Mitochondria, oxidative stress and Non-Alcoholic Fatty Liver Disease: a complex relationship

Running Title: Mitochondria, ROS and NAFLD - complex relations

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ABSTRACT

According to the "multiple-hit" hypothesis, several factors can act simultaneously in non-alcoholic fatty liver disease (NAFLD) progression. Increased nitro-oxidative (nitroso-oxidative) stress may be considered the main contributor involved in the development and risk of NAFLD progression to non-alcoholic steatohepatitis (NASH) characterised by inflammation and fibrosis. Moreover, it has been repeatedly postulated that mitochondrial abnormalities are also closely related to the development and worsening of liver steatosis and NAFLD pathogenesis. However, it is difficult to determine with certainty whether mitochondrial dysfunction or oxidative stress are primary events or a simple consequence of NAFLD development. On the one hand, increasing lipid accumulation in hepatocytes could cause a wide range of effects from mild to severe mitochondrial damages with a negative impact on cell fate. This can start the cascade of events, including an increase of cellular reactive nitrogen species (RNS) and reactive oxygen species (ROS) production that promotes disease progression from simple steatosis to more severe NAFLD stages. On the other hand, progressing mitochondrial bioenergetic catastrophe and oxidative stress manifestation could be considered accompanying events in the vast spectrum of abnormalities observed during the transition from NAFL to NASH and cirrhosis. This review updates our current understanding of NAFLD pathogenesis and clarifies whether mitochondrial dysfunction and ROS/RNS are culprits or bystanders of NAFLD progression.

Key words: mitochondria, ROS, NAFLD, NASH, mitochondrial dysfunction, oxidative stress

1. NAFLD definition, description and epidemiology

Non-alcoholic fatty liver disease (NAFLD) has an increasing incidence, commonly attributed to unhealthy lifestyles. NAFLD diagnosis is based on the presence of hepatic steatosis documented by imaging or histology and exclusion of excessive alcohol consumption, chronic or acute liver diseases, and other secondary causes of steatosis. Moreover, NAFLD is frequently referred to as a hepatic manifestation of metabolic syndrome. The prevalence of NAFLD and non-alcoholic steatohepatitis (NASH) has increased over the past decades and exceeded 25% of the adult population worldwide. There are differences between regions but NAFLD cannot be considered a Western countries disease exclusively anymore. NAFLD prevalence ranges from 13% in Africa to 30% in Asia and 32% in the Middle East. Europe and North America have reported a prevalence of 24%. Regional leaders have even higher prevalence, such as 30% in China, 33% in India and 51% in Indonesia.[1, 2] The incremental trends of the NAFLD epidemic in Western countries have slowed down compared to Asia but more significant numbers of patients with chronic disease account for a high incidence of progression into more advanced stage. It is reflected by an increase in disease burden measures related to cirrhosis and hepatocellular carcinoma (HCC) in patients with NAFLD or NASH compared to other chronic liver diseases. NASH has also gained importance as an underlying cause of end-stage liver disease and HCC among liver transplant candidates in Western countries.[3, 3, 4] Projections for the NAFLD epidemic up to 2030 have been reported recently. Estimates show that the total prevalence of NAFLD will increase by 13-20% in Europe, 18% in the United States and 29% in China. The prevalence of NASH will increase by 43-49% in Europe and China and 56% in the United States.

Genetic analyses of patients with fatty liver demonstrated that inherited predisposition also plays a vital role in the development and progression of hepatic steatosis.[5] Indeed, familial clustering and the result of twin studies showing greater concordance between monozygotic compared to dizygotic twins underscore the role of genetics in NAFLD.[6] In recent years, we have learned that carriers of the common adiponutrin a.k.a. patatin-like phospholipase domain-containing protein 3 (PNPLA3) variant p.I148M are at increased risk of developing NAFLD[7], liver fibrosis and cirrhosis[8] as well as hepatocellular carcinoma (HCC).[9] Other variants, for example, membrane-bound O-acyltransferase domain-containing 7 gene (MBOAT7) variant rs641738 C>T, transmembrane 6 superfamily member 2 gene (TM6SF2) variant p.

E167K or glucokinase regulatory protein gene (GCKR) variant p. P446L have also been shown to enhance the NAFLD risk.[10] PNPLA3, which is highly expressed on the intrahepatic lipid droplets, is involved in hydrolysis of triglycerides. This risk variant is most prevalent in Hispanics and is the least common in African Americans (17%). Previous studies indicate the PNPLA3 might have a lysophosphatidic acyltransferase activity[11] or function as a lipase.[12] It was also postulated that in hepatic stellate cells PNPLA3 has retinyl-palmitate lipase activity.[13] TM6SF2 is, in turn, involved in the hepatic secretion of VLDL (very low-density lipoproteins). Carriers of the TM6SF2 p.E167K variant have lower circulating VLDL, resulting in diminished cardiovascular risk and increased lipid contents in the liver.[14] MBOAT7 possesses a lysophosphatidylinositol acyltransferase activity and is involved in anti-inflammatory processes by regulating arachidonic acid levels.[15] NAFLD-associated genetic variant of MBOAT7 was linked to lower expression of MBOAT7 and its decreased function.[16] This already somewhat complicated picture of NAFLD genetics gained recently another level of complexity: variants in two genes, namely mitochondrial amidoxime reducing component 1 (MARC1)[17] and hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13)[18], were shown to have protective effects on liver status in the setting of NAFLD. Not surprisingly, polygenic risk scores, including the above-listed polymorphisms have been developed (for example as shown lately by Bianco et al.).[19] According to their inherited predisposition, these polygenic scores facilitate stratification of patients with fatty liver to develop progressive liver disease. However, genetic analyses of patients with NAFLD have not gained much attention in the clinic.[5]

2. Diagnosis, Classification, Staging and Non-invasive Biomarkers of NAFLD

2.1. Diagnosis and classification

NAFLD diagnosis is based on the presence of hepatic steatosis documented by imaging or histology, and exclusion of excessive alcohol consumption or other secondary causes of steatosis. Clinical description broadens the perspective, and NAFLD is frequently referred to as a hepatic manifestation of metabolic syndrome. Liver biopsy is considered the gold standard in diagnosing of NASH and differentiation between various stages of the disease characterized by steatosis, inflammation, steatohepatitis, fibrosis, and cirrhosis. Non-invasive tests such as imaging studies and various biochemical indexes are less accurate and cannot replace biopsy. However,

ultrasound examination is used to diagnose of hepatic steatosis in more than 90% of participants in extensive cohort studies.[20]

2.2. Staging

Depending on the pathological stage of the disease, the risk of progression and complications of NAFLD increases. Simple steatosis without hepatocyte injury that is a predominant presentation of the disease is associated with a limited risk of progression into more advanced stages, including steatohepatitis, fibrosis and cirrhosis. By definition, at least 5% of hepatic steatosis is required to establish the diagnosis and less than 5% of fatty infiltration is not considered NAFLD. NASH is defined as fatty infiltration exceeding 5% with concomitant inflammation and hepatocyte injury. At the time of diagnosis, various fibrosis degrees are present, ranging from no fibrosis up to cirrhosis.[1] The staging of NASH is most commonly based on the pathological scores such as NAFLD Activity Score (NAS) or Steatosis Activity Fibrosis (SAF).[21, 22] In NAS score each feature adds points to 0-8 total including 0-3 points for steatosis (in the range of <5%, 5-33%, 33-66% and >66%), 0-3 for lobular inflammation and 0-2 for ballooning. The diagnosis is NASH ≥5 points and not-NASH ≤2 points and a borderline diagnosis of NASH for 3-4 points. Fibrosis is classified as stages 0-4. Disease activity variations assessed with NAS correlate with progression or regression of fibrosis.[23]

2.3. Non-invasive biomarkers of NAFLD

Considering the fact that the alteration of specific proteins or peptides in patients' serum may be related to a specific disease, several groups also try to identify novel diagnostic biomarkers characteristic for different stages of NAFLD. To avoid an invasive liver biopsy, new reliable, non-invasive biomarkers that identify the disease's progression are urgently needed. Unfortunately, liver enzymes per se are not reliable and accurate predictors of NAFLD. Circulating extracellular vesicles (EVs), cell-derived small membrane-surrounded structures seem to be a promising NAFLD and NASH biomarker. Hepatocyte-derived EVs contain hepatocyte markers as asialoglycoprotein receptor 1 (ASGPR1) and bile acyl-CoA synthetase (SLC27A5). It has been demonstrated that the level of hepatocyte-derived EVs correlates with NASH severity.[24]

Many proteins[25], and metabolites[26] or lipidomic signatures[27, 28] may act as NAFLD biomarker at different stages of the disease. For more information about them, see the most recent reviews.[29, 30] Several studies demonstrated that circulating molecules, such as microRNA (miRNA) and cell-free nuclear material DNA or RNA,

can also be considered as potential promising biomarkers. Mitochondria contain their own extranuclear genome, mitochondrial DNA (mtDNA), a 16.5Kb circular DNA molecule present as multiple copies in cells.[31] MtDNA copies in single cells can range from hundreds to thousands of copies depending on the cell's bioenergetic needs. Moreover, mtDNA can dynamically change in response to physiological stimuli and under disease conditions. As part of the mitochondrial cell cycle, cellular mtDNA is constantly replicated and replaced via degradation. However, if the mitochondrial life cycle is disrupted and degradation of damaged mtDNA is impaired, mtDNA can leak out of the mitochondria into the cytosol or the peripheral circulation. Due to its resemblance to bacterial genomes, this cell-free (cf) mtDNA can activate inflammation via the TLR-9 pathway, leading to activation of TNF-alpha and a downstream inflammatory cytokine response.[32] Dysregulation of circulating mitochondrial DNA (mtDNA) has been widely reported in the literature, and circulating mtDNA has been proposed to be a minimally invasive biomarker of mitochondrial dysfunction.[32] Elevated levels of cell-free (cf)-mtDNA in diabetic patients and changes in both hepatic and circulating mtDNA have been reported in both animal models and human studies of NAFLD.[33, 34] A link between oxidative stress and leukocyte mtDNA was suggested in a cross-sectional study of a Chinese cohort showing that elevated mtDNA copy numbers in NAFLD patients positively correlated with the oxidative stress marker 8-oxo-2'deoxyguanosine.[35] Leakage of damaged mtDNA into the periphery from both hepatic tissue and fat cells has been shown to cause enhanced inflammation.[34, 36] Therefore, combining the impact of oxidative stress on inducing maladaptive mtDNA replication, together with the inflammatory properties of (cf)-mtDNA in circulation, suggesting that circulating (cf)-mtDNA is a potential mediator of the chronic inflammation seen in NAFLD and could be a useful biomarker. Additionally, miRNAs, especially miR-122 and miR34a, are also considered a promising diagnostic biomarker for NAFLD.[29] However, robust cross-sectional and longitudinal human studies are needed to understand the time course of (cf)-mtDNA and mi RNAs to evaluate their use as potential biomarkers of NAFLD.

2.4. Dietary and animal models to study NAFLD development and progression

The complicated issue of NAFLD progression has been studied in in vitro model and in laboratory animals and humans. Studies performed in rodents or humans revealed the complexity of the factors involved in NAFLD development and progression. Several

animal models and NAFLD-inducing diets are useful to study the pathogenesis of NAFLD progression to the more severe stages. Among the available diets the most popular are Choline-Deficient (CD) Diets, Semisynthetic Choline-Deficient L-Amino Acid-Defined (CDAA) Diet, Methionine- and Choline-Deficient (MCD) Diet, High-Fat (HF) Diets, High-Fat Diets combined with choline deficiency, Western Diets and Cholesterol supplemented Western diet.

2.4.1. Choline deficiency-based diets: Choline-Deficient (CD) Diets, Semisynthetic Choline-Deficient L-Amino Acid-Defined (CDAA) Diet, Methionine- and Choline-Deficient (MCD) Diets

Choline deficiency-based diets induce a significant increase in liver triglycerides in rodents after some weeks of feeding.[37, 38] Moreover, in rats, moderate periportal micro- and macrovesicular liver steatosis can be visible already after 4 weeks and could be further worsened by a prolonged feeding up to 12 weeks[39] and slight signs of inflammation and fibrosis.[39] Animals feed with CD diet very often do not show significantly increased weight gain.[38, 38] Moreover, an increased incidence (~15%) of hepatocarcinogenesis has been observed in rats fed with CD diets for 52 weeks.[38, 40] Unfortunately, this model poorly reflects metabolic phenotype in patients. CDAA Diet represents a variation of choline deficiency diet, which is also deprived of L-amino acids. Compared to the choline-deficient diet, this diet is more effective inducing steatotic phenotype and liver triglyceride content.[41] Longer feeding time with CDAA diet (up to 22 weeks) causes inflammation and pronounced fibrosis in mice[42] as well as increased body weight, plasma triglycerides, and insulin resistance.[42, 43] It has been observed that a combination of a CDAA diet with a fat-enriched diet (6–9 weeks of feeding) significantly worsens the fibrotic NASH phenotype in mice.[44] Combination of choline deficiency with methionine deficiency (MCD diet) is one of the most popular, rapid, and reproducible nutritional rodent models of a NASH, however, as in the case of CD diet, the MCD model does not exhibit any of the metabolic features of human NAFLD. In mice, MCD diet causes weight loss (up to 40% in 10 weeks). The animals showed also low fasting blood sugar, peripheral insulin sensitivity, low serum insulin, and decreased blood triglyceride and cholesterol levels.[45, 46, 47] In C57BL/6 mice MCD diet induces rapid and severe lobular inflammation and hepatocyte ballooning (already after 2–8 weeks) and early-onset fibrosis at 8–10 week of feeding.[45] Macrovesicular steatosis, perisinusoidal fibrosis, hepatocyte ballooning, apoptosis and

necroinflammation, as well as mitochondrial anomalies are also haracteristic for MCD-induced NASH phenotype.[48] Also as in the case of CD diet, its combination with HF diet shortens the time to 17 days to the appearance of extensive steatohepatitis with macro- and microvesicular steatosis and inflammatory foci.[49]

