



# Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease

Adel Hammoutene, Pierre-Emmanuel Rautou

## ► To cite this version:

Adel Hammoutene, Pierre-Emmanuel Rautou. Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. *Journal of Hepatology*, 2019, 70, pp.1278 - 1291. 10.1016/j.jhep.2019.02.012 . hal-03484914

**HAL Id: hal-03484914**

**<https://hal.science/hal-03484914>**

Submitted on 20 Dec 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

**Role of liver sinusoidal endothelial cells in nonalcoholic fatty liver disease**

**Authors:** Adel Hammoutene<sup>1,2</sup>, & Pierre-Emmanuel Rautou<sup>1,3,4,5</sup>

1- Inserm, UMR-970, Paris Cardiovascular Research Center, PARCC, Paris, France

2- University Paris Descartes, Paris, France

3- INSERM, UMR1149, Centre de Recherche sur l'Inflammation, Paris, France

4- University Paris Diderot, Paris, France

5- Service d'Hépatologie, Centre de Référence des Maladies Vasculaires du Foie,

DHU Unity, Pôle des Maladies de l'Appareil Digestif, Hôpital Beaujon, AP-HP,

Clichy, France

**Corresponding author:**

Prof. Pierre-Emmanuel Rautou, MD, PhD

Service d'hépatologie, Hôpital Beaujon,

100 boulevard du Général Leclerc, 92100 Clichy, France

Tel: +33.1.40. 87.52.83

Fax: +33.1. 40 87 55 30

pierre-emmanuel.rautou@inserm.fr

**Key words:** endothelium; steatosis; capillarization; endothelial dysfunction;

angiogenesis

**Abbreviations:** cGMP: cyclic guanosine monophosphate; eNOS: endothelial nitric oxide synthase; FABP4: fatty acid binding protein 4; FFA: free fatty acids; HCC: hepatocellular carcinoma; HIF: hypoxia inducible factors; ICAM-1: intercellular adhesion molecule 1; IL: interleukin; iNOS: inducible nitric oxide synthase; KLF: krüppel-like Factor; LSECs: liver sinusoidal endothelial cells; MAPK: mitogen activated protein kinase; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; NK: natural killer; ox-LDL: oxidized low density lipoprotein; PLVAP: plasmalemma vesicle-associated protein; ROS: reactive oxygen species; TGF: transforming growth factor; TLR: toll like receptor; TNF: tumor necrosis factor; VAP-1: vascular adhesion protein 1; VCAM-1: vascular cell adhesion molecule 1; VEGF: vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor.

**Words:** 5269

**References:** 147

**Financial support:** This work was supported by the “Institut National de la Santé et de la Recherche Médicale” (ATIP Avenir), Paris Descartes University, the “Agence Nationale pour la Recherche” (ANR-14-CE12-0011, ANR-14-CE35-0022, ANR-18-CE14-0006-01) and by the “Association Française pour l’Etude du foie” (AFEF 2014). A.H. was supported by the “Ministère de l’Enseignement Supérieur et de la Recherche”.

**Conflict of interest:** none

**Authors’ contributions:** A.H. and P-E.R wrote the manuscript.

1 **Summary:** (233 words)

2 Nonalcoholic fatty liver disease (NAFLD) and its complication is an expanding health  
3 problem associated with the metabolic syndrome. Liver sinusoidal endothelial cells  
4 (LSECs) are highly specialized endothelial cells localized at the interface between the  
5 blood derived from the gut and the adipose tissue on the one side, and other liver cells  
6 on the other side. In physiological conditions, LSECs are gatekeepers of liver  
7 homeostasis. LSECs display anti-inflammatory and anti-fibrogenic properties by  
8 preventing Kupffer cells and hepatic stellate cells activation and regulate intrahepatic  
9 vascular resistance and portal pressure. This review focuses on changes occurring in  
10 LSECs in NAFLD and on their consequences on NAFLD progression and complications.  
11 Capillarization, *i.e.* loss of LSECs *fenestrae*, and LSECs dysfunction, *i.e.* the loss of the  
12 ability of LSECs to generate vasodilator agents in response to increased shear stress, are  
13 two events occurring early in NAFLD. These LSECs changes favor steatosis development  
14 and set the stage for NAFLD progression. At the stage of non-alcoholic steatohepatitis,  
15 altered LSECs release inflammatory mediators and contribute to the recruitment of  
16 inflammatory cells, thus promoting liver injury and inflammation. Altered LSECs also fail  
17 to maintain hepatic stellate cells quiescence and release fibrogenic mediators, including  
18 Hedgehog signaling molecules, promoting liver fibrosis. Liver angiogenesis is increased  
19 in NAFLD and contributes to liver inflammation and fibrosis, but also to hepatocellular  
20 carcinoma development. Improving LSECs health thus appears as a promising approach  
21 to prevent NAFLD progression and complications.

1 **Lay summary**

2 Liver sinusoidal endothelial cells are highly specialized endothelial cells integrating  
3 signals derived from the gut and the adipose tissue, and interacting with other liver  
4 cells.

5 In nonalcoholic liver disease, liver sinusoidal endothelial cells alterations occur early in  
6 disease progression, at the stage of simple steatosis.

7 In nonalcoholic liver disease, changes in liver sinusoidal endothelial cells contribute to  
8 liver angiogenesis, inflammation, fibrosis and to hepatocellular carcinoma.

9

Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of conditions including simple steatosis and nonalcoholic steatohepatitis (NASH), defined as the association of steatosis, hepatocellular damage, inflammation and varying degree of fibrosis [1]. NAFLD is associated with obesity, insulin resistance and/or type 2 diabetes and other metabolic abnormalities, collectively termed the metabolic syndrome [2,3]. NAFLD is an expanding health problem with an estimated global prevalence of 25% [2,4]. A recent modeling approach estimated that NAFLD cases in the United States will expand from 83 million in 2015, corresponding to about 25% of the population, to 100 million in 2030, corresponding to more than 33% of the population [4]. While simple steatosis is generally benign, NASH can progress to both cirrhosis and end-stage liver disease. NASH is currently a leading cause of liver disease among adults awaiting liver transplantation in Europe and in the United States and is projected to become the most common indication for liver transplantation in the next decade [2,4]. Importantly, patients with metabolic syndrome and NASH also develop hepatocellular carcinoma (HCC) in the absence of cirrhosis [5,6]. Despite its prevalence and severity, there is no approved therapy for NASH and available treatments only aim to control associated conditions [1]. Understanding the mechanisms of NAFLD, and in particular how simple steatosis progresses to NASH and then to cirrhosis and/or liver cancer, is of utmost importance.

Current view of the pathogenesis of NASH centers on the response of hepatocytes to insulin resistance and lipotoxicity. Immune system and hepatic stellate cell activation are regarded as secondary events [1]. Vascular endothelium, representing the interface between blood and other tissues of the body, is not only a physical barrier but is implicated in different physiological roles, such as hemostasis, metabolites

1 transportation, inflammation, thrombosis, angiogenesis and vascular tone. Liver  
2 endothelium is mainly formed of liver sinusoidal endothelial cells (LSECs) which are  
3 highly specialized endothelial cells at the interface between blood derived from the  
4 visceral adipose tissue and the gut, on the one side, and hepatic stellate cells and  
5 hepatocytes, on the other side. LSECs have a unique phenotype in the human body as  
6 they lack a basement membrane and have a multitude of *fenestrae* organized in sieves,  
7 that regulate the transport of macromolecules, including lipids and lipoproteins, across  
8 the sinusoid [7,8]. This review will specifically focus on the role of LSECs in the  
9 pathophysiology of NAFLD and its complications.

## 11 **LSECs and simple steatosis**

### 12 *Role of LSECs in lipid transfer in the normal liver*

13 Dietary lipids present in the circulation have to be transported through the vascular  
14 endothelium to be metabolized by tissues. In physiological conditions, LSECs are major  
15 regulators of the bidirectional lipid exchange between the blood and the liver  
16 parenchyma in two manners. First, LSECs *fenestrae* allow for efficient transfer of  
17 lipoproteins, chylomicron remnants (small lipoproteins derived from chylomicrons  
18 generated by enterocytes from dietary lipids), and other macromolecules, from the  
19 sinusoidal blood to the space of Disse, where they are taken up by hepatocytes [9–11].  
20 LSECs *fenestrae* form a selective barrier for lipids. Indeed, older studies using  
21 radiolabeled lipoproteins showed that larger lipoproteins do not cross LSECs *fenestrae*  
22 and remain in the lumen of the sinusoid [12]. Moreover, when LSECs lose their  
23 *fenestrae* following VEGF pathway disruption, [11,13,14], uptake of fluorescently

labelled lipids is impaired [11,15]. Second, LSECs also regulate lipid transfer through their high endocytosis capacity, as attested by their high ability to rapidly take-up oxidized or acetylated-low density lipoproteins [8,16,17].

#### LSECs capillarization occurs early in NAFLD and promotes steatosis

LSECs undergo morphological and functional changes during liver steatosis [18–21]. One of the most remarkable phenotypic changes is the loss of *fenestrae*, also called defenestration or sinusoidal capillarization, associated with the formation of a basement membrane on the abluminal surface of LSECs. Several independent groups reported that sinusoidal capillarization appears very early in NAFLD [18–20]. Miyao and colleagues observed that defenestration begins after 1 week of choline-deficient, L-amino acid-defined diet in mice [18]. Similar observations were made in rats fed a high-fat diet for 3 weeks [20].

Triggers for sinusoidal capillarization are not fully identified, but we can speculate that excessive dietary macronutrients, including lipids, carbohydrates, and gut microbiota-derived products play a role [19]. Cogger and coworkers demonstrated in mice challenged with several diets varying in content of macronutrients and energy that LSECs porosity and *fenestrae* frequency are inversely correlated with dietary fat intake, while *fenestrae* diameter is inversely correlated with protein or carbohydrate intake [19]. In this study, authors also found a negative correlation between LSECs *fenestrae* (frequency, porosity and diameter) and circulating free fatty acids (FFA) levels [19]. *In vitro* studies suggested that defenestration occurs following excessive lipids exposition. For instance, exposure of human primary LSECs to oxidized-LDL (ox-LDL) reduces the diameter and the porosity of the *fenestrae* [21]. The effect of FFA on *fenestrae* has also



1 been tested in primary rat LSECs, but firm conclusions cannot be drawn since authors  
2 did not test several concentrations of FFA but rather presence vs. absence of FFA, which  
3 does not adequately mimic *in vivo* conditions [22]. Recent evidence point to gut  
4 microbiota in the pathogenesis of NAFLD [3,23]. Cogger and colleagues showed that  
5 LSECs *fenestrae* inversely correlated with the abundance of *Bacteroidetes* in the gut and  
6 positively correlated with the abundance of *Firmicutes* [19]. Moreover, it has been  
7 shown that a single injection of endotoxin in rats induces a decrease in both diameter  
8 and number of *fenestrae* suggesting that gut microbiota-derived products may  
9 contribute to LSECs capillarization, although caution is needed since concentration of  
10 endotoxin used in that study was high [24].

11 In turn, capillarization favors liver steatosis (Figure 1), as observed in mice deficient in  
12 plasmalemma vesicle-associated protein (PLVAP), an endothelial-specific integral  
13 membrane glycoprotein required for the formation of endothelial *fenestrae* [25]. These  
14 mice exhibit a pronounced reduction in the number of LSECs *fenestrae*, associated with a  
15 decrease in sinusoidal permeability [25] and spontaneously develop extensive steatosis  
16 [25]. These mice also have hyperlipoproteinemia and increased triglyceridemia due to  
17 chylomicron remnants retention in the blood. As mentioned above, *vegfb*<sup>-/-</sup> mice also  
18 exhibit reduction in the number of LSECs *fenestrae* and less uptake of labeled oleic acid  
19 due to capillarization, but steatosis was not evaluated [15].

20 A first hypothesis explaining this consequence of capillarization on steatosis could be  
21 that reduced LSECs permeability impairs the passage of hepatocytes-derived very low-  
22 density lipoprotein toward the sinusoidal lumen, thus inducing cholesterol and  
23 triglycerides retention in the liver. However, these lipoproteins may escape the liver  
24 through the lymphatic system [26]. As an alternative explanation, Herrnberger and

collaborators proposed that chylomicron remnants originating from the blood and required for synthesis of very low-density lipoprotein by hepatocytes can not reach hepatocytes due to LSECs capillarization; their absence in hepatocytes might then stimulate hepatic *de novo* lipid synthesis and induce steatosis, as a compensatory mechanism [25,27]. However, there is no available data to ascertain this hypothesis. Similarly, Fraser and collaborators postulated that, following LSECs capillarization, chylomicron remnants and dietary cholesterol do no longer pass *fenestrae* and inhibit HMGCoA reductase, the rate limiting enzyme for hepatocyte cholesterol biosynthesis, consequently activating endogenous cholesterol synthesis in hepatocytes [28]. Taken together, these data suggest that features of metabolic syndrome are associated with LSECs capillarization, which promotes steatosis (Figure 1).

#### *LSECs dysfunction occurs early in NAFLD and promotes steatosis*

Liver steatosis is associated with an increased portal pressure and increased intrahepatic vascular resistance [29–32]. In patients, hepatic venous pressure gradient correlates with the degree of steatosis [29]. Using Doppler flowmetry, Seifalian and colleagues observed an impaired sinusoidal perfusion in human fatty liver grafts compared with normal liver grafts [33]. Similar results were obtained in rabbits and rats with diet-induced steatosis, with an inverse correlation between hepatic parenchymal microcirculation and the severity of steatosis [34,35].

This increased intrahepatic vascular resistance has a mechanical and a dynamic component. The mechanical part is due to the compression of the sinusoidal lumen by enlarged fat-laden hepatocytes [29,36–38]. The dynamic part is due to a liver endothelial dysfunction. Endothelial dysfunction is a pathological condition, common to

all vascular beds, defined as the inability of blood vessels to dilate in response to increased blood flow. Endothelial dysfunction is generally indicated by the loss of nitric oxide bioavailability due to eNOS inhibition [39]. Several lines of evidence show that LSECs dysfunction occurs in fatty livers and is involved in the increased intrahepatic vascular resistance associated with steatosis [40–43]. eNOS activation and liver nitric oxide content are reduced in mice and rats fed a high-fat diet or a rich saturated fatty acids diet for 4 weeks [40,41]. Isolated-perfused liver experiments performed in these animals showed augmented portal perfusion pressure and reduced vasodilatory response to acetylcholine, attesting liver endothelial dysfunction. These changes were observed in the absence of inflammation and fibrosis, suggesting that endothelial dysfunction is an early feature associated with steatosis in NAFLD [41,42].

