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NUTRITIONAL REGULATION OF GASTROINTESTINAL GROWTH

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1. ABSTRACT

Nutrition can control gastrointestinal (GI) tract growth at many stages of development. Fetal growth of the GI tract can be inhibited by restriction of the maternal diet, decrease of blood supply to the placenta, or partial obstruction of amniotic fluid swallowing. In most species there is an immature appearance of the GI mucosa that is characterized by large, long villi extending into the proximal colon. This pattern usually changes around the time of weaning and can be modified by manipulation of the diet. While total nutrition has a profound effect on GI development, there are specific nutrients that influence the epithelium during adult life. In the small intestine, glutamine has the most important effects and this amino acid is now considered conditionally essential. In the colon, dietary fiber has the strongest influence on mucosal structure and turnover. While it has been assumed that concentrations of bile acids and/or short chain fatty acids are the mediating factors, there is substantial evidence that mitigates against this conclusion. A better understanding of the molecular changes accompanying alterations in GI growth may lead to more comprehensive strategies for improving intestinal function and decreasing the risk of colon cancer.

2. INTRODUCTION

Just as the body and its organs respond to nutritional changes with differential growth, the gastrointestinal (GI) tract also reflects alterations in diet. In fact, the tissues making up the GI tract are quite sensitive to qualitative and quantitative changes in nutrition. Overlaid on this responsiveness are levels of various growth factors and hormones as well as the age of the individual animal, all of which determine the nature of the GI response to changes in diet. Much of what we know about GI growth has been learned from studies on a number of mammalian species (generally excluding humans) and on chickens and frogs. Unfortunately, many of the species studied are herbivores and results from those animals may not be applicable to the human situation; the relative size of various parts of the GI tract and microscopic anatomy of herbivores' GI mucosa differs from those seen in carnivores and omnivores. Fortunately, the majority of experimental studies have been carried out using rats and swine, both of which are omnivores. However, even with these species there are significant differences from humans in gestation periods, developmental patterns, and intestinal anatomy. This does not mean that studies on other species are of no value but one must be aware of the limitations that must be remembered when making cross species comparisons.

The GI mucosa is exposed to more noxious stimuli than any other tissues of the body. This includes strongly acidic conditions, proteases, lipases, and by-products of bacterial fermentation. Not only is there a physical barrier of mucin coating the epithelium but turnover of the cells is extremely rapid with complete replacement every 3.5 days in most species. Only the bone marrow approaches this rapid rate of growth. The GI tract can grow in length and diameter; both the mucosa and muscle layers can expand in response to proper stimuli. After a certain age mucosal growth is limited. However, there is good evidence that proliferation rates of GI epithelium is increased in older animals (1).

A variety of experimental techniques have been used for measuring GI growth and lack of comparability has lead to discrepancies concerning qualitative and quantitative stimuli that affect GI cytokinetics. Many methods for measurement of cell proliferation correlate but do not give identical results. The most specific is incorporation of a labeled precursor for incorporation into DNA during S phase, with tritiated thymidine being the

most common example. Bromodeoxyuridine is commonly used as a substrate that can be disclosed without radiolabeling. PCNA and Ki67 are kinases that are disclosed immunohistochemically but stain a greater proportion of cells. Crypt cell production rate usually refers to the counting of cells arrested in metaphases per crypt by treatment with colchicine. One must also be concerned with cell death in the intestine and several assays are available for measurement of apoptosis. A limitation of all the above methods is that they give a snapshot of a very brief period in the intestine. In some studies that have reported microscopic morphological parameters in addition to the proliferation indices, there has not been good correlation between these measures. Therefore, descriptions of GI mucosal villous height, crypt depth, mucosal volume, and similar parameters probably give a better picture of net GI growth over time. Under proper stimuli, there can be comparable increases in both cell growth and death that would not yield a change in intestinal morphology but would properly be considered a change in GI growth.

