Nutritional Approaches and Gastrointestinal Health and Physiology

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Take-Home Message

The epithelium of the gastrointestinal tract is responsible for nutrient absorption, regulating transport of drugs and other compounds into the body and mediating the response to microbes and any other stimuli in the lumen. Diet and inflammation are the main signals that regulate the cellular composition and integrity of the gastrointestinal epithelium. During the early stages of life, diet and the environment will define the gut microbiota, epithelial composition and integrity that in turn modulate animal growth and metabolism. In order to define diets and supplements that optimize health and performance, we should take into consideration the animal developmental stage and the particular effect of nutrients on the different cell types of the intestinal epithelium.

Introduction

The gastrointestinal tract (GI) is a major player in animal performance. We constantly hear that a healthy gut is the key for proper development. Therefore, gut health is a familiar but hard to define term. Gut health refers to the complex response of the intestinal epithelium to luminal stimuli (diet, drugs, commensal and pathogenic microbiota, viruses, etc.) that may result in changes in epithelial integrity and composition, nutrient absorption, inflammation and metabolic disorders. Gut health is affected by numerous stimuli and the animal response to those is dependent of the developmental and health state of the animal and the environmental conditions.

The focus of this manuscript is to review the GI response to nutrients and changes in diet emphasizing the changes in the epithelial cell compartment and the overall consequences of those changes. Aware of the significant differences in the structure of the alimentary tract among animal species, in this review the word stomach refers only to the acid-secreting digestive compartment and we will discuss the cell types and structures that are present in the stomach, small and large intestine of most common species, including fish.

The epithelium of the GI is a formed of a single columnar cell layer from the esophagogastric junction to the anus. This epithelial lining has different cell type composition and organization along the GI. In general, the stomach epithelium is organized in glands that can be simple or have convoluted deep pockets. The stomach mucosa formed of the columnar secretory epithelium, has two morphological and functionally different parts: the proximal oxyntic mucosa of the fundus and corpus (for simplicity, we will refer to this part as corpus) characterized by acid and ghrelin secretion, and the distal antrum, distinguished by the secretion of the hormone gastrin. From the pyloric sphincter, the small intestine epithelium arranges in invaginations called crypts and finger-like structures called villi. The crypts contain the stem cells niche, transient amplifying and Paneth cells. The villi are lined by differentiated cells, most of them the absorptive enterocytes. The length and width of the villi change along the duodenum, jejunum and ileum to maximize the area for nutrient absorption. The epithelium of the colon is formed of

crypts that increase in length in the distal colon. Each fragment of the GI tract then has specific compartmentalization and cell type composition that defines the organ function. This specificity is also reflected in the epithelial response to different conditions and stimuli, and is dependent of the animal developmental stage and environment. Figure 1 presents a schematic view of the organization of the GI tract and the cell types found in each segment.

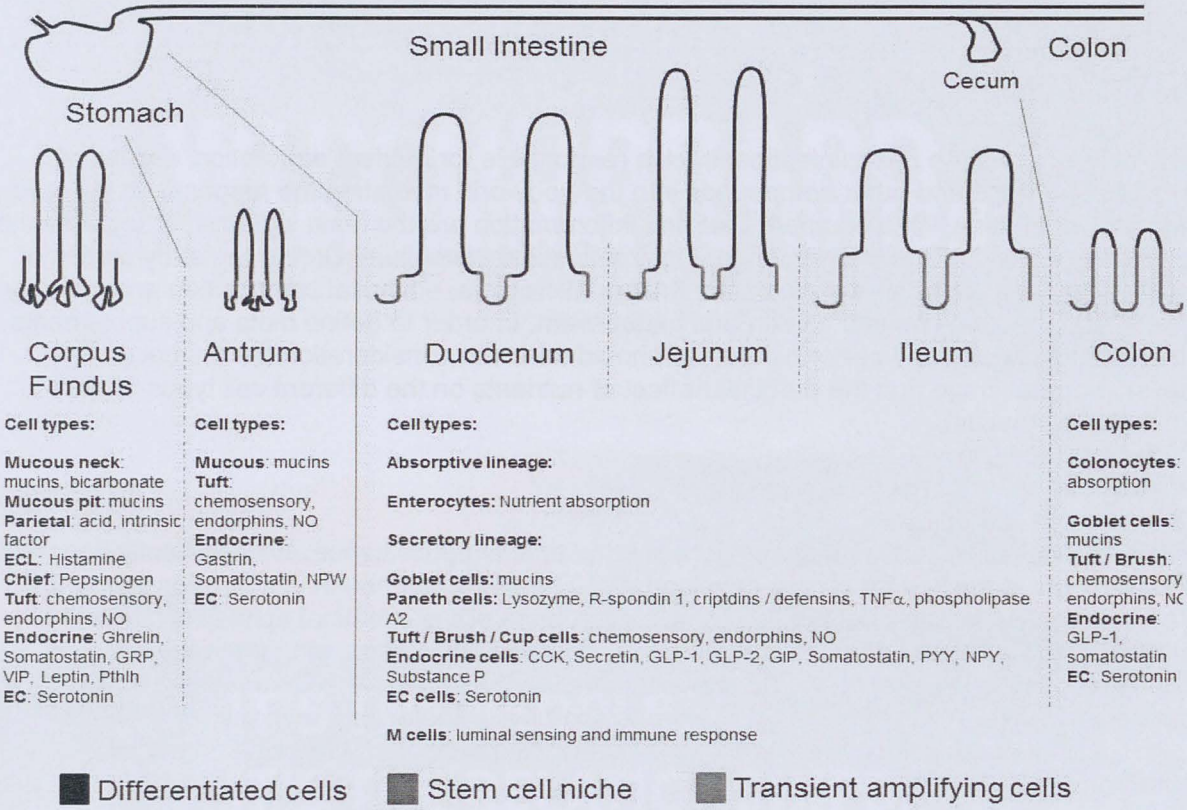

Figure 1: Schematic representation of the glandular organization along the gastrointestinal tract. The glands of the stomach have simple or convoluted deep pockets. Gastric glands have stem cell niches at the neck (corpus and fundus oxyntic mucosa) or at the bottom (antrum) of the glands. The intestinal epithelium organizes in villi and crypts that have different height and deep ratios along the small and large intestine. The stem cell niche is located at the bottom of the crypt and lined by the transient amplifying cells that give rise to the differentiated cells that populate the villus. Abbreviations: NO: Nitric oxide, GRP: Gastrin releasing peptide (bombesin), VIP: Vasointestinal peptide, Pthlh: Parathyroid hormone-like hormone, NPW: Neuropeptide W, TNFa: Tumor necrosis factor alpha, CCK: Cholecystokinin, GLP-1, -2: Glucagon-like peptide-1, -2, PYY: Peptide YY, NPY: Neuropeptide Y.

