Jejunum: the understudied meeting place of dietary lipids and the microbiota

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ABSTRACT
Although the jejunum is the main intestinal compartment responsible for lipid digestion and absorption, most of the studies assessing the impact of dietary lipids on the intestinal microbiota have been performed in the ileum, colon and faeces. This lack of interest in the jejunum is due to the much lower number of microbes present in this intestinal region and to the difficulty in accessing its lumen, which requires invasive methods. Recently, several recent publications highlighted that the whole jejunal microbiota or specific bacterial members are able to modulate lipid absorption and metabolism in enterocytes. This information reveals new strategies in the development of bacterial- and metabolite-based therapeutic interventions or nutraceutical recommendations to treat or prevent metabolic-related disorders, including obesity, cardiovascular diseases and malnutrition.
This review is strictly focused on the following triad: dietary lipids, the jejunal epithelium and the jejunal microbiota. First, we will describe each member of the triad: the structure and functions of the jejunum, the composition of the jejunal microbiota, and dietary lipid handling by enterocytes and by microorganisms. Then, we will present the mechanisms leading to lipid malabsorption in small intestinal bacterial overgrowth (SIBO), a disease in which the jejunal microbiota is altered and which highlights the strong interactions among this triad. We will finally review the recent literature about the interactions among members of the triad, which should encourage research teams to further explore the mechanisms by which specific microbial strains or metabolites, alone or in concert, can mediate, control or modulate lipid absorption in the jejunum.

Abbreviations: AMP, antimicrobial peptide; CDCA, chenodeoxycholic acid; CFU, colony forming units; DGAT, diacylglycerol: acylCoA transferase; endoplasmic reticulum, ER; FATP4, fatty acid transport protein 4; FXR, farnesoid X receptor; GF, germ free; GI, gastrointestinal; HF, high fat; LCFA, long-chain fatty acid; LF, low fat; MGAT, monoacylglycerol: acylCoA transferase; pIgA, polymeric immunoglobulin A; SCFA, short-chain fatty acid; SIBO, small intestinal bacterial overgrowth; SIFO, small intestinal fungal overgrowth; sIgA, secretory immunoglobulin A; SPF, specific pathogen-free; TAG, triacylglycerol/triglyceride; TGR5, Takeda G Protein-coupled receptor 5

Keywords:
Jejunum, microbiota, lipid absorption, fatty acid, epithelium, small intestine

1. Introduction

Dietary lipids are mainly composed of triacylglycerols (TAGs) as well as phospholipids and cholesterol, free or esterified. They are one of the main energetic nutrients for the organism and are also important for membrane structure and function, as well as for organ communication through steroid hormones. The vast majority of dietary lipids are digested and absorbed in the jejunum, the middle part of the small intestine.

Because of the worldwide increase in obesity, which can lead to cardiovascular diseases, a major public health problem, numerous studies have explored the consequences of a long-term diet containing a high amount of lipids on the whole organism, organs, tissues, and cells and analysed the mechanisms involved. In particular, after a high-fat (HF) diet, modifications of the colon microbiota have been described (for recent reviews, see [1, 2]). These findings follow the pioneering study of Jeffrey Gordon's team on commensal bacteria from the colon, showing that the commensals modulate the expression of genes involved in several important intestinal functions, including nutrient absorption, mucosal integrity, xenobiotic metabolism, angiogenesis and postnatal intestinal maturation [3]. In the colon, the underlying mechanisms of the interactions among lipids, the microbiota and the host are starting to be deciphered (for reviews, see [4, 5]).

Because the microbiota from the colon has been deeply studied so far, when one refers to the intestinal microbiota, one usually only thinks of the microbiota present in the large intestine (colon) or in faeces. However, the microbiota is present all along the gastrointestinal (GI) tract, from mouth to anus, and exhibits considerable variations among the different GI parts [6, 7].

Until now, in part because the jejunum is not readily accessible *in vivo* and because the number of microorganisms in the jejunum is small compared to that in the ileum or colon, the impact of dietary lipids on the jejunal microbiota has attracted little attention, although it is in this specific part of the small intestine that the vast majority of nutrients, including dietary lipids, are absorbed.

Recently, several papers demonstrated the existence of crosstalk among dietary lipids, the jejunum microbiota and the jejunal epithelium [8-13]. This review aims to highlight this currently understudied topic and to provide arguments regarding the need for future studies.

We will focus on the triad of dietary lipids, the jejunal epithelium, and the jejunal microbiota. We will first describe the structure of the jejunum and the composition of the microbiota present in its lumen, highlighting the specificities of the jejunum in comparison with the other parts of the small intestine. Then, we will describe the fate of dietary lipids reaching the jejunum, including lipid absorption by enterocytes and current knowledge about lipid handling by microorganisms. Next, because this is a good example showing the reality of interactions among dietary lipids, the jejunal epithelium, and the jejunal microbiota, we will present the mechanisms leading to lipid malabsorption in small intestinal bacterial overgrowth (SIBO), a disease in which the jejunal microbiota is altered. We will then review the existing literature studying the interactions among dietary lipids, the jejunal epithelium and the jejunal microbiota. In the conclusion, we will briefly point out the recent methodological developments allowing us to obtain additional information on this triad, although improvements are still needed.

2. The jejunum: the absorptive part of the small intestine for dietary lipids

2.1. The epithelium of the jejunum: the host compartment

The small intestine is composed of three parts: the duodenum, the jejunum and the ileum. There is continuity between the three portions of the small intestine, and although the frontiers between them are a rather grey area, there are tremendous differences regarding dietary lipid absorption. Briefly, the duodenum receives the alimentary bolus from the stomach, and at this stage, dietary lipids, which are mainly triglycerides, have not yet been digested. Bile and pancreatic enzymes that flow into the duodenum allow dietary lipid digestion into fatty acids that are absorbed all along the length of the jejunum. Under normal conditions, all the lipids have been absorbed when the alimentary bolus reaches the ileum. If one step does not occur properly, this defect will impact the rest of the process downstream (see below).

In the literature, data concerning the physical characteristics of the GI tract (length, surface) in humans are variable. In humans, the small intestine is meant to be between 3 and 8 m long, and because of the presence of folds (*plicae circulares*), villous structures and microvilli, the absorptive surface is enlarged [14]. It has been calculated to reach up to 200-400 m^2, the size of a tennis court, a surface size that has been taught to generations of students. More recently, Helander and Fandriks recalculated the surface of the human GI tract: it is closer to an average of 32 m^2, of which 95% belongs to the small intestine, which is still quite large but "closer to a half a badminton court than of a tennis lawn" [14]. However, the jejunum remains the longest part and the largest surface of the small intestine and of the whole GI tract.

The small intestine, including the jejunum, is composed of repeating crypt-villus units. The intestinal epithelium lining the crypts and the villi of the jejunum is composed of several cell types (Fig. 1). The stem cells, localized at the bottom of the crypts, produce the four cell types of the intestinal epithelium that, except Paneth cells, migrate along the intestinal villi up to the apex. On the villus epithelium, enterocytes, absorptive cells, represent approximately 90% of the cells; goblet cells, which produce mucus, represent approximately 10% of the cells; and enteroendocrine cells, which represent only 1% of the cells, produce various enterohormones all along the GI tract. Paneth cells, localized at the bottom of the crypt, produce various antimicrobial peptides (AMPs). In mice, Paneth cells are exclusively found in the small intestine, while in humans, they are also found in the caecum and proximal colon [4]. As in any epithelium, the cells interact physically with their neighbouring cells via tight junctions that physically separate the internal milieu of the organism from the external environment and that control exchanges between these two compartments via the paracellular route.

At the jejunum level, the barrier protecting the internal milieu from the external milieu is usually subdivided into four categories.

