REVIEW

Multiple organs involved in the pathogenesis of non-alcoholic fatty liver disease

Xiaoyan Li[1] and Hua Wang[1,2]*

Abstract

Non-alcoholic fatty liver disease (NAFLD) represents the leading cause of chronic liver disease worldwide and the anticipated health burden is huge. There are limited therapeutic approaches for NAFLD now. It's imperative to get a better understanding of the disease pathogenesis if new treatments are to be discovered. As the hepatic manifestation of metabolic syndrome, this disease involves complex interactions between different organs and regulatory pathways. It's increasingly clear that brain, gut and adipose tissue all contribute to NAFLD pathogenesis and development, in view of their roles in energy homeostasis. In the present review, we try to summarize currently available data regarding NAFLD pathogenesis and to lay a particular emphasis on the inter-organ crosstalk evidence.

Keywords: Non-alcoholic fatty liver disease, Energy metabolism, Lipid, Hormone, Inter-organ crosstalk

Introduction

Non-alcoholic fatty liver disease (NAFLD), has been commonly considered as the leading cause of chronic liver diseases in Western countries over the last decade [1]. In the meantime, urbanization in many developing countries has recently led to a sedentary lifestyle and overnutrition, which contribute to obesity, metabolic syndrome and the emerging condition of NAFLD [2]. NAFLD currently has a reported prevalence of 12–38% worldwide and the number is growing inexorably and steadily along with the unprecedented levels of obesity in human society [3]. On account of the radical cure of hepatitis C, the imminent disappearance of hepatitis B and the uncontrolled energy-dense lifestyle, NAFLD would undoubtedly become the mainspring for liver related morbidity and mortality very soon. As estimated, NAFLD should be the most frequent indication for liver transplantation by 2030 [4]. To date, no drug for NAFLD

has received FDA approval, giving rise to insufficient pharmacotherapeutic interventions in clinical practice [5]. These alarming situations necessitate the extension of our understanding towards pathogenic mechanisms of NAFLD.

NAFLD could be simply interpreted as the condition where excess fat is stored in the liver, and this secondary fat accumulation is not the consequence of other factors like heavy alcohol consumption, drug side effects or genetic variations [6]. Whether liver inflammation exists is the basis to subdivide NAFLD into two types, fatty liver and non-alcoholic steatohepatitis (NASH), and the latter is the progressive phenotype of NAFLD spectrum [7]. Persistent inflammation will jeopardize liver homeostasis and activate the repair processes. Activated hepatic stellate cells (HSCs) secrete extracellular matrix (ECM), including type 1 collagen, to support the injured region and structure the regeneration scaffold for subsequent hepatocyte proliferation (i.e., liver fibrosis formation), along with the clearance of necrotic tissues by infiltrated leukocytes [8]. This 'wound-healing' process will end with the settlement of activated HSCs, ECM resolution

*Correspondence: wanghua@ahmu.edu.cn
[1] Department of Oncology, the First Affiliated Hospital of Anhui Medical University, Hefei 230022, China
Full list of author information is available at the end of the article

and revascularization, given the detrimental effects are only transient [9]. Hence, it's objective to regard liver fibrosis as a well-orchestrated advantageous response towards hepatic injury, while the ongoing insults against liver should be to blame for disease progression to cirrhosis or hepatocellular carcinoma (HCC) [10]. Specifically, for NAFLD, the persistence of liver steatosis derives from the overload of free fatty acids (FFAs) influx, which is the coefficient result of high fat diet, obesity, IR, gut microbiota alteration, and other potential risk factors. Furthermore, high levels of FFAs will exert the lipotoxic effects, causing endoplasmic reticulum (ER) stress and mitochondrial dysfunction (oxidative stress, production of reactive oxygen species (ROS), etc.) in the liver [11]. IR not only motivates hepatic de novo lipogenesis (DNL), but also causes adipose tissue dysfunction with consequent adipokines and inflammatory cytokines production [12]. Gut microbiota dysregulation could lead to the increase of intestine permeability and the release of proinflammatory cytokines into circulation [13]. Multiple pathways synergistically create a vicious cycle which slowly exacerbates the disbalance of liver homeostasis and induces the shift from simple steatosis to chronic inflammatory state of NASH. Based on evidences above, NAFLD is definitely not a single-organ disease, but more like the hepatic manifestation of a variety of complicated metabolic disorders involving different organs and systems. Here we will review recent findings about brain, gut, adipose tissue and liver interactions in NAFLD pathogenesis and try to get a relatively comprehensive understanding of the disease mechanism.

Hepatic pathogenesis of NAFLD
The initiating events in NAFLD arise from the development of obesity and IR at the level of the adipose tissue and liver. The dysregulation of peripheral lipolysis, DNL, and dietary fat cause the increased FFA flux within the liver and then place hepatocytes under the lipotoxic condition. These lipotoxic FFAs are partitioned into inert intracellular triacylglycerol (TAG) for storage via acyl-CoA synthetic activity and mitochondrial β-oxidation, and the accumulation of TAG in hepatocyte cytoplasm, which is also called steatosis, is the generally accepted histological hallmark of NAFLD [14]. Many patients could stay in the NAFL stage for years, while the chronic insults would ultimately exceed the hepatic capacity to deal with the overload of fatty acids (FA) [5]. Significant hepatocyte injury leads to cell injury and inflammation, subsequently bringing Kupffer cells and other immune cells to the battlefield. Uncontrollable lipotoxicity facilitates ROS formation, ER stress, and hepatocellular dysfunction. Immune and apoptotic pathway activation

results in cell death, which further drives fibrosis development over time [15].

Two important enzyme systems, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), play vital roles in Hepatic FA synthesis. Insulin and glucose could regulate both enzymes by activating sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate-responsive element-binding protein (ChREBP), two important transcriptional factors in DNL, respectively. Liver X receptor (LXR), a nuclear receptor, also directly controls the activity of both SREBP-1c and ChREBP by binding to response elements in the promoters of genes [16]. Deletion of LXR leads to decreased SREBP-1c expression and less lipogenesis in mice. The induction effects of LXR on ACC and FAS could also be indirectly influenced by insulin and glucose [17]. As hyperinsulinemia and hyperglycemia is commonly seen in NAFLD population, the link between glucose and lipid metabolism is conspicuous. Excess glucose is usually stored as glycogen, mediated by insulin, while it could also be esterified into TAGs via DNL. Hepatic IR in NAFLD is found mediated by proinflammatory cytokines, ER stress, apoptosis pathways, and even lipid metabolites. Accumulation of diacylglycerol (DAG), a lipotoxic intermediate of FA synthesis, within cytosolic lipid droplets induces translocation of protein kinase C (PKC)-ε to the plasma membrane and inhibits the intracellular kinase domain of the insulin receptor. In a NAFLD cohort, DAG content and PKCε activation are the most significant predictors for hepatic IR and associate with 60% of the variability in hepatic insulin sensitivity [18]. Glucocorticoid receptor (GR) of hepatocyte is found critical for both direct and indirect transcriptional regulations of IR, hyperglycemia, gluconeogenesis and fatty liver [19]. A recent study targeting 17-hydroxyprogesterone (17-OHP), an intermediate product of steroid synthesis, pointed out the involvement of hepatic Cyp17A1/17-OHP/GR pathway in the development of IR [20].

The FA β oxidation process includes three major steps, activation, transportation and oxidation. Fatty acids are activated by cytosolic and ER acyl-CoA-synthetase to acyl-CoA for β oxidation or TAG synthesis. In charge of transporting acyl-CoA to mitochondria, carnitine palmitoyl transferase 1 (CPT1) serves as the rate-limiting enzyme for FA β oxidation and it could be inhibited by an important DNL intermediate, malonyl-CoA. This process could be inhibited by insulin and activated by peroxisome proliferator-activated receptor (PPAR)-α. In mitochondria, fatty acids are oxidized to produce energy for vital activities, and incidentally, to provide fatty acids with shorter chains and acetyl-CoA for other metabolic processes. Long-chain fatty acids are re-esterified in hepatocytes to produce TAG and stored in lipid droplets

(LDs) or coupled to apolipoproteins and further secreted as very low-density lipoprotein (VLDL) [21]. When the TAG incorporation into VLDL is blocked by microsomal TAG transport protein (*MTTP*) and apolipoprotein B (*APOB*) mutations in patients, TAG accumulates in the liver and consequently causes hepatic steatosis and NASH development [22]. Another mechanism to remove heatic fatty acids is TAG synthesis. In this process, stearoyl-CoA desaturase 1 (SCD1), diglyceride acyltransferase (DGAT), ACC, and FAS are major enzymes whose expressions and activities account for disturbed TAG, fatty acids accumulation and NAFLD development. In the methionine-choline deficient (MCD) diet NASH model, the deletion of *Scd1* gene impairs TAG synthesis and induces hepatocyte apoptosis [23]. It's also reported that inhibition of DGAT also leads to lipotoxicity [24] and liver specific DGAT overexpression increases VLDL secretion [25].

ROS production in hepatocyte is an incidental consequence of FAs oxidation. FAs overloading leads to upregulation of minor pathways (such as peroxisomal oxidation, microsomal oxidation and ER ω-oxidation) and mitochondrial respiratory chain. Consequent increase of ROS production surpasses normal antioxidative mechanisms like superoxide dismutase or glutathione, leading to oxidative stress and the initiation of NASH. NAFLD patients have lower hepatic expressions of genes related to mitochondrial biogenesis, peroxisomal proliferator-activated receptor gamma coactivator 1α (PGC1α), nuclear respiratory factor 1 (NRF1), and mitochondrial transcription factor A (mtTFA), which paves the way for disease progression [26]. ROS induces lipid peroxidation, damages plasma/intracellular membranes, and causes cell necrosis. 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) produced by lipid peroxidation promote inflammation and influence the posttranslational modifications (PTMs) of multiple hepatic proteins [27]. Many studies have discussed the ameliorating effects of radical-scavenging antioxidants in hospitalized NASH patients [28], backward suggesting the critical role of oxidative stress in NAFLD.

Evidence suggests that ER stress is among the most important factors for NAFLD pathogenesis [11]. The ER, an intracellular organelle, is sensitive to lipotoxicity. Dysregulation of ER function is represented by disturbed unfolded protein response (UPR), an adaptively orchestrated arrest of protein synthesis, which can further perpetuate ER stress. Subsequent oxidative and inflammatory signaling pathways trigger apoptosis and autophagy via PERK-mediated C/EBP homologous protein (CHOP), inositol-requiring enzyme 1α (IRE1α)-mediated recruitment of tumor necrosis factor receptor-associated factor 2 (TRAF2) and

signal-regulated kinase 1/c-Jun N-terminal kinase (JNK). IRE1 also splices the transcription factor X-box binding protein 1 (XBP1), which activates JNK and inhibitor of κB kinase (IKK)-NFκB signaling, to modulate inflammatory cascades and ROS production [29]. Liver biopsy samples from NAFLD patients show a specific association between disease severity, spliced XBP1 mRNA and JNK phosphorylation. Compared with healthy individuals, NAFLD patients have a variable degree of UPR activation [30].

Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3Ks) family and could activate downstream JNK and p38 mitogen-activated protein kinase (MAPK) signaling cascades to regulate autophagy, apoptosis, and inflammation. ASK1 itself could be activated by oxidative stress, ER stress and inflammatory cytokines [31]. Hepatic ASK1 activation is a key process in the progression of NASH and a promising target for treatment of the condition [32]. By modulating downstream p38-JNK1 and JNK2 signaling, ASK1 aggravates metabolic dysregulation of lipid and glucose, and precipitates hepatic inflammation [33, 34]. In the murine NASH model, one selective ASK1 inhibitor improves not only metabolic parameters but also hepatic steatosis, inflammation, and fibrosis [35]. A recent publication reported that ablation of *p38* gene in the liver could increase simple steatosis but attenuate oxidative stress-induced injury and fibrosis during NAFLD [36]. The detrimental and protective roles of p38 in different disease stages remind us that NAFLD therapies targeting ASK1-p38 pathway have to proceed with caution.

Hepatokines have also received considerable attention from researchers, considering their role in NAFLD pathogenesis [37]. Fetuin-A has long been considered as a liver-derived regulator for metabolic balance and is reported correlated with NAFLD in humans [38]. Fetuin-A contributes to the activation of Toll-like receptor 4 by fatty acids, which induces inflammation and IR. Hepatic fetuin-A binds to peripheral insulin receptors to inhibit insulin signaling [39] and correlates with key enzymes for lipid and glucose metabolism, such as SREBP-1c, CPT1, and phosphoenolpyruvate carboxy kinase 1 (PEPCK1) [40]. Many publications show significantly higher serum fibroblast growth factor 21 (FGF21) in NAFLD patients compared to healthy controls [41, 42]. FGF21 directly regulates lipid metabolism and reduces hepatic lipid accumulation in an insulin-independent manner. Toxic lipid accumulation in the MCD diet NASH model induces early FGF21 expression [43]. Adenovirus-mediated knockdown or genetic deletion of FGF21 cause lipotoxic damage, hepatic steatosis and dyslipidemia [44, 45]. FGF21 is also reported to mediate energy homeostasis via

regulation of sweet taste [46]. Another major hepatokine, angiopoietin-like 8 (ANGPTL8), correlates with hepatic lipid content independent of IR in NAFLD patients. ANGPTL8 is activated by ER stress or hyperlipidemia, and hence leads to the inhibition of lipoprotein lipase activity, and the activation of autophagic process [47].

Finally, autophagy has also been suggested participating in NAFLD. In murine models and patients of NAFLD, autophagy activity decreases [48–51]. Loss of autophagic modulation against cell death leads to hepatic steatosis and the shift from NAFLD to NASH. Overexpression of *autophagy-related 7* (*Atg7*) or *Atg14*, key autophagy mediators, eliminates steatosis in high fat diet (HFD)-fed mice, while specific deletion of *Atg7* gene in the liver alters TAG secretion and increases hepatic lipid contents. Hyperinsulinemia and IR suppress hepatic autophagy and key autophagy genes such as *Atg12*, and *Gamma-aminobutyric acid type A receptor associated protein like 1* (*Gabarapl1*) [52]. Conversely, autophagy induction also ameliorates ER stress and IR in NAFLD mice [53, 54]. Here exists a vicious circle, in which hepatic steatosis and lipotoxicity-induced IR synergistically impair autophagy which further exacerbates steatosis and insulin sensitivity. Through autophagy, damaged mitochondria containing excessive abnormal intracellular components could be removed to prevent ROS production and apoptosis [55]. Once this process gets dampened, e.g., by *Atg5* knockdown, ROS and energy imbalance activates upstream JNK/c-Jun signaling, which sensitizes hepatocytes to cell death [54].

Adipose tissue and NAFLD

Adipose tissue was considered originally as an inert organ only for fat storage, while recent studies have revealed its crucial roles participating in both energy homeostasis and immune regulation [56]. Altered adipose tissue biology has been recognized as a key early event in the initiation of NAFLD. During obesity progression, adipocytes gradually become hypertrophied in association with macrophages infiltration and subsequent adipokines, cytokines and chemokines alteration [57]. The expansion of adipose tissue also causes hypoxia and subsequent adipocyte death, triggering low-grade chronic inflammation, accumulation of ECM, and eventually IR. Local IR leads to more lipolysis in the adipose tissue and excess release of FFAs into the circulation [58]. The origin of hepatic TAG in NAFLD have been analyzed and indicate that adipose tissue lipolysis, DNL and dietary intake contribute to 60%, 25% and 15% of total hepatic TAGs, respectively [59]. Enhancement of ECM component level may incur progressive fibrosis in the adipose tissue, thereby limiting the fat storage capacity of adipocytes and promoting ectopic fat uptake [60].

Proinflammatory cytokines and chemokines produced by infiltrated macrophages could exacerbate IR and recruit more immune cells to the inflamed adipocytes [61]. It was reported in NAFLD patients that liver necroinflammation and fibrosis increased significantly with visceral fat in a dose-dependent manner [62]. Meanwhile, the adipose tissue from NAFLD patients were found with an increased expression of genes that regulate inflammation, and the adipose tissue macrophages produced increased levels of inflammatory cytokines, compared with control [63]. Inflamed adipose tissue macrophages were showed to signal to bone marrow and to stimulate production of myeloid cells, resulting in an exacerbation of inflammation and associated obesity processes [64, 65]. In a recent study, transplantation of adipose tissue from obese mice rapidly induced hepatic neutrophil recruitment by upregulating CXCL chemokine family genes and secondary macrophage accumulation [66]. Several studies have reported associations between adipocyte death and the pathogenesis of NFALD [67, 68]. After globally deleting the *Bid* gene or specifically deleting the *Fas* gene of adipocytes in HFD-fed mice, adipocyte death was mitigated, and as a result, IR and fatty liver were ameliorated. Correspondingly, adipocyte apoptosis caused by adipocyte-specific deletion of the synaptosomal-associated protein 23 (*Snap23*) gene led to IR and hepatic steatosis [69, 70]. The induction of adipocyte death in HFD-fed mice caused marked upregulation of *Mcp1*, which is important for macrophage recruitment in both adipose tissue and liver, at a very early time point. This rapid elevation of *Mcp1* was found to be mainly contributed by adipocytes, other than macrophages. After adipocyte death and macrophage activation, the elevation of epinephrine (EPI) and norepinephrine (NE) and the subsequent activation of lipolysis were observed [61].

The adipose tissue also serves as an important endocrine organ participating in energy balance by sensing metabolic signals and secreting a number of adipokines such as leptin, adiponectin, and resistin [71, 72]. The 'energy expenditure hormone' leptin is mainly secreted by visceral adipocytes and it's involved in a range of energy modulating activities including hunger, food energy utilization, physical exercise, thermogenesis, and fat mass regulation [73, 74]. Leptin downregulates the transcription of the preproinsulin gene and insulin excretion, and high levels of leptin were documented in obese individuals or during IR [75, 76]. Proinflammatory cytokines such as IL-1 and TNF-α could increase leptin levels and subsequently perpetuate the loop of chronic inflammation in obesity [77, 78]. Especially in the liver, leptin suppresses the lipogenic process by lowering the expression of SREBP-1 [79, 80]. It exerts a permissive role in promoting liver inflammation and fibrosis by

enhancing the production of type 1 collagen, upregulating tissue inhibitor of metalloproteinase (TIMP)-1 expression and downregulating matrix metalloproteinase 1 (MMP1) expression [81]. Leptin could also upregulate the expression of transforming growth factor (TGF)-β in Kupffer cells and sinusoidal endothelial cells, which contributes to liver fibrogenesis [82]. Although leptin has been reported contributing to IR, steatosis development and fibrogenesis, there are still disputes on its association with NASH severity in different cohorts [83, 84]. Hence, more studies with careful designs and strict criteria are needed to elucidate the role of leptin in NAFLD.

Another major adipokine is adiponectin, which is an adipocyte-derived anti-inflammatory mediator eliciting AMP-activated protein kinase (AMPK) signaling. Adiponectin suppresses adipose TNF-α expression and induces anti-inflammatory gene expression in leukocytes [85]. Suppression of adiponectin secretion by selectively deleting conventional kinesin heavy chain in adipose tissue exacerbates HFD-induced obesity and its associated metabolic disorders [86]. In NASH patients, circulating adiponectin levels were remarked diminished, and the downregulation of hepatic adiponectin could be reserved by weight loss [87]. A meta-analysis showed that hypoadiponectinemia serves as a critical feature of NASH patients and the reduction of adiponectin correlates closed with disease progression [88]. In an animal model of deleting C-terminus Hsc70-Interacting protein (CHIP), oxidative stress, IR, and hepatic inflammation had been achieved only except for steatosis because of a compensatory upregulation of adiponectin. By activating the AMPK-forkhead box O (FOXO)-signaling axis, adiponectin could override oxidative stress and JNK signaling, resulting in the counteracting progression of hepatic steatosis [89].

Resistin is mainly produced by adipocytes in mice and ATMs in humans [90]. Treatment of resistin in mice induced IR by impairing glucose tolerance and insulin action, and also upregulated suppressor of cytokine signaling 3 (SOCS3) expression, which further inhibited insulin signaling [91]. Knockout of resistin decreased hepatic steatosis via downregulating genes related to in hepatic lipogenesis and VLDL export [49]. Resistin has a proinflammatory role by not only stimulating multiple inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-12) [92], but also activating the MAPK pathway and the coagulation cascade. [93] Moreover, resistin could induce the production of TGF-β and type I collagen [94]. It was also reported that resistin could upregulate expressions of several chemokines [95]. Data about other adipokines (visfatin, retinol-binding protein 4, chemerin, acylation-stimulating protein, adipsin, apelin, obestatin, omentin, vaspin nesfatin-1, neopterin, neuregulin-4, etc.)

are inconclusive or limited. Further studies may elucidate their roles in NAFLD.

In mammals, the adipose-tissue pool consists of white adipose tissue (WAT) and brown adipose tissue (BAT). Compared to WAT, BAT plays a distinct role in maintaining energy homeostasis because of its abundance in mitochondria and capillaries. BAT generates heat by catabolizing glucose and fatty acid, hence it acts as a protector against obesity and diabetes because thermogenesis dissipates excess energy from high-calorie intake [96]. Decrease of BAT activities could cause metabolic disorders including IR and type 2 diabetes mellitus (T2DM) [97]. In the subcutaneous inguinal WAT, there is a group of brown fat-like adipocytes called browning of WAT, which also contribute to thermogenesis. Transgenic expression or deletion of PR domain containing 16 (PRDM16) in WAT, a transcription coregulator for brown adipocyte development, could bi-directionally attenuate diet-induced obesity (DIO) or lead to obesity [98]. Reported in a recent study, deletion of AMPK in adipocytes led to BAT mitochondrial dysfunction, and secondarily exacerbated hepatic steatosis, IR, and glucose intolerance [99]. Reduction of adipose AMPK commonly observed in NAFLD patients, hence this study offered mechanism for NAFLD pathogenesis and a potential therapeutic target [100].