2.4.2. High-Fat and Western Diets

A major advantage of these models is their high similarity to the metabolic profile observed in humans suffering by NAFLD. In contrast to the above-described diets, in the case of HF diets, the progression of NAFLD is visible only after extensive feeding time (>34 weeks) and is characterized by less pronounced signs of inflammation.[50] Only mice feed for a much longer time (approx. 50 weeks) developed increased inflammatory liver infiltration and minimal fibrosis. As in other diets, time of appearance NAFLD phenotype and degree of accompanying metabolic alterations depend on species, strain and sex of animals Moreover, high impact on observed NAFLD phenotype can have the FFA composition of HF diet (the content of saturated and unsaturated FFAs). For example, AKR/J mice fed with HF diet enriched in a trans-fatty acid developed more pronounced steatosis and liver damage after 8–16 weeks compared to the mice feed with non-modified HF.[51] Moreover, trans-fat-enriched HF diet significantly increased insulin resistance in mice.[51] In rats, trans-fat-enriched HF diet also develops a more pronounced NAFL profile in comparison to standard HF diet, but without differences in liver damage.[52] Western diets (WDs) mimics in rodents our Western dietary habits especially taking into account a high concentration of saturated fats and simple carbohydrates. It has been shown that a high intake of simple carbohydrates (fructose, sucrose or glucose) without combination with a high-fat diet is responsible for obesity and NAFL development in humans. Especially fructose, known for lipogenic properties, leads to visceral fat deposition, liver TG accumulation, and insulin resistance.[53, 54] The combination of fructose and a HF diet provided to mice for 8 weeks much faster induces steatosis than the HF diet alone. Such combined diet resulted in significant inflammation in the liver, however, without visible progression of liver damage.[55, 56] Mice feed with sucrose-supplemented HF diet for 15 weeks exhibited similar NAFL pattern (as HF diet alone), but increased levels of AST and ALT suggested more serious liver damage.[57] A study by Bortolin et al. (2018) performed on rats showed that 16 weeks of feeding with HF diet combined with sucrose caused significantly more pronounced steatosis, increased liver triglycerides, and obesity in

comparison to the rats fed with the HF diet alone.[58] In contrast to the study presented by Sampey et al.[59], Bortolin and colleagues observed that in their study, rats fed with HF diet alone had the same body weight or fat white deposits as a control chow-diet group.[58] The authors explained the importance of choosing the right control diet for the comparative experiments.[59] In the literature, several other varieties of Western diets have been found in the context of NAFLD induction in rodents. For example, a) the "American Lifestyle-Induced Obesity Syndrome" (ALIOS) diet, being the combination of a HF diet (45 kcal% with 30% fat content from trans fatty acids) and fructose present in drinking water, induces significant steatosis, inflammation, and liver damage in male C57BL/6 mice fed with this diet for 16 weeks. In mice, no fibrosis was observed in liver histology. However, the fibrogenic response in the liver could be detected at the molecular level[60]; b) another example can be a combination of Western diet (HF diet – where 12% of FFA in which saturated combined with fructose in drinking water) supplemented with 2% cholesterol. The results observed for this diet were comparable to those for HF diet-fed only[61]; c) HF diet supplemented with cholesterol and cholate. In rodents, this diet leads to the development of NASH-like liver phenotype, including MDBs and ballooned hepatocytes. However, observed weight loss, increased insulin sensitivity, and lower serum TG levels are opposite to what is observed NAFLD/NASH patients[48]; d) Charlton's fast-food model. This diet is based on a high trans-fat-HF diet (40 kcal% of which 18% is trans-fat), 2% cholesterol, and 20% sucrose present in the food.[62, 63]

It is important to mention that the diet composition impacts the observed phenotype, NAFLD progression, and its transition to NASH. Scientists should always consider an interplay between the genetic background, diet composition, and health conditions, including gut microbiota.[64] Evidence shows that NAFLD is also associated with promoting abnormal gut microorganisms colonization, which may promote liver condition deterioration.[65] Mice fed with High-fat/high-cholesterol (HFHC) diet for 14 months suffered from gut dysbiosis, similar to the observed in hypercholesterolemic patients. Germ-free mice that have been gavage with stools from mice fed HFHC manifested hepatic lipid accumulation, inflammation, and enhanced cell proliferation. This suggests that the microbiota condition may already affect liver response to the studied diet and may impact the disease progression.[66]

The rate of NAFLD manifestation and its progression in animal models also depends on their genetic background. Although most of the human NAFL predisposing

genetic variants are not present and cannot mimic human disease in rodents, Newberry and colleagues recently created a mouse devoid of Tm6sf2 gene (human TM6SF2 variant rs58542926) to study its impact on the development and progression of NAFLD.[67] Tm6 LKO mice fed a high-fat diet for 3 weeks exhibited increased steatosis and fibrosis. This NAFLD phenotype was further exacerbated when mice were fed with high fat/fructose diet for 20 weeks.[67] Among many different mice strains used in NAFLD studies, those naturally predisposed to diabetes type 2 (DM2) and NAFLD development e.g., Lepob/Lepob (ob/ob) or Leprfa/Leprfa rat model (fa/fa, also known as Zucker rats) do not need any particular treatment to observe NAFLD related changes in the liver.[68, 69] Another genetic DM2 model is based on mutation in the Alms1 gene, which leads to the increased food intake, increased body weight and DM2. When these animals are fed for 20–24 weeks with HFD, NASH phenotype with signs of fibrosis was observed.[70, 71]

It is necessary to underline that the above described dietary NAFLD models focus mostly on the metabolic situation observed in patients and may differ regarding clinical or morphologic aspects. Moreover, the accompanying diseases in humans can impact the rate of NAFLD progression, worsening liver function, and prognosis. More information about the above-described diets, their impact on metabolism, mitochondrial function and their efficacy to induce a NASH phenotype, as well as features that are similar in rodents and humans, is summarized in other sources e.g., in the review by Simoes et al (2019).[72]

3. Metabolic comorbidities of NAFLD – mitochondrial abnormalities

NAFLD is common among patients with metabolic syndrome. NAFLD prevalence rises with increasing body mass index (BMI) and the number of criteria defining metabolic syndrome.[20] Type 2 diabetes is an independent risk factor for severe steatosis and fibrosis. Contrarily NAFLD has been shown to more than double the risk of type 2 diabetes.[73] The association between NAFLD and other conditions has been reported including obstructive sleep apnea[74], hyperuricaemia[75] and even neurodegenerative disease[76], and the list is not exhaustive. Therefore, a new definition of metabolic dysfunction-associated liver disease (MAFLD) has been recently proposed and combines hepatic steatosis with overweight or obesity, type 2 diabetes or two or more metabolic abnormalities.[77]

3.1. Mitochondrial abnormalities in early NAFLD

Steatosis *per se* represents a result of storing lipotoxic free fatty acids (FFAs) as stable intracellular triglyceride stores and seem to be an adaptive response of hepatocyte to excessive stress caloric supply. Hepatic lipid accumulation results from a balance between the mechanisms governing lipid intake and lipid clearance. The main pathways involved in these processes are the uptake of circulating lipids, de novo lipogenesis (DNL), fatty acid oxidation (FAO) and very low-density lipoprotein (VLDL) export.[78] In a NAFLD context, free fatty acids (FFAs) uptake is increased due to a higher amount of FFA influx from lipolysis in adipose tissue.[79, 80] Accordingly, fatty acid transporter (FATP2 and FATP5) and translocase proteins (CD36) have been found increased in NAFLD and NASH patients.[81, 82] Moreover, a higher FAs uptake and its intracellular transport inside hepatocytes is correlated with the upregulation of fatty acid-binding proteins FABP1, FABP4 and FABP5[83, 84], which thereby promotes the storage of harmful FAs and subsequent steatosis. A study using stable isotope traces has showed that 60% of hepatic lipid accumulation is derived from adipose tissue lipolysis. Although, other sources have to be taken into account as mentioned above, namely DNL (26%) and the diet (15%).[85] DNL is a condition associated with hyperglycaemia and hyperinsulinemia[86, 87], under the regulation of sterol regulatory element binding protein-1c and carbohydrate responsive element binding protein (ChREBP) in response to glucose and insulin.[88, 89] Therefore, once active, these transcription factors induce de novo synthesis associated-FAs enzymes - acetyl-CoA carboxylase (ACC) and fatty acid synthase, as showed in NAFLD patients and in animal models.[90, 91, 92] Importantly, it was showed that knockout of both ACC-1 and -2 isoforms caused a decrease in hepatic lipid accumulation, thereby protecting against the development of obesity, diabetes and NAFLD.[93, 94] Interestingly, several works have associated ChREBP with higher mitochondrial oxidative phosphorylation efficiency and increased mitochondrial biogenesis.[95] Another lipogenic enzyme that was showed to have a critical role during hepatic lipid accumulation is stearoyl-CoA desaturase 1 (SCD-1).[96] By catalyzing the biosynthesis of monounsaturated fatty acids, SDC-1 prevents the intracellular accumulation of saturated fatty acids, which are described to promote endoplasmic reticulum (ER) stress, cellular apoptosis and in later stages, fibrosis.[97, 98, 99]

A major transcriptional factor involved in regulating hepatic lipid metabolism is peroxisome proliferator-activated receptor-α (PPAR-α). Upon binding to FAs, activated PPAR-α promotes FAs consumption through FAO and ketogenesis.[100] Although different NAFLD studies have reported FAO either increased, unchanged or

decreased[101, 102, 103, 104, 105], there is evidence that liver mitochondria are able to boost FAO in order to compensate for hepatic fat accumulation (DOI: 10.1016/j.livres.2019.06.001). Additionally, the export of triglycerides in the form of VLDL particles also contributes to decreasing fat content. However, this process tends to stabilize and fail to prevent steatosis when hepatic total fat content reaches 10%.[106]

Of note, mitochondria are not passive players in these scenarios, and they actively respond with several alterations to an increased lipid accumulation. During the early phase of adaptive responses to excessive lipids accumulation in hepatocyte cytosol, increased mitochondrial fatty acid oxidation (mFAO)[107], induction of tricarboxylic acid (TCA) cycle and stimulation of oxidative phosphorylation (OXPHOS)[108] could serve as a protective strategy to keep/control low-level FFA in the cytosol. Moreover, 5' AMP-activated protein kinase (AMPK), energy status sensor, inhibits de novo lipogenesis and increases fatty acid oxidation by decreasing malonyl-CoA levels and preventing carnitine palmitoyltransferase 1 (CPT-1) inhibition.[109] Mitochondrial adaptation in NAFL can also be manifested as an increased mitochondrial mass in the liver.[110] At the level of mitochondria, increased FAO is correlated with higher reactive oxygen species production, which may contribute to mitochondrial oxidative damage and subsequent mitochondrial impairment.[111, 112] In steatotic livers, the activity of complex I is reduced approximately 35% and is accompanied with increased H_2O_2 generation. The inhibition of complex I can be explained by oxidization of cardiolipin, which is required for the proper function of complex I.[113, 114]

Mitochondrial dysfunction can be also related to de-regulation of lipid homeostasis e.g., caused by carnitine palmitoyl transferase 1 (CPT1) inhibition in the presence of higher malonyl-CoA levels generated at the DNL pathway.[115] Such de-regulation is associated with the accumulation of lipid-derived toxic metabolites such as ceramides, diacylglycerols and dicarboxylic acids. These molecules are known to interfere with the mitochondrial function, insulin signaling pathway, and the induction of pro-inflammatory cytokines and ER stress. In particular, ceramides and dicarboxylic acids can inhibit electron transport chain and deplete cellular ATP levels, being ceramides depletion associated with higher OXPHOS complexes activities.[116] Along NAFLD development, there is a direct correlation between mitochondrial FAO dysfunction and the development of hepatic steatosis[117, 118], being PPAR-α found downregulated with disease progression and its severity.[119, 120]

3.2. Mitochondrial involvement in progression to NASH

Several factors have been proposed to participate in the pathogenesis of NAFLD. The most important among them seem to be genetic factors, nutrition habits, lipogenesis, insulin resistance, gut microbiota, inflammation, oxidative stress, and mitochondrial/metabolic remodeling. However, our knowledge about the factors responsible for the transition mode in each stage of NAFLD is still incomplete. Garcia-Martinez et al., (2016), highlighted the potential involvement of mitochondria in the disease progression to NASH. The authors suggested mtDNA, considered a pro-inflammatory molecule[121, 122] when released from fatty liver-damaged hepatocytes, causes liver inflammation by TRL-9 activation. In this scenario, mtDNA-induced liver inflammation could be an important factor responsible for the transition to NASH.[34] Interestingly, Fu et al. (2017) proposed that replacement of dysfunctional mitochondria by exogenous HepG2-derived mitochondria may recover hepatocyte function in high-lard-fat- and high-cholesterol feed mice.[123] The experimental approach used by authors comprised intravenous injection of mitochondria isolated from HepG2 cells; however, how mitochondria entered the cells in different tissues and were able to maintain the integrity and restore metabolic activity was not explained .[123]

Metabolic changes resulting from increasing hepatocyte FFA influx can be harmful and damage mitochondria through several mechanisms, including mitochondrial uncoupling and the induction of the mitochondrial permeability transition pore (mPTP) opening and oxidative stress. Chronic FFAs overload and disease progression is responsible for decreased CPT1-mediated FFA transport into mitochondria and defective mFAO. Alterations in mitochondrial respiratory chain complexes' level and activity have been observed in different NAFLD models[124, 112, 125] translates into decreased ATP level. Decreased ATP level could be responsible for the induction of endoplasmic reticulum (ER) stress and unfolded protein response (UPR) activation, which stimulates de novo lipogenesis pathways and further aggravates liver steatosis.[126] Progression of NAFL to NASH is also accompanied by increased mitochondrial cholesterol accumulation[127], leading to the cholesterol-induced alterations in the inner mitochondrial membrane's permeability. Such alterations in the properties of mitochondrial membranes can be a cause of mitochondrial glutathione (GSH) depletion described in NASH patients.[128] In patients with more advanced forms of NAFLD decreased of mtDNA levels have been observed.[34, 129]

It is not surprising that alterations in mitochondrial morphology and function can impact liver physiology. The direct link between remodeling of mitochondrial structure, metabolic dysfunction, and clinical phenotype development has been repeatedly demonstrated in several pathologies. In the range of adaptative response to the excessive FFA accumulation in the liver, in the initial NAFLD stages, observed metabolic changes are associated with an increase in mitochondrial mass, with or without increased mitochondrial fatty acid oxidation. Koliaki at al. (2015) described that maximal respiration rates measured in isolated mitochondria from obese patients with or without NAFL was 4.3- to 5.0-fold higher than lean individuals.[130] This was also confirmed by Sunny et al [108], who showed that mitochondrial oxidative metabolism was increased in the livers of subjects with elevated intrahepatic triglycerides. The increase in mitochondrial oxidative metabolism involved a 2~fold induction of oxidative fluxes through the TCA cycle. This finding also demonstrated that even with a large accumulation of triglycerides in the liver, the TCA cycle is functional. Interestingly, increase TCA fluxes were associated with gluconeogenesis, which, according to the authors, could account for the increased energy demand observed in individuals with NAFLD. The results from this study also dismiss the notion that mitochondrial dysfunction is a primary event in the progression from steatosis to more severe forms. Still, mitochondrial substrate overload could contribute to ROS generation or possibly to a cellular metabolic unbalance, which can prime hepatocytes for a pro-inflammatory state. Interestingly, even with increased mitochondrial substrate oxidation via the TCA cycle, lipotoxicity and incomplete fat oxidation typical of NAFLD progression to NASH were not avoided [131], contributing to inflammation and fibrosis. Agreeing with a progressive failure to maintain an effective lipid oxidation profile with increase intra-hepatic steatosis is the fact that mitochondrial biogenesis is inhibited and decreases with progression towards NASH. Interestingly, simultaneously with altered mitochondrial biogenesis, mitochondrial mass increases[110], although such mitochondria were swollen and showed a loss of cristae structure and were characterized by 31-40% lower maximal respiration and mitochondrial uncoupling.[130] This suggests that higher mitochondrial mass detected in NASH patients could result from defective removal of damaged mitochondria.[130]

It has been demonstrated that incubation of HepG2 cells with saturated fatty acids (a model resembling NASH), causes mitochondrial abnormalities accompanied with inhibition of mtDNA gene expression and accelerated degradation of respiratory

chain subunits.[132] Sequential exposure of hepatocytes to high concentrations of fatty acids and TNF-α mimic *in vitro* the progression of NAFLD from simple steatosis to steatohepatitis. In such a condition, the damage could be observed not only at a mitochondrial level but also elsewhere in the hepatocyte. Among them are increased apoptosis, reduced hepatocyte viability, increased oxidative stress, reduction in lipid droplet size, and up-regulation of IkappaB kinase beta-interacting protein and adipose triglyceride lipase expressions.[133]

More details about the interplay between mitochondrial dynamics and NAFLD can be found in a recent review by Longo et al.[134] In more advanced disease stages, the presence of megamitochondria (giant mitochondria) was also described.[135] Their presence has been also reported in other tissues with a high degree of metabolic activity.[136] However, it is still unknown whether the presence of megamitochondria in the liver is an adaptation or a consequence of NAFLD development.[135] Up to now, there is no clear explanation for the progressive decline of OXPHOS during NASH. Begriche et al, proposed possible mechanisms explaining OXPHOS dysfunction: lipotoxicity, oxidative stress and effect of interferons, adiponectin, and forkhead box protein O1 (FoxO1). For a detailed description of relations between abovementioned factors and mitochondrial dysfunction, see their comprehensive review.[137] A summary of the described alterations in the mitochondrial function has been presented in **Figure 1**.

