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Intestinal plasticity and metabolism as regulators of organismal energy homeostasis

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Abstract

The small intestine displays marked anatomical and functional plasticity that includes adaptive alterations in adult gut morphology, enteroendocrine cell profile and their hormone secretion, as well as nutrient utilization and storage. In this Perspective, we examine how shifting dietary and environmental conditions bring about changes in gut size, and describe how the intestine adapts to changes in internal state, bowel resection and gastric bypass surgery. We highlight the critical importance of these intestinal remodelling processes in maintaining energy balance of the organism, and in protecting the metabolism of other organs. The intestinal reshaping is supported by changes in the microbiota composition, and by activation of carbohydrate and fatty acid metabolism, which govern the intestinal stem cell proliferation, intestinal cell fate, as well as survivability of differentiated epithelial cells. The discoveries that intestinal remodelling is part of the normal physiological adaptations to various triggers, and the potential for harnessing the reversible gut plasticity, in our view, hold extraordinary promise for developing therapeutic approaches against metabolic and inflammatory diseases.

Introduction

Intestinal anatomy.

Principal function of the intestine is to complete the digestion of food, absorb nutrients, and excrete unused remnants while providing selective barrier between the organism and its environment. Through the enteroendocrine cells scattered in the intestinal epithelium, the gut further contributes to the organism's systemic homeostasis by secreting gut hormones, thus serving as a major endocrine organ in the body.

In mammals, the intestine is divided into small intestine - subsectioned into duodenum, jejunum, and ileum - and large intestine, including caecum, ascending, transverse, descending and sigmoidal colon, and rectum (**Fig. 1**). The duodenum in adult humans receives between six to twelve litres of liquified and broken up food (chyme) from the stomach. The nutritional content from the chyme is largely absorbed by the small intestine. What is left for the large intestine is undigested plant fibre, dead cells from intestinal mucosa, salt, water and bile pigments.

Small intestinal length is 6-7 m (large intestine is 1.5 m) in cadavers[1], but estimates of length in living humans vary widely, ranging from 2.5 to 7 meters[2-4]. The inner surface of the intestine contains permanent circular folds of mucous membrane projecting into the lumen, which start from the second part of the duodenum and are present up to the distal part of the ileum (**Fig. 1**), being most pronounced in the distal duodenum and jejunum (this is a feature of human anatomy that is lacking in mice). These folds are called plicae circulares (also known as valves of Kerckring) and they multiply the absorptive surface by 1.6X compared to a surface of smooth tube without them.

The whole epithelial surface is convoluted into villi – dense, finger-like projections of the mucosa. Finally, the cells in the villi contain microvilli (200 million per square millimetre), which are thin actin-based projections resolvable by electron microscopy. Together with the villi and plicae circulares, current estimates suggest that surface multiplication goes up to 60–120 times, for the total absorptive surface of ~30 m^2 in the small, and 2 m^2 in the large intestine, though older literature suggests higher estimates[5].

In *Drosophila*, the midgut is functionally analogous to the small intestine. There are no villi and crypts in the fruit fly—intestinal stem cells (ISCs) are dispersed on the smooth single-cell layered epithelial surface. This feature, together with tools that allow spatiotempotal genetic targeting of individual cell types, facilitates studies of intercellular communication in this model[6 7 8].

In this perspective, we examine how different dietary, environmental and internal triggers contribute to the adaptive non-pathological anatomical and functional intestinal alterations with a focus on the epithelial plasticity in the small intestine, and place these morphological changes in a whole-body metabolic perspective.

Intestinal renewal and differentiation.

The intestinal epithelium is the fastest renewing tissue in mammals. It is constantly and rapidly turned over through divisions of columnar base cells, which function as ISCs. They are located at the bottom of the epithelial invaginations called intestinal crypts (**Fig. 2**). Each villus is built up by several surrounding crypts. During postnatal development, the crypts undergo multiple replication rounds in a process called crypt fission, which plays a positive role in elongation of the intestinal tract. Crypt fusion, on the other hand is an event where two crypts fuse into one daughter crypt, which may be a homeostatic mechanism to maintain intestinal epithelium integrity [9,10].

The ISCs are typically identified by their specific marker, the Leucine-rich repeat-containing G-protein coupled receptor (LGR5), which is a Wnt target gene[11]. ICSs divide to both maintain their lineage, and to give rise to transit-amplifying cells (also called progenitor cells)[12,13,14,15]. The transit-amplifying cells divide 4 to 5 times over two days, commiting to either secretory or absorptive cell lineage (reviewed in[16,17]). From the exit of the crypt, cells move upward along the villus, which is an active process that involves actin protrusion on the basal side by which cells are dragged along the villus[18]. The enterocyte progenitors fully differentiate into enterocytes (main absorptive cells and bulk of the epithelium), while the secretory progenitors finally give rise to enteroendocrine cells (EEC, secretors of gut hormones), goblet cells (involved in secretion of mucus), very rare tuft cells (role in chemosensing and parasite defence), and Paneth cells, which remain in the crypt. Paneth cells surround the ISCs[11] at the bottom of the crypts (**Fig. 2**) , and in addition to their role in secretion of antimicrobial enzymes and peptides[19], express signals such as epidermal growth factor (EGF), transforming growth factor (TGF)-α, Wnt family member 3 (WNT3) and the delta like canonical Notch ligand 4 (DLL4), which establish niche for rise, maintenance and proliferation of the ISCs[20]. Also present in a specialized epithelium that overlies the Peyer's patches are the M cells, which take up and present microbial antigens to underlying lymphoid follicles in the small and large intestine[21].

EECs comprise only 1% of all intestinal cells but collectively, as mentioned earlier, form a major endocrine organ in the body. Gut hormones secreted by the EECs, glucagon-like peptide (GLP)-1, GLP-2, gastric inhibitory polypeptide (GIP), gut hormone peptide YY (PYY), cholecystokinin, oxyntomodulin and others) act systemically on the central nervous system (CNS), on the pancreas, stomach, and other organs in response to food ingestion [22,23]. A subset of gut hormones, most notably GLP-1 and GIP, contribute to the reduction of blood glucose after a meal in part by potentiating insulin secretion, which is why they are also called incretins[23].

The survival potential of the LGR5+ cells may vary. LGR5+ cells located further away are more susceptible to passive displacement and subsequent loss compared to the LGR5+ cells placed at the base of crypts, and therefore have a lower chance to form clones over the long term[24]. These cells are passively displaced by a conveyor-belt-like upward movement; however, they can also travel back

through a Wnt-dependent retrograde cell transport, which determines the number of long-term (effective) stem cells[25]. While the LGR5$^+$ ISCs are key drivers of the intestinal renewal and homeostasis, there are ample of redundant mechanisms in the crypts that can promote intestinal regeneration after damage from inflammation, resection or even severe irradiation[26]. In such case of a damage, a population of rare quiescent, transcriptionally distinct stem cells[27] is activated and helps to reconstitute LGR5$^+$ niche and other cell types. Both secretory[28,29] and enterocyte[30] lineages of progenitors can revert to stem cell phenotype and repopulate the LGR5$^+$ ISC pool in case of their depletion due to irradiation (a typical model of intestinal injury). The potential role of these mechanisms in non-damaging conditions is yet to be established.

Metabolic landscape of gut epithelium.

The small intestine displays marked differences in morphology, nutrient absorption and cellular physiology along its length, as well as the height of its villi[31]. In part, this compartmentalization of the gut is linked to the nutrient gradient in its lumen, formed by digestion and increased microbiota density towards the lower intestine (**Box 1**). In addition, the intestinal stem cell-intrinsic DNA methylation was proposed to also establish and maintain regional gut specification, which are independent of the cellular environment: organoids derived from epithelial samples from adults and children keep the regional identity of their source site during long-term cultures[32,33].

The contrast between the proliferative and the mature cell types in the gut also creates spatially restricted substrate preferences and energy conversion pathways. Regional diversities are present both at transcriptional and protein levels[34,35]. The fuel preferences vary between different cell types, as well as among cells of a single type as we move along the length of the intestine and the crypt-villus axis[36]. The bottom of the villus, similarly to crypts, is heavily dedicated to growth and biosynthesis, and abundantly expresses ribosomal components, splicing machinery, and aminoacyl-tRNA synthetases. The entire gastrointestinal tract retains about half of the absorbed dietary amino acids, of which about one third is recycled back into the circulation, and the rest is catabolized or used for protein synthesis[37].

