Liver macrophages and inflammation in physiology and physiopathology of non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome, being a common comorbidity of type 2 diabetes and with important links to inflammation and insulin resistance. NAFLD represents a spectrum of liver conditions ranging from steatosis in the form of ectopic lipid storage, to inflammation and fibrosis in nonalcoholic steatohepatitis (NASH). Macrophages that populate the liver play important roles in maintaining liver homeostasis under normal physiology and in promoting inflammation and mediating fibrosis in the progression of NAFLD toward to NASH. Liver macrophages are a heterogenous group of innate immune cells, originating from the yolk sac or from circulating monocytes, that are required to maintain immune tolerance while being exposed portal and pancreatic blood flow rich in nutrients and hormones. Yet, liver macrophages retain a limited capacity to raise the alarm in response to danger signals. We now know that macrophages in the liver play both inflammatory and noninflammatory roles throughout the progression of NAFLD. Macrophage responses are mediated first at the level of cell surface receptors that integrate environmental stimuli, signals are transduced through multiple levels of regulation in the cell, and specific transcriptional

Abbreviations

AP-1, activator protein 1; BMDM, bone marrow-derived macrophages; CCL, chemokine ligand 2; CCR, C-C motif chemokine receptor; CD, cluster of differentiation; CD-HFD, choline-deficient high-fat diet; CETP, cholesteryl ester transfer protein; CLEC4F, C-type lectin domain family 4 member F; CXCL, C-X-C motif ligand; CXCR, C-X-C chemokine receptor; DAMP, damage-associated molecular patterns; DR5, death receptor 5; ER, endoplasmic reticulum; GLUT4, glucose transporter type 4; GM-CSF, granulocyte-macrophage colony-stimulating factor; HCC, hepatocellular carcinoma; HFD, high-fat diet; HIF, hypoxia-inducible factor; HRG, histidine-rich glycoprotein; HSC, hepatic stellate cell; IFN, interferon; IGFBP7, insulin-like growth factor-binding protein 7; IL, interleukin; iNOS, inducible nitric oxide synthase; Insr, insulin receptor knockout; IRF, interferon regulatory factor; JNK, c-Jun N-terminal kinase; KC, Kupffer cell; LDLR, low density lipoprotein receptor; LPS, lipopolysaccharides; LSEC, liver sinusoidal epithelial cells; LXR, liver X receptor; Ly6, lymphocyte antigen; MAPK, mitogen-activated protein kinase; MCD, methionine-choline deficient; MCP-1, monocyte chemotactic protein 1; M-CSF, macrophage colony-stimulating factor; Mo, monocyte-derived; MP, macrophage; MUP-uPA, methionine adenosyl transferase 1A knockout mouse with high transient expression of urokinase plasminogen activator in hepatocytes; MyD88, myeloid differentiation primary response 88; NAFLD, non-alcoholic fatty liver disease; NAM, Nash-associated macrophage; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor kappa-B; PD-L1, programmed death ligand 1; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome proliferator-activated receptor; PTPROt, protein tyrosine phosphatase receptor type O truncated isoform; RHM, recruited hepatic macrophages; scRNA-seq, single-cell RNA sequencing; SREBP1c, sterol regulatory element-binding protein 1c; STAT, signal transducer and activator of transcription; STING, stimulator of interferon genes; T2D, type 2 diabetes; TGF, transforming growth factor; TN, helper T-cell; TH, thyroid hormone/thyroid hormone receptor; TIM4, T-cell immunoglobulin and mucin domain containing 4; TLR, Toll-like receptor; TNF, tumour necrosis factor; Treg, regulatory T cell; TREM2, triggering receptor expressed on myeloid cells; UPR, unfolded protein response; WD, Western diet; XBP1, X-box binding protein 1.
programmes dictate effector functions. These effector functions play paramount roles in determining the course of disease in NAFLD and even more so in the progression towards NASH. The current review covers recent reports in the physiological and pathophysiological roles of liver macrophages in NAFLD. We emphasise the responses of liver macrophages to insulin resistance and the transcriptional machinery that dictates liver macrophage function.

Introduction: Inflammation and metabolic decline in non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease with an estimated worldwide prevalence of 25% [1,2]. NAFLD is the hepatic manifestation of metabolic syndrome and common comorbidity of type 2 diabetes (T2D), obesity and hypertension. Indeed, around 55% of patients with T2D also have NAFLD [3]. Metabolic and inflammatory disturbances are important parts of the aetiology of NAFLD and of its comorbidities [4,5].

Non-alcoholic fatty liver disease represents a spectrum of conditions ranging from fatty liver, relatively benign steatosis in the form ectopic lipid storage, to nonalcoholic steatohepatitis (NASH) where inflammation and tissue remodelling can impair tissue function and whole-body metabolism. NASH represents the last reversible step of NAFLD, before progression to hepatocellular carcinoma (HCC) [6,7] (Fig. 1).

Over recent decades, considerable progress has been made in understanding the mechanisms of NAFLD development and progression [5]. An important milestone was published by Day and James in 1998 when they put forward their ‘two-hit’ hypothesis. In this hypothesis, steatosis was considered the first hit and inflammation the second, causing progression through the spectrum of NAFLD towards NASH [8].

Given that T2D and NAFLD are frequent comorbidities, a relationship with insulin sensitivity or secretion was sought in the earliest studies [9]. Initial clinical work found associations between insulin resistance and NAFLD, even in the absence of frank T2D (compromised insulin secretion). Glucose disposal and insulin sensitivity were also found to be progressively impaired going from healthy subjects, to patients with steatotic livers and then in patients with NASH [10,11]. At the cellular level, insulin resistance also contributes to steatosis through two main mechanisms: increased hepatic de novo lipogenesis [12] and ectopic lipid storage in response to systemic dyslipidaemia [13]. Dyslipidaemia arises early in disease course from increased lipolysis in adipose tissue [13].

When hepatocytes reach their lipid storage threshold, lipotoxicity and hepatocellular stress lead to apoptosis [14,15]. Lipid overload and insulin resistance are associated with endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) [15]. Physiologically, X-box binding protein (XBP)-1 mRNA splicing responds to ER stress and promotes cell survival by increasing ER protein folding capacity [16]. However, XBP-1-mediated cell survival fails in NAFLD, resulting in hepatocellular stress, inflammation, further loss of insulin sensitivity and apoptosis [17,18].

The above step is key in initiating inflammation and the transition from benign steatosis to NASH. The initial inflammatory response is largely mediated by tissue-resident macrophages [19]. Upon inflammatory signalling, tissue-resident macrophages recruit other immune cells from circulation, including monocytes that differentiate into macrophages in situ and amplify inflammatory signalling [20]. When this cycle is sustained under chronic hepatocellular stress, a macrophage pro-resolution response is also initiated. The resolution of inflammation is beneficial in response to acute inflammation; however, in response to chronic inflammation in the liver the resolution phase is associated with excessive deposition of collagen in extracellular matrix [21]. Fibrosis in later stages of NASH, from excessive collagen deposition, is the result of an exuberant scarring response, which over time significantly remodels the tissue and impedes liver function [21,22].

Macrophages are central to the progression of NAFLD, and their proliferation, differentiation and polarisation are tightly controlled and dependent on extracellular stimuli as well as intracellular signalling cascades [23]. While initially acting as sentinel cells, macrophages are also very important effectors cells that secrete cytokines and chemokines, influencing cells in the microenvironment. This review covers the mechanisms of how liver macrophages undergo activation and contribute to the development and progression of NAFLD. Given the importance of insulin resistance in
In the pathogenesis of disease, we also address the role of insulin signalling, and insulin action on liver macrophages.

**Insulin signalling and NAFLD**

Insulin is an anabolic hormone secreted by pancreatic beta cells and is widely recognised for its role in regulating glucose homeostasis, lipid metabolism and cell growth. The effects of insulin are mediated through the insulin receptor [24,25] and the insulin-like growth factor 1 receptor [26]. When insulin binds to its receptor it activates two major downstream pathways: the phosphoinositide 3-kinase (PI3K) pathway and the mitogen-activated protein kinase (MAPK) pathway [26,27]. The PI3K pathway mediates insulin’s metabolic effects including the translocation of glucose transporter (GLUT)-4 in metabolic tissues such as muscle, liver and adipose [26], while the MAPK pathway regulates mitogenesis and growth [27]. Recently, the insulin receptor has also been shown to directly interact with transcriptional machinery, an additional mechanism for effects in normal physiology and disease [28].

Insulin resistance is the term given to the lack of an appropriate response to physiological levels of insulin, typically determined through systemic metabolic measures such as blood glucose. Insulin resistance is a precursor syndrome to T2D and its comorbidities [29]. In humans, patients presenting with NAFLD are often insulin resistant; however, it is unclear whether insulin resistance is compensatory rather than causal – the challenges to addressing this important question have been recently reviewed [30]. Various murine models of NAFLD have been proposed (detailed below), and in order to reproduce human disease, the model applied will ideally display obesity, insulin resistance and NAFLD concurrently [31]. One of the early mouse models investigating insulin’s function, through global targeted disruption of the insulin receptor (Insr−/−)...

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**Fig. 1.** NAFLD progression. Benign steatosis (fat accumulation in hepatocytes) can trigger inflammation in the liver (starting point for NASH). As inflammation worsens, hepatic stellate cell activation leads to extracellular matrix deposition and fibrosis. Eventually, this process facilitates tumorigenesis and development of hepatocellular carcinoma. A tumour mass can also arise directly from NASH without need for progressive fibrosis. Fibrosis in NASH is the last reversible step of NAFLD.
mouse), reported liver steatosis and hepatic insulin resistance. The model initially exhibits dramatic metabolic insulin resistance, which is followed by age-dependent morphological and functional changes in the liver [32,33]. This early model suggested that changes in insulin sensitivity are sufficient to initiate NAFLD [32,33].

**Insulin and macrophages**

While macrophages are less associated with the roles of insulin compared with the majority cells of the metabolic tissues, macrophages do express insulin receptors and downstream intracellular signalling pathways [34,35]. *In vitro* studies investigating the direct effects of insulin on macrophages have shown insulin to have a profound effect on macrophage activation including inflammatory or M1-like polarisation (Table 1) or anti-inflammatory or M2-like polarisation (Table 2). Discrepancies in reports may be due to a lack of consistency in the model of macrophage investigated (different species/tissue type/cell line), concentration or duration of insulin used. The effects of insulin on macrophages are seemingly wide-ranging and thus may reflect macrophage plasticity and ability

### Table 1. Evidence supporting pro-inflammatory role of insulin in macrophages. Where insulin resistance is anti-inflammatory.

| Model | Summary | Ref |
|-------|---------|-----|
| Cell lines ML-1, THP-1, PL-21 | Insulin enhances LPS-stimulated IL-1β | [333] |
| Cell line THP-1 | Insulin upregulates TNFα | [334] |
| Mouse myeloid/macrophage insulin resistance | Protects against atherosclerosis | [37] |
| Mouse myeloid/macrophage insulin resistance | Protects against obesity-induced inflammation | [335] |
| Human macrophages | Insulin promotes foam cell formation | [336] |
| Mouse insulin-resistant macrophages | Attenuation of atherosclerosis, promotion of M2-type phenotype when stimulated with pro-inflammatory cytokines | [337] |
| Mouse macrophages | Insulin and IL-1β synergistically promote inflammation | [338] |
| Diabetic mouse bone marrow-derived macrophages | Insulin increases TNFα and IL-6 secretion in LPS-stimulated macrophages | [339] |
| Mouse macrophages | Insulin resistance promotes M2-like phenotype and reduced LPS responses | [340] |

### Table 2. Evidence supporting anti-inflammatory role of insulin in macrophages. Where insulin resistance is pro-inflammatory.

| Model | Summary | Ref |
|-------|---------|-----|
| Rat peritoneal macrophages | Insulin enhances phagocytosis capacity and production of H2O2 | [341] |
| Obese human mononuclear cells | Insulin inhibits NFκB and stimulates iκB | [342] |
| Cell line THP-1 | Insulin inhibits apoptosis | [343] |
| Cell line THP-1 | Insulin inhibits apoptosis and reduces TNF and IL-1β | [344] |
| Rat macrophages | Insulin suppresses LPS-induced iNOS and COX-2 expression and NK-κB activation | [345] |
| Mouse myeloid/macrophage insulin resistance | Increased macrophage apoptosis and atherosclerotic plaque necrotic core formation | [36] |
| Mouse insulin-resistant macrophages | Increased macrophage apoptosis | [346] |
| Cell line THP-1 | Insulin pretreatment delays endotoxin mediated macrophage activation | [347] |
| Mouse insulin-resistant macrophages | Increased LPS IL-1β production | [348] |
| Mouse insulin-resistant macrophages | Enhanced monocyte adhesion | [349] |
| Mouse insulin-resistant macrophages | Enhanced vascular wall adhesion and pro-inflammatory mediator adhesion | [350] |
| Mouse insulin-resistant macrophages | Increased apoptosis | [351] |
| Cell line RAW264.7 and high fat fed mice | Reduced foam cell formation, down-regulation of pro-inflammatory cytokines, decreased serum pro-inflammatory mediators and macrophage infiltration | [352] |
| | Insulin promotes IL-10 expression and attenuates LPS-induced Tnf-α, Il-1β and iNOS expression | [352] |
| | Insulin advances infiltration and resolution of macrophages | [355] |
| Diabetic mouse alveolar and peritoneal macrophages | Insulin reduces TNFα, IL-6 and IL-1β secretion in LPS-stimulated macrophages | [339] |
| Mouse macrophages | Insulin resistance impairs M2a activation | [356] |
| Rat macrophages and cell line THP-1 | Insulin polarises macrophages to M2 phenotype under high glucose conditions | [357] |
| Rat macrophages | Insulin restores abnormal macrophage infiltration, promotes efferocytosis and induces M1 to M2 transition | [358] |
to respond to the fluctuating nature of blood insulin levels.

**Insulin and liver macrophages**

Surprisingly, while the impact of macrophages on NAFLD development is appreciated, and the significance of insulin resistance on macrophages in cardiometabolic diseases such as atherosclerosis are recognised, studies investigating the specific role of insulin-resistant macrophages on NAFLD have yet to be reported [36–38]. In obesity-induced insulin resistance in mice, distinct subpopulations of hepatic macrophages, with Kupffer cells (KC), secrete high levels of chemokine ligand (CCL)-2/monocyte chemoattractant protein (MCP)-1. CCL2/MCP-1 acts to recruit ‘recruited hepatic macrophages’ (RHMs). RHMs in turn enhance the severity of obesity-induced inflammation and hepatic insulin resistance [39]. Recently, Morgantini et al. [40] have shown that in obesity-induced insulin resistance in flies, humans and mice; liver macrophages produce noninflammatory factors including insulin-like growth factor-binding protein (IGFBP)-7 that can bind to the insulin receptor, directly regulating liver metabolism independently of inflammation.

**Macrophages in liver physiology**

There are two major types of hepatic macrophages: monocyte-derived and tissue-resident macrophages. KCs, *bona fide* liver-resident macrophages, are by far the most abundant in the healthy liver. In mice, KCs are identified by their expression of the pan-macrophage marker F4/80, low expression of CD11b, as well as by expression of specific markers such as the C-Type Lectin Domain Family 4 Member F (CLEC4F) or T-Cell Immunoglobulin and Mucin Domain Containing 4 (TIM4) [41–43]. Their development occurs during embryogenesis, from yolk-sac precursors that populate the foetal liver [44–47]. Like other tissue-resident macrophages, KCs are thought to persist in adult mice by self-renewal [48]. From surveillance, to recycling iron and promoting immune tolerance, KCs play important homeostatic roles in normal liver physiology (Fig. 2A).

KCs are located in liver sinusoids, and they continuously survey blood for metabolites and microbial products [41]. Mice lacking KCs show impaired survival following *Listeria monocytogenes* infection, emphasising their importance for the depletion of blood-borne bacteria [49,50]. Similarly, KCs remove damaged or apoptotic cells [51,52]. KCs also have important roles in iron and cholesterol metabolism. They are able to detect and phagocytose damaged erythrocytes and erythrocyte-derived vesicles containing haemoglobin [53,54]. KCs also influence iron reabsorption by regulating hepatocyte hepcidin expression [55]. With regard to cholesterol, all macrophages metabolise lipids, as required by their canonical function of phagocytosing cellular debris and processing lipid-rich elements such as membranes. However, relative to other tissue-resident macrophages, the KC transcriptome is enriched with genes that uptake, process and export cholesterol to extracellular high-density lipoprotein acceptors [42]. Indeed, KCs highly express cholesteryl ester transfer protein (CETP) amongst other genes in lipid processing, which are controlled by well-known transcription factors that regulate cellular lipids (e.g. PPARs, LXR) [42,56]. Physiologically, KCs may require this high lipid processing capacity to cope with dynamic cholesterol synthesis in the liver or to cope with exposure to systemic lipids packaged into lipoproteins in the liver. While KCs are clear drivers of inflammation in NAFLD, [57] their activation spectrum remains to be defined (in the context of M1-/M2-like polarisation), similarly questions remain unanswered with regards to their capacity to accumulate lipids, such as adipose or vascular foams cells, and with regard to their persistence in later stages of NAFLD [58–61].