Gut and NAFLD

Gut, or the gastrointestinal tract, plays a critical role in human physiology in terms of digestion, absorption of nutrients and the excretion of waste. Its mucosal barrier protects the body from pathogens and extrinsic antigens, and gut also secrets hormones to communicate with other organs. Another important profile is that trillions of microorganisms inhabit in the gut and they are closely involved in human metabolism, immune regulation, and behavior modulation. Recently, gut and gut microbiome are linked with the pathogenesis of NAFLD by more and more studies, and 'gut-liver axis' or 'gut-brain-liver' axis have also become hot topics among scholars [13, 101, 102].

Gut could initiate complex hormonal responses to changes in the nutritional status by secreting peptides like ghrelin, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), fibroblast growth factor 19 (FGF19), etc., and some of these gut signals have also been considered as important mediators in NAFLD development [103].

Ghrelin is a small peptide mainly synthesized by the stomach and released into the circulation in two isoforms, the acylated ghrelin (AG) and the des-acyl ghrelin [104]. Ghrelin could stimulate appetite and act with the growth hormone secretagogue receptor (GHS-R). Apart from its unique role in the orexigenic central circuit,

ghrelin has recently received considerable attention in studies of metabolic diseases and liver diseases due to the regulatory functions on immunity and disease pathogenesis independent of its effects on food intake [105]. The association between elevated AG levels and hepatic steatosis was documented in NAFLD patients [106]. Both animal and human studies showed that ghrelin could directly promote lipogenesis and inhibit lipolysis in adipocytes. Administration of AG inactivated AMPK signaling, stimulated TAG storage in the liver and caused dysregulation of lipid oxidation and mitochondrial function [107]. By deleting ghrelin or ghrelin receptor genes in mice, the facilitation on de novo lipogenesis was eliminated, and the resistance towards obesity and hepatic steatosis was observed. Involving the interaction between PPARγ and mammalian target of rapamycin (mTOR), ghrelin could activate its receptor on hepatocytes to promote lipogenesis as well [108]. Ghrelin also regulates insulin secretion and sensitivity in pancreatic β-cells and stimulates glucose output from primary hepatocytes [109]. Reported in a recent study [110], ghrelin reduced apoptosis induced by TNF-α and autophagy in human hepatocytes via AMPK/mTOR, consistent with another research where ghrelin showed anti-fibrosis effects by downregulating the TGF-β1/Smad3 signaling pathways and inhabiting autophagy [111].

Glucagon-like peptide-1 (GLP-1) is a hormone secreted by the enteroendocrine L-cells of the intestine, which causes potentiation of glucose-stimulated insulin secretion after nutrient intake. GLP-1 could downregulate the expressions of SREBP-1c, ACC, SCD-1, and FAS, which are all important players for de novo lipogenesis [112]. Hence, low level of GLP-1 in NAFLD populations possibly derived from producing dysfunction or cleavage enhancement is considered as one predisposing factor for NAFLD development [113]. Treatment of GLP-1 receptor agonists (GLP-1RA) significantly reduced blood glucose level, IR and hepatic lipid concentration in mouse steatohepatitis model [114]. GLP-1RA downregulated expression of SREBP-1c and SCD-1 and upregulated the expression of PPARα in hepatocytes, which could further suppress de novo lipogenesis and induce β-oxidation of free fatty acids [115]. The majority of clinical trials investigating the effects of GLP-1RAs on NAFLD patients has shown promising results [116, 117]. GLP-1RAs were able to significantly reduce hepatic steatosis as well as control blood glucose in NAFLD patients [118]. In a Chinese cohort, Liraglutide, a recombinant polypeptide analogue of GLP-1, was found superior to traditional therapies including metformin and gliclazide, in ameliorating hepatic steatosis, improving liver function, and controlling body weight [119]. Bile acids are released into the duodenum for lipid digestion and 95% of them

are absorbed back into the portal circulation within the ileum for recycle. Increased bile acids in the intestine could activate the farnesoid X receptor (FXR) signaling in enterocytes and thereby induce FGF19 production and secretion. Besides their roles in regulating hepatic bile acid synthesis, FXR and FGF19 are also involved in metabolic homeostasis. Deletion of FXR led to significant increases in hepatic cholesterol and triglycerides [120, 121]. On contrary, the activation of FXR could prevent lipid accumulation in the liver by regulating hepatic de novo lipogenesis and fatty acid β-oxidation [122]. FGF19 inhibits the expression of lipogenic enzymes by increasing phosphorylation of signal transducer and activator of transcription 3 (STAT3) and decreasing the expression of PGC1β [123]. Peripheral administration of FGF19 could improve glucose tolerance in both HFD-feeding mice and ob/ob mice [124], possibly because of its motivating effects on hepatic protein and glycogen synthesis [125]. Although hepatic FXR protein contents and circulating FGF19 levels were found inversely associated with NASH severity in patients [126], more mechanistic studies are needed to clarify their roles in NAFLD development. As the agonist of FXR, obeticholic acid, has recently been studied in a multicenter, randomized, double-blind phase III study, and showed benefits in fibrosis improvement [127].

Gut harbors the most abundant bacterial populations in the body, while the importance of these 'guests' in human physiology has long been ignored. Recent studies have discovered more and more functional roles of gut microbiota, such as nutrient metabolism [129–131], xenobiotic metabolism [131, 132], antimicrobial protection [133], immunomodulation [134, 135] and gut integrity maintenance [136]. On this basis, the change in gut microbiota, or dysbiosis, is presumed associated with etiologies of many metabolic diseases, including NAFLD. Comparing individuals with NAFLD and non-NAFLD controls, both microbiome and bacterial density are different [137]. In NAFLD patients, increased intestinal permeability and bacterial overgrowth were reported positively correlated with the severity of steatosis [138, 139]. Germ-free mice were found resistant to DIO, in contrast to mice with the normal gut microbiota. Transplantation of stool from individuals with IR to healthy controls transfer the insulin resistant phenotype as well, which further highlights the contributions gut microbiota could make to host metabolic disorders [140].

Gut microbiota could ferment excess food that the host is not able to digest, and further produce short-chain fatty acids (SCFAs), including acetate, propionate, butyrate, etc., mostly in colon. Increased total SCFAs level is one common metabolic profile of both animal model and NAFLD patients, but precise production of SCFA is hard

to measure so far in patients due to technical difficulties. One human study showed a significant association between the presence of steatohepatitis and an increased percentage Firmicutes and a reduced percentage of Bacteroidetes (two predominant bacterial phyla colonizing the healthy human large intestine) [141], and the increase of the Firmicutes/Bacteroidetes ratio was reported associated with increased energy harvest from the diet [142]. SCFAs not only provide extra energy to the host (about 30% of hepatic energy supply) [143] but also impact satiety and insulin signaling by stimulating the production of peptide YY (PYY) and GLP-1 in the intestine [144]. The insulin-mediated fat accumulation could be suppressed by the interaction of SCFAs and their receptors, G-protein-coupled receptors (GPCRs), in the gut, skeletal muscle, adipose tissue and the liver [145]. SCFAs stimulate the leptin production [146], increase adipogenesis and suppress lipolysis in adipose tissue [147]. Administration of SCFAs to mice and patients leads to increased energy expenditure [148, 149], thermogenesis [150] and fatty acids oxidation secondary to AMPK activation [151], but SCFAs also serve as substrates for lipogenesis and gluconeogenesis. There have been mixed reports regarding the roles of SCFAs in regulating inflammation. GPCRs could activate signaling pathways such as RAS, protein kinase A, phosphoinositide 3-kinases (PI3K), and extracellular regulated protein kinases (ERK1/2) and further upregulate the expressions of IL-1, IL-6, TNF-α, CXCL1, and CXCL2, in favor of the contribution of SCFAs in NASH pathogenesis [153–156]. Increased acetate level was reported responsible for production of inflammatory cytokines in macrophages and even inflammatory responses in the liver [156]. While under inflammatory conditions, administration of SCFAs reduces NF-κB activity via the inhibition of histone deacetylase (HDAC), which suppresses the production of inflammatory cytokines such as IL-6, IL-8, and TNF-α [155, 157, 158]. Furthermore, SCFAs could enhance differentiation of anti-inflammatory regulatory T cells (Tregs) in the colon, and also stimulate the NLRP3 inflammasome. In vitro, SCFAs also attenuate PPARγ activity and reduce the expression of lymphocyte function-associated antigen 3 (LFA3) and intercellular adhesion molecule 1 (ICAM1). The beneficial functions of SCFAs to prevent NAFLD was discussed by a variety of animal studies [160–163], but there still need to be direct evidences from clinical trials.

From saccharolytic fermentation, Proteobacteria (e.g., *Escherichia coli*) also produce a decent amount of ethanol and this ability could be increased by dysbiosis. In both obese mice and human subjects, higher breath ethanol contents were detected compared to controls, which could be abrogated by antibiotic treatment [163, 164]. In addition, elevated circulating ethanol levels and increased hepatic alcohol metabolism (in terms of alcohol dehydrogenase 1, aldehyde dehydrogenase 2 and Cytochrome P450 2E1) were also reported in NALFD patients [165]. Such increases of the endogenous ethanol not only negatively regulate gut environment, but also supply a constant source of ROS to the liver [166]. In HFD-fed mice, ethanol and free fat acids were showed to synergistically promote liver injury through the elevation of hepatic/serum free fatty acids and upregulation of the hepatic expression of several chemokines [167]. A recent work published in *Cell Metabolism* introduced a high-alcohol-producing *Klebsiella pneumoniae* (HiAlc *Kpn*) strain in a rare NASH case with bacterial auto-brewery syndrome and reported a strong correlation between HiAlc *Kpn* between NAFLD in a Chinese cohort. When a HiAlc-*Kpn*-strain-containing fecal microbiota was transplanted into normal mice, NAFLD also developed. These novel findings all together suggested the alteration in the gut microbiome could to some extent facilitate NAFLD development via excess production of endogenous ethanol [168].

Increased intestine permeability is another important alteration caused by gut dysbiosis. The relationship between gut permeability and NAFLD is highlighted by the finding in a high-fat dietary model of NAFLD that increased circulating Lipopolysaccharide (LPS) levels correlated with worsened steatohepatitis, as measured by the NAFLD Activity Score and liver enzyme levels [111]. Impaired gut barrier (unsealed junctions between intestinal endothelial cells) allows gut mucosal cells and the liver exposed to harmful substances derived from the gut, including translocated bacteria, LPS and endotoxins as well as secreted cytokines. Gut-derived bacterial products, especially LPS, could be recognized by Toll-like receptors (TLRs), which are important players in innate and adaptive immune responses [101]. Increased TLR ligands could be detected in the portal system in the presence of gut dysbiosis, which contributes to the activation of TLR4 on Kupfer cells and stellate cells and subsequent stimulation of pro-inflammatory and profibrotic pathways [13, 102, 169]. A range of signaling cascades, including MAPK, c-Jun N-terminal kinase (JNK), p38 mitogen-activated kinases and NF-κB, are involved in this process and lead to activation of proinflammatory genes, production of inflammasomes and release of ROS [13]. Activation of the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome by LPS from gut microbiota via TLR4 and TLR9 was reported necessary for NASH development since it led to the early onset of steatohepatitis. In liver samples from NASH patients, NLRP3 inflammasome was also found positively correlated with hepatic collagen type 1α expression

[170]. Activation of TLRs could also contribute to hepatic steatosis via intestinal epithelial myeloid differentiation primary response gene 88 (MyD88) which is a central adaptor molecule for TLRS and is responsible for switching metabolism towards obesity [171].