4. ROS and RNS culprits or bystanders of NAFLD progression

Under physiological conditions, ROS are continuously produced in the liver due to intracellular metabolism, although kept under a certain threshold for redox signalling pathways, e.g., cell proliferation and differentiation.[138] Moreover, ROS like H_2O_2 regulates the expression of many genes, including AP-1, CREB, HSF1, NRF2, HIF-1, TP53, NF-κB, NOTCH, SP1 or SCREB-1.[139, 140] Due to their chemical structure, ROS are divided into two main categories: free radicals (superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($HO^{\bullet}$), nitric oxide ($NO^{\bullet}$, that we will describe in more detail below), nitrogen dioxide ($NO_2^{\bullet}$), carbonate radical anion ($CO_3^{\bullet-}$), and alkoxyl/alkyl peroxyl ($RO^{\bullet}$/$ROO^{\bullet}$)) and non-radicals (hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and peroxynitrite ($ONOO^-$)/peroxynitrous acid (ONOOH)). For a detailed description of chemical ROS features, we refer to a comprehensive review.[141] Within the different species, $O_2^{\bullet}$, $NO^{\bullet}$, and H_2O_2 are the primary molecules produced within the cell. These low reactivity molecules can readily generate other ROS species (HOCl, $ONOO^-$, and

ONOOH), which ultimately can form $NO_2^{\bullet}$, $CO_3^{\bullet-}$, and $RO^{\bullet}/ROO^{\bullet}$, known as powerful inducers of cellular oxidative damage.

To overcome nitroso-oxidative stress, cells possess a vast panel of enzymatic and non-enzymatic antioxidant defense systems. Enzymatic mechanisms include superoxide dismutase (SOD1-3), which converts $O_2^{\bullet-}$ into H_2O_2, which can be further converted into H_20 and O_2 by catalase. Moreover, H_2O_2 is also converted into H_20 by the action of glutathione peroxidase (GPX) or peroxiredoxin (PRDX), with oxidized glutathione (GSSG) converted back to its reduced form (GSH) by the action of glutathione reductase (GR). The cell's redox status regulation can be restrained due to GSH depletion and GSSG accumulation, as shown in NAFLD/NASH primary hepatocytes and NAFLD rodent models.[127, 125] H_2O_2 can also be reduced by the action of PRDX, using reduced thioredoxin as the electron donor. Lastly, glutathione-S-transferase can detoxify xenobiotic compounds through its conjugation with GSH. RNS/ROS can freely diffuse within various cell organelles, their signalling or damage effects within specific cell compartments constraints are limited to their half-life.[142]

Taking into account several controversies, mitochondria are considered one of the important sources of ROS and these, when produced extensively during pathological conditions.[143, 144] So far, several distinct sites of ROS production in mammalian mitochondria have been identified. NADH:ubiquinone oxidoreductase (complex I) is considered a major source of reactive oxygen species in mitochondria. Several sites of $O_2^{\bullet-}$ production in mitochondrial complex I have been proposed including: flavin[145, 146, 147], bound reduced nucleotide [148], FeS clusters N2[149] and N1a[150], and a semiquinone radical.[151, 152] Complex II is another source of mitochondrial ROS, involving the release of electrons from the flavin site or in a RET mode from a reduced ubiquinone pool.[153] Interestingly, a mutation in complex II might also result in $O_2^{\bullet-}$overproduction.[154] At Complex III, $O_2^{\bullet-}$ can be generated from the ubisemiquinone site and released to the intermembrane space, which can permeate into the matrix in the form of H_2O_2.[155] Other mitochondrial enzymes that have been associated with ROS production are glycerol 3-phosphate dehydrogenase considered as donor of electrons to ETC; α-ketoglutarate dehydrogenase and pyruvate dehydrogenase involved in TCA cycle.[156] In addition, the flavoprotein acyl-CoA dehydrogenase can produce ROS during oxidation of lipid-derived substrates.[157, 158]. Other documented sources of ROS in mitochondria include two other enzymes: monoamine oxidase and dihydroorotate dehydrogenase.[159, 160] It is important to underline that also microsomes (cytochrome P450, diamine oxidase),

peroxisomes (enzymes involved in fatty acid oxidation) and enzymes in the plasma membrane (like e.g., NADPH oxidase and lipoxygenase) have been identified as ROS generators.

Increased mitochondrial activity in the early phase of NAFL protects hepatocytes from lipotoxicity, while under excessive lipid influx increased activity can extensively generate ROS.[137] Increased ROS generation is caused by the fact that continuous supply of reduced substrates to the electron transport chain (ETC) promotes the leak of electrons from reduced flavin mononucleotide (FMN) or by reverse electron transfer (RET) at Complex I, resulting in $O_2^{\bullet-}$ release into the mitochondrial matrix.[161] Several reports have highlighted that accelerated β-oxidation of short-, medium- and long-chain saturated FAs with augmented CPT-1α gene expression, causes excessive mitochondrial electron flux, resulting in increased mitochondrial $O_2^{\bullet-}$ production in *in vitro* and *in vivo* models of steatosis.[162, 112] Moreover, UCP-2 up-regulation can protect hepatocytes from deleterious ROS-effects.[163] Additionally, the nuclear factor erythroid-derived 2 like 2 (NRF2) in response to the elevated level of $O_2^{\bullet-}$, activates expression of a compensatory antioxidant defence response.[164] A mitochondrial pro-oxidant state can overwhelm the mitochondrial antioxidant system (decreased SOD, GPX1 activity, and GSH/GSSG ratio levels) and result in oxidative injury in different mitochondrial structures containing proteins and lipids[165], depletion of mtDNA copy number and higher mtDNA damage during the progression of NAFLD towards NASH and beyond.[166, 167] Damaged mitochondria with loss of FAO and respiratory complexes activities were described in later stages of the disease.[163] At high concentration, some ROS, such as the hydroxyl radical, a highly-reactive molecule, can cause oxidative modification of lipids (lipid peroxidation - malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE))[168], proteins (carbonylation and nitration)[169], and nucleic acids (mtDNA depletion and DNA damage – 8-hydroxy-2'-deoxyguanosine (8-OHdG)).[130] The notion of nitroso-oxidative stress involves different markers ranging from alterations in ROS/RNS production to oxidative damage and altered antioxidant enzymes' activity. Several pieces of evidence suggest that the accumulation of hepatocyte damage is in the origin of the maladaptive response of hepatocytes to fat accumulation, thereby leading to hepatic metabolic impairment and NASH.[34, 170]

In the context of hepatic steatosis, there is a pro-oxidant state with activation of ROS-generating mechanisms while the levels/ activities of antioxidants are impaired, as identified in NAFLD animal models and NAFLD/ NASH clinical cohorts.[171, 172, 125]

Some data suggest that, NAD^+ dependent deacetylase, sirtuin 3 (SIRT3) is implicated in the modulation of mitochondrial ROS response and in the modulation of hepatocyte susceptibility to cell death/ autophagy in the presence of a high-fat (HF) diet.[173, 174] One pathway by which oxidative stress might cause the disruption of the mitochondrial network and function is through cardiolipin (CL) oxidation.[114] CL, an exclusive mitochondrial phospholipid, is highly sensitive to unsaturated bond-ROS attack, and its oxidation may lead to cytochrome c release from mitochondria, thereby triggering apoptotic cell death.[166]

It has been proposed that the accumulation of ROS-related damages is in the origin of the maladaptive response of hepatocytes to fat accumulation, thereby leading to hepatic metabolic impairment and NASH.[34, 170] Nitric oxide (NO•) is a RNS/ROS whose contribution for NAFLD progression is still obscure, ranging from the possible culprit, or at least contributor, to liver/vascular protectant in NAFLD. The role of (NO•) in NAFLD-related inflammation has been previously described.[175] Moreover, an increase in NO• production in NASH patients compared to more benign NAFLD phenotypes was observed suggesting that it can be considered as an inflammation marker in patients progressing from liver steatosis to steatohepatitis.[175] In contrast, Gu et al. described NO• as a protective agent for NAFLD progression towards more severe disease forms.[176] However, this observation is quite controversial because an increase, rather than a decrease of NO• production was shown to accompany NAFLD progression.[177] Indeed, NO•-mediated nitration of hepatocellular proteins, visualized by 3-nitrotyrosine level is markedly induced in the inflamed liver tissue from patients with chronic liver disease.[178] Interestingly, NAFLD has been linked with altered blood pressure, suggesting endothelial NO• synthase (eNOS) dysfunction in the disease's pathogenesis. Chronic inhibition of eNOS via N(ω)-nitro-L-arginine methyl ester (L-NAME) increased liver injury in a rat model of obesity, insulin resistance, and NAFLD.[179] Indeed, NAFLD patients show a marked eNOS dysfunction in platelets and liver tissue[180], associated with S1177 phosphorylation.[181]

Although excessive cellular ROS and /-or oxidative damage levels in NAFLD are associated with a compromised mitochondrial structure and function, most NAFLD studies lack experimental evidence to support the primary role of mitochondria as an essential source of ROS.[125, 166, 182] In fact, a comparative study investigating the contribution of different cellular organelles to ROS production demonstrated that endoplasmic reticulum (ER) and peroxisomes produce more H_2O_2 than mitochondria in

rodents liver.[183] Furthermore, recent studies by Einer et al. described a mitochondrial adaptation linked to fat accumulation without evidence of mitochondrial oxidative stress markers during steatosis.[55] This work was supported by another study in which it was shown that oxidative stress in steatotic mice was caused by peroxisomes and not by mitochondria, in which no or very reduced ROS production and oxidative damage were observed.[184] These observations are consistent with the up-regulation of peroxisome proliferator-activated receptor alpha (PPAR-α) and ACOX genes, as well as with higher levels of peroxisomal-related proteins in livers of HF fed-mice[185, 184] and in NAFLD patients.[186, 187]. Furthermore, a higher peroxisomal β-oxidation rate is responsible for A/J mice resistance to diet-induced steatosis and obesity.[188] This included the upregulation of genes involved in peroxisomal structure (Pex11a), VLCFAs uptake (Abcd3), FAO (Slc27a2, Acsl4, Ehhadh, Ech1, Crat), and detoxification (Cat, Alas1). Accordingly, an increased peroxisomal oxidative activity seems to represent an alternative pathway to support mitochondrial function, especially when its oxidative capacity is overloaded, as described in high-fat diet-induced steatosis.[189] This role might explain the proximity between peroxisomes and lipid droplets in the cytosol.[190] However, peroxisomal FAO is associated with an exacerbation of oxidative stress in microvesicular steatosis.[191] Other organelles that can counteract lipid accumulation, such as peroxisomes and microsomes or even cytosolic ROS-generating enzymes as NADPH oxidase (NOX) xanthine oxidase have emerged as central ROS producers during the onset of NAFLD.

Another example can be the 66kDa isoform of Shc (p66shc), an adaptor and redox protein that has been already linked to the lipid metabolism regulation by numerous reports.[192] It has been demonstrated that p66Shc participates in various mechanisms of liver injury.[193, 194] In livers of NAFLD patients, expression levels of apopotosis regulating proteins p53, p21, and p66Shc were significantly increased.[195] p53/p66Shc associated pathway was shown to play a significant regulatory role in NASH progression.[195] Similar observation comes from rodent NAFLD model. [196] Zhang et al. described a correlation between increased hepatic p66shc expression and upregulated expression of senescence markers: heterochromatin protein-1-beta (HP1b), p16, p21, and p53.[196] Slowed down steatosis development has been observed for p66Shc knockdown condition and on the other hand, overexpression of p66Shc promoted senescence and steatosis in L02 cells.[196] Moreover, targeting the p66Shc - cytochrome c cascade by catalpol can prevent the development of NAFLD

complications, which could be mediated by a specific microRNA, miR-96-5p effect.[197] Interestingly, it has been also demonstrated that the sirtuin 1 (SIRT1) / p66Shc anti-apoptosis pathway is a good target to prevent NAFLD-related injury.[198] A negative effect of p66Shc activation on liver mitochondria function can be additionaly prevented by exercise which , promotes liver mitochondria adaptive remodeling and hepatocyte renewal.[199] Recently, it was also shown that the p66Shc oxidative and ER stress pathway can be a potential therapeutic target in NAFLD as Isosteviol (ISV) prevents FFA-/HFD-induced hepatic injury. In rat model, ISV specifically inhibits expression, activation and translocation of p66Shc to mitochondria protecting against FFA-/HFD-induced hepatic injury.[198]

Peroxisomes are responsible for α-oxidation of branched-chain FAs and β-oxidation of very-long chain FAs (VLCFA), and other processes as amino acid metabolism and biosynthesis of glycerophospholipids and bile acids. These organelles act as a chain shortening system, producing the short and medium FAs further diverted to mitochondria to complete their oxidation. Acyl-CoA oxidases (ACOX), D-amino acid oxidase, D-aspartate oxidase, L-α-hydroxyacid oxidase, polyamine oxidase, and xanthine oxidase can generate ROS in peroxisomes.[200] Peroxisomes can further detoxify ROS by the most pre-eminent enzyme – catalase or by other peroxisomal resident antioxidant enzymes. Importantly, catalase silencing caused significantly more lipid accumulation, oxidative stress, and inflammation in high-fat fed mice when compared to matched controls.[201] Furthermore, a higher peroxisomal β-oxidation rate is responsible for A/J mice resistance to diet-induced steatosis and obesity.[188]

