Intestine-Liver Cross-talk in Type 2 Diabetes and Non-Alcoholic Fatty Liver Disease

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Abstract:

Type 2 diabetes (T2D) and Non-Alcoholic Fatty Liver Disease (NAFLD) are pathologies whose prevalence continues to increase worldwide. Both diseases are precipitated by an excessive caloric intake, which promotes insulin resistance and fatty liver. The role of the intestine and its crosstalk with the liver in the development of these metabolic diseases is receiving increasing attention. Alterations in diet-intestinal microbiota interactions lead to the dysregulation of intestinal functions, resulting in altered metabolite and energy substrate production and increased intestinal permeability. Connected through the portal circulation, these changes in intestinal functions impact the liver and other metabolic organs, such as visceral adipose tissue, hence participating in the development of insulin resistance, and worsening T2D and NAFLD. Thus, targeting the intestine may be an efficient therapeutic approach to cure T2D and NAFLD.

In this review, we will first introduce the signaling pathways linking T2D and NAFLD. Next, we will address the role of the gut-liver crosstalk in the development of T2D and NAFLD, with a particular focus on the gut microbiota and the molecular pathways behind the increased intestinal permeability and inflammation. Finally, we will summarize the therapeutic strategies which target the gut and its functions and are currently used or under development to treat T2D and NAFLD.

Abbreviations:

ALT: alanine aminotransferase

BA: bile acids

CYP7A1: cholesterol 7 alpha-hydroxylase

DDP-4: dipeptidyl peptidase-4

FGF: fibroblast growth factor

FMT: fecal microbiota transplantation

FXR: farnesoid X receptor

GLP-1: glucagon-like peptide-1

GLP-1R: glucagon-like peptide-1 receptor

HFD: high fat diet

HOMA-IR: homeostatic model assessment of insulin resistance

IgA: immunoglobulin A

IR: insulin resistance

JAM-A: junctional adhesion molecule A

LPS: lipopolysaccharide

MRI: magnetic resonance imaging

NAFL /NAFLD: non-alcoholic fatty liver/disease

NAS: non-alcoholic fatty liver disease activity score

NASH: non-alcoholic steatohepatitis

OCA: obeticholic acid

SCFA: short chain fatty acid

T2D: type 2 diabetes

TG: triglycerides

TGR5: takeda g-protein-coupled receptor 5

1. T2D and NAFLD, two closely linked multi-organ pathologies

Diabetes is the most common metabolic disease worldwide. With the western lifestyle pandemic, this multifactorial disease has a worldwide prevalence estimated at 500 million patients [1]. The majority

of diabetic patients suffer from Type 2 Diabetes (T2D), which can be classified in different etiological subgroups [2]. T2D is closely related to other metabolic diseases linked to excessive caloric intake and sedentary life style, such as obesity [1]. The liver plays a central role in lipid and glucose metabolism, and hepatic insulin resistance (IR) is a hallmark of both T2D and NAFLD [3,4]. Since >70% of T2D patients have NAFLD [5–7], it has been recently proposed to rename NAFLD to "Metabolic Associated Fatty Liver Disease" to highlight this association [6].

NAFLD is a progressive disease initiated by an increase of hepatic lipid content, termed steatosis, the "Non-Alcoholic Fatty Liver" (NAFL) stage. When steatosis becomes chronic, a more complicated histological pattern appears characterized by an increased number of inflammatory *foci* associated with hepatocyte balloonisation, the "Non-Alcoholic SteatoHepatitis" (NASH) stage. NAFL and NASH are reversible but, without life-style or medical intervention, liver fibrosis progressively appears developing over decades to cirrhosis and hepatocarcinoma [8,9].

The development of diagnostic tools to detect the presence of NASH and its progression to fibrosis is crucial given its increasing prevalence. Today, the gold standard of NASH diagnosis is based on scoring of the histological features of NASH on hepatic biopsies [9]. However, needle biopsies are not without risk and reader variability exists, precluding its application to large numbers of patients. Thus, non-invasive techniques based on biomarkers are being developed to facilitate diagnosis and stage identification [10]. In addition, Magnetic Resonance Imaging (MRI) is used to estimate the hepatic fat content and liver ultrasound elastography to determine the degree of fibrosis [11]. As NAFLD is linked to other metabolic diseases, these new diagnostic tools can be more easily applied to patients suffering from common NAFLD co-morbidities, such as T2D and cardiovascular disease [8].

It is still unclear whether T2D and NAFLD develop independently or not, but several studies indicate that the hepatic IR observed in T2D promotes NAFLD progression. Both metabolic diseases imply a suspended dialogue between the different metabolic organs (Figure 1). In the initial phase of glucose intolerance, the chronic hyperinsulinemia leads to decreased hepatic insulin receptor signaling due to reduced receptor synthesis and signal transduction defects , hence resulting in hepatic IR [12]. Moreover, the dialogue between adipose tissue and liver is impaired in obesity and NAFLD, with decreased adiponectin secretion and increased release of free fatty acids by adipose tissue, which worsen hepatic steatosis and fibrosis [13,14]. As a consequence to the loss of insulin sensitivity, hepatic steatosis increases, associated with a dysregulation of liver glycogenesis, gluconeogenesis and lipid synthesis [3]. The sustained increase of bioactive lipids, such as ceramides and diacylglycerols, increase IR by altering hepatic insulin receptor signaling. Indeed, diacylglycerols activate protein kinase C isoforms which phosphorylate insulin receptor substrates [15,16]. Moreover, ceramides produced in hepatocytes or released by the intestine [17,18] enhance protein kinase Cζ and phosphatase A2 which inhibit Akt2 [19], hence aggravating hepatic IR. Ceramides and diacylgycerols drive hepatocyte endoplasmic reticulum stress, induce liver inflammation, via pathways including the Nucleotide-binding oligomerization domain-Like Receptor Pyrin domain-containing-3 (NLRP3) inflammasome, and apoptosis [20–22]. These mechanisms promote the hepatic recruitment of immune cells, including macrophages, dendritic cells and $CD8^+$ T lymphocytes [23–25], maintaining inflammation and activating hepatic stellate cells resulting in fibrosis [26,27]. Muscle IR is linked to a decrease of muscular glycogen synthesis and an increase of hepatic *de novo* lipogenesis after carbohydrate ingestion [28]. In NAFLD patients, low skeletal muscle mass is associated with liver fibrosis development [29] and an increase of muscle fat content is associated with NASH [30]. Furthermore, sarcopenia independently associates with NAFLD in T2D,

suggesting that it may contribute to hepatic impairment in these patients [31]. Moreover, the hepatic damages in diabetic patients with advanced NAFLD may further promote organ dysfunctions, such as chronic kidney disease, cardiovascular complications or neurodegenerative diseases [32–36]. Thus, the dysregulation of cross-talk between liver and other metabolic organs plays a crucial role in the development of these metabolic diseases

In this review, we will discuss the role of the disrupted gut-liver crosstalk in T2D and NAFLD, focusing on changes in gut microbiota, gut permeability, the entero-hepatic cycle, the endocrine intestine and the immune system. Finally, we will discuss the intestine and its functions as potential therapeutic targets for the treatment of T2D and NAFLD.