Glutamate is the major energy sourse used by the enterocytes, which accounts for up to 10% of the total amino acids content in the human diet[38]. The amino acid absorption and metabolism preferentially take place along the mid-part of villi[35]. The mRNA level of genes regulating glucose metabolism, such as TCA cycle, glycolysis and gluconeogenesis, dominate the middle of the villi, though on a protein level the peak of glucose metabolism is closer to the tip[34]. The tip of the villi is marked by high expression of proteins for fatty acid metabolism controlled by the peroxisome proliferator-activated receptors (PPARs), factors involved in peroxisomal oxidation as opposed to mitochondrial one, and by sugar, peptide and fatty acid transporters[34]. Apolipoproteins, important for chylomicron synthesis and lipid transport, are also concentrated on the tip[35]. Villus zonation is not limited to the epithelial layer but extends to the mesenchymal cells. Villus tip telocytes (the only LGR5 expressing cells outside the

crypts) influence zonation changes in enterocytes as they approach the tip of the villus and prepare to shed off[39].

Adaptations of the absorptive surface

The gut plasticity is critical for adaptation to extreme nutritional stress incurred by the loss of significant fraction of absorptive surface[40,41]. However, the intestinal adaptations are also part of normal physiology and are of fundamental importance in the response to the constant environmental or nutritional challenges **(Fig. 2)**.

Food amount and intestinal surface.

Studies in pigs, rats and sheep in the 1980s with radioactive tracers consistently showed that size of visceral organs, including intestines, correlated with food intake[42]. The genetically modified obese *db/db*[43 44] and *ob/ob* mice[44], which eat twice as much as normal mice due to the lack of leptin receptor or leptin, respectively, have increased small intestinal length and villus height[44]. Importantly, the intestinal expansion is disproportional to the lean mass, while it correlates with the fat mass of mice[44,45].

Systematic analysis of mice that substantially differed in their food intake due to different housing temperature (cold exposure at 6°C[45]), appetite (*ob/ob* and *db/db*[44]) or caloric density of given food (diets rich in fat, or indigestible fibre, summarised below[44]), revealed that of the intestinal absorptive surface (length, villi and microvilli) correlated with the bulk amount of the eaten food[44,45]. Regardless of the reason for the increased food intake, the intestinal enlargement was reversible by restricting food intake to that of wild-type mice on standard chow diet kept at room temperature (RT)[44]. These findings point to a general interdependence between food intake and gut size, which is also affected by the composition of the food and the host microbiota (**Fig. 2**).

Germ-free and antibiotic-treated mice have massive increase of small intestine length and absorptive surface, which combined with cold exposure almost doubles villi and microvilli length in the duodenum and jejunum[45]. Likely explanation for this phenomenon, at least in part, is the microbiota importance in food digestion, causing intestines of the microbiota-depleted mice to contain a large volume of chyme and undigested food (**Box 1**). These observations indicate that the volume of food present in the lumen impacts the size of the absorptive surface on multiple levels (length – villus – microvillus), which may be linked, at least in part, to the mechanical pressure that food exercises on its surrounding tissue.

Several possible mechanisms could serve as a starting point for molecular explanation of the food volume – driven intestinal expansion. The ability to sense compressional and tensional forces that the luminal content exercises on the intestinal tissue is critical for proper gut motility, which consists of radial and longitudinal distensions and contractions of the gut wall[46]. Two independent studies showed

that the different fluid pressure between the apical side of crypt cells and the tension forces in villus domain and extracellular matrix regulate crypt morphogenesis[47,48].

In *Drosophila* adult midgut (which can also grow and shrink in response to diet through ISC-based plasticity[49]), genetic studies have linked food ingestion and epithelial stretching with the Hippo pathway: a mechanosensitive and evolutionarily conserved pathway that controls organ size[50,51]. Specifically, epithelial stretching resulting from food ingestion reduces Misshapen phosphorylation and membrane association, probably through a mechanical signal. Changes in Misshapen activity ultimately lead to increased ISC proliferation through Wts/Yki (LAT2 and YAP/TAZ homologues respectively). *Drosophila* studies have also identified the stretch-activated ion channel Piezo as a key mechanosensor that controls both intestinal progenitor proliferation and their differentiation into enteroendocrine cells[52]. Delineating the potential involvement of these pathways in regulating the intestinal plasticity in mammals awaits further attention.

Coping with thermal challenges.

Producing heat to maintain a constant body temperature is energetically expensive[53]. In mice, rising the body temperature by 1°C requires a 10% increase in the metabolic rate[54]. Part of the immediate response to exposure of temperatures well below thermoneutrality is the rapid increase in appetite, which approximately doubles within days[24]. Strikingly, cold exposure causes intestines to grow longer by 10-20% over few days to weeks. In jejunum of mice, this growth is coupled to over 30% increase of the villi length along the mucosal surface, and to a 50% rise of the microvilli, leading to a profound cumulative increase of the small intestine weight[45,55]. Moreover, glucose absorption capacity elevates by 5% per milligram of intestinal tissue, on top of the increase provided by the absorptive surface enlargement[15,55], pointing to an enhanced uptake machinery in addition to the anatomical enlargement. While it remains to be investigated whether cold exposure induces similar changes in humans, these adaptations indicate a remarkable enlargement capacity of the intestine to compensate for the increased energetic demands in conditions of high nutritional abundance. In line with this concept, future studies need to address whether decreased energy needs, e.g. by housing at temperatures closer to the thermoneutrality[56] leading to lowered thermogenesis, would restrict the intestinal surface.

Remodelling during reproduction.

It has been known for decades that the gastrointestinal tract grows both in length and absorptive capacity during pregnancy and lactation[57-59]. Although characterisation of this process at the cellular or molecular levels has not been extensive, changes in both progenitor proliferation and cell size have been described. While some aspects of intestinal remodelling may result from changes in feeding and/or microbiota[57,60], work in *Drosophila* suggests that anticipatory mechanisms may also be at play. Despite obvious differences in reproductive strategy, the *Drosophila* intestine also grows after mating: a process in part driven by a reproductive hormone, which acts directly on intestinal progenitors to increase their

proliferation, and on enterocytes to promote lipogenesis. In flies, intestinal remodelling during reproduction is adaptive: if genetically prevented, reproductive output is reduced[61,62].

These findings indicate that, while different environmental or internal states lead to intestinal growth, the underlying mechanisms may differ. Therefore, exploring how the reproductive remodelling of the mouse intestine compares to that resulting from dietary interventions or changes in intestinal microbiota will be of interest. Work in flies also indicates that epithelial remodelling is the tip of a bigger iceberg involving reproductive remodelling of other intestinal cell types such as gut-innervating neurons[63]. How these different cell types interact to enable reproductive remodelling therefore deserves further investigation.

Adaptation to bowel resection and gastric bypass surgery.

Bowel resection is a procedure most often undertaken due to cancer, blockage, severe inflammation, bleeding, or as a part of the gastric bypass surgery. The intestinal surface can easily regenerate and compensate for the loss of absorptive capacity following such surgical interventions[41,64]. Numerous clinical studies indicated that partial bowel removal causes the remaining part to adapt and start absorbing nutrients beyond its normal capacity. These changes occur within a timespan of a few months to 2-3 years in humans[65], and only weeks to months in mice and rats[66,67].

Depending on the type of bowel resection, as well as on the region of the excised fragment, short bowel syndrome may develop if the remaining small intestine is less than 150-200 cm long (reviewed in[65]). During the short bowel syndrome, crypts in the colon deepen and the tissue starts absorbing medium-chain fatty acids (MCFAs)[68], in addition to the short-chain fatty acids (SCFAs) that are normally absorbed there. Similarly, following jejunal resection the ileum undergoes hyperplasia with a more than two-fold increase in villus height[66] and ileal diameter[67], contributing to increased distal absorption.

In response to bowel resection and short bowel syndrome, secretion of enteroendocrine hormones increases, stimulated by hyperphagia and increased glucose load distally. Glucagon-like peptide (GLP)-1 and pancreatic peptide YY (PYY) slow down gut motility to extend the time that digested juices spend in the intestine in individuals with short bowel syndrome, while GLP-2 contributes to increased intestinal proliferation and growth, coupling nutrient intake to villus height[69,70]. The changes in gut hormone secretion contribute to upregulation of nutrient receptors, particularly sodium dependent glucose co-transporter (SGLT1) on the luminal, and glucose transporter (GLUT2) on the basolateral side.[71,72]

Roux-en-Y gastric bypass (RYGB), is the most effective bariatric surgery intervention used to induce weight loss and ameliorate type 2 diabetes mellitus in morbidly obese patients. During this surgical procedure, the proximal part of the stomach is joined to the distal small intestine, bypassing duodenum and parts of the jejunum. As mentioned above, to compensate the absence of proximal jejunum that

normally absorbs most of sugar and fat, the ileum shifts its absorption machinery and enteroendocrine cell repertoire towards more proximal characteristics[73-76]. The joined part undergoes hypertrophy and shows enhanced luminal and basolateral glucose transport, as well as glucose utilization for intestinal tissue growth.[75] GLP-1 and PYY secretion is increased up to tenfold after surgery, due to the rapid delivery of nutrients to the distal parts of the intestine[23,74]. Increased GLP-1, secreted from the GLP-1 and -2 producing enteroendocrine L-cells, contributes to the improved insulin sensitivity and ameliorated type 2 diabetes following the surgery. Peptide YY regulates ion-coupled absorption of glucose and dipeptides in paracrine fashion in response to nutrients[77]. It also stimulates release of endothelial nitric oxide, which increases blood flow in the enteric circulation.

