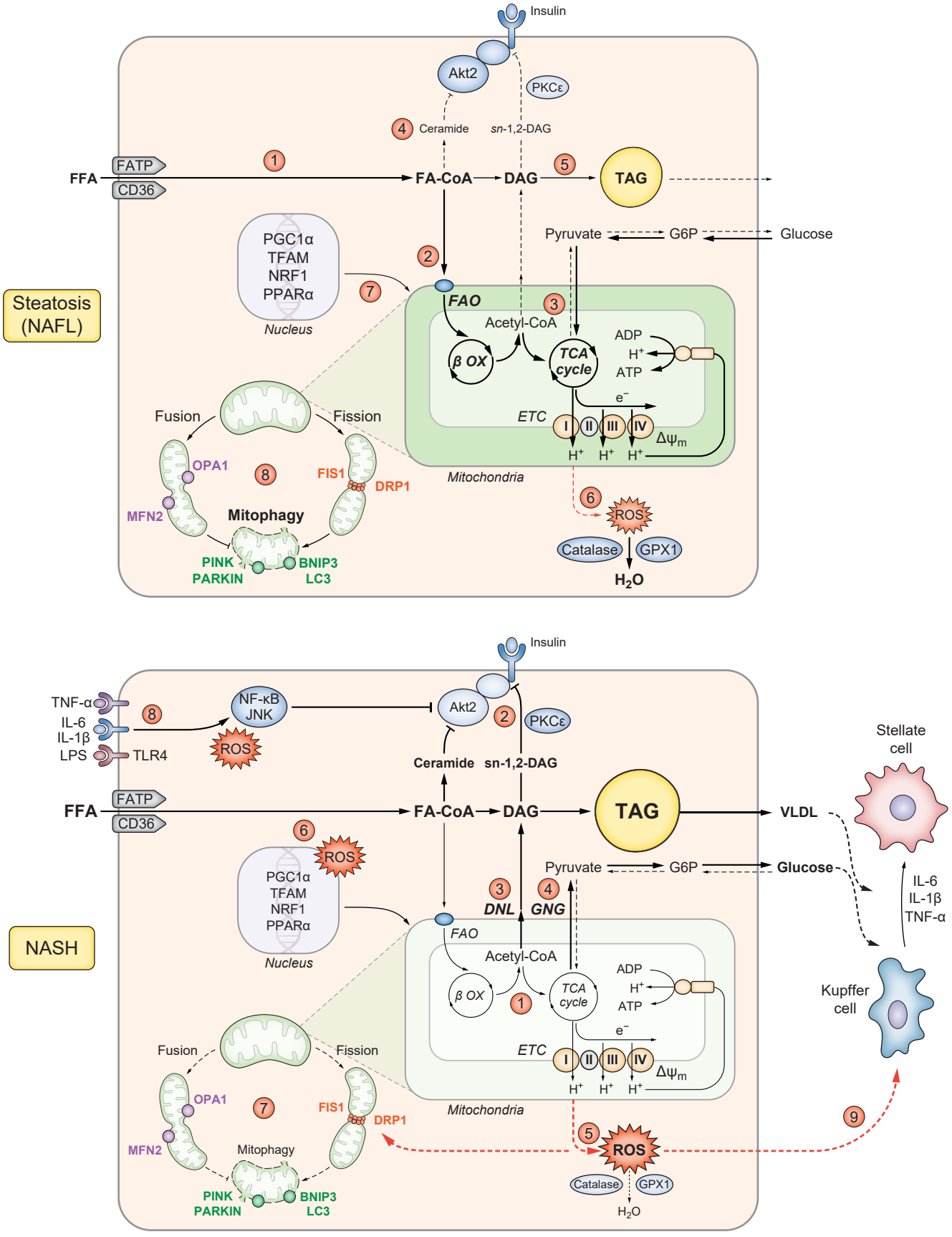

Fig. 2. Mitochondrial alterations in obesity, NAFL and NASH. Top panel: In obesity without or with NAFL (steatosis), greater availability of FFAs (1) increases the intracellular pool of FA-CoA, which (2) stimulates mitochondrial FAO and may also (3) increase TCA cycle and ETC activity. Upregulated mitochondrial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

