Non-alcoholic fatty liver disease and insulin resistance: From bench to bedside

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is now the most frequent chronic liver disease in the developed countries. There is also growing evidence from basic and clinical research that NAFLD has a strong relationship to insulin resistance, which is a key factor in the development of type 2 diabetes. The aim of this review is to summarize the recent important findings linking NAFLD and insulin resistance. Lipid accumulation, particularly of diacylglycerol, appears to be of major importance in this process. Mitochondrial dysfunction, through decreased mitochondrial biogenesis, increases oxidative stress, and ageing also plays an important role. Finally, endoplasmic reticulum stress and inflammation also probably contribute to the development of insulin resistance via mechanisms that are still not well understood. Clinical aspects of NAFLD, such as its diagnosis and management, are also investigated in this review.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD), a disorder characterized by fat accumulation in the liver, encompasses simple hepatic steatosis, non-alcoholic steatohepatitis (NASH) and cirrhosis. Fibrosis is a complication of NASH [1]. Its prevalence is constantly on the rise and is now approaching a pandemic state, particularly in Western countries, with a prevalence of 46% in a recent US population-based study of 328 individuals using ultrasound as a diagnostic tool. The prevalence of NAFLD varies across different populations, with an estimated prevalence of 58.3% in Hispanics, 44.4% in Caucasians and 35.1% in African-Americans. The prevalence of NASH was 12.2%, and 2.7% for significant liver fibrosis [2].

These differences can be attributed to variations in visceral adiposity distribution, genetic background, dietary habits and physical activity. NAFLD also affects children and adolescents with an estimated prevalence of 10% in the US [3].

NAFLD is defined as either excessive fat accumulation in the liver with more than 5% of hepatocytes containing...
visible intracellular triglycerides, or steatosis affecting at least 5% of the liver volume or weight in patients consuming less than 30 g (three units) of alcohol per day for men and less than 20 g (two units) of alcohol per day for women. One unit of alcohol (10 g) is defined as one glass of beer (25 cl), one glass of wine (20 cl) or one glass of whisky (3 cl). Simple hepatic steatosis may be reversible or progress to NASH if the causal factors persist. NASH is characterized morphologically by steatosis, ballooning hepatocytes, inflammation and fibrosis. NASH itself can continue to progress to cirrhosis and hepatocellular carcinoma (Fig. 1) [4,5]. NAFLD is characterized histologically by the presence of micro- and macrovesicular fatty changes in hepatocytes. Macrovesicular steatosis leads to displacement of the nucleus to the edge of the cell, creating the characteristic signet ring appearance [6].

NAFLD can be considered as the hepatic manifestation of the metabolic syndrome. The metabolic syndrome is defined as the presence of any three of the following five risk factors: large waist circumference; elevated triglycerides (≥ 1.7 mmol/L); reduced high-density lipoprotein cholesterol (HDL-C; < 1.0 mmol/L in men and < 1.3 mmol/L in women); raised blood pressure (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg); and elevated fasting glucose ≥ 100 mg/dL) [7].

Links between NAFLD and insulin resistance have been increasingly demonstrated through numerous studies [8,9]. The aim of the present review is to discuss the role of lipid accumulation and decreased mitochondrial function, as well as other concepts such as endoplasmic reticulum (ER) stress and inflammation, in the development of hepatic insulin resistance associated with NAFLD. To this end, this report describes the different mechanisms involved in the development of NAFLD according to recent research in animal models of NAFLD as well as in humans. Also highlighted are the most relevant and recent clinical updates to help practitioners in the management of NAFLD, particularly when the condition is associated with hepatic insulin resistance.

2. Clinical aspects of NAFLD

Clinically, NAFLD is most commonly silent, but can manifest with non-specific symptoms such as right upper quadrant discomfort or fatigue. Liver enzymes are usually minimally perturbed with mostly increased levels of alanine aminotransferase and gamma-glutamyl transpeptidase. NAFLD is a diagnosis of exclusion, so its workup needs to exclude other causes such as a significant alcohol consumption (defined as > 30 g/day of ethanol for men and 20 g/day of ethanol for women), hepatitis B and/or C infection, drug abuse, autoimmune liver disease, haemochromatosis or Wilson’s disease.