Immunologically, KCs promote immune tolerance by diverse mechanisms, and their capacity to present antigens and activate T cells is very limited [62]. In mice, as well as in humans, KCs secrete anti-inflammatory cytokines such as interleukin (IL)-10 [63,64]. They also express co-inhibitory molecule Programmed Death Ligand (PD-L)-1, a potent inhibitor of T-cell activity [64]. They can also induce regulatory T-cell (TReg) differentiation through secretion of prostaglandins [62,64]. Monocyte-derived macrophages (Mo-MPs) or RHMs can also populate the liver and differentiate from C-C motif chemokine receptor (CCR)-2+ C-X-C 3 chemokine receptor (CX3CR)-1+ lymphocyte antigen (Ly)-6C+ monocytes. Mo-MPs account for a minority of macrophages in the healthy liver [65] but can be rapidly recruited upon liver injury [66] and can persist in chronic diseases such as NAFLD. Like KCs, they are important for erythrocyte clearance and iron recycling during homeostasis [67]. More recently, a third type of macrophage was reported in the liver capsule and these capsular macrophages are derived from bone marrow and play a role in peritoneal-derived pathogen clearance [68].
Modelling NAFLD physiopathology

To study NAFLD physiopathology and allow the isolation of different cell fractions, including macrophages, murine models are indispensable. Modelling NAFLD in mice comes with its challenges and opportunities. Resistance of mice to spontaneously develop NAFLD upon high-fat feeding had initially led scientists to develop various models that recapitulate isolated events in the disease. In this light, a high-fat diet (HFD) can recapitulate simple steatosis and insulin resistance, while carbon tetrachloride (CCL4) induces inflammation and fibrosis without steatosis and a methionine-and-choline-deficient (MCD) diet results in fibrosis, inflammation and steatosis without insulin resistance [31,69,70]. Similarly, surgical ligation of the bile-duct induces cholestatic injury, inflammation and fibrosis in mice, without insulin resistance [71]. Genetic models such as the widely adopted ob/ob or db/db mice can recapitulate obesity, insulin resistance and to a slight degree liver inflammation, but do not progress beyond steatosis [69]. These above models may be
considered extreme and can only be interpreted as models due to their lacking holistic systemic representation of NAFLD and its comorbidities.

More holistic models exist today that recapitulate a larger part of the NAFLD spectrum, such as the choline-deficient HFD (CD-HFD), high fructose-HFD (HF-HFD) or genetic-based models including the sterol regulatory element-binding protein-1c (SREBP1c) transgenic mouse, methionine adenosyl transferase 1A knockout mouse with high transient expression of urokinase plasminogen activator in hepatocytes (MUP-uPA Tg) and the DIAMOND model [69]. These models, and others, have been recently reviewed in-depth by Febbraio et al. [69]. Briefly, through different mechanisms, these models have been shown to recapitulate obesity, insulin resistance, steatosis, inflammation, ER stress and fibrosis in NASH, including a transition towards HCC [69]. Of these models, CD-HFD is gaining popularity, where the lack of choline prevents cholesterol export from hepatocytes, resulting in lipotoxicity and progression of NAFLD. Mice on CD-HFD develop obesity, insulin resistance, glucose intolerance and NAFLD. However, whether the status of other tissues is modified by the lack of choline has not been investigated. The MUP-uPA model recapitulates obesity, insulin resistance and glucose intolerance on HFD, where the key mechanism of hepatocyte ER stress (due to uPA over-expression) leads to mice consistently developing NASH, and up to 85% spontaneously progressing to HCC [69,72].

Choice of model and understanding the mechanisms by which NAFLD and NASH develop are critical to correct interpretation of results. In the case of macrophage responses, most models recapitulate the inflammatory hit, to varying degrees, and thus most are applicable. However, results must always be interpreted within the constraints and contexts of the given model, especially in the case of toxic models of fibrosis (CCl4) or in models of global knockout or knockin (e.g. ob/ob, db/db or SREBP1c Tg).

Macrophages in NAFLD physiopathology

Macrophages are drivers of NAFLD, and human studies show positive correlations between macrophage numbers in the liver and NAFLD severity [73,74]. In mouse models, early depletion of KCs prevents progression of the disease, as well as insulin resistance [75–77]. In macrophage-depleted mice, IL-1β production was decreased while levels of the protective factor peroxisome proliferator-activated protein (PPAR)-α was increased in hepatocytes [78,79]. Additionally, preventing monocyte entry into the liver through CCR2 blockade improves NASH [74,80] and it is now widely accepted that monocyte recruitment and in situ differentiation into macrophages fuels NAFLD progression. In NASH, macrophages replace a fraction of the KC pool by differentiating into monocyte-derived KCs (Mo-KCs) [42,43] which express KC markers, but are functionally different. Mo-KCs express more inflammatory genes potentially contributing to disease progression [43].

Macrophages are at the heart of intense cellular crosstalk in NAFLD, interacting with many liver cell types (Fig. 2B). Macrophages recognise hepatocyte-derived Danger-Associated Molecular Patterns (DAMPs), they secrete cytokines that may alter hepatocyte physiology and promote NAFLD progression [79]. Macrophage-derived cytokines also target hepatic stellate cells (HSCs). Tumour necrosis factor (TNF)-α, IL-1β and transforming growth factor (TGF)-β can all induce HSC activation [81,82]. In turn, HSCs up-regulate several ligands able to attract macrophages and regulate their activity (like CCL2) in NASH [83]. Liver sinusoidal endothelial cells (LSECs) drive anti-inflammatory polarisation of macrophages and down-regulate cytokine and chemokine secretion through nitric oxide production [84]. However, LSECs can also promote monocyte infiltration and contribute to liver inflammation [85,86]. Finally, macrophages can interact with other immune cells. Cytokine secretion by activated T cells can then reinforce macrophage pro-inflammatory phenotype in a feed-forward loop [87]. Additionally, chemokine secretion by activated macrophages leads to recruitment of several immune cell types in the liver [88].

**Macrophage subtypes in the liver**

The optimisation of single-cell RNA sequencing (scRNA-seq) in recent years has allowed the more precise identification of macrophage subpopulations. Several macrophage subpopulations have been defined in NAFLD. One study identified KCs and three different populations of Mo-MPs. While it is surprising to see the presence of such Mo-MPs already under a normal diet, these cells were enriched in mice fed a western diet (WD). They express less calprotectin, a marker of inflammation, in WD-fed mice, suggesting that these subsets may be protective [65]. Another study in amylin diet-induced NASH showed both KCs and Mo-MPs displayed a pro-inflammatory phenotype compared with controls. Two KC subsets were identified and segregated based on triggering receptor expressed...
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on myeloid cells (TREM)-2 expression. TREM2-low KCs were predominant in mice fed a normal diet. TREM2-high KCs were almost exclusively found in NASH and were therefore called ‘NASH-associated macrophages’ (NAMs) [83]. These two reports independently identified different functionally and phenotypically diverse subsets of macrophages in NAFLD. Interestingly however, subsequent reanalysis demonstrated that similar macrophage subsets were found in sequencing data from both studies. This highlights the divergent views in the field with regard to the identity of resident macrophages [89]. Specifically addressing fibrosis, two populations of Mo-MPs have been described based on Ly6C expression. Ly6C<sup>hi</sup> cells are pro-fibrogenic and express cytokines that are able to activate HSCs such as IL-1β and TGFβ whereas Ly6C<sup>lo</sup> cells derived from Ly6<sup>hi</sup> monocytes promote resolution of fibrosis and express matrix-degrading factors such as metalloproteinases [90].

**Macrophage polarisation in NAFLD**

Depending on microenvironmental cues, macrophages display different functional phenotypes. Typically, macrophages have been divided into classically activated M1-like or alternatively activated M2-like macrophages. Macrophages adopt a M1 phenotype in response to Toll-like receptor (TLR) stimulation for example with lipopolysaccharide (LPS), and in response to type 1 cytokines such as interferon (IFN)-γ. These macrophages produce pro-inflammatory cytokines such as IL-1β or TNF-α and are potent antigen presenting cells that are able to induce T<sub>H</sub>1/T<sub>H</sub>17 cell responses. As a consequence, they are involved in inflammatory responses and are responsible for pathogen killing. On the contrary, M2 macrophages secrete anti-inflammatory cytokines such as IL-10 or TGF-β and elicit T<sub>H</sub>2/T<sub>R</sub>E<sub>reg</sub> responses. They respond to extracellular pathogens, are more tolerogenic and are associated with resolution of inflammation and tissue repair [91].

In a number of tissues, including the liver, M1 macrophages are generally characterised by the expression of CD11c, while CD206 is the common marker of M2 macrophages [92]. With increasing mechanistic studies and novel technologies, this framework has now developed to offer a more comprehensive representation of macrophage heterogeneity and functional diversity. For example, functionally diverse M2 macrophage subtypes have now been identified: M<sub>2a</sub>, M<sub>2b</sub>, M<sub>2c</sub> and M<sub>2d</sub> [93]. The emergence of transcriptomics and scRNA-seq technologies has further refined that description by emphasising the diversity of tissue macrophages and the highly spectral nature of macrophage polarisation [94,95].

Macrophage polarisation is an important parameter influencing NAFLD progression. Reports with regard to polarisation of liver macrophages in metabolic diseases have been conflicting. While high-fat diet (HFD) has been predominantly associated with M1 polarisation of liver macrophages [58,96], other groups have recently demonstrated that liver macrophages regulate systemic metabolism upon HFD through predominantly non-inflammatory signalling [40]. Impaired M2 polarisation was associated with impaired hepatic lipid metabolism and steatosis [97]. In addition, M2 macrophages were shown to induce M1 macrophage apoptosis [96]. Overall, studies suggest that pro-inflammatory macrophages are mostly detrimental in NAFLD while anti-inflammatory macrophages are protective, this remains highly dependent on the stage of NAFLD progression. Indeed, studies have pointed out detrimental roles for M2 macrophages, for example during long-term HFD [60,98]. Polarisation state has different effects depending on disease stage, for example M2-like macrophages may mitigate inflammation in the early stages of NAFLD but promote matrix deposition and fibrosis at later stages.

**Molecular mechanisms of macrophage polarisation in NAFLD**

**Molecular drivers of macrophage inflammatory status**

Macrophage polarisation, and more generally the role of macrophages in liver inflammation during NAFLD, is controlled by multiple pathways and transcription factors. The activity of these transcription factors is triggered by molecular cues coming from the liver but also from other organs of the body. While diverse in nature, in the context of NAFLD, these cues can be divided into two main categories: DAMPs and cytokines, both of which act through cell surface receptors and lead to transcriptional reprogramming in macrophages (Fig. 3).

**TLRs and TLR ligands in liver macrophage polarisation**

Toll-like receptors are transmembrane proteins expressed by cells of the innate immune system, notably macrophages, and that are activated in response to DAMPs, thirteen TLRs have been identified in mice [99]. Several TLRs play important roles in macrophages during NAFLD progression. TLR4, which
responds to bacterial LPS, is pivotal in KCs activation. Steatosis promotes lipid accumulation in macrophages, which become more sensitive to LPS-mediated TLR4 stimulation, promoting inflammation [100]. This is of particular relevance since microbial dysbiosis and microbial products coming from the gut are increasingly shown to potentiate NAFLD [101]. Other TLRs and their ligands play a part in macrophage polarisation during NAFLD. Dying hepatocytes, for example, release DAMPs that are recognised by TLRs. Histidine-rich glycoprotein (HRG), a protein that is abundantly produced by hepatocytes, induces pro-inflammatory cytokines in macrophages, even though the receptor and transduction pathway implicated are yet to be discovered. As a result, HRG-deficient mice were partially protected against steatohepatitis induced by a MCD diet [102]. Hepatocyte-derived mitochondrial DNA and extracellular vesicles also trigger a pro-inflammatory phenotype through respective ligation of TLR9 and Death receptor 5 (DR5) and therefore also drive NAFLD progression [103,104]. Finally, free fatty acids, especially saturated fatty acids, have been associated with macrophage-driven inflammation. Saturated fatty acids induce IL-1 and inducible nitric oxide synthase (iNOS) expression in macrophages in vitro through TLR4-dependent nuclear factor (NF)-κB.
activity while unsaturated fatty acids inhibit this process [105]. In another report, palmitate was shown to induce IL-1 expression after TLR2 stimulation [106]. TLR4-palmitate interaction in infiltrating macrophages has also been shown to drive NAFLD, indicating that different macrophage subsets may have specificities in recognising different sources of free fatty acids [107].

Cytokine signalling and liver macrophage polarisation

During NAFLD, activated KCs become pro-inflammatory and secrete cytokines such as IL-1β, IL-6 or TNF-α which promote inflammation and monocyte recruitment to the liver [108]. Several cytokines play key roles in NAFLD. Early TNF-α secretion by KCs drives hepatic steatosis and inflammation [108,109]. Similarly, IL-1β is a potent driver of NAFLD and produced by KCs in the early phases of the disease [108,110]. It has an important role in promoting monocyte recruitment, inflammation and steatosis [79,110]. In addition, both TNF-α and IL-1β, as well as TGFβ, promote liver fibrosis by activating HSCs and promoting their survival [81,82]. IL-6 has a more complex role in the course of NAFLD. It seems to have a protective effect against liver injury and hepatic death but may promote NASH progression and fibrosis at high levels [111,112].

Other cytokines favouring M1 polarisation, while less studied, also contribute to NAFLD progression [113]. Interestingly, IFNγ deficiency has been associated with decreased production of pro-inflammatory cytokines, decreased inflammation and fibrosis in a mouse model of NASH [114]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) promotes M1 polarisation and subsequent fibrosis in a model of virus-related fibrosis, suggesting that it could also play a similar role during NAFLD [115]. However, M2-polarising cytokines such as IL-10 also have a role in NAFLD. IL-10 production was reported in livers from mice fed a HFD alongside pro-inflammatory cytokines. Its blockade is associated with increased TNF-α and IL-1β levels and impaired insulin sensitivity [116].

Transcriptional control of liver macrophage polarisation

Transcriptional control downstream of TLR ligation

In response to their respective ligands, TLRs can trigger different intracellular pathways. TLR3 and TLR4 activate NF-κB, Activator protein (AP)-1 and Interferon Regulatory Factors (IRF)-3 and -5 while TLRs 7, 8 and 9 activate IRF7 instead of IRF3 (Fig. 3) [99].

In quiescent macrophages, NF-κB activity is hindered by inhibitor of κB (IκB). Upon TLR stimulation, phosphorylation of IκB releases NF-κB, and NF-κB then translocates to induce transcription of target genes [117]. NF-κB is a key regulator of M1 polarisation [118,119]. It is responsible for production of pro-inflammatory cytokines such as IL-1, IL-6, IL-12 or TNF-α [118]. During NAFLD, NF-κB seems to have an important role in triggering inflammation. Indeed, one upstream regulator of NF-κB, glucocorticoid-induced leucine zipper (Gilz), has been shown to be down-regulated in macrophages during NAFLD. Its overexpression in macrophages results in decreased pro-inflammatory cytokine secretion and decreased hepatic inflammation [120]. Decreased activity of NF-κB due to loss of Protein tyrosine phosphatase receptor type O truncated isoform (PTPROt) activity in liver macrophages is also associated with decreased inflammation [121]. AP-1 is a complex formed of 2 proteins, c-Jun and c-Fos. After TLR stimulation, AP-1 is activated through c-Jun phosphorylation by MAPK, specifically by p38 and c-Jun N-terminal kinase (JNK). AP-1 and NF-κB are closely linked and regulate similar transcriptional programmes [122]. JNK has been shown to promote M1 polarisation of adipose tissue macrophages, while pro-inflammatory cytokine production in response to palmitate in vitro is JNK-dependent [123,124]. Studies in other macrophages populations indicate a strongly pro-inflammatory role for AP-1, yet its role and regulation in liver macrophages remains to be entirely elucidated. A recent study has reported that c-Jun/AP-1 plays different roles in hepatic cells and in nonparenchymal liver cells (NPLCs, a significant proportion of which is macrophages) [125]. Schulien et al report that while expression in hepatocytes correlated with transition from steatosis to NASH, c-Jun expression in NPLCs specifically correlated with fibrosis. In hepatocytes, c-Jun promotes survival, preventing the regenerative ductal reaction and fibrosis, whereas in NPLCs c-Jun promotes ductal regeneration and fibrosis through regulating both osteopontin and CD44 expression [125]. Whether this mechanism is mediated wholly or in part by macrophages remains to be demonstrated. A recent study of macrophage-specific p38 deficiency demonstrated that, as canonically described, p38 maintains its pro-inflammatory actions in liver macrophages and this promotes the progression of NASH [126].

IRF3 has a more complex role in macrophage polarisation. BMDM differentiated with Macrophage Colony-Stimulating Factor (M-CSF) display are
predominantly M2-like and activate IRF3 [127]. Its overexpression in microglia blunts the production of IL-1β and TNF-α in response to IL-1β and IFNγ, while boosting IL-10 secretion [128]. However, IRF3 also triggers the expression of pro-inflammatory factors CCL5 [129] and IFN-β, as well as CXCL9 and CXCL10 [130]. Studies showed that the Stimulator of Interferon Genes (STING)-IRF3 cascade is activated in livers of mice fed a HFD [131]. Upon inactivation of STING in myeloid cells, inflammation and steatosis are decreased, suggesting a potential role for this axis in regulating liver macrophage inflammatory status [132]. Likewise, IRFs 5 and 7 has been associated with M1 polarisation in response to LPS [133,134]. Following TLR signalling, IRFs 3, 5 and 7 are responsible for type I IFNs production [99,135]. Type I IFNs are increasingly regarded as important players in NAFLD. In particular, they have been shown to induce T-cell recruitment, secretion of pro-inflammatory cytokines and subsequent insulin resistance [92,136].