A defective peroxisomal β-oxidation and a sustained peroxisome proliferator-activated receptor alpha (PPAR-α) activation seem to contribute to cytochrome P450 (CYP4A) induction and hepatic oxidative injury.[202] These observations provide evidence that if a VLCFAs oxidative pathway as peroxisomal FAO is defective or insufficient, the microsomal pathway may act as another alternative oxidative pathway. In ω-oxidation of VLCFA in microsomes, the first step of the reaction is catalysed by oxido-reductase CYP2E1/CYP4A enzymes in the presence of NADPH and O_2 with the generation of $O_2^{\bullet-}$ and H_2O_2 as byproducts.[203] Even though ω-oxidation is considered a minor FAO pathway under basal conditions[204], up-regulation of this pathway may occur in the context of hepatic fat accumulation in rodents and humans.[205, 206] Moreover, CYP2E1-mediated oxidative stress could induce insulin resistance development and inflammation, thereby aggravating NAFLD severity.[207] Although there are some

discrepancies regarding the participation of CYP2E1 and NOX in cellular ROS production[208, 209], a few other studies have also confirmed the role of NOX in oxidative stress of fatty liver rats and NASH patients.[208, 210] It was shown that hepatocyte-NOX4 deletion reduced oxidative stress, improved insulin sensitivity and decreased liver inflammation and fibrosis in a NASH-induced mouse model.[210]

Mitochondria, peroxisomes, microsomes, and RNS/ROS-generating enzymes such as NOX seem to play a role in ROS production during an early stage of NAFLD. Control of RNS/ROS for improving NAFLD/NASH phenotype may require a multi-organelle targeting approach. A potential clue for the role of ROS in NAFLD progression arises from the therapeutic use of antioxidants. Antioxidants such as sterol ester of alpha-linolenic acid[211], physalin B[212], N-acetyl cysteine[213], or vitamin E and vitamin C[214] showed beneficial effects against liver injury, including fibrosis, in the context of NAFLD. Still, a meta-analysis of randomized trials for treating NAFLD found that those including antioxidants gave mixed results.[215] Interestingly, not only coenzyme Q10, a component of the mitochondrial respiratory chain, prevented some of the hallmarks of the NAFLD phenotype[216], but its mitochondrial-targeted form, MitoQ (or mitoquinone), also showed some benefits in multiple models of fatty liver.[217, 218] This demonstrates that ROS (and probably RNS) do have an essential role in NAFLD progression, and that mitochondrial-derived ROS can play, at least in part, a role in key time-points of the disease progression. A schematic representation of hepatocyte cellular pathways involved in ROS generation in a NAFLD context is presented in **Figure 2**.

For example, vitamin E – a fat-soluble antioxidant was analyzed in the PIVENS trial published in 2010. Histological assessment of patients taking part in PIVENS study showed that vitamin E at the dose of 800 IU/day led to reduced hepatocyte ballooning ($p = 0.005$) and lobular inflammation ($P = 0.02$) but had no significant effects on liver fibrosis.[219] The subsequent TONIC trial involving 173 children and adolescents in NAFLD disappointingly showed that vitamin E (400 IU/twice daily for 96 weeks) did not reduce liver steatosis, fibrosis or lobular inflammation.[220] According to current recommendations, vitamin E can be considered as a short-term therapy in patients with biopsy-proven NASH. However, safety concerns (e.g., increased all-cause mortality[221] as well as enhances risk for prostate cancer[222]) need to be discussed with the patient before starting the therapy. The trials investigating resveratrol – a polyphenol with antioxidant properties found in fruits and vegetables did not show major effects on fatty

liver.[223] To date, obeticholic acid (OCA) is one of the few drugs that showed beneficial effects on NASH. Interim analysis of a phase 3 trial demonstrated that OCA at the dose of 25 mg can significantly improve fibrosis and components of NASH[224]. However, these results have not led to the approval of OCA as therapy for patients with fatty liver. Given the paucity of medications (acting as antioxidants), which are tackling NASH, current recommendations for patients with hepatic steatosis include lifestyle changes, weight loss, and correction of risk factors for progressive NAFLD.

5. Conclusion

Several contradictory observations describing mitochondrial response at different NAFLD stages reported in the literature makes it very difficult to define with certainty the time course of changes in mitochondrial parameters during NAFLD progression. Fromenty's group in their elegant review proposed several factors that could be responsible for the observed discrepancies between the studies investigating mitochondrial function along with the NAFLD progression.[137] Among them are imperfect histological classification, nutritional and genetic factors and methodology used.[137] Moreover, ambiguous results from antioxidant administration may result from different treatment protocols, the specific disease stages at which the treatment is initiated, and the antioxidant molecule's potential to have a multi-targeting activity, for example by reducing inflammation in the liver.

Hence, based on our recent knowledge, it is difficult to answer whether mitochondrial abnormalities and or mitochondrial/peroxisomal related oxidative stress are culprits or bystanders of NAFLD development and progression. Further carefully designed mitochondrial studies involving patients at different NAFLD stages and animal models of NAFLD are necessary to demonstrate the relationship between mitochondrial bioenergetics, oxidative metabolism, metabolic syndrome, oxidative stress and progression of NAFL to more severe NAFLD stages.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology (Baltimore, Md).* 2018;67(1):328-357.
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology (Baltimore, Md).* 2016;64(1):73-84.
3. Wong RJ, Aguilar M, Cheung R, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology.* 2015;148(3):547-555.
4. Haldar D, Kern B, Hodson J, et al. Outcomes of liver transplantation for non-alcoholic steatohepatitis: A European Liver Transplant Registry study. *Journal of hepatology.* 2019;71(2):313-322.
5. Krawczyk M, Liebe R, Lammert F. Toward Genetic Prediction of Nonalcoholic Fatty Liver Disease Trajectories: PNPLA3 and Beyond. *Gastroenterology.* 2020;158(7):1865-1880.e1861.
6. Caussy C, Soni M, Cui J, et al. Nonalcoholic fatty liver disease with cirrhosis increases familial risk for advanced fibrosis. *The Journal of clinical investigation.* 2017;127(7):2697-2704.
7. Dai G, Liu P, Li X, Zhou X, He S. Association between PNPLA3 rs738409 polymorphism and nonalcoholic fatty liver disease (NAFLD) susceptibility and severity: A meta-analysis. *Medicine.* 2019;98(7):e14324.
8. Lauridsen BK, Stender S, Kristensen TS, et al. Liver fat content, non-alcoholic fatty liver disease, and ischaemic heart disease: Mendelian randomization and meta-analysis of 279 013 individuals. *European heart journal.* 2018;39(5):385-393.
9. Huang Z, Guo X, Zhang G, Liang L, Nong B. Correlation between PNPLA3 rs738409 polymorphism and hepatocellular carcinoma: a meta-analysis of 10,330 subjects. *The International journal of biological markers.* 2019;34(2):117-122.
10. Carlsson B, Lindén D, Brolén G, et al. Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis. *Alimentary pharmacology & therapeutics.* 2020;51(12):1305-1320.
11. Kumari M, Schoiswohl G, Chitraju C, et al. Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. *Cell metabolism.* 2012;15(5):691-702.
12. Zechner R, Zimmermann R, Eichmann TO, et al. FAT SIGNALS--lipases and lipolysis in lipid metabolism and signaling. *Cell metabolism.* 2012;15(3):279-291.
13. Pirazzi C, Valenti L, Motta BM, et al. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. *Human molecular genetics.* 2014;23(15):4077-4085.
14. Kahali B, Liu YL, Daly AK, Day CP, Anstee QM, Speliotes EK. TM6SF2: catch-22 in the fight against nonalcoholic fatty liver disease and cardiovascular disease? *Gastroenterology.* 2015;148(4):679-684.
15. Johansen A, Rosti RO, Musaev D, et al. Mutations in MBOAT7, Encoding Lysophosphatidylinositol Acyltransferase I, Lead to Intellectual Disability Accompanied by Epilepsy and Autistic Features. *American journal of human genetics.* 2016;99(4):912-916.
16. Mancina RM, Dongiovanni P, Petta S, et al. The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent. *Gastroenterology.* 2016;150(5):1219-1230.e1216.

17. Emdin CA, Haas ME, Khera AV, et al. A missense variant in Mitochondrial Amidoxime Reducing Component 1 gene and protection against liver disease. *PLoS genetics.* 2020;16(4):e1008629.
18. Abul-Husn NS, Cheng X, Li AH, et al. A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. *The New England journal of medicine.* 2018;378(12):1096-1106.
19. Bianco C, Jamialahmadi O, Pelusi S, et al. Non-invasive stratification of hepatocellular carcinoma risk in non-alcoholic fatty liver using polygenic risk scores. *Journal of hepatology.* 2020.
20. Wong WK, Chan WK. Nonalcoholic Fatty Liver Disease: A Global Perspective. *Clinical therapeutics.* 2021.
21. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md).* 2005;41(6):1313-1321.
22. Bedossa P, Poitou C, Veyrie N, et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology (Baltimore, Md).* 2012;56(5):1751-1759.
23. Kleiner DE, Brunt EM, Wilson LA, et al. Association of Histologic Disease Activity With Progression of Nonalcoholic Fatty Liver Disease. *JAMA network open.* 2019;2(10):e1912565.
24. Povero D, Yamashita H, Ren W, et al. Characterization and Proteome of Circulating Extracellular Vesicles as Potential Biomarkers for NASH. *Hepatology communications.* 2020;4(9):1263-1278.
25. Lădaru A, Bălănescu P, Stan M, Codreanu I, Anca IA. Candidate proteomic biomarkers for non-alcoholic fatty liver disease (steatosis and non-alcoholic steatohepatitis) discovered with mass-spectrometry: a systematic review. *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals.* 2016;21(2):102-114.
26. Mayo R, Crespo J, Martínez-Arranz I, et al. Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: Results from discovery and validation cohorts. *Hepatology communications.* 2018;2(7):807-820.
27. Loomba R, Quehenberger O, Armando A, Dennis EA. Polyunsaturated fatty acid metabolites as novel lipidomic biomarkers for noninvasive diagnosis of nonalcoholic steatohepatitis. *Journal of lipid research.* 2015;56(1):185-192.
28. Puri P, Wiest MM, Cheung O, et al. The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology (Baltimore, Md).* 2009;50(6):1827-1838.
29. Piazzolla VA, Mangia A. Noninvasive Diagnosis of NAFLD and NASH. *Cells.* 2020;9(4).
30. Singh SP, Barik RK. NonInvasive Biomarkers in Nonalcoholic Fatty Liver Disease: Are We There Yet? *Journal of clinical and experimental hepatology.* 2020;10(1):88-98.
31. Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. *Nature.* 1981;290(5806):457-465.
32. Malik AN, Czajka A. Is mitochondrial DNA content a potential biomarker of mitochondrial dysfunction? *Mitochondrion.* 2013;13(5):481-492.
33. Malik AN, Simões ICM, Rosa HS, Khan S, Karkucinska-Wieckowska A, Wieckowski MR. A Diet Induced Maladaptive Increase in Hepatic Mitochondrial DNA Precedes OXPHOS Defects and May Contribute to Non-Alcoholic Fatty Liver Disease. *Cells.* 2019;8(10).

34. Garcia-Martinez I, Santoro N, Chen Y, et al. Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9. *The Journal of clinical investigation.* 2016;126(3):859-864.
35. Ma C, Liu Y, He S, et al. Association Between Leukocyte Mitochondrial DNA Copy Number and Non-alcoholic Fatty Liver Disease in a Chinese Population Is Mediated by 8-Oxo-2'-Deoxyguanosine. *Frontiers in medicine.* 2020;7:536.
36. Nishimoto S, Fukuda D, Higashikuni Y, et al. Obesity-induced DNA released from adipocytes stimulates chronic adipose tissue inflammation and insulin resistance. *Science advances.* 2016;2(3):e1501332.
37. Kulinski A, Vance DE, Vance JE. A choline-deficient diet in mice inhibits neither the CDP-choline pathway for phosphatidylcholine synthesis in hepatocytes nor apolipoprotein B secretion. *The Journal of biological chemistry.* 2004;279(23):23916-23924.
38. da Costa KA, Cochary EF, Blusztajn JK, Garner SC, Zeisel SH. Accumulation of 1,2-sn-diradylglycerol with increased membrane-associated protein kinase C may be the mechanism for spontaneous hepatocarcinogenesis in choline-deficient rats. *The Journal of biological chemistry.* 1993;268(3):2100-2105.
39. Oliveira CP, da Costa Gayotto LC, Tatai C, et al. Oxidative stress in the pathogenesis of nonalcoholic fatty liver disease, in rats fed with a choline-deficient diet. *J Cell Mol Med.* 2002;6(3):399-406.
40. da Costa KA, Garner SC, Chang J, Zeisel SH. Effects of prolonged (1 year) choline deficiency and subsequent re-feeding of choline on 1,2-sn-diradylglycerol, fatty acids and protein kinase C in rat liver. *Carcinogenesis.* 1995;16(2):327-334.
41. Nakae D, Mizumoto Y, Andoh N, et al. Comparative changes in the liver of female Fischer-344 rats after short-term feeding of a semipurified or a semisynthetic L-amino acid-defined choline-deficient diet. *Toxicol Pathol.* 1995;23(5):583-590.
42. Kodama Y, Kisseleva T, Iwaisako K, et al. c-Jun N-terminal kinase-1 from hematopoietic cells mediates progression from hepatic steatosis to steatohepatitis and fibrosis in mice. *Gastroenterology.* 2009;137(4):1467-1477 e1465.
43. Miura K, Kodama Y, Inokuchi S, et al. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology.* 2010;139(1):323-334 e327.
44. Matsumoto M, Hada N, Sakamaki Y, et al. An improved mouse model that rapidly develops fibrosis in non-alcoholic steatohepatitis. *Int J Exp Pathol.* 2013;94(2):93-103.
45. Itagaki H, Shimizu K, Morikawa S, Ogawa K, Ezaki T. Morphological and functional characterization of non-alcoholic fatty liver disease induced by a methionine-choline-deficient diet in C57BL/6 mice. *Int J Clin Exp Pathol.* 2013;6(12):2683-2696.
46. Lau JK, Zhang X, Yu J. Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances. *J Pathol.* 2017;241(1):36-44.
47. Ibrahim SH, Hirsova P, Malhi H, Gores GJ. Animal Models of Nonalcoholic Steatohepatitis: Eat, Delete, and Inflame. *Dig Dis Sci.* 2016;61(5):1325-1336.
48. Denk H, Abuja PM, Zatloukal K. Animal models of NAFLD from the pathologist's point of view. *Biochim Biophys Acta Mol Basis Dis.* 2019;1865(5):929-942.
49. Kudo H, Takahara T, Yata Y, Kawai K, Zhang W, Sugiyama T. Lipopolysaccharide triggered TNF-alpha-induced hepatocyte apoptosis in a murine non-alcoholic steatohepatitis model. *Journal of hepatology.* 2009;51(1):168-175.
50. Ito M, Suzuki J, Tsujioka S, et al. Longitudinal analysis of murine steatohepatitis model induced by chronic exposure to high-fat diet. *Hepatol Res.* 2007;37(1):50-57.