Several mechanisms could account for this liver endothelial dysfunction associated with steatosis (Figure 2). First, LSECs dysfunction can be induced by overabundance of lipids during steatosis. *In vitro* experiments showed that stimulation of human primary LSECs with ox-LDL downregulates eNOS expression through the ox-LDL receptor, LOX1 [21]. In addition, exposure of primary LSECs to palmitic acid also attenuates nitric oxide bioavailability through peroxynitrite production by NOX1, a nitric oxide consuming enzyme highly expressed in LSECs of mice fed a high-fat diet [44]. Second, steatosis induces insulin resistance in LSECs, leading to impairment of insulin-dependent vasodilation [41,45,46]. This effect is due, on the one hand, to the downregulation of eNOS activity [41], and on the other hand to the upregulation of iNOS, the inducible form of NOS which can cause endothelial dysfunction through increased nitrooxidative stress [46–48]. Interestingly, V-PYRRO/NO, a diazeniumdiolate ion metabolized in the liver that spontaneously decomposes to nitric oxide with a very short half-life at physiological

pH and triggers cyclic guanosine 3',5'-monophosphate (cGMP) synthesis, improves hepatic microcirculation in mice with high-fat diet-induced steatosis [49]. Third, gut microbiota also seems to contribute to liver endothelial dysfunction. Indeed, Garcia-Lezana and colleagues demonstrated that restoration of a healthy microbiota via fecal transplantation normalizes portal hypertension by improving intrahepatic vascular resistance and endothelial dysfunction in rats [43].

In turn, LSECs dysfunction favors steatosis (Figure 2). Indeed, deficiency in nitric oxide in *eNos*<sup>-/-</sup> mice results in massive fat droplets deposition and increases liver triglyceride content [40,50]. Nitric oxide contributes to the regulation of hepatic lipid content by limiting citrate synthesis in mitochondria, which is involved in fatty acids production [50]. Nitric oxide also attenuates synthesis of fatty acids in isolated cultured rat hepatocytes by nitrosylating acetyl-CoA [51] and activating AMP-activated protein kinase [52–54], an inhibitor of glycerol-3-phosphate acyltransferase and thus of triacylglycerol synthesis [55,56]. In addition, nitric oxide also allows efficient fatty acids beta-oxidation through s-nitrosylation of very long-chain acyl-CoA dehydrogenase in hepatocytes [57]. Interestingly, therapies augmenting nitric oxide availability in the liver improve steatosis. The V-PYRRO/NO or the improvement of nitric oxide/cGMP signaling with the phosphodiesterase-5 inhibitor sildenafil protect against liver steatosis in high-fat diet-fed mice [40,58,59]. Moreover, treatment with simvastatin, a drug able to increase expression and activity of eNOS expression in the liver, decreases high-fat diet-induced steatosis in rats [60].

To summarize, steatosis is associated with LSECs dysfunction which in turn worsens steatosis (Figure 2).

## 1 Angiogenesis and steatosis

2 Angiogenesis, defined as the formation of new vessels from preexistent vessels, is a key  
3 event in the progression of NAFLD [61–65]. Vascular endothelial growth factor (VEGF) is  
4 the master pro-angiogenic regulator of this process supported by activation of hypoxia  
5 inducible factors (HIF) in hypoxic areas [64].

6 Serum VEGF levels are higher in patients with biopsy-proven steatosis than in healthy  
7 individuals [62,63,65]. In animal models, liver expression of VEGF and CD105, an  
8 endothelial cell marker, increase after 3 days of methionine and choline deficient diet in  
9 obese and diabetic *db/db* transgenic mice and after 1 week of this diet in C57BL6/J mice,  
10 before NASH appears [63]. However, three studies reported that new vessels develop in  
11 the liver of patients with NASH, but not in the liver of individuals with simple steatosis  
12 or with a normal liver [66–68]. This suggests that molecular events associated with up-  
13 regulation of angiogenic factors start early in the course of NAFLD, while angiogenesis  
14 appears later, as detailed below.

### 15 **Key points:**

- 16 • *LSECs capillarization and dysfunction occur very early in NAFLD progression and*  
17 *contribute to dietary induced steatosis.*
- 18 • *Intrahepatic vascular resistance is increased even when steatosis is the only*  
19 *histological feature of NAFLD. This is due to the combination of a compression of*  
20 *sinusoids by fat-laden enlarged hepatocytes and of a dysfunction of LSECs due to*  
21 *reduced nitric oxide bioavailability.*
- 22 • *Molecular events associated with angiogenesis are initiated during simple steatosis,*  
23 *but angiogenesis itself is only detected in NASH.*

## **LSECs in NASH**

### ***LSECs contribute to oxidative stress in NASH***

In response to lipotoxicity, hepatocytes generate reactive oxygen species (ROS) and initiate a robust inflammatory response that accentuates liver injury [1,3]. In addition to hepatocytes [1,3], lipotoxic response also occurs in LSECs contributing to ROS generation [44,69]. Indeed, ROS have been detected not only in hepatocytes but also in sinusoidal cells in patients with NASH [70]. Circulating lipids seem to account for this LSECs oxidative stress. Indeed, exposure of murine LSECs to palmitic acid upregulates NOX1 expression, an enzyme implicated in ROS production [44]. In addition, stimulation of human primary LSECs with ox-LDL increases ROS generation after binding to LOX1 [21]. This LSECs oxidative stress contributes to NASH. Indeed, mice with a global deficiency in NOX1, which is highly expressed in LSECs in NAFLD, and fed a high-fat diet display attenuated liver lesions attested by lower serum ALT level and lower hepatic cleaved caspase-3 expression [44]. Therefore, in NASH, ROS production takes place not only in hepatocytes but also to some extent in LSECs and seems to contribute to hepatocyte injury.

### ***Anti-inflammatory role of LSECs at initial stages of NASH***

Progression of simple steatosis to steatohepatitis is accompanied with adhesion of leukocytes to the sinusoidal endothelium followed by infiltration of leukocytes within liver parenchyma to form inflammatory foci [71]. Moderate and resolved inflammatory responses are beneficial to the liver as they promote the re-establishment of homeostasis, contribute to tissue repair and exert hepato-protective effects [72].

1 However, chronic inflammation, as seen in NASH, is accompanied with death of  
2 hepatocytes and causes damage to liver parenchyma [72]. In physiological conditions,  
3 LSECs constitute a barrier regulating the entry of circulating leukocytes within liver  
4 parenchyma and play an anti-inflammatory role [73–76]. At early stages of NAFLD  
5 progression, some evidence indicates that LSECs also exhibit anti-inflammatory function  
6 [40,77]. Indeed, Tateya and colleagues elegantly demonstrated that nitric oxide derived  
7 from LSECs inhibits Kupffer cells activation in mice fed a high-fat diet for a short period  
8 of time (8 weeks) [40]. *In vitro*, both human and murine LSECs exposed to FFA for a  
9 short period (16 hours) exhibit a downregulation of pro-inflammatory chemokines  
10 involved in monocyte and macrophage recruitment, through a MAPK dependent  
11 pathway [77].

#### 12 *LSECs promotes liver inflammation at more advanced stages of NASH*

13 As mentioned above, LSECs alterations arise early in NAFLD progression, prior to liver  
14 inflammation [18,41]. Indeed, LSECs capillarization precedes Kupffer cells activation  
15 [18,41] and liver nitric oxide content falls before liver NF- $\kappa$ B activation and TNF $\alpha$ , IL6  
16 and ICAM-1 up-regulation [30,40,41]. LSECs capillarization and dysfunction are  
17 permissive for establishment of liver inflammation. Indeed, mice deficient in eNOS  
18 exhibit an accelerated hepatic inflammatory response, while improving nitric  
19 oxide/cGMP signaling with the phosphodiesterase-5 inhibitor sildenafil or with  
20 simvastatin prevents liver inflammation in rodents fed a high-fat diet [40,60].

21 During NAFLD progression, LSECs then acquire pro-inflammatory phenotype and  
22 functions (Figure 3).

LSECs pro-inflammatory phenotype during NASH is characterized by progressive overexpression of adhesion molecules at LSECs surface including ICAM-1, VCAM-1 and VAP-1, as observed in mouse models of NASH [68,78–82]. LSECs also produce a number of pro-inflammatory mediators in NASH, including TNF $\alpha$ , IL-6, IL-1 and MCP1 [68,82–84].

This pro-inflammatory phenotype of LSECs in NASH is associated with pro-inflammatory functions (Figure 3). First, dysfunctional LSECs fail to maintain Kupffer cells quiescence [40]. Second, the release of inflammatory mediators by LSECs contributes to the inflammatory response by activating neighboring Kupffer cells, and by favoring blood leukocytes recruitment, adhesion and transmigration [82,85,86]. Mechanisms of the interactions between leucocytes and LSECs in NASH have been reviewed elsewhere in detail and are summarized in Figure 3 [80,87–89]. LSECs expression of ICAM-1, VCAM-1 and VAP-1 is crucial for these interactions since *in vivo* and *in vitro* studies showed reduced leukocytes adhesion to hepatic sinusoids when these receptors are blocked or not functional [80,90]. Moreover, inhibition of the VCAM-1 ligand, VLA-4, on monocytes using an anti-VLA-4 antibody inhibits adhesion and transendothelial migration of monocytes across LSECs from wild-type mice fed a high-fat diet fed mice and from *ob/ob* obese mice, and improves liver inflammation [82].

Although stimuli responsible for LSECs inflammatory phenotype and functions in NASH are not firmly identified, several mediators are potential candidates. This includes products derived from the visceral adipose tissue, such as ox-LDL, FFA and adipokines. Indeed, *in vitro* studies showed that stimulation of LSECs with ox-LDL and FFA (palmitate) activates NF-kB and toll-like receptor-4 (TLR-4), respectively [21,44,91]. Moreover, circulating concentrations of several adipokines, including TNF $\alpha$  and IL6, are



increased in the portal vein in the context of metabolic syndrome, and may contribute to LSECs inflammatory phenotype [92]. Gut microbiota also has an emerging role in NASH as a source for liver inflammation [1,3]. Increased intestinal permeability and elevated plasma concentrations of lipopolysaccharide (LPS) [93,94] observed in NASH may also contribute to LSECs inflammation [83]. Besides mediators derived from the portal vein, hepatocytes and liver inflammatory cells also release inflammatory mediators in NASH that can activate LSECs [1,95].

To summarize, while at initial stages of NAFLD LSECs play an anti-inflammatory role, a switch towards pro-inflammatory functions occurs during the course of NAFLD development, paving the way for NASH progression.

#### Angiogenesis in liver inflammation in NASH

Pathologic angiogenesis increases with NASH [61,68,96,97] (Figure 4). Indeed, several studies reported the formation of new vessels in the liver of patients with NASH. Moreover, serum VEGF and sVEGFR1 levels are higher in patients with steatosis and biopsy-proven NASH than in healthy individuals [62,66–68,98]. Similarly, in animal models of NASH, liver vasculature is disrupted and hepatic expression of VEGF and CD105 is increased [63].

Several mechanisms trigger angiogenesis during NASH. First, chronic inflammation promotes angiogenesis. Indeed, chronic inflammation sustains tissue hypoxia and induces transcription of angiogenic genes modulated by HIF-1 $\alpha$  [61,99]. Pro-inflammatory mediators also elicit a direct angiogenic response through the induction of HIF-1 $\alpha$  transcriptional activity and VEGF production [61]. Moreover, cytokines and ROS released during NASH can activate the MAPK/ERK pathway, a signaling pathway

involved in cell migration and angiogenesis [61]. Second, hepatocyte-derived microvesicles link lipotoxicity with angiogenesis. Indeed, hepatocytes exposed *in vitro* to excessive amounts of saturated FFA, that mimics steatosis, release microvesicles with a pro-angiogenic activity [100]. Likewise, mice fed a methionine and choline deficient diet have high circulating levels of hepatocyte-derived microvesicles able to induce angiogenesis. Third, angiopoietin-2 is another mechanism of liver angiogenesis in NASH. Angiopoietins are key regulators of angiogenesis. Although angiopoietins-1 and 2 contribute to vascular stability and quiescence in physiological conditions, angiopoietin-2 promotes pathological angiogenesis in inflammatory conditions [101]. Lefere and coworkers recently showed that serum angiopoietin-2 levels are increased in patients with NASH and correlate with liver steatosis, inflammation and hepatocyte ballooning, but not with liver fibrosis [68]. Similar findings were observed with two murine models of NASH, namely mice fed a methionine and choline deficient diet and mice with neonatal injection of streptozotocin followed by 16 weeks of western diet [68]. The main source of hepatic angiopoietin-2 was LSECs [68]. Inhibiting angiopoietin-2 levels using the angiopoietin-2/Tie2 receptor inhibiting peptibody L1-10 reduced hepatic angiogenesis and normalized vascular microarchitecture [68].

In turn, angiogenesis promotes inflammation since various strategies of inhibition of angiogenesis all improve liver inflammation (Figure 4). Coulon and colleagues showed in a mouse model of NASH that treatment with anti-VEGFR2 antibody improves liver vasculature and decreases liver inflammatory gene expression, both using preventive and therapeutic approaches [63]. Lefere and colleagues showed that blocking angiopoietin-2/Tie2 interaction with the L1-10 peptibody also alleviates liver injury and inflammation in mice fed a methionine and choline deficient diet [68]. Importantly, this

effect of L1-10 therapy is at least partly mediated by an effect on LSECs since L1-10 treatment downregulates VCAM-1, ICAM-1 and MCP1 expression in liver endothelial cells isolated from mice fed a methionine and choline deficient diet [68]. This anti-inflammatory effect of anti-angiogenic treatment is not specific for NASH, as it is observed in most models of chronic liver disease, namely carbon tetrachloride, and bile duct ligation [102–106].

To summarize, inflammation stimulates angiogenesis that in turn worsens inflammation, as attested by the anti-inflammatory effect of anti-angiogenic therapies (Figure 4).

#### **Key points:**

- *Lipotoxicity and inflammation induce endothelial inflammation. Activated LSECs release cytokines and chemokines and overexpress adhesion molecules, thus sustaining liver inflammation.*
- *Inflammation promotes angiogenesis that in turn worsens liver inflammation as attested by the anti-inflammatory effect of anti-angiogenic therapies.*

#### **LSECs in NASH-related liver fibrosis**

Liver fibrosis is defined as the excessive deposition of extracellular matrix in liver parenchyma. The main mechanism leading to liver fibrosis is a long-standing wound healing process caused by hepatocellular injury and inflammation and mediated by hepatic stellate cells activation [1,107]. Hepatic stellate cells are nonparenchymal cells close to LSECs, in the space of Disse, which store retinoids in physiological conditions

1 and shift their phenotype to an activated myofibroblastic state during liver injury and  
2 inflammation to secrete large amounts of extracellular matrix compounds [107]. As  
3 detailed above, LSECs are major effectors of liver inflammation in NASH, and  
4 consequently also promote hepatic fibrosis. For example, LSECs overexpress VAP-1  
5 during inflammation which, in addition to its pro-inflammatory functions in NASH, is  
6 directly involved in hepatic stellate cells activation [80]. Inhibition or deficiency in VAP-  
7 1 in mice fed a methionine and choline deficient diet or a high-fat diet attenuates liver  
8 fibrosis [80]. LSECs also contribute to liver fibrosis through capillarization and  
9 endothelial dysfunction, as detailed below.