Other transcription factors, such as hypoxia inducible factor (HIF)-1α, can be indirectly involved in TLR-mediated macrophage polarisation. HIF-1α is stabilised and activated in response to hypoxia [137,138]. It was shown to induce M1 polarisation in vitro and to have an important role in macrophage function, both under normoxic or hypoxic conditions [139,140]. TLR stimulation can also induce HIF-1α activity though transcriptional control by NF-κB [141,142]. Liver macrophages from mice fed a MCD diet have enhanced HIF-1α expression. Mice overexpressing HIF-1α in myeloid cells display increased levels of pro-inflammatory cytokines and increased steatosis compared with controls, both under a chow diet or MCD [143,144]. Moreover, palmitate was shown to induce HIF-1α activity [143,144]. These results suggest a macrophage-specific role for TLR-induced HIF-1α in liver inflammation and pathology in NASH. A role for HIF-1α in liver fibrosis has also been reported even if the precise mechanisms still remain to be elucidated [145]. Additionally, HIF-1α is able to increase TLR4 in response to hypoxia, thereby sensitising macrophages to LPS stimulation [137]. Hypoxia has been reported to happen during NAFLD [146], which suggests that HIF-1α may also increase hepatic inflammation indirectly through macrophage sensitisation to TLR ligands.

Transcriptional control through cytokine signalling

Cytokines such as IFN-γ, IL-1β, IL-4 or IL-10 can activate different transcription factors to orient macrophage polarisation, among which NF-κB, AP-1 or members of the IRF and Signal Transducer and Activator of Transcription (STAT) families.

Interferon regulatory factors

The IRF family comprises nine members of transcription factors [147]. IRFs 3, 5 and 7 have critical roles in M1 polarisation. GM-CSF-treated macrophages display an M1-like phenotype and highly express IRF5 in particular. Macrophages transfected in vitro with a siRNA targeting IRF5 lose their ability to produce pro-inflammatory cytokines like IL-12 in response to LPS [148]. Alzaid et al. showed that IRF5 is also metabolically responsive and its expression in macrophages during NAFLD was responsible for M1 polarisation and secretion of pro-inflammatory and pro-apoptotic mediators. This translated into liver inflammation, Fas-dependent hepatocyte death and fibrosis [92,149].

Other members of the IRF family can induce M1 polarisation. IRF1 expression is induced in vitro in macrophages treated with IFN-γ. IRF1 can then synergise with NF-κB to trigger IL-12, iNOS and IFN-β expression [150]. IRF8 is activated through the Notch pathway during LPS stimulation and is crucial for transcription of typical M1-related genes in this context [151]. It was shown to collaborate with other transcription factors, such as IRF1 or STAT1, to drive pro-inflammatory genes transcription in response to IFN-γ [152]. On the contrary, IRF4 has a clear role in M2 polarisation in response to IL-4. IRF4-deficient macrophages secrete more cytokines such as TNF-α, IL-6 but also IL-10 [153]. Lysine demethylase 6B (KDM6B) was shown to enhance IRF4 production in IL-4-stimulated macrophages, an event directly promoting M2 polarisation [154]. Additionally, IRF4 can suppress IRF5 activity by competing for binding to the Myeloid differentiation primary response 88 (MyD88), a crucial adaptor protein in TLR signalling [155].

Signal transducers and activators of transcription

The STAT family is composed of seven members [156]. STAT1 is phosphorylated and activated in response to IFN-γ, one of the canonical stimuli of M1 polarisation [157]. STAT1-deficient macrophages lose the induction of IFN-γ-activated genes such as inducible nitric oxide synthase (iNOS) or Class II major histocompatibility complex transactivator (CIITA) [158]. STAT1 also plays a key role in type I IFNs ability to induce M1 macrophages through STAT1:STAT2 dimers and IFN-stimulated gene factor 3 (ISGF3)
[159,160]. Likewise, STAT5 is classically regarded as inducing M1 polarisation in response to GM-CSF [127]. However, broader assessment of GM-CSF-responsive genes has revealed that STAT5 may in fact induce both M1- and M2-related genes, resulting in an intermediate phenotype [161]. STAT3 and STAT6 promote M2-like macrophage polarisation. STAT6-deficient macrophages lose their ability to respond to IL-4 [162] and the ability to induce a number of M2-related genes [160]. IL-6 and IL-10 induce STAT3, and STAT3-deficiency leads to greater accumulation of pro-inflammatory macrophages and susceptibility to inflammatory conditions, namely enterocolitis [163–165].

**Nuclear receptors in gene regulation and as therapeutic targets in NAFLD and NASH**

The nuclear receptor (NR) superfamily of transcription factors control transcription in response to specific ligands [166]. Agonists for these receptors range from hormones to vitamins, to fatty acids and cholesterol [167] as well as synthetic and pharmaceutical ligands that currently represent about 16% of all approved drugs [168]. NRs play important functions in regulating hepatic lipid and glucose metabolism as well as multiple inflammatory pathways and immune responses. As such, they are prime candidates to modulate NAFLD development [169]. Indeed, many NRs have shown promising potential as targets for anti-NAFLD therapeutics. To date, 48 NRs that share structural and functional characteristics have been described in humans [166]. Of these, 17 have been linked to NAFLD, either using synthetic ligands that target them in experimental models of disease or in models of global, hepatic- or, in few cases, myeloid-specific NR deficiency showing changes in liver steatosis and/or the development of steatohepatitis. For a detailed description of how these receptors function and the roles they play, we refer the reader to a recent comprehensive review [169]. Here, we focus on receptors that are or have been drug targets in clinical trials for NAFLD and NASH.

**Thyroid hormone receptor β**

Thyroid hormone (TH) receptor β (TRβ) is the TR isoform thought to be responsible for the main beneficial effects of TH on liver [170]. TRβ regulates gene expression by binding to TH response elements (TREs) in regulatory regions within target genes, mostly as heterodimers with the retinoid X receptor or RXR [171]. Unliganded TR represses basal gene expression by recruiting a corepressor complex [172]. Ligand (TH) binding then leads to the dissociation of corepressors and favours the recruitment of coactivators promoting chromatin accessibility thereby increasing gene transcription [173]. In this manner, TR enhances the expression of genes involved in fatty acid metabolism [174]. TR also inhibits the expression of lipogenic genes promoting steatosis [175].

TH metabolism and TH status have been linked to various aspects of the immune response [176] and recent reports suggest that innate immune cells are important TH targets and that intracellular TH plays essential roles on several innate immune cell types, including monocytes and liver macrophages [176]. Functional studies have shown TH pro-inflammatory actions in macrophages. A shift towards an M1 phenotype alongside an inhibition of M2 polarisation was reported in bone marrow-derived macrophages [177]. Intriguingly, polarisation was associated with changes in TRβ1/2 ratio, suggesting the relative abundance of TR isoforms may be linked to macrophage phenotype [177]. However, this study contrasts with reports that found no effect on macrophage polarisation [178] and it has been speculated that this could be due to differences in the hormone concentrations used [179].

Some TH actions are mediated through signal transduction mechanisms, for instance, through cell surface integrins leading to PI3K activation followed by the iNOS upregulation, nitrite production and bacterial killing [180]. Other studies have concluded that higher levels of bioavailable TH increase macrophage phagocytic capacity [181]. The effects of intracellular TH are partly mediated via TRα [182] and unstimulated macrophages deficient in TRα show low-grade inflammation suggesting a TRα-mediated anti-inflammatory response [182]. TH stimulation leads to KC hyperplasia and enhanced phagocytosis [183]. TH has shown pro-inflammatory actions in KCs involving NFκB activation [184] and acute-phase responses in liver involving increased STAT3 activation [185], in turn increasing hepatic iNOS activity, enhancing production of reactive oxygen species and hepatic oxidative stress [186]. However, another study showed conflicting results in models of endotoxemia [187]. Clearly, more research is needed to establish whether similar mechanisms occur in other inflammatory contexts including NAFLD and to establish better in vitro models to replicate not only the inflammatory, but also dyslipidaemic environment in this disease.

Overall, THs have been shown to be beneficial for liver metabolism through: (a) an increase in energy
expenditure via ATP consumption, membrane permeability and effects on mitochondrial biogenesis and activation [188], and (b) lipid metabolism such as cholesterol clearance by LDLR, cholesterol biosynthesis and metabolism through regulation of CYP7A1, a key bile acid synthesis enzyme [189]. In addition, hypothyroidism has been considered a risk factor for NAFLD [190], while TH administration improves lipid profiles in experimental models of NAFLD [191]. Unfortunately, these beneficial effects are accompanied by thyrotoxicosis and harmful effects in the brain [192]. Work on animal models stresses the importance of TRs for the hepatic actions of TH [193]. Using individual TRα1 and TRβ, knockout mice treated with TH and dietary cholesterol showed that CYP7A1 regulation was lost only in TRβ knockout mice and that TH administration was not able to modulate cholesterol levels [194], suggesting a key role for TRβ. Consistently, TRβ mutant mice are unable to bind TH and develop liver steatosis [195].

As TRβ is the predominant isoform in liver, efforts have focussed on the development of TH analogs capable of uncoupling beneficial liver actions (triglyceride and cholesterol lowering) from deleterious effects [196]. TRβ agonists modulate lipid metabolism pathways and reduce hepatic steatosis and inflammation in animal models [197] as well as improve liver function in clinical trials in patients with NAFLD and NASH [191]. Unfortunately, Sobetirome (GC-1) and Eprotirome (KB2115), early examples of TRβ-selective thyromimetics showing encouraging effects against hypercholesterolemia and NASH, in the absence of adverse side effects, were stopped after Phase 1 and Phases 2–3 clinical trials, respectively [170]. Resmetirom (MGL-3196), another liver-directed and TRβ-selective agonist, successfully reduced steatosis and was advanced to Phase 3 trials. Other compounds are being evaluated in Phase 2 trials [198] or have shown promising preclinical effects [199]. These studies suggest that the most recent classes of thyromimetics are promising alternatives to existing NASH therapies.

**Peroxisome proliferator-activated receptors**

Peroxisome proliferator-activated receptors (PPARs) are a nuclear receptor subfamily with key actions on glucose and lipid metabolism as well as on inflammatory and fibrotic processes. Thus, PPARs are considered interesting NAFLD therapeutic targets for improving liver function and showing beneficial liver, cardiovascular and diabetes-related outcomes [200]. The role of PPARs in the development of NAFLD has been recently reviewed in detail [201].

PPARs were first described as ligand-activated transcription factors that promote peroxisome proliferation [202], and subsequently, they have been shown to be involved in function of other organelles, mainly mitochondria, showing pleiotropic actions [203]. Three PPAR isotypes have been described – α, β/δ and γ, with two subtypes: γ1 and γ2, and with each isotype showing a specific pattern of tissue and cell-type expression [203]. Additionally, substantial species-specific differences, especially for PPARα, exist and must be considered when translating findings from experimental models [204]. Specifically, PPARα activity in human liver is lower compared with rodents with reported differences in PPARα expression, ligand activation and biological responses [205].

**PPARα**

PPARα, encoded by the NR1C1 gene, binds to several saturated and unsaturated fatty acids, whereas the other isotypes show affinity mostly restricted to polyunsaturated fatty acids [206]. PPARα is predominantly expressed in tissues with high fatty acid oxidation rates such as skeletal muscle, liver – mostly in hepatocytes – heart, kidney and brown adipose tissue [207]. Besides hepatocytes, PPARα is expressed in sinusoidal endothelial cells and in HSCs [208]. In the liver, this nuclear receptor acts as a nutrient sensor and its expression and activity are stimulated by fasting or a fat-rich diet [209]. PPARα functions as a transcription factor mostly as a heterodimer with RXR and, upon ligand binding, activates genes associated with mitochondrial and peroxisomal fatty acid oxidation [210]. PPARα can also repress gene expression, by interfering with the glucocorticoid receptor [211] or by tethering to other transcription factors [212]. Regarding NAFLD development, it is worth noting that a fat-rich diet elevates hepatic PPARα expression in a circadian rhythmic manner and that the lipid-lowering effect of a PPARα agonist is more prominent when PPARα expression peaks [212,213]. Additionally, PPARα dampens NASH fibrotic and inflammatory gene expression through protein–protein interactions with pro-inflammatory transcription factors NF-kB and AP-1 [210,214].

Multiple studies in preclinical models or PPARα-deficient mice show PPARα is a critical regulator of target genes involved in fatty acid metabolism and ketogenesis. Specifically, it regulates fatty acid transport, peroxisomal and mitochondrial β-oxidation and lipolysis, and influences the production of apolipoproteins [215]. This reduces triglyceride-rich lipoproteins.
and triglyceride accumulation in the liver, whereas plasma HDL cholesterol is increased [215]. Consistently, preclinical studies show that deficiency in PPAR\(\alpha\), either in global or liver-specific-deficient mice, leads to more severe NASH [216,217], which can be improved or prevented by specific PPAR\(\alpha\) ligands [217–219]. Interestingly, expression of a PPAR\(\alpha\) mutant that only shows transrepressive activity in mice confers protection against NASH but not steatosis, whereas mice expressing wild-type PPAR\(\alpha\) are protected from both NASH and steatosis [210] highlighting the importance of this activity in the overall effects of PPAR\(\alpha\).

Considering that approximately 50% of PPAR\(\alpha\) target genes are conserved between mice and humans [220], it is relevant that this experimental evidence agrees with existing clinical findings (see below). Additionally, hepatic PPAR\(\alpha\) expression inversely correlates with severity in patients with NASH, visceral fat and insulin resistance, and improved liver histology positively correlates with increased PPAR\(\alpha\) expression [221]. Accordingly, PPAR\(\alpha\) was considered a promising therapeutic target for NAFLD, although the number of clinical studies evaluating single PPAR\(\alpha\) ligands is low [201,218]. Drugs of the fibrate class that predominantly act as PPAR\(\alpha\) ligands such as Clofibrate and Fenofibrate have been used clinically to treat hypertriglyceridemia, without affecting insulin sensitivity or hepatic steatosis [222–225]. Disappointingly, their effect on NASH was not proven [226,227], which could be due in part to the species-specific differences mentioned above. Exploiting the concept of selective PPAR modulators based on differences in receptor and coactivator binding, other fibrate compounds (Gemfibrozil, Pemafibrate) are currently being tested in clinical studies based on their promising clinical profiles [228–230]. In addition, targeting both PPAR\(\alpha\) and PPAR\(\beta/\delta\) with Elafibranor has shown promising anti-NASH properties in a clinical trial [231], reporting improved glycaemic control and lipid profile, reduction in hepatic and muscle insulin resistance and steatohepatitis [232]. Recruitment was recently terminated on the phase III RESOLVE-IT clinical trial (NCT02704403) assessing Elafibranor for NASH resolution [233]. Results at termination of the study have not yet been published; however, interim analyses in May 2020 revealed a near significant \((P = 0.066)\) resolution of NASH without worsening fibrosis in patients treated with Elafibranor compared with placebo [234]. Elafibranor was found to be safe and well tolerated, consistent with a previous study in biliary cholangitis that reported improvement in a number of disease markers [234,235].

**PPAR\(\beta/\delta\)**

PPAR\(\beta/\delta\) which is encoded by NR1C2 and expressed in hepatocytes, sinusoidal endothelial cells, HSCs and KCs also has an important role in liver metabolism [236]. This receptor activates glucose utilisation, hepatic lipogenesis and lipoprotein metabolism, as confirmed by transcriptomic analyses in PPAR\(\beta/\delta\)-deficient mice [237]. In addition, PPAR\(\beta/\delta\) increases the production of monounsaturated fatty acids and protects against lipotoxicity and saturated fatty acid cytotoxicity *in vitro* [238]. It appears PPAR\(\alpha\) is predominant in the fasting state whereas PPAR\(\beta/\delta\) is equally involved in both fasting and fed states [239].

PPAR\(\beta/\delta\) also regulates the expression of key genes in innate immunity and inflammation [237,240]. In the absence of ligand, PPAR\(\beta/\delta\) has pro-inflammatory effects mainly in atherosclerotic models. Ligand binding exerts anti-inflammatory effects, such as the suppression of pro-inflammatory adhesion molecules on endothelial cells [241,242]. PPAR\(\beta/\delta\) ligands promote a more anti-inflammatory phenotype in KCs resulting in improved metabolic and hepatic dysregulation [97]. In addition, PPAR\(\beta/\delta\) may have a potential role in wound healing, as its activation in fibroblasts increases \(\alpha\)-smooth muscle actin production and myofibroblast differentiation [243,244]. Importantly, synthetic PPAR\(\beta/\delta\) ligands mimic the endogenous activation of PPAR\(\beta/\delta\), although different responses have been reported for different ligands [245]. Additionally, the selective PPAR\(\beta/\delta\) agonist Seladelpar improves dyslipidemic lipid profiles in overweight or obesity patients at risk of CVD [246] although these compounds have not been as broadly tested as the fibrate PPAR\(\alpha\) agonists.

**PPAR\(\gamma\)**

Finally, PPAR\(\gamma\) is encoded by NR1C3 and is expressed in the liver, yet less than adipose tissue where it is a master regulator of multiple metabolic pathways. As other PPARs, PPAR\(\gamma\) forms a heterodimer with RXR to control gene expression. In addition, as shown in cistrome studies in peritoneal macrophages, macrophage lineage factors SPI1 (PU.1) and CEBP\(\beta\) are present with PPAR\(\gamma\) in regulatory sites [247,248], enhancing permissive chromatin configurations. In liver, PPAR\(\gamma\) is induced by obesity in mice [249] although this is not seen in patients with NASH [221]. Hepatocyte-specific PPAR\(\gamma\) deficiency protects mice from steatosis in diet-induced or genetic obesity in mice by reducing expression of genes promoting lipogenesis and lipid transport [250,251]. In contrast, PPAR\(\gamma\) agonists, including antidiabetic thiazolidinedione drugs (TZDs), improve
NAFLD partly by reshuffling fatty acids and triglycerides to privilege storage in adipose tissue [252].