51. Koppe SW, Elias M, Moseley RH, Green RM. Trans fat feeding results in higher serum alanine aminotransferase and increased insulin resistance compared with a standard murine high-fat diet. *American journal of physiology Gastrointestinal and liver physiology.* 2009;297(2):G378-384.
52. Longhi R, Almeida RF, Machado L, et al. Effect of a trans fatty acid-enriched diet on biochemical and inflammatory parameters in Wistar rats. *Eur J Nutr.* 2017;56(3):1003-1016.
53. Softic S, Cohen DE, Kahn CR. Role of Dietary Fructose and Hepatic De Novo Lipogenesis in Fatty Liver Disease. *Dig Dis Sci.* 2016;61(5):1282-1293.
54. Sanches SC, Ramalho LN, Augusto MJ, da Silva DM, Ramalho FS. Nonalcoholic Steatohepatitis: A Search for Factual Animal Models. *Biomed Res Int.* 2015;2015:574832.
55. Einer C, Hohenester S, Wimmer R, et al. Mitochondrial adaptation in steatotic mice. *Mitochondrion.* 2018;40:1-12.
56. Sellmann C, Priebs J, Landmann M, et al. Diets rich in fructose, fat or fructose and fat alter intestinal barrier function and lead to the development of nonalcoholic fatty liver disease over time. *The Journal of nutritional biochemistry.* 2015;26(11):1183-1192.
57. Ishimoto T, Lanaspa MA, Rivard CJ, et al. High-fat and high-sucrose (western) diet induces steatohepatitis that is dependent on fructokinase. *Hepatology (Baltimore, Md).* 2013;58(5):1632-1643.
58. Bortolin RC, Vargas AR, Gasparotto J, et al. A new animal diet based on human Western diet is a robust diet-induced obesity model: comparison to high-fat and cafeteria diets in term of metabolic and gut microbiota disruption. *Int J Obes (Lond).* 2018;42(3):525-534.
59. Sampey BP, Vanhoose AM, Winfield HM, et al. Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. *Obesity (Silver Spring).* 2011;19(6):1109-1117.
60. Tetri LH, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. *American journal of physiology Gastrointestinal and liver physiology.* 2008;295(5):G987-995.
61. Charlton M, Krishnan A, Viker K, et al. Fast food diet mouse: novel small animal model of NASH with ballooning, progressive fibrosis, and high physiological fidelity to the human condition. *American journal of physiology Gastrointestinal and liver physiology.* 2011;301(5):G825-834.
62. Kristiansen MN, Veidal SS, Rigbolt KT, et al. Obese diet-induced mouse models of nonalcoholic steatohepatitis-tracking disease by liver biopsy. *World J Hepatol.* 2016;8(16):673-684.
63. Clapper JR, Hendricks MD, Gu G, et al. Diet-induced mouse model of fatty liver disease and nonalcoholic steatohepatitis reflecting clinical disease progression and methods of assessment. *American journal of physiology Gastrointestinal and liver physiology.* 2013;305(7):G483-495.
64. Yu J, Marsh S, Hu J, Feng W, Wu C. The Pathogenesis of Nonalcoholic Fatty Liver Disease: Interplay between Diet, Gut Microbiota, and Genetic Background. *Gastroenterol Res Pract.* 2016;2016:2862173.
65. Ruuskanen MO, Aberg F, Mannisto V, et al. Links between gut microbiome composition and fatty liver disease in a large population sample. *Gut Microbes.* 2021;13(1):1-22.

66. Zhang X, Coker OO, Chu ES, et al. Dietary cholesterol drives fatty liver-associated liver cancer by modulating gut microbiota and metabolites. *Gut.* 2021;70(4):761-774.
67. Newberry EP, Hall Z, Xie Y, et al. Liver specific deletion of mouse Tm6sf2 promotes steatosis, fibrosis and hepatocellular cancer. *Hepatology (Baltimore, Md).* 2021.
68. Lindstrom P. The physiology of obese-hyperglycemic mice [ob/ob mice]. *ScientificWorldJournal.* 2007;7:666-685.
69. Van Herck MA, Vonghia L, Francque SM. Animal Models of Nonalcoholic Fatty Liver Disease-A Starter's Guide. *Nutrients.* 2017;9(10).
70. Jacobs A, Warda AS, Verbeek J, Cassiman D, Spincemaille P. An Overview of Mouse Models of Nonalcoholic Steatohepatitis: From Past to Present. *Curr Protoc Mouse Biol.* 2016;6(2):185-200.
71. Williams KH, Shackel NA, Gorrell MD, McLennan SV, Twigg SM. Diabetes and nonalcoholic Fatty liver disease: a pathogenic duo. *Endocr Rev.* 2013;34(1):84-129.
72. Simoes ICM, Janikiewicz J, Bauer J, et al. Fat and Sugar-A Dangerous Duet. A Comparative Review on Metabolic Remodeling in Rodent Models of Nonalcoholic Fatty Liver Disease. *Nutrients.* 2019;11(12).
73. Cariou B, Byrne CD, Loomba R, Sanyal AJ. Nonalcoholic fatty liver disease as a metabolic disease in humans: A literature review. *Diabetes, obesity & metabolism.* 2021.
74. Petta S, Marrone O, Torres D, et al. Obstructive Sleep Apnea Is Associated with Liver Damage and Atherosclerosis in Patients with Non-Alcoholic Fatty Liver Disease. *PloS one.* 2015;10(12):e0142210.
75. Brennan P, Clare K, George J, Dillon JF. Determining the role for uric acid in non-alcoholic steatohepatitis development and the utility of urate metabolites in diagnosis: An opinion review. *World journal of gastroenterology.* 2020;26(15):1683-1690.
76. Beller E, Lorbeer R, Keeser D, et al. Hepatic fat is superior to BMI, visceral and pancreatic fat as a potential risk biomarker for neurodegenerative disease. *European radiology.* 2019;29(12):6662-6670.
77. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *Journal of hepatology.* 2020;73(1):202-209.
78. Ipsen DH, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell Mol Life Sci.* 2018;75(18):3313-3327.
79. Falcon A, Doege H, Fluitt A, et al. FATP2 is a hepatic fatty acid transporter and peroxisomal very long-chain acyl-CoA synthetase. *American journal of physiology Endocrinology and metabolism.* 2010;299(3):E384-393.
80. Doege H, Grimm D, Falcon A, et al. Silencing of hepatic fatty acid transporter protein 5 in vivo reverses diet-induced non-alcoholic fatty liver disease and improves hyperglycemia. *The Journal of biological chemistry.* 2008;283(32):22186-22192.
81. Miquilena-Colina ME, Lima-Cabello E, Sanchez-Campos S, et al. Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C. *Gut.* 2011;60(10):1394-1402.
82. Greco D, Kotronen A, Westerbacka J, et al. Gene expression in human NAFLD. *American journal of physiology Gastrointestinal and liver physiology.* 2008;294(5):G1281-1287.

83. Westerbacka J, Kolak M, Kiviluoto T, et al. Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes.* 2007;56(11):2759-2765.
84. Higuchi N, Kato M, Tanaka M, et al. Effects of insulin resistance and hepatic lipid accumulation on hepatic mRNA expression levels of apoB, MTP and L-FABP in non-alcoholic fatty liver disease. *Exp Ther Med.* 2011;2(6):1077-1081.
85. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *The Journal of clinical investigation.* 2005;115(5):1343-1351.
86. Kumashiro N, Erion DM, Zhang D, et al. Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *Proceedings of the National Academy of Sciences of the United States of America.* 2011;108(39):16381-16385.
87. Foufelle F, Ferre P. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *The Biochemical journal.* 2002;366(Pt 2):377-391.
88. Ferramosca A, Zara V. Modulation of hepatic steatosis by dietary fatty acids. *World journal of gastroenterology.* 2014;20(7):1746-1755.
89. Dentin R, Girard J, Postic C. Carbohydrate responsive element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c): two key regulators of glucose metabolism and lipid synthesis in liver. *Biochimie.* 2005;87(1):81-86.
90. Higuchi N, Kato M, Shundo Y, et al. Liver X receptor in cooperation with SREBP-1c is a major lipid synthesis regulator in nonalcoholic fatty liver disease. *Hepatol Res.* 2008;38(11):1122-1129.
91. Xie Z, Li H, Wang K, et al. Analysis of transcriptome and metabolome profiles alterations in fatty liver induced by high-fat diet in rat. *Metabolism: clinical and experimental.* 2010;59(4):554-560.
92. Kohjima M, Enjoji M, Higuchi N, et al. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med.* 2007;20(3):351-358.
93. Mao J, DeMayo FJ, Li H, et al. Liver-specific deletion of acetyl-CoA carboxylase 1 reduces hepatic triglyceride accumulation without affecting glucose homeostasis. *Proceedings of the National Academy of Sciences of the United States of America.* 2006;103(22):8552-8557.
94. Savage DB, Choi CS, Samuel VT, et al. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *The Journal of clinical investigation.* 2006;116(3):817-824.
95. Kumar A, Katz LS, Schulz AM, et al. Activation of Nrf2 Is Required for Normal and ChREBPalpha-Augmented Glucose-Stimulated beta-Cell Proliferation. *Diabetes.* 2018;67(8):1561-1575.
96. Dobrzyn P, Bednarski T, Dobrzyn A. Metabolic reprogramming of the heart through stearoyl-CoA desaturase. *Prog Lipid Res.* 2015;57:1-12.
97. Wei Y, Wang D, Topczewski F, Pagliassotti MJ. Saturated fatty acids induce endoplasmic reticulum stress and apoptosis independently of ceramide in liver cells. *American journal of physiology Endocrinology and metabolism.* 2006;291(2):E275-281.

98. Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. *Endocrinology.* 2006;147(2):943-951.
99. Rizki G, Arnaboldi L, Gabrielli B, et al. Mice fed a lipogenic methionine-choline-deficient diet develop hypermetabolism coincident with hepatic suppression of SCD-1. *Journal of lipid research.* 2006;47(10):2280-2290.
100. Fougerat A, Montagner A, Loiseau N, Guillou H, Wahli W. Peroxisome Proliferator-Activated Receptors and Their Novel Ligands as Candidates for the Treatment of Non-Alcoholic Fatty Liver Disease. *Cells.* 2020;9(7).
101. Dasarathy S, Kasumov T, Edmison JM, et al. Glycine and urea kinetics in nonalcoholic steatohepatitis in human: effect of intralipid infusion. *American journal of physiology Gastrointestinal and liver physiology.* 2009;297(3):G567-575.
102. Miele L, Grieco A, Armuzzi A, et al. Hepatic mitochondrial beta-oxidation in patients with nonalcoholic steatohepatitis assessed by 13C-octanoate breath test. *Am J Gastroenterol.* 2003;98(10):2335-2336.
103. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology.* 2001;120(5):1183-1192.
104. Kotronen A, Seppala-Lindroos A, Vehkavaara S, et al. Liver fat and lipid oxidation in humans. *Liver Int.* 2009;29(9):1439-1446.
105. Croci I, Byrne NM, Choquette S, et al. Whole-body substrate metabolism is associated with disease severity in patients with non-alcoholic fatty liver disease. *Gut.* 2013;62(11):1625-1633.
106. Fabbrini E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology.* 2008;134(2):424-431.
107. Hodson L, McQuaid SE, Humphreys SM, et al. Greater dietary fat oxidation in obese compared with lean men: an adaptive mechanism to prevent liver fat accumulation? *American journal of physiology Endocrinology and metabolism.* 2010;299(4):E584-592.
108. Sunny NE, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell metabolism.* 2011;14(6):804-810.
109. Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. *Free radical biology & medicine.* 2012;52(1):59-69.
110. Mansouri A, Gattolliat CH, Asselah T. Mitochondrial Dysfunction and Signaling in Chronic Liver Diseases. *Gastroenterology.* 2018;155(3):629-647.
111. Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, Lesnefsky EJ. Production of reactive oxygen species by mitochondria: central role of complex III. *The Journal of biological chemistry.* 2003;278(38):36027-36031.
112. Eccleston HB, Andringa KK, Betancourt AM, et al. Chronic exposure to a high-fat diet induces hepatic steatosis, impairs nitric oxide bioavailability, and modifies the mitochondrial proteome in mice. *Antioxidants & redox signaling.* 2011;15(2):447-459.
113. Paradies G, Petrosillo G, Pistolese M, Ruggiero FM. Reactive oxygen species affect mitochondrial electron transport complex I activity through oxidative cardiolipin damage. *Gene.* 2002;286(1):135-141.
114. Petrosillo G, Portincasa P, Grattagliano I, et al. Mitochondrial dysfunction in rat with nonalcoholic fatty liver Involvement of complex I, reactive oxygen species and cardiolipin. *Biochimica et biophysica acta.* 2007;1767(10):1260-1267.

115. Weber M, Mera P, Casas J, et al. Liver CPT1A gene therapy reduces diet-induced hepatic steatosis in mice and highlights potential lipid biomarkers for human NAFLD. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 2020;34(9):11816-11837.
116. Chaurasia B, Tippetts TS, Mayoral Monibas R, et al. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science.* 2019;365(6451):386-392.
117. Diao L, Auger C, Konoeda H, Sadri AR, Amini-Nik S, Jeschke MG. Hepatic steatosis associated with decreased beta-oxidation and mitochondrial function contributes to cell damage in obese mice after thermal injury. *Cell death & disease.* 2018;9(5):530.
118. Barbier-Torres L, Fortner KA, Iruzubieta P, et al. Silencing hepatic MCJ attenuates non-alcoholic fatty liver disease (NAFLD) by increasing mitochondrial fatty acid oxidation. *Nat Commun.* 2020;11(1):3360.
119. Francque S, Verrijken A, Caron S, et al. PPARalpha gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. *Journal of hepatology.* 2015;63(1):164-173.
120. Fujita K, Nozaki Y, Wada K, et al. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. *Hepatology (Baltimore, Md).* 2009;50(3):772-780.
121. Zhang L, Deng S, Zhao S, et al. Intra-Peritoneal Administration of Mitochondrial DNA Provokes Acute Lung Injury and Systemic Inflammation via Toll-Like Receptor 9. *Int J Mol Sci.* 2016;17(9).
122. Boyapati RK, Tamborska A, Dorward DA, Ho GT. Advances in the understanding of mitochondrial DNA as a pathogenic factor in inflammatory diseases. *F1000Res.* 2017;6:169.
123. Fu A, Shi X, Zhang H, Fu B. Mitotherapy for Fatty Liver by Intravenous Administration of Exogenous Mitochondria in Male Mice. *Front Pharmacol.* 2017;8:241.
124. Teodoro JS, Rolo AP, Duarte FV, Simões AM, Palmeira CM. Differential alterations in mitochondrial function induced by a choline-deficient diet: understanding fatty liver disease progression. *Mitochondrion.* 2008;8(5-6):367-376.
125. Rector RS, Thyfault JP, Uptergrove GM, et al. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. *Journal of hepatology.* 2010;52(5):727-736.
126. Lee J, Homma T, Fujii J. Mice in the early stage of liver steatosis caused by a high fat diet are resistant to thioacetamide-induced hepatotoxicity and oxidative stress. *Toxicology letters.* 2017;277:92-103.
127. Gan LT, Van Rooyen DM, Koina ME, McCuskey RS, Teoh NC, Farrell GC. Hepatocyte free cholesterol lipotoxicity results from JNK1-mediated mitochondrial injury and is HMGB1 and TLR4-dependent. *Journal of hepatology.* 2014;61(6):1376-1384.
128. Marí M, Caballero F, Colell A, et al. Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. *Cell metabolism.* 2006;4(3):185-198.
129. Aubert J, Begriche K, Delannoy M, et al. Differences in early acetaminophen hepatotoxicity between obese ob/ob and db/db mice. *The Journal of pharmacology and experimental therapeutics.* 2012;342(3):676-687.