The Early Stages of Life

In contrast to most organs and systems of the body, the GI tract is not fully developed at the time of birth (de Santa Barbara et al., 2003, Keeley and Samuelson, 2010). The primitive gut tube derives from the dorsal part of the yolk sac and it is one of the first structures established

during embryogenic development. The muscular, neural and stromal components and accessory organs of the GI tract are completely developed and functional long before birth. In humans, around the end of the fourth week of gestation, the stomach is evident and its vagal innervation established; by the twelfth week, the GI tube has its mature form and accessory organs, bile synthesis begins on this stage (Sargiacomo et al., 1998). The oral cavity and anal canal derive from the ectoderm while the rest of the tract has components of different origin: the lining or epithelium is of endodermal while the muscular and connective tissues are of mesodermal derivation. The endodermal component of the GI tract achieves maturity after birth in a process driven mostly by luminal stimuli, i.e. presence of food (Zhang et al., 1997). In most animals, the villi formation and epithelial cytodifferentiation of the intestine starts later at embryonic development, E14 for the mouse and the gross structure of crypt and villi is evident by E18.5. However, even when all the cell lineages of the intestine are present at the time of birth (Madison et al., 2005), endocrine and Paneth cells do not reach functional maturity until 16 days after birth (Suzuki et al., 2005). In contrast, the stomach gland morphogenesis occurs only after birth, and adult epithelial cell lineages appear during the first postnatal weeks achieving maturity only around day 21 (Keeley and Samuelson, 2010, Saqui-Salces et al., 2011). The significance of this delayed gastric maturation has been highlighted in many studies, mostly regarding adaptation of the brood to adult diets and modeling pediatric GI pathology (Cranwell et al., 1976, Sangild et al., 2013).

The need for treatment of GI disorders in human pediatrics and the survival and successful adaptation of preterm animals to growing diets are the main forces driving the identification of factors that may participate in intestinal epithelial cell maturation and recovery from damage. Humans and animals on total parenteral nutrition (TPN) present significant atrophy of the gastric and intestinal mucosa (Furumoto, 1992, Goldstein et al., 1985, Heneghan et al., 2013) and their recovery is dependent of the intestinal maturity at the time of TPN, having a milder effect when TPN was administrated to preterm compared to preterm piglets (Oste et al., 2010a). Different studies have shown that feeding colostrum promotes cell proliferation and differentiation of damaged intestine while formula administration after TPN resulted in increased damage (Oste et al., 2010b, Støy et al., 2014). Treatment with bile acids and glucagon-like peptide 2 (GLP-2) induce intestinal cell proliferation favoring adaptation in young animals (Thymann et al., 2014, Ipharraguerre et al., 2013) but a similar treatment may have adverse results in piglets older than 4 weeks (Pereira-Fantini et al., 2008), further underscoring the importance of GI maturity for animal health and performance.

The GI developmental process has numerous implications on gut health. During the first few weeks of life, the stomach does not have sufficient acid secretion capability (Keeley and Samuelson, 2010, Saqui-Salces et al., 2011), compromising not only digestion but also the immune response of the animal by allowing the survival of bacteria that otherwise would be killed by the acidic gastric environment. In addition, at this stage the Paneth cells are just initiating their function as producers of antimicrobial products (Clevers, 2012, Farin et al., 2014), further limiting the innate immune response of the newborn. The neonatal stage is critical for the establishment of adequate gut microbiota and the lifelong health of the animal. We will expand on this important issue in another section.

The GI development, as well as the patterning and programming of the newborn for growth, health and even predisposition to metabolic diseases is determined by the environment *in utero* and during early life (Fernandez-Twinn and Ozanne, 2010). Substantial evidence is available supporting the importance of the environment in reproduction, growth, fat deposition, obesity, metabolic and cardiovascular disease and even mental development. The hypothesis behind

this is that at embryonic stages, in response to the environment, epigenetic adaptation occurs in order to warrant the survival and development of the product in the conditions cued by the intrauterine environment. When there is a significant difference between the offspring and the mother environments, the adaptations programmed *in utero* may result disadvantageous for the product (Gluckman et al., 2008). To mention one of the possible targets, the type of diet the animal receives in early life defines the insulin-like growth factor 2 (Igf2) gene promoter methylation pattern, and thus gene expression (Waterland et al., 2006). Studies in experimental animal models emphasize how proper diet supplementation to the mother and offspring with choline, folate and vitamin B12 may counteract some of the epigenetic imprinting of early development (for a review see (Vickers, 2014). How much of the mother and early life imprinting occurs in production animals is just starting to be analyzed and considered as a factor in animal performance.

Diet composition and some nutrients in particular have been associated with changes in the cell populations or cell function of the intestine. Glutamine has been identified as a key amino acid that participates in protein synthesis in the enterocyte, the regulation of tight junction proteins and maintenance of epithelial integrity in the small intestine (for a comprehensive review see (Wang et al., 2014). Dietary supplementation with sodium butyrate is a good example of a component that is of importance for the development of the GI epithelium but of limited efficiency in overall performance. Butyrate, as other bacterial produced short chain fatty acids in the large intestine, is a well-known energy source for the distal intestinal epithelium (Donohoe et al., 2011, Leonel and Alvarez-Leite, 2012) and supplemented in the feed can improve gain:feed ratio in pigs in the first 2 weeks postweaning (Manzanilla et al., 2006). This limited effect may be explained by butyrate promoting the development of gastric glands, acid secreting parietal cells and endocrine cells of the stomach in the weaned pig (Mazzoni et al., 2008). Once the stomach is functionally mature, butyrate supplementation is negligible for epithelial differentiation.

Stem and Differentiated Cells in the Adult

The last decade of research on stem cells has generated significant understanding of how cell renewal and differentiation occurs in the adult intestine and a recent report finally defined the markers for the elusive stem cells of the gastric corpus and fundus. The stem cell niche of the gastric glands is located close to the neck of the oxyntic glands and towards the base in the antral glands (el-Alfy et al., 1987, Karam and Leblond, 1995). Stem cells of the antrum express the leucine-rich repeat-containing-G-protein coupled receptor 5 (Lgr5) (Barker et al., 2007) while the reserve stem cells of the oxyntic mucosa seem to be chief cells that also express the marker Troy (Stange et al., 2013). The stem cells of the stomach divide and the resulting trans-differentiating and differentiated cells move up and down from the stem niche to populate the glands. Cells that move towards the tip of the glands undergo anoikis and are shed off to the lumen; cells at the bottom of the glands are eliminated by autophagy (Karam and Leblond, 1993a, Karam and Leblond, 1993b).

The intestinal stem cell niche is located at the base of the crypt. Lgr5 positive stem cells are intercalated with Paneth cells and divide each day to maintain the stem cell population and produce the differentiated cell types that will rise from the transient amplifying compartment in the crypt (Clevers, 2013a, Clevers, 2013b, Middendorp et al., 2014). The villi are populated only by differentiated cells that move along the villus until they undergo anoikis. Differentiated cell types have different life spans: gastric mucous cells, enterocytes and goblet cells are short-lived (4-5 days) while other secretory cells, Paneth and tuft cells may live up to 8 weeks.

Accumulating evidence suggests that the epithelia of the small intestine and colon have more than one cell type with stemness capacity and that although cells expressing Lgr5 are the main progenitors of the intestinal cell lineages in the normal conditions, certain insults or damage activate other subsets of progenitor cells to restitute the intestinal epithelium (Yan et al., 2012).

Damage to the intestinal epithelium is caused by numerous factors with a wide range of effects that may include inflammation, loss of cell contact, changes in epithelial cell population, changes in epithelial exchange rate and destruction of the epithelial and glandular structures. The capacity to recover from damage depends on the insult extend and the effects on the stem cell niches. The mesenchymal cells and the extracellular matrix in close proximity to the stem cell niche provide the signaling factors that modulate the mitogenic activity of the stem cells and mediate the differentiation process (Simons and Clevers, 2011, van der Flier and Clevers, 2009). In this way, both luminal and body signals modulate the regeneration and integrity of the epithelium, and its ability to recover from damage.