2. The role of gut-liver cross-talk disruption in T2D and NAFLD

The intestine is a complex tissue with many functions: it is not only a physical barrier controlling the absorption of nutrients, but also a site of hormone production and an immunological checkpoint. The gut *lumen* also hosts the microbiota, which are essential in the maintenance of a proper energy whole body balance. Through its direct anatomical connection with the liver via the portal vein, the intestine also directly regulates hepatic metabolism. Disruption of the dialogue between the intestine and the liver is likely a major contributor to the development of metabolic diseases such as T2D and NAFLD (Figure 2).

2.1 Dysregulation of intestinal lipid metabolism promotes dyslipidemia and hepatic steatosis

122 The main function of the intestine is the absorption of nutrients. For instance, dietary lipids like
123 triglycerides (TG) are hydrolyzed by pancreatic lipase. Released fatty acids are then transported in
124 enterocytes by specific intestinal fatty acid carriers. In the enterocyte, re-assembled TG are packaged
125 into triglyceride-rich lipoproteins called chylomicrons, which are excreted at the basolateral side of
126 enterocytes. Furthermore, the gut also plays a buffering role by storing lipids after high fat meals in
127 enterocytes, which are then secreted at later stages depending on the metabolic conditions [37].
128 T2D patients display a postprandial dyslipidemia due to a combination of elevated chylomicron
129 secretion and decreased intra-vascular clearance. Obese and T2D individuals exhibiting systemic IR
130 display also lower insulin-responses in the intestine. The resulting decreased Akt phosphorylation in
131 enterocytes results in an increased chylomicron production [38,39]. Moreover, obesity and IR lead to
132 intestinal inflammation and oxidative stress, as demonstrated by higher intestinal levels of tumor
133 necrosis factor-α (TNF-α) and cyclooxygenase-2 (COX-2) as well as increased basal and
134 lipopolysaccharide (LPS)-induced interleukin 6 (IL-6) secretion [38].
135 NAFLD patients display also often a dyslipoproteinemia characterized by increased
136 apolipoprotein (Apo) B-containing lipoproteins [40]. An elevated TG absorption, associated with high
137 plasma free fatty acid levels, contributes to an increase of *de novo* hepatic ceramide synthesis, hepatic
138 steatosis and IR in NAFLD patients [41,42].

139 **2.2 Disruption of the intestinal barrier increases low grade inflammation and liver injury**

140 The intestine is highly selective for the absorption of dietary nutrients and metabolites, while
141 acting as a barrier to protect the organism against harmful molecules and bacterial translocation. The
142 luminal mucus layer, which is mainly produced by mucin-producing goblet cells, prevents bacterial
143 adhesion. The intestinal epithelial cells, which transport the luminal metabolites, also react to noxious

144 stimuli by secreting antimicrobial peptides [43]. The epithelial cell monolayer is glued together by inter-
145 cell junctions, notably the tight junctions, which are composed of several proteins, such as zonula
146 occludens 1 (ZO-1), junctional adhesion molecule A (JAM-A) and occludin. These cell junctions establish
147 intestinal integrity and allow specific permeability to control the passage from the *lumen* to the systemic
148 or lymphatic circulation. In the *lamina propria*, the intestinal immune system (see next section for
149 details) produces chemokines, cytokines and immunoglobulins (Ig) in response to danger stimuli [44,45].
150 Finally, beyond the *lamina propria*, the junctions between endothelial cells around the portal vein are
151 increasingly considered as the last layer of the intestinal barrier, called the gut vascular barrier [46].

152 In healthy conditions, most dietary nutrients cross the intestinal barrier through enterocytes,
153 while fluids are mainly passively absorbed in a para-cellular manner [47]. In inflammatory states and
154 metabolic diseases, tight junctions are disrupted leading to the uncontrolled passage of several
155 microbiota-derived molecules, such as LPS, leading to low grade systemic inflammation and
156 endotoxemia. Although there are only limited studies addressing whether intestinal permeability is
157 dysregulated in T2D patients, a leaky gut has been shown to play a role in type 1 diabetes and NAFLD
158 onset [48,49].

159 Miele *et al.* found an association between increased gut permeability and liver steatosis in biopsy-
160 proven NAFLD patients [50]. The urinary excretion of orally administrated Chromium-51 Ethylene
161 Diamine Tetra-acetate, as an estimate of gut permeability, was higher in NAFLD patients and correlated
162 positively with liver steatosis. Immunohistochemically assessed ZO-1 protein was also decreased in
163 duodenal biopsies of the NAFLD patients [50]. JAM-A mRNA and protein expression levels are lower in
164 colon biopsies from ultrasonography diagnosed NAFLD subjects [51]. In obese children with NAFLD,
165 diagnosed using MRI and liver biopsy, the intestinal permeability increase positively correlated with liver

steatosis, but not with liver inflammation, ballooning nor NASH [52]. A large meta-analysis of adult and pediatric NAFLD cohorts did not observe a link between increased intestinal permeability and liver inflammation and fibrosis in NAFLD patients [49]. Thus, whereas steatosis and intestinal permeability appear connected, the role of intestinal permeability in inflammation and fibrosis is still under debate.

Feeding mice a high fat diet (HFD) increases hepatic steatosis and IR. These mice show body and liver weight increases, fasting hyperglycemia glucose intolerance and hyperinsulinemia [53]. Intestinal JAM-A deficient mice developed severe liver damage, illustrated by elevated plasma transaminase levels, when fed a NAFLD-inducing high fructose-high sucrose-high cholesterol diet. These mice also showed a rise of systemic LPS exposure, associated with exacerbated hepatic stellate cell activation, fibrosis and inflammation due to hepatic immune cell recruitment [51]. Moreover, HFD-feeding rapidly induced gut vascular barrier damage related to decreased Wnt/b-catenin signaling in endothelial cells [46].

Gut barrier integrity thus likely plays an important role in the development of metabolic diseases such as T2D and NAFLD. Although the chronology of events is still unclear, gut microbiota-derived endotoxemia may contribute to the altered gut liver cross-talk.

2.3 Gut microbiota dysbiosis increases gut permeability and liver damage

The healthy human intestinal microbiota is composed of a large diversity of microorganisms, mainly anaerobic bacteria [54]. In a healthy state, the microbiota have symbiotic interactions with its host. It participates in nutrient absorption, assists in intestinal barrier function protection to prevent pathogen invasion, improves the function of the intestinal immune system and modulates metabolism [54]. The ability of the gut microbiota to process non-digestible components of the diet results in the production of key metabolites for the host such as short-chain fatty acids (SCFA; butyrate, propionate

188 and acetate) from dietary fibers. These SCFA induce intestinal L-cell release of Glucagon-Like Peptide-1
189 (GLP-1) [55] and butyrate improves the maintenance of the intestinal barrier and the intestinal immune
190 system [56]. Gut bacteria also enzymatically modify host products, such as bile acids (BA), hence
191 impacting on host metabolism [57,58]. Simultaneously, certain gut microbiota can produce harmful
192 components, such as LPS by Gram-negative bacteria, or ethanol and acetaldehyde. In the healthy state,
193 low production of these compounds may contribute to the prevention of intestinal toxicity [59].
194 Quantitative or qualitative changes in the composition of the microbiota, called dysbiosis, may alter this
195 fine-tuned balance. Changes in the host's diet, administration of antibiotics, an opportunistic pathogen
196 or a pathology can disrupt this equilibrium [59].