130. Koliaki C, Szendroedi J, Kaul K, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell metabolism.* 2015;21(5):739-746.
131. Patterson RE, Kalavalapalli S, Williams CM, et al. Lipotoxicity in steatohepatitis occurs despite an increase in tricarboxylic acid cycle activity. *American journal of physiology Endocrinology and metabolism.* 2016;310(7):E484-494.
132. Garcia-Ruiz I, Solis-Munoz P, Fernandez-Moreira D, Munoz-Yague T, Solis-Herruzo JA. In vitro treatment of HepG2 cells with saturated fatty acids reproduces mitochondrial dysfunction found in nonalcoholic steatohepatitis. *Dis Model Mech.* 2015;8(2):183-191.
133. Vecchione G, Grasselli E, Cioffi F, et al. The Nutraceutic Silybin Counteracts Excess Lipid Accumulation and Ongoing Oxidative Stress in an In Vitro Model of Non-Alcoholic Fatty Liver Disease Progression. *Front Nutr.* 2017;4:42.
134. 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: clinical and experimental.* 2021;117:154708.
135. 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. *Scientific reports.* 2021;11(1):3319.
136. Boengler K, Kosiol M, Mayr M, Schulz R, Rohrbach S. Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. *Journal of cachexia, sarcopenia and muscle.* 2017;8(3):349-369.
137. Begriche K, Massart J, Robin MA, Bonnet F, Fromenty B. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md).* 2013;58(4):1497-1507.
138. Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK. Reactive Oxygen Species in Metabolic and Inflammatory Signaling. *Circulation research.* 2018;122(6):877-902.
139. Marinho HS, Real C, Cyrne L, Soares H, Antunes F. Hydrogen peroxide sensing, signaling and regulation of transcription factors. *Redox Biol.* 2014;2:535-562.
140. Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol.* 2017;11:613-619.
141. Zhang L, Wang X, Cueto R, et al. Biochemical basis and metabolic interplay of redox regulation. *Redox Biol.* 2019;26:101284.
142. Cardoso AR, Chausse B, da Cunha FM, et al. Mitochondrial compartmentalization of redox processes. *Free radical biology & medicine.* 2012;52(11-12):2201-2208.
143. Fridovich I. Mitochondria: are they the seat of senescence? *Aging cell.* 2004;3(1):13-16.
144. Brown GC, Borutaite V. There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells. *Mitochondrion.* 2012;12(1):1-4.
145. Galkin A, Brandt U. Superoxide radical formation by pure complex I (NADH:ubiquinone oxidoreductase) from Yarrowia lipolytica. *The Journal of biological chemistry.* 2005;280(34):30129-30135.
146. Liu Y, Fiskum G, Schubert D. Generation of reactive oxygen species by the mitochondrial electron transport chain. *J Neurochem.* 2002;80(5):780-787.
147. Kudin AP, Bimpong-Buta NY, Vielhaber S, Elger CE, Kunz WS. Characterization of superoxide-producing sites in isolated brain mitochondria. *The Journal of biological chemistry.* 2004;279(6):4127-4135.

148. Krishnamoorthy G, Hinkle PC. Studies on the electron transfer pathway, topography of iron-sulfur centers, and site of coupling in NADH-Q oxidoreductase. *The Journal of biological chemistry.* 1988;263(33):17566-17575.
149. Genova ML, Ventura B, Giuliano G, et al. The site of production of superoxide radical in mitochondrial Complex I is not a bound ubisemiquinone but presumably iron-sulfur cluster N2. *FEBS Lett.* 2001;505(3):364-368.
150. Kushnareva Y, Murphy AN, Andreyev A. Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)+ oxidation-reduction state. *The Biochemical journal.* 2002;368(Pt 2):545-553.
151. Lambert AJ, Brand MD. Inhibitors of the quinone-binding site allow rapid superoxide production from mitochondrial NADH:ubiquinone oxidoreductase (complex I). *The Journal of biological chemistry.* 2004;279(38):39414-39420.
152. Ohnishi ST, Ohnishi T, Muranaka S, et al. A possible site of superoxide generation in the complex I segment of rat heart mitochondria. *J Bioenerg Biomembr.* 2005;37(1):1-15.
153. Quinlan CL, Orr AL, Perevoshchikova IV, Treberg JR, Ackrell BA, Brand MD. Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions. *The Journal of biological chemistry.* 2012;287(32):27255-27264.
154. Ishii T, Yasuda K, Akatsuka A, Hino O, Hartman PS, Ishii N. A mutation in the SDHC gene of complex II increases oxidative stress, resulting in apoptosis and tumorigenesis. *Cancer Res.* 2005;65(1):203-209.
155. Bleier L, Drose S. Superoxide generation by complex III: from mechanistic rationales to functional consequences. *Biochimica et biophysica acta.* 2013;1827(11-12):1320-1331.
156. Quinlan CL, Perevoshchikova IV, Hey-Mogensen M, Orr AL, Brand MD. Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. *Redox Biol.* 2013;1:304-312.
157. St-Pierre J, Buckingham JA, Roebuck SJ, Brand MD. Topology of superoxide production from different sites in the mitochondrial electron transport chain. *The Journal of biological chemistry.* 2002;277(47):44784-44790.
158. Lambertucci RH, Hirabara SM, Silveira Ldos R, Levada-Pires AC, Curi R, Pithon-Curi TC. Palmitate increases superoxide production through mitochondrial electron transport chain and NADPH oxidase activity in skeletal muscle cells. *J Cell Physiol.* 2008;216(3):796-804.
159. Lenaz G. The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. *IUBMB Life.* 2001;52(3-5):159-164.
160. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. *Free radical biology & medicine.* 2000;29(3-4):222-230.
161. Kussmaul L, Hirst J. The mechanism of superoxide production by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria. *Proceedings of the National Academy of Sciences of the United States of America.* 2006;103(20):7607-7612.
162. Lockman KA, Baren JP, Pemberton CJ, et al. Oxidative stress rather than triglyceride accumulation is a determinant of mitochondrial dysfunction in in vitro models of hepatic cellular steatosis. *Liver Int.* 2012;32(7):1079-1092.
163. Serviddio G, Bellanti F, Tamborra R, 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(7):957-965.

164. Lohr K, Pachl F, Moghaddas Gholami A, et al. Reduced mitochondrial mass and function add to age-related susceptibility toward diet-induced fatty liver in C57BL/6J mice. *Physiol Rep.* 2016;4(19).
165. Mendez L, Pazos M, Molinar-Toribio E, et al. Protein carbonylation associated to high-fat, high-sucrose diet and its metabolic effects. *The Journal of nutritional biochemistry.* 2014;25(12):1243-1253.
166. Wang L, Liu X, Nie J, et al. ALCAT1 controls mitochondrial etiology of fatty liver diseases, linking defective mitophagy to steatosis. *Hepatology (Baltimore, Md).* 2015;61(2):486-496.
167. Valdecantos MP, Perez-Matute P, Gonzalez-Muniesa P, Prieto-Hontoria PL, Moreno-Aliaga MJ, Martinez JA. Lipoic acid improves mitochondrial function in nonalcoholic steatosis through the stimulation of sirtuin 1 and sirtuin 3. *Obesity (Silver Spring).* 2012;20(10):1974-1983.
168. Tong W, Ju L, Qiu M, et al. Liraglutide ameliorates non-alcoholic fatty liver disease by enhancing mitochondrial architecture and promoting autophagy through the SIRT1/SIRT3-FOXO3a pathway. *Hepatol Res.* 2016;46(9):933-943.
169. Videla LA, Rodrigo R, Orellana M, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. *Clin Sci (Lond).* 2004;106(3):261-268.
170. Simões ICM, Fontes A, Pinton P, Zischka H, Wieckowski MR. Mitochondria in non-alcoholic fatty liver disease. *The international journal of biochemistry & cell biology.* 2018;95:93-99.
171. Kumar A, Sharma A, Duseja A, et al. Patients with Nonalcoholic Fatty Liver Disease (NAFLD) have Higher Oxidative Stress in Comparison to Chronic Viral Hepatitis. *Journal of clinical and experimental hepatology.* 2013;3(1):12-18.
172. Koruk M, Taysi S, Savas MC, Yilmaz O, Akcay F, Karakok M. Oxidative stress and enzymatic antioxidant status in patients with nonalcoholic steatohepatitis. *Annals of clinical and laboratory science.* 2004;34(1):57-62.
173. Bao J, Scott I, Lu Z, et al. SIRT3 is regulated by nutrient excess and modulates hepatic susceptibility to lipotoxicity. *Free radical biology & medicine.* 2010;49(7):1230-1237.
174. Li S, Dou X, Ning H, et al. Sirtuin 3 acts as a negative regulator of autophagy dictating hepatocyte susceptibility to lipotoxicity. *Hepatology (Baltimore, Md).* 2017;66(3):936-952.
175. Nilova TV, Zvenigorodskaia LA, Cherkashova EA. [Diagnostic value of nitric oxide and endotoxins at non-alcoholic fatty liver disease]. *Eksperimental'naia i klinicheskaia gastroenterologiia = Experimental & clinical gastroenterology.* 2010(7):38-42.
176. Gu Q, Yang X, Lin L, et al. Genetic ablation of solute carrier family 7a3a leads to hepatic steatosis in zebrafish during fasting. *Hepatology (Baltimore, Md).* 2014;60(6):1929-1941.
177. Ibrahim M, Farghaly E, Gomaa W, Kelleni M, Abdelrahman AM. Nitro-aspirin is a potential therapy for non alcoholic fatty liver disease. *Eur J Pharmacol.* 2011;659(2-3):289-295.
178. Garcia-Monzon C, Majano PL, Zubia I, Sanz P, Apolinario A, Moreno-Otero R. Intrahepatic accumulation of nitrotyrosine in chronic viral hepatitis is associated with histological severity of liver disease. *Journal of hepatology.* 2000;32(2):331-338.
179. Sheldon RD, Padilla J, Jenkins NT, Laughlin MH, Rector RS. Chronic NOS inhibition accelerates NAFLD progression in an obese rat model. *American journal of physiology Gastrointestinal and liver physiology.* 2015;308(6):G540-549.

180. Persico M, Masarone M, Damato A, et al. "Non alcoholic fatty liver disease and eNOS dysfunction in humans". *BMC Gastroenterol.* 2017;17(1):35.
181. Sheldon RD, Laughlin MH, Rector RS. Reduced hepatic eNOS phosphorylation is associated with NAFLD and type 2 diabetes progression and is prevented by daily exercise in hyperphagic OLETF rats. *J Appl Physiol (1985).* 2014;116(9):1156-1164.
182. Braud L, Battault S, Meyer G, et al. Antioxidant properties of tea blunt ROS-dependent lipogenesis: beneficial effect on hepatic steatosis in a high fat-high sucrose diet NAFLD obese rat model. *The Journal of nutritional biochemistry.* 2017;40:95-104.
183. Boveris A, Oshino N, Chance B. The cellular production of hydrogen peroxide. *The Biochemical journal.* 1972;128(3):617-630.
184. Simoes ICM, Karkucinska-Wieckowska A, Janikiewicz J, et al. Western Diet Causes Obesity-Induced Nonalcoholic Fatty Liver Disease Development by Differentially Compromising the Autophagic Response. *Antioxidants (Basel, Switzerland).* 2020;9(10).
185. Matsuzawa-Nagata N, Takamura T, Ando H, et al. Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. *Metabolism: clinical and experimental.* 2008;57(8):1071-1077.
186. Collins JC, Scheinberg IH, Giblin DR, Sternlieb I. Hepatic peroxisomal abnormalities in abetalipoproteinemia. *Gastroenterology.* 1989;97(3):766-770.
187. De Craemer D, Pauwels M, Van den Branden C. Alterations of peroxisomes in steatosis of the human liver: a quantitative study. *Hepatology (Baltimore, Md).* 1995;22(3):744-752.
188. Hall D, Poussin C, Velagapudi VR, et al. Peroxisomal and microsomal lipid pathways associated with resistance to hepatic steatosis and reduced pro-inflammatory state. *The Journal of biological chemistry.* 2010;285(40):31011-31023.
189. Knebel B, Goddeke S, Hartwig S, et al. Alteration of Liver Peroxisomal and Mitochondrial Functionality in the NZO Mouse Model of Metabolic Syndrome. *Proteomics Clin Appl.* 2018;12(1).
190. Pu J, Ha CW, Zhang S, Jung JP, Huh WK, Liu P. Interactomic study on interaction between lipid droplets and mitochondria. *Protein Cell.* 2011;2(6):487-496.
191. Natarajan SK, Eapen CE, Pullimood AB, Balasubramanian KA. Oxidative stress in experimental liver microvesicular steatosis: role of mitochondria and peroxisomes. *Journal of gastroenterology and hepatology.* 2006;21(8):1240-1249.
192. Napoli C, Martin-Padura I, de Nigris F, et al. Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet. *Proceedings of the National Academy of Sciences of the United States of America.* 2003;100(4):2112-2116.
193. Wang Z, Zhao Y, Sun R, et al. circ-CBFB upregulates p66Shc to perturb mitochondrial dynamics in APAP-induced liver injury. *Cell death & disease.* 2020;11(11):953.
194. Huang P, Feng X, Zhao Z, et al. p66Shc promotes HCC progression in the tumor microenvironment via STAT3 signaling. *Experimental cell research.* 2019;383(2):111550.
195. Tomita K, Teratani T, Suzuki T, et al. p53/p66Shc-mediated signaling contributes to the progression of non-alcoholic steatohepatitis in humans and mice. *Journal of hepatology.* 2012;57(4):837-843.
196. Zhang J, Li Y, Wang B, Luo Y, Shi J, Zhao B. The p66shc-mediated Regulation of Hepatocyte Senescence Influences Hepatic Steatosis in Nonalcoholic Fatty Liver Disease. *Medical science monitor : international medical journal of experimental and clinical research.* 2020;26:e921887.