3. PRENATAL GROWTH

A large proportion of GI growth occurs during gestation and one might assume that there is little influence of diet, but this is not the case. The structure of the intestine evolves during this phase of development and terminally differentiated functions appear. Lactase develops in most species, with the exception of pinnipeds, in preparation for digestion of the carbohydrate in breast milk. Humans are the only mammalian species that develops sucrase early in gestation and late fetal levels of this enzyme equal those found in adults (2). Most other species express sucrase after birth and adult levels are usually achieved after weaning. Nutrient availability, peptide growth factors, and hormones in amniotic fluid (which is continuously swallowed by the fetus) can alter rates of GI growth *in utero.*

One example of how fetal exposure to factors that influence GI development is ethanol (3). Although direct organ culture of prenatal rat intestine in 25 mM ethanol showed no effect on several parameters of epithelial maturation, pups exposed in utero exhibited multiple signs of delayed mucosal development possibly from ethanol metabolites. Since one of the controls for this experiment was a diet diluted by half with cellulose, and this showed no influence on the parameters under study, it was concluded that maternal undernutrition was not responsible for the impaired intestinal development seen in fetuses from mothers consuming 25% of energy from alcohol. Nevertheless, nutrient restriction *in utero* seems to preferentially reduce GI growth (4). Parameters of growth of both the small and large intestine as well as indicators or GI epithelial maturation were delayed in fetuses of sheep with food restriction. However, it is clear that fetal ingestion of nutrients in amniotic fluid also plays a critical role in growth of the GI tract. Experimental obstruction of the fetal small intestine of sheep results in massive hypertrophy of the section proximal to ligation with grossly abnormal villus appearance and distal to the obstruction, a marked loss of microvilli (5).

Almost nothing is known about the growth of the esophagus. The only report found in the literature described a marked reduction in the thickness of the epithelium and increased proliferation of basal epithelial cells on the first postnatal day when suckling began (6). Bottle feeding with a variety of diets gave the same result but bottle feeding with water did not cause any changes in the esophageal structure. It was concluded that physical stimulation by food ingestion was the major stimulus for changes in growth of the newborn esophagus.

The stomach enlarges as the body grows to serve primarily as a reservoir where food begins to be digested. Ingestion of repeated large meals results in increased growth that enlarges the capacity of the stomach. Atrophy of the gastric mucosa is common in older humans and a variety of animals but there is not necessarily decreased growth of the mucosa. In fact, most parameters of cell proliferation are elevated in senescent rats (1). These last two paradoxical facts can be reconciled if apoptosis is also increased.

4. SMALL INTESTINE

In the perinatal period, there are significant and rapid changes in the function and morphology of the GI tract (7). This time is critical because of the shift from placental nutrition to oral intake. While all levels of the GI tract are affected by growth, most research studies have focused on the intestines. In some species such as swine and dogs, the GI tract mucosa doubles in weight within one day of birth (8); growth thereafter is markedly reduced. Expansion of the number of intestinal epithelial cells is accompanied by elongation of the villi and a large increase in the absorptive surface. Trophic factors for the gut include intestinal secretions such as bile and pancreatic juice, polyamines, and GI hormones such as gastrin, cholecystokinin, epidermal growth factor, insulin-like growth factors, etc. Identification of specific growth factors can be exploited therapeutically in infants born prematurely or with prenatal growth inhibition and in older individuals who have been fed via total parenteral nutrition for an extended period. The rate of GI growth correlates roughly with how quickly a particular species develops the ability to feed itself. During suckling, the principal source of nutrients for mammals is milk. Although milk composition varies with duration of suckling, there do not appear to be major changes in GI growth or function during this time, excluding the postnatal period.

In most newborn animals, the intestinal epithelium is permeable to macromolecules such as immunoglobulins, albumin, and dextrans (9). Depending on the species, passage of these molecules across the epithelium usually stops at 1-2 days after birth but in rats this takes about 21 days. Ingestion of food, including colostrum, is one of the controlling factors for the phenomenon of “gut closure” which is thought to occur when fetal cells are replaced by postnatal enterocytes. This

may be linked to growth factors in colostrum such as corticosterone or various peptide hormones.

A wide variety of nutrient deficiencies can impair growth of the animal after weaning and some of these deficiencies preferentially target GI development. Weanling rats fed a diet deficient in riboflavin for five weeks display significant changes in morphology and cytokinetics in the small intestine which are not reversed at three weeks of repletion even though biochemical measures of riboflavin status are normalized (10).