In addition to the L-cell gut hormones, global metabolomics of serum have identified numerous metabolites that are changed after gastric bypass, including bile acids (BAs), lipids, amino acids, inflammation markers and microbial metabolites[78]. BAs are synthesised by the hepatocytes and as conjugates (glycine in humans and taurine in rodents) secreted into the biliary system. These primary BAs, such as cholic and chenodeoxycholic acid, then transit to the intestinal tract where they play a critical role in solubilization and absorption of dietary fat and fat-soluble micronutrients. The primary BAs are modified by the microbiota into secondary bile acids such as deoxycholic and lithocholic acid, of which the majority (about 90%) are re-absorbed by the enterocytes in the distal intestine and transported back to the liver in a feedback mechanism that controls BAs synthesis.

Bariatric surgery markedly increases the circulating BA levels, and restores the obesity-induced ablation of their post-prandial rise[79,80]. Ileo-colonic delivery of conjugated BAs, and mimicking the physiological effects of BA signalling improves glycaemic control in patients with obesity and diabetes, despite the limited effects on the food intake and body weight[81]. Studies in mice revealed that the nuclear farnesoid X receptor (FXR) and the membrane G protein-coupled bile acid receptor 1 (GPBAR1, or TGR5) are required for the improved glucose tolerance caused by the elevated BAs levels following bariatric surgery[82,83]. It is not known what causes the marked increase in the BAs synthesis following bariatric surgery, nor whether BAs can modulate intestinal plasticity.

Several other mechanisms may contribute to the weight loss and improved glucose tolerance following bariatric surgery. Ablation of melanocortin signalling through whole-body deletion of melanocortin-4 receptor (MC4R), or its pharmacological blockade in the brain indicates its necessity for the bariatric surgery-induced weight loss[84]. Similarly, deletion of insulin-like growth factor binding protein-2 (IGFBP2) leads to approximately 30% less bariatric surgery-induced weight loss[85]. Interestingly, insulin-like growth factor-1 (IGF-1) is also increased in the intestine two weeks after bariatric surgery (RYGB) in rats, and IGF-1 enhances proliferation of the intestinal crypts (including stem cells and other crypt cells) isolated from obese humans[86]. Finally, while leptin levels are decreased after bariatric surgery[87], leptin-signalling is required for the full effects of gastric bypass on the body weight[87,88].

Counterintuitively, leptin increases enterocyte proliferation and reduces apoptosis[89], suggesting dominant role of additional gastric bypass-driven mechanisms in mediating the compensatory enlargement of the remaining part of the intestine.

Dietary modification.

Intestinal changes during consumption of a high-fat diet (40-60% of calories derived from dietary fat), high-fiber diet and upon caloric restriction in mice illustrate the complex relationship between stem cell proliferation and gut or villus length. A fibre-enriched diet elongates the gut, as reported in zebrafish[90] and rodents[91], and in agreement with the common observations that herbivores tend to have longer guts than carnivores[45]. Conversely, a low-fibre diet shortens gut and villi length in mice[92,93].

During caloric restriction, stem cell numbers and proliferation increase within the crypts[94-96] (**Fig. 2**), but paradoxically the intestines of calorically restricted mice are slightly shorter or not changed compared to *ad libitum* fed controls[44,94,95]. Numerous studies reported that fat-rich diets in various formulations increase ISC proliferation in small and large intestines in rodents[92,97-104], as measured by mitotic cell labelling as Ki-67 or BrdU and/or morphometry (addressing crypt depth increase). Puzzlingly, the reported effects on gut size vary. Feeding mice with high-fat diet for several weeks to months increases ISC proliferation through activation of PPAR-δ[99]. In these high-fat diet fed mice, villi length is reported to be increased[43,105], unchanged[99], or decreased[44,92,102]. It is likely that the specific nutrient (triglyceride, carbohydrate and/or amino acid) composition and source, formulation, and microbiota of the experimental mice all partially factor in to establish the specific gut size in fat-rich diets. For example, addition of medium chain triglycerides (C6-C12), which are passively taken up by enterocytes and readily oxidized, increases villus height in rats[106] and piglets[107]. Dietary or endogenously synthetized cholesterol promotes ISC and crypt proliferation, in the process that is connected to phospholipid remodelling in the cell membrane[108].

Two recent studies in mice shed light on the effects of the Western diets rich in fats and sugars towards homeostasis of the gut epithelium and cell-type composition[109,110]. High-fat diet enriched in sucrose caused hyperproliferation of ISC and transient progenitors, accelerated ISC differentiation, and enhanced cell turnover, coupled to elongation of villi and small intestine[109,44]. Similar intestinal and villus growth after sucrose addition was observed in rats[100]. Transcriptomics analysis of the guts from mice fed with sucrose-enriched diet revealed a shift in enteroendocrine cell profile and enterocyte absorptive and metabolic characteristics[109]. Enterocytes assumed phenotype increasingly more typical of proximal jejunum where the main part of lipid absorption takes place, contributing to worsening of the obese phenotype, in line with the increased lipid uptake and accumulation. Similarly, addition of dietary fructose in a form of high-fructose corn syrup - a common sweetener added to food in many countries - to the high-fat diet led to longer intestinal villi[110]. The villus elongation in presence of fructose is based on increased cell survivability on the top of the villi. Mechanistically, dietary fructose

is converted by ketohexokinase to fructose 1-phosphate (F1P). F1P inhibits the M2 isoform of pyruvate kinase (PKM2) in these hypoxic cells[110], diverting intermediates toward biosynthetic and antioxidant pathways, thus promoting cell survival[111]. Deletion of ketohexokinase prevents the elongation of villi, thereby lowering the caloric uptake and tumour growth induced by high-fructose corn syrup. These observations are consistent with our studies in cold exposed and germ-free or antibiotic treated mice, which showed a low rate of apoptosis on the top of the villi as a consequence of a decrease in pro-apoptotic and an increase in anti-apoptotic gene expression[45].

Taken together, the evidence shows that villus size in the intestine depends on the rates of at least three processes: ISC proliferation in the crypts, differentiation of these progenitors and apoptosis at the top of the villus. Finally, work in *Drosophila* underscores the need to consider additional macronutrients and even micronutrients in the context of intestinal plasticity – for example specific dietary amino acids such as glutamate and methionine)[58,112,113]. *Drosophila* gut enterocytes can sense the levels of essential amino acids, both diet- and microbiome-derived, and communicate the macronutrient-deprived condition to the brain through (unexpectedly for enterocytes) production of a peptide hormone: CNMamide[114]. Moreover, the intestinal zinc sensor Hodor activates Tor signalling in a subset of enterocytes[112], promoting food intake and sustaining developmental growth, together suggesting that the intestinal nutrient detection may not only be mediated by sensors residing in enteroendocrine cells (**Box 2**).

The question remains as to what the physiological rationale is that high-fat diet feeding drives ISC renewal and that glucose favors differentiation. The increased proliferation is possibly a compensation for an increased oxidative stress during high-fat diet. High fat content leads to increased oxidative phosphorylation and generation of reactive oxygen species (ROS). ROS cause oxidative stress in the ISCs[115], and promote their increased proliferation, apoptosis and turnover[116]. This increase in the turnover rates may help replenishing and healing the epithelium that is prone to inflammatory damage and loss of the barrier integrity during the high-fat diet feeding and in obesity[117-119]. Conversely, activation of glycolysis by sugar-rich diets relieves the oxidative stress and improves differentiation and lifetime of villi[110], eventually leading to their elongation.

From metabolites to intestinal growth

Metabolic machinery of intestinal cell proliferation and differentaiton.

Intestinal cell metabolism is central for the balance between cell division and cell death in the gut. Proliferation and differentiation of ISCs and progenitors in response to injury, nutrients or inflammation is determined by the intricate interplay between the core signalling pathways involved in intestinal

specification: Wnt, Notch, EGF/EphB, BMP and Hippo[26]. To influence the gut size, metabolic signals need to be transmitted to the core machinery of crypt proliferation and differentiation.

The evolutionary conserved Wnt/β-catenin signalling pathway determines the cellular intestinal specification in the dividing stem cells and progenitors[120,121]. Wnts are paracrine signalling proteins, secreted in the gut mainly by mesenchymal[122,123], as well as by Paneth cells (with the Paneth cells being the source in organoids, **Box 3**), and they trigger canonical and non-canonical pathways in surrounding cells. In the canonical pathway, through its interactions with FZD and LRP5/6 receptors, Wnt stabilizes β-catenin, which then translocates to the nucleus and transactivates expression of the Wnt target genes, many of them implicated in cell cycle regulation. As an example of a coordinated response between the uptake machinery and intestinal proliferation, TMED9, a modulator of Wnt/β-catenin signalling in Paneth cells, is repressed by miR-802, which in turn also down-regulates glucose (SGLT1, GLUT2) and fructose (GLUT5) transporters[124].