Brain and NAFLD

The central nerve system plays a predominating role in energy regulation as neuronal networks and nuclei in certain brain regions crosstalk and integrate peripheral signals like plasma nutrients and key metabolic hormones to coordinate adaptive changes in food intake and energy expenditure [172]. In particular, the hypothalamic arcuate nucleus (ARC) is considered as the most important central sensor for signals in circulation and cerebrospinal fluid because it is anatomically adjacent to the median eminence and the third ventricle [173]. Neurons in ARC are the first-order neurons on which peripheral metabolic hormones, including leptin, insulin, ghrelin and nutrients primarily act, and, they are each responsible for expressing orexigenic neuropeptides like neuropeptide Y (NPY) and agouti-related peptide (AgRP), or anorexigenic neuropeptides like proopiomelanocortin (POMC). Then, second-order neurons in the paraventricular nucleus (PVN), ventromedial hypothalamus (VMH) and lateral hypothalamus (LH) could receive signals from first-order neurons via axonal transport. AgPR released from NPY/AgRP neurons or α-melanocyte-stimulating hormone (α-MSH) released from POMC neurons could bind to the melanocortin-3 and -4 receptors (MC3R and MC4R) on second-order neurons. α-MSH activates catabolic pathways to modulate food intake and energy expenditure while AgPR competes with α-MSH for MC3Rs and MC4Rs and antagonizes its effects [174]. Deletion of MC4R in mice causes hyperphagia and obesity [175], and MC4R gene mutation is also associated with severe early-onset obesity in human study [176]. A novel mouse model composing both MC4R knockout and HFD feeding successfully simulates the clinical and pathologic features of human NASH. The loss of hypothalamic MC4R function accounts for the development of IR, dyslipidemia, liver fibrosis and HCC [177]. When ablating NPY/AgRP neurons in young mice, researchers find significant food intake inhibition and weight loss [178], and direct administration of NPY and AgRP also stimulate feeding in animal models [179]. PVN neurons synthesize catabolic neuropeptides and control autonomic outflow to peripheral metabolic organs, which increases fatty acid oxidation and lipolysis. Consistently, lesion study on PVN shows overeating and obesity in rats [180]. Neurons in the VMH could sense glucose and leptin and produce anorexigenic neuropeptides like brain-derived neurotrophic factor (BDNF). Destruction of VMH leads

to hyperphagia, obesity and hyperglycemia [181]. In contrast, electronic or chemical ablation of the LH lead to anorexia and weight loss because LH neurons produce orexigenic neuropeptides melanin-concentrating hormone (MCH) and orexin which are responsible for interactions between ARC and LH. Chronic infusion of MCH causes obesity [182], and MCH deletion causes resistance to diet-induced obesity in mice [183, 184]. Recently, a selective MCH receptor 1 antagonist is reported successfully ameliorating obesity and hepatic steatosis in mouse NAFLD models [185].

By modulating energy consumption pathways (e.g., locomotor activity, fatty acid oxidation or thermogenesis), the brain also regulates energy expenditure of the body. The suprachiasmatic nucleus (SCN) produces tumor growth factor-α in a circadian manner to mobilize growth factor receptors in the hypothalamus paraventricular nucleus and subsequently inhibit [186]. LH neurons and POMC neurons also control locomotor activity by producing orexin and recognizing circulating signals like leptin level [173]. As mentioned above, thermogenesis is mainly carried out by BAT. The brain regulates BAT activity via the interaction of norepinephrine and β-adrenergic receptors. Downstream activation of cyclic-adenosine monophosphate (cAMP) signaling then upregulates mitochondrial uncoupling protein-1 (Ucp1), a key molecule for metabolic thermogenesis to avoid an excess of fat accumulation [187]. Hypothalamus integrates body temperature sensation and modulates thermogenesis with excitatory neurons in VMH and efferent sympathetic outflow. Abnormal hormonal signals could trick hypothalamus and influence sympathetic outflow to BAT [188]. Central administration of leptin, MC3/4R agonists, glucagon or GLP-1 could stimulate BAT activity. Intracerebroventricular co-injection of leptin and insulin induces WAT browning and then increases energy expenditure [189].

Particular of note, SCN is the master cerebral clock coordinating all biological clocks in the body and controlling [190]. The SCN pace the self-sustained and cell-autonomous molecular oscillators in the peripheral tissues through autonomic neural outputs and humoral signals such as melatonin and glucocorticoids [191]. A loss-of-function mutation in the *Clock* gene, a critical transcriptional factor affecting both the persistence and period of circadian rhythms, leads to hyperglycemia and dyslipidemia, and mice develop adipocyte hypertrophy and hepatic steatosis [192]. Under the circumstance of hepatic steatosis, clock-related gene alterations are found associated with pathways regarding fatty acid oxidation, lipoprotein, fatty acid synthesis and cholesterol metabolism. In the liver, immediate early genes are regulated by systematic signals and then

communicate rhythmic signals to the hepatic molecular clockworks. The molecular clockwork provokes the expression of genes encoding the enzymatic and transport proteins managing lipogenesis and lipolysis, such as hepatic cytochrome P450 cholesterol 7α-hydroxylase, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), FAS, lipolytic enzymes, apoA-IV and C-III, low-density lipoprotein receptor, FA transport protein 1 (FATP1), fatty acyl-CoA synthetase 1 and adipocyte differentiation-related protein [193].

Both the liver and the gut are rich in vagal afferent fibers which transmit local information to the brain stem, another key area involved in the central regulation of energy balance. In the brain stem, the nucleus tractus solitaries (NTS) is predominantly in charge of receiving afferent inputs indicating parameters of splanchnic organs and then processing extensive signals before delivering them to higher brain regions [194]. The NTS receives neuronal projections from the hypothalamus, the amygdala and the nucleus accumbens, and vice versa, hence, it serves as a relay station and presides over orchestrating a coherent output reflex to the periphery [195]. During HFD-induced obesity, the vagal afferent pathway becomes dysfunctional as the neuronal excitability is reduced. As a result, higher levels of stimulations (such as stomach distension or hormones) are needed to activate vagal afferents with reduced responding abilities [196]. Nevertheless, neurons of obese animal or human remain the normal phenotype as lean subjects irrespective of abundant energy stores, hence improper afferent signals could not be appropriately interpreted in the brain and will inevitably exacerbate hyperphagia and obesity by increasing meal consumptions and blunting satiety signals [197]. After phenol-induced hepatic denervation in rats, CPT1 is suppressed. As a mitochondrial transport protein that traverses the outer mitochondrial membrane, CPT1 motivates fatty acids to enter the mitochondria, where the β-oxidation takes place [198]. In the hypothalamus, AMPK phosphorylates ACC, lowers malonyl-CoA production, modulate CPT1 activity and thereby regulate energy balance. The AMPK-malonyl-CoA-CPT1 axis has already been targeted for the therapy of NASH [199]. Interestingly, gut dysbiosis and relevant endotoxemia are considered partially contributing to the dysregulation of gut-brain vagal communication and subsequent outcomes such as obesity and NAFLD [200, 201]. In HFD-fed mice and obese patients, multiple hormones (CCK, PYY, GLP-1, etc.) become scarce capable to activate vagal afferents and their receptors in vagal neurons also reduce [196, 202, 203]. A posteriori, the benefits of bariatric procedures on NAFLD patients are also ascribed to the remediation of vagal nerve circuits [204].

Leptin activates receptors in many brain regions, among which ARC is the most important area to regulate appetite, thermogenesis, and locomotor activity [173]. Leptin drives hypothalamic signaling cascades such as the Janus kinase-STAT pathway, the insulin receptor substrate (IRS)-PI3K signaling, mTOR-S6 kinase signaling, AMPK signaling and ERK signaling [205]. Meanwhile, ghrelin also acts through central mechanisms to increase caloric intake and functions through hypothalamic neuronal circuits to increase lipogenesis and decreasing β-oxidation in WAT. By activating GHS-R1a on hypothalamic NPY/AgRP neurons, ghrelin promotes the blockade of MC3/4R and regulates peripheral lipogenesis through the sympathetic nervous system [206].

Hypothalamic neurons (such as orexin-producing neurons in the LH) could be activated by sweet foods [207], while one unique point to mention here is, besides the caloric and glycemic aspects, sweet taste itself should also be discussed when we talk about the role of brain in NAFLD and other metabolic disorders. When assigned to diet soda with aspartame, a nonnutritive sweetener worldwide consumed, rats develop hyperglycemia and fat accumulation in two months compared to their water drinking counterparts [208]. The aspartame exposure downregulates adiponectin and PPAR and increases leptin production, which may potentially contribute to the pathogenesis of NAFLD. A number of studies reported that consumption of artificial sweeteners brings great risks of obesity, metabolic syndrome and type 2 diabetes [209–212]. Reminded by these studies, the learned behavior of sweet taste is associated with disease pathogenesis. Oral but not gastric administration of glucose or saccharin leads to decreased GLP-1 release [213], which subsequently disrupts the satiety process and increases food intake and weight gain.