The principal risk factors for developing NAFLD are the presence of insulin resistance and obesity. More generally, any elements constituting the metabolic syndrome, such as type 2 diabetes, hypertension and dyslipidaemia, are linked to the development of NAFLD, and approximately 85% of patients with NAFLD have at least one such constituent. The metabolic syndrome itself is present in 30% of patients with NAFLD.

It is estimated that around 55% of patients with type 2 diabetes have NAFLD but, because insulin resistance and obesity can also be seen in type 1 diabetes, NAFLD is also found in approximately 45% of patients with type 1 diabetes [10,11]. The major causes of death in NAFLD are, on one hand, linked to the development of cirrhosis and subsequently to hepatocellular carcinoma and, on the other hand, related to the presence of cardiovascular diseases such as ischaemic heart disease and cerebrovascular disease [12].

Imaging tests can be helpful as a non-invasive diagnostic approach. Ultrasonography and computed tomography (CT) have a sensitivity greater than 90% when fatty liver infiltration is above 30%. Magnetic resonance imaging (MRI) has a better performance record and also has the advantage of no irradiation. FibroScan has interesting features particularly as a screening test to exclude advanced fibrosis in NAFLD in view of its high negative predictive value. For stage 3 liver fibrosis according to METAVIR score, FibroScan performance in terms of sensitivity, specificity, and positive and negative predictive values is 91%, 75%, 52% and 97%, respectively [13].

However, the limitation of all these radiological techniques is the lack of validation to differentiate between fatty liver, NASH and hepatic fibrosis [14,15]. This means that the “gold standard” for the diagnosis of NAFLD remains liver biopsy. The procedure permits evaluation of the presence and degree of steatosis, inflammation and fibrosis, and can also differentiate simple steatosis from NASH and cirrhosis. Its limitations, however, include its invasiveness, risk of complications such as bleeding, and high cost because of the ever-growing number of people with NASH; thus, liver biopsy cannot be used routinely.

The management of NAFLD [16] should be based on the diagnosis and treatment of the constituents of the metabolic syndrome such as obesity or type 2 diabetes. First, lifestyle modifications including weight reduction and physical activity should be considered. However, evidence of their positive impact on NAFLD remains limited as there are only short-term trials showing that weight loss is associated with a reduction in plasma transaminases and steatosis, and with minimal decreases in inflammation and fibrosis [17]. Medical treatments that have been the most studied in NAFLD are those acting on insulin resistance, such as metformin and thiazolidinedione (TZD) therapies. Unfortunately, these studies have included only a few patients, so limiting their conclusions. Regarding metformin, a positive impact has been observed on plasma transaminases, but no significant improvement in histological steatosis, inflammation or ultrasound imaging of NAFLD have been reported [18,19]. TZDs have been shown to improve plasma
transaminases, and also inflammation and steatosis, but not liver fibrosis, but this was probably due to the short duration of the trials [20, 21].

A recent meta-analysis including 49 randomized clinical trials (RCT) evaluated most of the treatments indicated for NAFLD and NASH [22]. Weight-loss resulted in a reduction in histological involvement, but less than 50% of the participants reached their target weight loss. TZDs also led to improvements in steatosis and inflammation, but caused weight gain. Vitamin E proved to be superior in improving histological alterations in NASH compared with placebo whereas, in the same study, pioglitazone showed no advantage compared with placebo [23]. Conclusions on antioxidants such as vitamin C were not possible because of the heterogeneity across studies, and other hepatoprotective agents such as telmisartan, pentoxifylline and L-carnitine showed positive impacts on steatosis in at least one RCT [22]. Ursodeoxycholic acid has been found to improve markers of glycemic control, insulin resistance and serum fibrosis [24].

Clinical trials using glucagon-like peptide-1 agonists are currently ongoing and may represent a promising approach in the treatment of NAFLD. Bariatric surgery may also be considered a promising approach for NAFLD, although the data supporting the technique are only in the form of prospective observational studies, retrospective studies and case series, with improvement observed in histological lesions. However, in general, there is a need for more large-scale well-designed trials of longer follow-up durations to be carried out before any definitive recommendations can be made.