197 Demonstration of the involvement of dysbiosis in the development of metabolic diseases has
198 prompted studies aimed at understanding these variations and to develop new strategies to treat T2D
199 and/or NAFLD through modulation of the gut microbiota. However, a "healthy or normal" microbiota is
200 under the influence of the environment, genetics and geographical location of its host [60,61]. The gut
201 microbiota of patients is therefore very heterogeneous, both at the phyla and family levels, which
202 explains the complexity of studying the signature of a specific microbiome [62–64].

203 In humans, lower microbial diversity associates positively with dyslipidemia, IR, chronic
204 inflammation, diabetes and obesity [60,61,65]. Furthermore, transplantation of fecal microbiota (FMT)
205 from NAFLD patients into germ-free mice induces hepatic steatosis and inflammation, which are
206 exacerbated by HFD-feeding [66]. The richness of only a few bacteria may vary specifically in metabolic
207 diseases. For instance, *Akkermansia muciniphila* are decreased in obese and T2D patients [67,68] and
208 high levels of *E. coli* correlate positively with hepatic fibrosis [69]. The accompanying increase in systemic
209 LPS correlates with increased hepatic nuclear factor-kappa B (NF-kB) and Toll-Like Receptor 4 (TLR4)

positive macrophages in biopsy-proven NASH patients. Circulating bacterial 16S rDNA levels correlate positively with liver damage in obese patients and measurement of its plasma concentrations has been suggested as a marker of liver fibrosis [70]. Although there is a strong assumption that these bacterial components originate from the gut, there is no clear physiological evidence that microbiota exist in blood and 16s rDNA levels may only reflect the increased intestinal permeability. Moreover, their exclusive intestinal origin is not unequivocally proven [70].

In rodents with metabolic disorders, a strong link between dysbiosis and increased intestinal permeability has been established. HFD-feeding of mice induces dysbiosis with gut vascular barrier damage and bacterial translocation to the liver. FMT from healthy mice to HFD mice restored intestinal permeability, as evidenced by the FITC-dextran assay, and metabolic disorders improved [46]. The same protection of the gut barrier is observed upon antibiotic treatment of HFD-fed mice [71]. Treatment with the TLR4 inhibitor improves the liver of mice on a NAFLD-inducing diet [72].

Other products of Gram-negative bacteria, such as endogenous ethanol, from carbohydrate fermentation, and acetaldehyde, an ethanol metabolite, stimulate the release of pro-inflammatory cytokines from enterocytes and disrupt gut tight junctions [73,74]. In rodent models of NAFLD, endogenous gut-produced ethanol increases hepatic TNF-α expression and inhibits the tricarboxylic acid cycle, resulting in TG accumulation in the liver. Ethanol may increase the activity of cytochrome P450 2E1 which produces free radicals through the its oxidation in the liver [59,75,76]. This observation challenged the name “NAFLD”, as alcohol may contribute to the disease without exogenous intake [77].

In T2D and NAFLD, other metabolites, such as SCFA, are produced and/or modified by the microbiota and have an impact on disease progression. An overall decrease in butyrate-producing bacteria is found in NAFLD and T2D [78]. In humans, low levels of butyrate are associated with decreased

mucus production and a leaky gut [65]. In the colonic *lamina propria* of mice, butyrate has been shown to induce the expression of Foxp3 in $CD4^+$ lymphocytes, inducing a T-regulatory profile [79]. *In vitro* butyrate treatment of dendritic cells reduced the expression of pro-inflammatory cytokines in response to LPS [80]. Treatment of HFD-fed mice with butyrate improved villi morphology and gut tight junction protein expression [81], which may be due to the regulation of IgA production by B cells, hence protecting the mucus layer [82]. Butyrate-treated HFD-fed mice have lower levels of hepatic TG and cholesterol associated with decreased expression of fibrosis genes (TGF-β, SMAD2, SMAD7, and α-SMA) and pro-inflammatory (MCP-1, TNF-α, IL-1, IL-2, IL-6, IFN-γ). Thus, decreased systemic butyrate appears to exacerbate NAFLD progression [81]. On the other hand, propionate and acetate increase liver TG storage and hepatic gluconeogenesis in a NAFLD mouse model [59].

Another microbial product with effects on cardio-metabolic diseases is the choline-derivative trimethylamine, which increases liver glucose intolerance in mice [83]. In the liver, TMA is oxidized to Trimethylamine N-oxide (TMAO), which has been associated to increased atherosclerosis and cardiovascular risks [83]. Moreover, diabetic patients display increased blood levels of imidazole propionate, a microbiota-derived metabolite of histidine, which induces liver IR [84]. Imidazole propionate alters insulin signaling in the liver through activation of p62 and the mechanistic target of rapamycin complex 1 (mTORC1) resulting in insulin receptor degradation, thus exacerbating IR in T2D patients [85].

Overall, the gut microbiota, through modulation of the gut barrier and through the beneficial effects of its products, plays a key role in liver health and dysbiosis may promote the progression of T2D and NAFLD.

2.4 Alterations of the entero-hepatic cycle of bile acids in NAFLD

Bile acids (BA) are amphipathic molecules acting as detergents to facilitate lipid absorption in the intestine. BA are produced in the liver from cholesterol, secreted in bile and stored in the gallbladder to be released in the intestine after meal ingestion to participate to lipid absorption. In the intestine, the microbiota generates the secondary BA after deconjugation by bacterial bile salt hydrolase, dehydroxylation and epimerization. The majority of BA (95%) are re-absorbed in the distal intestine and recycled to the liver via the portal vein, the remainder being lost in feces [86].

Several studies reported changes in BA composition under different metabolic situations, with results often differing between studies possibly due to differences between the cohorts [87]. In T2D patients, both increased [88] or unchanged [89] plasma BA concentrations have been reported. Higher total plasma BA concentrations have been found in diabetic and non-diabetic NASH patients [87], but the NASH-related increases in plasma BA levels depend on the severity of insulin resistance [90]. Differences between conjugated and unconjugated BA found in NAFLD patients may be due, at least in part, to the decreased colonization of bile salt hydrolase expressing bacteria in the intestine [78]. Interestingly, plasma BA concentrations also increase with progression of hepatic fibrosis [91].

Besides their role in lipid absorption, BA signal via several receptors, amongst which the nuclear receptor Farnesoid X Receptor (FXR) and the Takeda G-protein-coupled Receptor 5 (TGR5) which are expressed in gut and liver. Both FXR and TGR5 regulate, in an opposite manner, GLP-1 secretion by enteroendocrine L cells. TGR5 activation hence improves glucose homeostasis through the release of pancreatic β-cell insulin [86]. Treatment of western diet-fed obese mice with the TGR5 agonist RDX8940 improved IR and hepatic steatosis [92]. TGR5 activation in rodents also improved hepatic inflammation by mechanisms involving reduced NF-κB signaling [93]. Furthermore, TGR5 is expressed in various

276 immune cells, including dendritic cells, Kupffer cells and macrophages, and its activation decreases
277 cytokine production and reduces the low grade inflammation observed in metabolic diseases [86].