Summary

NAFLD is a rising health problem worldwide and related medical burden is also increasing at an alarming rate. Recently, an international expert group has announced the consensus of renaming NAFLD as metabolic (dysfunction) associated fatty liver disease (MAFLD), in two position papers [214, 215]. Although there is still some feeling in academia that the new acronym is premature [216], the effort to bring this disease to a more practical status and closer to metabolic disorders is not doubt destined. The new terminology and corresponding diagnostic criteria explicitly highlight overweight/obesity, type 2 diabetes mellitus, and metabolic dysregulation, which shall bring more targets for mechanism research and intervention. Without
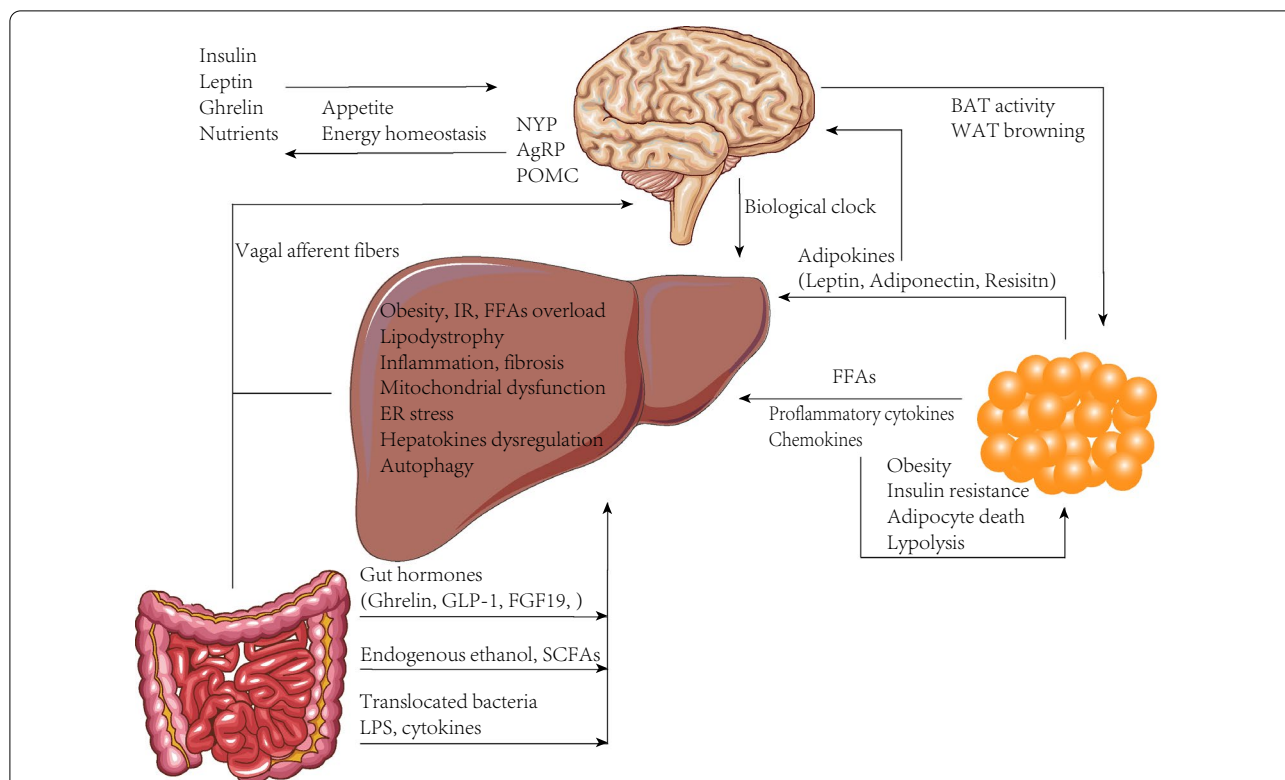
Fig. 1 Inter-organ crosstalk regarding the pathogenesis mechanism of NAFLD. The adipose tissue contributes fatty acids to facilitate hepatic steatosis and also produces cytokines to impact proinflammatory pathways, which in turn exacerbates obesity, insulin resistance, adipocyte death and lipolysis. Adipokines deliver metabolic signals to the brain as well. Gut dysbiosis affects gut hormones, metabolites and bacterial components, which subsequently influence the development and progression of NAFLD. Moreover, gut hormones could also interact with different neurons of the brain, to influence appetite and energy homeostasis. The central nerve system integrates hormonal and neurol signals from peripheral organs to control energy balance, which when impaired leads to obesity and NAFLD. It also remarkably influences lipogenesis and lipolysis by mastering biological clocks and modulating adipose activities. Consequently, in the liver, lipodystrophy may result in ROS production, mitochondrial dysfunction, ER stress, apoptosis, inflammation, hepatokine dysregulation, and autophagy, thus collectively inducing the development of NAFLD

doubt, the pathogenesis of NAFLD (or MAFLD) is complex and evidently involves multiple organs and diverse mechanisms (Fig. 1). The adipose tissue not only contributes fatty acids to facilitate hepatic steatosis but also produces hormones and cytokines to influence proinflammatory pathways. The gut directly takes charge of energy absorption and it communicates with brain to modulate food intake. Gut microbiota has become a hot topic recently as it participates in NAFLD pathogenesis via the gut-liver axis. Altered gut permeability also exposes liver to bacterial components and further triggers immune responses. The central nerve system integrates hormonal and neurol signals to control energy balance, which when impaired leads to obesity and NAFLD. Finally, in the liver, key mechanisms include lipotoxicity, IR, ROS production, ER stress, apoptosis, inflammation, autophagy, etc. Based on researches emphasizing aforementioned mechanisms, quests for novel therapies are underway and many candidates have already entered clinical trials. Further

work is still urgently needed to find detailed pathogenic mechanisms of NAFLD, especially the inter-organ crosstalk aspect.

Histone deacetylase; HiAlc *Kpn*: High-alcohol-producing *Klebsiella pneumoniae*; HMGCR: 3-Hydroxy-3-methylglutaryl coenzyme A reductase; HSCs: Hepatic stellate cells; ICAM1: Intercellular adhesion molecule 1; IKK: Inhibitor of κB kinase; IR: Insulin resistance; IRE1α: Inositol-requiring enzyme 1α; IRS: Insulin receptor substrate; JNK: Jun N-terminal kinase; LDs: Lipid droplets; LFA3: Lymphocyte function-associated antigen 3; LH: Lateral hypothalamus; LPS: Lipopolysaccharide; LXR: Liver X receptor; MAFLD: Metabolic (dysfunction) associated fatty liver disease; MAP3K: Mitogen-activated protein kinase kinase kinase; MAPK: Mitogen-activated protein kinase; MC3R: Melanocortin-3 receptor; MC4R: Melanocortin-4 receptor; MCD: Methionine-choline deficient; MCH: Melanin-concentrating hormone; MDA: Malondialdehyde; MMP1: Matrix metalloproteinase 1; mTOR: Mammalian target of rapamycin; mtTFA: Mitochondrial transcription factor A; MTTP: Microsomal TAG transport protein; MyD88: Myeloid differentiation primary response gene 88; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; NE: Norepinephrine; NLRP3: NACHT, LRR and PYD domains-containing protein 3; NPY: Neuropeptide Y; NRF1: Nuclear respiratory factor 1; NTS: Nucleus tractus solitaries; PEPCK1: Phosphoenolpyruvate carboxy kinase 1; PGC1α: Peroxisomal proliferator-activated receptor gamma coactivator 1α; PI3K: Phosphoinositide 3 kinases; PKC: Protein kinase C; POMC: Proopiomelanocortin; PPAR: Proliferator-activated receptor; PRDM16: PR domain containing 16; PTMs: Post-translational modifications; PVN: Paraventricular nucleus; PYY: Peptide YY; ROS: Reactive oxygen species; SCD1: Stearoyl-CoA desaturase 1; SCFAs: Short-chain fatty acids; SCN: Suprachiasmatic nucleus; Snap23: Synaptosomal associated protein 23; SOCS3: Suppressor of cytokine signaling 3; SREBP-1c: Sterol regulatory element-binding protein 1c; STAT3: Signal transducer and activator of transcription 3; T2DM: Type 2 diabetes mellitus; TAG: Triacylglycerol; TGF: Transforming growth factor; TIMP1: Tissue inhibitor of metalloproteinase 1; TLR: Toll-like receptor; TRAF2: Tumor necrosis factor receptor-associated factor 2; Tregs: Regulatory T cells; Ucp1: Uncoupling protein 1; UPR: Unfolded protein response; VLDL: Very low-density lipoprotein; VMH: Ventromedial hypothalamus; WAT: White adipose tissue; XBP1: X-box binding protein 1.

Author details
[1] Department of Oncology, the First Affiliated Hospital of Anhui Medical University, Hefei 230022, China. [2] Inflammation and Immune Mediated Diseases Laboratory of Anhui Province, Hefei 230032, China.

References
1. Armstrong MJ, Adams LA, Canbay A, et al. Extrahepatic complications of nonalcoholic fatty liver disease. Hepatology. 2014;59:1174–97.
2. Younossi ZM, Blissett D, Blissett R, et al. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. Hepatology. 2016;64:1577–86.
3. Chacko KR, Reinus J. Extrahepatic complications of nonalcoholic fatty liver disease. Clin Liver Dis. 2016;20:387–401.
4. Masuoka HC, Chalasani N. Nonalcoholic fatty liver disease: an emerging threat to obese and diabetic individuals. Ann N Y Acad Sci. 2013;1281:106–22.
5. 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. 2018;67:328–57.
6. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002;346:1221–31.
7. Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. Gastroenterology. 2002;122:1649–57.
8. Oseini AM, Sanyal AJ. Therapies in non-alcoholic steatohepatitis (NASH). Liver Int. 2017;37(Suppl 1):97–103.
9. Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. Gut. 2015;64:830–41.
10. Zhou F, Zhou J, Wang W, et al. Unexpected rapid increase in the burden of NAFLD in China from 2008 to 2018: a systematic review and meta-analysis. Hepatology. 2019;70:1119–33.
11. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65:1038–48.
12. Bril F, Sninsky JJ, Baca AM, et al. Hepatic steatosis and insulin resistance, but not steatohepatitis, promote atherogenic dyslipidemia in NAFLD. J Clin Endocrinol Metab. 2016;101:644–52.
13. Leung C, Rivera L, Furness JB, et al. The role of the gut microbiota in NAFLD. Nat Rev Gastroenterol Hepatol. 2016;13:412–25.
14. Leoni S, Tovoli F, Napoli L, et al. Current guidelines for the management of non-alcoholic fatty liver disease: a systematic review with comparative analysis. World J Gastroenterol. 2018;24:3361–73.
15. Hardy T, Oakley F, Anstee QM, et al. Nonalcoholic fatty liver disease: pathogenesis and disease spectrum. Annu Rev Pathol. 2016;11:451–96.
16. Repa JJ, Liang G, Ou J, et al. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. Genes Dev. 2000;14:2819–30.
17. Vacca M, Degirolamo C, Mariani-Costantini R, et al. Lipid-sensing nuclear receptors in the pathophysiology and treatment of the metabolic syndrome. Wiley Interdiscip Rev Syst Biol Med. 2011;3:562–87.
18. Kumashiro N, Erion DM, Zhang D, et al. Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. Proc Natl Acad Sci USA. 2011;108:16381–5.
19. Bose SK, Hutson I, Harris CA. Hepatic glucocorticoid receptor plays a greater role than adipose GR in metabolic syndrome despite renal compensation. Endocrinology. 2016;157:4943–60.
20. Lu Y, Wang E, Chen Y, et al. Obesity-induced excess of 17-hydroxyprogesterone promotes hyperglycemia through activation of glucocorticoid receptor. J Clin Invest. 2020;130:3791–804.
21. Canbay A, Bechmann L, Gerken G. Lipid metabolism in the liver. Z Gastroenterol. 2007;45:35–41.
22. Di Filippo M, Moulin P, Roy P, et al. Homozygous MTTP and APOB mutations may lead to hepatic steatosis and fibrosis despite metabolic differences in congenital hypocholesterolemia. J Hepatol. 2014;61:891–902.
23. Li ZZ, Berk M, McIntyre TM, et al. Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: role of stearoyl-CoA desaturase. J Biol Chem. 2009;284:5637–44.
24. Yamaguchi K, Yang L, McCall S, et al. Diacylglycerol acyltranferase 1 antisense oligonucleotides reduce hepatic fibrosis in mice with nonalcoholic steatohepatitis. Hepatology. 2008;47:625–35.
25. Yamazaki T, Sasaki E, Kakinuma C, et al. Increased very low density lipoprotein secretion and gonadal fat mass in mice overexpressing liver DGAT1. J Biol Chem. 2005;280:21506–14.
26. Girodet PO, Ozier A, Bara I, et al. Airway remodeling in asthma: new mechanisms and potential for pharmacological intervention. Pharmacol Ther. 2011;130:325–37.
27. Long EK, Olson DM, Bernlohr DA. High-fat diet induces changes in adipose tissue trans-4-oxo-2-nonenal and trans-4-hydroxy-2-nonenal levels in a depot-specific manner. Free Radic Biol Med. 2013;63:390–8.