3. Pathogenesis

One hypothesis for the pathogenesis of NASH from NAFLD is the “two-hit” theory. The first “hit” is the accumulation of triglycerides (TG), leading to the development of NAFLD, while the second hit is the generation of free radicals after the release of cytokines and inflammatory mediators [25]. However, one recent theory regarding the pathophysiological model of development of NASH from NAFLD argues in favour of multiple parallel hits that are derived simultaneously from adipose tissue and the gut to promote liver inflammation. According to this idea, cellular dysfunction and insulin resistance are acting simultaneously [26].

Other studies suggest that hepatic insulin resistance is the first step in the development of peripheral insulin resistance and type 2 diabetes. Mice lacking the muscle-specific insulin receptor alone (MIRKO) have been shown to present with decreased insulin-stimulated muscle glucose uptake with no modification of total body glucose homeostasis [27]. Moreover, mouse knockout (KO) for the adipose tissue insulin receptor (FIRKO) has reduced adipose tissue glucose uptake, yet appears to have a lower risk of developing obesity, dyslipidaemia and glucose intolerance; these findings were also associated with an increased lifespan [28,29]. However, mouse KO for the insulin receptor specifically in the liver (LIRKO) has shown both fasting and postprandial hyperglycaemia associated with hepatic insulin resistance and an increase in muscle insulin resistance [30]. These findings suggest that hepatic insulin resistance could be the first step in the development of peripheral insulin resistance in skeletal muscle and adipose tissue.

In accordance with the concept of primary hepatic insulin resistance, a recent study has also suggested that the development of hepatic insulin resistance could be due to mitochondrial dysfunction in the liver. When sedentary hyperphagic obese rats were compared with non-hyperphagic control rats, the authors observed that, at 5 weeks of age, serum insulin, glucose concentrations and hepatic TG content were similar between both groups whereas the hyperphagic rats showed significant hepatic mitochondrial dysfunction, as measured by decreased fatty acid (FA) oxidation, hepatic carnitine palmitoyl-CoA transferase-1 activity and cytochrome c protein content compared with control rats. Insulin resistance developed within 13 weeks in the hyperphagic rats and was associated with NAFLD progression. The study showed that decreased hepatic FA oxidation capacity and enzyme activity preceded the development of NAFLD and insulin resistance [31].

In addition, white adipose tissue most likely plays an important role in NAFLD and insulin resistance. Indeed, white adipose tissue secretes different molecules, called “adipokines”, such as leptin, tumour necrosis factor (TNF)-α, interleukin (IL)-6 and adiponectin. These molecules appear to modulate insulin resistance via different mechanisms [32].

Autophagy may be another factor in the pathogenesis of insulin resistance. Autophagy normally regulates intracellular lipid stores. Its inhibition has been seen in insulin-resistant states and appears to increase TG storage in liver, thus promoting lipid accumulation [33,34].

Gut microbiota has also been identified as an important environmental factor affecting the predisposition towards obesity and energy storage in the host [35].

4. Genetic background

There is evidence that NAFLD can develop without obesity perhaps due to genetic susceptibility. For example, Asian Indian men show an increased prevalence of hepatic steatosis despite having normal body mass index (BMI) [36]. Petersen et al. [37] observed that the presence of the ApoC3 polymorphism (T482/C455) conferred a risk for NAFLD and insulin resistance. In that study, carriers of the ApoC3 variant alleles had, in comparison to those with wild-type homozygotes, an approximately 60% increase in fasting plasma TG concentration, a doubled plasma TG level following an oral fat tolerance test and a 46% reduction in plasma TG clearance. The prevalence of NAFLD was 38% among variant-allele carriers and 0% among wild-type homozygotes. A subgroup of seven individuals with hepatic steatosis was put under caloric restriction (~1200 kcal/day) over a period of 3 to 6 months. After modest weight reduction of approximately 6 kg, a marked improvement was observed in glucose tolerance on oral glucose tolerance tests with reductions in both plasma insulin and glucose concentrations, suggesting that a decrease in hepatic steatosis can improve insulin sensitivity in these individuals.