PPARγ is also present in macrophages, KCs and HSCs. In liver and other tissues, PPARγ binds to the p65 subunit of the NF-xB complex to dampen NF-xB-driven inflammatory gene expression [253]. A PPARγ sumoylation-dependent pathway was described to mediate some of the anti-inflammatory actions of this receptor [254]. In KCs, PPARγ agonists inhibit pro-inflammatory gene expression leading to lower inflammation and hepatosteatosis [58]. Consistently, inhibition of PPARγ with a specific antagonist promotes the M2c anti-inflammatory phenotype in human monocyte-derived macrophages [255], although despite the concomitant induction of MerTK expression, cells do not show enhanced efferocytosis. In HSCs, PPARγ is predominantly expressed in the quiescent state and lowered in the activated state. Ligand activation in these cells or in experimental models of fibrosis reduces collagen levels, but the mechanism underlying this regulation still need to be refined [256,257]. Finally, PPARγ also improves endothelial cell inflammation and function in patients with diabetes and atherosclerosis [258], controls vascular homeostasis and decreases blood pressure in patients with diabetes, leading to reduced CVD risk [259].

Hepatic PPARγ expression is elevated in patients with NAFLD and NASH [260], and PPARγ agonists are promising therapeutics. The TZD class of PPARγ agonist antidiabetics, including rosiglitazone and pioglitazone. TZDs ameliorate steatosis and inflammation, but have shown only minimal reduction in fibrosis [258,259,261–263]. A PPARα/γ dual agonist Saroglitazar improves cardiovascular risk profiles in diabetics [264,265] and after promising results in animal models of NASH [266] is being tested in a randomised clinical trial [258].

This subfamily of nuclear receptors represents a great example of how simultaneous activation of multiple isotypes could be a more efficacious therapeutic approach by targeting multiple pathways that contribute to the development and progression of NASH. Early studies with Lanifibranor (IVA337), which activates all three PPAR subtypes and acts on multiple NASH-affected pathways [267,268], showed it was effective at preventing and even inducing regression of pre-existing fibrotic lesions in different organs [269,270]. This occurred in the absence of deleterious effects of TZDs while improving insulin sensitivity and lipid profiles in NASH [267]. Remarkably, Lanifibranor actions on inflammation, fibrosis and macrophage accumulation and activation seem stronger than single and dual PPAR agonists in several models of NASH [271]. Lanifibranor is now part of a phase IIb trial in patients with NASH without cirrhosis. To date, significant reductions in steatohepatitis, regression of fibrosis and improved glycemic control and lipid profile have been reported [272], suggesting pan-PPAR agonism could have a strong therapeutic potential and be a promising therapeutic strategy for NASH.

**Farnesoid X receptor**

Farnesoid X receptor (FXR), encoded by *NR1H4* gene, whose expression is attenuated in NASH patients [273], was originally labelled as an orphan receptor and subsequently considered ‘adopted’ as free or conjugated bile acids were recognised to be endogenous ligands [274–276]. Its impact on the regulation of key aspects of metabolic, inflammatory and fibrotic pathways has been recently covered in detail [277]. FXR is highly expressed in liver [274] and acts, through FXR response elements, mainly as a heterodimer with RXR [278]. The liver receptor homolog-1 (LRH-1) is also present in a substantial number of FXR-binding sites and induces gene transcription mostly in lipid metabolic pathways [279,280]. Other studies have proposed direct transcriptional repression by FXR in the regulation of lipoprotein metabolism and as an important contributor to its anti-inflammatory effects through a motif independent of the canonical one [281–284].

FXR is a well-established regulator of bile acid homeostasis showing tissue-specific roles in the liver and intestine [285]. Upon activation, FXR reduces the levels of its ligands by suppressing bile acid synthesis through CYP7A1 [275], an example of a negative feedback regulatory loop. In addition, FXR is critical in regulating the enterohepatic circulation of bile acids by affecting the expression of several transporters [286–289] and in regulating lipid and glucose homeostasis. FXR activation lowers blood lipid levels as it inhibits fatty acid synthesis [290,291], decreases hepatic secretion of VLDL [292] and increases triglyceride hydrolysis and clearance as well as fatty acid oxidation [293–296]. Conflicting evidence exists regarding FXR actions in glucose homeostasis [277] which could be due to species differences between humans and mice [297,298]. Nevertheless, FXR likely plays an important role as FXR-deficient mice develop steatosis, show elevated circulating FFAs and glucose levels, and are insulin resistant [299]. In addition, FXR activation may improve glucose dysregulation, as either FXR activation or hepatic overexpression significantly lowers blood glucose levels and FFA levels, and improves...
insulin sensitivity in both db/db diabetic and wild-type mice [300].

FXR is also a homeostatic regulator that suppresses liver inflammation and fibrosis. Pretreatment of HepG2 cells and primary hepatocytes with FXR agonists suppresses NF-κB-mediated inflammation in an FXR-dependent manner [301]. In NASH models, synthetic FXR agonists lower MCP-1 chemokine expression leading to significantly reduced hepatic inflammatory cell infiltration [301,302]. Moreover, FXR-deficient mice display strong hepatic inflammation in response to LPS, concomitant liver necrosis and a significant increase in inflammatory molecules such as iNOS, COX-2 and IFN-γ [301]. A growing body of evidence suggests that bile acids modulate intestinal and liver immune cells [303–305] and the role played by bile acid receptors has been reviewed in detail [303]. Briefly, FXR is expressed by circulating monocytes and both intestinal and liver macrophages [306]. FXR activation in human and rodent macrophages shows effective anti-inflammatory activities, and FXR is required for the TLR9-dependent inhibition of pro-inflammatory responses of intestinal macrophages [307]. Transrepression of inflammatory genes in macrophages by FXR ligands involves complex mechanisms that are both SHP-dependent and SHP-independent [306–308]. In addition, ligand-induced sumoylation of FXR has also been implicated in the regulation of NF-κB and API1-driven gene expression [309]. Beyond NF-κB-mediated mechanisms, FXR may exert anti-inflammatory actions indirectly, for instance by reducing cholestasis and the levels of toxic bile acid production and accumulation in the liver [277].

Macrophage phenotypic shift has also been described for FXR. Treatment of an obese and diabetic mouse model of NAFLD mice with the semi-synthetic bile acid and FXR agonist obeticholic acid (OCA) improves liver histology and increases expression of M2 markers and the proportion of intrahepatic anti-inflammatory monocytes [310]. In addition, non-specific ligands for FXR also acting on another bile acid receptor [311], reverse liver steatosis and fibrosis along with markers of inflammation, shifting macrophage polarisation towards an M2-like phenotype [311,312]. Whether these modulatory effects of the hepatic immune system add to the metabolic effects of FXR ligands in the clinic requires further investigations. Moreover, FXR activation suppresses the development of hepatic fibrosis both by reducing fibrosis and by inducing antifibrotic gene expression in HSCs. HSC inactivation is also achieved by ligand-activated FXR inducing a transcriptional regulatory cascade involving other nuclear receptors, namely the small heterodimeric partner SHP and PPARγ [256,313,314].

Both steroidal and nonsteroidal FXR agonists have been developed for the treatment of liver diseases. Based on previous favourable results [315], OCA was investigated in the phase IIb Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) multicentre trial in patients with noncirrhotic NASH [316]. OCA improved biochemical and histological features of NASH when compared with placebo without the worsening of fibrosis. Unfortunately, no difference was observed on the resolution of NASH and effects on ALP, lipids and blood glucose observed in the placebo group associated with weight loss were absent or even reversed in OCA-treated patients [317]. In addition, unfavourable dyslipidaemia occurred in the OCA treatment group [316]. OCA, FDA approved for biliary cholangitis therapy, was further evaluated in a NASH phase III trial REGENERATE [318], with a disappointing outcome [319]. Nevertheless, FXR remains an attractive target for NAFLD. For instance, safety and efficacy of the nonsteroidal FXR agonist Cilofexor (GS-9674) was evaluated in a phase II study for other liver conditions [320]. Cilofexor improved inflammatory biomarkers alongside significant reductions in serum markers of liver injury [320]. In a phase II trial in NASH noncirrhotic patients, Cilofexor significantly improved hepatic steatosis, liver biochemistry and bile acids without affecting serum lipids [321]. Moreover, Tropifexor significantly reduced oxidative stress, steatosis, inflammation and fibrosis in mouse models of NASH [322]. Time will tell whether this nonsteroidal FXR agonist also proves beneficial in NASH patients.

**Closing remarks: Challenges and future perspectives**

NAFLD is extremely complex, due to its multiple aetiologies and the large spectrum of liver states that ranges from steatosis to inflammation and fibrosis. The complexity of NAFLD is also present at the cellular and molecular levels, where we now know that macrophages play an important role in both maintaining normal physiology and in the pathophysiology of NAFLD. As reviewed, liver macrophages are central actors of NAFLD progression, they receive and respond to signals from systemic circulation, such as insulin or lipolysis products from adipose tissue, and they are exposed to nutrient-rich blood from portal circulation as well as a multitude of signals from the liver microenvironment. As part of the innate immune system, macrophages must act primarily as sentinels,
keeping the peace then raising the alarm when homeostasis is disturbed. Raising this alarm is a tightly regulated process where a number of molecular actors within the cell integrate afferent signals and coordinate efferent responses. The responses of these very important cells can dictate disease course in NAFLD. While recent decades have accumulated a wealth of knowledge, much is yet to be learned about how therapeutically target these cells in NAFLD.

**In insulin dynamics, NAFLD and liver macrophages**

Strong mechanistic associations have been drawn between insulin resistance and NAFLD, yet the physiological and physiopathological adaptations of liver macrophages remain to be fully understood. Insulin levels oscillate from low levels when fasting, to higher postprandial levels. Basal levels of insulin in the blood are also different in healthy people compared with people with prediabetes or diabetes (reviewed recently [323]). An added layer of complexity for investigating the physiological and pathophysiological levels of insulin on liver macrophage function include the location and action of the liver. The liver is immediately downstream of the pancreas and clears ~100-fold higher in the portal vein than in the systemic circulation [326]. The amplitude of insulin’s oscillations are thought to be ~100-fold higher in the portal vein than in the systemic circulation [326]. Therefore, even in health, liver macrophages are exposed to higher levels of insulin compared with other tissue-resident macrophages which may have consequences, affecting trained immunity in macrophages for example, these effects of insulin have been recently reviewed [327].

**Macrophage heterogeneity and challenges in therapeutic targeting**

The plasticity of macrophage terminal differentiation is now widely recognised, similarly macrophage polarisation states and effector functions now exist on a sliding scale with the classical and alternative states being at the extremes. The technical advances of recent years, namely single-cell sequencing and high-density cytometric methods, have allowed this appreciation of macrophage heterogeneity. Novel functional classification of macrophage subsets is an area of active research, booming in a number of fields and specially in study of the liver and pathogenesis of NAFLD [94,328]. Despite the currently wide application of these technologies, one technical hurdle that has been a long-standing subject of discussion in the field is the in vivo modelling of NAFLD [31]. Given the range of models available that reproduce the different components of NAFLD, macrophage populations are also very likely to vary across these numerous in vivo models. Future studies are working towards consistency and specificity with regard to the different models available and in the way that data are reported, with the multiple models being increasingly applied as mechanistic representations of different stages of disease. This trend enables a more thorough understanding of the importance of different macrophage subsets in NAFLD. Accordingly, it is necessary to decipher macrophage heterogeneity across different models of NAFLD and to understand cellular and molecular drivers of this intra- and inter-model heterogeneity. The respective role of embryonic KCs, inflammatory KCs and Mo-MPs, and their different subsets, in liver disease can now be investigated with great precision. Understanding macrophage heterogeneity in kinetic studies will also be of value. Such an approach will allow understanding of how cellular diversity arises, leading to the therapeutic targeting of detrimental subsets at the appropriate time without influencing other potentially beneficial subsets.

**Challenges in translatability and NAFLD clinical evaluation**

One of the most important milestones of research in NAFLD was the proposal of the two-hit hypothesis in 1998. Since then, clear experimental evidence has implicated insulin resistance, lipotoxicity and inflammation in the pathogenesis of NAFLD. Yet a number of well-known barriers exist in the field with regard to the translatability of certain findings from basic research to clinical practice, which in itself has clear priorities to improve staging and diagnosis of NAFLD. The main barrier to translatability is in the complex modelling of NAFLD. Today no single murine model recapitulates the whole spectrum of NAFLD, the models applied will allow at best the integration of one or two stages, taking into account one or two factors. For example, a high-fat diet will robustly reproduce insulin resistance; however, the liver is not affected beyond simple steatosis. Diets deficient in certain amino acids (methionine/choline) may induce moderate steatohepatitis and when combined with high fat will also induce obesity and insulin resistance; however, the depletion of amino acids reduces the physiological relevance to human disease. Similarly, surgical (bile-duct ligation) and toxic (CCL₄) models that mimic steatohepatitis and fibrosis are far from physiological. Taking into account the above models and other
genetic models, reviewed by [31,69], the scientific community can still gain a lot of mechanistic insight with regard to discrete components of the disease that can be currently reproduced. While challenging to encapsulate the entire spectrum of NAFLD as well as its comorbidities, it is also of mechanistic value that these models allow the isolated study of the different components of NAFLD. The application of these models must in future be interpreted as such, until a holistic and physiologically relevant model is developed.

Two major clinical barriers that are areas of active work are the noninvasive staging and the detection of NAFLD. Currently, a very widely used and accepted staging method is the SAF score, which histologically grades steatosis, activity and fibrosis in NAFLD. However, establishing a SAF score requires an invasive biopsy and a recognised limit to this method is considerable heterogeneity in the staging of advanced fibrosis [329]. It is for this reason that a future priority of large-scale trials are currently tackling this issue (LITMUS, NIMBLE, QUID-NASH) [330–332].

Acknowledgements

This work is supported by The French National Research Agency (Agence National de la Recherche) ANR-JCJC grant for the MitoFLAME Project ANR-19-CE14-0005, the French Society for Diabetes (Société Francophone du Diabète; SFD) Allocation Exceptionnelle and the European Foundation for the Study of Diabetes grant to FA.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

RT, MCG, IP-T, GC, NV and FA wrote the manuscript.

References

1 Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM & Ahmed A (2015) Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology 148, 547–555.

2 Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J & Bugianesi E (2018) Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 15, 11–20.

3 Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, Qiu Y, Burns L, Afendy A & Nader F (2019) The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. J Hepatol 71, 793–801.

4 Gehrek N & Schattenberg JM (2020) Metabolic inflammation—a role for hepatic inflammatory pathways as drivers of comorbidities in nonalcoholic fatty liver disease? Gastroenterology 158, 1929–1947.e6.

5 Sheka AC, Adeyi O, Thompson J, Hameed B, Crawford PA & Ikramuddin S (2020) Nonalcoholic steatohepatitis: a review. JAMA 323, 1175–1183.

6 Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L & Wymer M (2016) Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 64, 73–84.

7 Huang DQ, El-Serag HB & Loomba R (2021) Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 18, 223–238.

8 Day CP & James OF (1998) Hepatic steatosis: innocent bystander or guilty party? Hepatology 27, 1463–1466.

9 Amiri Dash Atan N, Koushki M, Motedayen M, Dousti M, Sayehmiri F, Vafaee R, Norouzian M & Gholami R (2017) Type 2 diabetes mellitus and non-alcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterol Hepatol Bed Bench 10, S1–S7.

10 Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G & Melchionda N (1999) Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 107, 450–455.

11 Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML & Clore JN (2001) Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 120, 1183–1192.

12 Smith GI, Shankaran M, Yoshino M, Schweitzer GG, Chondronikola M, Beals JW, Okunade AL, Patterson BW, Nyangau E, Field T et al. (2020) Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. J Clin Invest 130, 1453–1460.

13 Cedo L, Santos D, Roglans N, Julve J, Pallares V, Rivas-Urbina A, Llorente-Cortes V, Laguna JC, Blanco-Vaca F & Escola-Gil JC (2017) Human hepatic
lipase overexpression in mice induces hepatic steatosis and obesity through promoting hepatic lipogenesis and white adipose tissue lipolysis and fatty acid uptake. PLoS One 12, e0189834.

14 Mota M, Banini BA, Cazanave SC & Sanyal AJ (2016) Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. Metabolism 65, 1049–1061.

15 Kim OK, Jun W & Lee J (2015) Mechanism of ER stress and inflammation for hepatic insulin resistance in obesity. Ann Nutr Metab 67, 218–227.

16 Aachard CS & Laybutt DR (2012) Lipid-induced endoplasmic reticulum stress in liver cells results in two distinct outcomes: adaptation with enhanced insulin signaling or insulin resistance. Endocrinology 153, 2164–2177.

17 Puri P, Mirshahi F, Cheung O, Natarajan R, Maher JW, Kellum JM & Sanyal AJ (2008) Activation and dysregulation of the unfolded protein response in nonalcoholic fatty liver disease. Gastroenterology 134, 568–576.

18 Brown M, Dainty S, Strudwick N, Mihai AD, Watson JN, Dendooven R, Paton AW, Paton JC & Schroder M (2020) Endoplasmic reticulum stress causes insulin resistance by inhibiting delivery of newly synthesized insulin receptors to the cell surface. Mol Biol Cell 31, 2597–2629.

19 Davies LC & Taylor PR (2015) Tissue-resident macrophages: then and now. Immunochemistry 144, 541–548.

20 Wen Y, Lambrecht J, Ju C & Tacke F (2021) Hepatic macrophages in liver homeostasis and disease diversity, plasticity and therapeutic opportunities. Cell Mol Immunol 18, 45–56.

21 Bataller R & Brenner DA (2005) Liver fibrosis. J Clin Invest 115, 209–218.

22 Weiskirchen R & Tacke F (2016) Liver fibrosis: from signaling or insulin resistance. distinct outcomes: adaptation with enhanced insulin signaling or insulin resistance. Endocrinology 153, 2164–2177.