With the use of mouse models, the mediators of intestinal cell proliferation and differentiation that commit precursor cells to a differentiated phenotype have been identified (VanDussen et al., 2012, Gerbe et al., 2011, Mori-Akiyama et al., 2007, Petersen et al., 2014). It is now well stablished that due to lateral inhibition, the cell commitment to differentiate into absorptive enterocytes or to secretory cells is mutually exclusive (Figure 2). This means that the expansion of one cell type occurs only at the expense of other cell types, unless there is a significant increase in the proliferative rate and/or decreased anoikis of the intestinal epithelium. Overall increase in the proliferative rate and decreased anoikis will result in a total elongation of the villi. The lengthening of the crypt suggests an increase in proliferation or arrested differentiation, a process that is associated to the development of intestinal adenomas. The presence of proliferating cells above the crypt area indicates the activation of progenitor cells different from the Lgr5 niche or that the differentiation signaling is compromised. Any of these events will result in a loss of differentiated cell lineages and the consequent alteration in function associated to the lineages affected.

Different studies have shown that some diets induce changes in GI epithelial cell numbers. The most common observation is the increase of goblet cells in animals fed high fiber diets (Ito et al., 2009). A recent report shows that feeding rats with high fat diet resulted in impaired endocrine cell differentiation that favored enterocyte differentiation via the modulation of the expression of factors like Math1, neurogenin 3 and neuro D1 (see Figure 2) (Sakar et al., 2014). This work provides an explanation to the increased enterocyte numbers observed in obese humans and animals by different groups (Mao et al., 2013, Gniuli et al., 2010) and changes in endocrine cells induced by fat in the diet (Saqui-Salces et al., 2012). Strangely, in production animals the effects of the feed on GI epithelial cell population have not been properly analyzed. The first stage to establish gut health is the maintenance of the epithelial population. More research is needed to understand how diet participated in this maintenance and whether feed changes result in decreased energy efficiency due to loss of absorptive capacity or decreased endocrine function of the intestine.

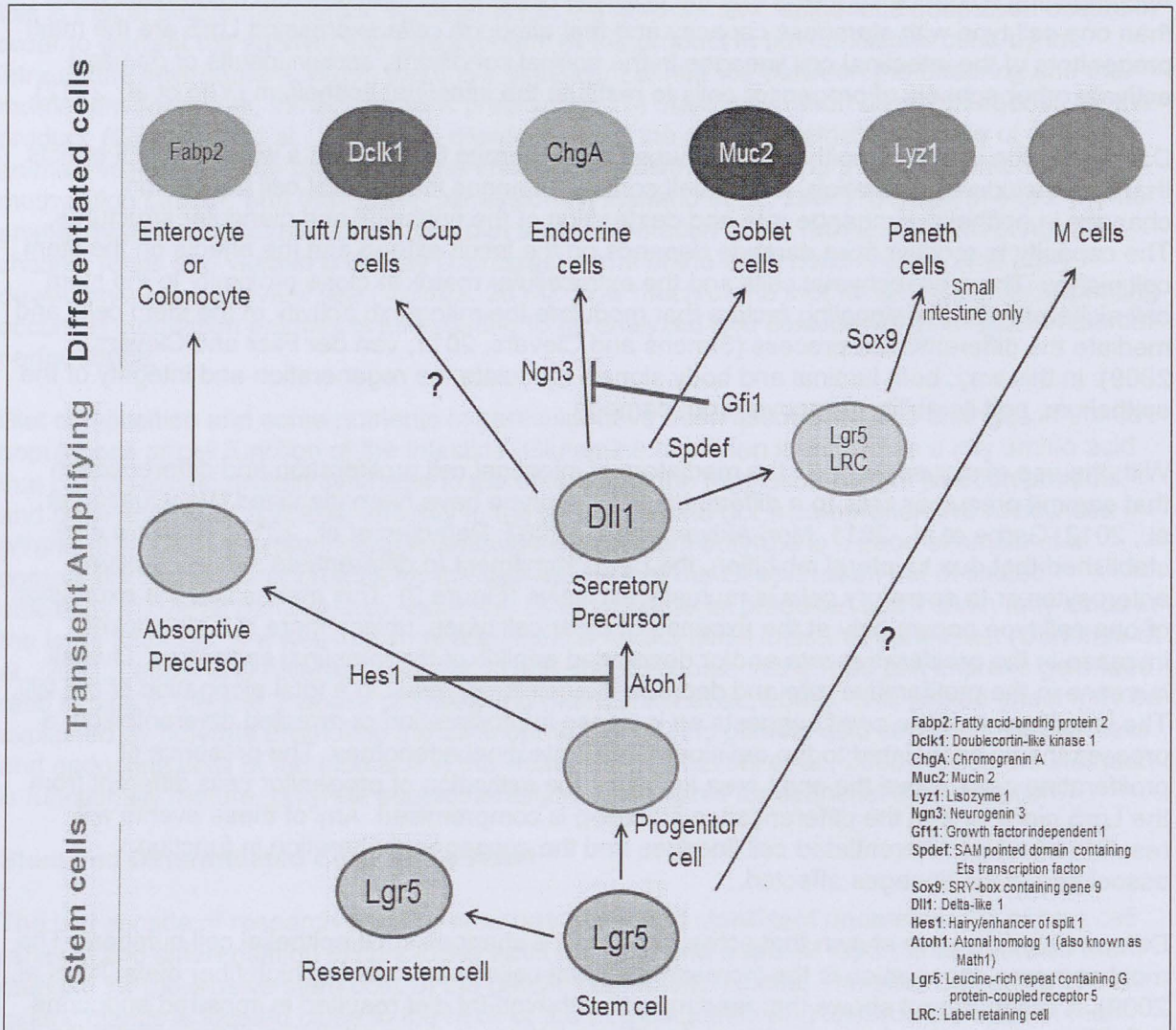

Figure 2: Intestinal stem cells (identified by Lgr5) give rise to transient amplifying progenitor cells that differentiate into all the mature cell types (specific markers are indicated for each cell type). There are different steps that regulate the final cell type populations. The expression of Hes1 or Atoh1 define the absorptive or secretory lineage commitment. The secretory lineage is further segregated to endocrine or goblet / Paneth cells by the action of Gfi1.

Nutrient Sensing and Endocrine Secretion

During the last decade, the finding that the GI tract is populated with cells that express receptors to nutrients and food components similar to those that trigger taste and olfactory sensations (Tolhurst et al., 2012) has generated new understanding on how diet can affects whole body metabolism and performance (Janssen and Depoortere, 2013). Most of the luminal GI chemosensing occurs in endocrine cells. Diet regulates the signaling and expression of chemosensory receptors and changes in GI hormones that regulate appetite, satiation, insulin secretion and sensitivity, nutrient absorption, liver function and gut microbiota composition,

overall modulating weight gain, muscular, adipose, bone, cardiac and immune function (Diakogiannaki et al., 2012, Gribble, 2012).