Another study performed a genome-wide association scan of non-synonymous sequence variations (n = 9229) in a multiethnic
population aiming to identify genetic variants causing hepatic fat accumulation. An allele of the PNPLA3 gene (rs738409; I148M) was strongly linked to more hepatic inflammation and fat content. Homozygote individuals for this allele had a twofold increase in hepatic fat content compared with non-carriers. The allele was also more common in Hispanics, the population most prone to develop NAFLD [38]. Petta et al. [39] showed in a recent study that, in NAFLD patients, II28B rs12979860 CC and PNPLA3 rs738409 GG genotypes were associated with the severity of histological features of liver disease.

5. Lipid intermediates accumulation

Accumulation of TG in liver and skeletal muscle cells is due to various factors creating a positive balance between TG synthesis and influx vs TG degradation and outflow. These factors are increased by dietary TG coming from the intestines as chylomicrons, lipolysis of insulin-resistant adipose tissue leading to FA influx to the liver, and decreased FA oxidation and reduced outflow of lipids from the liver [9,40].

It has been observed that increasing the plasma FA serum rate through lipid plus heparin infusions in lean humans can lead to insulin resistance. However, this procedure does not precisely imitate the obese state, but instead reproduces the high lipid flow in obese subjects with no other confounding factors linked to elevated adipose tissue [41–44]. Also, intramyocellular lipid stores have been clearly associated with insulin resistance except in the so-called “athlete’s paradox”, where increased lipid accumulation in muscle cells leads to increased insulin sensitivity [45–48].

Normally, FA is integrated in muscle or liver and may follow two principal pathways, one consisting of β-oxidation in the mitochondria and the other with storage as TG. Overload of these pathways leads to accumulation of lipid intermediates such as phosphatidic acid, lysophosphatidic acid, diacylglycerol (DAG) and ceramide [9]. These lipid intermediates indirectly affect insulin action through their activation of different inhibitory serine kinases such as Jun N-terminal kinase (JNK), mammalian target of rapamycin (mTOR), inhibitor of kappa-B kinase (IKK) and novel protein kinase C (nPKC), thereby promoting insulin resistance (Fig. 2) [49–54].

Several studies using rodent models of insulin resistance have indicated a critical role for DAG and PKCe in the development of hepatic insulin resistance in hepatic steatosis [54–60]. Samuel et al. [55] elaborated a model where suppression of PKCe expression, using an antisense oligonucleotide, protected rats against developing fat-induced hepatic insulin resistance, thereby identifying PKCe, which is activated by DAG, as a key mediator of insulin resistance. Similarly, infusion of lipids provoked insulin resistance in muscle with accumulation of intracellular DAG and specific activation of PKC9 [61,62].

These findings in rodents were also confirmed in a recent study in humans. Kumashiro et al. [8] sought to determine which cellular mechanism in humans was responsible for the development of hepatic insulin resistance in NAFLD. The authors studied different hepatic lipid intermediates, and ER stress and inflammation. Using the homoeostasis model assessment for insulin resistance (HOMA-IR) as a surrogate marker of insulin resistance, they found that the hepatic DAG content in lipid droplets in hepatocytes was the best predictor of insulin resistance. There was no association or only partial correlation observed between markers of inflammation or ER stress and insulin resistance as determined by the HOMA-IR index. In addition, the authors showed that PKCe activation was strongly associated with hepatic DAG content in insulin-resistant non-diabetic obese subjects. Thus, the study confirmed previous findings in rodent models of hepatic insulin resistance and indicated that, in humans, hepatic DAG content also plays a key role in the pathogenesis of hepatic insulin resistance [8].

Bandyopadhyay et al. [63] found that insulin-resistant subjects present with elevated malonyl-CoA levels probably caused by a decrease in AMP-activated protein kinase (AMPK) activity and an increase in acetyl-CoA carboxylase (ACC), resulting in lower FA oxidation rates. Furthermore, increased expression of FA transporters in the muscle of insulin-resistant subjects was noted, thus promoting accumulation of long-chain fatty acyl-CoA and TG. Subsequently, during hyperinsulinaemic–euglycaemic clamp tests, muscle FA oxidation was reduced in lean vs insulin-resistant subjects. In contrast, isolated muscle mitochondria from type 2 diabetic patients exhibited greater rates of FA oxidation compared with the lean group. These findings suggest that increased levels of
and malonyl-CoA could lead to insulin resistance by lowering the rate of FA oxidation [63].