At weaning, there is the opportunity to evoke a variety of additional adaptive changes in GI structure and function. This is also the time when the intestinal microflora are changing toward adult patterns, although it may take years for stable establishment of this and there are variations in microflora based on changes in diet, infections, etc. Additional factors involved at weaning are psychological, nutritional, and environmental stresses. It has recently been established that the transition at weaning from milk to a solid diet containing a variety of nutrients, including dietary fiber, results in significantly shorter villi and deeper crypts in the small intestinal mucosa of swine. However, continuation of animals on amounts of milk sufficient to equal growth on the weaning diet resulted in morphology similar to that found with the solid diet indicating that level of energy intake is a greater determinant of changes in villous growth than composition of the diet (11). In rat pups whose dams were subjected to protein deficiency, the heights of villi at weaning were less in all segments of the intestine (12). Feeding a normal diet after weaning led to ileal villi that were longer than normal; continuing the protein deficient diet resulted in short villi. Rats that are unweaned have leaf-like jejunal villous morphology which changes at weaning to long, ridged villi on the usual commercial diet (13). Rats weaned to a fiber-free diet maintain the immature villous appearance; addition of cellulose or the bile acid binding resin cholestyramine has no effect, while pectin has some effect on development of the adult villous pattern. This change is accompanied by an increase in the concentration of villi in the intestine.

4.1. GLUTAMINE

One nutrient, often considered as conditionally essential for normal GI function and growth, is glutamine. This amino acid is a major substrate for enterocytes from the small intestine. Pigs weaned prematurely develop small intestinal atrophy. Supplementation of the diet with glutamine corrects the changes seen in glutamine deprived swine (14). Similarly, glutamine provided parenterally has the same effects on improved mucosal growth and function in models of small bowel resection or transplantation (15-17). Patients with short bowel syndrome following massive intestinal resection show improved nutrient absorption and indices of bowel function when provided with supplemental glutamine (18).

5. LARGE INTESTINE

There are marked differences in structure and function between colons of newborns and adults. A major difference is the transient appearance of colonic villi in the proximal colon of almost all mammalian species (9). In humans, the villi have all but disappeared at the time of birth but most other species display this characteristic for 3-10 days postnatally. Cells in the villi display biochemical and ultrastructural characteristics of ileal epithelium. These cells are capable of nutrient absorption and presumably function to increase the absorptive surface area during gestation. At the end of the period in which villi are replaced by the flat mucosa characteristic of adult colon the major functions of the colon are absorption of water and salts, in addition to the obvious purpose as a reservoir for intestinal waste. The colon also is active in absorbing short chain fatty acids (SCFA) along its length; these are produced by the bacteria acting on dietary fiber and other fermentable substrates. Since these substrates for the colonic microflora control both their abundance and physiology, fiber and related compounds have been studied extensively for effects on growth of the colonic mucosa.

The role of the bacterial flora has been studied extensively for effects on colonic growth. Much of this work has been done in conjunction with studies of dietary fat or fiber. When conventional and germ-free mice were fed high or low fat diets containing either cellulose or guar gum, the diet having viscous fiber (guar) and high fat stimulated cell proliferation in the cecum and proximal colon (19). The presence of bacteria had no statistically significant effect on any parameters measured. In another study comparing conventional and germ-free rats fed diets containing cellulose, guar gum, “simulated Western diets,” or standard commercial diet, it was also observed that the presence of bacteria had little impact on the volume of colonic mucosa (20). The alterations in colonic growth did not correlate with concentrations of SCFA, ammonia, or bile acids in the colonic contents. These studies strongly suggest that the fermentation of dietary fiber to SCFA is not a simple predictor of colonic growth.

6. DIETARY FIBER

Dietary fiber can be defined as those components of plants that are resistant to digestion by endogenous enzymes. However, the majority of fiber is fermented in the colon, primarily to SCFA. Butyrate is a preferred fuel of colonocytes while acetate and propionate pass into the bloodstream for utilization by other tissues. It is often considered that soluble fibers are completely fermented while insoluble ones are resistant to fermentation. This simplification is probably too vague to be useful. Some soluble fibers are rapidly fermented (e.g., guar gum), some fermented at a moderate rate (pectin), and a few are fermented slowly (psyllium). Most insoluble fibers are moderately or slowly fermented; those highly resistant to fermentation include isolated cellulose and its derivatives that may not be acted upon at all by the microflora. Lignin is also a component of fiber that is not fermented due to its composition as a phenylpropane polymer and not a carbohydrate. Most foods contain varying mixes of both soluble and insoluble fibers. However, these designations are primarily useful for physical characterization and should probably not be used for physiological effects.

Overall, on a typical western diet 75% of fiber is fermented by the microflora of the colon.