278 Under physiological conditios, FXR in enterocytes induces the expression of Fibroblast Growth
279 Factor 19 (FGF19) (and its rodent ortholog FGF15). The peptide secreted in the portal circulation targets
280 FGFR4/βKlotho receptor on hepatocytes to repress cholesterol 7 alpha-hydroxylase (CYP7A1) expression
281 and subsequently BA synthesis. Inhibition of BA synthesis reduces hepatic BA toxicity and protects
282 against inflammation [94]. In rodents, FGF15-deficiency increases hepatic steatosis and ER stress, which
283 is reversed by adeno-associated virus-mediated expression of FGF19 in the liver [94]. Moreover, FGF19
284 overexpression reduces plasma alanine aminotransferase (ALT) concentrations and improves NASH and
285 fibrosis in a diet-induced NASH model [95]. These mice also lose weight and display improved glucose
286 tolerance [95]. Direct FXR activation in the liver also decreases BA synthesis due to the induction of the
287 Cyp7a1 inhibitor Small Heterodimer Partner (SHP) [86]. Finally, with the FGF19 analogue aldafermin
288 improves liver histology in NASH patients (see the next chapter).

289 Thus, increasing hepatic and intestinal FXR activation, which represses BA synthesis and hepatic
290 fibrosis, is a promising therapeutic strategy to treat NAFLD, which explains the development of
291 numerous FXR agonists. In addition to its approved use in the treatment of primary biliary cholangitis
292 [96], the FXR agonist obeticholic acid (OCA) is the most advanced compound for fibrosis treatment in
293 NASH [97], whereas tropifexor and nidufexor are other FXR agonists under development [98,99].
294 Although FXR activation may be a promising strategy to treat fibrosis, the role of FXR in T2D or NAFLD
295 progression remains unclear. Prawitt and colleagues have shown that whole-body FXR knock-out mice
296 are protected against HFD-induced obesity [100]. The improved glycemic control observed in these mice
297 is due, at least in part, to a de-repression of GLP-1 secretion by gut L-cells [101]. To decipher the specific

298 role of FXR in the gut, several studies used intestine-specific FXR-deficient mice. Similarly to what is
299 observed in whole-body FXR knock-out mice, intestinal FXR knock-out mice are protected against HFD-
300 induced obesity [102]. These mice show lower HFD-induced hepatic SREBP1C-dependent steatosis due
301 to a decrease in intestinal ceramide synthesis [18,103]. However, whether these mice are also protected
302 from NASH and fibrosis is unknown at present. All these data highlight the complex role of intestinal FXR
303 in NAFLD progression, also illustrated by the fact that whole-body FXR knock-out mice present gut barrier
304 defects when fed a normal chow diet [104] and hepatic steatosis when fed a cholesterol-supplemented
305 diet [105].
306
307 **2.5 Gut immune system disruption increases liver inflammation**
308 The intestinal immune system, which is an integral part of the gut barrier, contributes to shape
309 gut microbiota tolerance and to maintain gut integrity. Found in intestinal crypts, Paneth cells are
310 specialized cells that secrete antimicrobial peptides which modulate the gut microbiome and the local
311 inflammatory response and contribute to the protection of the organism [106]. Moreover, secondary
312 lymphoid organs, the Peyer's patches, are structures localized in the small intestine that contribute to
313 immune system education and tolerance towards food-derived antigens. Specialized M epithelial cells
314 allow antigen transcytosis from the intestinal lumen to the subepithelial dome of Peyer's patches , where
315 they induce, via dendritic cell-mediated antigen presentation, the production of immunoglobulin IgA and
316 IgM by plasmocytes [107,108]. IgAs are dimeric immunoglobulins secreted in the mucus layer to protect
317 the mucosa against pathogens [109]. Lymphoid cells are also found outside of Peyer's patches in the
318 intestinal tissue. Indeed, intra-epithelial lymphocytes localized between the enterocytes are resident
319 cells contributing to the intestinal barrier [110], and regulatory lymphocytes promoting immune

tolerance [111] as well as resident self-maintaining macrophages which control gut homeostasis [112] are also found in the *lamina propria*. Finally, other immune cells such as innate lymphoid cells and mucosal-associated invariant T cells are found in the intestine and participate in the mucosal defense against pathogens and in the regulation of tissue inflammation [113,114].

The intestinal immune system equilibrium, however, is challenged in obesity and T2D leading to an increased low grade chronic inflammation, dysbiosis and gut permeability [115]. In rodents, HFD feeding modifies the gut immune system through changes in intestinal Pattern Recognition Receptor (PRR) expression, cytokine production and immune cell populations [116]. Moreover, intestinal IgA^+ B cells are important for the maintenance of glucose tolerance: loss of IgA production results in an increased intestinal pro-inflammatory T cell response in HFD-fed mice [109]. HFD feeding also enhances the pool of $F4/80^+CD11b^+CD11c^+$ pro-inflammatory macrophages in the colonic *lamina propria* [117]. Experiments in macrophage $CCR2^-$ and epithelial CCL2-deficient mice showed that colonic macrophage infiltration increases IR independently of body weight gain [117].

Obese patients display an increased infiltration of $CD68^+$ macrophages in the jejunal epithelium, which correlates with decreased enterocyte insulin signaling [118]. Obese patients also show jejunal mucosa alterations in $CD8^+$ T cell subtypes, with a decrease of $CD8\alpha\alpha^+$ and an increase of $CD8\alpha\beta^+$ T cells, resulting in enhanced pro-inflammatory cytokine secretion [118]. In NAFLD patients, diagnosed using ultrasonography and elevated transaminases, an increased immune cell infiltration in the colonic mucosa has been observed [51]. The epithelial density of $CD8^+$ T cells also correlated positively with the NAS score, although NAS scores were low excluding the presence of overt NASH [118]. Furthermore, bariatric surgery-induced weight loss and improved metabolism are accompanied by increased fecal IgA levels [109].

342 Whether intestinal immune system dysregulation is a cause or a consequence of gut barrier
343 disruption remains unclear [116]. As gut immune population homeostasis impacts on IR, targeting
344 immune cell populations in the intestinal tissue may be a promising strategy for both T2D and NAFLD.
345
346 ## 3 Targeting the intestine to treat T2D and NAFLD
347 As first-line treatment of T2D, weight-reducing strategies, essentially by lifestyle interventions,
348 also efficiently cure NAFLD. A reduction of calorie intake together with increased physical activity
349 improves NAFL, as assessed by MRI liver fat and plasma ALT level analysis [119], as well as later stage
350 biopsy-confirmed NASH and fibrosis [120].
351 Here, we discuss therapeutic approaching targeting the intestine and the gut-liver axis, already
352 used in the clinic or in a late stage clinical development (Table 1). It should be noted that these studies
353 are difficult to compare due to the heterogeneity of patients and diagnostic methods used for NASH
354 patient stratification.
355
356 ### 3.1 Bariatric surgery
357 Bariatric surgery is a therapeutic option for obese patients with BMI>40, or >35 when co-
358 morbidities are present, such as T2D or NASH. These surgical interventions, beyond their effect on
359 weight, cure not only T2D [121], but also improve NASH [122]. Indeed, bariatric surgery induces
360 histological reversion of NASH in 85% of patients one year after the intervention [123], an effect which
361 is sustained 5 years after surgery [124]. Similar results were obtained in patients with non-invasively
362 diagnosed for NASH, combining elastography and MRI [11]. In addition, results from a retrospective
363 study revealed that patients are less likely to progress to cirrhosis after bariatric surgery [125].