23 Alisi A, Carpino G, Oliveira FL, Panera N, Nobili V, Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Shen E et al. (1986) Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J 5, 2503–2512.

24 Ullrich A, Bell JR, Chen EJ, Lamotho B, Cordonnier N, Mesbah K, Monthioux E, Jamil J & Bucchini D (1996) Targeted disruption of the insulin receptor gene in the mouse results in neonatal lethality. EMBO J 15, 1542–1547.

25 Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA & Kahn CR (2000) Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 6, 87–97.

26 Bar RS, Kahn CR & Koren HS (1977) Insulin inhibition of antibody-dependent cytotoxicity and insulin receptors in macrophages. Nature 265, 632–635.

27 O’Rourke L, Yeaman SJ & Shepherd PR (2001) Insulin and leptin acutely regulate cholesterol ester metabolism in macrophages by novel signaling pathways. Diabetes 50, 955–961.

28 Han S, Liang CP, DeVries-Seimon T, Ranalletta M, Welch CL, Collins-Fletcher K, Accili D, Tabas I & Tall AR (2006) Macrophage insulin receptor deficiency increases ER stress-induced apoptosis and necrotic core formation in advanced atherosclerotic lesions. Cell Metab 3, 257–266.

29 Baumgartl J, Bauder S, Scherner M, Babaei V, Makowski L, Suttles J, McDuffie M, Tobe K, Kadowaki T, Fazio S et al. (2006) Myeloid lineage cell-restricted insulin resistance protects apolipoproteinE-deficient mice against atherosclerosis. Cell Metab 3, 247–256.
(2019) The role of macrophages in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Nat Rev Gastroenterol Hepatol* **16**, 145–159.
39 Morinaga H, Mayoral R, Heinrichsdorf J, Osborn O, Franck N, Hah N, Walenta E, Bandyopadhyay G, Pessentheiner AR, Chi TJ et al. (2015) Characterization of distinct subpopulations of hepatic macrophages in HFD/obese mice. *Diabetes* **64**, 1120–1130.
40 Morgantini C, Jager J, Li X, Levi L, Azzimato V, Sulen A, Barreby E, Xu C, Tencerova M, Naslund E et al. (2019) Liver macrophages regulate systemic metabolism through non-inflammatory factors. *Nat Metab* **1**, 445–459.
41 Bleriot C & Ginhoux F (2019) Understanding the heterogeneity of resident liver macrophages. *Front Immunol* **10**, 2694.
42 Scott CL, Zheng F, De Baetselier P, Martens L, Saeyes Y, De Prijck S, Lippens S, Abels C, Schoonoghe S, Raes G et al. (2016) Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. *Nat Commun* **7**, 10321.
43 Tran S, Baba I, Poupel L, Dussaud S, Moreau M, Gelineau A, Marcelin G, Magreau-Daye E, Ouhachi M, Lesnik P et al. (2020) Impaired Kupffer cell self-renewal alters the liver response to lipid overload during non-alcoholic steatohepatitis. *Immunity* **53**, 627–640.e5.
44 Schulz C, Gomez Perdiguero E, Chorro L, Szabó-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW et al. (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* **336**, 86–90.
45 Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, Strauss-Ayali D, Viukov S, Guilliams M, Misharin A et al. (2013) Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* **38**, 79–91.
46 Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, Garner H, Trouillet C, de Bruijn MF, Geissmann F et al. (2015) Tissue-resident macrophages originate from yolk-sac-derived erythromyeloid progenitors. *Nature* **518**, 547–551.
47 Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, Beaudin AE, Lum J, Low I, Forsberg EC et al. (2015) C-Myb(+) erythromyeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* **42**, 665–678.
48 Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, Becker CD, See P, Price J, Lucas D et al. (2013) Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* **38**, 792–804.
49 Ebe Y, Hasegawa G, Takatsuka H, Umez U, Mitsuyama M, Arakawa M, Mukaida N & Naito M (1999) The role of Kupffer cells and regulation of neutrophil migration into the liver by macrophage inflammatory protein-2 in primary listeriosis in mice. *Pathol Int* **49**, 519–532.
50 Gregory SH, Sagnimeni AJ & Wing EJ (1996) Bacteria in the bloodstream are trapped in the liver and killed by immigrating neutrophils. *J Immunol* **157**, 2514–2520.
51 Shi J, Fujieda H, Kokubo Y & Wake K (1996) Apoptosis of neutrophils and their elimination by Kupffer cells in rat liver. *Hepatology* **24**, 1256–1263.
52 Shi J, Gilbert GE, Kokubo Y & Ohashi T (2001) Role of the liver in regulating numbers of circulating neutrophils. *Blood* **98**, 1226–1230.
53 Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK & Moestrup SK (2001) Identification of the haemoglobin scavenger receptor. *Nature* **409**, 198–201.
54 Willekens FL, Werme JM, Krujit JK, Roerdinkholder-Stoelwinder B, Groenen-Dopp YA, van den Bos AG, Bosman GJ & van Berkel TJ (2005) Liver Kupffer cells rapidly remove red blood cell-derived vesicles from the circulation by scavenger receptors. *Blood* **105**, 2141–2145.
55 Theurl M, Theurl I, Hochegger K, Obrist P, Subramaniam N, van Rooijen N, Schuemann K & Weiss G (2008) Kupffer cells modulate iron homeostasis in mice via regulation of hepcidin expression. *J Mol Med (Berl)* **86**, 825–835.
56 Yang W, van der Tuin S, Tjeerdema N, van Dam AD, Rensen SS, Hendrikk T, Berbee JF, Atanasovska B, Fu J, Hoekstra M et al. (2015) Plasma cholesterol ester transfer protein is predominantly derived from Kupffer cells. *Hepatology* **62**, 1710–1722.
57 Krenkel O & Tacke F (2017) Liver macrophages in tissue homeostasis and disease. *Nat Rev Immunol* **17**, 306–321.
58 Luo W, Xu Q, Wang Q, Wu H & Hua J (2017) Effect of modulation of PPAR-gamma activity on Kupffer cells M1/M2 polarization in the development of non-alcoholic fatty liver disease. *Sci Rep* **7**, 44612.
59 Han YH, Kim HJ, Na H, Nam MW, Kim JY, Kim JS, Koo SH & Lee MO (2017) RORalpha induces KLF4-mediated M2 polarization in the liver macrophages that protect against nonalcoholic steatohepatitis. *Cell Rep* **20**, 124–135.
60 Hart KM, Fabre T, Sciaruba JC, Gieseck RL, Borthwick LA, Vannella KM, Acciani TH, de Queiroz Prado R, Thompson RW, White Sandra, et al. (2017) Type 2 immunity is protective in metabolic disease but exacerbates NAFLD collaboratively with TGF-β. *Sci Transl Med* **9**, eaal3694. doi: 10.1126/scitranslmed.aal3694.
61 Devisser L, Scott CL, Lefere S, Raevens S, Bogaerts E, Paridaens A, Verhelst X, Geerts A, Guilliams M &
Van Vlierberghe H (2017) Non-alcoholic steatohepatitis induces transient changes within the liver macrophage pool. Cell Immunol 322, 74–83.

You Q, Cheng L, Kedl RM & Ju C (2008) Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. Hepatology 48, 978–990.

Knolle P, Schlaak J, Uhrig A, Kempf P, Meyer zum Buschenfelde KH & Gerken G (1995) Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. J Hepatol 22, 226–229.

Heymann F, Peusquens J, Ludwig-Portugall I, Kohlhepp M, Ergen C, Niemietz P, Martin C, van Rooijen N, Ochando JC, Randolphi GJ et al. (2015) Liver inflammation abrogates immunological tolerance induced by Kupffer cells. Hepatology 62, 279–291.

Krenkel O, Hundertmark J, Abdallah AT, Kohlhepp M, Puengel T, Roth T, Branco DPP, Mossanen JC, Luedde T, Trautwein C et al. (2020) Myeloid cells in liver and bone marrow acquire a functionally distinct inflammatory phenotype during obesity-related steatohepatitis. Gut 69, 551–563.

Dal-Secco D, Wang J, Zeng Z, Kolaezkowska E, Wong CH, Petri B, Ransohoff RM, Charo IF, Jenne CN & Kubes P (2015) A dynamic spectrum of monocytes arising from the in situ reprogramming of CCR2+ monocytes at a site of sterile injury. J Exp Med 212, 447–456.

Theurl I, Hilgendorf I, Schlaak J, Uhrig A, Kempf P, Meyer zum Buschenfelde KH & Gerken G (1995) Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. J Hepatol 22, 226–229.

Heymann F, Peusquens J, Ludwig-Portugall I, Kohlhepp M, Ergen C, Niemietz P, Martin C, van Rooijen N, Ochando JC, Randolphi GJ et al. (2015) Liver inflammation abrogates immunological tolerance induced by Kupffer cells. Hepatology 62, 279–291.

Krenkel O, Hundertmark J, Abdallah AT, Kohlhepp M, Puengel T, Roth T, Branco DPP, Mossanen JC, Luedde T, Trautwein C et al. (2020) Myeloid cells in liver and bone marrow acquire a functionally distinct inflammatory phenotype during obesity-related steatohepatitis. Gut 69, 551–563.

Dal-Secco D, Wang J, Zeng Z, Kolaezkowska E, Wong CH, Petri B, Ransohoff RM, Charo IF, Jenne CN & Kubes P (2015) A dynamic spectrum of monocytes arising from the in situ reprogramming of CCR2+ monocytes at a site of sterile injury. J Exp Med 212, 447–456.

Theurl I, Hilgendorf I, Naar M, Tymoszk J, Haschka D, Asshoff M, He S, Gerhardt LM, Holderried TA, Seifert M et al. (2016) On-demand erythrocyte disposal and iron recycling requires transient macrophages in the liver. Nat Med 22, 945–951.

Siero F, Evrard M, Rizzetto S, Melino M, Mitchell AJ, Florido M, Beattie L, Walters SB, Tay SS, Lu B et al. (2017) A liver capsular network of monocyte-derived macrophages restricts hepatic dissemination of intraperitoneal bacteria by neutrophil recruitment. Immunity 47, 374–388.e6.

Febbraio MA, Reibe S, Shalapour S, Ooi GJ, Watt MJ & Karin M (2019) Preclinical models for studying NASH-driven HCC: how useful are they? Cell Metab 29, 18–26.

Oligschlager Y, Shiri-Sverdlov R. (2020) NAFLD preclinical models: more than a handful, less of a concern?. Biomedicines 8, 28. doi: 10.3390/biomedicine.s8020028

Tag CG, Sauer-Lehnen S, Weiskirchen S, Borkham-Kamphorst E, Tolba RH, Tacke F, Weiskirchen R (2015) Bile duct ligation in mice: induction of inflammatory liver injury and fibrosis by obstructive cholestasis. J Vis Exp. doi: 10.3791/52438

Nakagawa H, Umemura A, Taniguchi K, Font-Burgada J, Dhar D, Ogata H, Zhong Z, Valasek MA, Seki E, Hidalgo J et al. (2014) ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. Cancer Cell 26, 331–343.

Park JW, Jeong G, Kim SJ, Kim MK & Park SM (2007) Predictors reflecting the pathological severity of non-alcoholic fatty liver disease: comprehensive study of clinical and immunohistochemical findings in younger Asian patients. J Gastroenterol Hepatol 22, 491–497.

Baeck C, Wehr A, Karlmark KR, Heymann F, Vucur M, Gassler N, Huss S, Klussmann S, Eulberg D, Luedde T et al. (2012) Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. Gut 61, 416–426.

Huang W, Metlakunta A, Dedousis N, Zhang P, Sipula I, Dube JJ, Scott DK & O’Doherty RM (2010) Depletion of liver Kupffer cells prevents the development of diet-induced hepatic steatosis and insulin resistance. Diabetes 59, 347–357.

Neyrinck AM, Cani PD, Dewulf EM, De Backer F, Bindels LB & Delzenne NM (2009) Critical role of Kupffer cells in the management of diet-induced diabetes and obesity. Biochem Biophys Res Commun 385, 351–356.

Reid DT, Reyes JL, McDonald BA, Vo T, Reimer RA & Eksteen B (2016) Kupffer cells undergo fundamental changes during the development of experimental NASH and are critical in initiating liver damage and inflammation. PLoS One 11, e0159524.

Stienstra R, Mandard S, Patsouris D, Maass C, Kersten S & Muller M (2007) Peroxisome proliferator-activated receptor alpha protects against obesity-induced hepatic inflammation. Endocrinology 148, 2753–2763.

Stienstra R, Saudale F, Duval C, Keshhtkar S, Groener JE, van Rooijen N, Staels B, Kersten S & Muller M (2010) Kupffer cells promote hepatic steatosis via interleukin-1beta-dependent suppression of peroxisome proliferator-activated receptor alpha activity. Hepatology 51, 511–522.

Krenkel O, Puengel T, Govaere O, Abdallah AT, Mossanen JC, Kohlhepp M, Liepelt A, Lefebvre E, Luedde T, Hellerbrand C et al. (2018) Therapeutic inhibition of inflammatory monocyte recruitment reduces steatohepatitis and liver fibrosis. Hepatology 67, 1270–1283.

Pradere JP, Kluwe J, De Minicis S, Jiao JJ, Gwak HY, Dapito DH, Ang KC, Guenther ND, Mederacke I, Friedman R et al. (2013) Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice. Hepatology 58, 1461–1473.

Tsuchida T & Friedman SL (2017) Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol 14, 397–411.
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83 Xiong X, Kuang H, Ansari S, Liu T, Gong J, Wang S, Zhao XY, Ji Y, Li C, Guo L et al. (2019) Landscape of intercellular crosstalk in healthy and NASH liver revealed by single-cell secretome gene analysis. *Mol Cell* 75, 644–660.e5.

84 Tateya S, Rizzo NO, Handa P, Cheng AM, Morgan-Stevenson V, Daum G, Clowes AW, Morton GJ, Schwartz MW & Kim F (2011) Endothelial NO/cGMP/VASP signaling attenuates Kupffer cell activation and hepatic insulin resistance induced by high-fat feeding. *Diabetes* 60, 2792–2801.

85 Weston CJ, Shepherd EL, Claridge LC, Rantakari P, Reynolds GM, Aalto K, Anstee QM et al. (2015) Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis. *J Clin Invest* 125, 501–520.

86 Hammoutene A & Rautou PE (2019) Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. *J Hepatol* 70, 1278–1291.

87 Sutti S & Albano E (2020) Adaptive immunity: an emerging player in the progression of NAFLD. *Nat Rev Gastroenterol Hepatol* 17, 81–92.

88 Heymann F & Tacke F (2016) Immunology in the liver—from homeostasis to disease. *Nat Rev Gastroenterol Hepatol* 13, 88–110.

89 Remmerie A, Martens L & Scott CL (2020) Macrophage subsets in obesity, aligning the liver and adipose tissue. *Front Endocrinol (Lausanne)* 11, 259.

90 Ramachandran P, Pellecoro A, Vernon MA, Boulter L, Reynolds GM, Aalto K, Anstee QM et al. (2015) Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the spectrum of macrophage activation. *Mol Ther* 1–61.

91 Kolodziejczyk AA, Zheng D, Shibolet O, Elinav E et al. (2018) Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. *Nat Commun* 9, 4383.

92 Alzaid F, Lagadec F, Albuquerque M, Ballaire R, De Nardo D, Gohel TD, Emde M, Schmidleithner L et al. (2014) Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* 40, 274–288.

93 Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, De Nardo D, Gohel TD, Emde M, Schmidleithner L et al. (2014) M2 Kupffer cells promote M1 Kupffer cell apoptosis: a protective mechanism against alcoholic and nonalcoholic fatty liver disease. *Hepatology* 59, 130–142.

94 MacParland SA, Liu JC, Ma XZ, Innes BT, Bartczak AM, Gage BK, Manuel J, Khoo N, Echeverri J, Linares I et al. (2018) Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. *Nat Commun* 9, 4383.
through Toll-like receptor 4. *J Biol Chem* **276**, 16683–16689.

106 Snodgrass RG, Huang S, Choi IW, Rutledge JC & Hwang DH (2013) Inflammamosome-mediated secretion of IL-1beta in human monocytes through TLR2 activation; modulation by dietary fatty acids. *J Immunol* **191**, 4337–4347.

107 Kim SY, Jeong JM, Kim SJ, Seo W, Kim MH, Choi WM, Yoo W, Lee JH, Shim YR, Yi HS et al. (2017) Pro-inflammatory hepatic macrophages generate ROS through NADPH oxidase 2 via endocytosis of monomeric TLR4-MD2 complex. *Nat Commun* **8**, 2247.

108 Tosello-Trampont AC, Landes SG, Novitskaya T, McGuinness OP, De Taeye BM, Novobrantseva TI, Voican CS, Bouchet-Delbos L, Tran T, Hemon P et al. (2016) Decreased expression of the glucocorticoid receptor-GILZ pathway in Kupffer cells promotes liver inflammation in obese mice. *J Hepatol* **64**, 916–924.

109 Baker RG, Hayden MS & Ghosh S (2011) NF-kappaB, inflammation, and metabolic disease. *Cell Metab* **13**, 11–22.

110 Liu CP, Zhang X, Tan QL, Xu WX, Zhou CY, Luo M, Li X, Huang RY & Zeng X (2017) NF-kappaB pathways are involved in M1 polarization of RAW macrophage by polyporus polysaccharide in the tumor microenvironment. *PLoS One* **12**, e0188317.

111 Miyamoto M, Terczynska-Dyla E, Thomsen KL, de Araujo EP, Cintra DE, Pauli JR, Araujo EP, Moraes JC, de Souza CT, Milanski M, Morari J, Gambero A, Saad MJ & Velloso LA (2008) Interleukin-10 is a protective factor against diet-induced insulin resistance in liver. *J Hepatol* **48**, 628–637.