A significant number of receptors and signaling molecules associated with chemosensing have been identified to date in the luminal GI (Reimann et al., 2012, Tolhurst et al., 2012, Nøhr et al., 2013). We have previously reported that these chemosensory molecules are expressed in tuft and endocrine cells of the mouse stomach and intestine. These markers appear late in development and are expressed once the organs are fully mature (around 3 weeks after birth) (Saqui-Salces et al., 2011). Only a partial characterization of some chemosensory markers in the pig stomach has been reported (Widmayer et al., 2011, Mazzoni et al., 2013, Colombo et al., 2012) and identification of chemosensory cells and mediators in most other production animals is still lacking. This lack of basic information limits our capacity to modulate sensory signals to improve desired physiological responses to increase nutrient absorption and growth efficiency while reducing costs and environmental impact.

Food is the main trigger of endocrine secretion in the luminal GI and the main signals for GI hormone secretion are peptides, fatty acids and sugars; minerals as vanadium (Thompson et al., 2009) and selenium (Pinto et al., 2012) modulate insulin sensitivity and glucose metabolism. In the stomach, food induces gastric acid, pepsin, intrinsic factor, gastrin-releasing peptide (bombesin) and approximately twenty different peptides and hormones; the functions of most are currently undefined. The small intestine secretes, among other, cholecystokinin (CCK), secretin, glucose-dependent insulinotropic peptide (also called gastric inhibitory polypeptide) (GIP), polypeptide YY (PYY), somatostatin, serotonin and the glucagon-like peptides 1 and 2 (GLP-1 and GLP-2)

Gastrin is a peptide hormone whose main function is to induce pepsinogen and acid secretion in the stomach. Gastrin is also an incretin (GI hormones that in response to feeding induce insulin secretion to decrease blood glucose levels) and a differentiation factor for gastric and colonic cells (Wang et al., 2000, Singh et al., 2000). Gastrin stimulates the endocrine and secretory pancreas (Tellez et al., 2011, Boushey et al., 2003), the gall bladder (Valenzuela et al., 1976), the heart (Grossini et al., 2011) and cardiovascular system (Charlot et al., 2011). Gastrin secretion is regulated mainly by peptides in the diet (Kidd et al., 2009) and fat (Saqui-Salces, 2012).

Ghrelin is secreted by the endocrine X (also called A-like and P/D1) cells of the gastric corpus and fundus. Ghrelin is the ligand for the growth hormone secretagogue receptor and modulates food intake (Bewick et al., 2009, Ueno et al., 2005), the accumulation of fat mass (Perez-Tilve et al., 2011), glucose homeostasis (Dezaki et al., 2011), the immune (Xia et al., 2004) and central nervous systems (Steiger et al., 2011). Its expression follows a circadian pattern (LeSauter et al., 2009) and circulating ghrelin peaks just prior to food intake (Barrachina et al., 1997) but the signals that regulate its expression and secretion are not well defined.

CCK is secreted by I-cells in the duodenum in response to peptides and free amino acids in the chyme. CCK (originally named pancreozymin) took its name after one of its specific functions, the induction of gall bladder emptying (EDHOLM, 1960), playing a significant role in the first step for intestinal fat absorption. CCK also modulates GI motility (Johnson and Magee, 1965), stimulates the release of digestive enzymes from the exocrine pancreas (Zieve et al., 1966), and counteracts ghrelin-induced appetite signaling thus contributing to satiety and reduction of food intake (Sayegh et al., 2014, Overduin et al., 2014). As other GI hormones, CCK also participates in the differentiation and modulation of cells of the immune system (Zhang et al., 2014, Jia et al., 2014). Although CCK secretion is stimulated by peptides (Cuber et al., 1990,

Nishi et al., 2001) and aromatic amino acids in particular (Meyer and Grossman, 1972), the specific peptides and receptors involved in CCK secretion are not defined to date.

GIP is another incretin secreted in K-cells of the duodenum and ileum. For many years, GIP was thought to inhibit gastric acid and motility, now it is clear that the responsible for such actions is another GI hormone: secretin. GIP induces insulin secretion in response to luminal glucose (Holst and Gromada, 2004), it stimulates glucose uptake and has lipogenic acitvity (Hauner et al., 1988). GIP secretion is triggered by glucose and dietary fats (Falko et al., 1975), facilitating glucose absorption from fat/carbohydrate mixed diets (Collier et al., 1988) and thus increasing metabolizing energy intake in monogastric animals and ruminants (Miyawaki et al., 2002, Relling et al., 2014).

By far, the most studied incretin is the ileal GLP-1. While glucagon (secreted in the pancreas) regulates blood glucose by stimulating gluconeogenesis and decreasing glycolysis in the liver as a counterpart of insulin, GLP-1 decreases glucagon secretion and stimulates insulin secretion in response to glucose in the diet (Lamont et al., 2012). GLP-1 secretion is stimulated by glucose and galactose but not by fructose, mannose, xylose or 2-deoxyglucose (Shima et al., 1990), highlighting the importance of the carbohydrate sources in the diet on the modulation of insulin secretion and insulin actions. Monosaturated fatty acids and bile acids also stimulate GLP-1 secretion. While GLP-2 is important for fat absorption (Hsieh et al., 2009) and enterocyte differentiation as we mentioned before (Ipharraguerre et al., 2013), GLP-1 modulates glucose metabolism (Chai et al., 2012), reduces appetite, gastric emptying (Baggio et al., 2004) and increases insulin sensitivity and microvascularization in skeletal muscle (Subaran et al., 2014).

Although there is enough information on the importance of GI endocrine function for growth and development, reports on how real-life diets modulate glucose, GI hormones and energy homeostasis are scarce (Forbes et al., 2013). In particular, the use of GI hormones in regulating appetite and satiation may be limited because most experimental models use intracerebral infusion of peptides that may or not reach the brain in normal conditions. The use of diet as a tool for modulating physiology in order to improve nutrient absorption and favor production traits is currently being explored in humans (Viladomiu et al., 2013) and extensively in domestic animals. However, most studies overlook the metabolic characterization of the tested diets, limiting our capacity to evaluate and further improve the overall responses with minimal modifications.

The Gut Microbiota

Numerous studies have shown gut microbiota establishes in the early months of life. The microbial profile is defined by maternal health and nutrition, hygiene, geography, environment and especially diet composition and feeding timing. Gut microbiota affect the immune system, metabolism and nutrient utilization and thus whole body performance in previously unsuspected ways that include growth, metabolic control, response to inflammation and stress and even aggressive behavior. The importance of the gut microbiome and bacteria species that have beneficial and detrimental effects on health has been widely reviewed. However, strategies attempting to improve health and growth by modulating the gut microbiota (via probiotics, prebiotics or diet changes) have resulted in limited or no success. Recent studies show that there is significant heterogeneity in the gut microbiota among individuals of a species in the same environment and genotype (Annalisa et al., 2014, Schloss et al., 2014), sex and diet interact significantly in such modulation (Bolnick et al., 2014).

The complexty of the microbial community, its relationship with the host and its environment limits our capacity to use the gut microbiota as a tool to improve animal performance for now. However, there are important considerations on the establishment of gut microbiota that can definitely optimize health and performance. In addition to maternal proper nutritional status and an adequate transition of the offspring to adult feed, the microbial community that populates the intestine is defined by the sanitary conditions of the animal in early life. Studies in humans and animals have clearly shown that immature and juvenile individuals exposed to unsanitary conditions, i.e. contamination with feces, result in the development of enteropathy that leads to impaired intestinal barrier function, endotoxemia and chronic inflammation (Campbell et al., 2003, Lunn et al., 1991, Le Floc'h et al., 2014). Although hygiene is not directly a nutritional approach to health, it may make the difference between a healthy growing and a poor performing animal, independently of diet.