Lipodystrophy is a heterogeneous group of disorders in which the primary defect is a generalized or partial absence of adipose tissue with the possible presence of a large increase in visceral fat, which may be either congenital or acquired. Several studies have clearly shown that human immunodeficiency virus (HIV) patients receiving retroviral therapy with lipodystrophy syndrome present with higher rates of insulin resistance, hyperlipidaemia and liver lesions consistent with NASH [64,65]. One hypothesis to explain these findings was that HIV patients with lipodystrophy may have overexpression of sterol regulatory element-binding protein-1 (SREBP-1) and decreased expression of peroxisome proliferator-activated receptor (PPAR)-γ1 and PPAR-γ2. Lipodystrophy allows evaluation of the consequences of insulin resistance with ectopic fat accumulation in the absence of peripheral adiposity. Patients with severe lipodystrophy show major decreases in subcutaneous fat, hypertriglyceridaemia and ectopic fat deposition together with hepatic steatosis and insulin resistance. Mouse models of lipodystrophy have revealed important hepatic and peripheral insulin resistance with fat accumulation in skeletal muscle and liver [66,67]. It has also been shown that transplantation of fat pads in lipodystrophic mice leads to normalization of hepatic and muscle insulin sensitivity [68]. Another interesting finding was the observation that leptin administration in patients with congenital generalized lipodystrophy led to a reduction of approximately 90% of hepatic TG content and 30% of muscle TG content with an increase in hepatic insulin sensitivity [69]. These findings in patients with lipodystrophy suggest that ectopic accumulation of lipids can induce insulin resistance despite the lack of peripheral adiposity.

Sphingolipid ceramide also appears to play an important role in the development of insulin resistance. Ceramide is produced in the presence of stress stimuli linked to obesity such as saturated FAs, an inflammatory state because of Toll-like receptor 4 (TLR4) and ER stress [70]. Intracellular accumulation of ceramide provokes disruption of insulin signalling through different mechanisms, such as activation of the PKCζ isofrom that causes inhibition of Akt phosphorylation, resulting in an increase of protein phosphatase 2A that, in turn, also acts upon Akt, thus leading to mitochondrial dysfunction and ER stress [71–76].

Also, the idea of a protective role of steatosis has recently been suggested. Yamaguchi et al. [77] showed that, by inhibiting the enzyme that catalyzes the final step in hepatic TG biosynthesis known as “diacylglycerol acyltransferase 2” (DGAT 2), liver steatosis was reduced while hepatic free FAs were increased, as were lobular necroinflammation, fibrosis and markers of lipid peroxidation/oxidative stress. In fact, the relationship between liver steatosis and insulin resistance may not be obvious. Carbohydrate responsive element-binding protein (ChREBP) is involved in the control of glucose and lipid homeostasis in a complex manner. In ChREBP-overexpressing mice fed a high-fat diet, normal insulin levels and improved insulin signalling were observed compared with the control group, despite hepatic steatosis [78].

In general, the accumulation of lipid intermediates, particularly DAG and ceramide, whether in the liver or in muscle is a major contributing factor to the development of insulin resistance via various actions on intracellular insulin signalling. The example of lipodystrophy shows that ectopic lipid accumulation plays a major role in the development of insulin resistance even in the absence of subcutaneous fat tissue.

6. Mitochondrial dysfunction

The main function of mitochondria is the production of energy in the form of adenosine triphosphate (ATP) by oxidative cellular respiration metabolism of nutrients. Two major steps are needed for ATP production: oxidation of NADH (or FADH2); and phosphorylation of adenosine diphosphate (ADP) to ATP (oxidative phosphorylation, or OXPHOS). The oxidative power of mitochondria is determined by their size, number and rate of expression of OXPHOS subunits [79].

There is growing evidence that type 2 diabetes and insulin resistance are secondary to diminished mitochondrial function that may lead to decreased mitochondrial biogenesis, increased oxidative stress and ageing [14,80,81]. The mitochondrial genome is particularly vulnerable to mutations, as it is not protected by histones and is closer to radical oxidative stress production [82]. For example, OXPHOS genes can themselves be the target of cellular stress and ageing that can lead to alterations in their expression and, consequently, to mitochondrial dysfunction via a reduced rate of OXPHOS, thereby leading to insulin resistance [79].