First, the physical barrier consists of epithelial cells interacting with each other via tight junctions. The tight junctions can be more or less leaky and therefore prone to let exogenous peptides and bacterial components penetrate into the internal milieu so that the immunological cells are educated. However, this leakiness is thought to be responsible for lipopolysaccharide entry into the internal milieu, leading to an inflammatory response that causes the onset of insulin resistance, obesity and diabetes. This phenomenon was first and clearly shown by Cani *et al* (2007) in mouse models fed a high-fat (HF) diet for 4 weeks [15]. An increased level of circulating LPS, called metabolic endotoxaemia, results in low-grade chronic inflammation throughout the body, including in the liver, adipose tissue and muscles. Metabolic endotoxaemia is closely associated with obesity in both mice and humans. However, in humans, long-term high-fat feeding does not consistently lead to increased plasma endotoxin levels (for review, see [16]). Recently, the permeability of human jejunal explants to molecules of various sizes (0.4 to 10 kDa) was assessed in Ussing chambers, which showed that jejunal permeability was not significantly different between obese and non-obese patients [17]. However, the permeability of the jejunum from obese patients to small molecules (0.4 kDa) was positively

correlated with systemic inflammation markers, such as haptoglobin and C-reactive protein. Interestingly, the addition of lipid micelles (composition similar to a mixture of digested dietary lipids [18]) at the apical pole of jejunal explants mounted in Ussing chambers increased their permeability to 4 kDa molecules. Moreover, in these conditions, jejunal samples from obese patients exhibited increased permeability compared to that of explants from non-obese patients. These results suggest that digested dietary lipids increase the permeability of the human jejunal epithelium and that jejunal samples from obese patients are more susceptible than samples from non-obese patients to the lipid effect. It would be interesting to determine whether and how long this effect lasts after the end of the contact between the jejunal epithelium and lipids. Although the bacterial content in the jejunum is low, the jejunum surface is large, and the lipid effect may contribute significantly to the metabolic endotoxaemia of obese subjects.

The chemical barrier is made in particular of the mucus layer produced by goblet cells and of the AMPs secreted by Paneth cells. The main physiological role of the continuously produced mucus is to protect the epithelium of the GI tract from its luminal content due to its gel structure with controlled mesh size and ion exchange properties that create a stable unstirred water layer lining the epithelium (for review, see [19]). There are multiple protective effects, and their relative importance varies depending on the portion of the GI tract. In the stomach and duodenum, a major role of the mucus is to protect the epithelium from the luminal acidic pH by providing unstirred water that is neutralized by mucosal secretion of bicarbonate. It protects the epithelium from toxic small molecules that may come from the diet (xenobiotics) or the organism itself (bile compounds, for example) or that may have been secreted by microorganisms present in the GI tract. It also protects the epithelium from enzymes/proteins (mainly digestive hydrolases secreted by the stomach and pancreas in the upper GI tract and from enzymes secreted by the commensal microorganisms in the lower GI tract). Last, mucus also protects epithelial cells from microorganisms present in the lumen of the GI tract, mainly in the ileum and colon. The description below comes from studies performed in rats or mice. The secreted mucus adopts two forms by the action of various enzymes secreted by epithelial cells: a firmly adherent mucus layer lining the epithelial cells and, further, a loosely adherent mucus layer whose mesh size is larger and can accommodate large particles, such as bacteria [20-23]. The thickness of each subtype varies as a function of the GI segment [20] and provides a functional organization of the intestinal mucus system. In the upper part of the small intestine, the thickness of the mucus layer is the lowest (less than 200 microns) and made, in rats, of 15 microns of firmly adherent mucus in both the duodenum and jejunum and of approximately 150 and 100 microns of loosely adherent mucus in the duodenum and jejunum, respectively [20]. Subsequently, Ermund *et al* (2013) found in mice only a single mucus layer in the small intestine that was loosely adherent [22]. Because nutrient absorption occurs mainly in the jejunum and mucus functions as a filter with ion exchange properties, it makes sense that at this location, the mucus layer is the thinnest. In addition, the mucus of the small intestine was shown to be permeable to beads the size of bacteria, highlighting the importance of efficient intestinal peristalsis to regularly flush out mucus that may contain bacteria [22]. In the ileum, the mucus thickness increases up to 500 microns (30 and 450 microns of firmly and loosely adherent mucus, respectively) and reaches more than 800 microns in the colon (110 and 700 microns, respectively) [20]. Under normal conditions, bacteria are found in the loose mucus layer but not in the firmly adherent mucus layer [22, 24]. The loose mucus layer is flushed out regularly due to intestinal peristalsis. A lower pH, a higher oxygen level and the presence of conjugated biliary acids provide different growth conditions for microbes in the jejunum than in the colon [25, 26] (Fig. 2A).

The immunological barrier consists of the immune cells that are present in the lamina propria but also between epithelial cells. In particular, B lymphocytes secrete polymeric immunoglobulin A (pIgA), which transcytoses across the epithelium due to the polymeric

immunoglobulin receptor (pIgR) to yield secretory IgA (sIgA), which is very resistant to proteases. SIgA favours both maintenance of non-invasive commensal bacteria and neutralization of invasive pathogens through multiple mechanisms (for reviews, see [27-29]). Several grams are secreted in the intestinal lumen per day [30].

The fourth barrier is the microbial barrier itself, where commensal microbes prevent colonization by pathogenic microbes by competing for nutrients or producing inhibitory substances, such as bacteriocins [31, 32]. Alterations in the microbiota composition could therefore favour pathogenic microbes.

2.2. The lumen of the jejunum: the microbiota compartment

The gut microbiota resides within the lumen of the gastrointestinal tract and is a complex microbial consortium composed of bacteria as well as fungi, viruses, protists and archaea, which have key roles in several physiological host functions, including metabolic and nutritional homeostasis, immune system maturation and development, and even brain activity. The composition and therefore the functions of the intestinal microbiota are influenced by several factors, including diet, environment, drugs, antibiotics and genetics. A loss of the fragile equilibrium within the microbiota, termed dysbiosis, has been implicated in numerous human diseases, and extensive research is dedicated to identifying microbiota alterations that are associated with these diseases [33, 34]. To date, most of the work has been done on the large intestine and luminal faecal microbiota and has focused on bacteria, with relatively little known about the mucosal microbiota, other microorganisms, or microbial interactions and their consequences on host physiology. The small intestine microbiota, which may also have an impact on physiology, has been understudied in humans, partially because the small intestine is poorly accessible in healthy individuals [35]. Most available data come from mouse studies or from endoscopic samples of patients having some underlying condition, which limits knowledge on the healthy small intestine microbiota. Therefore, accurate information about the number and types of microbial species present in the three parts of the human small intestine is not readily available. However, any alteration may disrupt this equilibrium and may lead to intestinal disorders, such as small intestinal bacterial overgrowth (SIBO) or small intestinal fungal overgrowth (SIFO) (see below). Importantly, compared to the colonic microbiota, which is relatively stable, the small intestine microbiota displays more pronounced compositional fluctuations, adding another layer of complexity to its study and hindering the ability to make comparisons. Microbiota composition analysis of ileostomy effluents revealed, for example, large fluctuations per individual during the course of several days and even between the morning and afternoon, which are most likely consequences of diet [36]. In addition, a high strain-level richness has been observed in the small intestine, as highlighted by a study looking specifically at *Streptococcus* and *Veillonella* populations from ileostomies: a total of 160 *Streptococcus* and 37 *Veillonella* isolates were obtained within a 3-day time frame [37].
The concentration of fungi in the gut seems relatively stable [38]. The fungal population variations along the gastrointestinal tract have been poorly described so far, and no information is yet available for the fungal community in the jejunum.