112 Nishimura T, Itoh Y, Shi Y, Zhang H, Sun Y, Zangyan G, Wang F, Yu W, Wang J, Tao X et al. (2020) PTPROt aggravates inflammation by enhancing NF-kappaB activation in liver macrophages during nonalcoholic steatohepatitis. *Theranostics* **10**, 5290–5304.

113 Ji Z, He L, Regev A & Struhl K (2019) Inflammatory regulatory network mediated by the joint action of NF-kB, STAT3, and AP-1 factors is involved in many human cancers. *Proc Natl Acad Sci USA* **116**, 9453–9462.

114 Solinas G, Vilcu C, Neels JG, Bandyopadhyay GK, Luo JL, Naugler W, Grivennikov S, Wynshaw-Boris A, Scadeng M, Olefsky JM et al. (2007) JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell Metab* **6**, 386–397.

115 Cintra DE, Pauli JR, Araujo EP, Moraes JC, de Souza CT, Milanski M, Morari J, Gambero A, Saad MJ & Velloso LA (2008) Interleukin-10 is a protective factor against diet-induced insulin resistance in liver. *J Hepatol* **48**, 628–637.

116 Hwang DH (2013) Inflammasome-mediated secretion of IL-1beta in human monocytes through TLR2 activation; modulation by dietary fatty acids. *J Immunol* **191**, 4337–4347.

117 Kawai T & Akira S (2007) Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med* **13**, 460–469.

118 Toonen EJM (2018) IL-1 family cytokine pathways underlying NAFLD: towards new treatment strategies. *J Hepatol* **64**, 916–924.

119 Liu CP, Zhang X, Tan QL, Xu WX, Zhou CY, Luo M, Li X, Huang RY & Zeng X (2017) NF-kappaB pathways are involved in M1 polarization of RAW macrophage by polyporus polysaccharide in the tumor microenvironment. *PLoS One* **12**, e0188317.

120 Robert O, Boujedidi H, Bigorgne A, Ferrere G, Voican CS, Vettorazzi S, Tuckermann JP, Bouchet-Delbos L, Tran T, Hemon P et al. (2016) Decreased expression of the glucocorticoid receptor-GILZ pathway in Kupffer cells promotes liver inflammation in obese mice. *J Hepatol* **64**, 916–924.

121 Jin K, Liu Y, Shi Y, Zhang H, Sun Y, Zangyan G, Wang F, Yu W, Wang J, Tao X et al. (2020) PTPROt aggravates inflammation by enhancing NF-kappaB activation in liver macrophages during nonalcoholic steatohepatitis. *Theranostics* **10**, 5290–5304.

122 Ji Z, He L, Regev A & Struhl K (2019) Inflammatory regulatory network mediated by the joint action of NF-kB, STAT3, and AP-1 factors is involved in many human cancers. *Proc Natl Acad Sci USA* **116**, 9453–9462.

123 Solinas G, Vilcu C, Neels JG, Bandyopadhyay GK, Luo JL, Naugler W, Grivennikov S, Wynshaw-Boris A, Scadeng M, Olefsky JM et al. (2007) JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell Metab* **6**, 386–397.

124 Hao J, Hu Y, Li Y, Zhou Q & Lv X (2017) Involvement of JNK signaling in IL4-induced M2 macrophage polarization. *Exp Cell Res* **357**, 155–162.

125 Schulien I, Hockenjos B, Schmitt-Graeff A, Perdekamp MG, Follo M, Thimme R & Hasselblatt P (2019) The transcription factor c-Jun/AP-1 promotes liver fibrosis during non-alcoholic steatohepatitis by regulating Osteopontin expression. *Cell Death Differ* **26**, 1688–1699.

126 Zhang X, Fan L, Wu J, Xu H, Leung WY, Fu K, Wu J, Liu K, Man K, Yang X et al. (2019) Macrophage p38alpha promotes nutritional steatohepatitis through M1 polarization. *J Hepatol* **71**, 163–174.

127 Fleetwood AJ, Dinh H, Cook AD, Hertzog PJ & Hamilton JA (2009) GM-CSF- and M-CSF-dependent macrophage phenotypes display differential dependence on type I interferon signaling. *J Leukoc Biol* **86**, 411–421.
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128 Tarassishin L, Suh HS & Lee SC (2011) Interferon regulatory factor 3 plays an anti-inflammatory role in microglia by activating the PI3K/Akt pathway. J Neuroinflammation 8, 187.

129 Lin R, Heylbroke C, Genin P, Pitha PM & Hiscott J (1999) Essential role of interferon regulatory factor 3 in direct activation of RANTES chemokine transcription. Mol Cell Biol 19, 959–966.

130 Fleetwood AJ, Lawrence T, Hamilton JA & Cook AD (2007) Granulocyte-macrophage colony-stimulating factor (CSF) and macrophage CSF-dependent macrophage phenotypes display differences in cytokine profiles and transcription factor activities: implications for CSF blockade in inflammation. J Immunol 178, 5245–5252.

131 Qiao JT, Cui C, Qing L, Wang LS, He TY, Yan F, Luo X, Li H, Ma L, Zhou J, Guo X, Woo SL, Pei Y, Tanaka T, Murakami K, Bando Y & Yoshida S (2015) Interferon regulatory factor 7 participates in the landscape of IRF3, IRF5 and IRF7 dimers: One implication for dimer-specific gene regulation. Nucleic Acids Res 46, 2509–2520.

132 Tanaka T, Murakami K, Bando Y & Yoshida S (2015) Interferon regulatory factor 7 participates in the M1-like microglial polarization switch. Glia 63, 595–610.

133 Pinilla-Vera M, Xiong Z, Zhao Y, Donahoe MP, Barge S, Horne WT, Kolls JK, McVerry BJ, Birukova A et al. (2016) Full spectrum of LPS activation in alveolar macrophages of healthy volunteers by whole transcriptomic profiling. PLoS One 11, e0159329.

134 Andrilenas KK, Ramlall V, Kurland J, Leung B, Harbaugh AG & Siggers T (2018) DNA-binding landscape of IRF3, IRF5 and IRF7 dimers: implications for dimer-specific gene regulation. Nucleic Acids Res 46, 259–262.

135 Ghazarian M, Revelo XS, Nøhr MK, Luck H, Zeng K, Lei H, Tsai S, Schroer SA, Park YJ, Chng MHY et al. (2017) Type I interferon responses drive intrahepatic T cells to promote metabolic syndrome. Sci Immunol 2, eaai7616. doi: 10.1126/sciimmunol.aai7616.

136 Kim SY, Choi YJ, Joung SM, Lee BH, Jung YS & Lee JY (2010) Hypoxia stress up-regulates the expression of Toll-like receptor 4 in macrophages via hypoxia-inducible factor. Immunology 129, 516–524.

137 Wang T, Liu H, Lian G, Zhang SY, Wang X & Jiang C (2017) HIF1alpha-induced glycolysis metabolism is essential to the activation of inflammatory macrophages. Mediators Inflamm. 2017, 9029327.

138 Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Dormer S, Nizet V et al. (2003) HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell 112, 645–657.

139 Werno C, Menrad H, Weigert A, Dehne N, Goerdt S, Schledzewski K, Kryzhowskaja J & Brune B (2010) Knockout of HIF-1alpha in tumor-associated macrophages enhances M2 polarization and attenuates their pro-angiogenic responses. Carcinogenesis 31, 1863–1872.

140 Andrilenas KK, Ramlall V, Kurland J, Leung B, Harbaugh AG & Siggers T (2018) The impact of interferon-regulatory factors to macrophage differentiation and polarization into M1 and M2. Immunobiology 223, 101–111.

141 Krausgruber T, Saliba D, Ryzhakov G, Lanfrancotti A, Blazek K & Udalova IA (2010) IRF5 is required.
for late-phase TNF secretion by human dendritic cells. *Blood* **115**, 4421–4430.

149 Dalmas E, Toubal A, Alzaïd F, Blazek K, Eames HL, Lebozec K, Pini M, Hainault I, Montastier E, Denis RG et al. (2015) Irf5 deficiency in macrophages promotes beneficial adipose tissue expansion and insulin sensitivity during obesity. *Nat Med* **21**, 610–618.

150 Liu J, Cao S, Herman LM & Ma X (2003) Differential regulation of interleukin (IL)-12 p35 and p40 gene expression and interferon (IFN)-gamma-mediated IL-12 production by IFN regulatory factor 1. *J Exp Med* **198**, 1265–1276.

151 Xu H, Zhu J, Smith S, Foldi J, Zhao B, Chung AY, Outt H, Kitajewski J, Shi C, Weber S et al. (2012) Notch-RBP-J signaling regulates the transcription factor IRF8 to promote inflammatory macrophage polarization. *Nat Immunol* **13**, 642–650.

152 Langlais D, Barreiro LB & Gros P (2016) The macrophage IRF8/IRF1 regulome is required for protection against infections and is associated with chronic inflammation. *J Exp Med* **213**, 585–603.

153 Honma K, Udono H, Kohno T, Yamamoto K, Ogawa A, Takemori T, Kumatari A, Suzuki S, Matsuyama T & Yui K (2005) Interferon regulatory factor 4 negatively regulates the production of proinflammatory cytokines by macrophages in response to LPS. *Proc Natl Acad Sci USA* **102**, 16001–16006.

154 Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, Miyake T, Matsushita K, Okazaki T, Saitoh T et al. (2010) The JmjD3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol* **11**, 936–944.

155 Negishi H, Ohba Y, Yanai H, Takaoka A, Honma K, Yui K, Matsuyama T, Taniguchi T & Honda K (2005) Negative regulation of Toll-like-receptor signaling by IRF-4. *Proc Natl Acad Sci USA* **102**, 15989–15994.

156 Loh CY, Arya A, Naema AF, Wong WF, Sethi G & Looi CY (2019) Signal transducer and activator of transcription (STATs) proteins in cancer and inflammation: functions and therapeutic implication. *Front Oncol* **9**, 48.

157 Kovarik P, Stoiber D, Novy M & Decker T (1998) Stat1 combines signals derived from IFN-gamma and LPS receptors during macrophage activation. *EMBO J* **17**, 3660–3668.

158 Meraz MA, White JM, Sheehan KC, Bach EA, Rodig SJ, Dighe AS, Kaplan DH, Riley JK, Greenlund AC, Campbell D et al. (1996) Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. *Cell* **84**, 431–442.

159 Hong HJ, Lee JW, Park SS, Kang YJ, Chang SY, Kim KM, Kim JO, Murchy KK, Payne JS, Yoon SK et al. (2000) A humanized anti–4-1BB monoclonal antibody suppresses antigen-induced humoral immune response in nonhuman primates. *J Immunother* **23**, 613–621.

160 Lawrence T & Natoli G (2011) Transcriptional regulation of macrophage polarization: enabling diversity with identity. *Nat Rev Immunol* **11**, 750–761.

161 Dabritz J, Weinlage T, Varga G, Wirth T, Walscheid K, Brockhausen A, Schwarzmaier D, Bruckner M, Ross M, Bettenworth D et al. (2015) Reprogramming of monocytes by GM-CSF contributes to regulatory immune functions during intestinal inflammation. *J Immunol* **194**, 2424–2438.

162 Szanto A, Balint BL, Nagy ZS, Barta E, Dezső B, Pap A, Széles L, Poliska S, Oros M, Evans RM et al. (2010) STAT6 transcription factor is a facilitator of the nuclear receptor PPARgamma-regulated gene expression in macrophages and dendritic cells. *Immunity* **33**, 699–712.

163 Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Forster I & Akira S (1999) Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* **10**, 39–49.

164 Lang R, Patel D, Morris JJ, Rutschman RL & Murray PJ (2002) Shaping gene expression in activated and resting primary macrophages by IL-10. *J Immunol* **169**, 2253–2263.

165 Yin Z, Ma T, Lin Y, Lu X, Zhang C, Chen S & Jian Z (2018) IL-6/STAT3 pathway intermediates M1/M2 macrophage polarization during the development of hepatocellular carcinoma. *J Cell Biochem* **119**, 9419–9432.

166 Chawla A, Repa JJ, Evans RM & Mangelsdorf DJ (2001) Nuclear receptors and lipid physiology: opening the X-files. *Science* **294**, 1866–1870.

167 Evans RM & Mangelsdorf DJ (2014) Nuclear receptors, RXR, and the big bang. *Cell* **157**, 255–266.

168 Santos R, Ursu O, Gaulton A, Bento AP, Donadi RS, Bologa CG, Karlsson A, Al-Lazikani B, Hersey A, Oprea TI et al. (2017) A comprehensive map of molecular drug targets. *Nat Rev Drug Discov* **16**, 19–34.

169 Xiao Y, Kim M, Lazar MA (2020) Nuclear receptors and transcriptional regulation in non-alcoholic fatty liver disease. *Mol Metab* **10**, doi: 10.1016/j.molme t.2020.101119

170 Saponaro F, Sestito S, Runfola M, Rapposelli S & Chiellini G (2020) Selective thyroid hormone receptor-beta (TRbeta) agonists: new perspectives for the treatment of metabolic and neurodegenerative disorders. *Front Med (Lausanne)* **7**, 331.
171 Yen PM (2001) Physiological and molecular basis of thyroid hormone action. *Physiol Rev* 81, 1097–1142.
172 Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK *et al.* (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* 377, 397–404.
173 Xu L, Glass CK & Rosenfeld MG (1999) Coactivator Radenne A, Akpa M, Martel C, Sawadogo S, Hashimoto K, Yamada M, Matsumoto S, Monden T, Ortega E, Forner MA, Garcia JJ, Rodriguez AB & van der Spek AH, Fliers E & Boelen A (2017) Thyroid hormone action. *Physiol Rev* 97, 140–147.
174 Radenne A, Akpa M, Martel C, Sawadogo S, Mauvoisin D & Mounier C (2008) Hepatic regulation of fatty acid synthase by insulin and T3: evidence for T3 genomic and nongenomic actions. *Am J Physiol Endocrinol Metab* 295, E884–E894.
175 Hashimoto K, Yamada M, Matsumoto S, Monden T, Satoh T & Mori M (2006) Mouse sterol response element binding protein-1c gene expression is negatively regulated by thyroid hormone. *Endocrinology* 147, 4292–4302.
176 De Vito P, Incerpi S, Pedersen JZ, Luly P, Davis FB & Davis PJ (2011) Thyroid hormones as modulators of immune activities at the cellular level. *Thyroid* 21, 879–890.
177 Perrotta C, Buldorini M, Assi E, Cazzato D, De Palma C, Clementi E & Cervia D (2014) The thyroid hormone triiodothyronine controls macrophage maturation and functions: protective role during inflammation. *Am J Pathol* 184, 230–247.
178 Ortega E, Forner MA, Garcia JJ, Rodriguez AB & Barriga C (1999) Enhanced chemotaxis of macrophages by strenuous exercise in trained mice: thyroid hormones as possible mediators. *Mol Cell Biochem* 201, 41–47.
179 van der Spek AH, Fliers E & Boelen A (2017) Thyroid hormone metabolism in innate immune cells. *J Endocrinol* 232, R67–R81.
180 Chen Y, Sjölander M, Wang X, Altenbacher G, Hagner M, Berglund P, Gao Y, Lu T, Jonsson AB & Sjölander H (2012) Thyroid hormone enhances nitric oxide-mediated bacterial clearance and promotes survival after meningococcal infection. *PLoS One* 7, e41445.
181 Forner MA, Barriga C & Ortega E (1995) Exercise-induced stimulation of murine macrophage phagocytosis may be mediated by thyroxine. *J Appl Physiol* 80, 899–903.
182 Billon C, Canaple L, Fleury S, Deloire A, Beylot M, Dombrowicz D, Del Carmine P, Samarut J & Gauthier K (2014) TR alpha protects against atherosclerosis in male mice: identification of a novel anti-inflammatory property for TR alpha in mice. *Endocrinology* 155, 2735–2745.
183 Tapia G, Pepper I, Smok G & Videla LA (1997) Kupffer cell function in thyroid hormone-induced liver oxidative stress in the rat. *Free Radic Res* 26, 267–279.
184 Valencia C, Cornejo P, Romanque P, Tapia G, Varela P, Videla LA & Fernandez V (2004) Effects of acute lindane intoxication and thyroid hormone administration in relation to nuclear factor-kappaB activation, tumor necrosis factor-alpha expression, and Kupffer cell function in the rat. *Toxicol Lett* 148, 21–28.
185 Tapia G, Fernandez V, Pino C, Ardiles R & Videla LA (2006) The acute-phase response of the liver in relation to thyroid hormone-induced redox signaling. *Free Radic Biol Med* 40, 1628–1635.
186 Fernandez V, Tapia G, Varela P & Videla LA (2005) Redox regulation of thyroid hormone-induced Kupffer cell-dependent IkappaB-alpha phosphorylation in relation to inducible nitric oxide synthase expression. *Free Radic Res* 39, 411–418.
187 Contreras-Jurado C, Alonso-Merino E, Saiz-Ladera C, Valino AJ, Regadera J, Alemany S & Aranda A (2016) The thyroid hormone receptors inhibit hepatic interleukin-6 signaling during endotoxemia. *Sci Rep* 6, 30990.
188 Vaitkus JA, FARRAR JS & CELI FS (2015) Thyroid hormone-mediated modulation of energy expenditure. *Int J Mol Sci* 16, 16158–16175.
189 Gullberg H, Rudling M, Salto C, Forrest D, Angelin B & Vennstrom B (2002) Requirement for thyroid hormone receptor beta in T3 regulation of cholesterol metabolism in mice. *Mol Endocrinol* 16, 1767–1777.
190 Mantovani A, Nascimbeni F, Lonardo A, Zoppini G, Bonora E, Mantzoros CS & Targher G (2018) Association between primary hypothyroidism and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Thyroid* 28, 1270–1284.
191 Sinha RA, Bruinstroop E, Singh BK & Yen PM (2019) Nonalcoholic fatty liver disease and hypercholesterolemia: roles of thyroid hormones, metabolites, and agonists. *Thyroid* 29, 1173–1191.
192 Accorroni A, Saponaro F & Zucchi R (2016) Tissue thyroid hormones and thyronamines. *Heart Fail Rev* 21, 373–390.
193 Gullberg H, Rudling M, Forrest D, Angelin B & Vennstrom B (2000) Thyroid hormone receptor beta-deficient mice show complete loss of the normal cholesterol 7alpha-hydroxylase (CYP7A) response to thyroid hormone but display enhanced resistance to dietary cholesterol. *Mol Endocrinol* 14, 1739–1749.
194 Johansson C, Vennstrom B & Thoren P (1998) Evidence that decreased heart rate in thyroid hormone receptor-alpha1-deficient mice is an intrinsic defect. *Am J Physiol* 275, R640–R646.
195 Araki O, Ying H, Zhu XG, Willingham MC & Cheng SY (2009) Distinct dysregulation of lipid metabolism by unliganded thyroid hormone receptor isoforms. *Mol Endocrinol* 23, 308–315.
196 Zucchi R (2020) Thyroid hormone analogues: an update. *Thyroid* 30, 1099–1105.
The role and regulation of the peroxisome proliferator-activated receptor alpha in human liver. *Biochimie* **136**, 75–84.