#### 10 *LSECs capillarization promotes liver fibrosis*

11 Capillarization is observed in patients and animal models of NASH and precedes fibrosis  
12 [18,41,108–112], but also promotes its development. Indeed, PLVAP deficient mice,  
13 displaying a pronounced reduction in the number of LSECs *fenestrae*, spontaneously  
14 develop perisinusoidal liver fibrosis [25].

15 Experiments performed using cultured LSECs and hepatic stellate cells highlighted an  
16 important cross-talk between LSECs and hepatic stellate cells to regulate each other  
17 phenotype. While healthy LSECs maintain hepatic stellate cells quiescent, capillarized  
18 LSECs lose this ability [112,113] (Figure 5). A vicious cycle between LSECs  
19 capillarization and hepatic stellate cells activation then occurs during the fibrotic  
20 process.

21 In NASH, ballooned hepatocytes produce Hedgehog molecules [114]. Hedgehog pathway  
22 regulates capillarization, as inhibition of Hedgehog signaling prevents capillarization  
23 and partially reverts LSECs phenotype from dedifferentiated to their differentiated state  
24 [115]. LSECs are thus Hedgehog-sensitive cells, but they are also Hedgehog producing

cells. Similarly, quiescent hepatic stellate cells are Hedgehog-sensitive cells, while activated hepatic stellate cells become Hedgehog-producing cells able also to release microvesicles loaded with Hedgehog signaling molecules that interact with LSECs [116,117]. It is thus tempting to speculate that during NASH, Hedgehog ligands are released by hepatocytes and LSECs, thus activating LSECs themselves, as well as quiescent hepatic stellate cells, by autocrine and paracrine effects. Activated hepatic stellate cells can then secrete Hedgehog molecules, promoting LSECs capillarization which in turn favors hepatic stellate cells activation, promoting the fibrogenic process.

#### LSECs dysfunction promotes liver fibrosis

Endothelial dysfunction appears very early in the course of NAFLD and precedes fibrosis in animal models of NASH [30,40,41]. Several lines of evidence suggest that liver endothelial dysfunction contributes to liver fibrosis. First, in rats fed a high-fat diet, simvastatin increases liver eNOS expression and ameliorates liver fibrosis [60]. Second, eNOS inhibition using L-NAME blocks the ability of healthy LSECs to keep hepatic stellate cells quiescent [118,119]. Third, an activator of soluble guanylate cyclase, a receptor for nitric oxide, can recapitulate the effect of healthy LSECs and reverse hepatic stellate cells activation [13]. A limitation of these studies is however that most approaches might not only act on endothelial function but also on LSECs capillarization [112,118]. Indeed, although L-NAME blocks LSECs-induced hepatic stellate cells quiescence, addition of a nitric oxide donor does not directly reverse hepatic stellate cells activation *in vitro* [13], suggesting that additional LSECs-derived factors could be responsible for the reversion of activated hepatic stellate cells to quiescence.

To summarize, these data demonstrate that capillarization and LSECs dysfunction not only precedes liver fibrosis, but are also permissive for it (Figure 5). LSECs are able to maintain hepatic stellate cells quiescent as long LSECs are differentiated, so that differentiated LSECs are gatekeepers for fibrosis in NASH, like in other chronic liver diseases.

#### Liver endothelial-to-mesenchymal transition: a process promoting liver fibrosis?

Another important process that links endothelial cells to organ fibrosis is endothelial-to-mesenchymal transition, *i.e.* a mechanism by which endothelial cells convert into myofibroblasts and contribute to extracellular matrix deposition [120,121]. Endothelial-to-mesenchymal transition occurs in various fibrotic cardiovascular and pulmonary diseases [120–122]. *In vitro* studies showed that healthy LSECs produce a modest amount of collagen and fibronectin [123]. Capillarized LSECs secrete fibrogenic factors, such as TGF- $\beta$ 1, and extracellular matrix proteins, such as fibronectin and laminin, that may be considered as an endothelial-to-mesenchymal transition, and also stimulates activation of neighboring hepatic stellate cells [8,124,125]. *In vivo*, in the liver disease field, only one study described endothelial-to-mesenchymal transition in patients with alcohol or hepatitis C virus-related cirrhosis and in mice treated with carbon tetrachloride [126]. This process might also happen during liver fibrosis in NASH, but will deserve further studies.

#### Angiogenesis in NASH-related liver fibrosis

Liver angiogenesis correlates with liver fibrosis in patients with NASH [66,67]. The mechanisms leading to liver angiogenesis in NASH-related fibrosis include those mentioned above, namely tissue hypoxia [61], hepatocytes-derived microvesicles

1 [100,127] and angiopoietin-2 [68], but also leptin. Leptin concentrations are increased  
2 in the serum of patients with NAFLD [128]. This adipocytokine has both pro-angiogenic  
3 effects [129], and direct pro-fibrogenic effects, through the upregulation of TGF- $\beta$  in  
4 LSECs and Kupffer cells [130].

5 In turn, angiogenesis promotes liver fibrosis since several approaches inhibiting liver  
6 angiogenesis prevents NASH-related fibrosis (Figure 4). First, in the study by Kitade and  
7 colleagues, neither angiogenesis nor fibrosis was observed in the absence of leptin  
8 signaling in a rat model of NASH [129]. Second, blocking the release of pro-angiogenic  
9 microvesicles from fat laden-hepatocytes or inhibiting their binding to their target cells  
10 protects mice from steatohepatitis-induced pathologic angiogenesis and results in a  
11 reduction in liver fibrosis [100]. Third, Zhou and coworkers recently showed that a  
12 specific deletion of the physiological inhibitor of angiogenesis, prolyl-hydroxylase-2, in  
13 endothelial cells results in an overexpression of angiopoietin-2 and TGF- $\beta$ 1 in the liver  
14 and promotes dietary-induced liver fibrosis in mice [131,132]. Whether this pro-fibrotic  
15 effect of endothelial prolyl-hydroxylase-2 deficiency is directly induced by promoting  
16 angiogenesis remains to be demonstrated. Fourth, two studies reported that inhibiting  
17 angiotensin-II receptor using telmisartan or candesartan inhibits liver angiogenesis and  
18 fibrosis in rats fed a choline-deficient, L-amino acid-defined diet [96,97]. Finally, Lefere  
19 and colleague demonstrated that blocking angiogenesis by inhibiting angiopoietin-  
20 2/Tie2 interaction using the L1-10 peptibody improves liver fibrosis in preventive and  
21 therapeutic strategies in mice fed a methionine and choline deficient diet. Therapeutic  
22 application of L1-10 peptibody also prevents liver fibrosis in diabetic mice with NASH  
23 (streptozotocin and western diet model) [68]. It should be noted that an anti-fibrotic

effect of anti-angiogenic treatments has also been observed in models of chronic liver disease without NASH [102–106,133–139].

**Key points:**

- *LSECs capillarization and dysfunction precede liver fibrosis and are permissive for it, through the loss of the ability of LSECs to maintain quiescence of hepatic stellate cells.*
- *Liver angiogenesis correlates with the severity of liver fibrosis and promotes its development. Once established, fibrosis stimulates angiogenesis by increasing tissue hypoxia. Blocking pathologic angiogenesis prevents liver fibrosis.*

**LSECs in NASH-induced hepatocellular carcinoma**

In most cases, hepatocellular carcinoma (HCC) develops within a background of chronic liver disease (70–90% of all patients). The role of liver endothelial cells in HCC development, outside the NAFLD setting, has been reviewed elsewhere and is summarized in Figure 6 [8,76,140–143].

Patients with metabolic syndrome and NAFLD also develop HCC in the absence of underlying cirrhosis, suggesting oncogenic pathways specific for NAFLD [5,6]. Adipokines and angiogenesis associated with NAFLD seem to account –at least partly– for this specific link between NAFLD and HCC.

Circulating concentrations of the adipokine FABP4 are increased in patients with NAFLD without HCC and correlate with liver inflammation and fibrosis [144]. Interestingly,



Laouirem and colleagues recently demonstrated that LSECs exposed to conditions mimicking NAFLD -namely high concentrations of glucose, insulin, or VEGFA- release FABP4. They also observed that FABP4 released by LSECs exerts pro-oncogenic effects, since it induces hepatocyte proliferation. In mice fed a high-fat diet, specific inhibition of FABP4 reduces HCC growth [145]. We can speculate that FABP4 from LSECs not only contributes to HCC progression but also to HCC development in a NAFLD setting.

In NAFLD, angiogenesis is highly stimulated and promotes NAFLD-associated HCC, since various inhibitions of angiogenesis all prevent HCC development. First, leptin-mediated angiogenesis has been demonstrated to be involved in HCC development as neither angiogenesis nor HCC develop in the absence of leptin signaling in Zucker rats fed a choline-deficient, L-amino acid-defined diet [129]. Second, Yoshiji and colleagues showed that a conventional anti-angiogenic treatment with sorafenib inhibits preneoplastic lesions appearance in rats fed a choline-deficient, L-amino acid-defined diet [146]. In this study, authors also demonstrated that a treatment combining low doses of sorafenib with the angiotensin-II receptor inhibitor losartan also successfully inhibits preneoplastic lesions [146]. Third, Tamaki and colleagues demonstrated that inhibition of angiotensin-II receptor with telmisartan inhibits HIF-1 $\alpha$  activity and VEGF expression and prevent HCC development in the liver of rats fed a choline-deficient, L-amino acid-defined diet for 48 weeks [96]. Finally, Lefere and coworkers recently showed that therapeutic inhibition of angiopoietin-2 alleviates steatohepatitis and prevents NASH-associated HCC progression in mice [68].

### ***Key point:***

- *Adipokines contribute to HCC development in NAFLD.*

- *NAFLD associated angiogenesis promotes HCC. Blocking pathologic angiogenesis prevents HCC development and progression.*

#### **Gaps in knowledge and future directions**

Even if our understanding of the role of LSECs in NAFLD has progressed over the last years, several aspects remain elusive. First, triggers responsible for LSECs alterations in NAFLD are mostly unknown. Mediators derived from the visceral adipose tissue and the gut have been speculated, but not convincingly established. Indeed, available *in vitro* studies considered each mediator individually and not in combination as *in vivo* in the portal venous blood [21,44,77]. Second, mechanisms underlying endothelial changes in NAFLD, including capillarization, need to be defined which might provide new therapeutic targets for NAFLD. Third, the role of LSECs in NASH-related cirrhosis has not been specifically investigated. Whether LSEC function and phenotype differ in cirrhosis related to NASH from cirrhosis related to other causes remains to be assessed [147]. Fourth, although NAFLD is well recognized as favoring HCC development, we are still at a very early stage of understanding how LSECs changes might favor HCC development.

#### **Conclusion**

LSECs are gatekeepers of liver homeostasis in physiological conditions. In NAFLD, sinusoidal endothelial alterations, including capillarization and LSECs dysfunction, occur early in disease progression, at the stage of simple steatosis. These initial changes, triggered by lipotoxicity, adipokines, and inflammation and gut microbiota-derived

1 products are associated with a loss of the ability of LSECs to prevent liver inflammation  
2 and fibrosis associated with NASH. Indeed, altered LSECs fail to maintain Kupffer cells  
3 and hepatic stellate cells quiescence. At the stage of NASH, altered LSECs contribute to  
4 liver angiogenesis, inflammation, fibrosis and HCC. Improving LSECs health has high  
5 therapeutic potential for NAFLD. Current challenge is the identification of strategies to  
6 target specifically LSECs to modulate their activity.

7

8

## 1 **References**

- 2 [1] Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of  
3 NAFLD development and therapeutic strategies. Nat Med 2018;24:908–22.  
4 doi:10.1038/s41591-018-0104-9.
- 5 [2] Younossi ZM. Non-Alcoholic Fatty Liver Disease-A Global Public Health  
6 Perspective. J Hepatol 2018. doi:10.1016/j.jhep.2018.10.033.
- 7 [3] Marra F, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH  
8 pathogenesis. J Hepatol 2018;68:280–95. doi:10.1016/j.jhep.2017.11.014.
- 9 [4] Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of  
10 nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease.  
11 Hepatology 2018;67:123–33. doi:10.1002/hep.29466.
- 12 [5] Paradis V, Zalinski S, Chelbi E, Guedj N, Degos F, Vilgrain V, et al. Hepatocellular  
13 carcinomas in patients with metabolic syndrome often develop without significant liver  
14 fibrosis: a pathological analysis. Hepatology 2009;49:851–9. doi:10.1002/hep.22734.
- 15 [6] Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA,  
16 et al. Risk of Hepatocellular Cancer in Patients With Non-Alcoholic Fatty Liver Disease.  
17 Gastroenterology 2018;155:1828-1837.e2. doi:10.1053/j.gastro.2018.08.024.
- 18 [7] Zapotoczny B, Szafranska K, Kus E, Braet F, Wisse E, Chlopicki S, et al. Tracking  
19 Fenestrae Dynamics in Live Murine Liver Sinusoidal Endothelial Cells. Hepatology 2018.  
20 doi:10.1002/hep.30232.
- 21 [8] **Poisson J, Lemoinne S**, Boulanger C, Durand F, Moreau R, Valla D, et al. Liver  
22 sinusoidal endothelial cells: Physiology and role in liver diseases. J Hepatol 2017;66:212–27.  
23 doi:10.1016/j.jhep.2016.07.009.
- 24 [9] Hilmer SN, Cogger VC, Fraser R, McLean AJ, Sullivan D, Le Couteur DG. Age-  
25 related changes in the hepatic sinusoidal endothelium impede lipoprotein transfer in the rat.