197. Zhang Y, Wang C, Lu J, et al. Targeting of miR-96-5p by catalpol ameliorates oxidative stress and hepatic steatosis in LDLr-/- mice via p66shc/cytochrome C cascade. *Aging (Albany NY).* 2020;12(3):2049-2069.
198. Yi H, Xu D, Wu X, Xu F, Lin L, Zhou H. Isosteviol Protects Free Fatty Acid- and High Fat Diet-Induced Hepatic Injury via Modulating PKC-β/p66Shc/ROS and Endoplasmic Reticulum Stress Pathways. *Antioxidants & redox signaling.* 2019;30(17):1949-1968.
199. Santos-Alves E, Marques-Aleixo I, Rizo-Roca D, et al. Exercise modulates liver cellular and mitochondrial proteins related to quality control signaling. *Life Sci.* 2015;135:124-130.
200. Fransen M, Nordgren M, Wang B, Apanasets O. Role of peroxisomes in ROS/RNS-metabolism: implications for human disease. *Biochimica et biophysica acta.* 2012;1822(9):1363-1373.
201. Piao L, Choi J, Kwon G, Ha H. Endogenous catalase delays high-fat diet-induced liver injury in mice. *The Korean journal of physiology & pharmacology : official journal of the Korean Physiological Society and the Korean Society of Pharmacology.* 2017;21(3):317-325.
202. Hashimoto T, Fujita T, Usuda N, et al. Peroxisomal and mitochondrial fatty acid beta-oxidation in mice nullizygous for both peroxisome proliferator-activated receptor alpha and peroxisomal fatty acyl-CoA oxidase. Genotype correlation with fatty liver phenotype. *The Journal of biological chemistry.* 1999;274(27):19228-19236.
203. Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Seminars in liver disease.* 2001;21(1):27-41.
204. Sanders RJ, Ofman R, Duran M, Kemp S, Wanders RJA. Omega-oxidation of very long-chain fatty acids in human liver microsomes. Implications for X-linked adrenoleukodystrophy. *The Journal of biological chemistry.* 2006;281(19):13180-13187.
205. Bell LN, Temm CJ, Saxena R, et al. Bariatric surgery-induced weight loss reduces hepatic lipid peroxidation levels and affects hepatic cytochrome P-450 protein content. *Annals of surgery.* 2010;251(6):1041-1048.
206. Abdelmegeed MA, Banerjee A, Yoo SH, Jang S, Gonzalez FJ, Song BJ. Critical role of cytochrome P450 2E1 (CYP2E1) in the development of high fat-induced non-alcoholic steatohepatitis. *Journal of hepatology.* 2012;57(4):860-866.
207. Kathirvel E, Morgan K, French SW, Morgan TR. Overexpression of liver-specific cytochrome P4502E1 impairs hepatic insulin signaling in a transgenic mouse model of nonalcoholic fatty liver disease. *European journal of gastroenterology & hepatology.* 2009;21(9):973-983.
208. Carmiel-Haggai M, Cederbaum AI, Nieto N. A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 2005;19(1):136-138.
209. Nakamura S, Takamura T, Matsuzawa-Nagata N, et al. Palmitate induces insulin resistance in H4IIEC3 hepatocytes through reactive oxygen species produced by mitochondria. *The Journal of biological chemistry.* 2009;284(22):14809-14818.
210. Bettaieb A, Jiang JX, Sasaki Y, et al. Hepatocyte Nicotinamide Adenine Dinucleotide Phosphate Reduced Oxidase 4 Regulates Stress Signaling, Fibrosis, and Insulin Sensitivity During Development of Steatohepatitis in Mice. *Gastroenterology.* 2015;149(2):468-480 e410.
211. Han H, Li X, Guo Y, Zheng M, Xue T, Wang L. Plant sterol ester of alpha-linolenic acid ameliorates high-fat diet-induced nonalcoholic fatty liver disease in mice:

association with regulating mitochondrial dysfunction and oxidative stress via activating AMPK signaling. *Food & function.* 2021;12(5):2171-2188.
212. Zhang MH, Li J, Zhu XY, et al. Physalin B ameliorates nonalcoholic steatohepatitis by stimulating autophagy and NRF2 activation mediated improvement in oxidative stress. *Free radical biology & medicine.* 2021;164:1-12.
213. Dludla PV, Nkambule BB, Mazibuko-Mbeje SE, et al. N-Acetyl Cysteine Targets Hepatic Lipid Accumulation to Curb Oxidative Stress and Inflammation in NAFLD: A Comprehensive Analysis of the Literature. *Antioxidants (Basel, Switzerland).* 2020;9(12).
214. Oliveira CP, Gayotto LC, Tatai C, et al. Vitamin C and vitamin E in prevention of Nonalcoholic Fatty Liver Disease (NAFLD) in choline deficient diet fed rats. *Nutr J.* 2003;2:9.
215. Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md).* 2010;52(1):79-104.
216. Chen K, Chen X, Xue H, et al. Coenzyme Q10 attenuates high-fat diet-induced non-alcoholic fatty liver disease through activation of the AMPK pathway. *Food & function.* 2019;10(2):814-823.
217. Fink BD, Yu L, Coppey L, et al. Effect of mitoquinone on liver metabolism and steatosis in obese and diabetic rats. *Pharmacology research & perspectives.* 2021;9(1):e00701.
218. Turkseven S, Bolognesi M, Brocca A, Pesce P, Angeli P, Di Pascoli M. Mitochondria-targeted antioxidant mitoquinone attenuates liver inflammation and fibrosis in cirrhotic rats. *American journal of physiology Gastrointestinal and liver physiology.* 2020;318(2):G298-g304.
219. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *The New England journal of medicine.* 2010;362(18):1675-1685.
220. Lavine JE, Schwimmer JB, Van Natta ML, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *Jama.* 2011;305(16):1659-1668.
221. Miller ER, 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med.* 2005;142(1):37-46.
222. Klein EA, Thompson IM, Jr., Tangen CM, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *Jama.* 2011;306(14):1549-1556.
223. Rafiee S, Mohammadi H, Ghavami A, Sadeghi E, Safari Z, Askari G. Efficacy of resveratrol supplementation in patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis of clinical trials. *Complementary therapies in clinical practice.* 2021;42:101281.
224. Younossi ZM, Ratziu V, Loomba R, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet.* 2019;394(10215):2184-2196.

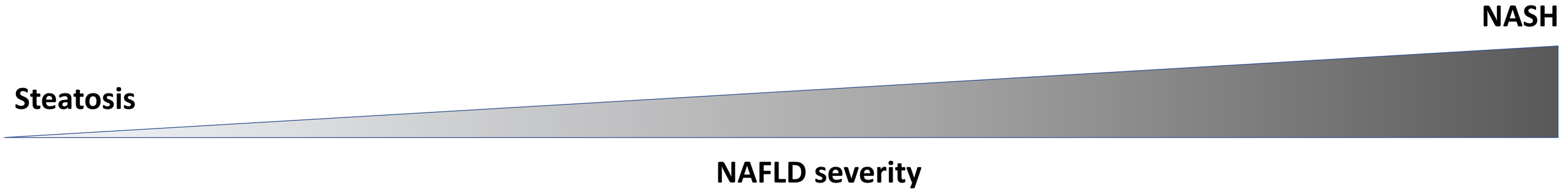
NAFL		
decreased	**no changes detected**	**increased**
CI activity	CI activity	CIV activity
CIV activity	CIV activity	O_2 consumption
O_2 consumption	O_2 consumption	FAO and ketogenesis
FAO and ketogenesis	TCA cycle activity	TCA cycle activity
mtDNA content:	mtDNA content	mtDNA content
		Mito fission

Genetic predisposition

PROGRESSION

Exposome

NASH		
decreased	**no changes detected**	**increased**
CI activity	CIV activity	CIV activity
CIV activity	O_2 consumption	O_2 consumption
OXPHOS subunits level	ATPase activity	Mito uncoupling
O_2 consumption	FAO and ketogenesis	FAO and ketogenesis
ATP level		TCA cycle fluxes
FAO and ketogenesis		mtDNA damage
		UCP2 level



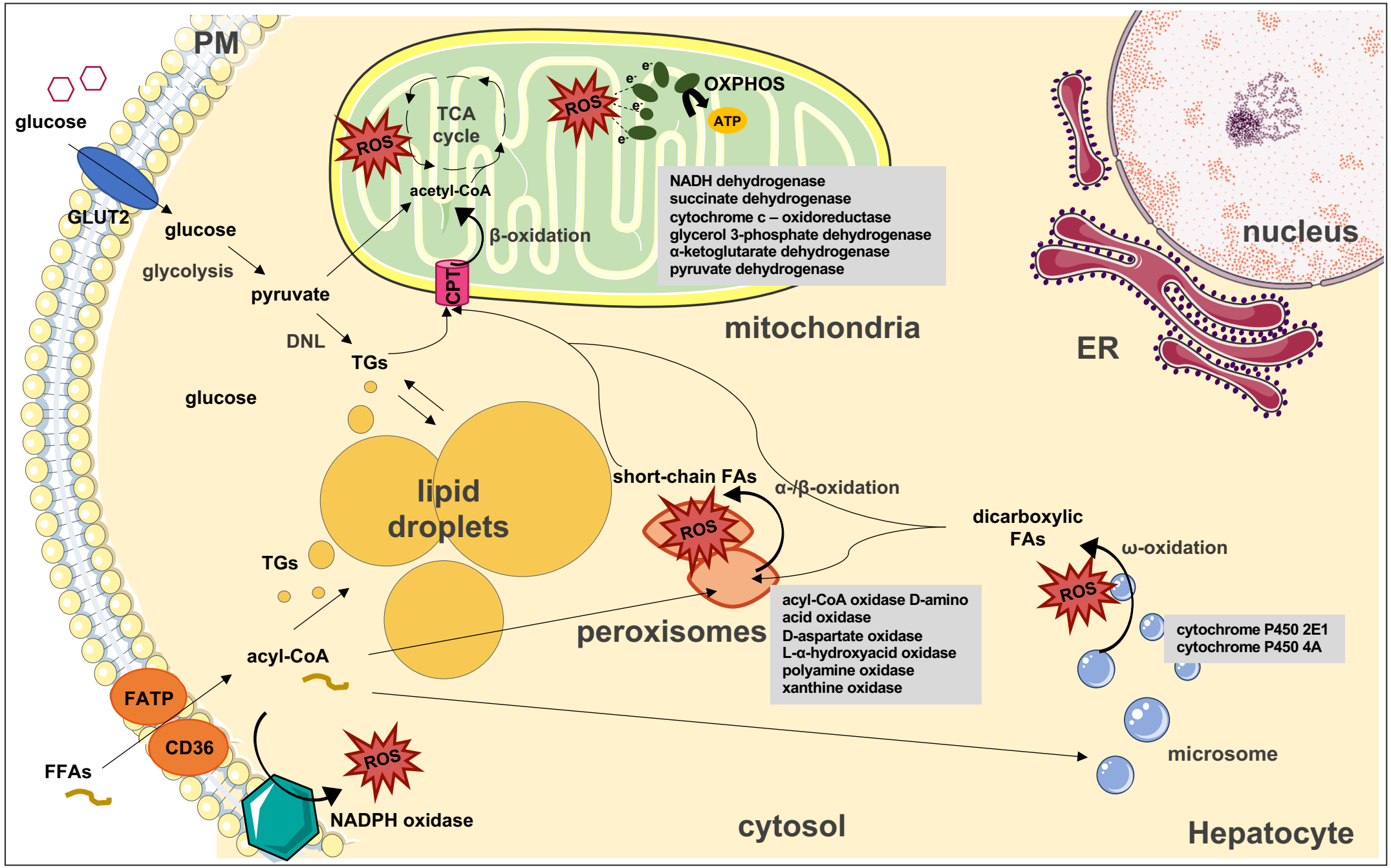

PM
glucose
GLUT2
glucose
glycolysis
pyruvate
DNL
TGs
glucose
TCA cycle
ROS
acetyl-CoA
β-oxidation
CPT
OXPHOS
ATP
NADH dehydrogenase
succinate dehydrogenase
cytochrome c – oxidoreductase
glycerol 3-phosphate dehydrogenase
α-ketoglutarate dehydrogenase
pyruvate dehydrogenase
mitochondria
nucleus
ER
lipid droplets
TGs
short-chain FAs
α-/β-oxidation
dicarboxylic FAs
ω-oxidation
peroxisomes
acyl-CoA oxidase D-amino acid oxidase
D-aspartate oxidase
L-α-hydroxyacid oxidase
polyamine oxidase
xanthine oxidase
cytochrome P450 2E1
cytochrome P450 4A
microsome
acyl-CoA
FATP
CD36
FFAs
NADPH oxidase
cytosol
Hepatocyte

Figure 2.

Figure 1. Outcomes regarding mitochondrial parameters and related metabolic processes in NAFLD. The figure was prepared initially based on Begriche et al.[58] and Simoes et al.[66] and updated accounting for subsequent studies. Disease stage is according to Begriche et al.[58] and Koliaki et al.[54]. ACC, acetyl-CoA carboxylase; FAO, fatty acid oxidation; FAS, fatty acid synthase; OXPHOS, oxidative phosphorylation; PPARγ, peroxisome proliferator-activated receptor γ; SCD-1, stearoyl-CoA desaturase-1; SREBP-1c, sterol regulatory element-binding protein-1c; TCA, tricarboxylic acid cycle; UCP2, mitochondrial uncoupling protein 2; β-HAD, β-hydroxyacyl-CoA dehydrogenase; Due to the limit of references number published reports cited in the Figure in [ ] are available and listed in Supplementary Material.

Figure 2. Schematic representation of hepatocyte-related organelles involved in ROS generation in a NAFLD context. Circulating levels of glucose and FFAs are taken by hepatocytes. In an early NAFLD stage, hepatic adaptation includes a series of molecular pathways in order to cope with the excess of available nutrients. One main pathway involved is the activation of FAO in mitochondria but also in peroxisomes, microsomes and NADPH oxidase. Despite the protective role of FAO by decreasing fat accumulation, the upregulation of this pathway induce the production of ROS. When present in excess, ROS play a role as culprits in the induction of hepatic oxidative damage of molecules and organelles, thereby contributing to organelles malfunction (e.g. mitochondrial dysfunction) and further disease progression. ATP, adenosine triphosphate; CD36, cluster of differentiation; CPT, carnitine palmitoyltransferase; DNL, *de novo* lipogenesis; ER, endoplasmic reticulum; FAO, faty aci oxidation; FAs, fatty acids; FATP, fatty acid transporter protein; FFAs, free fatty acids; GLUT2, glucose transporter 2; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; OXPHOS, oxidative phosphorylation; PM, plasma membrane; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle; TGs, triglycerides.