Overall, the main limitation of our current knowledge on how nutrition, diet and environment affect gut health is that most of the basic research use genetically controlled rodents in laboratory settings. The rodent GI tract has some important differences from the GI tract of some production animals, for example, the presence of tuft cells, that rose significant attention when it was suggested tuft cells may constitute a progenitor cell population. Evidence supports that tuft cells are chemosensory and signaling cells and not progenitor cells but recent studies show that although tuft cells are abundant in the rodent stomach and intestine, they are hard to find in humans and pigs, and their presence has not been explored in other species. The significance of the function of these chemosensory cells and their participation in response to diet and damage then cannot be extrapolated to animals different from rodents without proper characterization. Another important difference is the abundance of Paneth cells in the small intestine of most species while other, i.e. pigs, lack of classical Paneth cells (van Es and Clevers, 2014).

Another important issue to take into account is the tightly controlled genetic background of research animal strains and the controlled experimental environment. Even feeding diets with 60% of calories provided by fat (rodent high-fat standard research diet) results in significant differences in weight gain and adiposity in different mouse strains. In addition, experimental diets, i.e. high-fat, are usually compared to a standard grain based, high-fiber diet as control, masking the effects that changes in single diet components may have on the GI epithelium. On the other hand, research on production animals tends to miss the evaluation of molecular and metabolic markers that may lead into linking basic science to other models and evaluating its applicability. Even well designed studies evaluating the effect of dietary components on the GI constitution and physiology should be taken with diffidence and animal species, genetics and experimental conditions considered as variables. Ultimately, diet composition and feeding schemes for optimal health and growth are highly dependent of the individual and environmental specific conditions that may need to be determined on a case-by-case basis.

References

Annalisa, N., Alessio, T., Claudette, T. D., Erald, V., Antonino, D. L. & Nicola, D. D. 2014. Gut Microbioma Population: An Indicator Really Sensible to Any Change in Age, Diet, Metabolic Syndrome, and Life-Style. *Mediators Inflamm,* 2014, 901308.

Baggio, L. L., Huang, Q., Brown, T. J. & Drucker, D. J. 2004. A recombinant human glucagon-like peptide (GLP)-1-albumin protein (albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. *Diabetes,* 53, 2492-500.

Barker, N., van Es, J. H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P. J. & Clevers, H. 2007. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature,* 449, 1003-7.

Barrachina, M. D., Martinez, V., Wang, L., Wei, J. Y. & Tache, Y. 1997. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. *Proc Natl Acad Sci U S A,* 94, 10455-60.

Bewick, G. A., Kent, A., Campbell, D., Patterson, M., Ghatei, M. A., Bloom, S. R. & Gardiner, J. V. 2009. Mice with hyperghrelinemia are hyperphagic and glucose intolerant and have reduced leptin sensitivity. *Diabetes,* 58, 840-6.

Bolnick, D. I., Snowberg, L. K., Hirsch, P. E., Lauber, C. L., Org, E., Parks, B., Lusis, A. J., Knight, R., Caporaso, J. G. & Svanbäck, R. 2014. Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nat Commun,* 5, 4500.

Boushey, R. P., Abadir, A., Flamez, D., Baggio, L. L., Li, Y., Berger, V., Marshall, B. A., Finegood, D., Wang, T. C., Schuit, F. & Drucker, D. J. 2003. Hypoglycemia, defective islet glucagon secretion, but normal islet mass in mice with a disruption of the gastrin gene. *Gastroenterology,* 125, 1164-74.

Campbell, D. I., Elia, M. & Lunn, P. G. 2003. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. *J Nutr,* 133, 1332-8.

Chai, W., Dong, Z., Wang, N., Wang, W., Tao, L., Cao, W. & Liu, Z. 2012. Glucagon-like peptide 1 recruits microvasculature and increases glucose use in muscle via a nitric oxide-dependent mechanism. *Diabetes,* 61, 888-96.

Charlot, M., Grove, E. L., Hansen, P. R., Olesen, J. B., Ahlehoff, O., Selmer, C., Lindhardsen, J., Madsen, J. K., Køber, L., Torp-Pedersen, C. & Gislason, G. H. 2011. Proton pump inhibitor use and risk of adverse cardiovascular events in aspirin treated patients with first time myocardial infarction: nationwide propensity score matched study. *BMJ,* 342, d2690.

Clevers, H. 2012. The Paneth cell, caloric restriction, and intestinal integrity. *N Engl J Med,* 367, 1560-1.

Clevers, H. 2013a. Stem Cells: A unifying theory for the crypt. *Nature,* 495, 53-4.

Clevers, H. 2013b. The intestinal crypt, a prototype stem cell compartment. *Cell,* 154, 274-84.

Collier, G. R., Greenberg, G. R., Wolever, T. M. & Jenkins, D. J. 1988. The acute effect of fat on insulin secretion. *J Clin Endocrinol Metab,* 66, 323-6.

Colombo, M., Trevisi, P., Gandolfi, G. & Bosi, P. 2012. Assessment of the presence of chemosensing receptors based on bitter and fat taste in the gastrointestinal tract of young pig. *J Anim Sci,* 90 Suppl 4, 128-30.

Cranwell, P. D., Noakes, D. E. & Hill, K. J. 1976. Gastric secretion and fermentation in the suckling pig. *Br J Nutr,* 36, 71-86.

Cuber, J. C., Bernard, G., Fushiki, T., Bernard, C., Yamanishi, R., Sugimoto, E. & Chayvialle, J. A. 1990. Luminal CCK-releasing factors in the isolated vascularly perfused rat duodenojejunum. *Am J Physiol,* 259, G191-7.

de Santa Barbara, P., van den Brink, G. R. & Roberts, D. J. 2003. Development and differentiation of the intestinal epithelium. *Cell Mol Life Sci,* 60, 1322-32.

Dezaki, K., Damdindorj, B., Sone, H., Dyachok, O., Tengholm, A., Gylfe, E., Kurashina, T., Yoshida, M., Kakei, M. & Yada, T. 2011. Ghrelin Attenuates cAMP-PKA Signaling to Evoke Insulinostatic Cascade in Islet {beta}-Cells. *Diabetes,* 60, 2315-24.

Diakogiannaki, E., Gribble, F. M. & Reimann, F. 2012. Nutrient detection by incretin hormone secreting cells. *Physiol Behav,* 106, 387-93.

Donohoe, D. R., Garge, N., Zhang, X., Sun, W., O'Connell, T. M., Bunger, M. K. & Bultman, S. J. 2011. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab,* 13, 517-26.

Edholm, P. 1960. Gallbladder evacuation in the normal male induced by cholecystokinin. *Acta radiol,* 53, 257-65.

el-Alfy, M., Leblond, C. P. & Lee, E. R. 1987. Dynamic histology of the antral epithelium in the mouse stomach: V. Proliferation gradient from the gland base to the isthmus at various times of day. *Am J Anat,* 178, 65-71.

Falko, J. M., Crockett, S. E., Cataland, S. & Mazzaferri, E. L. 1975. Gastric inhibitory polypeptide (GIP) stimulated by fat ingestion in man. *J Clin Endocrinol Metab,* 41, 260-5.

Farin, H. F., Karthaus, W. R., Kujala, P., Rakhshandehroo, M., Schwank, G., Vries, R. G., Kalkhoven, E., Nieuwenhuis, E. E. & Clevers, H. 2014. Paneth cell extrusion and release of antimicrobial products is directly controlled by immune cell-derived IFN-γ. *J Exp Med,* 211, 1393-405.