Mutations in mitochondrial DNA have been identified as causes of the metabolic syndrome. The A3243G mutation in the mitochondrial DNA-encoded tRNA(Leu)(UUR) gene, for example, results in progressive impairment of insulin secretion by pancreatic β cells. Carriers of this mutation show significant decreases in first- and second-phase insulin secretion in comparison to non-carriers during hyperglycaemic clamp testing. The pathophysiological mechanism of impaired insulin secretion with this mutation is hypothesized to be a decreased cytosolic ATP/ADP ratio causing a reset of glucose-sensing levels by β cells [83]. It was also observed that, in a population of non-diabetic Pima Indians, a polymorphism in the PPAR-γ coactivator-1α (PGC-1α), a transcriptional coactivator implicated in lipid metabolism, was associated with a decrease in insulin secretion and lipid oxidation. Despite being encoded by nuclear genes, PGC-1α plays a crucial role in mitochondrial biogenesis [84]. These studies showing genetic alterations in the mitochondrial genome suggest that mitochondrial dysfunction is related to the development of insulin resistance.

The observation that hepatic mitochondria are reduced in number, but increased in size and swollen because of matrix hypodensity and paracrystalline inclusions, is an argument supportive of the idea that mitochondrial biogenesis is affected in NALFD [85–87]. These morphological anomalies suggest a deficiency in normal mitochondrial activity such as ATP synthesis and respiratory chain activities [88,89]. Mitochondria in skeletal muscle cells also show alterations related to insulin resistance with reductions in both size and number. One study
showed a 38% reduction in mitochondrial density in a cohort of young lean, normoglycaemic, insulin-resistant offspring of patients with type 2 diabetes compared with individuals matched for age and BMI; this finding was associated with a similar reduction in ATP synthesis [90,91].

Theoretically, mitochondrial dysfunction could lead to a reduced capacity of muscle cells to oxidize FAs particularly when FA dietary intake is high, thereby leading to insulin resistance. Nevertheless, fundamental and clinical studies have shown discrepancies in their results. In some studies, insulin-resistant obese type 2 diabetic subjects had a similar capacity for FA oxidation as the lean controls and possibly better mitochondrial function [63,92,93]. These findings suggest that patients with insulin resistance have a decreased OXPHOS capacity that may be attributed to a reduced number of mitochondria.

Regarding muscle mitochondria and insulin resistance, one study showed that rats fed a high-fat diet developed a gradual increase in muscle mitochondria along with insulin resistance [94]. This was thought to be due to the activation of PPAR-δ by FAs, leading to a post-transcriptional increase in PGC-1α expression. These mechanisms permit increases in mitochondrial biogenesis and mitochondrial activities despite the development of insulin resistance. However, while these findings are in contradiction to the theory that insulin resistance is provoked by a decrease in muscle mitochondria, they suggest a compensatory mechanism by which muscle increases mitochondrial oxidative function to overcome the insulin resistance [94]. Nevertheless, other studies have shown the opposite results with reduced FA oxidation and mitochondrial function in obese type 2 diabetic individuals [95,96].

Different factors regulating mitochondrial biogenesis have been identified and may be altered by NAFLD. PGC-1α, a co-transcriptional factor, has an important role in activating transcription factors such as nuclear respiratory factors 1 (NRF-1) and 2 (NRF-2). The latter regulates the expression of many mitochondrial genes, including mitochondrial transcriptional factor A (TFAM) and OXPHOS genes that play a major role in mitochondrial genome expression and replication [97–102]. The expression of PGC-1α and NFRs is reduced in diabetic and insulin-resistant subjects, and probably leads to a decreased number of skeletal muscle mitochondria [103]. However, in the offspring of type 2 diabetes, the level of mRNA expression of PGC-1α and NFRs was not lower than that of normal subjects, although mitochondrial function was reduced, suggesting that a decrease in mitochondrial biogenesis alone cannot explain mitochondrial dysfunction [91]. AMPK also has an important role in the regulation of mitochondrial biogenesis, and is a key regulator of cellular and whole-body metabolism. AMPK can be stimulated by the pharmacological activator β-guanidinopropionic acid (βGPA), which results in increased mitochondrial biogenesis through PGC-1α and NRFs [104–106].