28. Sumida Y, Niki E, Naito Y, et al. Involvement of free radicals and oxidative stress in NAFLD/NASH. Free Radic Res. 2013;47:869–80.
29. Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell. 2010;140:900–17.
30. Puri P, Mirshahi F, Cheung O, et al. Activation and dysregulation of the unfolded protein response in nonalcoholic fatty liver disease. Gastroenterology. 2008;134:568–76.
31. Challa TD, Wueest S, Lucchini FC, et al. Liver ASK1 protects from non-alcoholic fatty liver disease and fibrosis. EMBO Mol Med. 2019;11:e10124.
32. Zhang P, Wang PX, Zhao LP, et al. The deubiquitinating enzyme TNFAIP3 mediates inactivation of hepatic ASK1 and ameliorates nonalcoholic steatohepatitis. Nat Med. 2018;24:84–94.
33. Lawan A, Zhang L, Gatzke F, et al. Hepatic mitogen-activated protein kinase phosphatase 1 selectively regulates glucose metabolism and energy homeostasis. Mol Cell Biol. 2015;35:26–40.
34. Xiang M, Wang PX, Wang AB, et al. Targeting hepatic TRAF1-ASK1 signaling to improve inflammation, insulin resistance, and hepatic steatosis. J Hepatol. 2016;64:1365–77.
35. Loomba R, Lawitz E, Mantry PS, et al. The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: a randomized, phase 2 trial. Hepatology. 2018;67:549–59.
36. Hwang S, Wang X, Rodrigues RM, et al. Protective and detrimental roles of p38alpha MAPK in different stages of nonalcoholic fatty liver disease. Hepatology 2020.
37. Lebensztejn DM, Flisiak-Jackiewicz M, Bialokoz-Kalinowska I, et al. Hepatokines and non-alcoholic fatty liver disease. Acta Biochim Pol. 2016;63:459–67.
38. Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. Nat Rev Endocrinol. 2017;13:509–20.
39. Pal D, Dasgupta S, Kundu R, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med. 2012;18:1279–85.
40. Ou HY, Yang YC, Wu HT, et al. Increased fetuin-A concentrations in impaired glucose tolerance with or without nonalcoholic fatty liver disease, but not impaired fasting glucose. J Clin Endocrinol Metab. 2012;97:4717–23.
41. Li H, Fang Q, Gao F, et al. Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. J Hepatol. 2010;53:934–40.
42. Reinehr T, Woelfle J, Wunsch R, et al. Fibroblast growth factor 21 (FGF-21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: a longitudinal analysis. J Clin Endocrinol Metab. 2012;97:2143–50.
43. Tanaka N, Takahashi S, Zhang Y, et al. Role of fibroblast growth factor 21 in the early stage of NASH induced by methionine- and choline-deficient diet. Biochim Biophys Acta. 2015;1852:1242–52.
44. Hui E, Xu A, Bo Yang H, et al. Obesity as the common soil of non-alcoholic fatty liver disease and diabetes: role of adipokines. J Diabetes Investig. 2013;4:413–25.
45. Fisher FM, Chui PC, Nasser IA, et al. Fibroblast growth factor 21 limits lipotoxicity by promoting hepatic fatty acid activation in mice on methionine and choline-deficient diets. Gastroenterology. 2014;147(1073–83):e6.
46. von Holstein-Rathlou S, BonDurant LD, Peltekian L, et al. FGF21 Mediates endocrine control of simple sugar intake and sweet taste preference by the liver. Cell Metab. 2016;23:335–43.
47. Zhang R. The ANGPTL3-4-8 model, a molecular mechanism for triglyceride trafficking. Open Biol. 2016;6:150272.
48. Yang L, Li P, Fu S, et al. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell Metab. 2010;11:467–78.
49. Singhal NS, Patel RT, Qi Y, et al. Loss of resistin ameliorates hyperlipidemia and hepatic steatosis in leptin-deficient mice. Am J Physiol Endocrinol Metab. 2008;295:E331–8.
50. Gonzalez-Rodriguez A, Mayoral R, Agra N, et al. Impaired autophagic flux is associated with increased endoplasmic reticulum stress during the development of NAFLD. Cell Death Dis. 2014;5:e1179.
51. Kashima J, Shintani-Ishida K, Nakajima M, et al. Immunohistochemical study of the autophagy marker microtubule-associated protein 1 light chain 3 in normal and steatotic human livers. Hepatol Res. 2014;44:779–87.
52. Liu HY, Han J, Cao SY, et al. Hepatic autophagy is suppressed in the presence of insulin resistance and hyperinsulinemia: inhibition of FoxO1-dependent expression of key autophagy genes by insulin. J Biol Chem. 2009;284:31484–92.
53. Xiong X, Tao R, DePinho RA, et al. The autophagy-related gene 14 (Atg14) is regulated by forkhead box O transcription factors and circadian rhythms and plays a critical role in hepatic autophagy and lipid metabolism. J Biol Chem. 2012;287:39107–14.
54. Singh R, Kaushik S, Wang Y, et al. Autophagy regulates lipid metabolism. Nature. 2009;458:1131–5.
55. Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. Am J Gastroenterol. 2004;99:1497–502.
56. Grant RW, Dixit VD. Adipose tissue as an immunological organ. Obesity (Silver Spring). 2015;23:512–8.
57. Kang YE, Kim JM, Joung KH, et al. The roles of adipokines, proinflammatory cytokines, and adipose tissue macrophages in obesity-associated insulin resistance in modest obesity and early metabolic dysfunction. PLoS ONE. 2016;11:e0154003.
58. Gentile CL, Weir TL, Cox-York KA, et al. The role of visceral and subcutaneous adipose tissue fatty acid composition in liver pathophysiology associated with NAFLD. Adipocyte. 2015;4:101–12.
59. Donnelly KL, Smith CI, Schwarzenberg SJ, et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115:1343–51.
60. Bertolio R, Napoletano F, Mano M, et al. Sterol regulatory element binding protein 1 couples mechanical cues and lipid metabolism. Nat Commun. 2019;10:1326.
61. Kim SJ, Feng D, Guillot A, et al. Adipocyte death preferentially induces liver injury and inflammation through the activation of chemokine (C-C Motif) receptor 2-positive macrophages and lipolysis. Hepatology. 2019;69:1965–82.
62. Xu C, Ma Z, Wang Y, et al. Visceral adiposity index as a predictor of NAFLD: a prospective study with 4-year follow-up. Liver Int. 2018;38:2294–300.
63. du Plessis J, van Pelt J, Korf H, et al. Association of adipose tissue inflammation with histologic severity of nonalcoholic fatty liver disease. Gastroenterology. 2015;149(635–48):e14.
64. Takikawa A, Mahmood A, Nawaz A, et al. HIF-1alpha in myeloid cells promotes adipose tissue remodeling toward insulin resistance. Diabetes. 2016;65:3649–59.
65. Engin AB. Adipocyte-macrophage cross-talk in obesity. Adv Exp Med Biol. 2017;960:327–43.
66. Alicka M, Major P, Wysocki M, et al. Adipose-derived mesenchymal stem cells isolated from patients with type 2 diabetes show reduced "Stemness" through an altered secretome profile, impaired anti-oxidative protection, and mitochondrial dynamics deterioration. J Clin Med. 2019;8:765.
67. Choe SS, Huh JY, Hwang IJ, et al. Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. Front Endocrinol (Lausanne). 2016;7:30.
68. Alisi A, Carpino G, Oliveira FL, et al. The role of tissue macrophage-mediated inflammation on NAFLD pathogenesis and its clinical implications. Mediators Inflamm. 2017;2017:8162421.
69. Feng D, Amgalan D, Singh R, et al. SNAP23 regulates BAX-dependent adipocyte programmed cell death independently of canonical macroautophagy. J Clin Invest. 2018;128:3941–56.
70. Alkhouri N, Gornicka A, Berk MP, et al. Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis. J Biol Chem. 2010;285:3428–38.
71. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol Cell Endocrinol. 2010;314:1–16.
72. Ouchi N, Parker JL, Lugus JJ, et al. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11:85–97.
73. Park HK, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. Metabolism. 2015;64:24–34.
74. Dalamaga M, Chou SH, Shields K, et al. Leptin at the intersection of neuroendocrinology and metabolism: current evidence and therapeutic perspectives. Cell Metab. 2013;18:29–42.

75. Moon HS, Dalamaga M, Kim SY, et al. Leptin's role in lipodystrophic and nonlipodystrophic insulin-resistant and diabetic individuals. Endocr Rev. 2013;34:377–412.

76. Petersen KF, Oral EA, Dufour S, et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. J Clin Invest. 2002;109:1345–50.

77. Janik JE, Curti BD, Considine RV, et al. Interleukin 1 alpha increases serum leptin concentrations in humans. J Clin Endocrinol Metab. 1997;82:3084–6.

78. Zarrati M, Aboutaleb N, Cheshmazar E, et al. The association of obesity and serum leptin levels with complete blood count and some serum biochemical parameters in Iranian overweight and obese individuals. Med J Islam Repub Iran. 2019;33:72.

79. Yahagi N, Shimano H, Hasty AH, et al. Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lep(ob)/Lep(ob) mice. J Biol Chem. 2002;277:19353–7.

80. Sekiya M, Yahagi N, Matsuzaka T, et al. Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. Hepatology. 2003;38:1529–39.

81. Park HY, Kwon HM, Lim HJ, et al. Potential role of leptin in angiogenesis: leptin induces endothelial cell proliferation and expression of matrix metalloproteinases in vivo and in vitro. Exp Mol Med. 2001;33:95–102.

82. Honda H, Ikejima K, Hirose M, et al. Leptin is required for fibrogenic responses induced by thioacetamide in the murine liver. Hepatology. 2002;36:12–21.

83. Uygun A, Kadayifci A, Yesilova Z, et al. Serum leptin levels in patients with nonalcoholic steatohepatitis. Am J Gastroenterol. 2000;95:3584–9.

84. Polyzos SA, Aronis KN, Kountouras J, et al. Circulating leptin in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Diabetologia. 2016;59:30–43.

85. Wang ZV, Scherer PE. Adiponectin, the past two decades. J Mol Cell Biol. 2016;8:93–100.

86. Cui J, Pang J, Lin YJ, et al. Adipose-specific deletion of Kif5b exacerbates obesity and insulin resistance in a mouse model of diet-induced obesity. FASEB J. 2017;31:2533–47.