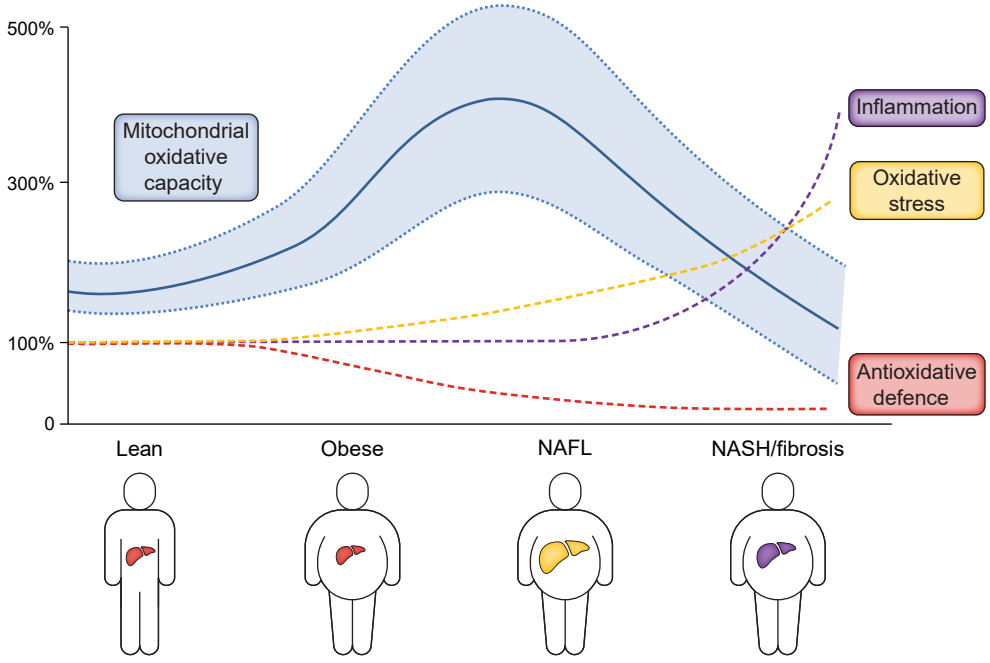

Fig. 3. Concept of hepatic mitochondrial adaption/plasticity during NAFLD development. Mitochondrial oxidative capacity varies broadly across the spectrum of obesity and NAFLD, mainly due to body mass, but also age, insulin sensitivity, concomitant type 2 diabetes, chronic alcohol abuse and possibly 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 content[30] and ATP synthase flux rates (V_{ATP})[31] between elderly obese people with NAFL and young lean volunteers. A study using [1-^{13}C]acetate infusion to label hepatic [5-^{13}C]glutamate and [1-^{13}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}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 individuals[24] (Fig. 2, top panel). Recent high-resolution respirometry studies also reported increased maximal coupled (significant) and uncoupled (p = 0.054) mtDNA/nDNA-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 substrate[37] 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-κB. Ongoing oxidative stress, hyperglycaemia and dyslipidaemia (9) activate Kupffer cells and stellate cells, which via cytokines (*e.g.* TNF-α, IL-1β 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; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PKCε, protein kinase Cε; PPARα, peroxisome proliferator-activated receptor-α; ROS, reactive oxygen species; TAG, triacylglycerol; TCA, tricarboxylic acid; TFAM, transcription factor A mitochondrial; TLR4, Toll-like receptor 4; TNFα, tumour necrosis factor-α; VLDL, very low-density lipoprotein.