Nevertheless, the type of surgical procedure seems to have an impact, since sleeve gastrectomy is less efficient than Roux-en-Y gastric bypass in reducing the Fatty Liver Index and BARD non-invasive scores of NAFL and fibrosis [126].

3.2 FGF19 analogs

As discussed above, several preclinical studies identified beneficial effects of the intestinal FGF19 pathway on liver health [94,95]. Therefore, aldafermin (NGM282), a recombinant non-tumorigenic modified FGF19 peptide, was developed. Twelve weeks of treatment with aldafermin reduced liver fat and ALT levels in biopsy-proven NASH patients, without improving IR [127]. Aldafermin treatment reduced the NAS score, and plasma markers of collagen in a dose-escalating phase 2 study, suggesting an improvement of hepatic fibrosis [127,128]. In addition to the reduction of liver fat, 24 week treatment with aldafermin (1 mg) resulted in histological improvement of NASH, i.e. lower steatosis, inflammation, ballooning and fibrosis scores without weight loss or effects on IR [129]. Unfortunately, the ALPINE 2/3 study did not meet its primary endpoint, liver fibrosis improvement, and, even though the secondary endpoint, *ie* NASH resolution without fibrosis worsening, was achieved, the clinical development of aldafermin for NASH treatment was stopped [130].

3.3 FXR agonists

Rather than injecting exogenous recombinant FGF19, endogenous enterocyte FGF19 production is induced by FXR agonists, such as OCA, hence increasing peripheral blood FGF19 levels in T2D and NAFLD patients [131]. This synthetic BA is presently the most clinically advanced FXR-targeting therapy.

In NASH patients, long-term OCA treatment improves hepatic histological markers of NASH [97,132]. However, glucose control and IR may deteriorate after OCA treatment and plasma LDL-cholesterol levels increased requiring statin treatment [97].

Other therapies targeting FXR with (semi-)synthetic compounds show interesting results in preclinical models and, at present, in a few clinical studies. INT-767, a modified BA dual FXR/TGR5 agonist with intestinal tropism, reduces histological features of NASH and lowers fibrosis [133], while promoting brown adipogenesis in rodents [134]. Other synthetic FXR agonists are tropifexor, which reduces plasma TG and induces intestinal FGF15 production in rats [99], and nidufexor, which improves hepatic steatosis and NASH in murine models of T2D and NASH murine model. These compounds are now under evaluation in phase 2 clinical trials for NASH treatment [98,135].

Finally, the intestine-restricted FXR agonist fexaramine[136] improves insulin sensitivity in mice through a microbiota-dependent increase of plasma GLP-1 concentrations [137], but its potential effect on NASH has not yet been reported.

3.4 Targeting the incretin pathway

Complementary to physical exercise and dietary intervention, activation of the GLP-1R either by subcutaneous injection of semi-synthetic recombinant GLP-1R agonists or *per os* treatment with a gliptin, which inhibits the GLP-1-degrading dipeptidyl peptidase-4 (DPP-4) hence increasing endogenous active GLP-1 concentrations, are largely used therapeutic options for the treatment of obesity and T2D. As these metabolic disorders are linked to NAFLD, they are also being tested for this indication in more recent clinical trials.

DPP-4 inhibitor monotherapy with sitagliptin did not improve hepatic steatosis in a two-year clinical trial [138]. In combination with metformin, sitagliptin was shown to decrease the hepatic fat fraction as assessed by MRI, without improving fibrosis in T2D NAFLD patients [139]. Therefore, the efficacy of DPP-4 inhibitors, if any, appears limited to steatosis, without effects on more advanced stages of NASH and fibrosis.

GLP-1R agonist treatment reduces body weight and improves glucose homeostasis. Therefore, they are interesting candidates for the management of NAFLD. The LEAN trial was the first study to show efficacy of liraglutide to lower NASH in biopsy-proven NASH patients [140]. More recent studies only show results in patients with less advanced hepatic disease [139,141–143]. In monotherapy, liraglutide was found as efficient as lifestyle interventions to improve NAFL, fasting glycemia and IR assessed by the homeostatic model assessment of insulin resistance (HOMA-IR) [141]. In T2D and NAFLD patients, daily liraglutide and weekly dulaglitide treatment also improved liver steatosis [139,142–144]. Moreover, weekly injections of semaglutide for 72 weeks reversed NASH and reduced ALT levels in NASH patients [145]. Although it is unclear at present whether fibrosis improves upon GLP-1R agonist treatment, this compound is of high interest given the fact that it can also be administered orally.

Finally, cotadutide, a dual GLP-1R/glucagon receptor agonist, which mimics the effects of oxyntomodulin, an intestinal peptide dual glucagon receptor and GLP-1R activator, reduced body weight and improved liver histology in mice [146].

3.5 Targeting microbiota

As discussed above, the gut microbiota plays an important role in health and metabolic diseases, as demonstrated by the fecal microbiota transplantation (FMT) in germ free mice [66]. Lean donor FMT to patients with metabolic syndrome improved peripheral insulin sensitivity [147]. However, FMT with

a healthy donor graft did not improve the hepatic fat fraction nor IR in NAFLD patients [148]. Nevertheless, improved intestinal barrier function was observed, as assessed by the lactulose/mannitol urinary test [148]. Except for the treatment of intestinal *C. difficile* infection, application of FMT is limited due to problems of patient acceptance and lack of definition of an healthy graft [149].

Since germ-free mice were protected against diet-induced weight gain and hyperglycemia, the use of antibiotics to modulate the microbiota may also be considered. However, antibiotic treatment increased TG levels [150] and penicillin treatment worsened IR and NAFLD in rodent models of HFD-induced obesity [151]. Such approach has only been tested in a limited manner in humans. Rifaximin, a broad spectrum antibiotic, lowered ALT levels, endotoxemia and HOMA-IR in histologically-diagnosed obese NASH patients [152]. Treatment combining metrodinazole, an antiprotozoal antibiotic, and the prebiotic inulin decreased transaminase levels in prediabetic patients, an effect not observed with inulin alone [153]. The lack of bacterial species-selective antibiotics and the apparition of antibiotic resistance strongly limit the use of antibiotics for metabolic disorders, which should be restricted to infections.