Supplementary Material:

Published reports used for preparation of Figure 1 (Panel NAFL):

NAFL		
decreased	**no changes detected**	**increased**
CI activity: [1]	**CI activity:** [2],[15]	**CIV activity:** [22]
CIV activity: [1],[2]	**CIV activity:** [5],[8], [15],[16]	**O_2 consumption:** [21], [23]
O_2 consumption: [2], [3],[4],[5],[6]	**O_2 consumption:** [8], [15],[16],[17],[18],[19]	**FAO and ketogenesis:** [3],[4],[21],[23],[24], [25],[26],[27],[28],[29], [30],[31],[32],[33]
FAO and ketogenesis: [2],[7],[8],[9],[10],[11]	**TCA cycle activity:** [20]	**TCA cycle activity:** [21]
mtDNA content: [12],[13],[14]	**mtDNA content:** [21]	**mtDNA content:** [5], [28],[32],[34],[35]
		Mito fission: [36]

1. Mingorance C, Duluc L, Chalopin M, et al. Propionyl-L-carnitine corrects metabolic and cardiovascular alterations in diet-induced obese mice and improves liver respiratory chain activity. PloS one. 2012;7(3):e34268.
2. Vial G, Dubouchaud H, Couturier K, et al. Effects of a high-fat diet on energy metabolism and ROS production in rat liver. Journal of hepatology. 2011;54(2):348-356.
3. Raffaella C, Francesca B, Italia F, Marina P, Giovanna L, Susanna I. Alterations in hepatic mitochondrial compartment in a model of obesity and insulin resistance. Obesity (Silver Spring, Md). 2008;16(5):958-964.
4. Mollica MP, Lionetti L, Moreno M, et al. 3,5-diiodo-l-thyronine, by modulating mitochondrial functions, reverses hepatic fat accumulation in rats fed a high-fat diet. Journal of hepatology. 2009;51(2):363-370.
5. Nadal-Casellas A, Amengual-Cladera E, Proenza AM, Lladó I, Gianotti M. Long-term high-fat-diet feeding impairs mitochondrial biogenesis in liver of male and female rats. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2010;26(3):291-302.
6. Teodoro J, Rolo AP, Oliveira PJ, Palmeira CM. Decreased ANT content in Zucker fatty rats: relevance for altered hepatic mitochondrial bioenergetics in steatosis. FEBS letters. 2006;580(8):2153-2157.
7. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology. 2001;120(5):1183-1192.
8. Wardlaw GM, Kaplan ML. Oxygen consumption and oxidative capacity of hepatocytes from young male obese and nonobese Zucker rats. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY). 1986;183(2):199-206.
9. Roglans N, Vilà L, Farré M, et al. Impairment of hepatic Stat-3 activation and reduction of PPARalpha activity in fructose-fed rats. Hepatology (Baltimore, Md). 2007;45(3):778-788.
10. Soh JR, Shin DH, Kwon DY, Cha YS. Effect of Cheonggukjang supplementation upon hepatic acyl-CoA synthase, carnitine palmitoyltransferase I, acyl-CoA oxidase and

uncoupling protein 2 mRNA levels in C57BL/6J mice fed with high fat diet. Genes & nutrition. 2008;2(4):365-369.
11. Kotronen A, Seppälä-Lindroos A, Vehkavaara S, et al. Liver fat and lipid oxidation in humans. Liver international : official journal of the International Association for the Study of the Liver. 2009;29(9):1439-1446.
12. Walker CS, Li X, Whiting L, et al. Mice lacking the neuropeptide alpha-calcitonin gene-related peptide are protected against diet-induced obesity. Endocrinology. 2010;151(9):4257-4269.
13. Aharoni-Simon M, Hann-Obercyger M, Pen S, Madar Z, Tirosh O. Fatty liver is associated with impaired activity of PPARγ-coactivator 1α (PGC1α) and mitochondrial biogenesis in mice. Laboratory investigation; a journal of technical methods and pathology. 2011;91(7):1018-1028.
14. Valdecantos MP, Pérez-Matute P, González-Muniesa P, Prieto-Hontoria PL, Moreno-Aliaga MJ, Martínez JA. Lipoic acid improves mitochondrial function in nonalcoholic steatosis through the stimulation of sirtuin 1 and sirtuin 3. Obesity (Silver Spring, Md). 2012;20(10):1974-1983.
15. Flamment M, Rieusset J, Vidal H, et al. Regulation of hepatic mitochondrial metabolism in response to a high fat diet: a longitudinal study in rats. Journal of physiology and biochemistry. 2012;68(3):335-344.
16. Flamment M, Arvier M, Gallois Y, et al. Fatty liver and insulin resistance in obese Zucker rats: no role for mitochondrial dysfunction. Biochimie. 2008;90(9):1407-1413.
17. Ciapaite J, Bakker SJ, Van Eikenhorst G, et al. Functioning of oxidative phosphorylation in liver mitochondria of high-fat diet fed rats. Biochimica et biophysica acta. 2007;1772(3):307-316.
18. Oliveira CP, Coelho AM, Barbeiro HV, et al. Liver mitochondrial dysfunction and oxidative stress in the pathogenesis of experimental nonalcoholic fatty liver disease. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 2006;39(2):189-194.
19. Petersen KF, Befroy DE, Dufour S, Rothman DL, Shulman GI. Assessment of Hepatic Mitochondrial Oxidation and Pyruvate Cycling in NAFLD by (13)C Magnetic Resonance Spectroscopy. Cell metabolism. 2016;24(1):167-171.
20. Patterson RE, Kalavalapalli S, Williams CM, et al. Lipotoxicity in steatohepatitis occurs despite an increase in tricarboxylic acid cycle activity. American journal of physiology Endocrinology and metabolism. 2016;310(7):E484-494.
21. Koliaki C, Szendroedi J, Kaul K, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell metabolism. 2015;21(5):739-746.
22. Valdecantos MP, Pérez-Matute P, González-Muniesa P, Prieto-Hontoria PL, Moreno-Aliaga MJ, Martínez JA. Lipoic acid administration prevents nonalcoholic steatosis linked to long-term high-fat feeding by modulating mitochondrial function. The Journal of nutritional biochemistry. 2012;23(12):1676-1684.
23. Lazarin Mde O, Ishii-Iwamoto EL, Yamamoto NS, et al. Liver mitochondrial function and redox status in an experimental model of non-alcoholic fatty liver disease induced by monosodium L-glutamate in rats. Experimental and molecular pathology. 2011;91(3):687-694.
24. 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. European journal of nutrition. 2013;52(2):537-545.

25. Bugianesi E, Gastaldelli A, Vanni E, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia. 2005;48(4):634-642.
26. Hodson L, McQuaid SE, Humphreys SM, et al. Greater dietary fat oxidation in obese compared with lean men: an adaptive mechanism to prevent liver fat accumulation? American journal of physiology Endocrinology and metabolism. 2010;299(4):E584-592.
27. Iozzo P, Bucci M, Roivainen A, et al. Fatty acid metabolism in the liver, measured by positron emission tomography, is increased in obese individuals. Gastroenterology. 2010;139(3):846-856, 856.e841-846.
28. Ciapaite J, van den Broek NM, Te Brinke H, et al. Differential effects of short- and long-term high-fat diet feeding on hepatic fatty acid metabolism in rats. Biochimica et biophysica acta. 2011;1811(7-8):441-451.
29. Iossa S, Lionetti L, Mollica MP, et al. Effect of high-fat feeding on metabolic efficiency and mitochondrial oxidative capacity in adult rats. The British journal of nutrition. 2003;90(5):953-960.
30. Stefanovic-Racic M, Perdomo G, Mantell BS, Sipula IJ, Brown NF, O'Doherty RM. A moderate increase in carnitine palmitoyltransferase 1a activity is sufficient to substantially reduce hepatic triglyceride levels. American journal of physiology Endocrinology and metabolism. 2008;294(5):E969-977.
31. Triscari J, Nauss-Karol C, Levin BE, Sullivan AC. Changes in lipid metabolism in diet-induced obesity. Metabolism: clinical and experimental. 1985;34(6):580-587.
32. Satapati S, Sunny NE, Kucejova B, et al. Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. Journal of lipid research. 2012;53(6):1080-1092.
33. Sunny NE, Satapati S, Fu X, et al. Progressive adaptation of hepatic ketogenesis in mice fed a high-fat diet. American journal of physiology Endocrinology and metabolism. 2010;298(6):E1226-1235.
34. Chiappini F, Barrier A, Saffroy R, et al. Exploration of global gene expression in human liver steatosis by high-density oligonucleotide microarray. Laboratory investigation; a journal of technical methods and pathology. 2006;86(2):154-165.
35. Carabelli J, Burgueño AL, Rosselli MS, et al. High fat diet-induced liver steatosis promotes an increase in liver mitochondrial biogenesis in response to hypoxia. Journal of cellular and molecular medicine. 2011;15(6):1329-1338.
36. Galloway CA, Lee H, Brookes PS, Yoon Y. Decreasing mitochondrial fission alleviates hepatic steatosis in a murine model of nonalcoholic fatty liver disease. American journal of physiology Gastrointestinal and liver physiology. 2014;307(6):G632-641.

Published reports used for preparation of Figure 1 (Panel NASH):

NASH		
decreased	**no changes detected**	**increased**
CI activity: [37],[38],[39],[40]	**CIV activity:** [38]	**CIV activity:** [48]
CIV activity: [37],[39],[41]	**O_2 consumption:** [40]	**O_2 consumption:** [48]
OXPHOS subunits level: [21]	**ATPase activity:** [45]	**Mito uncoupling:** [21],[45]
O_2 consumption: [21],[42]	**FAO and ketogenesis:** [46],[47]	**FAO and ketogenesis:** [7],[48],[49],[50],[51],[52],[53]
ATP level: [43]		**TCA cycle fluxes:** [20],[54]
FAO and ketogenesis: [20],[44]		**mtDNA damage:** [55],[56],[57],[58]
		UCP2 level: [45]

7. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology. 2001;120(5):1183-1192.
20. Patterson RE, Kalavalapalli S, Williams CM, et al. Lipotoxicity in steatohepatitis occurs despite an increase in tricarboxylic acid cycle activity. American journal of physiology Endocrinology and metabolism. 2016;310(7):E484-494.
21. Koliaki C, Szendroedi J, Kaul K, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell metabolism. 2015;21(5):739-746.
37. Pérez-Carreras M, Del Hoyo P, Martín MA, et al. Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. Hepatology (Baltimore, Md). 2003;38(4):999-1007.
38. Décordé K, Agne A, Lacan D, et al. Preventive effect of a melon extract rich in superoxide scavenging activity on abdominal and liver fat and adipokine imbalance in high-fat-fed hamsters. Journal of agricultural and food chemistry. 2009;57(14):6461-6467.
39. Bruce KD, Cagampang FR, Argenton M, et al. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. Hepatology (Baltimore, Md). 2009;50(6):1796-1808.
40. Serviddio G, Bellanti F, Tamborra R, et al. Alterations of hepatic ATP homeostasis and respiratory chain during development of non-alcoholic steatohepatitis in a rodent model. European journal of clinical investigation. 2008;38(4):245-252.
41. 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 radical biology & medicine. 2009;47(7):924-931.
42. Mantena SK, King AL, Andringa KK, Eccleston HB, Bailey SM. Mitochondrial dysfunction and oxidative stress in the pathogenesis of alcohol- and obesity-induced fatty liver diseases. Free radical biology & medicine. 2008;44(7):1259-1272.
43. 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(17):1659-1664.

44. Serviddio G, Giudetti AM, Bellanti F, 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(9):e24084.
45. Serviddio G, Bellanti F, Tamborra R, 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(7):957-965.
46. Schneider AR, Kraut C, Lindenthal B, Braden B, Caspary WF, Stein J. Total body metabolism of 13C-octanoic acid is preserved in patients with non-alcoholic steatohepatitis, but differs between women and men. European journal of gastroenterology & hepatology. 2005;17(11):1181-1184.
47. Mawatari H, Inamori M, Fujita K, et al. The continuous real-time 13C-octanoate breath test for patients with nonalcoholic steatohepatitis using the BreathID system. Hepato-gastroenterology. 2009;56(94-95):1436-1438.
48. Romestaing C, Piquet MA, Letexier D, et al. Mitochondrial adaptations to steatohepatitis induced by a methionine- and choline-deficient diet. American journal of physiology Endocrinology and metabolism. 2008;294(1):E110-119.
49. Chalasani N, Gorski JC, Asghar MS, et al. Hepatic cytochrome P450 2E1 activity in nondiabetic patients with nonalcoholic steatohepatitis. Hepatology (Baltimore, Md). 2003;37(3):544-550.
50. Miele L, Grieco A, Armuzzi A, et al. Hepatic mitochondrial beta-oxidation in patients with nonalcoholic steatohepatitis assessed by 13C-octanoate breath test. The American journal of gastroenterology. 2003;98(10):2335-2336.
51. Dasarathy S, Kasumov T, Edmison JM, et al. Glycine and urea kinetics in nonalcoholic steatohepatitis in human: effect of intralipid infusion. American journal of physiology Gastrointestinal and liver physiology. 2009;297(3):G567-575.
52. Dasarathy S, Yang Y, McCullough AJ, Marczewski S, Bennett C, Kalhan SC. Elevated hepatic fatty acid oxidation, high plasma fibroblast growth factor 21, and fasting bile acids in nonalcoholic steatohepatitis. European journal of gastroenterology & hepatology. 2011;23(5):382-388.
53. Rizki G, Arnaboldi L, Gabrielli B, et al. Mice fed a lipogenic methionine-choline-deficient diet develop hypermetabolism coincident with hepatic suppression of SCD-1. Journal of lipid research. 2006;47(10):2280-2290.
54. Kalavalapalli S, Bril F, Guingab J, et al. Impact of exenatide on mitochondrial lipid metabolism in mice with nonalcoholic steatohepatitis. The Journal of endocrinology. 2019;241(3):293-305.
55. Caldwell SH, Swerdlow RH, Khan EM, et al. Mitochondrial abnormalities in non-alcoholic steatohepatitis. Journal of hepatology. 1999;31(3):430-434.
56. Kawahara H, Fukura M, Tsuchishima M, Takase S. Mutation of mitochondrial DNA in livers from patients with alcoholic hepatitis and nonalcoholic steatohepatitis. Alcoholism, clinical and experimental research. 2007;31(1 Suppl):S54-60.
57. Nomoto K, Tsuneyama K, Takahashi H, Murai Y, Takano Y. Cytoplasmic fine granular expression of 8-hydroxydeoxyguanosine reflects early mitochondrial oxidative DNA damage in nonalcoholic fatty liver disease. Applied immunohistochemistry & molecular morphology : AIMM. 2008;16(1):71-75.
58. Garcia-Martinez I, Santoro N, Chen Y, et al. Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9. The Journal of clinical investigation. 2016;126(3):859-864.