Fernandez-Twinn, D. S. & Ozanne, S. E. 2010. Early life nutrition and metabolic programming. *Ann N Y Acad Sci,* 1212, 78-96.

Forbes, J. M., Cowan, S. P., Andrikopoulos, S., Morley, A. L., Ward, L. C., Walker, K. Z., Cooper, M. E. & Coughlan, M. T. 2013. Glucose homeostasis can be differentially modulated by varying individual components of a western diet. *J Nutr Biochem,* 24, 1251-7.

Furumoto, T. 1992. An experimental study on cell dynamic alteration in digestive organs following total parenteral nutrition in dogs. *Gastroenterol Jpn,* 27, 488-95.

Gerbe, F., van Es, J. H., Makrini, L., Brulin, B., Mellitzer, G., Robine, S., Romagnolo, B., Shroyer, N. F., Bourgaux, J. F., Pignodel, C., Clevers, H. & Jay, P. 2011. Distinct ATOH1 and Neurog3 requirements define tuft cells as a new secretory cell type in the intestinal epithelium. *J Cell Biol,* 192, 767-80.

Gluckman, P. D., Hanson, M. A., Beedle, A. S. & Spencer, H. G. 2008. Predictive adaptive responses in perspective. *Trends Endocrinol Metab,* 19, 109-10; author reply 112.

Gniuli, D., Calcagno, A., Dalla Libera, L., Calvani, R., Leccesi, L., Caristo, M. E., Vettor, R., Castagneto, M., Ghirlanda, G. & Mingrone, G. 2010. High-fat feeding stimulates endocrine, glucose-dependent insulinotropic polypeptide (GIP)-expressing cell hyperplasia in the duodenum of Wistar rats. *Diabetologia,* 53, 2233-40.

Goldstein, R. M., Hebiguchi, T., Luk, G. D., Taqi, F., Guilarte, T. R., Franklin, F. A., Niemiec, P. W. & Dudgeon, D. L. 1985. The effects of total parenteral nutrition on gastrointestinal growth and development. *J Pediatr Surg,* 20, 785-91.

Gribble, F. M. 2012. The gut endocrine system as a coordinator of postprandial nutrient homoeostasis. *Proc Nutr Soc,* 71, 456-62.

Grossini, E., Caimmi, P., Molinari, C., Uberti, F., Mary, D. & Vacca, G. 2011. Intracoronary gastrin 17 increases cardiac perfusion and function through autonomic nervous system, CCK receptors, and nitric oxide in anesthetized pigs. *J Appl Physiol,* 110, 95-108.

Hauner, H., Glatting, G., Kaminska, D. & Pfeiffer, E. F. 1988. Effects of gastric inhibitory polypeptide on glucose and lipid metabolism of isolated rat adipocytes. *Ann Nutr Metab,* 32, 282-8.

Heneghan, A. F., Pierre, J. F., Tandee, K., Shanmuganayagam, D., Wang, X., Reed, J. D., Steele, J. L. & Kudsk, K. A. 2013. Parenteral Nutrition Decreases Paneth Cell Function and Intestinal Bactericidal Activity While Increasing Susceptibility to Bacterial Enteroinvasion. *JPEN J Parenter Enteral Nutr.*

Holst, J. J. & Gromada, J. 2004. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol Endocrinol Metab,* 287, E199-206.

Hsieh, J., Longuet, C., Maida, A., Bahrami, J., Xu, E., Baker, C. L., Brubaker, P. L., Drucker, D. J. & Adeli, K. 2009. Glucagon-like peptide-2 increases intestinal lipid absorption and chylomicron production via CD36. *Gastroenterology,* 137, 997-1005, 1005.e1-4.

Ipharraguerre, I. R., Tedó, G., Menoyo, D., de Diego Cabero, N., Holst, J. J., Nofrarías, M., Mereu, A. & Burrin, D. G. 2013. Bile acids induce glucagon-like peptide 2 secretion with limited effects on intestinal adaptation in early weaned pigs. *J Nutr,* 143, 1899-905.

Ito, H., Satsukawa, M., Arai, E., Sugiyama, K., Sonoyama, K., Kiriyama, S. & Morita, T. 2009. Soluble fiber viscosity affects both goblet cell number and small intestine mucin secretion in rats. *J Nutr,* 139, 1640-7.

Janssen, S. & Depoortere, I. 2013. Nutrient sensing in the gut: new roads to therapeutics? *Trends Endocrinol Metab,* 24, 92-100.

Jia, X., Cong, B., Zhang, J., Li, H., Liu, W., Chang, H., Dong, M. & Ma, C. 2014. CCK8 negatively regulates the TLR9-induced activation of human peripheral blood pDCs by targeting TRAF6 signaling. *Eur J Immunol,* 44, 489-99.

Johnson, L. P. & Magee, D. F. 1965. Inhibition of gastric motility by a commercial duodenal mucosal extract containing cholecystokinin and pancreozymin. *Nature,* 207, 1401.

Karam, S. & Leblond, C. P. 1995. Origin and migratory pathways of the eleven epithelial cell types present in the body of the mouse stomach. *Microsc Res Tech,* 31, 193-214.

Karam, S. M. & Leblond, C. P. 1993a. Dynamics of epithelial cells in the corpus of the mouse stomach. II. Outward migration of pit cells. *Anat Rec,* 236, 280-96.

Karam, S. M. & Leblond, C. P. 1993b. Dynamics of epithelial cells in the corpus of the mouse stomach. III. Inward migration of neck cells followed by progressive transformation into zymogenic cells. *Anat Rec,* 236, 297-313.

Keeley, T. M. & Samuelson, L. C. 2010. Cytodifferentiation of the postnatal mouse stomach in normal and Huntingtin-interacting protein 1-related-deficient mice. *Am J Physiol Gastrointest Liver Physiol,* 299, G1241-51.

Kidd, M., Hauso, O., Drozdov, I., Gustafsson, B. I. & Modlin, I. M. 2009. Delineation of the chemomechanosensory regulation of gastrin secretion using pure rodent G cells. *Gastroenterology,* 137, 231-41, 241 e1-10.

Lamont, B. J., Li, Y., Kwan, E., Brown, T. J., Gaisano, H. & Drucker, D. J. 2012. Pancreatic GLP-1 receptor activation is sufficient for incretin control of glucose metabolism in mice. *J Clin Invest,* 122, 388-402.

Le Floc'h, N., Knudsen, C., Gidenne, T., Montagne, L., Merlot, E. & Zemb, O. 2014. Impact of feed restriction on health, digestion and faecal microbiota of growing pigs housed in good or poor hygiene conditions. *Animal,* 1-11.

Leonel, A. J. & Alvarez-Leite, J. I. 2012. Butyrate: implications for intestinal function. *Curr Opin Clin Nutr Metab Care,* 15, 474-9.

LeSauter, J., Hoque, N., Weintraub, M., Pfaff, D. W. & Silver, R. 2009. Stomach ghrelin-secreting cells as food-entrainable circadian clocks. *Proc Natl Acad Sci U S A,* 106, 13582-7.

Lunn, P. G., Northrop-Clewes, C. A. & Downes, R. M. 1991. Intestinal permeability, mucosal injury, and growth faltering in Gambian infants. *Lancet,* 338, 907-10.