The bacterial community varies in number and composition from the stomach to the colon in humans and in mice [6, 33], and this variation results from many factors, including lumen oxygen level, nutrient bioavailability, pH, bile acid contents, gastrointestinal transit time/motility, mucus and immune factors [7, 25, 39] (Fig. 2A). Indeed, bacterial abundance follows a gradient from the stomach (10^1-10^3 colony forming units (CFU)/ml) to the colon (10^{12}-10^{14} CFU/ml) [7, 40, 41]. The jejunal microbiota is estimated to consist of 10^4-10^7 CFU/ml. One possible reason why the microbial load is lower in the small intestine than in the colon is the faster transit time in the small intestine, which prevents microbial colonization and

stable establishment. Consistent with this idea, the small intestinal microbiota from surgically induced stasis exhibits a greater bacterial abundance and diversity and resembles a colonic microbiota [42]. In addition, the presence of oxygen, antimicrobial peptides, bile acids and secretory IgA in the small intestine favours the presence of aerobic microorganisms resistant to bile and acidic conditions (Table 1). Work using culture-dependent analyses revealed the presence of gram-positive bacteria (such as enterococci, lactobacilli, staphylococci, and streptococci) and limited gram-negative bacteria (such as *Escherichia coli* and *Klebsiella*) in the jejunum [43, 44]. More recently, Hayashi *et al* (2005) evaluated the diversity of bacterial community structure in the jejunum, ileum, caecum and recto-sigmoid colon of three elderly individuals at autopsy by molecular analysis. Although major inter-individual differences occur in the composition of the different microbiotas, facultative anaerobes, such as streptococci and lactococci, were the predominant species in the jejunal microbiota [45]. Seekatz *et al* (2019) published an analysis of the stomach and small intestinal microbiota (duodenum and multiple jejunum sites) in eight fasted healthy humans and showed that Firmicutes (*Streptococcus, Lactobacillus,* and *Veillonella*) represent between 60 and 80% of the jejunal bacterial community, Proteobacteria (*Pasteurellaceae* and Enterobacteria) between 10-25% and Bacteroidetes less than 3% [46]. These data are consistent with a previous report from Sundin *et al* comparing the human jejunal microbiota to those in the oral cavity and colon in 20 subjects [47]. This study reported that in the jejunum, *Streptococcus*, *Prevotella*, *Veillonella* and *Fusobacterium* were especially abundant, as well as non-oral genera, including *Escherichia*, *Klebsiella*, and *Citrobacter*, and that the jejunum was devoid of the genera *Alistipes*, *Ruminococcus*, and *Faecalibacterium* and other strict anaerobes abundant in the colon. The distal ileum has an estimated microbial load of 10^3-10^8 CFU/ml and is a transition zone between the sparse populations of aerobic bacteria of the jejunum and the very dense populations of strict anaerobes in the colon [48].

Firmicutes (*Lactobacillus*, *Veillonella*, *Enterococcus,* and *Clostridium*) and Proteobacteria (Enterobacteria) are the main bacteria in the ileum [45, 49], while the dominant bacterial phyla in the colon are Bacteroidetes (*Bacteroidaceae, Prevotellaceae*, etc.) and Firmicutes (*Lachnospiraceae* and *Ruminococcaceae*) [7, 25, 41].

The gut microbiota affects host metabolism through diverse physiological processes, including the production of short-chain fatty acids, tryptophan metabolism and conversion of primary bile acids into secondary bile acids [5, 50]. These bacterial metabolites within the intestinal environment are important in maintaining normal intestinal epithelial cell physiology in the small intestine as well as in the colon. Facultative anaerobes present in the jejunum produce lactate, which is an important energy source for small intestinal stem cells [51], while obligate anaerobes present in the colon produce the majority of butyrate, which is consumed by colonocytes [52]. The small intestine microbiota is therefore likely to play a pivotal role in metabolic regulation [39], and studies should pay greater attention to the jejunum to better understand the role of bacteria in lipid digestion and absorption [53, 54]. Indeed, microorganisms living in the jejunum might participate actively in the degradation of lipids and sugars. More particularly, *Lactobacillus* strains may play a substantial role in host lipid metabolism [54], as they produce bile salt hydrolases [55-58] or lipases [59]; short-chain fatty acids, including acetate, propionate and butyrate; and a significant amount of lactic acid [60]. Interestingly, bacterial lipases have been identified in other bacteria present in the jejunum, such as *Enterococcus* or *Staphylococcus* [61, 62]. It is also known that fungi secrete phospholipases (phospholipases B and D) [63], and one can speculate that these enzymes could contribute to lipid metabolism in the jejunum. Further work on lipid-degrading microorganisms is needed to decipher the role of the jejunal microbiota in fat metabolism.

3. Absorption of dietary lipids

In humans, the dietary recommendation for fat is 20 to 35% of the total energy intake per day, which is approximately 44 to 78 g/day for a 2000 kcal/day intake [64, 65]. However, the recommended percentage has been recently increased up to 35-40%, with better attention paid to the quality of the fatty acids, e.g., privilege the intake of unsaturated over saturated and trans fats. More than 95% of the ingested lipids are TAG, which are made of glycerol esterified with three fatty acids. In the diet of human adults, fatty acids are almost long-chain fatty acids (LCFAs). The "long-chain" term is defined as a chain length of 16 or 18 carbons. The rest of the dietary lipids are made of phospholipids, glycolipids, and sterols and contain fat-soluble vitamins. All these molecules have a very limited solubility in water. The scientific literature dealing with the colon microbiota often mentions short-chain fatty acids (SCFAs) as important gut metabolites, and it is important at this stage to highlight the differences between these two kinds of FAs. Short-chain fatty acids (SCFAs), e.g., acetate, propionate and butyrate, have a chain length of only 2 to 4 carbons and are thus freely soluble in water. Moreover, these molecules are produced in the colon by bacterial fermentation of dietary fibres made of sugar polymers. Therefore, in the gut ecosystem context, LCFAs and SCFAs have very little in common except their carboxyl group. To avoid confusion, SCFAs may be better called organic acids or volatile organic acids. Other organic acids, such as lactate and glycerate, were shown to be produced from fructose in the small intestine, as recently shown [66].

Lipid absorption requires prior digestion (Fig. 2A). While rodents exhibit strong lingual lipase activity, which starts the TAG digestion process in the oral cavity, lipase activity at this site is weak in humans. However, although weak, the low lipase activity is important since the effective stimulus for the taste of fat in the mouth through fat receptors, such as cluster of differentiation 36 (FAT/CD36) or G-protein coupled receptor 120 (GPCR120), is the fatty acid molecule that is generated from TAG hydrolysis [67, 68]. Gastric lipase is present in the stomach, but it is mainly useful for infants because it preferentially hydrolyses TAG with medium-chain fatty acids, which are abundant in milk. Therefore, in adults, TAG digestion truly starts in the duodenum and depends on an intricate interplay among pancreatic lipase, colipase, and bile salts (conjugated biliary acids) produced and secreted by the pancreas and the liver, respectively. Bile acids are synthesized from cholesterol, and in humans, the primary bile acids, i.e., cholic acid (CA) and chenodeoxycholic acid (CDCA), are conjugated to predominantly glycine or to taurine [69]. Conjugated bile salts, major bile components together with phospholipids and cholesterol, are stored in the gallbladder until secretion in the duodenum upon arrival of the alimentary bolus. Even if their primary functions shift to also be major metabolic integrators in physiology and metabolism (for review, see [70, 71]), bile acids are mandatory for TAG digestion because they are amphipathic biological detergents, therefore able to solubilize lipids and necessary for pancreatic lipase activation. During digestion, their concentration averages 10 mM in the lumen of the small intestine [72]. By the concerted action of conjugated bile acids and pancreatic lipase, dietary lipids emulsified as lipid droplets in the stomach due to mechanical division are digested and solubilized as lipid micelles, allowing lipid absorption by enterocytes. TAG digestion leads to fatty acid and 2-monoacylglycerol production, while phospholipids, mainly phosphatidylcholine, which is present in bile and the diet, are hydrolysed by pancreatic phospholipase A2 into fatty acids and lysophospholipids. Fatty acids are taken up by enterocytes by a simple diffusion mechanism when in the unionized form, but facilitated transport due to transmembrane proteins, such as CD36 and fatty acid transport protein 4 (FATP-4), has also been shown [73]. Because of their cytotoxicity due to their detergent properties, fatty acids are rapidly metabolized into phospholipids and TAG at the endoplasmic reticulum (ER) membrane. Monoacylglycerol:acylCoA transferase (MGAT) converts 2-monoacylglycerol and fatty acids into diacylglycerol, which is in turn converted into TAG by diacylglycerol: acylCoA transferases (DGATs). Because they are highly hydrophobic, newly synthesized TAGs accumulate as lenses between the two leaflets of the ER membrane