206 Xu HE, Lambert MH, Montana VG, Plunket KD, Moore LB, Collins JL, Oplinger JA, Kliwer SA, Gampe RT Jr, McKee DD et al. (2001) Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. *Proc Natl Acad Sci USA* **98**, 13919–13924.

207 Braissant O, Foufelle F, Scotto C, Dauca M & Wahl W (1996) Differential expression of peroxisome proliferator-activated receptor (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* **137**, 354–366.

208 Zardi EM, Navarini L, Sambataro G, Piccinni P, Sambataro FM, Spina C & Dobrana A (2013) Hepatic PPARs: their role in liver physiology, fibrosis and treatment. *Curr Med Chem* **20**, 3370–3396.

209 Chakravarthy MV, Lodhi HJ, Yin L, Malapaka RR, Xu HE, Turk J & Semenkovich CF (2009) Identification of a physiologically relevant endogenous ligand for PPARalpha in liver. *Cell* **138**, 476–488.

210 Pawlak M, Bauge E, Bourguet W, De Bosscher K, Lalloyer F, Tailleux A, Lebherz C, Lefebvre P & Staels B (2014) The transrepressive activity of peroxisome proliferator-activated receptor alpha is necessary and sufficient to prevent liver fibrosis in mice. *Hepatology* **60**, 1593–1606.

211 Bougarne N, Paumelle R, Caron S, Hennuyer N, Mansouri R, Gervois P, Staels B, Haegeman G & De Bosscher K (2009) PPARalpha blocks glucocorticoid receptor alpha-mediated transactivation but cooperates with the activated glucocorticoid receptor alpha for transrepression on NF-kappaB. *Proc Natl Acad Sci USA* **106**, 7397–7402.

212 Guan D, Xiong Y, Borec PC, Jang C, Doulias PT, Papazyan R, Fang B, Jiang C, Zhang Y, Briggs ER et al. (2018) Diet-induced circadian enhancer remodeling synchronizes opposing hepatic lipid metabolic processes. *Cell* **174**, 831–842.e12.

213 Chen L & Yang G (2014) PPARs integrate the mammalian Clock and energy metabolism. *PPAR Res* **2014**, 653017.

214 Gervois P, Vu-Dac N, Kleemann R, Kockx M, Dubois G, Laine B, Kosykh V, Fruchart JC, Kooistra T & Staels B (2001) Negative regulation of human fibrinogen gene expression by peroxisome proliferator-activated receptor alpha agonists via inhibition of CCAAT box/enhancer-binding protein beta. *J Biol Chem* **276**, 33471–33477.

215 Bougarne N, Weyers B, Desmet SJ, Deckers J, Ray DW, Staels B & De Bosscher K (2018) Molecular actions of PPARalpha in lipid metabolism and inflammation. *Endocr Rev* **39**, 760–802.

216 Patsouris D, Reddy JK, Muller M & Kersten S (2006) Peroxisome proliferator-activated receptor alpha mediates the effects of high-fat diet on hepatic gene expression. *Endocrinology* **147**, 1508–1516.

217 Montagner A, Polizzi A, Fouche E, Ducheix S, Lippi Y, Lasserre F, Barquissau V, Regnier M, Lukowicz C, Benhamed F et al. (2016) Liver PPARalpha is crucial for whole-body fatty acid homeostasis and is protective against NAFLD. *Gut* **65**, 1202–1214.

218 Bott A, Audano M, Sahebkar A, Sirtori C, Mitro N, Ruscica M (2018) PPAR agonists and metabolic syndrome: an established role?. *Int J Mol Sci* **19**, 1197. doi: 10.3390/ijms19041197.

219 Pawlak M, Lefebvre P & Staels B (2015) Molecular mechanism of PPARalpha action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J Hepatol* **62**, 720–733.
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222 Rakhshandehroo M, Hooiveld G, Muller M & Kersten S (2009) Comparative analysis of gene regulation by the transcription factor PPARalpha between mouse and human. PLoS One 4, e6796.

221 Francque S, Verriken A, Caron S, Prawitt J, Paumelle R, Derudas B, Lefebvre P, Taskinen MR, Van Hul W, Mertens I et al. (2015) PPARalpha gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. J Hepatol 63, 164–173.

222 Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E & Fruchart JC (1998) Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation 98, 2088–2093.

223 Rosenson RS (2008) Fenofibrate: treatment of hyperlipidemia and beyond. Expert Rev Cardiovasc Ther 6, 1319–1330.

224 Belfort R, Berria R, Cornell J & Cusi K (2010) Fenofibrate reduces systemic inflammation markers independent of its effects on lipid and glucose metabolism in patients with the metabolic syndrome. J Clin Endocrinol Metab 95, 829–836.

225 Fabbriini E, Mohammed BS, Korenblat KM, Magkos F, McCrea J, Patterson BW & Klein S (2010) Effect of fenofibrate and niacin on intrahepatic triglyceride content, very low-density lipoprotein kinetics, and insulin action in obese subjects with nonalcoholic fatty liver disease. J Clin Endocrinol Metab 95, 2727–2735.

226 Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, Rakela J & McGill DB (1996) Ursodeoxycholic acid or colchicine in the treatment of non-alcohol-induced steatohepatitis: a pilot study. Hepatology 23, 1464–1467.

227 Fernandez-Miranda C, Perez-Carreras M, Colina F, Lopez-Alonso G, Vargas C & Solis-Herruzo JA (2008) Ursodeoxycholic acid or colchicine in the treatment of patients with nonalcoholic steatohepatitis. J Hepatol 31, 384.

229 Araki E, Yamashita S, Arai H, Yokote K, Satoh J, Inoguchi T, Nakamura J, Maegawa H, Yoshioka N, Tanizawa Y et al. (2019) Efficacy and safety of pemafibrate in people with type 2 diabetes and elevated triglyceride levels: 52-week data from the PROVIDE study. Diabetes Obes Metab 21, 1737–1744.

230 Yokote K, Yamashita S, Arai H, Araki E, Suganami H, Ishibashi S & on behalf of the K-Study Group (2019) Long-term efficacy and safety of pemafibrate, a novel selective peroxisome proliferator-activated receptor-alpha modulator (SPPARMalpha), in dyslipidemic patients with renal impairment. Int J Mol Sci 20, 706.
242 Kilgore KS & Billin AN (2008) PPARbeta/delta ligands as modulators of the inflammatory response. Curr Opin Investig Drugs 9, 463–469.

243 Ham SA, Hwang JS, Yoo T, Lee WJ, Paek KS, Oh JW, Park CK, Kim JH, Do JT, Kim JH et al. (2015) Ligand-activated PPARdelta upregulates alpha-smooth muscle actin expression in human dermal fibroblasts: a potential role for PPARdelta in wound healing. J Dermatol Sci 80, 186–195.

244 Park JR, Ahn JH, Jung MH, Koh JS, Park Y, Hwang SI, Jeong YH, Kwak CH, Lee YS, Seo HG et al. (2016) Effects of peroxisome proliferator-activated receptor-delta agonist on cardiac healing after myocardial infarction. PLoS One 11, e0148510.

245 Iwaisako K, Haimerl M, Paik YH, Taura K, Kodama Y, Sirlin C, Yu E, Yu RT, Downes M, Evans RM et al. (2012) Protection from liver fibrosis by a peroxisome proliferator-activated receptor delta agonist. Proc Natl Acad Sci USA 109, E1369–E1376.

246 Choi YJ, Roberts BK, Wang X, Geaney JC, Naim S, Lefterova MI, Steger DJ, Zhuo D, Qatanani M, Pott S, Kamrani NK, Bourque G, Pettersson S & Liu Vidal-Puig A, Jimenez-Linan M, Lowell BB, Hamann Moran-Salvador E, Lopez-Parra M, Garcia-Alonso V, Matsusue K, Haluzik M, Lambert G, Yim SH, Wojnoonski K, Karpf DB & Krauss RM (2012) Effects of peroxisome proliferator-activated receptor-gamma in non-alcoholic fatty liver disease. Basic Clin Pharmacol Toxicol 124, 528–537.

253 Ricote M & Glass CK (2007) PPARs and molecular mechanisms of transrepression. Biochim Biophys Acta 1771, 926–935.

254 Pascual G, Fong AL, Ogawa S, Gamiel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG & Glass CK (2005) A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature 437, 759–763.

255 Zizzo G & Cohen PL (2015) The PPAR-gamma antagonist GW9662 elicits differentiation of M2c-like cells and upregulation of the MerTK/Gas6 axis: a key role for PPAR-gamma in human macrophage polarization. J Inflamm (Lond) 12, 36.

256 Miyahara T, Schrum L, Rippe R, Xiong S, Yee HF, Motomura K, Anania FA, Willson TM & Tsukamoto H (2000) Peroxisome proliferator-activated receptors and hepatic stellate cell activation. J Biol Chem 275, 35715–35722.

257 Liu X, Xu J, Rosenthal S, Zhang LJ, McCubbin R, Mesghin N, Shang L, Koyama Y, Ma HY, Sharma S et al. (2020) Identification of lineage-specific transcription factors that prevent activation of hepatic stellate cells and promote fibrosis resolution. Gastroenterology 158, 1728–1744.e14.

258 Liss KH & Finck BN (2017) PPARs and nonalcoholic fatty liver disease. Biochimie 136, 65–74.

259 Ratziu V, Giral P, Jacqueminet S, Charlotte F, Hartemann–Heurtier A, Serfaty L, Podevin P, Lacorte JM, Bernhardt C, Bruckert E et al. (2008) Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial. Gastroenterology 135, 100–110.

260 Pettinelli P & Videla LA (2011) Up-regulation of PPAR-gamma mRNA expression in the liver of obese patients: an additional reinforcing lipogenic mechanism to SREBP-1c induction. J Clin Endocrinol Metab 96, 1424–1430.

261 Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Jacobson S, Motgemperd J, Scicchitano P et al. (2006) A placebo-controlled trial of pioglitazone in patients with nonalcoholic steatohepatitis. N Engl J Med 355, 2297–2307.

262 Cusi K, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, Tio F, Hardies J, Darland C, Musi N et al. (2016) Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial. Ann Intern Med 165, 305–315.

263 Harrison SA, Alkhouri N, Davison BA, Sanyal A, Edwards C, Colca JR, Lee BH, Loomba R, Cusi K, Koltermann O et al. (2020) Insulin sensitizer MSDC-0602K in non-alcoholic steatohepatitis: a randomized,
266 Jain MR, Giri SR, Bhoi B, Trivedi C, Rath A, Wilding JP (2012) PPAR agonists for the treatment of cardiovascular disease in patients with diabetes. *Diabetes Obes Metab* **14**, 973–982.

267 Jani RH, Pai V, Jariwala G, Mukhopadhyay S, Bhansali A & Joshi S (2014) A multicenter, prospective, randomized, double-blind study to evaluate the safety and efficacy of Saroglitazar 2 and 4 mg compared with placebo in type 2 diabetes mellitus patients having hypertriglyceridemia not controlled with atorvastatin therapy (PRESS VI). *Diabetes Technol Ther* **16**, 63–71.

268 Jain MR, Giri SR, Bhoi B, Trivedi C, Rath A, Rathod R, Ranvir R, Kadam S, Patel H, Swain P et al. (2018) Dual PPARalpha/gamma agonist saroglitazar improves liver histopathology and biochemistry in experimental NASH models. *Liver Int* **38**, 1084–1094.

269 Wettstein G, Luccarini JM, Poекel T, Hundertmark J, Penners C, Avouac J, Konstantinova I, Guignabert C, Pezet S, Ruzehaji N, Frantz C, Ponsoye M, Avouac J, Pezet S, Boubia B, Poupardin O, Barth M, Binet J, Peralba P, Junie JL, Adarbes V, Defrene E, Tantzen I et al. (2017) The new-generation pan-peroxisome proliferator-activated receptor agonist IVA337 protects the liver from metabolic disorders and fibrosis. *Hepatol Commun* **1**, 524–537.

270 Boubia B, Poupardin O, Barth M, Binet J, Peralba P, Mounier L, Jacquier E, Gauthier E, Lepais V, Chatar C, Junie JL et al. (2018) Design, synthesis, and evaluation of a novel series of indole sulfonamide peroxisome proliferator activated receptor (PPAR) alpha/gamma/delta triple activators: discovery of lanifibranor, a new antifibrotic clinical candidate. *J Med Chem* **61**, 2246–2265.

271 Lefere S, Puengel T, Faye P, Kupkowski F, Adarbes V, Defrene E, Estivalet C, Gawronski X, Tantzen I et al. (2017) The new-generation pan-peroxisome proliferator-activated receptor agonist IVA337 protects the liver from metabolic disorders and fibrosis. *Hepatol Commun* **1**, 524–537.

272 Cren F (2020) Inventiva’s lanifibranor meets the primary and key secondary endpoints in the Phase IIb NATIVE clinical trial in non-alcoholic steatohepatitis (NASH). Inventiva Pharma.

273 Aguilar-Olivos NE, Carrillo-Cordova D, Oria-Hernandez J, Sanchez-Vallie V, Ponciano-Rodriguez G, Ramirez-Jaramillo M, Chable-Montero F, Chavez-Tapia NC, Uribe M & Mendez-Sanchez N (2015) The nuclear receptor FXR, but not LXR, up-regulates bile acid transporter expression in non-alcoholic fatty liver disease. *Ann Hepatol* **14**, 487–493.

274 Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliwer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD et al. (1999) Bile acids: natural ligands for an orphan nuclear receptor. *Science* **284**, 1365–1368.

275 Wang H, Chen J, Hollister K, Sowers LC & Forman BM (1999) Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol Cell* **3**, 543–553.

276 Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ & Shan B (1999) Identification of a nuclear receptor for bile acids. *Science* **284**, 1362–1365.

277 Stofan M & Guo GL (2020) Bile acids and FXR: novel targets for liver diseases. *Front Med (Lausanne)* **7**, 544.

278 Laffitte BA, Kast HR, Nguyen CM, Zavacki AM, Moore DD & Edwards PA (2000) Identification of the DNA binding specificity and potential target genes for the farnesoid X-activated receptor. *J Biol Chem* **275**, 10638–10647.

279 Chong HK, Infante AM, Seo YK, Jeon TI, Zhang Y, Edwards PA, Xie X & Osborne TF (2010) Genome-wide interrogation of hepatic FXR reveals an asymmetric IR-1 motif and synergy with LRH-1. *Nucleic Acids Res* **38**, 6007–6017.

280 Chong HK, Biesinger J, Seo YK, Xie X & Osborne TF (2012) Genome-wide analysis of hepatic LRH-1 reveals a promoter binding preference and suggests a role in regulating genes of lipid metabolism in concert with FXR. *BMC Genom* **13**, 51.

281 Li L, Zhang Q, Peng J, Jiang C, Zhang Y, Shen L, Dong J, Wang Y & Jiang Y (2015) Activation of farnesoid X receptor downregulates monocyte chemoattractant protein-1 in murine macrophage. *Biochem Biophys Res Commun* **467**, 841–846.

282 Claudel T, Sturm E, Duez H, Torra IP, Sirvent A, Kosykh V, Fruchart JC, Dallongeville J, Hum DW, Kuipers F et al. (2002) Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-I transcription via a negative FXR response element. *J Clin Invest* **109**, 961–971.

283 Barbier O, Torra IP, Sirvent A, Claudel T, Blanquart C, Duran-Sandoval D, Kuipers F, Kosykh V, Fruchart JC & Staels B (2003) FXR induces the UGT2B4 enzyme in hepatocytes: a potential mechanism of negative feedback control of FXR activity. *Gastroenterology* **124**, 1926–1940.
284 Chenamsetty I, Claudel T, Kostner KM, Baghdasaryan A, Kratky D, Levak-Frank S, Frank S, Gonzalez FJ, Trauner M & Kostner GM (2011) Farnesoid X receptor represses hepatic human APOA gene expression. J Clin Invest 121, 3724–3734.

285 Kim I, Ahn SH, Inagaki T, Choi M, Ito S, Guo GL, Kliwer SA & Gonzalez FJ (2007) Differential regulation of bile acid homeostasis by the farnesoid X receptor in liver and intestine. J Lipid Res 48, 2664–2672.