Differentiated cells complete oxidation of glucose by importing pyruvate into the mitochondria, a step catalysed by the Mitochondrial Pyruvate Carrier (MPC). While cancer and stem cells robustly consume glucose, they do not fully oxidize the pyruvate that is generated from glycolysis. Limiting mitochondrial pyruvate metabolism promotes the proliferative capacity of these cells (**Fig. 3**). Loss of MPC in LGR5-EGFP^{+} ISCs, or treatment of intestinal organoids with an MPC inhibitor, increases stem cell proliferation, while MPC overexpression in *Drosophila* suppresses it[125]. In the *Drosophila* midgut model, ISCs use the hexosamine biosynthesis pathway (HBP) to monitor the nutritional status. Higher HBP activity promotes Warburg effect-like metabolic reprogramming leading to increased ISC division rates according to the nutrient content[126]. The extent to which changes in the ISC proliferation rates that are regulated by these abovementioned pathways contribute to the alterations in the intestinal surface area remains to be established.

Notch signalling is another evolutionary-conserved pathway that plays a pivotal role in determining commitment to secretory, as opposed to absorptive lineage[127]. Work in *Drosophila* revealed that dietary cholesterol is coupled to stem cell differentiation. By altering Notch and Delta stability in the endoplasmic reticulum, dietary cholesterol changes fraction of enteroendocrine cells produced by the stem cells[128]. LGR5^{+} ISCs, but not differentiated intestinal cells, express *Hmgcs2*[103] (3-hydroxy-3-methylglutaryl-CoA synthetase 2) gene encoding rate-limiting enzyme for ketone body production. As a result, ketone bodies such as beta-hydroxybutyrate (βOHB) are produced in ISCs from the fatty acid-derived acetyl-CoA. βOHB in turn promotes Notch by inhibiting its transcriptional repressor, class I histone deacetylase (HDAC)[103]. Through this βOHB-Notch signalling axis, a ketogenic diet (low sugar, high fat and protein) increases ISC proliferation in mice (**Fig. 3a**). Notably, despite being calorically opposite conditions, both high-fat diet feeding[44,129] and fasting[94] robustly increase PPARα, PPARδ and CPT1α-dependant fatty acid oxidation in crypts, which generates acetyl-CoAs units necessary for

ketone body synthesis. Future work may address whether ketogenesis is indeed a common mechanism behind ISC proliferation in caloric restriction[94] and high-fat diet[129].

Energy used for gut growth.

Increasing gut size, as well as maintaining and renewing the enlarged absorptive surface requires extensive amounts of energy. The enlarged intestines of 30-day cold-exposed (6°C) or obese (*ob/ob*) mice show upregulation of several critical energy-converting metabolic pathways such as glycolysis, gluconeogenesis, glutamate and lipid oxidation[44]. Several enzymes of these pathways are involved in controlling intestinal tissue homeostasis, e.g. by mediating interactions with microbiota and inflammatory processes.

Hexokinase 2 (HK2), a mitochondria-associated glycolytic enzyme, is an example of this control as it mediates cell death in the ileum and large intestine, and may contribute to colitis and Crohn's disease (a subtype of inflammatory bowel disease of the small intestine and upper colon with immunological underpinnings)[130]. Deletion of hexokinase 2, which is upregulated in inflammatory bowel disease and ulcerative colitis, reduces mitochondrial respiration and suppresses intestinal cell death[130]. Interestingly, gut-specific genetic deletion of the rate-limiting enzymes or key regulatory nodes of most of the potentiated energetic pathways: hexokinase (HK2), phosphoenolpyruvate kinase (PCK1), and glutamate dehydrogenase (GLUD1) did not result in detectable alterations of the small intestinal morphology. On the other hand, intestinal knock-out (KO) of the transcriptional factor peroxisome proliferator-activated receptor alpha (PPARα) prevented the intestinal and villus elongation during cold exposure or high-fat high-sucrose diet[44], causing improved glucose tolerance, limited lipid uptake and reduced obesity. This finding indicates that while glucose and glutamate conversions are dispensable for adaptive intestinal growth in overeating, the PPARα-controlled intestinal lipid metabolism is critically involved in regulating the absorption and surface of the gut epithelium, providing the boost in fatty acid oxidation necessary for gut growth in times of need. (**Fig. 3b**). PPARα also stabilizes fatty acid uptake.

Mechanistically, PPARα deletion induces complete blunting of the *Plin2* expression and fully prevents its elevation normally driven by fatty acid uptake by the cell[44]. PLIN2 deletion in mice limits lipid absorption and largely abrogates the long-term deleterious effects of a high-fat diet[131]. Accordingly, the reduced lipid uptake following PPARα inhibition may be a result of three complementary effects: decreased intestinal surface due to shorter villi, downregulation of fatty acid transporters on the apical surface of brush border, and reduced expression of Perilipin 2 (*Plin2*) in the enterocytes[44] (**Fig. 4a**).

Multiple additional studies point towards the fatty acid metabolism as the central metabolic node that drives intestinal plasticity, with different transcription factors dominating the various regions of the intestine[94,97]. PPAR isoforms are critical for the obesogenic response in the intestine, with PPARα

driving jejunal expansion[44] and PPARβ/δ regulating duodenal adaptations[94]. The transcriptional factor PR domain containing 16 (PRDM16) is a PPARs binding partner that acts upstream of many fatty acid oxidation genes and drives progenitor differentiation in the upper intestine[97]. PPARs heterodimerize with their transcriptional co-activator retinoid X receptor (RXR). RXR is necessary for differentiation of progenitors into enterocytes, an important step in villus maturation and growth under normal conditions[132]. Its inhibition maintains crypts in regenerative mode by retaining YAP active, which helps tissue regeneration upon injury. Also, PPARα and PPARγ act as suppressors of the canonical Wnt pathway through inhibition of β-catenin, which would favour differentiation and villus growth[133]. Through these functions, the PPAR system (activated by FAs) integrates lipid sensing in the cell with the transcriptional machinery that regulates cell fate determination in the crypts.

Crosstalk of intestinal plasticity, nutrient uptake and systemic physiology

Beyond its major role in nutrient absorption, the gut participates in maintaining macro- and micro-nutrient homeostasis through neural and circulatory communication with other organs, particularly brain[134-136 137,138], as well liver, adipose tissues, or gonad. There is increasing evidence for key roles of intestinal nutrient sensors in the regulation of nutrient intake, choice and utilisitation (**Box 2**). Hence, besides intrinsic mechanisms of intestinal epithelial remodelling, we must also consider the roles for inter-cellular and inter-organ communication in enabling and mediating epithelial plasticity.

Interestingly, metabolic outcomes of the same energy pathways in the intestine and elsewhere often diverge. One of the best understood examples for such difference is the role of intestinal gluconeogenesis, studied in mice, which surges during periods of prolonged fasting[139] (over 24 hrs in rodents) when it accounts for 20 to 30% of the total endogenous glucose production. Intestinal gluconeogenesis depends on the levels of Phosphoenolpyruvate Carboxykinase (PEPCK) and Glucose 6-phosphatase (G6Pase), which are abundant in the duodenum and jejunum and normally diminish toward ileum. However, PEPCK and G6Pase are highly upregulated in ileum following entero-gastro anastomosis in RYGB[76]. Glucose derived from the intestinal gluconeogenesis is released through the GLUT2 transporter in the basolateral circulation and reaches the portal vein where it is sensed by a portal glucose sensor (**Fig. 4b**). This portal glucose sensing decreases the hepatic glucose production and signals to the brain to reduce eating, leading to improved hepatic insulin sensitivity[140,141] and lowering of the systemic glucose levels.

The microbiota also influences the intestinal gluconeogenesis. Microbiota-derived short-chain fatty acids propionate, butyrate and succinate trigger intestinal gluconeogenesis: butyrate directly upregulates PEPCK and G6Pase in the gut epithelium, while propionate acts through the G-protein-coupled receptor FFAR3 in the peripheral nervous system at the wall of the portal vein[142]. Butyrate also

limits hexokinase 2 expression by targeting histone deacetylase 8 (HDAC8), thus protecting against dextran sodium sulphate-induced colitis in mice. While butyrate directly impacts expression of several genes implicated in intestinal cell fitness, it also inhibits numerous strains from the Bacteroides phylum, a process that depends on the exact glycans used by the respective strains[143]. Investigating to which extent butyrate exerts microbiota remodelling *in vivo*, and whether such butyrate-induced microbiota alterations mediate some of the effects of fructose (as well as of other nutrients) on the intestine will be of interest.