Exercise can also activate AMPK through PGC-1α phosphorylation. AMPK and PGC-1α appear to have an interdependent relationship. It has been shown that PGC-1α protein is required for AMPK activity in gene expression and mitochondrial function. Likewise, direct phosphorylation of PGC-1α by AMPK on threonine-177 and serine-538 increases PGC-1α-dependent activation of its own promoter. Therefore, AMPK participates in the regulation of mitochondrial biogenesis, leading to greater mitochondrial oxidative capacity and, consequently, improved insulin sensitivity [107]. The effect of AMPK activation on hepatic lipid metabolism has been studied notably through the action of metformin. First, AMPK activation provokes inactivation of ACC. When activated, ACC induces the production of malonyl-CoA from acetyl-CoA. Malonyl-CoA leads to lipid synthesis, but also inhibits FA oxidation through its inhibitory effect on carnitine palmitoyl transferase (CPT-1) [108]. Another consequence of AMPK activation is the inhibition of SREBP-1c, an essential insulin-stimulated transcription factor. SREBP-1c induces genes encoding lipogenic enzymes such as FA synthase. Suppression of SREBP-1c by AMPK can lead to a decrease in hepatic FA accumulation, leading to improved hepatic insulin sensitivity [109]. Furthermore, AMPK may also contribute to liver insulin sensitivity through its regulation of hepatic gluconeogenesis. It has been observed that stimulation of AMPK by 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR) infusions in both obese insulin-resistant and normal rats provokes inhibition of hepatic glucose production [110].

Thus, AMPK participates in the regulation of mitochondrial biogenesis, mitochondrial oxidative capacity, FA oxidation and hepatic gluconeogenesis, ultimately leading to a reduction in hepatic insulin resistance. Other factors also appear to influence mitochondrial biogenesis, including calcium/calcmodulin-dependent protein kinase type IV (CaMKIV), nitric oxide (NO), sirtuin (silent mating type information regulation 2 homologue) 1 (SIRT1) and transducer of regulated CREB (cAMP response element-binding protein)-binding protein (TORC), mostly through activation of PGC-1α [111–116].

Mitochondria are considered the powerhouses of cells, and are also the principal site of reactive oxygen species (ROS) production mostly through OXPHOS and the respiratory chain. These mitochondrial processes, which produce ATP by electron transport, can also produce excess electrons that are transferred to oxygen-generating superoxide anion radicals ($O_2^−$) that can further react to form hydroxyl radicals (HO−). However, protective mechanisms that can reduce ROS production are represented by enzymes such as superoxide dismutase and catalase, but these are not sufficient to totally inhibit ROS production [117]. Lee et al. [118] studied mitochondrial function, intramyocellular lipid content and insulin action in lean healthy mice with targeted overexpression of the human catalase gene to mitochondria (MCAT). They found that MCAT mice were protected from age-associated reductions in muscle mitochondrial function and insulin resistance. This was associated with decreased skeletal muscle DAG content and reduced PKCθ activity. These findings support the hypothesis that the mitochondrial production of ROS causes reductions in mitochondrial function and participates in the pathogenesis of age-associated insulin resistance [118].
7. ER stress and inflammation

Growing evidence suggests a link between ER stress and the pathogenesis of insulin resistance. ER plays a central role in protein and lipid biosynthesis. A physiologically adaptive process known as the “unfolded protein response” (UPR) is activated by disordered protein maturation, folding or transfer that can be caused by either a high-fat diet or hypoxia [119]. The role of UPR is to maintain protein homoeostasis by reducing protein synthesis, increasing degradation of unfolded proteins and increasing ER chaperones. However, prolonged activation of UPR has negative effects, leading to perturbations of energy metabolism and triggering proapoptotic and inflammatory pathways. UPR activates at least three transmembrane signal transducers – namely, inositol-requiring protein-1 (IRE-1), protein kinase-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6) – resulting in ER stress [120–122]. Consequences of ER stress are, most notably, impairment of insulin signaling in the liver, apoptosis and decreased protein synthesis. JNK: Jun N-terminal kinase; XBP1: X-box protein; CHOP: C/EBP homologous protein; eIF2α: eukaryotic translation initiation factor 2α.