Madison, B. B., Braunstein, K., Kuizon, E., Portman, K., Qiao, X. T. & Gumucio, D. L. 2005. Epithelial hedgehog signals pattern the intestinal crypt-villus axis. *Development,* 132, 279-89.

Manzanilla, E. G., Nofrarías, M., Anguita, M., Castillo, M., Perez, J. F., Martín-Orúe, S. M., Kamel, C. & Gasa, J. 2006. Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. *J Anim Sci,* 84, 2743-51.

Mao, J., Hu, X., Xiao, Y., Yang, C., Ding, Y., Hou, N., Wang, J., Cheng, H. & Zhang, X. 2013. Overnutrition stimulates intestinal epithelium proliferation through β-catenin signaling in obese mice. *Diabetes,* 62, 3736-46.

Mazzoni, M., De Giorgio, R., Latorre, R., Vallorani, C., Bosi, P., Trevisi, P., Barbara, G., Stanghellini, V., Corinaldesi, R., Forni, M., Faussone-Pellegrini, M. S., Sternini, C. & Clavenzani, P. 2013. Expression and regulation of α-transducin in the pig gastrointestinal tract. *J Cell Mol Med,* 17, 466-74.

Mazzoni, M., Le Gall, M., De Filippi, S., Minieri, L., Trevisi, P., Wolinski, J., Lalatta-Costerbosa, G., Lallès, J. P., Guilloteau, P. & Bosi, P. 2008. Supplemental sodium butyrate stimulates different gastric cells in weaned pigs. *J Nutr,* 138, 1426-31.

Meyer, J. H. & Grossman, M. I. 1972. Comparison of D- and L-phenylalanine as pancreatic stimulants. *Am J Physiol,* 222, 1058-63.

Middendorp, S., Schneeberger, K., Wiegerinck, C. L., Mokry, M., Akkerman, R. D., van Wijngaarden, S., Clevers, H. & Nieuwenhuis, E. E. 2014. Adult stem cells in the small intestine are intrinsically programmed with their location-specific function. *Stem Cells,* 32, 1083-91.

Miyawaki, K., Yamada, Y., Ban, N., Ihara, Y., Tsukiyama, K., Zhou, H., Fujimoto, S., Oku, A., Tsuda, K., Toyokuni, S., Hiai, H., Mizunoya, W., Fushiki, T., Holst, J. J., Makino, M., Tashita, A., Kobara, Y., Tsubamoto, Y., Jinnouchi, T., Jomori, T. & Seino, Y. 2002. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med,* 8, 738-42.

Mori-Akiyama, Y., van den Born, M., van Es, J. H., Hamilton, S. R., Adams, H. P., Zhang, J., Clevers, H. & de Crombrugghe, B. 2007. SOX9 is required for the differentiation of paneth cells in the intestinal epithelium. *Gastroenterology,* 133, 539-46.

Nishi, T., Hara, H., Hira, T. & Tomita, F. 2001. Dietary protein peptic hydrolysates stimulate cholecystokinin release via direct sensing by rat intestinal mucosal cells. *Exp Biol Med (Maywood),* 226, 1031-6.

Nøhr, M. K., Pedersen, M. H., Gille, A., Egerod, K. L., Engelstoft, M. S., Husted, A. S., Sichlau, R. M., Grunddal, K. V., Poulsen, S. S., Han, S., Jones, R. M., Offermanns, S. & Schwartz, T. W. 2013. GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology,* 154, 3552-64.

Oste, M., De Vos, M., Van Haver, E., Van Brantegem, L., Thymann, T., Sangild, P., Weyns, A. & Van Ginneken, C. 2010a. Parenteral and enteral feeding in preterm piglets differently affects extracellular matrix proteins, enterocyte proliferation and apoptosis in the small intestine. *Br J Nutr,* 104, 989-97.

Oste, M., Van Haver, E., Thymann, T., Sangild, P., Weyns, A. & Van Ginneken, C. J. 2010b. Formula induces intestinal apoptosis in preterm pigs within a few hours of feeding. *JPEN J Parenter Enteral Nutr,* 34, 271-9.

Overduin, J., Gibbs, J., Cummings, D. E. & Reeve, J. R. 2014. CCK-58 elicits both satiety and satiation in rats while CCK-8 elicits only satiation. *Peptides,* 54C, 71-80.

Pereira-Fantini, P. M., Nagy, E. S., Thomas, S. L., Taylor, R. G., Sourial, M., Paris, M. C., Holst, J. J., Fuller, P. J. & Bines, J. E. 2008. GLP-2 administration results in increased proliferation but paradoxically an adverse outcome in a juvenile piglet model of short bowel syndrome. *J Pediatr Gastroenterol Nutr,* 46, 20-8.

Perez-Tilve, D., Heppner, K., Kirchner, H., Lockie, S. H., Woods, S. C., Smiley, D. L., Tschop, M. & Pfluger, P. 2011. Ghrelin-induced adiposity is independent of orexigenic effects. *FASEB J,* 25, 2814-22.

Petersen, N., Reimann, F., Bartfeld, S., Farin, H. F., Ringnalda, F. C., Vries, R. G., van den Brink, S., Clevers, H., Gribble, F. M. & de Koning, E. J. 2014. Generation of L cells in mouse and human small intestine organoids. *Diabetes,* 63, 410-20.

Pinto, A., Juniper, D. T., Sanil, M., Morgan, L., Clark, L., Sies, H., Rayman, M. P. & Steinbrenner, H. 2012. Supranutritional selenium induces alterations in molecular targets related to energy metabolism in skeletal muscle and visceral adipose tissue of pigs. *J Inorg Biochem,* 114, 47-54.

Reimann, F., Tolhurst, G. & Gribble, F. M. 2012. G-protein-coupled receptors in intestinal chemosensation. *Cell Metab,* 15, 421-31.

Relling, A. E., Crompton, L. A., Loerch, S. C. & Reynolds, C. K. 2014. Short communication: Plasma concentration of glucose-dependent insulinotropic polypeptide may regulate milk energy production in lactating dairy cows. *J Dairy Sci,* 97, 2440-3.

Sakar, Y., Duca, F. A., Langelier, B., Devime, F., Blottiere, H., Delorme, C., Renault, P. & Covasa, M. 2014. Impact of high-fat feeding on basic helix-loop-helix transcription factors controlling enteroendocrine cell differentiation. *Int J Obes (Lond).*

Sangild, P. T., Thymann, T., Schmidt, M., Stoll, B., Burrin, D. G. & Buddington, R. K. 2013. Invited review: the preterm pig as a model in pediatric gastroenterology. *J Anim Sci,* 91, 4713-29.

Saqui-Salces, M., Dowdle, W. E., Reiter, J. F. & Merchant, J. L. 2012. A high-fat diet regulates gastrin and acid secretion through primary cilia. *FASEB J,* 26, 3127-39.

Saqui-Salces, M., Keeley, T. M., Grosse, A. S., Qiao, X. T., El-Zaatari, M., Gumucio, D. L., Samuelson, L. C. & Merchant, J. L. 2011. Gastric tuft cells express DCLK1 and are expanded in hyperplasia. *Histochem Cell Biol,* 136, 191-204.

Sargiacomo, M., Onori, P., Bravo, E., Franchitto, A., Cantafora, A., Barca, S., Peschle, C. & Gaudio, E. 1998. Long-term cultures of human fetal liver cells: a three-dimensional experimental model for monitoring liver tissue development. *J Hepatol,* 28, 480-90.