Increased consumption of table sugar and high-fructose sweeteners is suspected to have risen the incidence of obesity and cancer[144]. Excess dietary fructose reaches the liver where it is used to synthesise fat, which contributes to non-alcoholic fatty liver disease, obesity and diabetes[145]. Through intestine-specific ketohexokinase (KHK-C), an enzyme that turns fructose into fructose-1-phosphate and can feed into the glycolytic pathway (**Fig 3b**), the gut takes over the surges of excessive dietary fructose and thereby reduces the load of fructose on the liver (**Fig. 4c**). Knock-out of KHK-C increases fructose absorption into the bloodstream and uptake by the liver[145]. Through this mechanism, the gut buffers fructose loads and shields circulation from detrimental levels of fructose, which is instead used for villi elongation[110].

Lipid absorption in enterocytes is linked with iron levels in white adipose tissue[146] (**Fig. 4a**). KO of transferrin receptor 1 in adipocytes reduces adiposity in both white fat and liver, and improves metabolism under high-fat diet. This is in part a result of the lower transport of lipids by the small intestine through the basolateral side of enterocytes. The precise molecular routes by which adipose tissue instructs this decrease in lipid transport remain to be uncovered[146].

Metabolites can also mediate intercellular and interorgan communication. ISCs and Paneth cells differ in their metabolic identity, and lactate derived from Paneth cells can sustain stem cell function by enhancing mitochondrial oxidative phosphorylation[147] **(Fig. 3a)**. Metabolic communication is not confined to the intestinal epithelium. Lactate is also generated by *Lactobacillus* and *Bifidobacillus* bacteria and binds the G-protein-coupled receptor Gpr81 in Paneth and stromal cells (situated in submucosa), which activates WNT3 secretion from these cells[148] and activation of β-catenin signalling in ISC. In *Drosophila* males, there is a sex-specific coordination between organs through metabolites emanating from the intestine. A cytokine released from testis stimulates intestinal citrate production through JAK-STAT signalling. Enterocyte-derived citrate is taken up by the testes, where it sustains spermatogenesis[149], while also increasing food intake through neuronal signalling to brain (**Fig. 4d**).

Both gut-derived and systemic hormones contribute to the remodelling of the intestinal epithelium in response to dietary changes or internal state. Besides the potential contributions of the reproductive hormones described above, an intriguing candidate in this regard is GLP-2, an enteroendocrine hormone that increases crypt cell proliferation, suppresses apoptosis and promotes gut and villi growth[150].

Collectively, these studies have increased our awareness that the sensors and mediators of intestinal epithelial plasticity often lie outside the intestinal epithelium itself, and involve a combination of systemic and paracrine signals and metabolites.

Human perspective and challenges

Obesity is caused by energetic disbalance between chronic excess in food intake and energy expenditure. Which of the two is more decisive in the modern human setting has long been debated[151,152]. Due to patient variability and inherent difficulties in measuring intestinal length in living people, reports on gut dimensions in subjects with obesity are scarce. Surgical measurements in patients undergoing bariatric surgery or laparoscopy suggested that intestinal length correlates with body weight either weakly[4] or not at all[2,3,153]. However, the recent work in mice[44,110] indicates that the gut size should not simply be associated with the BMI, but also with the etymology of the disease. It will be of critical importance to extend the intestinal measurements to perimeter, villi and microvilli assessments, couple them with careful dietary and life-style assessments, and integrate them with cell type, as well as with immunological profiling.

In this context, subjects with obesity have an increase in pro-inflammatory macrophages in the stomach, duodenum, and colon, coupled with rise in intermediate blood monocytes[154], as well as in intestinal CD8αβ$^+$ T cells[155]. This inflammatory environment in obesity is linked with intestinal dysfunction, facilitating dysregulated glucose homeostasis[156]. Moreover, studies in mice show that γδ T cells mediate the adaptive response of enterocytes to the increased abundance of sugars in the diet[157]. Intestinal γδ T cells regulate the carbohydrate transcriptional program by limiting interleukin-22 production from type 3 innate lymphoid cells, further pointing to the importance of the immune cells in modulating the epithelial function and plasticity.

As mentioned earlier, intestinal resection causes bowel lengthening, villi growth, and induction of transporter expression[40], which are processes linked to mucosa thickening. The PPARα-driven mechanism responsible for the overeating-induced villi growth[44] could explain why fatty acids such as palmitate or linoleate are the most effective dietary therapy that promotes mucosa growth after bowel resection[158,159], which can be further potentiated by feeding prostaglandins[160]. In line with this observation, disruption of PPARα and fatty acid oxidation is found in celiac disease, a condition characterized by shortened villi and malabsorption[161].

Application of gut hormones is another good example of how the gut-related findings can be used in the clinics. In this respect, pharmacological GLP-1 analogues have been in use for years as potent insulinotropic drugs, while GLP-2 analogues (such as teduglutide) have been approved for treatment of short bowel syndrome. Studies in rodents and humans used the GLP-2 analogues as an intestine-specific

growth factor that restores mucosa and blood supply[162,163], and suggest them as potential therapy of Crohn's disease[150], enteritis, and post-irradiation diarrhoea.

The discoveries that intestinal remodelling is a part of the normal physiological adaptations to environmental and dietary triggers[44,45,110] hold extraordinary promise to harness the reversible nature of gut plasticity for tackling metabolic and inflammatory diseases. It is tempting to envision a next generation of anti-obesity therapeutics that would target nutrient uptake by modifying the intestinal absorptive capacity, regulating the specific nutrient transport, or by altering the EEC activity and number.

Single-cell profiling of intestinal cells, organoids, and high-throughput sequencing of gut flora metagenome have advanced gut research over the past decade (**Box 3**), deepening our understanding of the spatial distribution in the cellular metabolism. However, challenges remain. We are currently limited in our ability to therapeutically target the intestinal machinery with a molecular precision needed to change the metabolic output of the gut, or its absorption of macronutrients. More research is also needed to identify how specific molecular signalling cascades govern nutrient uptake, conversion, and distribution by the intestine, and how can we externally control it. Finally, studies in humans are in practice limited by the availability of biopsies, which are typically derived from patients undergoing gastric bypass surgeries or cancer removal. Establishing a unified model of adaptive gut growth that links the intestinal environment (nutrients, microbiota, mechanical pressure) to the cell proliferation, survival and differentiation, as well as to the nutrient transport machinery, would facilitate discovery of approaches for therapeutic interventions in the gut.

Box 1. Microbiota and gut anatomy. Microbiota comprises trillions of microorganisms, including bacteria, fungi, viruses, protozoa, and archaea, with composition influenced by its environment or host[164]. The gut microbiota of humans (and other organisms) with obesity largely differs from that of lean subjects, the former harbouring an increased capacity to harvest energy from the diet[165]. Microbiota, as well as its absence or depletion[45,166], modifies gut structure, while microbiota products such as SCFAs regulate enzymatic activities in metabolic pathways in the gut[130,142]. Cold–exposure induced alterations in the microbiota composition are sufficient to increase the intestinal surface and caloric uptake[45]. Transplantation of the cold–adapted microbiota leads to altered intestinal gene expression promoting tissue remodelling and suppression of apoptosis, an effect that is diminished by co–transplanting the most cold–downregulated strain *Akkermansia muciniphila* during the cold microbiota transfer. Consecutively, supplementation of *Akkermansia muciniphila* to cold–exposed[45] or high-fat diet-fed mice[167] reduces the caloric uptake from the consumed food. Through its metabolites SCFAs, branched-chain amino acids, indole, secondary bile acids and lipopolysaccharides, microbiota can alter enteroendocrine cell number and secretion profile[23]. Such mechanisms operate in various

condition, e.g. during high nutritional availability and intake, where microbiota impacts appetite and secretion of insulin through signaling cascade that involves the parasympathetic nervous system through increased acetate turnover that potentiates secretion of the 'hunger hormone' ghrelin[168 169].

Box 2. Intestinal detection of nutrients. An active role for the gastrointestinal tract in sensing and relaying nutritional information was suggested decades ago, following the discovery that glucose triggered insulin secretion more effectively when supplied orally than when administered intravenously: the so-called incretin effect [170]. Subsequent studies naturally focused on the detection of sugars, leading to the current model whereby sweet taste receptors (T1R2 and T1R3 subunits) and the sodium-glucose co-transporter SGLT1 stimulate hormone secretion from enteroendocrine cells, contributing to the regulation of glucose storage and uptake[171 137,172,173].

Enteroendocrine cells also make local contacts with vagal neurons, and intestinal sugar detection via this route may control food choices, allowing mice to distinguish sugars from non-nutritive sweeteners [134,137,174]. Intestinal nutrient detection is not confined to sugars; there is also some evidence for sensing of peptides and lipids. Products of protein digestion are detected via multiple nutrient chemosensors including the proton-coupled peptide-transporter, PEPT1 (SLC15A1) and the calcium-sensing-receptor CaSR, both of which contribute to hormone release from enteroendocrine cells[172,175-177]. Fatty acid detection may also be mediated by several free fatty acid receptors as well as the fatty acid transporter, cluster of differentiation (CD) 36. G protein-coupled receptors GPR40 and GPR120/FFAR4 may detect medium/long-chain fatty acids, whereas GPR41 and FFAR2/GPR43 may detect microbiota-derived short-chain fatty acids[172,178-180]. Further work will be required to establish the physiological significance of these mechanisms beyond enteroendocrine hormone secretion and glucose homeostasis.

