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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.

30 **Abbreviations:**

31 ALT: alanine aminotransferase

32 BA: bile acids

33 CYP7A1: cholesterol 7 alpha-hydroxylase

34 DDP-4: dipeptidyl peptidase-4

35 FGF: fibroblast growth factor

36 FMT: fecal microbiota transplantation

37 FXR: farnesoid X receptor

38 GLP-1: glucagon-like peptide-1

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

40 HFD: high fat diet

41 HOMA-IR: homeostatic model assessment of insulin resistance

42 IgA: immunoglobulin A

43 IR: insulin resistance

44 JAM-A: junctional adhesion molecule A

45 LPS: lipopolysaccharide

46 MRI: magnetic resonance imaging

47 NAFL /NAFLD: non-alcoholic fatty liver/disease

48 NAS: non-alcoholic fatty liver disease activity score

49 NASH: non-alcoholic steatohepatitis

50 OCA: obeticholic acid

51 SCFA: short chain fatty acid

52 T2D: type 2 diabetes

53 TG: triglycerides

54 TGR5: takeda g-protein-coupled receptor 5

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

56 Diabetes is the most common metabolic disease worldwide. With the western lifestyle pandemic,

57 this multifactorial disease has a worldwide prevalence estimated at 500 million patients [1]. The majority

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

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

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

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

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

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

110

111 **2. <u>The role of gut-liver cross-talk disruption in T2D and NAFLD</u>**

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

119

120

121 **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

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

170 Feeding mice a high fat diet (HFD) increases hepatic steatosis and IR. These mice show body and
171 liver weight increases, fasting hyperglycemia glucose intolerance and hyperinsulinemia [53]. Intestinal
172 JAM-A deficient mice developed severe liver damage, illustrated by elevated plasma transaminase levels,
173 when fed a NAFLD-inducing high fructose-high sucrose-high cholesterol diet. These mice also showed a
174 rise of systemic LPS exposure, associated with exacerbated hepatic stellate cell activation, fibrosis and
175 inflammation due to hepatic immune cell recruitment [51]. Moreover, HFD-feeding rapidly induced gut
176 vascular barrier damage related to decreased Wnt/b-catenin signaling in endothelial cells [46].
177 Gut barrier integrity thus likely plays an important role in the development of metabolic diseases such
178 as T2D and NAFLD. Although the chronology of events is still unclear, gut microbiota-derived
179 endotoxemia may contribute to the altered gut liver cross-talk.

180

181 **2.3 Gut microbiota dysbiosis increases gut permeability and liver damage**

182 The healthy human intestinal microbiota is composed of a large diversity of microorganisms,
183 mainly anaerobic bacteria [54]. In a healthy state, the microbiota have symbiotic interactions with its
184 host. It participates in nutrient absorption, assists in intestinal barrier function protection to prevent
185 pathogen invasion, improves the function of the intestinal immune system and modulates metabolism
186 [54]. The ability of the gut microbiota to process non-digestible components of the diet results in the
187 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)

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

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

222 Other products of Gram-negative bacteria, such as endogenous ethanol, from carbohydrate
223 fermentation, and acetaldehyde, an ethanol metabolite, stimulate the release of pro-inflammatory
224 cytokines from enterocytes and disrupt gut tight junctions [73,74]. In rodent models of NAFLD,
225 endogenous gut-produced ethanol increases hepatic TNF-α expression and inhibits the tricarboxylic acid
226 cycle, resulting in TG accumulation in the liver. Ethanol may increase the activity of cytochrome P450
227 2E1 which produces free radicals through the its oxidation in the liver [59,75,76]. This observation
228 challenged the name “NAFLD”, as alcohol may contribute to the disease without exogenous intake [77].