Fig. 3. Schematic representation of endoplasmic reticulum (ER) stress. Prolonged activation of the unfolded protein response (UPR) causes activation of the three transmembrane signal transducers inositol-requiring protein-1 (IRE-1), protein kinase-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6), thereby leading to ER stress. Consequences of ER stress include impaired insulin signaling in the liver, apoptosis and decreased protein synthesis. JNK: Jun N-terminal kinase; XBP1: X-box protein; CHOP: C/EBP homologous protein; eIF2α: eukaryotic translation initiation factor 2α.

that Xbp1 deficiency led to alterations in de novo hepatic lipid synthesis that resulted in decreases in serum cholesterol, TG and FA without concomitant hepatic steatosis. These findings suggest that hepatic insulin resistance in models of ER stress may be due to defective lipid storage rather than ER-specific stress signals. Jurczak et al. [125] conducted a study aiming to dissociate hepatic lipid storage from activation of ER stress signaling pathways. Xbp1 KO mice were compared to control mice and fed a fructose diet. The authors measured hepatic lipids, TG and DAG as well as markers of ER stress, and also assessed whole-body and tissue-specific insulin sensitivity using the gold-standard hyperinsulinaemic–euglycaemic clamp. Xbp1 KO mice displayed an increase in hepatic ER stress signaling with, for example, twofold increased hepatic JNK activity, with concomitant increased hepatic sensitivity and reduced hepatic DAG content. These findings show that ER stress and JNK activation can be separated from hepatic insulin resistance, and support the theory that hepatic insulin resistance in models of ER stress may be secondary to ER stress alteration due to hepatic lipogenesis [125]. Other consequences of ER stress include apoptosis via various pathways, including PERK phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), thereby causing a global decrease in protein synthesis [88] and induction of proteins such as antiadaptive C/EBP homologous protein (CHOP), a proapoptotic factor [126,127]. It has also been shown that one target of CHOP is pancreatic β cells, therefore possibly involving this protein in diabetes pathogenesis [128]. In humans, a study of 11 obese subjects before and 1 year after gastric bypass surgery evaluated the effects of the intervention on ER stress. Systemic insulin sensitivity using hyperinsulinaemic–euglycaemic clamps was measured, as were ER stress markers in the liver and subcutaneous adipose tissue before and after surgery. The subjects showed a loss of approximately 40% of body weight 1 year after surgery. Markers of ER stress were reduced in the liver and insulin sensitivity was markedly improved after surgery, suggesting a link between ER stress, obesity and metabolic disturbances [129]. However, it is not clear whether weight loss per se contributed to the improvement in ER stress markers and insulin sensitivity.

Chronic inflammation observed in obese patients probably also plays an important role in the development of insulin resistance. Accumulation of adipose tissue causes an increase in total FA release associated with hyperperfusion of adipocytes leading to microhypoxia. These phenomena in turn provoke an increase in the expression of the hypoxia-inducible factor (HIF) gene programme as well as activation of the genes implicated in inflammation, ER stress, and the JNK and IκB-α kinase (IKK) pathways [130–132]. Activation of these pathways induces the production and release of chemokines and cytokines such as TNF-α, IL-6, leptin, resistin, monocyte chemotactic protein (MCP)-1 and plasminogen activator inhibitor (PAI)-1, thereby promoting the migration from bone marrow to adipocytes of monocyte-derived macrophages that secrete cytokines with paracrine effects, thus promoting insulin resistance [132–134].

These findings suggest that ER stress and inflammation are important contributors to the development of insulin resistance.
although the exact mechanisms of these interactions remain poorly understood.

8. Conclusion

NAFLD can now be considered a worldwide condition associated with the global obesity epidemic. A growing number of studies in both rodents and humans have led to a greater understanding of the complex pathophysiology involving different mechanisms such as intracellular lipid accumulation, mitochondrial dysfunction, and ER stress and inflammation. NAFLD is almost always associated with the development of hepatic insulin resistance and with serious morbidity and mortality. Lifestyle modifications, such as weight loss and exercise, are currently the most effective treatment of NAFLD.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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