and contribute to lipid droplet formation by budding into the ER lumen for chylomicron assembly or into the cytosol for cytosolic lipid droplet formation [74, 75]. The distribution of lipid droplets between the cytosol and the ER lumen in enterocytes has been shown to be modified by nutritional conditions [76]. In the secretory pathway, chylomicron assembly occurs by fusing luminal lipid droplets with dense apolipoprotein B48 phospholipid-rich particles that are formed independently, while cytosolic lipid droplets constitute a transient lipid storage pool [73, 77-81]. Chylomicrons, secreted at the basolateral pole of enterocytes, are too large to enter blood capillaries that lead to the liver via the portal vein. Instead, they enter the lymphatic circulation via the lacteals/lymphatics and flow into the general blood circulation via the thoracic duct before reaching the liver. During this body "tour", TAGs from chylomicrons are hydrolysed due to lipoprotein lipase present in the bloodstream. The fatty acids released are taken up by muscle cells for energy production or by adipocytes for storage [82, 83].

Overall, the intestine is able to absorb more than 95% of the triglycerides in the diet, and the faecal loss remains below a few percent of the total content. When the alimentary bolus reaches the ileum, almost all fatty acids are absorbed. There, while conjugated bile acids are reabsorbed by active transport, bacteria present in the ileum and especially in the colon are able to deconjugate bile salts and metabolize biliary acids by 7α-dehydroxylation, leading to secondary biliary acids, which are more hydrophobic [71, 84]. These molecules are absorbed by the ileal epithelium in a passive way and reach the liver, where they are conjugated again with glycine or taurine. The entero-hepatic cycle of biliary acids occurs 7-10 times a day. In humans, the biliary pool is 2-5 grams, and the daily faecal loss is approximately 10-15% of the total pool [70, 85]. Bile acids also regulate complex processes through binding receptors and activation of signal transduction pathways (for review, see [86]). Indeed, the potency of activation of a given receptor depends on the nature of the bile acid. For example, farnesoid X receptor (FXR), a nuclear receptor that has an important role in the regulation of bile acid homeostasis and lipid and glucose metabolism, is activated by primary bile acids, CDCA being the most potent activator, and, to a lesser extent, by secondary bile acids [87]. FXR is expressed in the jejunum, although at a lower level than in the ileum [88]. However, the unconjugated form is the active one [87], and in physiological conditions, FXR may thus not be as strongly stimulated in the jejunum as in the ileum. Takeda G Protein-coupled receptor 5 (TGR5), a G protein-coupled receptor widely expressed throughout the body, regulates a variety of processes ranging from glucose homeostasis to immune cell regulation. Its expression is low in the small intestine [89]. In CHO cells transfected with human TGR5, the potency of activation by bile acids increases with the hydrophobicity of the bile acids. In the intestine, TGR5 is expressed by L-cells that secrete a major enterohormone, glucagon-like peptide 1 (GLP-1), and TGR5 activation by bile acids induces GLP-1 secretion. However, in the intestine, L-cells are predominantly expressed in the ileum and colon [90].

Bile acids also have direct antimicrobial effects on gut microbes [91-93]. In the jejunum, bile acids are in the conjugated form and thus strongly acidic. They are fully ionized and do not cross membranes unless a transport system is available, while they may cross membranes if unconjugated. Indeed, bile acid binding to membranes and passive flip-flopping across the lipid bilayer correlates with hydrophobicity. Gram-positive bacteria seem to be more sensitive to the deleterious effect of bile than gram-negative bacteria [91].

Overall, the ileum and colon microbiota, by metabolizing bile acids, have an impact on the activation of bile acid receptors. However, additional studies are needed to obtain a clear and precise picture of the activation of these biliary receptors in the context of the jejunum specifically, in healthy as well as in pathological conditions.

4. SIBO: a disease that highlights the need to maintain a specific microbiota in the jejunum

The composition of the intestinal lumen milieu is highly dynamic in the jejunum, at least for nutrients, including lipids. In fact, the small intestine is able to adapt to a very large variety of dietary situations, whether they occur in the short or long term (for review, see [94]). Despite this adaptability, lipid malabsorption occurs in various circumstances, defined as a faecal loss above 5% of dietary fat, i.e., steatorrhea. If malabsorption occurs, lipids are available for microorganisms and can serve as energetic substrates for their proliferation [95].

SIBO is an acquired disorder that is well known for causing inflammation and nutrient and vitamin malabsorption. The diagnosis relies on a breath test that measures the concentration of hydrogen and methane in breath, representative of the break-down of carbohydrates in the gut and thus of the abundance of bacteria in the proximal intestine. In SIBO, the intestinal flora of the proximal intestine is altered in both quantity and quality; the microbiota adopts the characteristics of the large intestinal microbiota (Fig. 2B). The current accepted criteria for the diagnosis of SIBO is the presence of aerobic and anaerobic coliform bacteria isolated from the proximal jejunum at $>10^5$ CFU/ml. Pistiki *et al* isolated 170 aerobic microorganisms from 117 patients with SIBO. In 64 of the patients (54.7%), SIBO was due to the overgrowth of one isolate, and in the other 53 patients, it was due to the overgrowth of two isolates. These bacterial isolates were mainly *E. coli* (41%), *Klebsiella* (33%), *Enterobacter* (19,6%), *Enterococcus* (12%) and *Staphylococcus* (10%) [96]. Next-generation sequencing methods, such as 16S rRNA sequencing and shotgun metagenomics sequencing, should help to characterize the composition and function of the SIBO-associated microbiota.

Bacteria specific to the colon should not be found in abundance in the proximal small bowel. They may grow in the upper part of the small intestine as the result of a failure of small bowel clearance or anatomic alterations, which can develop after small bowel surgery, radiation or Crohn's disease [48]. Delayed transit may promote small intestinal bacterial overgrowth by facilitating colonization of the small bowel with ascending colonic bacteria [95]. In patients with stagnant-loop syndrome or partial gastrectomy, Tabaqchali *et al* clearly established the relationship among high bacterial counts in the jejunum, the presence of free bile acids (unconjuguated) in the jejunum and steatorrhea: oral broad-spectrum antibiotics resulted in an abrupt decrease in bacterial counts in the jejunal fluid, disappearance of free bile acids in the jejunum and strong improvement of steatorrhea [97]. As schematized in Fig. 2B, because of the presence of bacteria specific to the colon in the jejunum, bacterial deconjugation of bile salts already occurs in the small intestine. Deconjugated bile salts are reabsorbed in the jejunum rather than the ileum, impairing enterohepatic reabsorption and resulting in fat malabsorption and, consequently, deficiencies in fat-soluble vitamins. Bile acids (unconjugated) are toxic to the intestinal mucosa, leading to bile acid diarrhoea and further malabsorption [48].