Finally, it is important to note that intestinal nutrient detection may not only be mediated by sensors residing in enteroendocrine cells. *Drosophila* has recently provided examples of enterocyte-based mechanisms. A case in point is the CNMamide neuropeptide produced by enterocytes to drive protein appetite in response to protein restriction[114]. Work in *Drosophila* also led to the identification of Hodor: an intestinal zinc sensor that activates Tor signalling in a subset of enterocytes[112]. Hodor promotes food intake and sustains developmental growth from this small subset of enterocytes, underscoring the need to consider micronutrients such as metals in the context of intestinal plasticity and nutrient sensing.

Box 3. From organoids to physiology. Organoids are extensively used to investigate transcriptomic regulation during intestinal stem cell division and differentiation. They are an indispensable tool to study gut plasticity in isolation without interference of the complex *in vivo* physiological environment. Quantification of organoid size, crypt budding and formation of secondary colonies from passaged organoids provides a measure of intestinal proliferation using reductionist approaches. Growth in

presence of WNT (WENR medium) produces spherical organoids composed of stem cells, while removal of WNT initiates budding of crypts and differentiation of mini-villus domains.

The key comparative strength of organoids and their derivations based on WENR protocols is that they can be formed and subcultured directly from patient biopsies and from human iPSCs. They are the starting point for personalized medicine, drug screens and genetic perturbations using CRISPR approach, typically delivered by electroporations[181,182]. Organoids were used to measure osmo-mechanical forces that govern villus growth vs. cell division balance[47,48,132]. However, organoids have limited utility for physiological uptake tests due to their closed spherical anatomy, which makes them unsuitable to study luminal-to-basolateral transport[183,184]. Various workarounds have been tried out. Microinjections of metabolites and even microbes into luminal domain of organoids are possible[185]. Another approach is breaking up the organoids in extracellular matrix and fostering them to reform inside-out in suspension, with apical side facing outward[186,187]. Matthias Lüttoff's group developed bioprinted and microfabricated scaffolds and hydrogels, which restrict intestinal morphogenes to confined zones that direct tissue development into forming proper *in vitro* intestinal tubes with assessable lumen[188]. Monolayerized organoid cultures have been also used as a simpler version of intestinal cellular complexity for studying cell function and cell type changes in response to luminal nutrients or pharmacological stimuli[182,189], and can be grown directly on the plate or on transwell insets that provide access to both luminal and basolateral side. Establishing reliable cellular models derived from human biopsies (e.g. patient derived organoids) remains critical. Incorporation of non-epithelial cell types in organoid cultures (developed as 3D, 2D on cell culture insets or on organ chip or scaffolds), ranging from co-culturing in presence of bacteria[190], to reconstitution of the native anatomy with functional macrophages, neurons and other stromal cells developed in proper physiological relation to epithelial cells[191] is a priority for future research.

Figure Legends

Figure 1. Basic anatomy of small intestine and colon.

In mouse and human, the small intestine is divided into duodenum, jejunum and ileum, with most of the breakdown and absorption of macronutrients taking place in the proximal half of the small intestine. In mouse, the cecum is an enlarged blind sac between small and large intestine where fermentation of fibre-rich components takes place; in human, it is a small pouch at the beginning of the large intestine assisting in salt absorption and lubrication. In humans, mucosal membrane (epithelial cells, lamina

propria and loose connective tissue below) of small intestine (especially in jejunum) is folded into spiralling circular folds (plicae circulares). In mouse and human, the absorptive surface in increased by dense carpet of villi, finger-like projections of mucosa. Each villus is built up by several surrounding crypts, which are deep invaginations in the epithelium. The microvilli are thin actin-based projections present at the apical (luminal) side of the enterocytes (main absorptive cells), and express genes involved in nutrient degradation and uptake machinery. Colon is the longest part of the large intestine that absorbs water, as well as part of the remaining nutrients and electrolytes, solidifying the remnants to stool.

Figure 2. Dietary and microbial cues models the intestinal absorptive surface.

Villus is the basic functional unit of nutrient sensing and absorption in the small intestine. It is constantly renewed through division of $LGR5^{+}$ intestinal stem cells (ISCs) that are located in the crypts. The ISCs differentiate into enterocytes, mucus-secreting goblet cells, as well as enteroendocrine and tuft cells. Villus homeostasis is balanced by apoptosis and shredding of the cells located at the top of the villus. The intestinal anatomy and composition can be altered by environmental factors in the gut, with the volume of the luminal content playing a critical role driving tissue expansion. Increase in the luminal content through increased eating or lack of microbiota promotes elongation of the villi and the microvilli. By assisting in fibre breakdown, providing substrates, and possibly by other mechanisms including immunological regulation, the gut microbiota moderates gut, villi and microvilli length, with some species (e.g. *Akkermansia muciniphila*) exercising profound effects. In part, the intestinal morphogenesis is conveyed by mechanosensing through specialized and non-specialized mechanosensory cells. The composition of the diet influences the enteroendocrine cell number and function in secretion of hormones. The gut elongation results from crypt fission by which crypt bifurcates into two daughter crypts in a still poorly understood process, intense during infancy, but slowed down into adulthood[192]. In mice, 3-4% of the crypts at a given time undergo crypt fission, which is balanced out by similar fusion rates.

Figure 3. Interplay of metabolites controls the balance between intestinal cell division, differentiation and growth.

a, Intestinal stem cell (ISC) self-renewal is fuelled by glycolysis and mitochondrial respiration of fatty acid (FA)-derived (yellow arrows) acetyl-CoA[193]. Ketone bodies such as beta-hydroxybutyrate (βOHB) are produced in the intestinal stem cells from the FA-derived acetyl-CoA, thanks to the expression of

3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2). βOHB promotes cell proliferation by activating the Notch signalling (through suppression of its transcriptional repressor, class I histone deacetylase). A portion of the substrates for TCA cycle in the stem cells originates from lactate (green arrows) that is derived from the adjacent highly glycolytic Paneth cells. Lactate is converted into pyruvate before being channelled for oxidative phosphorylation (through Mitochondrial Pyruvate Carrier, MPC). This is a point of regulation of ISC activity, as the MPC is typically inefficient in the stem cells, thus favouring cell proliferation. Lactate also regulates ISC function indirectly as a signalling molecule. It increases WNT3 secretion from Paneth and intestinal stromal cells in the submucosa. WNT3 activates WNT/β-catenin signalling in ISC.

b, In progenitors and enterocytes, the oxidative phosphorylation of pyruvate-derived acetyl-COA (through MPC) is robust and favours intestinal cell differentiation. In both ISCs and enterocytes, the FA oxidation is driven by the transcription factors PRDM16, PPARα, PPARδ. High levels of dietary fructose are trapped in enterocytes by phosphorylation of fructose to fructose-1-phosphate (F1P) by ketohexokinase (see also Figure 4b). F1P inhibits the M2 isoform of the pyruvate kinase in hypoxic intestinal cells. This reduces generation of reactive oxygen species and promotes cell survival by dampening apoptosis and improved survivability of the cells along the villus leading to their net growth. Both proliferation and differentiation of ISCs, as well as increased survival of the differentiated cells contribute to the villus elongation.

Figure 4. Nutrient uptake in intestine is modified by circuitry of enteroendocrine cells, enterocytes, and peripheral organs.

a, Triacylglycerides (TAGs) are taken up by enterocytes as fatty acids (FA) by CD36 fat transporter and accumulate as cytosolic lipid droplets that are stabilized by perilipin 2 (PLIN2). In mice fed with high-fat diet, PPARα is critical for promoting lipid uptake: intestinal KO of PPARα or PLIN2 reduces villi height and lipid uptake, and ameliorates diet-induced obesity, glucose intolerance and liver steatosis. Through an unknown mechanism, the lipid uptake in intestines is correlated with the iron levels in white adipocytes (dashed lines): reduced iron in adipocyte limits adiposity in white fat and in liver of mice.

b, Intestinal gluconeogenesis is upregulated after bariatric surgery, as well as by short-chain fatty acids (SCFAs) and by succinate generated by microbiota during fermentation of fibre-rich food. Glucose is exported through GLUT2 on the basolateral side and released into the systemic circulation. This increase in glucose is sensed by the portal glucose sensor that promotes satiety signals, inhibits the

hepatic glucose production (HGP) and limits the *de novo* lipogenesis in the liver. These mechanisms contribute to the beneficial metabolic adaptations following gastric bypass surgery.

c, Enterocytes buffer the excess of dietary fructose from reaching the circulation. Fructose is transported through GLUT5 (not shown) on the apical side of the enterocyte brush border, and is trapped inside by its phosphorylation to fructose-1-phosphate (F1P) by ketohexokinase (KHK-C). This process diverts fructose towards the intestinal phosphate pentose pathway that supports villus growth, rather than enabling its extensive release into the circulation through GLUT2, thereby protecting the liver from fructose-driven steatosis.

d, The Upd1 cytokine secreted from the testis of *Drosophila* leads to activation of JAK-STAT signaling in posterior midgut enterocytes, which activates the expression of the male-specific genes of sugar metabolism. Citrate (green dots) secreted through Indy channel upregulates food intake and sustains spermatogenesis in the testis.