Rather than replacing or destroying the commensal bacterial ecosystem, gut microbiota can be more subtly modulated in different ways. Prebiotics are substrates selectively used by host micro-organisms which confer a health benefit by modulating bacterial metabolite production, *eg* from non-digestible carbohydrates that are transformed into SCFA [154]. Inulin treatment for 14 weeks decreased HFD-induced hepatic injury in mice, as illustrated by decreased ALT levels, lower numbers of hepatic macrophages, improved hepatic steatosis and improved glucose tolerance in HFD-fed mice [155]. Prebiotic treatment of non-diabetic patients lowered transaminases serum levels, serum TG and fasting glycemia [156]. As microbial selection by prebiotics is difficult to control and depends on dietary habits or geographic origin, exogenous intake of non-pathogenic bacteria can be an option. Probiotics are live

452 microorganisms which, when administered in proper doses, confer a health benefit to the host [157]. In
453 HFD-fed mice, *Lactobacillus acidophilus* NS1 supplementation improved hepatic steatosis and reduced
454 fatty acid synthesis [158]. Administration of *Akkermansia muciniphila* modestly improved hepatic
455 histology and reduced hepatic IL-6 expression [68]. Rather than using a single bacteria species, some
456 studies evaluated the efficiency of probiotic mixtures on NAFLD. A mixture of 6 probiotic agents lowered
457 hepatic fat content in 12 weeks-treated NAFL patients, without any effect on IR [159]. VSL#3, another
458 probiotic mixture, reduced hepatic steatosis and increased GLP-1 levels, without reducing ALT levels nor
459 IR in obese NAFLD children [160].

460 Finally, pre- and probiotics can be combined into symbiotics. Similar as in HFD-fed rodents [161],
461 an 8 week treatment with a symbiotic containing 7 probiotics and fructo-oligosaccharides improved
462 hepatic steatosis without decreasing ALT in NAFLD patients [162]. Twenty-eight weeks of treatment with
463 the same symbiotic improved insulin sensitivity, reduced ALT levels and improved fibrosis in Fibroscan
464 diagnosed NAFL patients [163]. However, a recent study reported that combination of *Bifidobacterium*
465 *animalis subspecies lactis* BB-12 and fructooligosaccharides for 10-14 months failed to reduce hepatic
466 fat content in NAFLD patients, whereas the degree of weight loss was the only variable correlated to
467 steatosis [164].

468 Overall, the benefits of microbiota-based approaches on NAFLD and T2D appear modest. The use
469 of these medications seems limited to the reduction of hepatic steatosis and cytolysis, with sometimes
470 benefits on IR. They thus appear indicated in the prevention and treatment of early, less severe forms of
471 NAFLD.

472

473 **4 Conclusion**

The metabolic consequences of an altered liver-intestine dialogue reveal its central role in maintenance of energy homeostasis. As T2D and NAFLD impact both organs, targeting the intestine and its functions seems to be a promising therapeutic strategy in the management of obesity, T2D and NAFLD. Further research on the intestine-liver connection is therefore warranted.

Disclosure of Interest

The authors report no conflict of interest

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Author contributions

Margaux Nawrot and Simon Peschard wrote the manuscript and made figures. Sophie Lestavel and Bart Staels revised the article critically for intellectual content and gave final approval of the submitted version.

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Figure 1: The cross-talk between liver and metabolic organs is altered in T2D and NAFLD. Common dysregulations observed in T2D and NAFLD highlighting the central role of the liver and its dialogue with metabolic organs.

(Abbreviation: FFA: Free Fatty Acid)

Figure 2: Gut dysfunctions alter liver health in T2D and/or NAFLD. Left panel shows the multiple pathways of intestine-liver cross-talk in the healthy state. Enterocytes mediate the post-prandial absorption of nutrients, such as fats, regulating TG storage and chylomicron synthesis. The mucus layer and TJ complexes protect against bacterial translocation and endotoxemia. SCFA produced by the intestinal microbiota improve the gut barrier through mucin and IgA production, by goblet cells and LB respectively. Conjugated and deconjugated BA via binding to TGR5 receptor enhance GLP-1 release to maintain glucose homeostasis, and to FXR increase FGF15/19 synthesis.

Right panel shows the dysregulations of these pathways in T2D and/or NAFLD in relation to excess calorie intake and their impact on the liver. Increased FA absorption promotes gut and liver inflammation, and liver steatosis. The gut barrier disruption is illustrated by a thinner mucus layer and a decrease of TJ, which is exacerbated by microbiota alterations. Dysbiosis, associated with increased LPS and ethanol levels, exacerbates gut inflammation, bacteria translocation in the portal circulation and endotoxemia increasing liver steatosis, inflammation and fibrosis. Moreover, the change of SCFA producing bacteria worsens gut permeability by decreasing mucin and IgA levels, promoting liver steatosis and IR. Simultaneously, alterations in intestinal BA metabolism, such as decreased BA deconjugation, in these pathological contexts may lead to reduced levels of GLP-1 and FGF15/19, hence impacting on liver injuries such as steatosis and fibrosis.

(Abbreviations: FA: Fatty Acid; LPS: lipopolysaccharide; EtOH: Ethanol; LT: T lymphocyte; LB: B Lymphocyte; DC: dendritic cell; BA: Bile Acids; SCFA: short chain fatty acid; TGR5: takeda g-protein-coupled receptor 5; FXR: farnesoid X receptor; GLP-1: Glucagon-like peptide-1; FGF15/19: Fibroblast growth factor 15 or 19; TG: triglycerides; TJ: Tight Junctions; IgA: Immunoglobulin A; ZO-1: Zonula Occludens 1; JAM-A: junctional adhesion molecule A; BSH: Bile Salt Hydrolase; ER stress: Endoplasmic Reticulum Stress; IR: Insulin resistance; T2D: Type 2 Diabetes; NAFLD: Non-Alcoholic Fatty Liver diseases)

Table 1: Clinical trials on NAFLD treatments targeting gut functions.

Abbreviations: ALT : alanine aminotransferase; AST : aspartate aminotransferase; BMI : body mass index, GGT : gamma-glutamyl transferase; MRI : magnetic resonance imaging; NAFLD : non-alcoholic fatty liver disease; NAS : NAFLD activity score; NASH : non-alcoholic steatohepatitis; OCA : obeticholic acid; OGTT : oral glucose tolerance test; T2D : type 2 diabetes; TG : triglyceride