- 1 Hepatology 2005;42:1349–54. doi:10.1002/hep.20937.
- 2 [10] Cogger VC, Hilmer SN, Sullivan D, Muller M, Fraser R, Le Couteur DG.  
3 Hyperlipidemia and surfactants: the liver sieve is a link. Atherosclerosis 2006;189:273–81.  
4 doi:10.1016/j.atherosclerosis.2005.12.025.
- 5 [11] **Carpenter B, Lin Y**, Stoll S, Raffai RL, McCuskey R, Wang R. VEGF is crucial for  
6 the hepatic vascular development required for lipoprotein uptake. Development  
7 2005;132:3293–303. doi:10.1242/dev.01902.
- 8 [12] Fraser R, Bosanquet AG, Day WA. Filtration of chylomicrons by the liver may  
9 influence cholesterol metabolism and atherosclerosis. Atherosclerosis 1978;29:113–23.
- 10 [13] Xie G, Wang X, Wang L, Wang L, Atkinson RD, Kanel GC, et al. Role of  
11 differentiation of liver sinusoidal endothelial cells in progression and regression of hepatic  
12 fibrosis in rats. Gastroenterology 2012;142:918-927.e6. doi:10.1053/j.gastro.2011.12.017.
- 13 [14] May D, Djonov V, Zamir G, Bala M, Safadi R, Sklair-Levy M, et al. A transgenic  
14 model for conditional induction and rescue of portal hypertension reveals a role of VEGF-  
15 mediated regulation of sinusoidal fenestrations. PLoS ONE 2011;6:e21478.  
16 doi:10.1371/journal.pone.0021478.
- 17 [15] Hagberg CE, Falkevall A, Wang X, Larsson E, Huusko J, Nilsson I, et al. Vascular  
18 endothelial growth factor B controls endothelial fatty acid uptake. Nature 2010;464:917–21.  
19 doi:10.1038/nature08945.
- 20 [16] Van Berkel TJ, De Rijke YB, Kruijt JK. Different fate in vivo of oxidatively modified  
21 low density lipoprotein and acetylated low density lipoprotein in rats. Recognition by various  
22 scavenger receptors on Kupffer and endothelial liver cells. J Biol Chem 1991;266:2282–9.
- 23 [17] Blomhoff R, Drevon CA, Eskild W, Helgerud P, Norum KR, Berg T. Clearance of  
24 acetyl low density lipoprotein by rat liver endothelial cells. Implications for hepatic  
25 cholesterol metabolism. J Biol Chem 1984;259:8898–903.

- 1 [18] Miyao M, Kotani H, Ishida T, Kawai C, Manabe S, Abiru H, et al. Pivotal role of liver  
2 sinusoidal endothelial cells in NAFLD/NASH progression. *Lab Invest* 2015;95:1130–44.  
3 doi:10.1038/labinvest.2015.95.
- 4 [19] Cogger VC, Mohamad M, Solon-Biet SM, Senior AM, Warren A, O'Reilly JN, et al.  
5 Dietary macronutrients and the aging liver sinusoidal endothelial cell. *Am J Physiol Heart*  
6 *Circ Physiol* 2016;310:H1064-1070. doi:10.1152/ajpheart.00949.2015.
- 7 [20] **Qiuxian Peng, Zhang Q, Xiao W**, Shao M, Fan Q, Zhang H, et al. Protective effects  
8 of *Sapindus mukorossi* Gaertn against fatty liver disease induced by high fat diet in rats.  
9 *Biochem Biophys Res Commun* 2014;450:685–91. doi:10.1016/j.bbrc.2014.06.035.
- 10 [21] **Zhang Q, Liu J**, Liu J, Huang W, Tian L, Quan J, et al. oxLDL induces injury and  
11 defenestration of human liver sinusoidal endothelial cells via LOX1. *J Mol Endocrinol*  
12 2014;53:281–93. doi:10.1530/JME-14-0049.
- 13 [22] Hang T-C, Lauffenburger DA, Griffith LG, Stolz DB. Lipids promote survival,  
14 proliferation, and maintenance of differentiation of rat liver sinusoidal endothelial cells in  
15 vitro. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G375-388.  
16 doi:10.1152/ajpgi.00288.2011.
- 17 [23] Soderborg TK, Clark SE, Mulligan CE, Janssen RC, Babcock L, Ir D, et al. The gut  
18 microbiota in infants of obese mothers increases inflammation and susceptibility to NAFLD.  
19 *Nat Commun* 2018;9:4462. doi:10.1038/s41467-018-06929-0.
- 20 [24] Dobbs BR, Rogers GW, Xing HY, Fraser R. Endotoxin-induced defenestration of the  
21 hepatic sinusoidal endothelium: a factor in the pathogenesis of cirrhosis? *Liver* 1994;14:230–  
22 3.
- 23 [25] Herrnberger L, Hennig R, Kremer W, Hellerbrand C, Goepferich A, Kalbitzer HR, et  
24 al. Formation of fenestrae in murine liver sinusoids depends on plasmalemma vesicle-  
25 associated protein and is required for lipoprotein passage. *PLoS ONE* 2014;9:e115005.

doi:10.1371/journal.pone.0115005.

[26] Tanaka M, Iwakiri Y. The Hepatic Lymphatic Vascular System: Structure, Function, Markers, and Lymphangiogenesis. *Cell Mol Gastroenterol Hepatol* 2016;2:733–49.

doi:10.1016/j.jcmgh.2016.09.002.

[27] Feingold KR, Grunfeld C. Introduction to Lipids and Lipoproteins. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, et al., editors. *Endotext*, South Dartmouth (MA): MDText.com, Inc.; 2000.

[28] Fraser R, Cogger VC, Dobbs B, Jamieson H, Warren A, Hilmer SN, et al. The liver sieve and atherosclerosis. *Pathology* 2012;44:181–6. doi:10.1097/PAT.0b013e328351bcc8.

[29] Francque S, Verrijken A, Mertens I, Hubens G, Van Marck E, Pelckmans P, et al. Noncirrhotic human nonalcoholic fatty liver disease induces portal hypertension in relation to the histological degree of steatosis. *Eur J Gastroenterol Hepatol* 2010;22:1449–57. doi:10.1097/MEG.0b013e32833f14a1.

[30] **Francque S, Laleman W**, Verbeke L, Van Steenkiste C, Casteleyn C, Kwanten W, et al. Increased intrahepatic resistance in severe steatosis: endothelial dysfunction, vasoconstrictor overproduction and altered microvascular architecture. *Lab Invest* 2012;92:1428–39. doi:10.1038/labinvest.2012.103.

[31] Mendes FD, Suzuki A, Sanderson SO, Lindor KD, Angulo P. Prevalence and indicators of portal hypertension in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2012;10:1028-1033.e2. doi:10.1016/j.cgh.2012.05.008.

[32] Baffy G. Origins of Portal Hypertension in Nonalcoholic Fatty Liver Disease. *Dig Dis Sci* 2018;63:563–76. doi:10.1007/s10620-017-4903-5.

[33] Seifalian AM, Chidambaram V, Rolles K, Davidson BR. In vivo demonstration of impaired microcirculation in steatotic human liver grafts. *Liver Transpl Surg* 1998;4:71–7.

[34] Seifalian AM, Piasecki C, Agarwal A, Davidson BR. The effect of graded steatosis on

1 flow in the hepatic parenchymal microcirculation. Transplantation 1999;68:780–4.

2 [35] Ijaz S, Yang W, Winslet MC, Seifalian AM. The role of nitric oxide in the modulation  
3 of hepatic microcirculation and tissue oxygenation in an experimental model of hepatic  
4 steatosis. Microvasc Res 2005;70:129–36. doi:10.1016/j.mvr.2005.08.001.

5 [36] Farrell GC, Teoh NC, McCuskey RS. Hepatic microcirculation in fatty liver disease.  
6 Anat Rec (Hoboken) 2008;291:684–92. doi:10.1002/ar.20715.

7 [37] Caldwell S, Lackner C. Perspectives on NASH Histology: Cellular Ballooning. Ann  
8 Hepatol 2017;16:182–4. doi:10.5604/16652681.1231550.

9 [38] Ijaz S, Yang W, Winslet MC, Seifalian AM. Impairment of hepatic microcirculation in  
10 fatty liver. Microcirculation 2003;10:447–56. doi:10.1038/sj.mn.7800206.

11 [39] Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, et al.  
12 The assessment of endothelial function: from research into clinical practice. Circulation  
13 2012;126:753–67. doi:10.1161/CIRCULATIONAHA.112.093245.

14 [40] Tateya S, Rizzo NO, Handa P, Cheng AM, Morgan-Stevenson V, Daum G, et al.  
15 Endothelial NO/cGMP/VASP signaling attenuates Kupffer cell activation and hepatic insulin  
16 resistance induced by high-fat feeding. Diabetes 2011;60:2792–801. doi:10.2337/db11-0255.

17 [41] Pasarín M, La Mura V, Gracia-Sancho J, García-Calderó H, Rodríguez-Vilarrupla A,  
18 García-Pagán JC, et al. Sinusoidal endothelial dysfunction precedes inflammation and fibrosis  
19 in a model of NAFLD. PLoS ONE 2012;7:e32785. doi:10.1371/journal.pone.0032785.

20 [42] **Gonzalez-Paredes FJ, Hernández Mesa G**, Morales Arraez D, Marcelino Reyes R,  
21 Abrante B, Diaz-Flores F, et al. Contribution of Cyclooxygenase End Products and Oxidative  
22 Stress to Intrahepatic Endothelial Dysfunction in Early Non-Alcoholic Fatty Liver Disease.  
23 PLoS ONE 2016;11:e0156650. doi:10.1371/journal.pone.0156650.

24 [43] García-Lezana T, Raurell I, Bravo M, Torres-Arauz M, Salcedo MT, Santiago A, et al.  
25 Restoration of a healthy intestinal microbiota normalizes portal hypertension in a rat model of



- 1 nonalcoholic steatohepatitis. *Hepatology* 2018;67:1485–98. doi:10.1002/hep.29646.
- 2 [44] **Matsumoto M, Zhang J, Zhang X, Liu J, Jiang JX, Yamaguchi K, et al.** The NOX1  
3 isoform of NADPH oxidase is involved in dysfunction of liver sinusoids in nonalcoholic fatty  
4 liver disease. *Free Radic Biol Med* 2018;115:412–20.  
5 doi:10.1016/j.freeradbiomed.2017.12.019.
- 6 [45] **Pasarín M, Abraldes JG, Rodríguez-Vilarrupla A, La Mura V, García-Pagán JC,**  
7 **Bosch J.** Insulin resistance and liver microcirculation in a rat model of early NAFLD. *J*  
8 *Hepatol* 2011;55:1095–102. doi:10.1016/j.jhep.2011.01.053.
- 9 [46] **Pasarín M, Abraldes JG, Liguori E, Kok B, La Mura V.** Intrahepatic vascular changes  
10 in non-alcoholic fatty liver disease: Potential role of insulin-resistance and endothelial  
11 dysfunction. *World J Gastroenterol* 2017;23:6777–87. doi:10.3748/wjg.v23.i37.6777.
- 12 [47] **Gunnnett CA, Lund DD, Chu Y, Brooks RM, Faraci FM, Heistad DD.** NO-dependent  
13 vasorelaxation is impaired after gene transfer of inducible NO-synthase. *Arterioscler Thromb*  
14 *Vasc Biol* 2001;21:1281–7.
- 15 [48] **Chauhan SD, Seggara G, Vo PA, Macallister RJ, Hobbs AJ, Ahluwalia A.** Protection  
16 against lipopolysaccharide-induced endothelial dysfunction in resistance and conduit  
17 vasculature of iNOS knockout mice. *FASEB J* 2003;17:773–5. doi:10.1096/fj.02-0668fje.
- 18 [49] **Kus E, Jasiński K, Skórka T, Czyzyska-Cichon I, Chlopicki S.** Short-term treatment  
19 with hepatoselective NO donor V-PYRRO/NO improves blood flow in hepatic  
20 microcirculation in liver steatosis in mice. *Pharmacol Rep* 2018;70:463–9.  
21 doi:10.1016/j.pharep.2017.11.019.
- 22 [50] **Schild L, Dombrowski F, Lendeckel U, Schulz C, Gardemann A, Keilhoff G.**  
23 Impairment of endothelial nitric oxide synthase causes abnormal fat and glycogen deposition  
24 in liver. *Biochim Biophys Acta* 2008;1782:180–7. doi:10.1016/j.bbadis.2007.12.007.
- 25 [51] **Roediger WE, Hems R, Wiggins D, Gibbons GF.** Inhibition of hepatocyte lipogenesis

1 by nitric oxide donor: could nitric oxide regulate lipid synthesis? IUBMB Life 2004;56:35–  
2 40. doi:10.1080/15216540310001649822.

3 [52] Winder WW, Hardie DG. Inactivation of acetyl-CoA carboxylase and activation of  
4 AMP-activated protein kinase in muscle during exercise. Am J Physiol 1996;270:E299-304.  
5 doi:10.1152/ajpendo.1996.270.2.E299.

6 [53] Fu WJ, Haynes TE, Kohli R, Hu J, Shi W, Spencer TE, et al. Dietary L-arginine  
7 supplementation reduces fat mass in Zucker diabetic fatty rats. J Nutr 2005;135:714–21.  
8 doi:10.1093/jn/135.4.714.

9 [54] Zou M-H, Hou X-Y, Shi C-M, Kirkpatrick S, Liu F, Goldman MH, et al. Activation of  
10 5'-AMP-activated kinase is mediated through c-Src and phosphoinositide 3-kinase activity  
11 during hypoxia-reoxygenation of bovine aortic endothelial cells. Role of peroxynitrite. J Biol  
12 Chem 2003;278:34003–10. doi:10.1074/jbc.M300215200.

13 [55] Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB, et al.  
14 Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphate  
15 acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in  
16 response to exercise. J Biol Chem 2002;277:32571–7. doi:10.1074/jbc.M201692200.

17 [56] Muoio DM, Seefeld K, Witters LA, Coleman RA. AMP-activated kinase reciprocally  
18 regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that  
19 sn-glycerol-3-phosphate acyltransferase is a novel target. Biochem J 1999;338 ( Pt 3):783–91.

20 [57] Doulias P-T, Tenopoulou M, Greene JL, Raju K, Ischiropoulos H. Nitric oxide  
21 regulates mitochondrial fatty acid metabolism through reversible protein S-nitrosylation. Sci  
22 Signal 2013;6:rs1. doi:10.1126/scisignal.2003252.

23 [58] Maslak E, Zabielski P, Kochan K, Kus K, Jaształ A, Sitek B, et al. The liver-selective  
24 NO donor, V-PYRRO/NO, protects against liver steatosis and improves postprandial glucose  
25 tolerance in mice fed high fat diet. Biochem Pharmacol 2015;93:389–400.