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Authors' contribution

All authors conceptualised, discussed and wrote the paper. O.S. prepared the figures. M.T. initiated the project.

Ethical declarations

Competing interests. O.S., I.M.A, and M.T. declare no competing interests.

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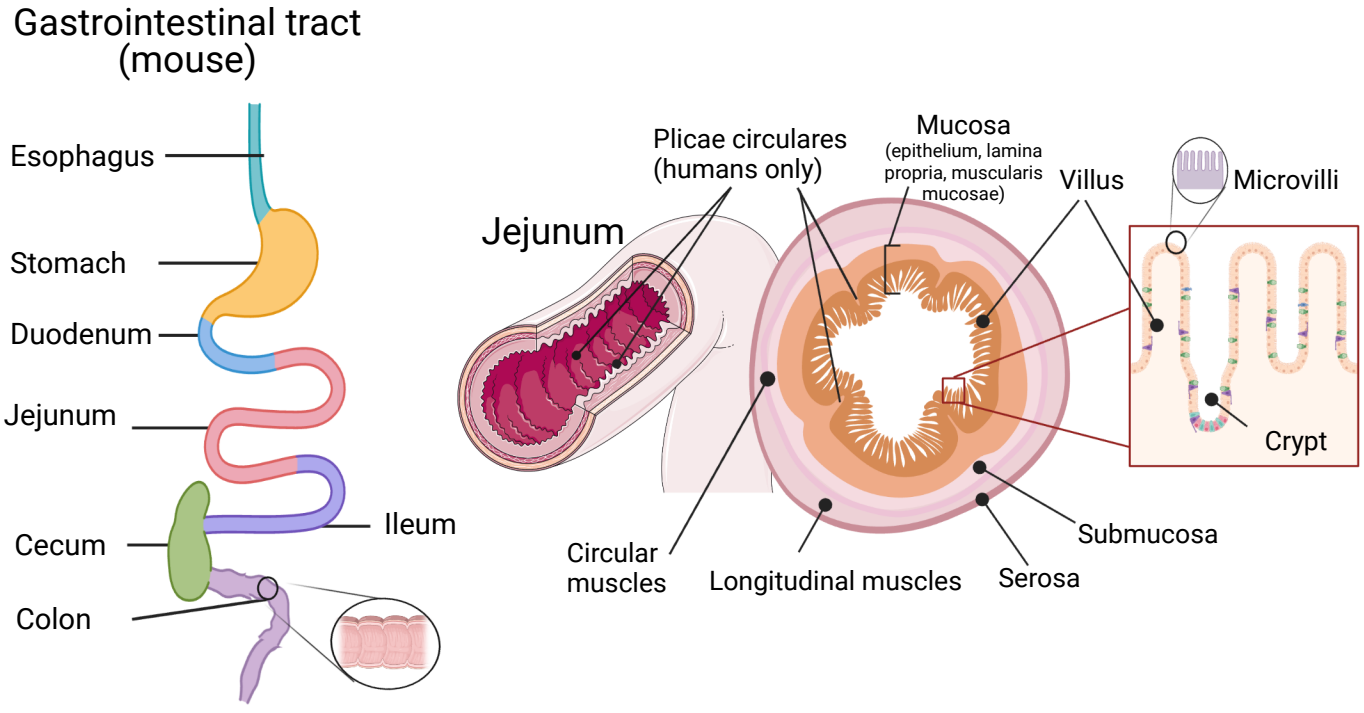
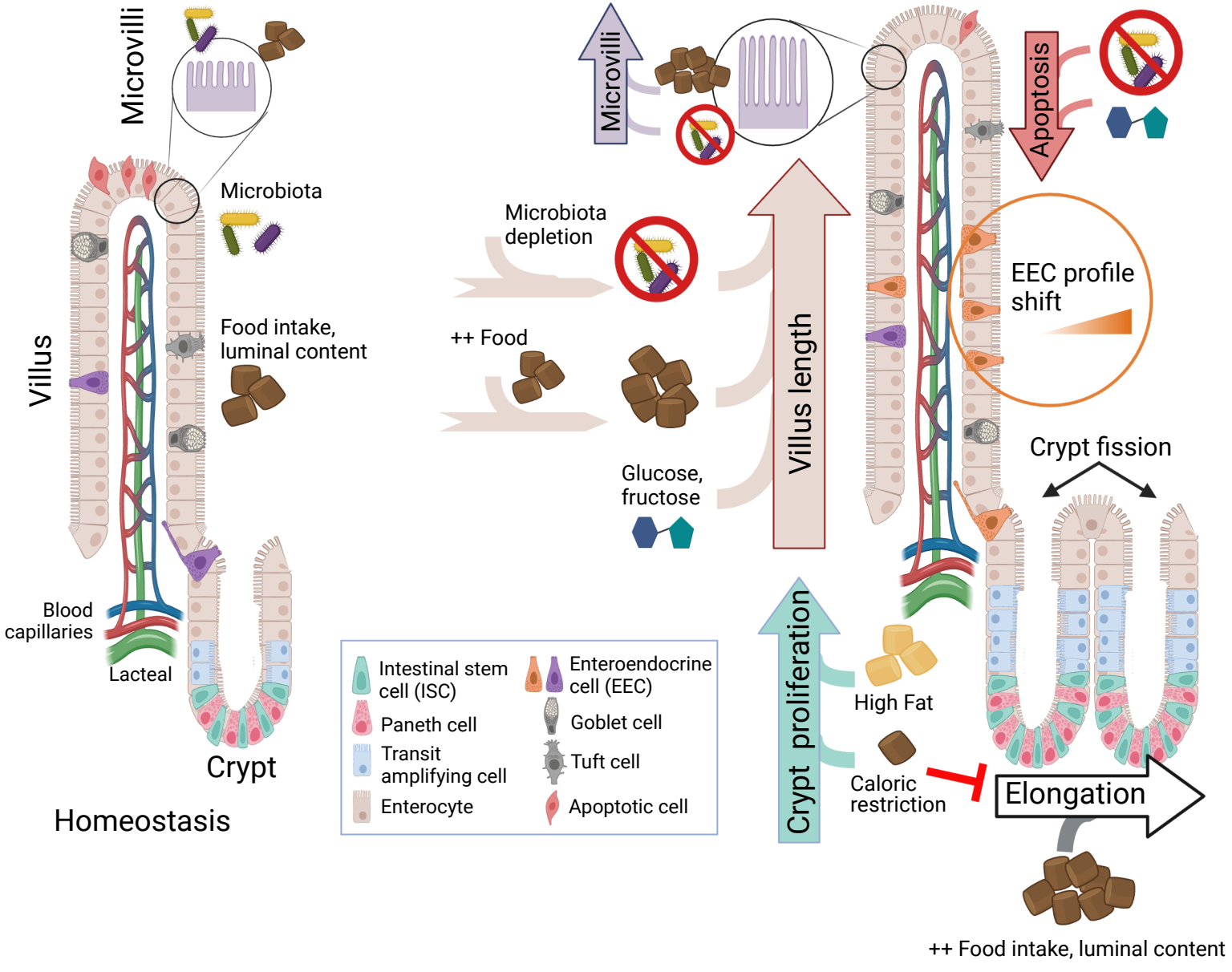
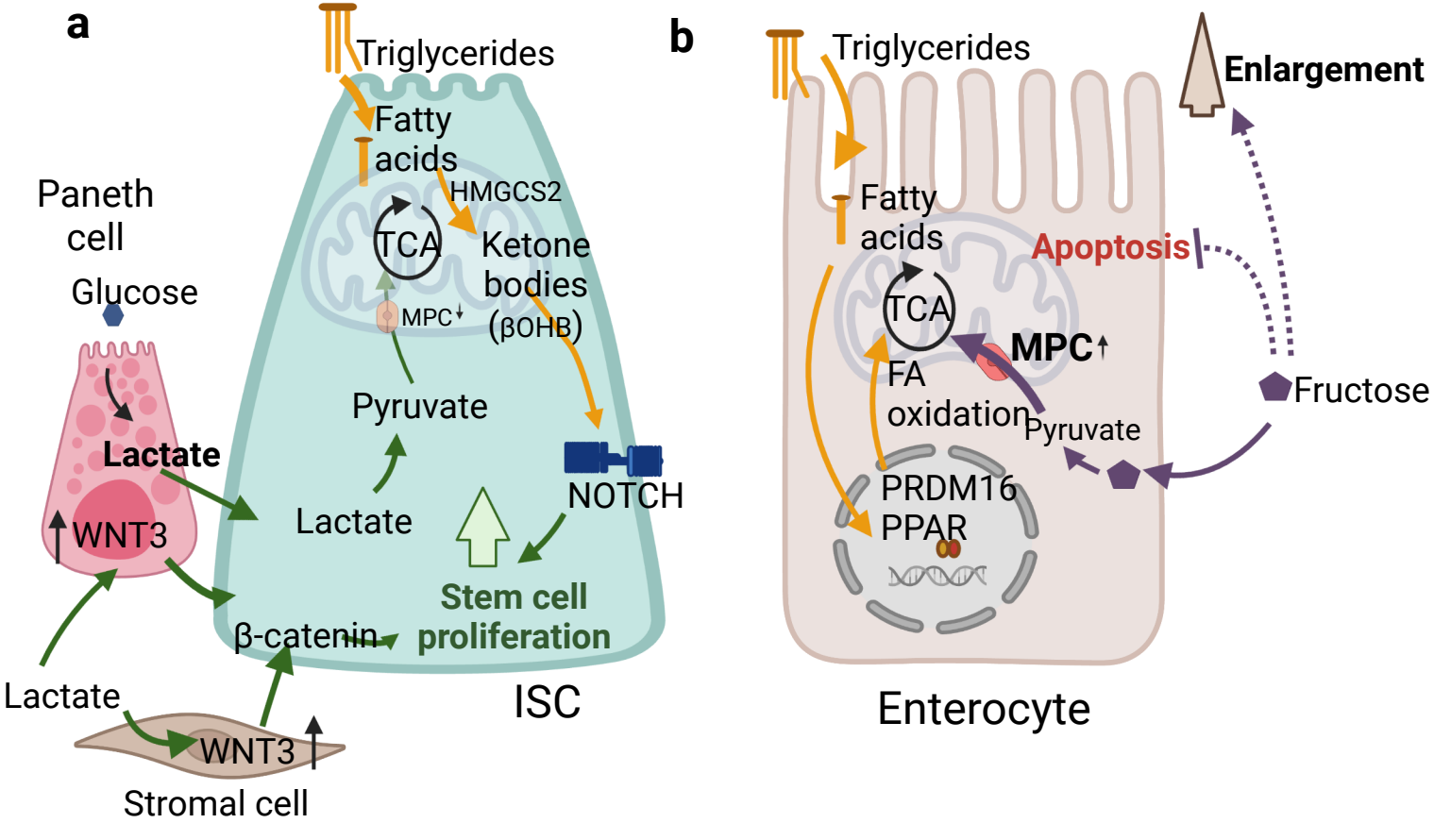
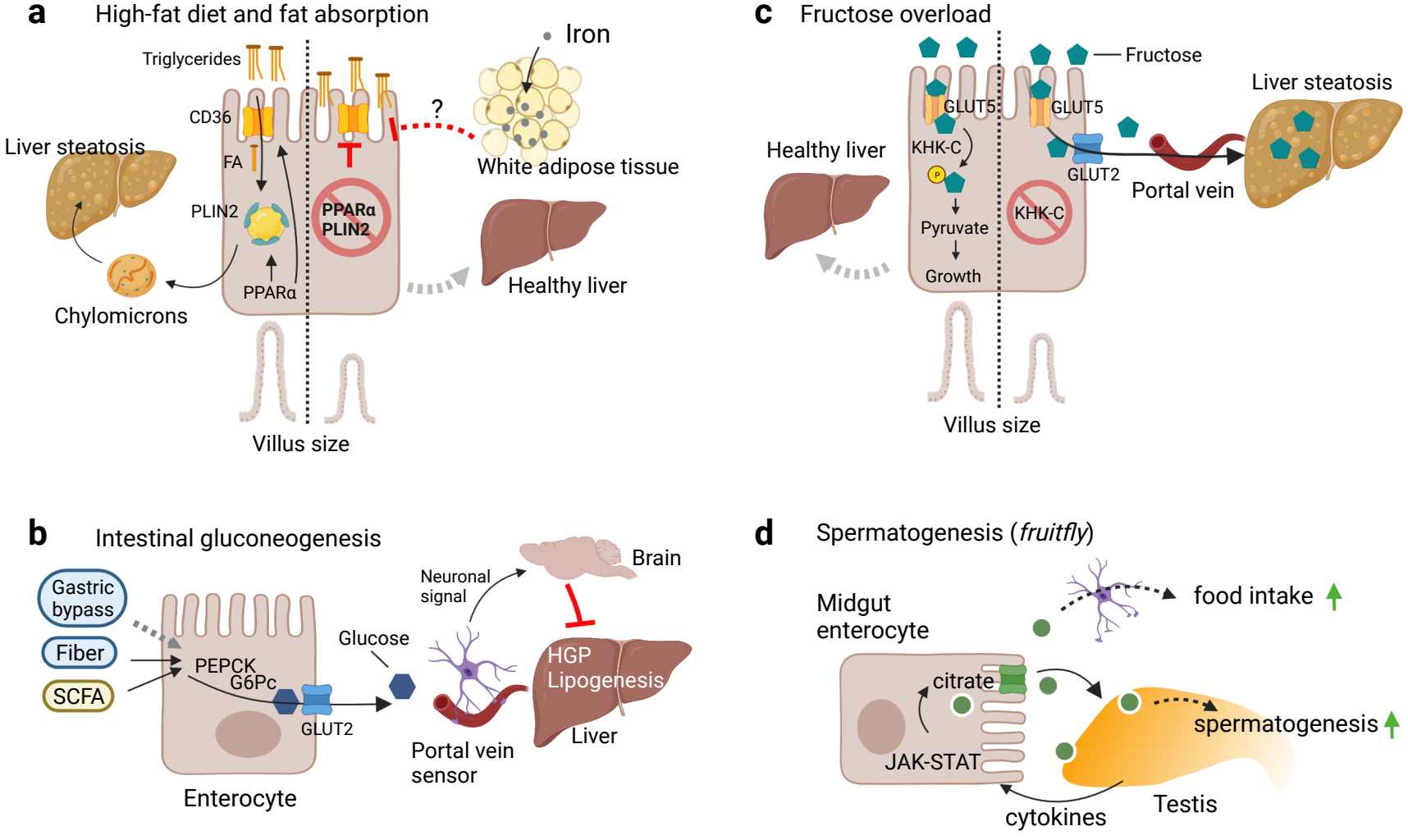