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

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

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

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

253

254 ### 2.4 Alterations of the entero-hepatic cycle of bile acids in NAFLD

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

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

269 Besides their role in lipid absorption, BA signal via several receptors, amongst which the nuclear
270 receptor Farnesoid X Receptor (FXR) and the Takeda G-protein-coupled Receptor 5 (TGR5) which are
271 expressed in gut and liver. Both FXR and TGR5 regulate, in an opposite manner, GLP-1 secretion by
272 enteroendocrine L cells. TGR5 activation hence improves glucose homeostasis through the release of
273 pancreatic β-cell insulin [86]. Treatment of western diet-fed obese mice with the TGR5 agonist RDX8940
274 improved IR and hepatic steatosis [92]. TGR5 activation in rodents also improved hepatic inflammation
275 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

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

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

333 Obese patients display an increased infiltration of $CD68^+$ macrophages in the jejunal epithelium,
334 which correlates with decreased enterocyte insulin signaling [118]. Obese patients also show jejunal
335 mucosa alterations in $CD8^+$ T cell subtypes, with a decrease of $CD8\alpha\alpha^+$ and an increase of $CD8\alpha\beta^+$ T cells,
336 resulting in enhanced pro-inflammatory cytokine secretion [118]. In NAFLD patients, diagnosed using
337 ultrasonography and elevated transaminases, an increased immune cell infiltration in the colonic
338 mucosa has been observed [51]. The epithelial density of $CD8^+$ T cells also correlated positively with the
339 NAS score, although NAS scores were low excluding the presence of overt NASH [118]. Furthermore,
340 bariatric surgery-induced weight loss and improved metabolism are accompanied by increased fecal IgA
341 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].

364 Nevertheless, the type of surgical procedure seems to have an impact, since sleeve gastrectomy is less
365 efficient than Roux-en-Y gastric bypass in reducing the Fatty Liver Index and BARD non-invasive scores
366 of NAFL and fibrosis [126].
367
368 **3.2 FGF19 analogs**
369 As discussed above, several preclinical studies identified beneficial effects of the intestinal FGF19
370 pathway on liver health [94,95]. Therefore, aldafermin (NGM282), a recombinant non-tumorigenic
371 modified FGF19 peptide, was developed. Twelve weeks of treatment with aldafermin reduced liver fat
372 and ALT levels in biopsy-proven NASH patients, without improving IR [127]. Aldafermin treatment
373 reduced the NAS score, and plasma markers of collagen in a dose-escalating phase 2 study, suggesting
374 an improvement of hepatic fibrosis [127,128]. In addition to the reduction of liver fat, 24 week treatment
375 with aldafermin (1 mg) resulted in histological improvement of NASH, i.e. lower steatosis, inflammation,
376 ballooning and fibrosis scores without weight loss or effects on IR [129]. Unfortunately, the ALPINE 2/3
377 study did not meet its primary endpoint, liver fibrosis improvement, and, even though the secondary
378 endpoint, *ie* NASH resolution without fibrosis worsening, was achieved, the clinical development of
379 aldafermin for NASH treatment was stopped [130].
380
381
382 **3.3 FXR agonists**
383 Rather than injecting exogenous recombinant FGF19, endogenous enterocyte FGF19 production
384 is induced by FXR agonists, such as OCA, hence increasing peripheral blood FGF19 levels in T2D and
385 NAFLD patients [131]. This synthetic BA is presently the most clinically advanced FXR-targeting therapy.

386 In NASH patients, long-term OCA treatment improves hepatic histological markers of NASH [97,132].
387 However, glucose control and IR may deteriorate after OCA treatment and plasma LDL-cholesterol levels
388 increased requiring statin treatment [97].
389
390 Other therapies targeting FXR with (semi-)synthetic compounds show interesting results in
391 preclinical models and, at present, in a few clinical studies. INT-767, a modified BA dual FXR/TGR5 agonist
392 with intestinal tropism, reduces histological features of NASH and lowers fibrosis [133], while promoting
393 brown adipogenesis in rodents [134]. Other synthetic FXR agonists are tropifexor, which reduces plasma
394 TG and induces intestinal FGF15 production in rats [99], and nidufexor, which improves hepatic steatosis
395 and NASH in murine models of T2D and NASH murine model. These compounds are now under
396 evaluation in phase 2 clinical trials for NASH treatment [98,135].
397 Finally, the intestine-restricted FXR agonist fexaramine[136] improves insulin sensitivity in mice
398 through a microbiota-dependent increase of plasma GLP-1 concentrations [137], but its potential effect
399 on NASH has not yet been reported.
400
401 **3.4 Targeting the incretin pathway**
402 Complementary to physical exercise and dietary intervention, activation of the GLP-1R either by
403 subcutaneous injection of semi-synthetic recombinant GLP-1R agonists or *per os* treatment with a
404 gliptin, which inhibits the GLP-1-degrading dipeptidyl peptidase-4 (DPP-4) hence increasing endogenous
405 active GLP-1 concentrations, are largely used therapeutic options for the treatment of obesity and T2D.
406 As these metabolic disorders are linked to NAFLD, they are also being tested for this indication in more
407 recent clinical trials.