SIBO can also be due to an increased number of gram-positive bacteria ($>10^5$ CFU/ml), but the clinical significance remains unclear. Under normal conditions, nearly 99% of all bacteria in the stomach are killed within 5 minutes under a physiologic gastric pH environment. Thus, under physiological conditions, very few bacteria coming from the oral cavity reach the jejunum and grow. A failure of the gastric acid barrier due to the use of proton pump inhibitors, which are potent inhibitors of gastric acid secretion, for example, may lead to gram-positive bacterial overgrowth in the jejunum [48]. Additionally, abnormal antibody or T cell responses, such as IgA deficiency, hypogammaglobulinemia, and T cell deficiency, may increase the risk of SIBO with gram-positive bacteria [48].

In addition to SIBO, examples of SIFO have been reported [98]. For example, Jacobs *et al* found that 24/150 patients with unexplained gastrointestinal symptoms had SIFO due to *Candida* [99]. SIFO symptoms are similar to those of SIBO. Small intestinal dysmotility and

the use of proton pump inhibitors might predispose patients to SIFO. The role of the fungal microbiota and the consequences of SIFO in lipid absorption have not yet been studied.

In conclusion, although little attention has been paid to the jejunal microbiota to date, any change in the bacterial or fungal flora of the jejunum (nature and number) may lead to a multitude of effects, such as competition with the host for critical nutrients, alteration of host metabolism, damage to the absorptive mucosa of the host, and gastrointestinal symptoms.

5. Lipid - epithelium - microbiota interactions: a ménage à trois to explore and to monitor

As discussed above, multiple factors contribute to maintaining a low bacterial content in the lumen of the small intestine: gastric acidity, the presence of sIgA and AMPs, and propulsive intestinal motility that periodically clears the jejunum lumen. Additionally, conjugated bile acids, probably together with fatty acids, inhibit bacterial growth directly by their pharmacological properties as well as by their signalling properties [72, 100, 101].

In this last section, to highlight the intricate crosstalk among lipids, the jejunum epithelium and microorganisms, we will review the impact of dietary lipids on 1) their own absorption, 2) the production of antibacterial molecules by the jejunum and 3) the bacterial content in the jejunum. Then, ultimately, we will review recent papers describing the impact of bacteria and bacterial products on intestinal lipid metabolism.

Research on metabolic diseases, such as obesity or diabetes, often relies on animal models that have been fed a high-fat diet for a long period of time. Therefore, most of the data concerning the impact of dietary lipids on their own absorption by the jejunum have usually been performed after long-term high-fat diet consumption rather than acute lipid challenge.

Reviews on *ex vivo* and *in vitro* models for the study of host-microbiota interactions, including 3 dimensional (3D) models, have been published recently [102, 103]. There have been tremendous advances due to intestinal organoid development over the past ten years, which allows the study of all the cell types of the intestinal epithelium, including Paneth cells, for which there are no cell lines available yet. However, studies are still hampered because of the lack of 2D organoid models, which would allow a polarized exposure of the epithelium to bacteria and/or lipids.

5.1. Impact of dietary lipids on the expression of genes and proteins involved in fatty acid metabolism in the jejunum

Adaptation of the jejunum occurs in response to a high-fat diet, which was shown a long time ago. After four weeks of a regular chow diet supplemented with 20% lipids via lard, lipid uptake, esterification and MGAT activity were increased in the jejunum of rats compared to those in the control animals. This adaptation was shown to occur primarily in the jejunum rather than in the ileum [104].

In mice fed a high-fat diet for 3 weeks (40% w/w), Petit *et al* (2007) showed greater fatty acid uptake by the intestinal mucosa, without steatorrhea. There was no intracellular triacylglycerol accumulation in enterocytes, suggesting an increased lipid absorption capacity. In the jejunum, the expression of genes involved in fatty acid uptake (FAT/CD36 and FATP4), trafficking [liver- and intestinal-fatty acid-binding proteins (L- and I-FABP)] and lipoprotein synthesis [microsomal transfer protein (MTP) and apolipoprotein A-IV (apoA-IV)] was increased. These changes were lipid-mediated since they were fully abolished in mice refed the control diet. They also showed increased cell proliferation [105]. Mice fed a high-fat diet for 8 weeks exhibited extended villus length [106]. This increased absorption surface probably contributes to the increased lipid absorption capacity observed.

Compared to those in low-fat diet-fed mice, genes related to lipid metabolism, cell cycle and inflammation/immune response were strongly modified in mice fed a high-fat diet for up to 8 weeks. The highest number of differentially expressed genes was found in the middle part of the small intestine, i.e., the jejunum [107].

By mass spectrometry, Wisniewski *et al* (2015) analysed the proteins present in the jejunum mucosa of mice fed a high-fat diet or a normal diet for two months. They observed that proteins engaged in fatty acid absorption [L-FABP, apolipoprotein B48 (apoB48), DGAT1], fatty acid activation [acyl-CoA synthetase5 (ACSL5)] and fatty acid-beta oxidation [carnitine palmitoyl transferase 1 (CPT1), hydroxyacyl-CoA dehydrogenase (HADH)] were increased in high-fat diet-fed mice [108].

More recently, Clara *et al* (2017) showed that in mice, a 3-day high-fat diet (60% of energy from fat) already led to an alteration of lipid metabolism-related genes in the jejunum, although weak, and that the adaptation of the small intestine occurred before the adaptation of the liver [109].

Overall, the jejunum is able to adapt its lipid absorption capacity in line with the amount of lipids present in the diet.

5.2. Impact of dietary lipids on the production of antimicrobial proteins and peptides by the jejunum

In the few studies published to date, the analysis of the jejunal mucosa of mice fed a high-fat diet showed alterations of the antimicrobial defence compared to that in low-fat or standard diet-fed mice [8, 107, 108, 110, 111].

In all the studies published so far, regenerating islet-derived 3-gamma (Reg3g) has been routinely found to be strongly decreased at the gene and/or at the protein expression level in the jejunum mucosa in models of high-fat diet-fed mice compared to that in control mice [8, 107, 110, 111]. Reg3g is an AMP produced by Paneth cells as well as by enterocytes that specifically targets gram-positive bacteria. In line with this finding, a high-fat diet should lead to an increase in gram-positive bacteria and a decrease in gram-negative bacteria in the jejunum. This effect was observed in the contents of the intestinal segments analysed (i.e., small intestine [111], caecum [8, 110], and faeces [8]). However, this analysis has not yet been performed on the jejunal microbiota, and this effect remains to be shown.

Most likely because the high-fat fed mouse models are different regarding the composition and duration of the diet, the results concerning other components of the antimicrobial defence are less consistent. Lysozyme is a muramidase secreted by Paneth cells that destroys the gram-positive bacterial wall. Its expression (mRNA and/or protein) was found to be significantly decreased or not modified by the high-fat diet [8, 110, 111], although the entire small intestine was used for the analysis in [111].