Gastrointestinal tract
(mouse)
Esophagus
Stomach
Duodenum
Jejunum
Ileum
Cecum
Colon
Jejunum
Plicae circulares
(humans only)
Mucosa
(epithelium, lamina
propria, muscularis
mucosae)
Villus
Microvilli
Crypt
Circular
muscles
Longitudinal muscles
Serosa
Submucosa


Microvilli
Microbiota
Villus
Food intake,
luminal content
Blood
capillaries
Lacteal
Crypt
Homeostasis
Intestinal stem
cell (ISC)
Paneth cell
Transit
amplifying cell
Enterocyte
Enteroendocrine
cell (EEC)
Goblet cell
Tuft cell
Apoptotic cell
Microvilli
Apoptosis
Microbiota
depletion
++ Food
Glucose,
fructose
Villus length
EEC profile
shift
Crypt fission
Crypt proliferation
High Fat
Caloric
restriction
Elongation
++ Food intake, luminal content


a
Triglycerides
Fatty acids
HMGCS2
TCA
Ketone bodies (βOHB)
MPC
Paneth cell
Glucose
Pyruvate
Lactate
WNT3
Lactate
NOTCH
Stem cell proliferation
β-catenin
ISC
Lactate
WNT3
Stromal cell
b
Triglycerides
Enlargement
Fatty acids
Apoptosis
TCA
MPC
FA oxidation
Pyruvate
Fructose
PRDM16
PPAR
Enterocyte


a
High-fat diet and fat absorption
Triglycerides
CD36
FA
PLIN2
PPARα
Chylomicrons
Liver steatosis
PPARα
PLIN2
?
Iron
White adipose tissue
Healthy liver
Villus size
b
Intestinal gluconeogenesis
Gastric bypass
Fiber
SCFA
PEPCK
G6Pc
GLUT2
Enterocyte
Glucose
Neuronal signal
Brain
HGP
Lipogenesis
Liver
Portal vein sensor
c
Fructose overload
Fructose
GLUT5
GLUT5
KHK-C
P
Pyruvate
Growth
KHK-C
GLUT2
Healthy liver
Liver steatosis
Portal vein
Villus size
d
Spermatogenesis (fruitfly)
Midgut enterocyte
food intake
citrate
JAK-STAT
spermatogenesis
cytokines
Testis