87. Qiu Y, Wang SF, Yu C, et al. Association of circulating adipsin, visfatin, and adiponectin with nonalcoholic fatty liver disease in adults: a case–control study. Ann Nutr Metab. 2019;74:44–52.

88. Polyzos SA, Mantzoros CS. Adiponectin as a target for the treatment of nonalcoholic steatohepatitis with thiazolidinediones: a systematic review. Metabolism. 2016;65:1297–306.

89. Kim SM, Grenert JP, Patterson C, et al. CHIP(−/−)-Mouse liver: adiponectin-AMPK-FOXO-activation overrides CYP2E1-elicited JNK1-activation, delaying onset of NASH: therapeutic implications. Sci Rep. 2016;6:29423.

90. Acquarone E, Monacelli F, Borghi R, et al. Resistin: a reappraisal. Mech Ageing Dev. 2019;178:46–63.

91. Pirvulescu M, Manduteanu I, Gan AM, et al. A novel pro-inflammatory mechanism of action of resistin in human endothelial cells: up-regulation of SOCS3 expression through STAT3 activation. Biochem Biophys Res Commun. 2012;422:321–6.

92. Silswal N, Singh AK, Aruna B, et al. Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. Biochem Biophys Res Commun. 2005;334:1092–101.

93. Shen YH, Zhang L, Gan Y, et al. Up-regulation of PTEN (phosphatase and tensin homolog deleted on chromosome ten) mediates p38 MAPK stress signal-induced inhibition of insulin signalling. A cross-talk between stress signaling and insulin signaling in resistin-treated human endothelial cells. J Biol Chem. 2006;281:7727–36.

94. Pagano C, Soardo G, Pilon C, et al. Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. J Clin Endocrinol Metab. 2006;91:1081–6.

95. Aquilante CL, Kosmiski LA, Knutsen SD, et al. Relationship between plasma resistin concentrations, inflammatory chemokines, and components of the metabolic syndrome in adults. Metabolism. 2008;57:494–501.

96. Virtanen KA, Lidell ME, Orava J, et al. Functional brown adipose tissue in healthy adults. N Engl J Med. 2009;360:1518–25.

97. Stanford KI, Middelbeek RJ, Townsend KL, et al. Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest. 2013;123:215–23.

98. Seale P, Conroe HM, Estall J, et al. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J Clin Invest. 2011;121:96–105.

99. Mottillo EP, Desjardins EM, Crane JD, et al. Lack of adipocyte AMPK exacerbates insulin resistance and hepatic steatosis through brown and beige adipose tissue function. Cell Metab. 2016;24:118–29.

100. Day EA, Ford RJ, Steinberg GR. AMPK as a therapeutic target for treating metabolic diseases. Trends Endocrinol Metab. 2017;28:545–60.

101. Gangarapu V, Yildiz K, Ince AT, et al. Role of gut microbiota: obesity and NAFLD. Turk J Gastroenterol. 2014;25:133–40.

102. Boursier J, Mueller O, Barret M, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatology. 2016;63:764–75.

103. Koukias N, Buzzetti E, Tsochatzis EA. Intestinal hormones, gut microbiota and non-alcoholic fatty liver disease. Minerva Endocrinol. 2017;42:184–94.

104. Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev. 2005;85:495–522.

105. Barazzoni R, Zanetti M, Ferreira C, et al. Relationships between desacylated and acylated ghrelin and insulin sensitivity in the metabolic syndrome. J Clin Endocrinol Metab. 2007;92:3935–40.

106. Estep M, Abawi M, Jarrar M, et al. Association of obestatin, ghrelin, and inflammatory cytokines in obese patients with non-alcoholic fatty liver disease. Obes Surg. 2011;21:1750–7.

107. Li Y, Hai J, Li L, et al. Administration of ghrelin improves inflammation, oxidative stress, and apoptosis during and after non-alcoholic fatty liver disease development. Endocrine. 2013;43:376–86.

108. Li Z, Xu G, Qin Y, et al. Ghrelin promotes hepatic lipogenesis by activation of mTOR-PPARgamma signaling pathway. Proc Natl Acad Sci USA. 2014;111:13163–8.

109. Poher AL, Tschop MH, Muller TD. Ghrelin regulation of glucose metabolism. Peptides. 2018;100:236–42.

110. Ezquerro S, Mocha F, Fruhbeck G, et al. Ghrelin reduces TNF-alpha-induced human hepatocyte apoptosis, autophagy, and pyroptosis: role in obesity-associated NAFLD. J Clin Endocrinol Metab. 2019;104:21–37.

111. Mao JW, Tang HY, Zhao T, et al. Intestinal mucosal barrier dysfunction participates in the progress of nonalcoholic fatty liver disease. Int J Clin Exp Pathol. 2015;8:3648–58.

112. Muller TD, Finan B, Bloom SR, et al. Glucagon-like peptide 1 (GLP-1). Mol Metab. 2019;30:72–130.

113. Petit JM, Verges B. GLP-1 receptor agonists in NAFLD. Diabetes Metab. 2017;43(Suppl 1):2S28–33.

114. Valdecantos MP, Pardo V, Ruiz L, et al. A novel glucagon-like peptide 1/glucagon receptor dual agonist improves steatohepatitis and liver regeneration in mice. Hepatology. 2017;65:950–68.

115. Patel V, Joharapurkar A, Kshirsagar S, et al. Coagonist of GLP-1 and glucagon receptor ameliorates development of non-alcoholic fatty liver disease. Cardiovasc Hematol Agents Med Chem. 2018;16:35–43.

116. Armstrong MJ, Hull D, Guo K, et al. Glucagon-like peptide 1 decreases lipotoxicity in non-alcoholic steatohepatitis. J Hepatol. 2016;64:399–408.

117. Yan J, Yao B, Kuang H, et al. Liraglutide, sitagliptin, and insulin glargine added to metformin: the effect on body weight and intrahepatic lipid in patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease. Hepatology. 2019;69:2414–26.

118. Gastaldelli A, Gaggini M, Daniele G, et al. Exenatide improves both hepatic and adipose tissue insulin resistance: a dynamic positron emission tomography study. Hepatology. 2016;64:2028–37.

119. Feng W, Gao C, Bi Y, et al. Randomized trial comparing the effects of gliclazide, liraglutide, and metformin on diabetes with non-alcoholic fatty liver disease. J Diabetes. 2017;9:800–9.

120. Arab JP, Karpen SJ, Dawson PA, et al. Bile acids and nonalcoholic fatty liver disease: Molecular insights and therapeutic perspectives. Hepatology. 2017;65:350–62.

121. den Besten G, van Eunen K, Groen AK, et al. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 2013;54:2325–40.

122. Moore DD. Nuclear receptors reverse McGarry's vicious cycle to insulin resistance. Cell Metab. 2012;15:615–22.

123. Miyata M, Sakaida Y, Matsuzawa H, et al. Fibroblast growth factor 19 treatment ameliorates disruption of hepatic lipid metabolism in farnesoid X receptor (Fxr)-null mice. Biol Pharm Bull. 2011;34:1885–9.

124. Fu L, John LM, Adams SH, et al. Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. Endocrinology. 2004;145:2594–603.

125. Kir S, Beddow SA, Samuel VT, et al. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. Science. 2011;331:1621–4.

126. Nobili V, Alisi A, Mosca A, et al. Hepatic farnesoid X receptor protein level and circulating fibroblast growth factor 19 concentration in children with NAFLD. Liver Int. 2018;38:342–9.

127. 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;394:2184–96.

128. Cantarel BL, Lombard V, Henrissat B. Complex carbohydrate utilization by the healthy human microbiome. PLoS ONE. 2012;7:e28742.

129. Hooper LV, Wong MH, Thelin A, et al. Molecular analysis of commensal host-microbial relationships in the intestine. Science. 2001;291:881–4.

130. Marin L, Miguelez EM, Villar CJ, et al. Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. Biomed Res Int. 2015;2015:905215.

131. Wallace BD, Wang H, Lane KT, et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science. 2010;330:831–5.

132. Carvalho FA, Aitken JD, Vijay-Kumar M, et al. Toll-like receptor-gut microbiota interactions: perturb at your own risk! Annu Rev Physiol. 2012;74:177–98.

133. Chung H, Pamp SJ, Hill JA, et al. Gut immune maturation depends on colonization with a host-specific microbiota. Cell. 2012;149:1578–93.

134. Sonnenberg GF, Artis D. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. Immunity. 2012;37:601–10.

135. Franchi L, Kamada N, Nakamura Y, et al. NLRC4-driven production of IL-1beta discriminates between pathogenic and commensal bacteria and promotes host intestinal defense. Nat Immunol. 2012;13:449–56.

136. Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58:1091–103.

137. Belei O, Olariu L, Dobrescu A, et al. The relationship between non-alcoholic fatty liver disease and small intestinal bacterial overgrowth among overweight and obese children and adolescents. J Pediatr Endocrinol Metab. 2017;30:1161–8.

138. Rivera CA, Adegboyega P, van Rooijen N, et al. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. J Hepatol. 2007;47:571–9.

139. Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology. 2009;49:1877–87.

140. Backhed F, Manchester JK, Semenkovich CF, et al. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci USA. 2007;104:979–84.

141. Mouzaki M, Comelli EM, Arendt BM, et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology. 2013;58:120–7.

142. Jumpertz R, Le DS, Turnbaugh PJ, et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr. 2011;94:58–65.

143. Wostmann BS, Larkin C, Moriarty A, et al. Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats. Lab Anim Sci. 1983;33:46–50.

144. Hong J, Jia Y, Pan S, et al. Butyrate alleviates high fat diet-induced obesity through activation of adiponectin-mediated pathway and stimulation of mitochondrial function in the skeletal muscle of mice. Oncotarget. 2016;7:56071–82.

145. Kimura I, Ozawa K, Inoue D, et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nat Commun. 2013;4:1829.

146. Zaibi MS, Stocker CJ, O'Dowd J, et al. Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. FEBS Lett. 2010;584:2381–6.

147. Ge H, Li X, Weiszmann J, et al. Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. Endocrinology. 2008;149:4519–26.

148. Gao Z, Yin J, Zhang J, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes. 2009;58:1509–17.

149. Canfora EE, van der Beek CM, Jocken JWE, et al. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. Sci Rep. 2017;7:2360.

150. Reynes B, Palou M, Rodriguez AM, et al. Regulation of adaptive thermogenesis and browning by prebiotics and postbiotics. Front Physiol. 2018;9:1908.

151. Demigne C, Morand C, Levrat MA, et al. Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. Br J Nutr. 1995;74:209–19.

152. Brown AJ, Goldsworthy SM, Barnes AA, et al. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem. 2003;278:11312–9.

153. Vinolo MA, Rodrigues HG, Nachbar RT, et al. Regulation of inflammation by short chain fatty acids. Nutrients. 2011;3:858–76.

154. Kim MH, Kang SG, Park JH, et al. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. Gastroenterology. 2013;145(396–406):e1-10.

155. Vinolo MA, Ferguson GJ, Kulkarni S, et al. SCFAs induce mouse neutrophil chemotaxis through the GPR43 receptor. PLoS ONE. 2011;6:e21205.