Sayegh, A. I., Washington, M. C., Raboin, S. J., Aglan, A. H. & Reeve, J. R. 2014. CCK-58 prolongs the intermeal interval, whereas CCK-8 reduces this interval: Not all forms of cholecystokinin have equal bioactivity. *Peptides,* 55C, 120-125.

Schloss, P. D., Iverson, K. D., Petrosino, J. F. & Schloss, S. J. 2014. The dynamics of a family's gut microbiota reveal variations on a theme. *Microbiome,* 2, 25.

Shima, K., Suda, T., Nishimoto, K. & Yoshimoto, S. 1990. Relationship between molecular structures of sugars and their ability to stimulate the release of glucagon-like peptide-1 from canine ileal loops. *Acta Endocrinol (Copenh),* 123, 464-70.

Simons, B. D. & Clevers, H. 2011. Stem cell self-renewal in intestinal crypt. *Exp Cell Res,* 317, 2719-24.

Singh, P., Dai, B., Wu, H. & Owlia, A. 2000. Role of autocrine and endocrine gastrin-like peptides in colonic carcinogenesis. *Curr Opin Gastroenterol,* 16, 68-77.

Stange, D. E., Koo, B. K., Huch, M., Sibbel, G., Basak, O., Lyubimova, A., Kujala, P., Bartfeld, S., Koster, J., Geahlen, J. H., Peters, P. J., van Es, J. H., van de Wetering, M., Mills, J. C. & Clevers, H. 2013. Differentiated Troy+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. *Cell,* 155, 357-68.

Steiger, A., Dresler, M., Schussler, P. & Kluge, M. 2011. Ghrelin in mental health, sleep, memory. *Mol Cell Endocrinol,* 340, 88-96.

Støy, A. C., Heegaard, P. M., Thymann, T., Bjerre, M., Skovgaard, K., Boye, M., Stoll, B., Schmidt, M., Jensen, B. B. & Sangild, P. T. 2014. Bovine colostrum improves intestinal function following formula-induced gut inflammation in preterm pigs. *Clin Nutr,* 33, 322-9.

Subaran, S. C., Sauder, M. A., Chai, W., Jahn, L. A., Fowler, D. E., Aylor, K. W., Basu, A. & Liu, Z. 2014. GLP-1 at physiological concentrations recruits skeletal and cardiac muscle microvasculature in healthy humans. *Clin Sci (Lond),* 127, 163-70.

Suzuki, K., Fukui, H., Kayahara, T., Sawada, M., Seno, H., Hiai, H., Kageyama, R., Okano, H. & Chiba, T. 2005. Hes1-deficient mice show precocious differentiation of Paneth cells in the small intestine. *Biochem Biophys Res Commun,* 328, 348-52.

Tellez, N., Joanny, G., Escoriza, J., Vilaseca, M. & Montanya, E. 2011. Gastrin treatment stimulates beta-cell regeneration and improves glucose tolerance in 95% pancreatectomized rats. *Endocrinology,* 152, 2580-8.

Thompson, K. H., Lichter, J., LeBel, C., Scaife, M. C., McNeill, J. H. & Orvig, C. 2009. Vanadium treatment of type 2 diabetes: a view to the future. *J Inorg Biochem,* 103, 554-8.

Thymann, T., Stoll, B., Mecklenburg, L., Burrin, D. G., Vegge, A., Qvist, N., Eriksen, T., Jeppesen, P. B. & Sangild, P. T. 2014. Acute effects of the glucagon-like peptide 2 analogue, teduglutide, on intestinal adaptation in short bowel syndrome. *J Pediatr Gastroenterol Nutr,* 58, 694-702.

Tolhurst, G., Reimann, F. & Gribble, F. M. 2012. Intestinal sensing of nutrients. *Handb Exp Pharmacol,* 309-35.

Ueno, H., Yamaguchi, H., Kangawa, K. & Nakazato, M. 2005. Ghrelin: a gastric peptide that regulates food intake and energy homeostasis. *Regul Pept,* 126, 11-9.

Valenzuela, J. E., Walsh, J. H. & Isenberg, J. I. 1976. Effect of gastrin on pancreatic enzyme secretion and gallbladder emptying in man. *Gastroenterology,* 71, 409-11.

van der Flier, L. G. & Clevers, H. 2009. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol,* 71, 241-60.

van Es, J. H. & Clevers, H. 2014. Paneth cells. *Curr Biol,* 24, R547-8.

VanDussen, K. L., Carulli, A. J., Keeley, T. M., Patel, S. R., Puthoff, B. J., Magness, S. T., Tran, I. T., Maillard, I., Siebel, C., Kolterud, Å., Grosse, A. S., Gumucio, D. L., Ernst, S. A., Tsai, Y. H., Dempsey, P. J. & Samuelson, L. C. 2012. Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. *Development,* 139, 488-97.

Vickers, M. H. 2014. Early life nutrition, epigenetics and programming of later life disease. *Nutrients,* 6, 2165-78.

Viladomiu, M., Hontecillas, R., Yuan, L., Lu, P. & Bassaganya-Riera, J. 2013. Nutritional protective mechanisms against gut inflammation. *J Nutr Biochem,* 24, 929-39.

Wang, B., Wu, G., Zhou, Z., Dai, Z., Sun, Y., Ji, Y., Li, W., Wang, W., Liu, C., Han, F. & Wu, Z. 2014. Glutamine and intestinal barrier function. *Amino Acids.*

Wang, T. C., Dangler, C. A., Chen, D., Goldenring, J. R., Koh, T., Raychowdhury, R., Coffey, R. J., Ito, S., Varro, A., Dockray, G. J. & Fox, J. G. 2000. Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer. *Gastroenterology,* 118, 36-47.

Waterland, R. A., Lin, J. R., Smith, C. A. & Jirtle, R. L. 2006. Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (Igf2) locus. *Hum Mol Genet,* 15, 705-16.

Widmayer, P., Breer, H. & Hass, N. 2011. Candidate chemosensory cells in the porcine stomach. *Histochem Cell Biol,* 136, 37-45.

Xia, Q., Pang, W., Pan, H., Zheng, Y., Kang, J. S. & Zhu, S. G. 2004. Effects of ghrelin on the proliferation and secretion of splenic T lymphocytes in mice. *Regul Pept,* 122, 173-8.

Yan, K. S., Chia, L. A., Li, X., Ootani, A., Su, J., Lee, J. Y., Su, N., Luo, Y., Heilshorn, S. C., Amieva, M. R., Sangiorgi, E., Capecchi, M. R. & Kuo, C. J. 2012. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. *Proc Natl Acad Sci U S A,* 109, 466-71.

Zhang, H., Malo, C. & Buddington, R. K. 1997. Suckling induces rapid intestinal growth and changes in brush border digestive functions of newborn pigs. *J Nutr,* 127, 418-26.

Zhang, J. G., Liu, J. X., Jia, X. X., Geng, J., Yu, F. & Cong, B. 2014. Cholecystokinin octapeptide regulates the differentiation and effector cytokine production of CD4(+) T cells in vitro. *Int Immunopharmacol.*

Zieve, L., Silvis, S. E., Mulford, B. & Blackwood, W. D. 1966. Secretion of pancreatic enzymes. I. Response to secretin and pancreozymin. *Am J Dig Dis,* 11, 671-84.

Notes