- 1    doi:10.1016/j.bcp.2014.12.004.
- 2    [59]    Kus K, Walczak M, Maslak E, Zakrzewska A, Gonciarz-Dytman A, Zabielski P, et al.
- 3    Hepatoselective Nitric Oxide (NO) Donors, V-PYRRO/NO and V-PROLI/NO, in
- 4    Nonalcoholic Fatty Liver Disease: A Comparison of Antisteatotic Effects with the
- 5    Biotransformation and Pharmacokinetics. *Drug Metab Dispos* 2015;43:1028–36.
- 6    doi:10.1124/dmd.115.063388.
- 7    [60]    Wang W, Zhao C, Zhou J, Zhen Z, Wang Y, Shen C. Simvastatin ameliorates liver
- 8    fibrosis via mediating nitric oxide synthase in rats with non-alcoholic steatohepatitis-related
- 9    liver fibrosis. *PLoS ONE* 2013;8:e76538. doi:10.1371/journal.pone.0076538.
- 10    [61]    **Coulon S, Heindryckx F**, Geerts A, Van Steenkiste C, Colle I, Van Vlierberghe H.
- 11    Angiogenesis in chronic liver disease and its complications. *Liver Int* 2011;31:146–62.
- 12    doi:10.1111/j.1478-3231.2010.02369.x.
- 13    [62]    Coulon S, Francque S, Colle I, Verrijken A, Blomme B, Heindryckx F, et al.
- 14    Evaluation of inflammatory and angiogenic factors in patients with non-alcoholic fatty liver
- 15    disease. *Cytokine* 2012;59:442–9. doi:10.1016/j.cyto.2012.05.001.
- 16    [63]    Coulon S, Legry V, Heindryckx F, Van Steenkiste C, Casteleyn C, Olievier K, et al.
- 17    Role of vascular endothelial growth factor in the pathophysiology of nonalcoholic
- 18    steatohepatitis in two rodent models. *Hepatology* 2013;57:1793–805. doi:10.1002/hep.26219.
- 19    [64]    Iwakiri Y, Shah V, Rockey DC. Vascular pathobiology in chronic liver disease and
- 20    cirrhosis - current status and future directions. *J Hepatol* 2014;61:912–24.
- 21    doi:10.1016/j.jhep.2014.05.047.
- 22    [65]    Tarantino G, Conca P, Pasanisi F, Ariello M, Mastrolia M, Arena A, et al. Could
- 23    inflammatory markers help diagnose nonalcoholic steatohepatitis? *Eur J Gastroenterol*
- 24    *Hepatol* 2009;21:504–11. doi:10.1097/MEG.0b013e3283229b40.
- 25    [66]    Kitade M, Yoshiji H, Kojima H, Ikenaka Y, Noguchi R, Kaji K, et al.

- 1 Neovascularization and oxidative stress in the progression of non-alcoholic steatohepatitis.  
2 Mol Med Rep 2008;1:543–8.
- 3 [67] Kitade M, Yoshiji H, Noguchi R, Ikenaka Y, Kaji K, Shirai Y, et al. Crosstalk  
4 between angiogenesis, cytokeratin-18, and insulin resistance in the progression of non-  
5 alcoholic steatohepatitis. World J Gastroenterol 2009;15:5193–9.
- 6 [68] Lefere S, Van de Velde F, Hoorens A, Raevens S, Van Campenhout S,  
7 Vandierendonck A, et al. Angiopoietin-2 promotes pathological angiogenesis and is a novel  
8 therapeutic target in murine non-alcoholic fatty liver disease. Hepatology 2018.  
9 doi:10.1002/hep.30294.
- 10 [69] **Peters KM, Wilson RB**, Borradaile NM. Non-parenchymal hepatic cell lipotoxicity  
11 and the coordinated progression of non-alcoholic fatty liver disease and atherosclerosis. Curr  
12 Opin Lipidol 2018. doi:10.1097/MOL.0000000000000535.
- 13 [70] Seki S, Kitada T, Yamada T, Sakaguchi H, Nakatani K, Wakasa K. In situ detection of  
14 lipid peroxidation and oxidative DNA damage in non-alcoholic fatty liver diseases. J Hepatol  
15 2002;37:56–62.
- 16 [71] Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA  
17 2015;313:2263–73. doi:10.1001/jama.2015.5370.
- 18 [72] **Brenner C, Galluzzi L**, Kepp O, Kroemer G. Decoding cell death signals in liver  
19 inflammation. J Hepatol 2013;59:583–94. doi:10.1016/j.jhep.2013.03.033.
- 20 [73] Bissell DM, Wang SS, Jarnagin WR, Roll FJ. Cell-specific expression of transforming  
21 growth factor-beta in rat liver. Evidence for autocrine regulation of hepatocyte proliferation. J  
22 Clin Invest 1995;96:447–55. doi:10.1172/JCI118055.
- 23 [74] Carambia A, Freund B, Schwinge D, Heine M, Laschtowitz A, Huber S, et al. TGF- $\beta$ -  
24 dependent induction of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs by liver sinusoidal endothelial cells. J  
25 Hepatol 2014;61:594–9. doi:10.1016/j.jhep.2014.04.027.

- 1 [75] **Limmer A, Ohl J**, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, et al. Efficient  
2 presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in  
3 antigen-specific T-cell tolerance. *Nat Med* 2000;6:1348–54. doi:10.1038/82161.
- 4 [76] **Berg M, Wingender G**, Djandji D, Hegenbarth S, Momburg F, Hämmerling G, et al.  
5 Cross-presentation of antigens from apoptotic tumor cells by liver sinusoidal endothelial cells  
6 leads to tumor-specific CD8+ T cell tolerance. *Eur J Immunol* 2006;36:2960–70.  
7 doi:10.1002/eji.200636033.
- 8 [77] McMahan RH, Porsche CE, Edwards MG, Rosen HR. Free Fatty Acids Differentially  
9 Downregulate Chemokines in Liver Sinusoidal Endothelial Cells: Insights into Non-Alcoholic  
10 Fatty Liver Disease. *PLoS ONE* 2016;11:e0159217. doi:10.1371/journal.pone.0159217.
- 11 [78] Ip E, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent  
12 PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice.  
13 *Hepatology* 2004;39:1286–96. doi:10.1002/hep.20170.
- 14 [79] Dela Peña A, Leclercq I, Field J, George J, Jones B, Farrell G. NF-kappaB activation,  
15 rather than TNF, mediates hepatic inflammation in a murine dietary model of steatohepatitis.  
16 *Gastroenterology* 2005;129:1663–74. doi:10.1053/j.gastro.2005.09.004.
- 17 [80] **Weston CJ, Shepherd EL, Claridge LC**, Rantakari P, Curbishley SM, Tomlinson  
18 JW, et al. Vascular adhesion protein-1 promotes liver inflammation and drives hepatic  
19 fibrosis. *J Clin Invest* 2015;125:501–20. doi:10.1172/JCI73722.
- 20 [81] Tomita K, Tamiya G, Ando S, Ohsumi K, Chiyo T, Mizutani A, et al. Tumour  
21 necrosis factor alpha signalling through activation of Kupffer cells plays an essential role in  
22 liver fibrosis of non-alcoholic steatohepatitis in mice. *Gut* 2006;55:415–24.  
23 doi:10.1136/gut.2005.071118.
- 24 [82] Miyachi Y, Tsuchiya K, Komiya C, Shiba K, Shimazu N, Yamaguchi S, et al. Roles  
25 for Cell-Cell Adhesion and Contact in Obesity-Induced Hepatic Myeloid Cell Accumulation

- 1 and Glucose Intolerance. *Cell Rep* 2017;18:2766–79. doi:10.1016/j.celrep.2017.02.039.
- 2 [83] **Wu J, Meng Z**, Jiang M, Zhang E, Trippler M, Broering R, et al. Toll-like receptor-  
3 induced innate immune responses in non-parenchymal liver cells are cell type-specific.  
4 *Immunology* 2010;129:363–74. doi:10.1111/j.1365-2567.2009.03179.x.
- 5 [84] Feder LS, Todaro JA, Laskin DL. Characterization of interleukin-1 and interleukin-6  
6 production by hepatic endothelial cells and macrophages. *J Leukoc Biol* 1993;53:126–32.
- 7 [85] Marra F, Tacke F. Roles for chemokines in liver disease. *Gastroenterology*  
8 2014;147:577-594.e1. doi:10.1053/j.gastro.2014.06.043.
- 9 [86] Roh Y-S, Seki E. Chemokines and Chemokine Receptors in the Development of  
10 NAFLD. *Adv Exp Med Biol* 2018;1061:45–53. doi:10.1007/978-981-10-8684-7\_4.
- 11 [87] Shetty S, Lalor PF, Adams DH. Lymphocyte recruitment to the liver: molecular  
12 insights into the pathogenesis of liver injury and hepatitis. *Toxicology* 2008;254:136–46.  
13 doi:10.1016/j.tox.2008.08.003.
- 14 [88] Lalor PF, Shields P, Grant A, Adams DH. Recruitment of lymphocytes to the human  
15 liver. *Immunol Cell Biol* 2002;80:52–64. doi:10.1046/j.1440-1711.2002.01062.x.
- 16 [89] Lalor PF, Edwards S, McNab G, Salmi M, Jalkanen S, Adams DH. Vascular adhesion  
17 protein-1 mediates adhesion and transmigration of lymphocytes on human hepatic endothelial  
18 cells. *J Immunol* 2002;169:983–92.
- 19 [90] **Edwards S, Lalor PF**, Nash GB, Rainger GE, Adams DH. Lymphocyte traffic  
20 through sinusoidal endothelial cells is regulated by hepatocytes. *Hepatology* 2005;41:451–9.  
21 doi:10.1002/hep.20585.
- 22 [91] Sutter AG, Palanisamy AP, Lench JH, Eskilsen S, Geng T, Lewin DNB, et al.  
23 Dietary Saturated Fat Promotes Development of Hepatic Inflammation Through Toll-Like  
24 Receptor 4 in Mice. *J Cell Biochem* 2016;117:1613–21. doi:10.1002/jcb.25453.
- 25 [92] Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine

secretion is associated with systemic inflammation in obese humans. *Diabetes* 2007;56:1010–3. doi:10.2337/db06-1656.

[93] **Harte AL, da Silva NF, Creely SJ, McGee KC, Billyard T, Youssef-Elabd EM, et al.** Elevated endotoxin levels in non-alcoholic fatty liver disease. *J Inflamm (Lond)* 2010;7:15. doi:10.1186/1476-9255-7-15.

[94] Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, et al. Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 2007;292:G518-525. doi:10.1152/ajpgi.00024.2006.

[95] Kazankov K, Jørgensen SMD, Thomsen KL, Møller HJ, Vilstrup H, George J, et al. The role of macrophages in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Nat Rev Gastroenterol Hepatol* 2018. doi:10.1038/s41575-018-0082-x.

[96] Tamaki Y, Nakade Y, Yamauchi T, Makino Y, Yokohama S, Okada M, et al. Angiotensin II type 1 receptor antagonist prevents hepatic carcinoma in rats with nonalcoholic steatohepatitis. *J Gastroenterol* 2013;48:491–503. doi:10.1007/s00535-012-0651-7.

[97] Yoshiji H, Kuriyama S, Noguchi R, Ikenaka Y, Kitade M, Kaji K, et al. Angiotensin-II and vascular endothelial growth factor interaction plays an important role in rat liver fibrosis development. *Hepatol Res* 2006;36:124–9. doi:10.1016/j.hepres.2006.07.003.

[98] Cayón A, Crespo J, Guerra AR, Pons-Romero F. [Gene expression in obese patients with non-alcoholic steatohepatitis]. *Rev Esp Enferm Dig* 2008;100:212–8.

[99] Kietzmann T, Görlach A. Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression. *Semin Cell Dev Biol* 2005;16:474–86. doi:10.1016/j.semcdb.2005.03.010.

[100] Povero D, Eguchi A, Niesman IR, Andronikou N, de Mollerat du Jeu X, Mulya A, et al. Lipid-induced toxicity stimulates hepatocytes to release angiogenic microparticles that

- 1 require Vanin-1 for uptake by endothelial cells. *Sci Signal* 2013;6:ra88.  
2 doi:10.1126/scisignal.2004512.
- 3 [101] Kim M, Allen B, Korhonen EA, Nitschké M, Yang HW, Baluk P, et al. Opposing  
4 actions of angiopoietin-2 on Tie2 signaling and FOXO1 activation. *J Clin Invest*  
5 2016;126:3511–25. doi:10.1172/JCI84871.
- 6 [102] Tugues S, Fernandez-Varo G, Muñoz-Luque J, Ros J, Arroyo V, Rodés J, et al.  
7 Antiangiogenic treatment with sunitinib ameliorates inflammatory infiltrate, fibrosis, and  
8 portal pressure in cirrhotic rats. *Hepatology* 2007;46:1919–26. doi:10.1002/hep.21921.
- 9 [103] **Van Steenkiste C, Ribera J**, Geerts A, Pauta M, Tugues S, Casteleyn C, et al.  
10 Inhibition of placental growth factor activity reduces the severity of fibrosis, inflammation,  
11 and portal hypertension in cirrhotic mice. *Hepatology* 2011;53:1629–40.  
12 doi:10.1002/hep.24238.
- 13 [104] **Thabut D, Routray C**, Lomberk G, Shergill U, Glaser K, Huebert R, et al.  
14 Complementary vascular and matrix regulatory pathways underlie the beneficial mechanism  
15 of action of sorafenib in liver fibrosis. *Hepatology* 2011;54:573–85. doi:10.1002/hep.24427.
- 16 [105] Öztürk Akcora B, Storm G, Prakash J, Bansal R. Tyrosine kinase inhibitor BIBF1120  
17 ameliorates inflammation, angiogenesis and fibrosis in CCl4-induced liver fibrogenesis  
18 mouse model. *Sci Rep* 2017;7:44545. doi:10.1038/srep44545.
- 19 [106] Yang L, Kwon J, Popov Y, Gajdos GB, Ordog T, Brekken RA, et al. Vascular  
20 endothelial growth factor promotes fibrosis resolution and repair in mice. *Gastroenterology*  
21 2014;146:1339-1350.e1. doi:10.1053/j.gastro.2014.01.061.
- 22 [107] Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev*  
23 *Gastroenterol Hepatol* 2017;14:397–411. doi:10.1038/nrgastro.2017.38.
- 24 [108] Schaffner F, Poper H. Capillarization of hepatic sinusoids in man. *Gastroenterology*  
25 1963;44:239–42.