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

413 GLP-1R agonist treatment reduces body weight and improves glucose homeostasis. Therefore,
414 they are interesting candidates for the management of NAFLD. The LEAN trial was the first study to show
415 efficacy of liraglutide to lower NASH in biopsy-proven NASH patients [140]. More recent studies only
416 show results in patients with less advanced hepatic disease [139,141–143]. In monotherapy, liraglutide
417 was found as efficient as lifestyle interventions to improve NAFL, fasting glycemia and IR assessed by the
418 homeostatic model assessment of insulin resistance (HOMA-IR) [141]. In T2D and NAFLD patients, daily
419 liraglutide and weekly dulaglitide treatment also improved liver steatosis [139,142–144]. Moreover,
420 weekly injections of semaglutide for 72 weeks reversed NASH and reduced ALT levels in NASH patients
421 [145]. Although it is unclear at present whether fibrosis improves upon GLP-1R agonist treatment, this
422 compound is of high interest given the fact that it can also be administered orally.

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

426 **3.5 Targeting microbiota**

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

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

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

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

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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929

930 **Figure 1: The cross-talk between liver and metabolic organs is altered in T2D and NAFLD.** Common
931 dysregulations observed in T2D and NAFLD highlighting the central role of the liver and its dialogue with
932 metabolic organs.
933 (Abbreviation: FFA: Free Fatty Acid)
934
935 **Figure 2: Gut dysfunctions alter liver health in T2D and/or NAFLD.** Left panel shows the multiple
936 pathways of intestine-liver cross-talk in the healthy state. Enterocytes mediate the post-prandial
937 absorption of nutrients, such as fats, regulating TG storage and chylomicron synthesis. The mucus layer
938 and TJ complexes protect against bacterial translocation and endotoxemia. SCFA produced by the
939 intestinal microbiota improve the gut barrier through mucin and IgA production, by goblet cells and LB
940 respectively. Conjugated and deconjugated BA via binding to TGR5 receptor enhance GLP-1 release to
941 maintain glucose homeostasis, and to FXR increase FGF15/19 synthesis.
942 Right panel shows the dysregulations of these pathways in T2D and/or NAFLD in relation to excess calorie
943 intake and their impact on the liver. Increased FA absorption promotes gut and liver inflammation, and
944 liver steatosis. The gut barrier disruption is illustrated by a thinner mucus layer and a decrease of TJ,
945 which is exacerbated by microbiota alterations. Dysbiosis, associated with increased LPS and ethanol
946 levels, exacerbates gut inflammation, bacteria translocation in the portal circulation and endotoxemia
947 increasing liver steatosis, inflammation and fibrosis. Moreover, the change of SCFA producing bacteria
948 worsens gut permeability by decreasing mucin and IgA levels, promoting liver steatosis and IR.
949 Simultaneously, alterations in intestinal BA metabolism, such as decreased BA deconjugation, in these
950 pathological contexts may lead to reduced levels of GLP-1 and FGF15/19, hence impacting on liver
951 injuries such as steatosis and fibrosis.

952 (Abbreviations: FA: Fatty Acid; LPS: lipopolysaccharide; EtOH: Ethanol; LT: T lymphocyte; LB: B
953 Lymphocyte; DC: dendritic cell; BA: Bile Acids; SCFA: short chain fatty acid; TGR5: takeda g-protein-
954 coupled receptor 5; FXR: farnesoid X receptor; GLP-1: Glucagon-like peptide-1; FGF15/19: Fibroblast
955 growth factor 15 or 19; TG: triglycerides; TJ: Tight Junctions; IgA: Immunoglobulin A; ZO-1: Zonula
956 Occludens 1; JAM-A: junctional adhesion molecule A; BSH: Bile Salt Hydrolase; ER stress: Endoplasmic
957 Reticulum Stress; IR: Insulin resistance; T2D: Type 2 Diabetes; NAFLD: Non-Alcoholic Fatty Liver diseases)
958