As explained above, pIgA is secreted in large amounts in the intestinal lumen as sIgA, which plays a crucial role in the maintenance of non-invasive commensal bacteria and neutralization of invasive pathogens. In pIgA and sIgA, antibody molecules are linked by a J chain. In contrast, de Wit *et al* (2008) found increased gene expression of the J chain in mRNA isolated from the jejunum [107], and Wisniewski *et al* (2015) found a lower protein amount of the J chain (and of other IgA subunits) by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis of the scraped jejunal mucosa [108]. These contrasting results do not come from the part of the small intestine that was used to perform the analysis (the jejunum in both cases), and in both cases, the tissue was obtained by scraping the mucosa. However, the high-fat diet composition was different and compared to control normal diets or low-fat diets. Additionally, mRNA expression and protein content do not always correlate, which is attributed to the involvement of other levels of regulation. Further work needs to be

done to obtain a complete and accurate picture of sIgA metabolism in the intestine and its modification upon high-fat diet consumption. In a recent study, the faecal IgA concentration was shown to decrease in faeces from high-fat fed mice [112]. If a lower faecal IgA concentration is a reflection of a lower IgA concentration in the lumen of the jejunum, a high-fat diet may thus favour invasion of the mucus by pathogenic microorganisms, which may thus reach epithelial cells.

The different studies reviewed here indicate that when fed for a few weeks with a high amount of dietary lipids, the production of antibacterial protein and peptides by the jejunal mucosa is usually decreased. Whether this effect leads to an increased susceptibility to bacterial infection needs to be further explored.

5.3. Impact of dietary lipids on the bacterial content in the jejunum

It is well established that a high-fat diet has an impact on gut microbiota composition and function; however, most studies focus on the faecal or colonic luminal microbiota. Too little is known about the impact of a high-fat diet on the jejunal microbiota, especially in humans.

In 15-day-old rat pups (overfed with milk rich in fats), Mozes *et al* (2008) and Sefcikova (2010) showed using the fluorescence in situ hybridization (FISH) technique reduced numbers of Bacteroidetes (*Bacteroides*/*Prevotella*) and increased numbers of Firmicutes (*Lactobacillus*/*Enterococcus*) in the jejunum compared to those in controls [113, 114].

Tomas *et al* (2016) demonstrated that a high-fat diet alters small intestinal defences in mice, disrupts epithelial integrity and induces an increased bacterial density in the lumen of the jejunum [8]. In addition to affecting the number and spatial distribution of the bacteria, the HF diet also drastically affected the microbiota composition, with an increase in Firmicutes (appearance of Erysipelotrichia), Proteobacteria (Desulfovibrionales) and Verrucomicrobia and a decrease in Bacteroidetes. However, these modifications were not specific to the jejunum. Moreover, a high-fat diet (fat 37,5% kCal for 4 weeks) was shown to lead to strong modifications of the murine jejunal microbiota [9, 115]. In this study, the relative abundance of the families Clostridiaceae and Peptostreptococcaceae (both belonging to the Firmicutes phylum) was increased, while that of the families of Bifidobacteriaceae (Actinobacteria phylum) and Bacteroidaceae (Bacteroidetes phylum) was decreased. Specific bacterial strains, such as *Clostridium*, influence processes underlying intestinal lipid absorption (see section 5.4). Recently, by comparing the effects of diet (high fat versus low fat), broad-spectrum antibiotics and antibiotic cocktails, Poteres *et al* (2020) showed that antibiotics had an effect on the murine small intestine, caecum and faeces microbiota composition, while diet significantly affected only the jejunum and caecum microbiota, suggesting that diet has a selective regional impact on the gut microbiota [13]. In addition, Turnbaugh *et al* (2009) showed using humanized mice that switching from a low-fat, plant polysaccharide-rich diet to a high-fat, high-sugar "Western" diet shifts the composition of the microbiota within a single day and changes microbial metabolic pathways and gene expression along the length of the gut, including in the small intestine [116]. A bloom in members of the Erysipelotrichia and Bacilli classes of Firmicutes occurred along the length of the gut. This work highlights that a changing diet in mice affects both microbiota composition and function. Further work is needed to determine whether the modifications are induced by fats, sugars or both. Similarly, Lacroix *et al* recently compared the effect of a high-fat, high-sucrose diet on jejunum, ileum and caecum microbiota composition. Their data suggested intestinal segment-specific bacterial responses to this diet, underlying the importance of studying different intestinal segments, at least in animal models where this approach is feasible [11]. Studies on the impact of lipids on the microbial content in the jejunum of humans are needed to decipher in detail the relationship among the diet, microbiota and host.

5.4. Impact of the whole microbiota or its specific bacterial members on small intestinal lipid absorption and metabolism

The first seminal reports linking the gut microbiota and host lipid absorption [9, 117] found that germ-free (GF) mice fed a low-fat (LF) or a high-fat (HF) diet had elevated faecal excretion of lipids (*e.g.,* cholesterol) compared to that of specific pathogen-free (SPF) or conventional mice. This effect is most likely due to an impairment in intestinal lipid absorption (lower TAG and cholesterol appearance in plasma) [9, 117] and/or lipid digestion (*e.g.*, a defect in cholecystokinin action, a hormone that plays a key role in facilitating digestion [118]) present in GF mice [9]. In agreement with these findings, Sato *et al*. observed that antibiotic-induced microbiota depletion in rats decreased chylomicron secretion into the lymph after an intraduodenal lipid challenge [119]. Altogether, these studies suggest that the presence of a regular gut microbiota is required for the efficient absorption of dietary lipids. Indeed, recent works using cell culture and animal models demonstrated that the small intestinal microbiota acts as a key regulator of dietary lipid absorption, storage, and secretion [9, 10, 12, 120]. Martinez-Guryn *et al*. showed that GF mice transplanted with a HF diet-shaped jejunal microbiota had higher plasma levels of orally administered ^{3}H-triolein (TAG containing oleic acid) and ^{14}C-cholesterol, regardless of the diet that the mice consumed after being transplanted (LF or HF diet). These results suggest that small intestinal microorganisms programme the intestine of GF mice to increase dietary lipid absorption regardless of the amount of lipids they ingest [9]. Following GF mouse experiments, the authors examined the ability of the commensal strain *Clostridium bifermentans* or its conditioned medium (bacterial culture supernatant containing secreted bioactive molecules) to affect lipid absorption in *in vitro* models of intestinal organoids [9]. Both *C. bifermentans* and its conditioned medium stimulated ^{3}H-oleic acid uptake and *Dgat2* mRNA expression (see section 3) in both jejunal and duodenal organoids. In agreement with these findings, the authors found that gavaging SPF mice (under a LF diet) with conditioned medium from *C. bifermentans* for 4 weeks increased jejunal mRNA levels of *Dgat2* [9]. These results were, however, not observed in SPF mice fed a HF diet, which may seem inconsistent with the data obtained with GF mice colonized with a HF diet-shaped microbiota. This discrepancy may be explained by differences between animal models: GF mice were not primed by a rich and diverse small intestinal microbiota prior to performing experiments, while SPF mice were.

By using other commensal bacteria, *i.e.,* the earliest intestinal colonizers *Lactobacillus paracasei* and *Escherichia coli* RB01*,* Tazi *et al*. reported in 2018 a distinct impact of native microbiota members on small intestinal lipid metabolism [10]. In their study, SPF mice fed normal chow and monocolonized with *L. paracasei* showed an increase in lipid storage in the form of larger jejunal lipid droplets when compared to those in SPF control mice. On the other hand, mice monocolonized with *E. coli* showed enhanced lipid catabolism due to a reduction in jejunal lipid droplet number and chylomicron appearance in the circulation. These observations were almost entirely confirmed *in vitro* by using a bacteria-murine enterocyte (m-ICcl2 cells) coculture system. Exposure of enterocytes to *L. paracasei* promoted lipid storage in cytosolic lipid droplets and inhibited chylomicron secretion, whereas *E. coli* RB01 enhanced lipid consumption and inhibited chylomicron secretion. Comparing these results with data presented in section 5.3, we can speculate that the presence of excess lipids might selectively promote the colonization and establishment of the Firmicutes and Proteobacteria phyla enriched in *E. coli* and *L. paracasei* species, respectively, allowing them to efficiently and distinctly drive lipid metabolism in enterocytes.