- 1 [109] Xu B, Broome U, Uzunel M, Nava S, Ge X, Kumagai-Braesch M, et al.  
2 Capillarization of hepatic sinusoid by liver endothelial cell-reactive autoantibodies in patients  
3 with cirrhosis and chronic hepatitis. *Am J Pathol* 2003;163:1275–89. doi:10.1016/S0002-  
4 9440(10)63487-6.
- 5 [110] Sørensen KK, Simon-Santamaria J, McCuskey RS, Smedsrød B. Liver Sinusoidal  
6 Endothelial Cells. *Compr Physiol* 2015;5:1751–74. doi:10.1002/cphy.c140078.
- 7 [111] DeLeve LD, Wang X, Kanel GC, Atkinson RD, McCuskey RS. Prevention of hepatic  
8 fibrosis in a murine model of metabolic syndrome with nonalcoholic steatohepatitis. *Am J*  
9 *Pathol* 2008;173:993–1001. doi:10.2353/ajpath.2008.070720.
- 10 [112] DeLeve LD. Liver sinusoidal endothelial cells in hepatic fibrosis. *Hepatology*  
11 2015;61:1740–6. doi:10.1002/hep.27376.
- 12 [113] Maslak E, Gregorius A, Chlopicki S. Liver sinusoidal endothelial cells (LSECs)  
13 function and NAFLD; NO-based therapy targeted to the liver. *Pharmacol Rep* 2015;67:689–  
14 94. doi:10.1016/j.pharep.2015.04.010.
- 15 [114] Rangwala F, Guy CD, Lu J, Suzuki A, Burchette JL, Abdelmalek MF, et al. Increased  
16 production of sonic hedgehog by ballooned hepatocytes. *J Pathol* 2011;224:401–10.  
17 doi:10.1002/path.2888.
- 18 [115] Xie G, Choi SS, Syn W-K, Michelotti GA, Swiderska M, Karaca G, et al. Hedgehog  
19 signalling regulates liver sinusoidal endothelial cell capillarisation. *Gut* 2013;62:299–309.  
20 doi:10.1136/gutjnl-2011-301494.
- 21 [116] Matz-Soja M, Gebhardt R. The many faces of Hedgehog signalling in the liver: recent  
22 progress reveals striking cellular diversity and the importance of microenvironments. *J*  
23 *Hepatol* 2014;61:1449–50. doi:10.1016/j.jhep.2014.06.041.
- 24 [117] Witek RP, Yang L, Liu R, Jung Y, Omenetti A, Syn W-K, et al. Liver cell-derived  
25 microparticles activate hedgehog signaling and alter gene expression in hepatic endothelial

cells. *Gastroenterology* 2009;136:320-330.e2. doi:10.1053/j.gastro.2008.09.066.

[118] Deleve LD, Wang X, Guo Y. Sinusoidal endothelial cells prevent rat stellate cell activation and promote reversion to quiescence. *Hepatology* 2008;48:920–30. doi:10.1002/hep.22351.

[119] **Marrone G, Russo L**, Rosado E, Hide D, García-Cardena G, García-Pagán JC, et al. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. *J Hepatol* 2013;58:98–103. doi:10.1016/j.jhep.2012.08.026.

[120] Li Y, Lui KO, Zhou B. Reassessing endothelial-to-mesenchymal transition in cardiovascular diseases. *Nat Rev Cardiol* 2018;15:445–56. doi:10.1038/s41569-018-0023-y.

[121] Piera-Velazquez S, Mendoza FA, Jimenez SA. Endothelial to Mesenchymal Transition (EndoMT) in the Pathogenesis of Human Fibrotic Diseases. *J Clin Med* 2016;5. doi:10.3390/jcm5040045.

[122] Singh KK, Lovren F, Pan Y, Quan A, Ramadan A, Matkar PN, et al. The essential autophagy gene ATG7 modulates organ fibrosis via regulation of endothelial-to-mesenchymal transition. *J Biol Chem* 2015;290:2547–59. doi:10.1074/jbc.M114.604603.

[123] Smedsrød B, De Bleser PJ, Braet F, Lovisetti P, Vanderkerken K, Wisse E, et al. Cell biology of liver endothelial and Kupffer cells. *Gut* 1994;35:1509–16.

[124] **Wang R, Ding Q**, Yaqoob U, de Assuncao TM, Verma VK, Hirsova P, et al. Exosome Adherence and Internalization by Hepatic Stellate Cells Triggers Sphingosine 1-Phosphate-dependent Migration. *J Biol Chem* 2015;290:30684–96. doi:10.1074/jbc.M115.671735.

[125] Wells RG. Cellular sources of extracellular matrix in hepatic fibrosis. *Clin Liver Dis* 2008;12:759–68, viii. doi:10.1016/j.cld.2008.07.008.

[126] Ribera J, Pauta M, Melgar-Lesmes P, Córdoba B, Bosch A, Calvo M, et al. A small

- 1 population of liver endothelial cells undergoes endothelial-to-mesenchymal transition in  
2 response to chronic liver injury. *Am J Physiol Gastrointest Liver Physiol* 2017;313:G492–  
3 504. doi:10.1152/ajpgi.00428.2016.
- 4 [127] Lemoine S, Thabut D, Housset C, Moreau R, Valla D, Boulanger CM, et al. The  
5 emerging roles of microvesicles in liver diseases. *Nat Rev Gastroenterol Hepatol*  
6 2014;11:350–61. doi:10.1038/nrgastro.2014.7.
- 7 [128] **Huang X-D, Fan Y**, Zhang H, Wang P, Yuan J-P, Li M-J, et al. Serum leptin and  
8 soluble leptin receptor in non-alcoholic fatty liver disease. *World J Gastroenterol*  
9 2008;14:2888–93.
- 10 [129] Kitade M, Yoshiji H, Kojima H, Ikenaka Y, Noguchi R, Kaji K, et al. Leptin-mediated  
11 neovascularization is a prerequisite for progression of nonalcoholic steatohepatitis in rats.  
12 *Hepatology* 2006;44:983–91. doi:10.1002/hep.21338.
- 13 [130] Marra F, Bertolani C. Adipokines in liver diseases. *Hepatology* 2009;50:957–69.  
14 doi:10.1002/hep.23046.
- 15 [131] Zhou L-Y, Zeng H, Wang S, Chen J-X. Regulatory Role of Endothelial PHD2 in the  
16 Hepatic Steatosis. *Cell Physiol Biochem* 2018;48:1003–11. doi:10.1159/000491968.
- 17 [132] Ozer A, Bruick RK. Regulation of HIF by prolyl hydroxylases: recruitment of the  
18 candidate tumor suppressor protein ING4. *Cell Cycle* 2005;4:1153–6.  
19 doi:10.4161/cc.4.9.2040.
- 20 [133] Kantari-Mimoun C, Castells M, Klose R, Meinecke A-K, Lemberger UJ, Rautou P-E,  
21 et al. Resolution of liver fibrosis requires myeloid cell-driven sinusoidal angiogenesis.  
22 *Hepatology* 2015;61:2042–55. doi:10.1002/hep.27635.
- 23 [134] Taura K, De Minicis S, Seki E, Hatano E, Iwaisako K, Osterreicher CH, et al. Hepatic  
24 stellate cells secrete angiopoietin 1 that induces angiogenesis in liver fibrosis.  
25 *Gastroenterology* 2008;135:1729–38. doi:10.1053/j.gastro.2008.07.065.

- 1 [135] Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Hicklin DJ, et al. Vascular  
2 endothelial growth factor and receptor interaction is a prerequisite for murine hepatic  
3 fibrogenesis. *Gut* 2003;52:1347–54.
- 4 [136] Zhao S, Zhang Z, Yao Z, Shao J, Chen A, Zhang F, et al. Tetramethylpyrazine  
5 attenuates sinusoidal angiogenesis via inhibition of hedgehog signaling in liver fibrosis.  
6 *IUBMB Life* 2017;69:115–27. doi:10.1002/iub.1598.
- 7 [137] Hennenberg M, Trebicka J, Kohistani Z, Stark C, Nischalke H-D, Krämer B, et al.  
8 Hepatic and HSC-specific sorafenib effects in rats with established secondary biliary  
9 cirrhosis. *Lab Invest* 2011;91:241–51. doi:10.1038/labinvest.2010.148.
- 10 [138] **Coch L, Mejias M**, Berzigotti A, Garcia-Pras E, Gallego J, Bosch J, et al. Disruption  
11 of negative feedback loop between vasohibin-1 and vascular endothelial growth factor  
12 decreases portal pressure, angiogenesis, and fibrosis in cirrhotic rats. *Hepatology*  
13 2014;60:633–47. doi:10.1002/hep.26995.
- 14 [139] Chatterjee S. Reversal of vasohibin-driven negative feedback loop of vascular  
15 endothelial growth factor/angiogenesis axis promises a novel antifibrotic therapeutic strategy  
16 for liver diseases. *Hepatology* 2014;60:458–60. doi:10.1002/hep.27061.
- 17 [140] Yang ZF, Poon RTP. Vascular changes in hepatocellular carcinoma. *Anat Rec*  
18 (Hoboken) 2008;291:721–34. doi:10.1002/ar.20668.
- 19 [141] **Géraud C, Mogler C**, Runge A, Evdokimov K, Lu S, Schledzewski K, et al.  
20 Endothelial transdifferentiation in hepatocellular carcinoma: loss of Stabilin-2 expression in  
21 peri-tumourous liver correlates with increased survival. *Liver Int* 2013;33:1428–40.  
22 doi:10.1111/liv.12262.
- 23 [142] Wu LQ, Zhang WJ, Niu JX, Ye LY, Yang ZH, Grau GE, et al. Phenotypic and  
24 functional differences between human liver cancer endothelial cells and liver sinusoidal  
25 endothelial cells. *J Vasc Res* 2008;45:78–86. doi:10.1159/000109079.

[143] Höchst B, Schildberg FA, Böttcher J, Metzger C, Huss S, Türler A, et al. Liver sinusoidal endothelial cells contribute to CD8 T cell tolerance toward circulating carcinoembryonic antigen in mice. *Hepatology* 2012;56:1924–33. doi:10.1002/hep.25844.

[144] Milner K-L, van der Poorten D, Xu A, Bugianesi E, Kench JG, Lam KSL, et al. Adipocyte fatty acid binding protein levels relate to inflammation and fibrosis in nonalcoholic fatty liver disease. *Hepatology* 2009;49:1926–34. doi:10.1002/hep.22896.

[145] Laouirem S, Sannier A, Norkowski E, Cauchy F, Doblas S, Rautou PE, et al. Endothelial fatty liver binding protein 4: a new targetable mediator in hepatocellular carcinoma related to metabolic syndrome. *Oncogene* 2018. doi:10.1038/s41388-018-0597-1.

[146] Yoshiji H, Noguchi R, Namisaki T, Moriya K, Kitade M, Aihara Y, et al. Combination of sorafenib and angiotensin-II receptor blocker attenuates preneoplastic lesion development in a non-diabetic rat model of steatohepatitis. *J Gastroenterol* 2014;49:1421–9. doi:10.1007/s00535-013-0906-y.

[147] Gracia-Sancho J, Marrone G, Fernández-Iglesias A. Hepatic microcirculation and mechanisms of portal hypertension. *Nat Rev Gastroenterol Hepatol* 2018. doi:10.1038/s41575-018-0097-3.

**Author names in bold designate shared co-first authorship.**

## **Figure legend**

**Figure 1: LSECs capillarization promotes steatosis. In physiological conditions,** chylomicron remnants cross LSECs *fenestrae* and provide cholesterol and triglycerides for VLDL synthesis. VLDL are then released by hepatocytes and reach blood flow through *fenestrae*. **In metabolic syndrome conditions,** LSECs capillarization arises early in the course of NAFLD, possibly because of exposure of LSECs to dietary macronutrients. In turn, LSECs capillarization promotes steatosis, possibly because capillarization blocks the transfer of chylomicron remnants to hepatocytes, thus stimulating endogenous cholesterol and triglycerides synthesis, as a compensatory mechanism for the synthesis of VLDL, wich reach blood flow through the lymphatic system. FFA, free fatty acids; LDL, low-density lipoprotein; LSECs, liver sinusoidal endothelial cells; ox-LDL, oxidized low-density lipoprotein; VLDL; very low-density lipoprotein.

**Figure 2: LSECs dysfunction promotes steatosis. In physiological conditions,** LSECs release NO which regulates intrahepatic vascular tone on the one hand, and hepatic lipid metabolism on the other hand. NO limits hepatic lipid content by inhibiting hepatic *de novo* lipogenesis, through a limitation of citrate synthesis in mitochondria, an inhibition of ACC and of GPAT, and by promoting fatty acids beta-oxidation. **In metabolic syndrome conditions,** overabundance of lipids and insulin resistance lead to downregulation of eNOS activity and to upregulation of iNOS and of NOX1 (a nitric oxide consuming enzyme), causing nitrooxidative stress through peroxynitrites production and eventually endothelial dysfunction. Reduced NO availability promotes steatosis. Liver steatosis is associated with an increased intrahepatic vascular resistance which has a mechanical component, due to the compression of the sinusoidal lumen by

enlarged fat-laden hepatocytes, and a dynamic component, due to a liver endothelial dysfunction. ACC, acetyl CoA carboxylase; AMPK, AMP-activated protein kinase; eNOS; endothelial nitric oxide synthase; GPAT, glycerol-3-phosphate acyltransferase; LSECs, liver sinusoidal endothelial cells; IHRV; intrahepatic vascular resistance; iNOS, inducible nitric oxide synthase; NO, nitric oxide; NOX1, NADPH oxidase 1.

**Figure 3: LSECs acquire pro-inflammatory functions in NASH.** Lipotoxicity, inflammation and gut microbiota-derived products induce LSECs inflammatory phenotype and function mediated by NFkB activation, which orchestrate the release of pro-inflammatory mediators and the overexpression of adhesion molecules. Inflammatory mediators and LSECs dysfunction promote Kupffer cells activation and leukocytes chemoattraction. Adhesion molecules overexpression allows adhesion and transendothelial migration of the recruited leukocytes in the hepatic parenchyma. ICAM-1, intercellular adhesion molecule-1; LSECs, liver sinusoidal endothelial cells; NF-kB, nuclear factor kappa B; NO, nitric oxide; VAP-1, vascular adhesion protein-1; VCAM-1, vascular cell adhesion molecule-1.

**Figure 4: Angiogenesis in NASH.** Hypoxia, liver injury, lipids, oxidative stress, inflammation and fibrosis induce the release of pro-angiogenic factors, such as hepatocyte-derived microvesicles, VEGF and Angiopoietin-2, from parenchymal and non-parenchymal cells including LSECs, therefore promoting pathologic angiogenesis. Adipokines, such as leptin, also exhibits pro-angiogenic activity contributing to pathologic angiogenesis in NASH. In turn, angiogenesis promotes liver inflammation and fibrosis as attested by anti-angiogenic therapies which prevent liver inflammation and fibrosis in experimental models of NASH. VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor2.