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

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

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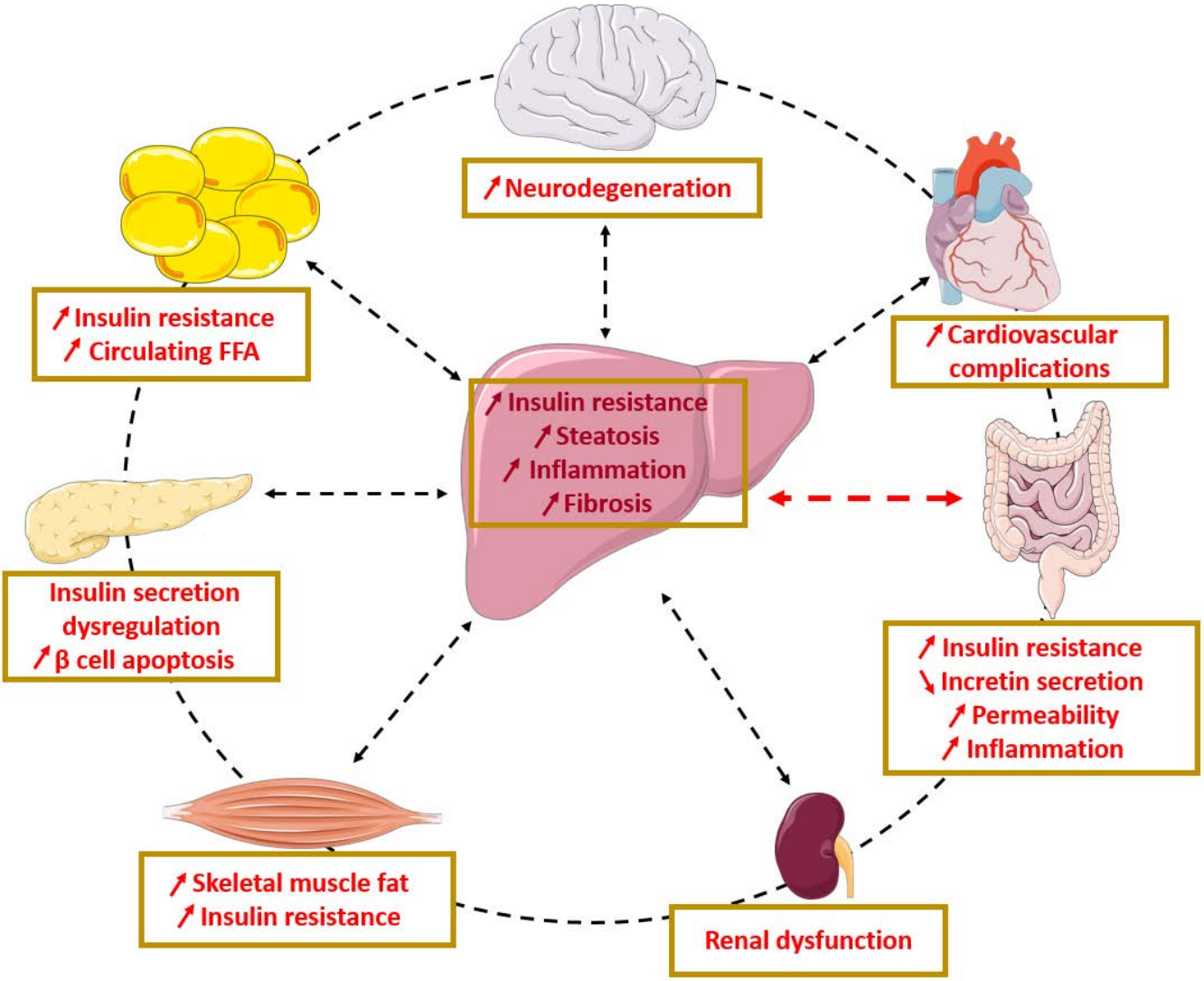
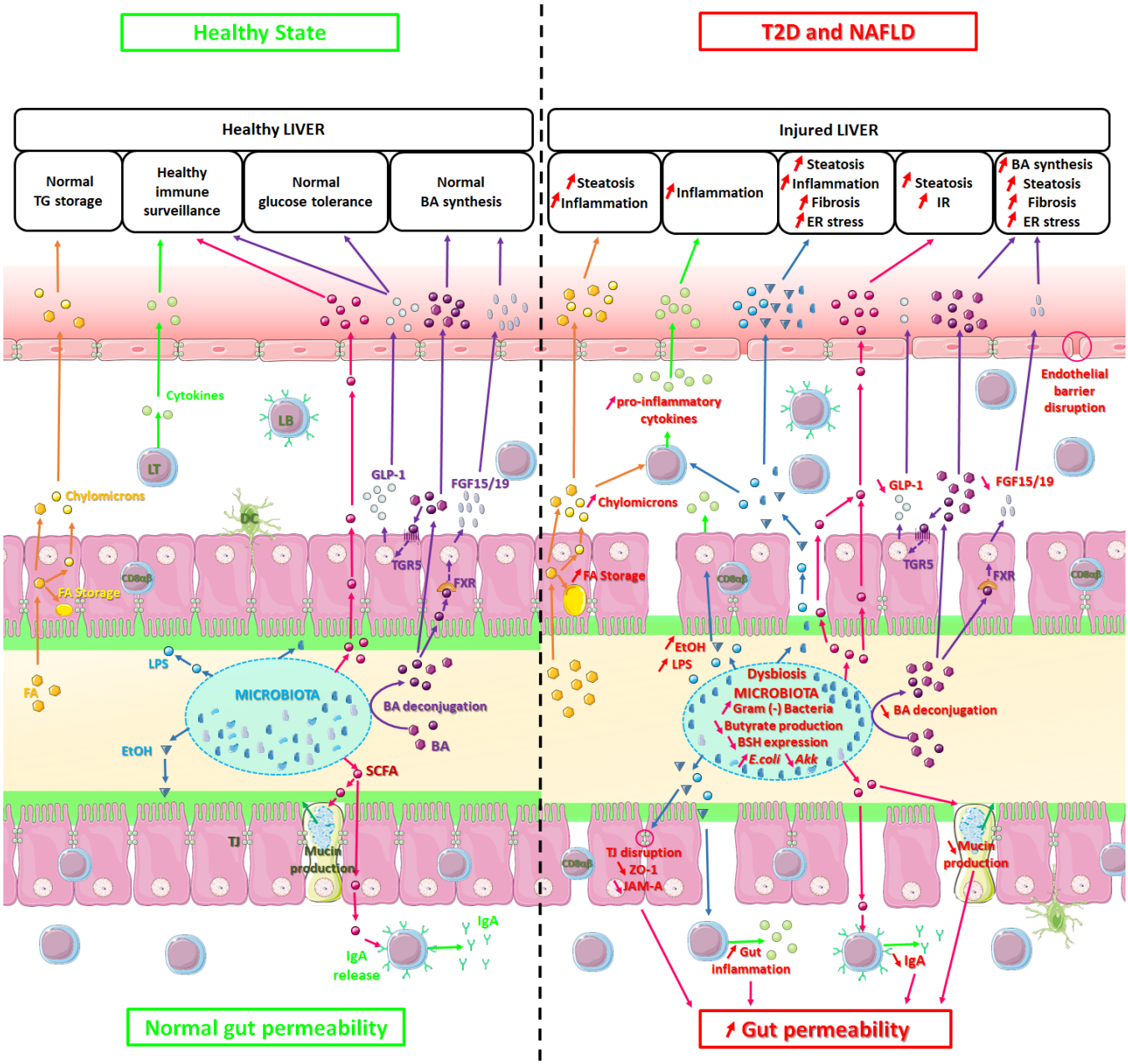

↗ Neurodegeneration
↗ Insulin resistance
↗ Circulating FFA
↗ Cardiovascular complications
↗ Insulin resistance
↗ Steatosis
↗ Inflammation
↗ Fibrosis
Insulin secretion dysregulation
↗ β cell apoptosis
↗ Insulin resistance
↘ Incretin secretion
↗ Permeability
↗ Inflammation
↗ Skeletal muscle fat
↗ Insulin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

Figure 1



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979 **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]

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

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984 **Table 1**

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