Following the work of Tazi *et al*., *L. paracasei* and *E. coli* RB01-derived molecules or effectors and the underlying molecular mechanisms by which they altered enterocyte lipid metabolism

were identified by Araújo *et al*. [12]. In both m-ICcl2 cells and SPF mice, the authors demonstrated that L-lactate secreted by *L. paracasei* elicited lipid storage by a mechanism involving L-lactate absorption by enterocytes, its conversion to malonyl-CoA and subsequent inhibition of lipid beta-oxidation. In contrast, acetate secreted by *E. coli* RB01 promoted lipid oxidation by a mechanism involving acetate absorption by enterocytes, its metabolism to acetyl-CoA and adenosine monophosphate and subsequent upregulation of the AMP-activated protein kinase (AMPK)/Peroxisome proliferator-activated receptor gamma coactivator-1-*alpha* (PGC-1α)/ Peroxisome Proliferator-Activated Receptor alpha (PPARα) signalling pathway [12].

Collectively, these studies highlight that the richness and diversity of the whole microbiota together with the complexity of its vast set of effectors impact small intestinal lipid metabolism (*e.g.*, increase in lipid absorption) in a different way than a single dominant bacterial species and its restricted set of effectors (*e.g.*, decrease or increase in lipid absorption). Data obtained from these studies may have important implications for the development of bacterial- and metabolite-based therapeutic interventions against obesity, atherosclerosis and/or malnutrition.

6. New tools to study the dietary lipid-microbiota-jejunum triad

Continuous technical improvements in the characterization of bacterial communities, such as high-throughput next-generation sequencing, metagenomics, metatranscriptomics, and metabolomics coupled to culturomics, will help to characterize jejunal microbiota composition and function. Intestinal *in vitro* and *ex vivo* models to study host-microbiome interactions have been reviewed recently [102]. Among them, organoids generated from jejunal tissue [121, 122] or stem cells [123] are promising, although organoid culture on 2D devices allowing polarized supply of nutrients or microorganisms is still challenging. Inside-out organoids may be an interesting alternative [124]. In addition, the use of a dynamic *in vitro* jejunum model could help to decipher the interactions between the microbiota and lipids. To date, TIM-1 (TNO-Intestinal Model-1 [125]) is one of the systems that allows the closest simulation of *in vivo* dynamic events occurring within the human upper gastrointestinal tract [126]). Recently, Stolaki *et al* (2019) developed a dynamic *in vitro* ileum model based on the same concept as TIM-1 and inoculated it with faecal and ileostomy effluents [127]. This study showed that the microbiota at steady state *in vitro* resembles that of the human ileum and paves the way for the development of an *in vitro* jejunum model inoculated with the jejunal microbiota or a bacterial consortium.

7. Conclusion

Although the jejunum is the major intestinal site for lipid absorption in the GI tract, studies aimed at deciphering interactions between dietary lipid metabolism and the intestinal microbiota in the GI tract have been almost all performed on the ileum and colon. However, recent papers, as reviewed here, highlight that the jejunum is worth studying. Mouse models (transgenic or obtained by dietary manipulation) that have already been well characterized regarding lower regions of the gut may be revisited by analysing the jejunum as well.

The poor interest in the jejunum may be explained by the low number of microbes present in this region, the difficulty of sampling the jejunal content and the poor access to jejunal tissue. In patients, small bowel samples can be obtained by catheter aspirations, but this invasive method limits their use. Intestinal waste from patients with ileostomy or jejunum pieces from patients subjected to gastric bypass surgery (Roux-en-Y) may provide useful samples. However, all these samples come from diseased patients, and these procedures are indeed not

applicable to healthy individuals. Less invasive procedures for sampling the lumen from the human jejunum, such as capsules, are thus needed [39, 128].

By using the recently available tools, it will be exciting to explore the mechanisms by which specific microbial strains or metabolites, alone or in concert, can mediate, control or modulate lipid absorption in the jejunum. These studies deciphering how the microbiome interacts with pathways of intestinal lipid and lipoprotein metabolism will allow the development of bacterial- and metabolite-based therapeutic interventions or nutraceutical recommendations to treat or prevent obesity, cardiovascular diseases or malnutrition, which are global public health problems.

Conflict of interest

The authors declare no conflicts of interest.

Author contribution

N.R and S.D designed the major concept of the article and supervised the study. All authors contributed to the literature search and the writing of the article. All authors have approved the final version of the article.

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Legends to Figures

Figure 1: The jejunum: Structure of the epithelium and composition of the luminal content during the fasting state. Three cell types are present on the intestinal villi of the jejunal epithelium: enterocytes, absorptive cells; goblet cells, which produce mucus into the lumen of the jejunum; and enteroendocrine cells, which secrete hormones into the internal milieu. Stem cells are present at the bottom of the crypts, and they generate Paneth cells, which produce many antimicrobial peptides (AMPs), as well as the other cell types of the intestinal epithelium that, after migration and differentiation along the intestinal villi, die by apoptosis and detachment from the epithelium into the lumen. In the lumen, a thin firmly adherent layer of mucus follows the epithelium, topped with a thicker loosely adherent mucus layer, into which bacteria can enter. Secretory IgA (sIgA) is secreted by enterocytes by transcytosis of polymeric IgA produced by lymphocytes present in the lamina propria. The jejunum exhibits low microbial density and diversity, and the jejunal microbiota community is composed of fast-growing facultative anaerobic (both gram-positive and gram-negative) bacteria and fungi.

Figure 2: Dietary lipid absorption in the small intestine in healthy conditions (A) and in small intestine bacterial overgrowth (SIBO) (B). A: In healthy conditions, triglycerides are hydrolysed by the combined action of bile and pancreatic lipase, leading to production of

fatty acid and 2-monoacylglycerol that are taken up by enterocytes and used for chylomicron synthesis. Almost all lipids are absorbed by the end of the jejunum, and in the ileum, conjugated bile acids are reabsorbed by active transport. Bacteria present in the ileum are able to deconjugate bile acids and metabolize them into more hydrophobic bile acids, which are called secondary bile acids. Unconjugated bile acids are absorbed by the intestinal epithelium in a passive way. **B:** In SIBO with ileum bacteria (here as an example, gram-negative bacteria such as *E. coli*), deconjugation of bile acids occurs already in the jejunum. Unconjugated bile acids are not as efficient for lipid micelle formation, which facilitates triacylglycerol hydrolysis. Thus, this results in lipid malabsorption and steatorrhea. Unconjugated bile salts are cytotoxic and are reabsorbed in a passive way in the jejunum.

Table 1: Predominant bacterial phyla and families found in the human jejunum.
Examples of bacterial species are indicated in brackets.