Feeding of fiber-free diets to animals is well documented as inducing atrophy of the intestinal mucosa and muscularis with the largest effects seen in the colon and distal small intestine; addition of dietary fiber reverses these effects (21, 22). Although most fiber sources have a trophic effect on colon, only the easily fermented ones have an effect on the small bowel which led to the conclusion that fermentation products, specifically SCFA, mediated this effect. Infusions of butyrate or SCFA mixtures are clearly trophic to the GI mucosa without the mechanical effects of including fiber in the diet (23). It was subsequently shown that both sympathetic and parasympathetic nervous pathways mediate the mucosal growth induced by infusions of SCFA (24).

A number of studies have looked at organ weights in the GI tract as a function of differences in specific nutrients, especially fiber. Measurements of total organ weights, however, reflect changes more in the muscle layer than in the mucosa. The stomach does not appear to be affected while small intestine, cecum and colon weights are all increased by inclusion of fermentable fiber (25). This effect may result from viscosity of soluble fibers or from bulking effects of insoluble fibers. The length of time and the amount of fiber fed are variables that have led to conflicting results in the literature. Addition of 10% fiber to swine diet for two weeks results in some increase in cell proliferation in both the small and large intestines as well as the width of small intestinal villi (26). Studies with humans and rats show that various parameters that measure adaptation to ingestion of dietary fiber take about three weeks to stabilize.

In a study of colonic mucosal volume, it was shown that increasing dietary cellulose or guar gum resulted in greater mucosal growth when corrected for body weight but increasing wheat bran did not expand the epithelial volume. There was, however, a strong correlation between fecal weight and mucosal growth but no clear relationship between colonic mucosa and fermentation of fiber (27). In a series of studies, Vahouny and colleagues found that adding fiber sources to the diets of rats induced varying effects on ultrastructure of the jejunum and colon and modified rates of synthesis of intestinal mucin (28-30). The ability of dietary fiber to induce deviations in normal appearance of the intestinal mucosa correlated with the bile acid binding affinity of the fiber. Therefore, the effects of the different fibers in decreasing order of disruption of ultrastructural appearance were: alfalfa > pectin > cellulose > wheat bran. The opposite relationship held for increasing synthesis of intestinal mucin, suggesting two reasons why wheat bran may be the most effective dietary fiber source in prevention of colon cancer (31).

7. OTHER NUTRIENTS

Fat has also been suggested to affect GI growth because it is a risk factor for development of colon cancer. Many, but not all, studies of cell proliferation in the colon suggest that increased dietary fat leads to elevated cytokinetics. The presumptive mediator of this is greater concentration of intestinal bile acids but the data on this point are highly contradictory (32, 33). Obesity is also associated with increased cell proliferation in the colon and weight reduction reduces this (34). Caloric restriction generally inhibits cell turnover throughout the GI tract (35) but when combined with increased dietary fat not only was there no decrease, there was a significant increase in colonic cytokinetics from rats fed a low calorie, high fat diet (36). Calcium can reduce proliferation of colonocytes if the starting level is above normal but has no effect if the initial rate is not elevated (37).

Protein is also implicated in modulation of colonic cytokinetics because of the strong effect of colonic ammonia concentration on shortening the life span of colonocytes (38). Although gastrointestinal physiologists claim that virtually no dietary protein reaches the colon, it is well documented that increasing dietary protein leads to elevations in colonic ammonia (39). While relatively little dietary protein reaches the colon, considerable amounts of sloughed intestinal cells, mucin, and dead bacteria provide significant amounts of protein as substrate to the microflora in the colon.

8. CONCLUSIONS

There can be little doubt that diet has a profound influence on GI growth at all stages of life, from fetal development through weaning to adulthood. The morphology and function of the mucosa is malleable through most stages of life; both cell proliferation and apoptosis can be altered at all phases of the life cycle. While total nutrient availability seems to be a dominant factor in growth of the GI tract, a limited number of individual nutrients affect the mucosa. Glutamine has the largest influence on the small bowel while dietary fiber primarily affects the colon. These two food components serve as preferred substrates, directly or indirectly, for the mucosal cells and this is presumably related to their effects although a number of experiments have challenged the importance of fiber fermentation in modulating colonic growth. These observations have profound importance for absorption of nutrients and risk of developing colon cancer later in life. Better understanding of nutrient interactions on these effects and of the mechanisms mediating the observations described above are still needed. Not only do these factors have importance for understanding of GI physiology but they are crucial for making public health recommendations.

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