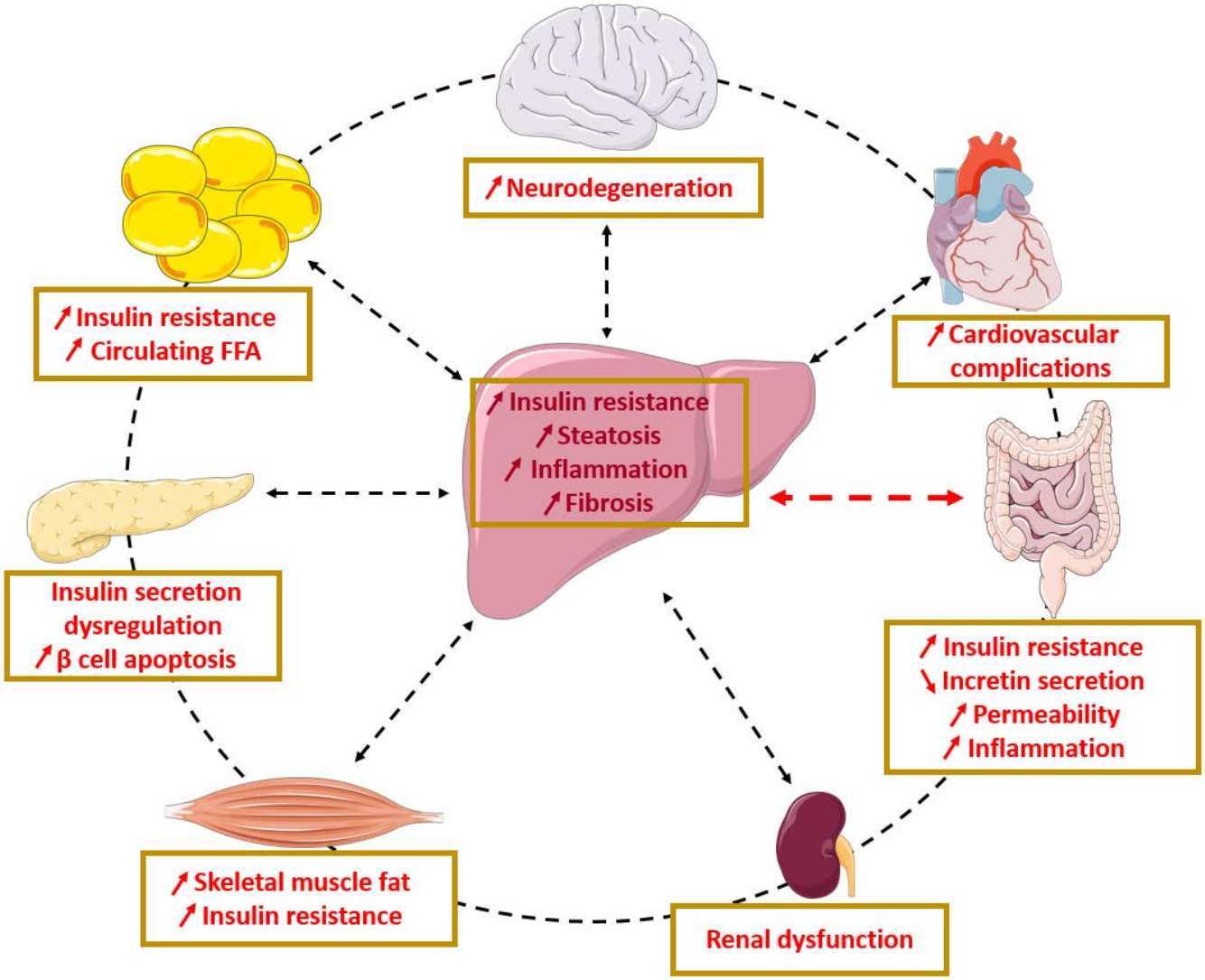
↗Neurodegeneration
↗Insulin resistance
↗Circulating FFA
↗Cardiovascular complications
↗Insulin resistance
↗Steatosis
↗Inflammation
↗Fibrosis
Insulin secretion dysregulation
↗β cell apoptosis
↗Insulin resistance
↘Incretin secretion
↗Permeability
↗Inflammation
↗Skeletal muscle fat
↗Insulin resistance
Renal dysfunction

Figure 1

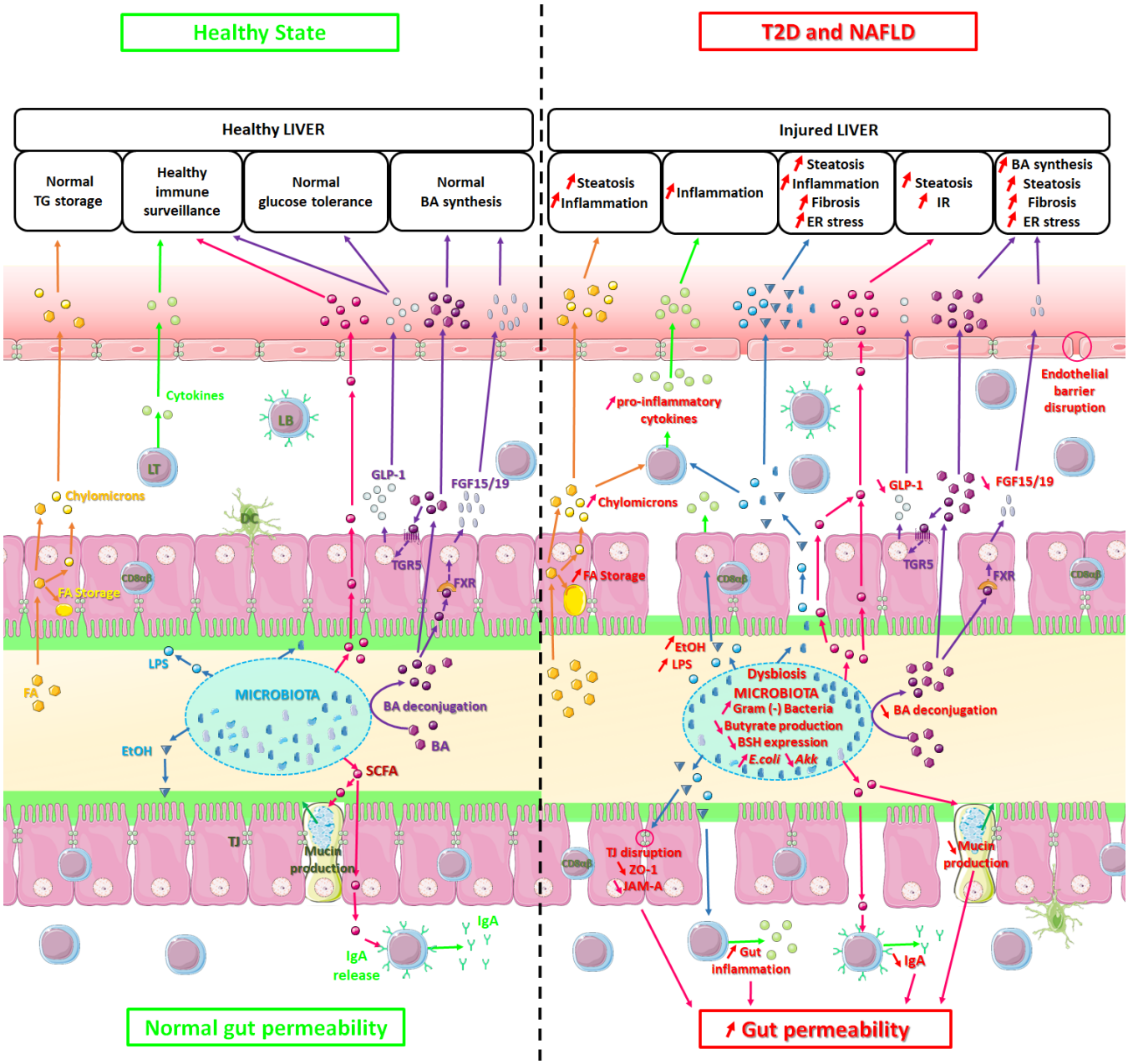
Healthy State
T2D and NAFLD
Healthy LIVER
Injured LIVER
Normal TG storage
Healthy immune surveillance
Normal glucose tolerance
Normal BA synthesis
Steatosis
Inflammation
Inflammation
Steatosis
Inflammation
Fibrosis
ER stress
Steatosis
IR
BA synthesis
Steatosis
Fibrosis
ER stress
Cytokines
LB
LT
Chylomicrons
DC
GLP-1
FGF15/19
TGR5
FXR
FA Storage
CD8αβ
LPS
FA
MICROBIOTA
BA deconjugation
BA
EtOH
SCFA
TJ
Mucin production
IgA
IgA release
pro-inflammatory cytokines
Endothelial barrier disruption
EtOH
LPS
Dysbiosis
MICROBIOTA
Gram (-) Bacteria
Butyrate production
BSH expression
E.coli
Akk
TJ disruption
ZO-1
JAM-A
Gut inflammation
Normal gut permeability
Gut permeability