	Gram + bacteria		**Gram – bacteria**	
Phylum	facultative anaerobes	anaerobes	facultative anaerobes	anaerobes
Firmicutes	Lactobacillaceae (*Lactobacillus*) Streptococcaceae (*Streptococcus*) Staphylococcaceae (*Staphylococcus*) Enterococcaceae (*Enterococcus*)			Veillonellaceae (*Veillonella*)
Proteobacteria			Enterobacteriaceae (*Escherichia*, *Klebsiella*) Pasteurellaceae (*Pasteurella*)	
Actinobacteria		Bifidobacteriaceae (*Bifidobacterium*)		
Bacteroidetes				Prevotellaceae (*Prevotella*)

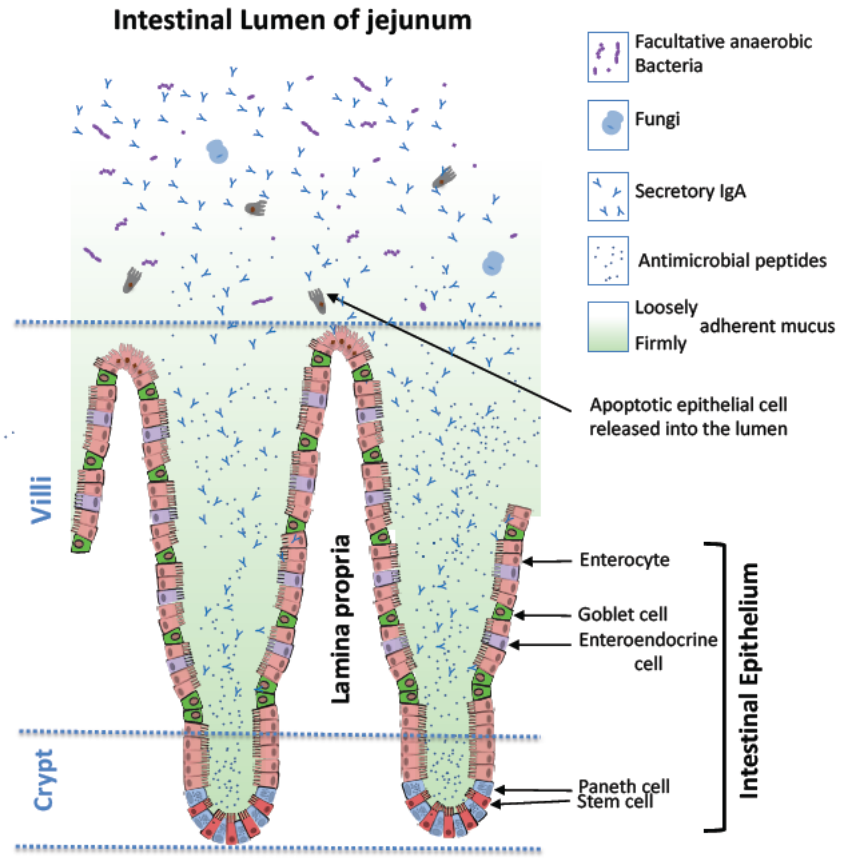
Intestinal Lumen of jejunum
Facultative anaerobic Bacteria
Fungi
Secretory IgA
Antimicrobial peptides
Loosely adherent mucus
Firmly
Apoptotic epithelial cell released into the lumen
Villi
Lamina propria
Crypt
Enterocyte
Goblet cell
Enteroendocrine cell
Paneth cell
Stem cell
Intestinal Epithelium

Figure 1

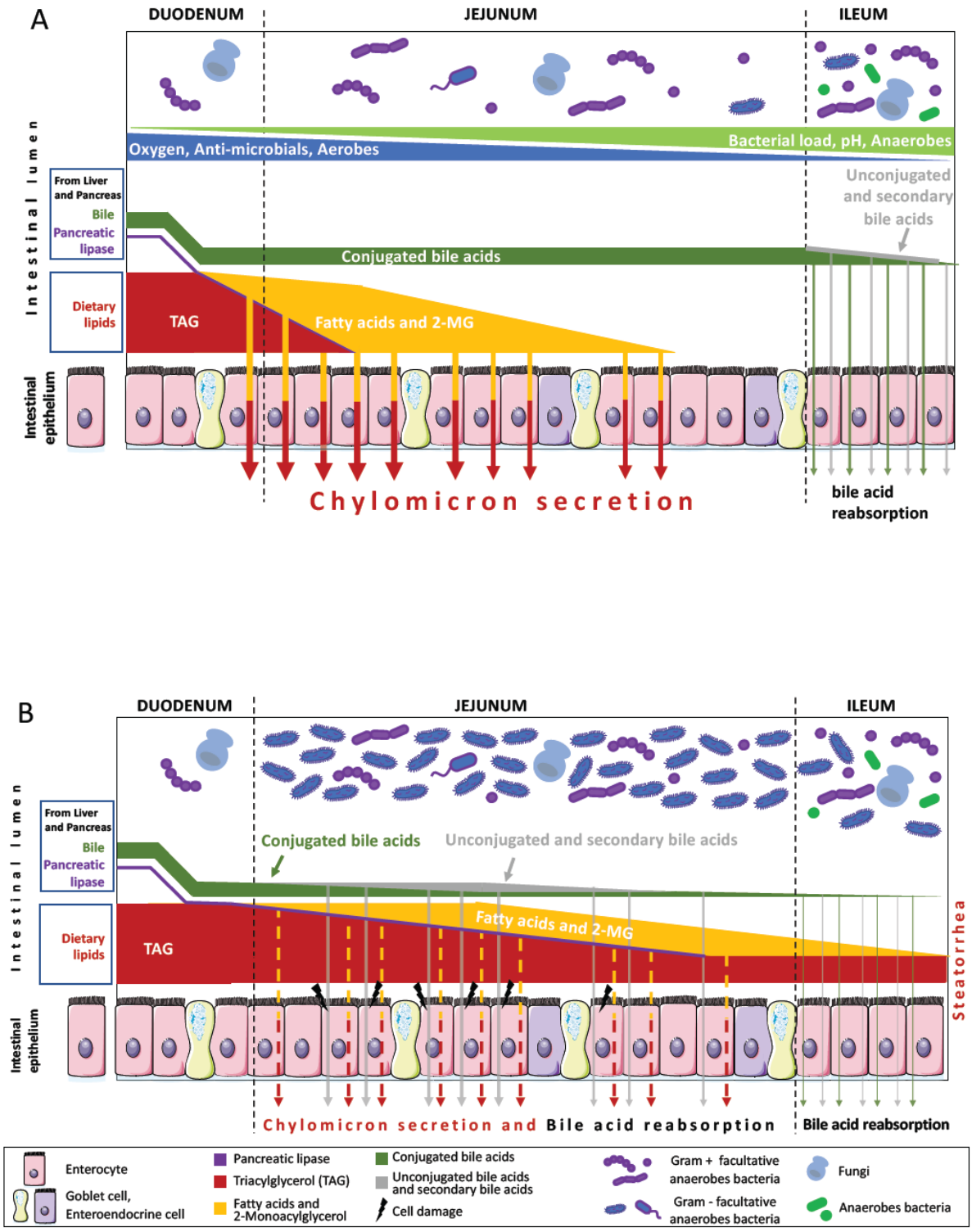
A
DUODENUM
JEJUNUM
ILEUM
Intestinal lumen
Oxygen, Anti-microbials, Aerobes
Bacterial load, pH, Anaerobes
From Liver and Pancreas
Bile
Pancreatic lipase
Unconjugated and secondary bile acids
Conjugated bile acids
Dietary lipids
TAG
Fatty acids and 2-MG
Intestinal epithelium
Chylomicron secretion
bile acid reabsorption
B
DUODENUM
JEJUNUM
ILEUM
Intestinal lumen
From Liver and Pancreas
Bile
Pancreatic lipase
Conjugated bile acids
Unconjugated and secondary bile acids
Dietary lipids
TAG
Fatty acids and 2-MG
Steatorrhea
Intestinal epithelium
Chylomicron secretion and Bile acid reabsorption
Bile acid reabsorption
Enterocyte
Goblet cell, Enteroendocrine cell
Pancreatic lipase
Triacylglycerol (TAG)
Fatty acids and 2-Monoacylglycerol
Conjugated bile acids
Unconjugated bile acids and secondary bile acids
Cell damage
Gram + facultative anaerobes bacteria
Gram - facultative anaerobes bacteria
Fungi
Anaerobes bacteria

Figure 2