**Figure 5: LSECs in NASH-related fibrosis.** Healthy LSECs are gatekeepers of liver fibrosis by maintaining HSCs quiescent through NO, while altered LSECs (following capillarization and LSECs dysfunction) lose this ability. In addition, altered LSECs release profibrogenic molecules such as TGF- $\beta$ , Hedgehog molecules, laminin and fibronectin, which activate HSCs. Activated HSCs produces Hedgehog molecules reinforcing their own activation and LSECs capillarization. Activated HSCs then produce large amounts of extracellular matrix thus inducing liver fibrosis. ECM, extracellular matrix; Hh, Hedgehog; HSC, hepatic stellate cell; LSECs, liver sinusoidal endothelial cell; NO, nitric oxide; TGF- $\beta$ , transforming growth factor- $\beta$ .

**Figure 6: Role of liver endothelial cells in hepatocellular carcinoma (HCC) development in chronic liver diseases (not specific for NAFLD).** During HCC progression, endothelial cells sequentially lose their specific markers including stabilin-1, stabilin-2, LYVE-1 and CD32b (SE-1). Conversely, endothelial expression of integrins increases, facilitating adhesion of liver cancer cells. In parallel, endothelial expression of ICAM-1 decreases, leading to a lower ability of leukocyte to adhere and infiltrate HCC. Endothelial cells within HCC can also alter tumor-associated immune responses *via* their ability to confer T cell tolerance towards cancer-associated antigens and to create an immunosuppressive environment.

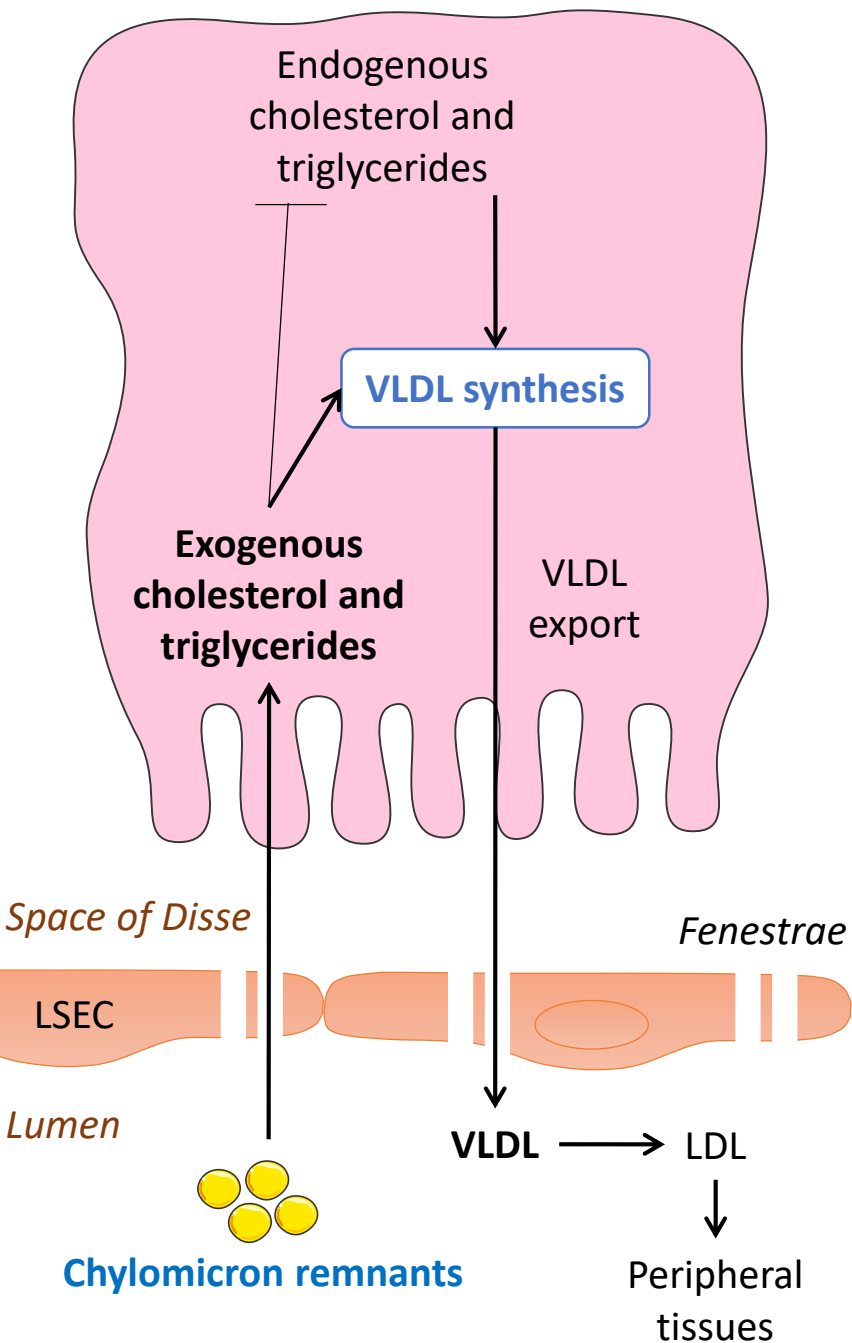
**Figure 7: Role of liver endothelial cells in hepatocellular carcinoma development in the NAFLD setting.** Circulating concentrations of angiocrine factors, such as VEGF and angiopoietin-2, and adipokines, such as leptin are increased in NAFLD. These mediators induce angiogenesis which promotes HCC growth. The adipokine FABP4 is released by adipose tissue and endothelial cells and contributes to HCC development and progression.



**BOX: Liver sinusoidal endothelial cell (LSEC) changes are not specific for certain animal models of NASH**

LSECs alterations are observed in various dietary or genetic models of NAFLD, without apparent specificity. Indeed, LSECs capillarization is observed in animals fed a choline-deficient, l-amino acid-defined diet or a high-fat diet [18–20]. LSECs dysfunction can be induced by a high-fat diet, or a rich saturated fatty acids diet, or a high-fat high-glucose and high-fructose diet [40,41,43]. Markers of LSECs inflammatory phenotype are observed in animals fed a high-fat diet or a methionine and choline deficient diet [68,82]. Markers of angiogenesis appear after methionine and choline deficient diet in *db/db* obese mice and in C57BL/6 mice and in rats fed a choline-deficient, l-amino acid-defined diet [63,129].