286 Plass JR, Mol O, Heegsma J, Geuken M, Faber KN, Jansen PL & Muller M (2002) Farnesoid X receptor and bile salts are involved in transcriptional regulation of the gene encoding the human bile salt export pump. Hepatology 35, 589–596.

287 Landrier JF, Eloranta JJ, Vavricka SR & Kullak-Ublick GA (2006) The nuclear receptor for bile acids, FXR, transactivates human organic solute transporter-alpha and -beta genes. Am J Physiol Gastrointest Liver Physiol 290, G476–G485.

288 Zollner G, Wagner M, Moustafa T, Faber KN, Kostner KM & Thibaut R. (2001) Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. Gastroenterology 125, 544–555.

289 Pineda Torra I, Claudel T, Duval C, Kosykh V, Frucht JC, Staub S, Kast HR, Nguyen CM, Sinal CJ, Jones SA, Lafitte BA, Reue K, Gonzalez FJ, Willson TM & Edwards PA (2001) Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. Mol Endocrinol 15, 1720–1728.

290 Claudel T, Inoue Y, Barbier O, Duran-Sandoval D, Kosykh V, Frucht JC, Gonzalez FJ & Staub S (2003) Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. Gastroenterology 125, 544–555.

291 Hang S, Paik D, Yao L, Kim E, Trinath J, Lu J, Ha S, Nelson BN, Kelly SP, Wu L et al. (2019) Bile acid metabolites control TH17 and Treg cell differentiation. Nature 576, 143–148.

292 Vaubourj P, Mencarelli A, Renga B, Disturbi E & Fiorucci S (2009) The bile acid receptor FXR is a modulator of intestinal innate immunity. J Immunol 183, 6251–6261.

293 Pineda Torra I, Claudel T, Duval C, Kosykh V, Frucht JC & Staub S (2003) Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor. Mol Endocrinol 17, 259–272.

294 Thomas AM, Hart SN, Kong B, Fang J, Zhong XB & Guo GL (2010) Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine. Hepatology 51, 1410–1419.

295 Zhan L, Liu HX, Fang Y, Kong B, He Y, Zhong XB, Fang J, Wan YJ & Guo GL (2014) Genome-wide binding and transcriptome analysis of human farnesoid X receptor in primary human hepatocytes. PLoS One 9, e105930.

296 Thomas AM, Hart SN, Kong B, Fang J, Zhong XB & Guo GL (2010) Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine. Hepatology 51, 1410–1419.

297 Thomas AM, Hart SN, Kong B, Fang J, Zhong XB & Guo GL (2010) Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine. Hepatology 51, 1410–1419.

298 Thomas AM, Hart SN, Kong B, Fang J, Zhong XB & Guo GL (2010) Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine. Hepatology 51, 1410–1419.

299 Thomas AM, Hart SN, Kong B, Fang J, Zhong XB & Guo GL (2010) Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine. Hepatology 51, 1410–1419.

300 Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, Willson TM & Edwards PA (2006) Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. Proc Natl Acad Sci USA 103, 1006–1011.

301 Wang YD, Chen WD, Wang M, Yu D, Forman BM & Huang W (2008) Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. Hepatology 48, 1632–1643.

302 Zhang S, Wang J, Liu Q & Harnish DC (2009) Farnesoid X receptor agonist WAY-362450 attenuates liver inflammation and fibrosis in murine model of non-alcoholic steatohepatitis. J Hepatol 51, 380–388.

303 Fiorucci S & Disturbi E (2015) Bile acid-activated receptors, intestinal microbiota, and the treatment of metabolic disorders. Trends Mol Med 21, 702–714.

304 Campbell C, McKenney PT, Konstantinovsky D, Isaeva OI, Schizas M, Verter J, Mai C, Jin WB, Guo CJ, Violante S et al. (2020) Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. Nature 581, 475–479.

305 Hang S, Paik D, Yao L, Kim E, Trinath J, Lu J, Ha S, Nelson BN, Kelly SP, Wu L et al. (2019) Bile acid metabolites control TH17 and Treg cell differentiation. Nature 576, 143–148.
The bile acid sensor farnesoid X receptor (FXR) is required for immune-regulatory activities of TLR-9 in intestinal inflammation. *PLoS One* **8**, e54472.

Wildenberg ME & van den Brink GR (2011) FXR activation inhibits inflammation and preserves the intestinal barrier in IBD. *Gut* **60**, 432–433.

Mencarelli A, Renga B, Migliorati M, Cipriani S, Distruiti E, Santucci L & Fiorucci S (2009) The bile acid sensor farnesoid X receptor is a modulator of liver immunity in a rodent model of acute hepatitis. *J Immunol* **183**, 6657–6666.

McMahan RH, Wang XX, Cheng LL, Smith M, El Kasm K, Pruzenski M, Adorini L, Golden-Mason L, Levi M et al. (2013) Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. *J Biol Chem* **288**, 11761–11770.

Carino A, Cipriani S, Marchiano S, Biagioli M, Santorelli C, Donini A, Zampella A, Monti MC & Fiorucci S (2017) BAR502, a dual FXR and GPBAR1 agonist, promotes browning of white adipose tissue and reverses liver steatosis and fibrosis. *Sci Rep* **7**, 42801.

Hogenauer K, Arista L, Schmiedeberg N, Werner G, Distrutti E, Santucci L & Fiorucci S (2011) SHP-1 and SHP-2 mediate cytokine-induced potentiation of TLR-9 and IFN-α-dependent antiviral responses in human myeloid cells. *J Immunol Ther* **6**, 10343–10354.

Fiorucci S, Rizzo G, Antonelli E, Renga B, Mencarelli A, Riccardi L, Morelli A, Pruzenski M & Pellicciari R (2005) Cross-talk between farnesoid-X-receptor (FXR) and peroxisome proliferator-activated receptor gamma contributes to the antifibrotic activity of FXR ligands in rodent models of liver cirrhosis. *J Pharmacol Exp Ther* **315**, 58–68.

Renga B, Mencarelli A, Migliorati M, Cipriani S, D’Amore C, Distruiti E & Fiorucci S (2011) SHP-dependent and -independent induction of peroxisome proliferator-activated receptor-gamma by the bile acid sensor farnesoid X receptor counter-regulates the pro-inflammatory phenotype of liver myofibroblasts. *Inflamm Res* **60**, 577–587.

Chapman RW & Lynch KD (2020) Obeticholic acid—a new therapy in PBC and NASH. *Br Med Bull* **133**, 95–104.

Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B et al. (2015) Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* **385**, 956–965.

Hameed B, Terrault NA, Gill RM, Loomba R, Chalasani N, Hoofnagle JH, Van Natta ML & Nash CRN (2018) Clinical and metabolic effects associated with weight changes and obeticholic acid in non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* **47**, 645–656.

Ratziu V, Sanyal AJ, Loomba R, Rinella M, Harrison S, Anstee QM, Goodman Z, Bedossa P, MacConell L, Shringarpure R et al. (2019) REGENERATE: Design of a pivotal, randomised, phase 3 study evaluating the safety and efficacy of obeticholic acid in patients with fibrosis due to nonalcoholic steatohepatitis. *Contemp Clin Trials* **84**, 105803.

(2020) Intercept’s NASH hopes dashed. *Nat Biotechnol* **38**, 911.

Trauner M, Gulamhusein A, Hameed B, Caldwell S, Shiffman ML, Landsis C, Eksteen B, Agarwal K, Muir A, Rushbrook S et al. (2019) The nonsteroidal farnesoid X receptor agonist cilofexor (GS-9674) improves markers of cholestasis and liver injury in patients with primary sclerosing cholangitis. *Hepatology* **70**, 788–801.

Patel K, Harrison SA, Elkhashab M, Trotter JF, Herring R, Rojter SE, Kayali Z, Wong VW, Greenbloom S, Jayakumar S et al. (2020) Cilofexor, a nonsteroidal FXR agonist, in patients with noncirrhotic NASH: a phase 2 randomized controlled trial. *Hepatology* **72**, 58–71.

Hernandez ED, Zheng L, Kim Y, Fang B, Liu B, Valdez RA, Dietrich WF, Rucker PV, Chianelli D, Schmeits J et al. (2019) Tropifexor-mediated abrogation of steatohepatitis and fibrosis is associated with the antioxidative gene expression profile in rodents. *Hepatol Commun* **3**, 1085–1097.

Orliaguet L, Dalmas E, Drareni K, Venteclef N & Alzaid F (2020) Mechanisms of macrophage polarization in insulin signaling and sensitivity. *Front Endocrinol (Lausanne)* **11**, 62.

Eaton RP, Allen RC & Schade DS (1983) Hepatic removal of insulin in normal man: dose response to endogenous insulin secretion. *J Clin Endocrinol Metab* **56**, 1294–1300.

Polonsky KS & Rubenstein AH (1984) C-peptide as a measure of the secretion and hepatic extraction of insulin. Pitfalls and limitations. *Diabetes* **33**, 486–494.

Song SH, McIntyre SS, Shah H, Veldhuis JD, Hayes PC & Butler PC (2000) Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. *J Clin Endocrinol Metab* **85**, 4491–4499.

Ieronymaki E, Daskalaki MG, Lyroni K & Tsatsanis C (2019) Insulin signaling and insulin resistance facilitate trained immunity in macrophages through...
metabolic and epigenetic changes. *Front Immunol* 10, 1330.

328 Artyomov MN & Van den Bossche J (2020) Immunometabolism in the Single-Cell Era. *Cell Metab* 32, 710–725.

329 Bedossa P, Poitou C, Veyrie N, Bouillot JL, Basdevant A, Paradis V, Tordjman J & Clement K (2012) Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology* 56, 1751–1759.

330 Poynard T, Peta V, Deckmyn O, Pais R, Ngo Y, Charlotte F, Ngo A, Munteanu M, Imbert-Bismut F, Monneret D et al. (2020) Performance of liver biomarkers, in patients at risk of nonalcoholic steatohepatitis, according to presence of type-2 diabetes. *Eur J Gastroenterol Hepatol* 32, 998–1007.

331 López-Sánchez G, Domínguez-Pérez M, Uribe M, Chávez-Tapia NC, Nuno-Lambarri N (2021) Nonalcoholic fatty liver disease and microRNAs expression, how it affects the development and progression of the disease. *Ann Hepatol* 21, 100212. doi: 10.1016/j.aohep.2020.04.012

332 Sanyal AJ (2019) Past, present and future perspectives in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 16, 377–386.

333 Holan V & Minowada J (1992) Selective enhancement of interleukin 1 beta production in myelomonocytic cell lines by insulin and its related cytokines. *Immunol Lett* 34, 243–247.

334 Iida KT, Shimano H, Kawakami Y, Sone H, Toyoshima H, Suzuki S, Asano T, Okuda Y & Yamada N (2001) Insulin up-regulates tumor necrosis factor-alpha production in macrophages through an extracellular-regulated kinase-dependent pathway. *J Biol Chem* 276, 32531–32537.

335 Mauer J, Chaurasia B, Plum L, Quast T, Hampel B, Bluher M, Kolanus W, Kahn CR & Bruning JC (2010) Myeloid cell-restricted insulin receptor deficiency protects against obesity-induced inflammation and systemic insulin resistance. *PLoS Genet* 6, e1000938.

336 Park YM, Kashyap SR, Major JA & Silverstein RL (2012) Insulin promotes macrophage foam cell formation: potential implications in diabetes-related atherosclerosis. *Lab Invest* 92, 1171–1180.

337 Rotllan J, Chamorro-Jorjanes A, Araldi E, Wanschel AC, Aryal B, Aranda JF, Goedeke L, Salerno AG, Ramirez CM, Sessa WC et al. (2015) Hematopoietic Akt2 deficiency attenuates the progression of atherosclerosis. *FASEB J* 29, 597–610.

338 Dör R, Dalmas E, Meier DT, Wueest S, Thevenet J, Thielen C, Timper K, Nordmann TM, Traub S, Schulze F et al. (2017) Postprandial macrophage-derived IL-1beta stimulates insulin, and both synergistically promote glucose disposal and inflammation. *Nat Immunol* 18, 283–292.

339 Tessaro FHG, Ayala TS, Nolasco EL, Bella LM & Martins JO (2017) Insulin influences LPS-induced TNF-alpha and IL-6 release through distinct pathways in mouse macrophages from different compartments. *Cell Physiol Biochem* 42, 2093–2104.

340 Ieronymaki E, Theodorakis EM, Lyroni K, Vergadi E, Lagoudaki E, Al-Qahtani A, Aznauorova M, Neofotistou-Themeli E, Eliopoulos AG, Vaporioti K et al. (2019) Insulin resistance in macrophages alters their metabolism and promotes an M2-like phenotype. *J Immunol* 202, 1786–1797.

341 Costa Rosa LF, Safi DA, Cury Y & Curi R (1996) The effect of insulin on macrophage metabolism and function. *Cell Biochem Funct* 14, 33–42.

342 Dandonia P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E & Ahmad S (2001) Insulin inhibits intranuclear factor kappab and stimulates Ikappab in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab* 86, 3257–3265.

343 Iida KT, Suzuki H, Sone H, Shimano H, Toyoshima H, Yatoh S, Asano T, Okuda Y & Yamada N (2002) Insulin inhibits apoptosis of macrophage cell line, THP-1 cells, via phosphatidylinositol-3-kinase-dependent pathway. *Arterioscler Thromb Vase Biol* 22, 380–386.

344 Leffler M, Hrach T, Stuerzl M, Horch RE, Herndon DN & Jeschke MG (2007) Insulin attenuates apoptosis and exerts anti-inflammatory effects in endotoxemic human macrophages. *J Surg Res* 143, 398–406.

345 Martins JO, Ferracini M, Ravanelli N, Landgraf RG & Jancar S (2008) Insulin suppresses LPS-induced iNOS and COX-2 expression and NF-kappaB activation in alveolar macrophages. *Cell Physiol Biochem* 22, 279–286.

346 Senokuchi T, Liang CP, Seimon TA, Han S, Matsumoto M, Banks AS, Paik JH, DePinho RA, Accili D, Tabas I et al. (2008) Forkhead transcription factors (FoxOs) promote apoptosis of insulin-resistant macrophages during cholesterol-induced endoplasmic reticulum stress. *Diabetes* 57, 2967–2976.

347 Cuschieri J, Bulger E, Grinsell R, Garcia I & Maier RV (2008) Insulin regulates macrophage activation through activin A. *Shock* 29, 285–290.

348 Su D, Coudriet GM, Hyun Kim D, Lu Y, Perdomo G, Qu S, Slusher S, Tse HM, Piganelli J, Giannoukakis N et al. (2009) FoxO1 links insulin resistance to proinflammatory cytokine IL-1beta production in macrophages. *Diabetes* 58, 2624–2633.

349 Mita T, Goto H, Azuma K, Jin WL, Nomiyama T, Fujitani Y, Hirose T, Kawamori R & Watada H (2010) Impact of insulin resistance on enhanced
monocyte adhesion to endothelial cells and atherosclerogenesis independent of LDL cholesterol level. *Biochem Biophys Res Commun* **395**, 477–483.

350 Mita T, Azuma K, Goto H, Jin WL, Arakawa M, Nomiyama T, Suzuki R, Kubota N, Tobe K, Kadowaki T *et al.* (2011) IRS-2 deficiency in macrophages promotes their accumulation in the vascular wall. *Biochem Biophys Res Commun* **415**, 545–550.

351 Liang CP, Han S, Li G, Tabas I & Tall AR (2012) Impaired MEK signaling and SERCA expression promote ER stress and apoptosis in insulin-resistant macrophages and are reversed by exenatide treatment. *Diabetes* **61**, 2609–2620.

352 Yan H, Ma Y, Li Y, Zheng X, Lv P, Zhang Y, Li J, Ma M, Zhang L, Li C *et al.* (2016) Insulin inhibits inflammation and promotes atherosclerotic plaque stability via PI3K-Akt pathway activation. *Immunol Lett* **170**, 7–14.

353 Reardon CA, Lingaraju A, Schoenfelt KQ, Zhou G, Cui C, Jacobs-El H, Babenko I, Hoofnagle A, Czyz D, Shuman H *et al.* (2018) Obesity and insulin resistance promote atherosclerosis through an IFNgamma-regulated macrophage protein network. *Cell Rep* **23**, 3021–3030.

354 Pal S, Nath P, Das D, Hajra S & Maitra S (2018) Cross-talk between insulin signalling and LPS responses in mouse macrophages. *Mol Cell Endocrinol* **476**, 57–69.

355 Yu T, Gao M, Yang P, Pei Q, Liu D, Wang D, Zhang X & Liu Y (2017) Topical insulin accelerates cutaneous wound healing in insulin-resistant diabetic rats. *Am J Transl Res* **9**, 4682–4693.

356 Kubota T, Inoue M, Kubota N, Takamoto I, Mineyama T, Iwayama K, Tokuyama K, Moroi M, Ueki K, Yamauchi T *et al.* (2018) Downregulation of macrophage Irs2 by hyperinsulinemia impairs IL-4-induced M2a-subtype macrophage activation in obesity. *Nat Commun* **9**, 4863.

357 Yu T, Gao M, Yang P, Liu D, Wang D, Song F, Zhang X & Liu Y (2019) Insulin promotes macrophage phenotype transition through PI3K/Akt and PPAR-gamma signaling during diabetic wound healing. *J Cell Physiol* **234**, 4217–4231.

358 Yang P, Wang X, Wang D, Shi Y, Zhang M, Yu T, Liu D, Gao M, Zhang X, Liu Y (2020) Topical insulin application accelerates diabetic wound healing by promoting anti-inflammatory macrophage polarization. *J Cell Sci* **133**, jcs235838. doi: 10.1242/jcs.235838