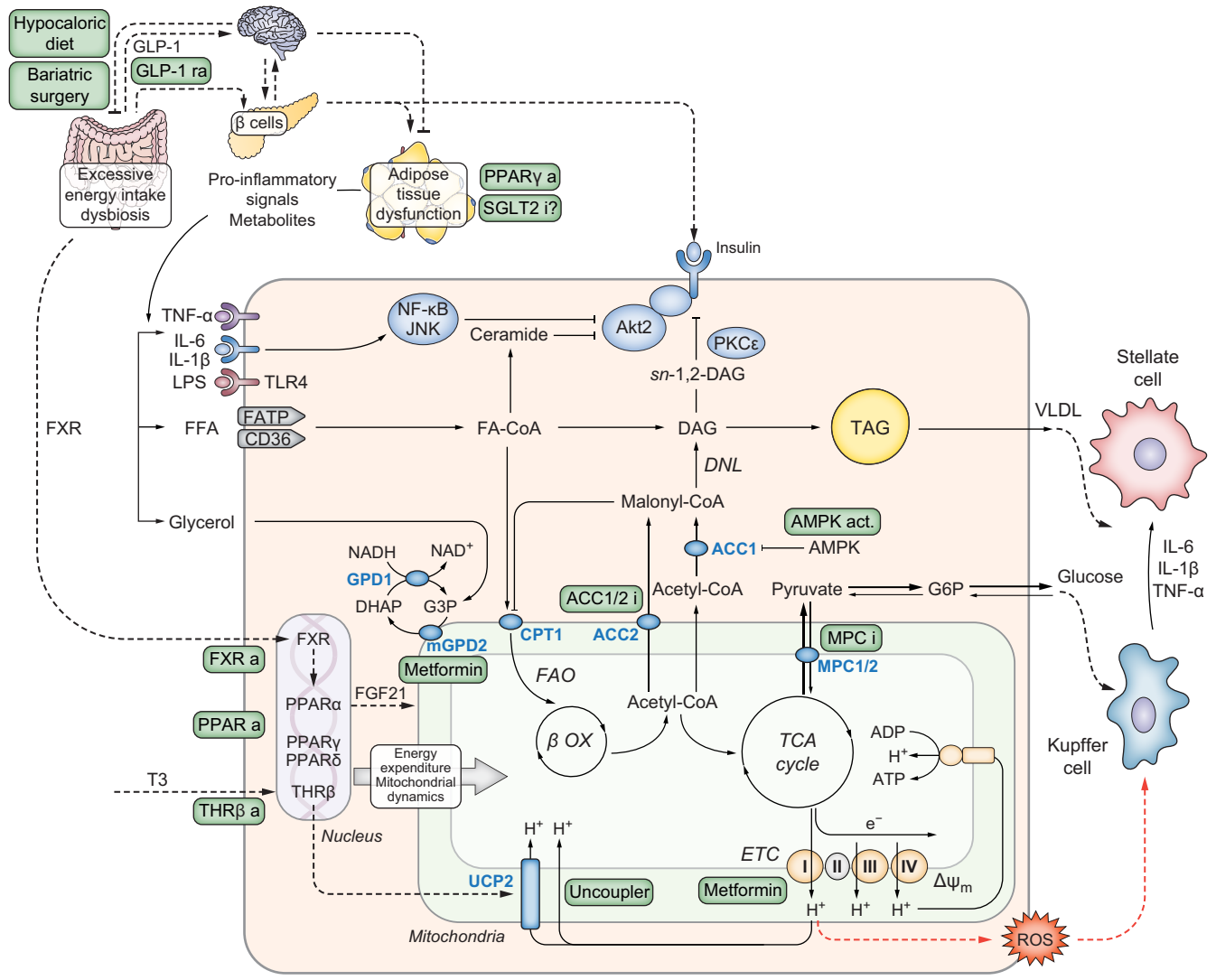

Fig. 4. Interventions (in)directly targeting liver mitochondria in obesity, type 2 diabetes and NAFLD. Aside from lifestyle (hypocaloric diet)- and surgically induced (bariatric surgery) weight loss, several drugs that are being assessed for the treatment of NAFLD target liver mitochondria directly or indirectly (green boxes) repisration. Metformin can inhibit ETC complex I and subsequentely 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 PPARα/δ/γ, FXR, THRβ, 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 PPARγ 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.

biogenesis[24] or activities of rate-controlling enzymes such as β-hydroxyacyl-CoA dehydrogenase.[38] Such mitochondrial alterations might then contribute to oxidative stress and lipid peroxidation[24] (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 ^{13}C-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 fibrosis[29] 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 γATP 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 activity[53] 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 NASH[24] (Fig. 2, bottom panel). In NASH, hepatic OXPHOS capacity was ∼40–51% lower than in obese individuals with/without steatosis and ∼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 ∼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* measurements[77] 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 NASH[53] 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] PGC1α 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 macrovacuolar steatosis is not a pure mitochondrial disease and is often asymptomatic in the short term.[7,83] Lipid accumulation in macrovacuolar 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 PPARα 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 (MATα1).[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 PGC1α) and *Ppara* (encoding PPARα).[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 [D_7]glucose, [$^{13}C_4$]β-hydroxybutyrate and [3-^{13}C]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 functionality[135] (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 THRβ 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 models[142] (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 vesicles[148] 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 PGC1α activity[73,79] and mitochondrial MATα1 levels[93] or reduce the expression of methylation-controlled J protein[150] and OPA1.[80,151]

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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; MATα1, 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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Author names in bold designate shared co-first authorship

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