## Physiological conditions



## Metabolic syndrome

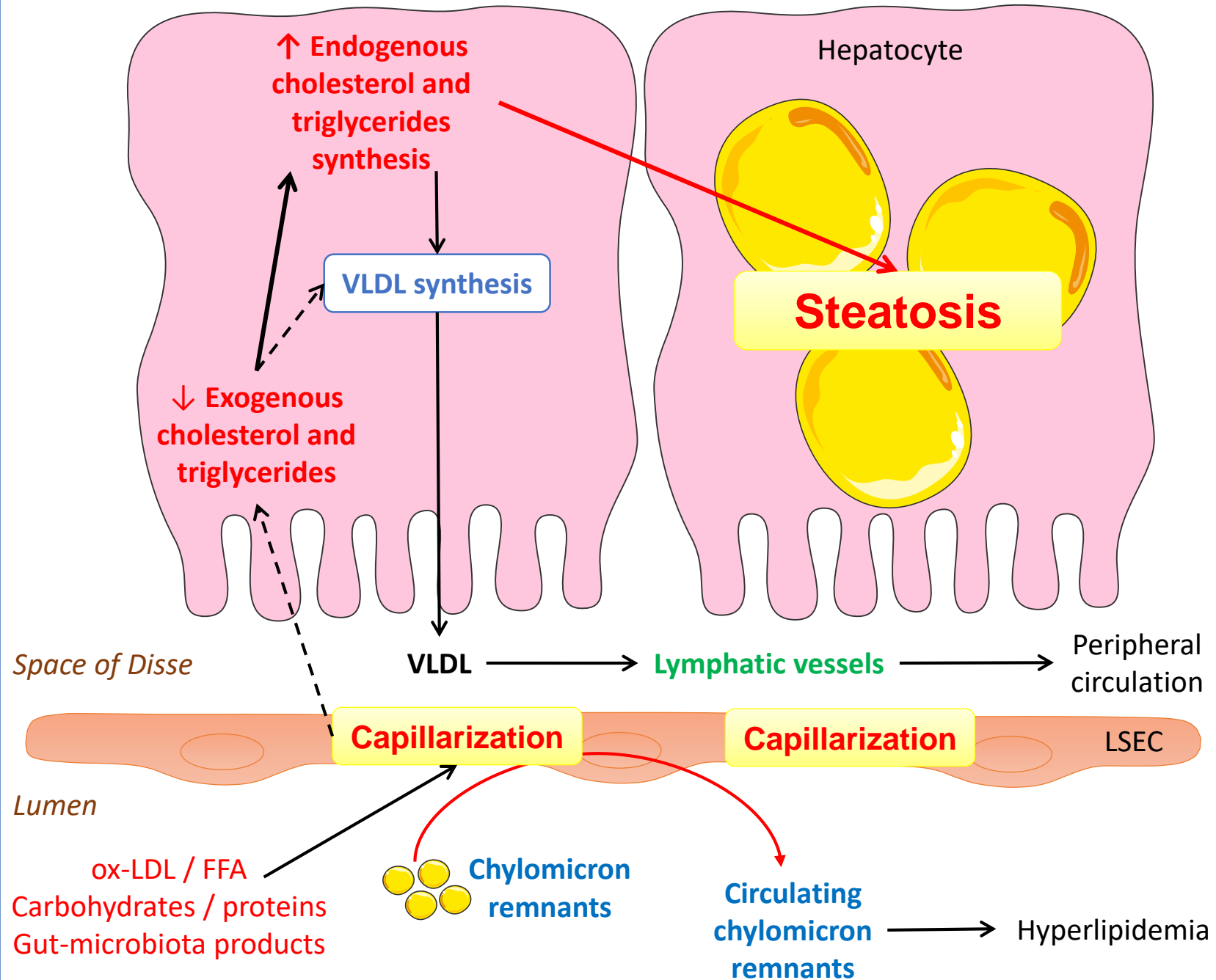


Figure 1

Figure 2

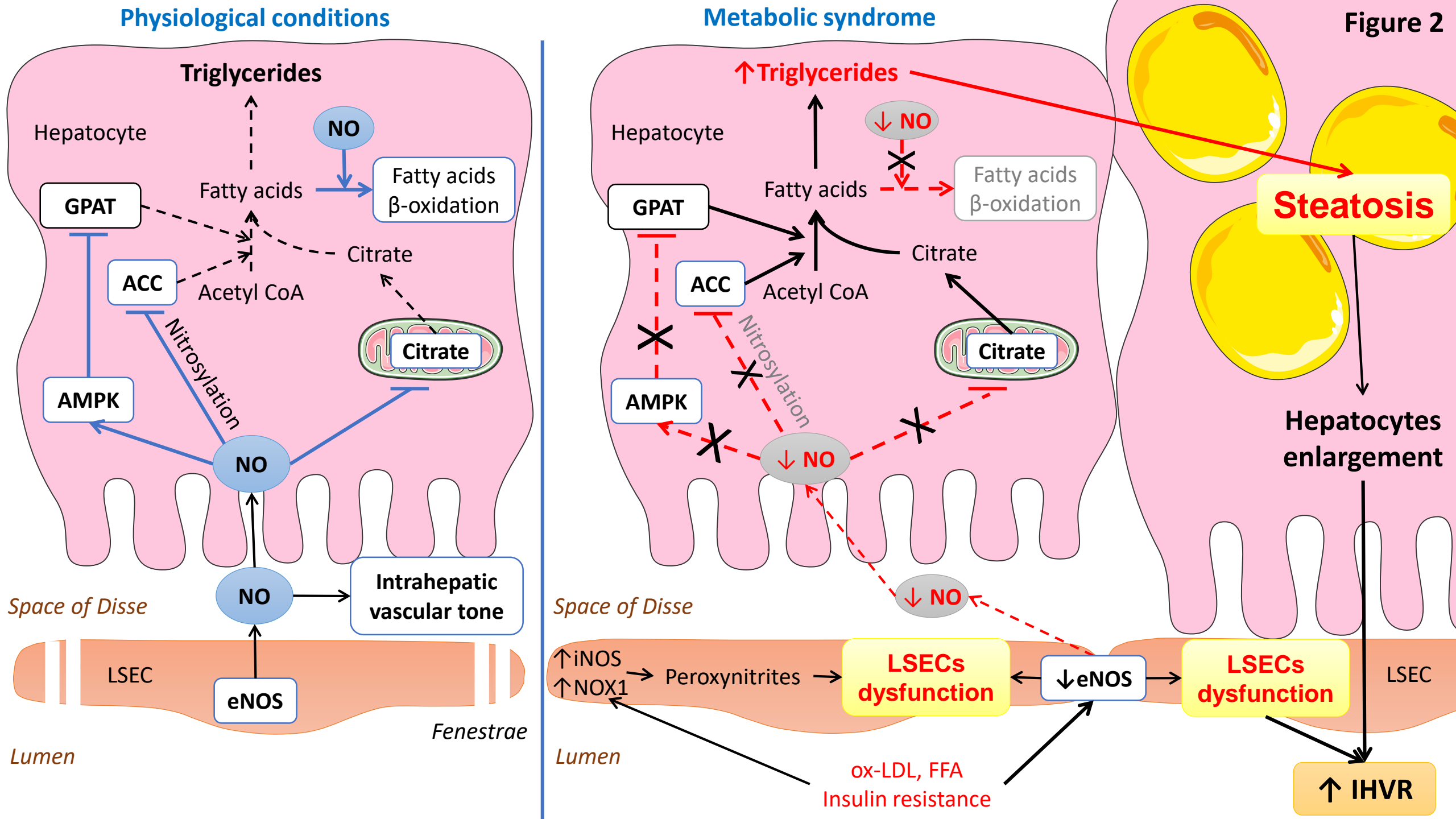


Figure 3

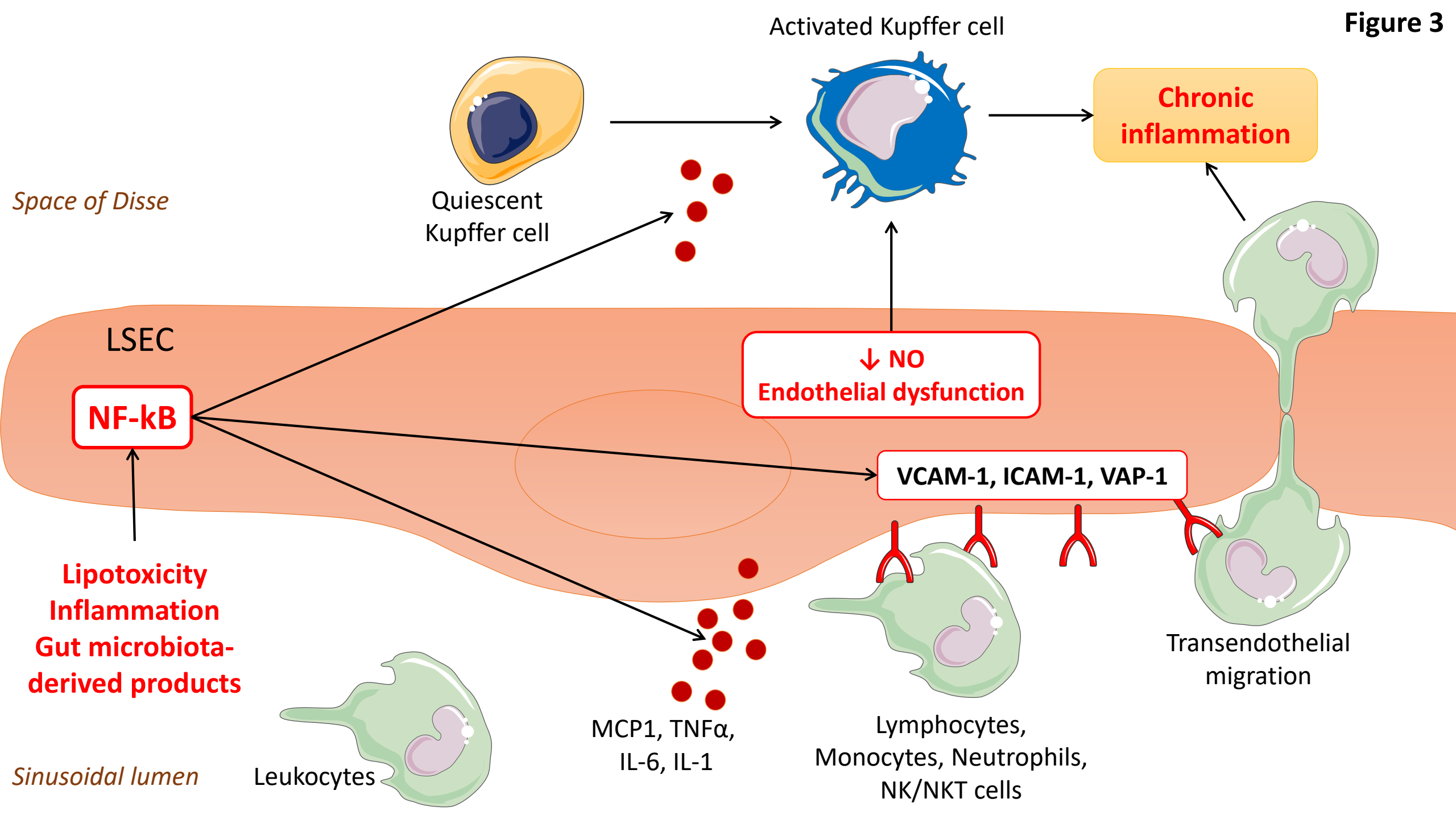


Figure 4

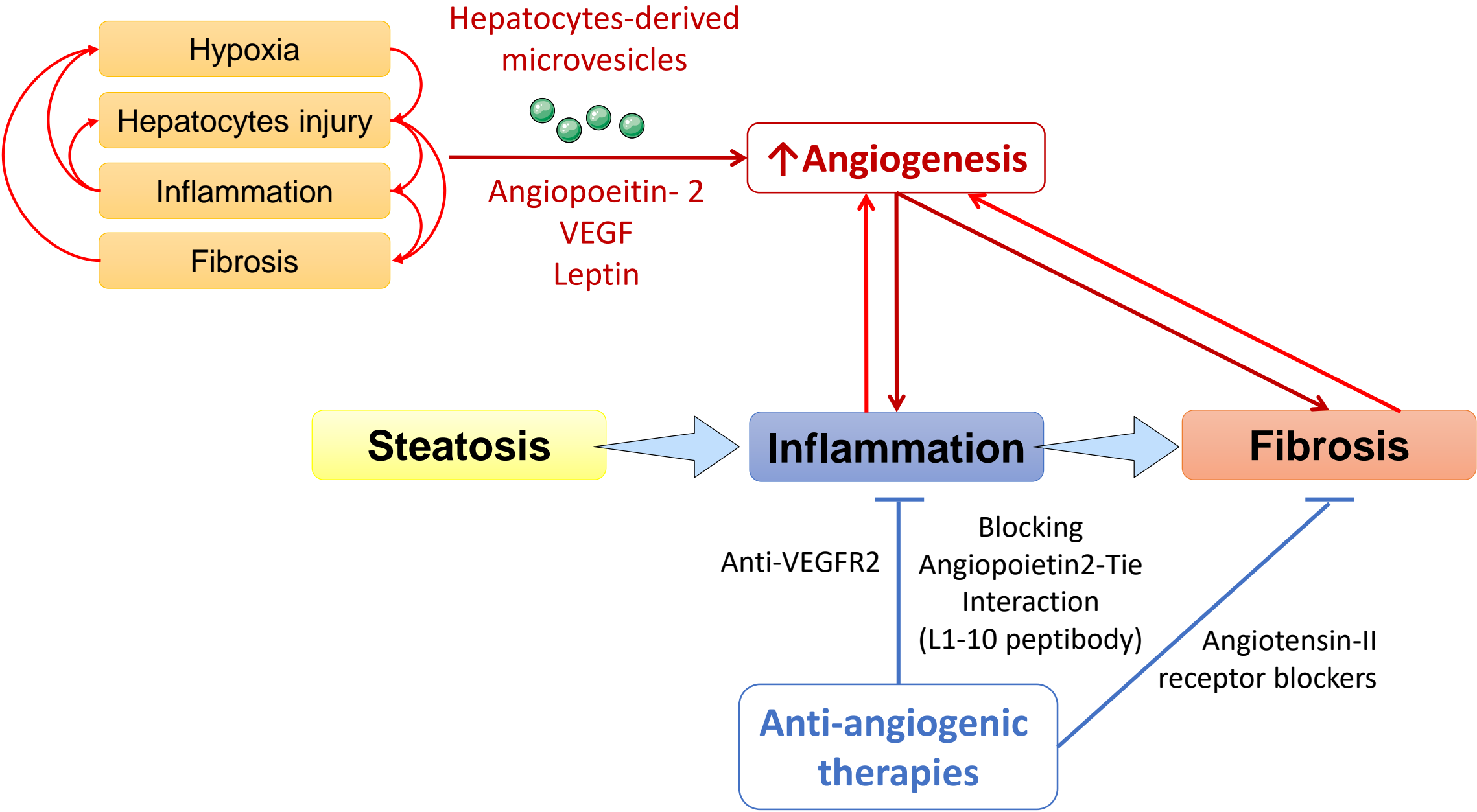


Figure 5

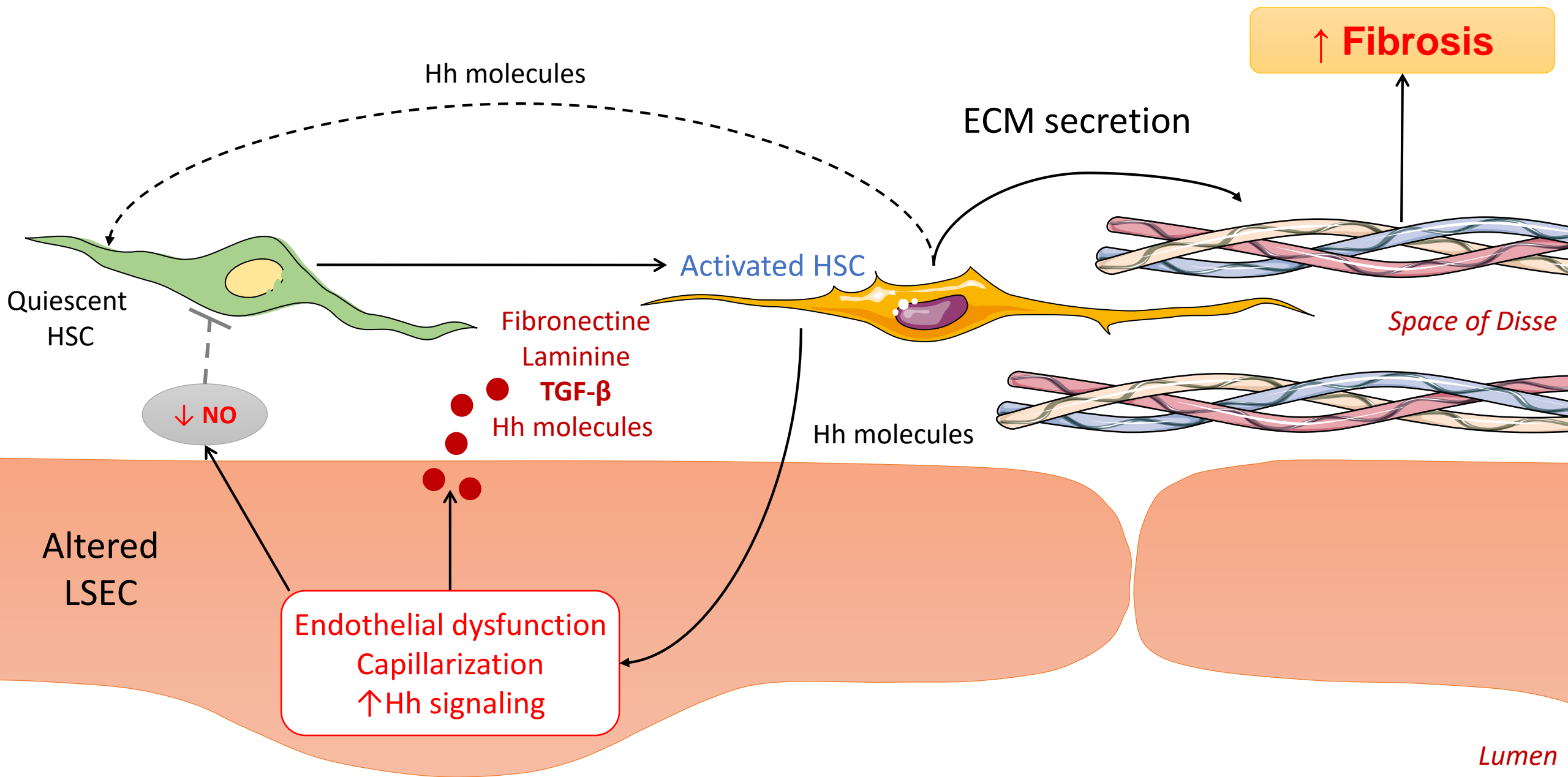


Figure 6

Tumor progression

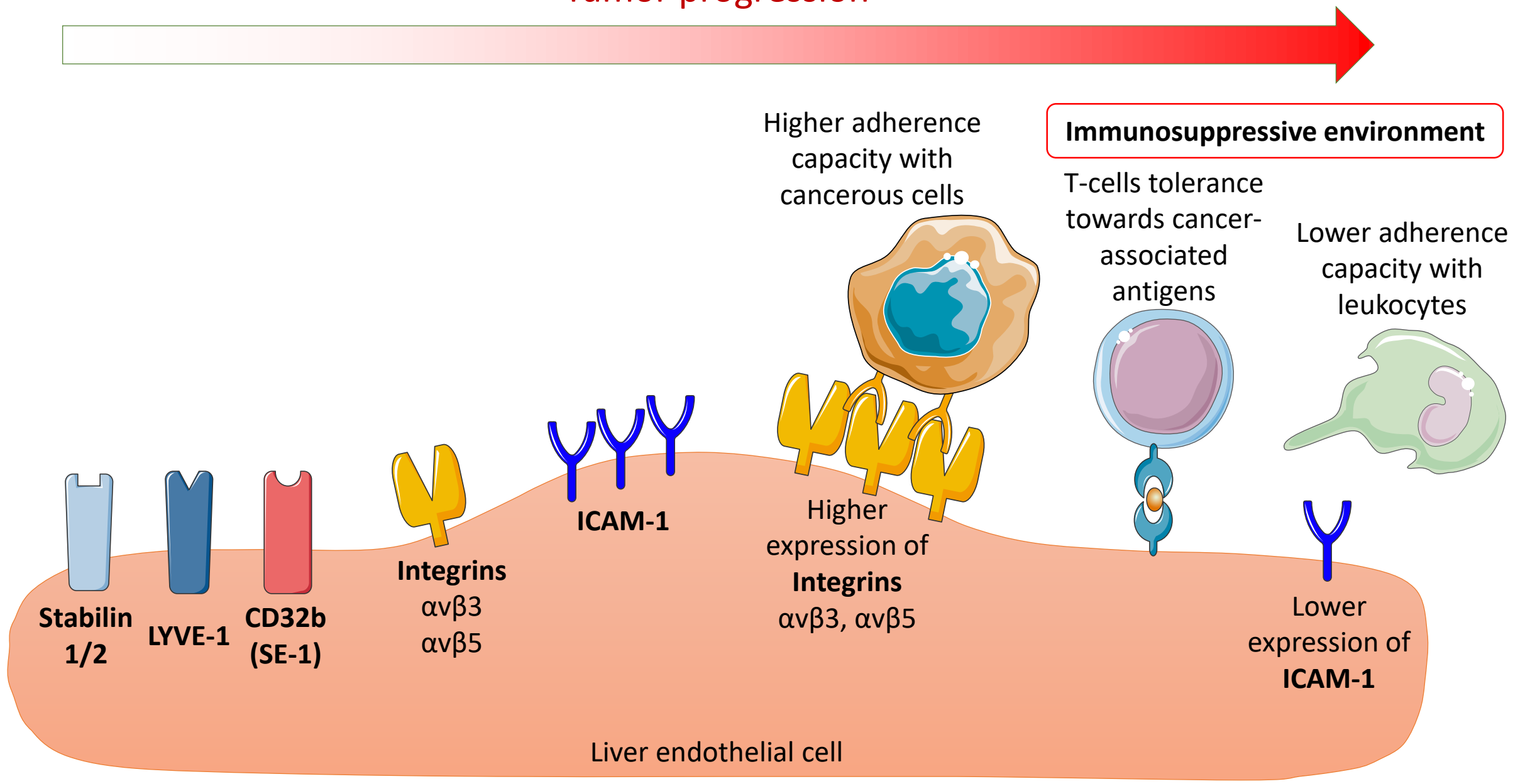


Figure 7