156. Kendrick SF, O'Boyle G, Mann J, et al. Acetate, the key modulator of inflammatory responses in acute alcoholic hepatitis. Hepatology. 2010;51:1988–97.

157. Usami M, Kishimoto K, Ohata A, et al. Butyrate and trichostatin A attenuate nuclear factor kappaB activation and tumor necrosis factor alpha secretion and increase prostaglandin E2 secretion in human peripheral blood mononuclear cells. Nutr Res. 2008;28:321–8.

158. Maslowski KM, Vieira AT, Ng A, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461:1282–6.

159. Li W, Zhang K, Yang H. Pectin alleviates high fat (lard) diet-induced nonalcoholic fatty liver disease in mice: possible role of short-chain fatty acids and gut microbiota regulated by pectin. J Agric Food Chem. 2018;66:8015–25.

160. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol. 2015;11:577–91.

161. Sahuri-Arisoylu M, Brody LP, Parkinson JR, et al. Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. Int J Obes (Lond). 2016;40:955–63.

162. Mollica MP, Mattace Raso G, Cavaliere G, et al. Butyrate regulates liver mitochondrial function, efficiency, and dynamics in insulin-resistant obese mice. Diabetes. 2017;66:1405–18.

163. Cope K, Risby T, Diehl AM. Increased gastrointestinal ethanol production in obese mice: implications for fatty liver disease pathogenesis. Gastroenterology. 2000;119:1340–7.

164. Nair S, Cope K, Risby TH, et al. Obesity and female gender increase breath ethanol concentration: potential implications for the pathogenesis of nonalcoholic steatohepatitis. Am J Gastroenterol. 2001;96:1200–4.

165. Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology. 2013;57:601–9.

166. Baker SS, Baker RD, Liu W, et al. Role of alcohol metabolism in non-alcoholic steatohepatitis. PLoS ONE. 2010;5:e9570.

167. Chang B, Xu MJ, Zhou Z, et al. Short- or long-term high-fat diet feeding plus acute ethanol binge synergistically induce acute liver injury in mice: an important role for CXCL1. Hepatology. 2015;62:1070–85.

168. Yuan J, Chen C, Cui J, et al. Fatty liver disease caused by high-alcohol-producing Klebsiella pneumoniae. Cell Metab. 2019;30(675–688):e7.

169. Than NN, Newsome PN. A concise review of non-alcoholic fatty liver disease. Atherosclerosis. 2015;239:192–202.

170. Wree A, McGeough MD, Pena CA, et al. NLRP3 inflammasome activation is required for fibrosis development in NAFLD. J Mol Med (Berl). 2014;92:1069–82.

171. Everard A, Geurts L, Caesar R, et al. Intestinal epithelial MyD88 is a sensor switching host metabolism towards obesity according to nutritional status. Nat Commun. 2014;5:5648.

172. Loh K, Herzog H, Shi YC. Regulation of energy homeostasis by the NPY system. Trends Endocrinol Metab. 2015;26:125–35.

173. Roh E, Song DK, Kim MS. Emerging role of the brain in the homeostatic regulation of energy and glucose metabolism. Exp Mol Med. 2016;48:e216.

174. Ollmann MM, Wilson BD, Yang YK, et al. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science. 1997;278:135–8.

175. Zhang Y, Kilroy GE, Henagan TM, et al. Targeted deletion of melanocortin receptor subtypes 3 and 4, but not CART, alters nutrient partitioning and compromises behavioral and metabolic responses to leptin. FASEB J. 2005;19:1482–91.

176. Tao YX. Molecular mechanisms of the neural melanocortin receptor dysfunction in severe early onset obesity. Mol Cell Endocrinol. 2005;239:1–14.

177. Itoh M, Suganami T, Nakagawa N, et al. Melanocortin 4 receptor-deficient mice as a novel mouse model of nonalcoholic steatohepatitis. Am J Pathol. 2011;179:2454–63.

178. Bewick GA, Gardiner JV, Dhillo WS, et al. Post-embryonic ablation of AgRP neurons in mice leads to a lean, hypophagic phenotype. FASEB J. 2005;19:1680–2.

179. Bingham NC, Anderson KK, Reuter AL, et al. Selective loss of leptin receptors in the ventromedial hypothalamic nucleus results in increased adiposity and a metabolic syndrome. Endocrinology. 2008;149:2138–48.

180. Foster MT, Song CK, Bartness TJ. Hypothalamic paraventricular nucleus lesion involvement in the sympathetic control of lipid mobilization. Obesity (Silver Spring). 2010;18:682–9.

181. Gonzalez JA, Reimann F, Burdakov D. Dissociation between sensing and metabolism of glucose in sugar sensing neurones. J Physiol. 2009;587:41–8.

182. Gomori A, Ishihara A, Ito M, et al. Chronic intracerebroventricular infusion of MCH causes obesity in mice melanin-concentrating hormone. Am J Physiol Endocrinol Metab. 2003;284:E583–8.

183. Kokkotou E, Jon JY, Wang X, et al. Mice with MCH ablation resist diet-induced obesity through strain-specific mechanisms. Am J Physiol Regul Integr Comp Physiol. 2005;289:R117–24.

184. Marsh DJ, Weingarth DT, Novi DE, et al. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. Proc Natl Acad Sci USA. 2002;99:3240–5.

185. Kawata Y, Okuda S, Hotta N, et al. A novel and selective melanin-concentrating hormone receptor 1 antagonist ameliorates obesity and hepatic steatosis in diet-induced obese rodent models. Eur J Pharmacol. 2017;796:45–53.

186. Kramer A, Yang FC, Snodgrass P, et al. Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. Science. 2001;294:2511–5.

187. Morrison SF, Madden CJ, Tupone D. Central neural regulation of brown adipose tissue thermogenesis and energy expenditure. Cell Metab. 2014;19:741–56.

188. Seoane-Collazo P, Ferno J, Gonzalez F, et al. Hypothalamic-autonomic control of energy homeostasis. Endocrine. 2015;50:276–91.

189. Lockie SH, Heppner KM, Chaudhary N, et al. Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. Diabetes. 2012;61:2753–62.

190. Wang L, Liangpunsakul S. Circadian clock control of hepatic lipid metabolism: role of small heterodimer partner (Shp). J Investig Med. 2016;64:1158–61.

191. Schibler U, Sassone-Corsi P. A web of circadian pacemakers. Cell. 2002;111:919–22.

192. Turek FW, Joshu C, Kohsaka A, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. Science. 2005;308:1043–5.

193. Mazzoccoli G, Vinciguerra M, Oben J, et al. Non-alcoholic fatty liver disease: the role of nuclear receptors and circadian rhythmicity. Liver Int. 2014;34:1133–52.

194. Lopez-Gambero AJ, Martinez F, Salazar K, et al. Brain glucose-sensing mechanism and energy homeostasis. Mol Neurobiol. 2019;56:769–96.

195. Blasi C. The role of the vagal nucleus tractus solitarius in the therapeutic effects of obesity surgery and other interventional therapies on type 2 diabetes. Obes Surg. 2016;26:3045–57.

196. Daly DM, Park SJ, Valinsky WC, et al. Impaired intestinal afferent nerve satiety signalling and vagal afferent excitability in diet induced obesity in the mouse. J Physiol. 2011;589:2857–70.

197. Kentish SJ, Page AJ. The role of gastrointestinal vagal afferent fibres in obesity. J Physiol. 2015;593:775–86.

198. Carreno FR, Seelaender MC. Liver denervation affects hepatocyte mitochondrial fatty acid transport capacity. Cell Biochem Funct. 2004;22:9–17.

199. Smith BK, Marcinko K, Desjardins EM, et al. Treatment of nonalcoholic fatty liver disease: role of AMPK. Am J Physiol Endocrinol Metab. 2016;311:E730–40.

200. de La Serre CB, de Lartigue G, Raybould HE. Chronic exposure to low dose bacterial lipopolysaccharide inhibits leptin signaling in vagal afferent neurons. Physiol Behav. 2015;139:188–94.

201. Sen T, Cawthon CR, Ihde BT, et al. Diet-driven microbiota dysbiosis is associated with vagal remodeling and obesity. Physiol Behav. 2017;173:305–17.

202. Meyer-Gerspach AC, Wolnerhanssen B, Beglinger B, et al. Gastric and intestinal satiation in obese and normal weight healthy people. Physiol Behav. 2014;129:265–71.

203. Duca FA, Sakar Y, Covasa M. Combination of obesity and high-fat feeding diminishes sensitivity to GLP-1R agonist exendin-4. Diabetes. 2013;62:2410–5.

204. Browning KN, Fortna SR, Hajnal A. Roux-en-Y gastric bypass reverses the effects of diet-induced obesity to inhibit the responsiveness of central vagal motoneurones. J Physiol. 2013;591:2357–72.

205. Oswal A, Yeo G. Leptin and the control of body weight: a review of its diverse central targets, signaling mechanisms, and role in the pathogenesis of obesity. Obesity (Silver Spring). 2010;18:221–9.

206. Al Massadi O, Lopez M, Tschop M, et al. Current understanding of the hypothalamic ghrelin pathways inducing appetite and adiposity. Trends Neurosci. 2017;40:167–80.

207. Disse E, Bussier AL, Veyrat-Durebex C, et al. Peripheral ghrelin enhances sweet taste food consumption and preference, regardless of its caloric content. Physiol Behav. 2010;101:277–81.

208. Lebda MA, Tohamy HG, El-Sayed YS. Long-term soft drink and aspartame intake induces hepatic damage via dysregulation of adipocytokines and alteration of the lipid profile and antioxidant status. Nutr Res. 2017;41:47–55.

209. Duffey KJ, Steffen LM, Van Horn L, et al. Dietary patterns matter: diet beverages and cardiometabolic risks in the longitudinal coronary artery risk development in young adults (CARDIA) study. Am J Clin Nutr. 2012;95:909–15.

210. Dhingra R, Sullivan L, Jacques PF, et al. Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. Circulation. 2007;116:480–8.

211. Nettleton JA, Polak JF, Tracy R, et al. Dietary patterns and incident cardiovascular disease in the multi-ethnic study of atherosclerosis. Am J Clin Nutr. 2009;90:647–54.

212. Fagherazzi G, Vilier A, Saes Sartorelli D, et al. Consumption of artificially and sugar-sweetened beverages and incident type 2 diabetes in the Etude Epidemiologique aupres des femmes de la Mutuelle Generale de l'Education Nationale-European Prospective Investigation into Cancer and Nutrition cohort. Am J Clin Nutr. 2013;97:517–23.

213. Swithers SE, Laboy AF, Clark K, et al. Experience with the high-intensity sweetener saccharin impairs glucose homeostasis and GLP-1 release in rats. Behav Brain Res. 2012;233:1–14.

214. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol. 2020;73:202–9.

215. Eslam M, Sanyal AJ, George J, et al. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology. 2020;158(1999–2014):e1.

216. Younossi ZM, Rinella ME, Sanyal A, et al. From NAFLD to MAFLD: implications of a premature change in terminology. Hepatology. 2020.

Publisher's Note