Figure 2

Treatment	n NAFLD patients	NAFLD type	Diagnostic	n T2D patients	Treatment effects on NAFLD	Treatment effects on T2D/IR	Study
Bariatric surgery							
	109	NASH	Liver biopsy	69	NASH reversion at 1 year post-surgery in 85% of patients	Reduction of fasting glycemia and insulin resistance from baseline	[123]
	64	NASH	Liver biopsy	?	NASH reversion at 5 year post surgery in 85% of patients	Reduction of fasting glycemia, HbA1c, Insulin resistance index from baseline	[124]
	2942	NAFL	?	987	Reduced risk to develop hepatic cirrhosis if patients underwent bariatric surgery		[125]
	1955	NAFL	FLI score : TG, BMI, GGT and waist circumference BARD score : BMI, AST/ALT, diabetes	522	Reduction of both FLI and BRAD score 1 year after surgery		[126]
FGF19 analogs							
Aldafermin (NGM282)							
	27 (3 mg) 28 (6 mg)	NASH	Liver biopsy MRI ALT	15 (3 mg) 17 (6 mg)	Decrease of hepatic fat content > 30% in 85% of patients, independently of dose Decrease of circulating fibrosis markers	No impact on fasting glycemia or insulin resistance	[127]
	24 (1 mg) 19 (3 mg)	NASH	Liver biopsy MRI ALT	9 (1 mg) 7 (3 mg)	Dose dependent improvement of NASH and fibrosis		[128]
	53 (1 mg)	NASH	Liver biopsy MRI ALT	32	Reduction of hepatic fat content, NAS score, fibrosis and ALT	No impact on fasting glycemia, HbA1c or HOMA-IR	[129]
FXR agonists							
Obeticholic Acid (OCA)							
	20 (25 mg) 21 (50 mg)	NAFL	Ultrasonography (17 + 18) ALT (3 + 5) Liver biopsy (0 + 2)	20 (25 mg) 21 (50 mg)	Increase plasma FGF19 Reduction of circulating fibrosis markers	Improve insulin sensitivity	[131]
	141	NASH	Liver biopsy	75	Liver histology improvement Decrease of weight loss, ALT, GGT	Increase of insulin resistance and fasting insulimemia	[165]
	312 (10 mg) 308 (25 mg)	NASH	Liver biopsy	171 (10 mg) 171 (25 mg)	Dose dependent fibrosis reduction Reduction of NAS score for the 25 mg dose	In T2D patients, early increase of glycemia and HbA1c, which return to placebo level by month 6	[132]

Targeting Incretin pathway							
Sitagliptin							
	25	NAFL	MRI + ALT	12	Not better than placebo to improve liver fat content	No effect on glycemia, insulinemia, HbA1c, IR	[138]
+ Metformin	27	NAFL	MRI	27	Reduction of hepatic fat fraction	Reduction of HbA1c	[139]
Liraglutide							
	23	NASH	Liver biopsy	9	NASH resolution Reduction of NAS score components	Reduction of HbA1c and fasting glycemia	[140]
	14	NAFL	MRI + ALT	?	Reduction of liver fat fraction from baseline Decrease of AST and ALT	Reduction of Insulin resistance, glycemia and insulinemia from baseline	[141]
	31	NAFL	Steatosis on liver biopsy or imaging technique	31	Reduction of intrahepatic content of lipids Decrease of AST and ALT from baseline	Reduction of insulin resistance	[142]
	29	NAFL	Ultrasonography	29	Reduction of hepatic fat Decrease of AST and ALT	Improvement in fasting glycemia, HbA1c and glucose tolerance after OGTT	[143]
+ Metformin	24	NAFL	MRI	27	Reduction of hepatic fat fraction	Reduction of HbA1c, fasting glycemia and post prandial plasma glucose	[139]
Semaglutide							
	80 (0.1 mg) 78 (0.2 mg) 82 (0.4 mg)	NASH	Liver biopsy	49 (0.1 mg) 51 (0.2 mg) 49 (0.4 mg)	NASH Resolution Reduction of ALT and AST		[145]
Targeting microbiota							
Allogenic FMT							
	15	NAFL	Liver biopsy + FibroScan + ultrasonography	?	No effect	No effect	[148]
Antibiotics							
Rifaximin	25	NASH	Liver biopsy + elevated ALT level	11	Reduction at 3 and 6 months of ALT, NAFLD-liver fat score, endotoxemia, IL6 Decrease at 6 months of AST Increase at 3 and 6 month of IL10	Reduction at 3 and 6 months of insulin resistance Reduction at 6 month of glycemia and insulinemia	[152]
Metrodinazole + inulin	20	?	Liver biopsy or BMI>27 + T2D or metabolic	7	Reduction of ALT	No effect	[153]

			syndrome with elevated ALT				
Prebiotics							
Inulin	29	NAFL	Ultrasonography + ALT	0	Reduction of ALT, AST and TG	Reduction of fasting glycemia	[156]
Probiotics							
Lactobacillus acidophilus, Lactobacillusrhamnosus, Lactobacillus paracasei, Pediococcus pentosaceus, Bifidobacterium lactis* & *Bifidobacterium breve	30	NAFL	MRI	?	Reduction of intrahepatic fat Reduction of TG	No changes in fasting glycemia, HOMA-IR or insulinemia	[159]
VSL#3	22 children	NASH	Liver biopsy + ALT	?	No effect on ALT Improvement of fatty liver	No effect on insulin resistance or fasting glycemia Increase of GLP-1	[160]
Symbiotics							
***Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus bulgaricus, Bifidobacterium longum, Bifidobacterium breve* & *Streptococcus thermophilus* + fructooligosaccharides**	38	NAFL	Ultrasonography	?	No effect on ALT Improvement of steatosis		[162]
	26	NAFL	Ultrasonography + elevated ALT	?	Decrease of ALT Improvement of fibrosis score	Reduction of insulin resistance, fasting glycemia and insulinemia	[163]
Bifidobacterium animalis sub lactis BB12 + **fructooligosaccharides**	45	NAFL	MRI	17	No improvement in fatty liver Reduction of NAFLD fibrosis score vs baseline, but not vs placebo		[164]

ALT : alanine aminotransferase, AST : aspartate aminotransferase, BMI : body mass index, GGT : gamma-glutamyl transferase, MRI : magnetic resonance imaging, NAFLD : non-alcoholic fatty liver disease, NAS : NAFLD activity score, NASH : non-alcoholic steatohepatitis, OCA : obeticholic acid, OGTT : oral glucose tolerance test, T2D : type 2 diabetes, TG : triglycerides

Table 1