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Recebido a 26 de Setembro de 2014
Aceite a 18 de Outubro de 2014

Effect of Diet on the Human Large Intestinal Microbiota

Efeito da Alimentação na Microbiota do Intestino Grosso Humano

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ABSTRACT

The impact of diet on the gastrointestinal microbiota has been discussed for several decades. However, advances in molecular microbiological techniques have expanded the knowledge on microbial ecology and the study of the effect of diet and dietary changes on the resident microbiota. It has become evident that diet influences the composition of the microbiota. This review aims to present an overview of the dietary effects on the human fecal microbiota.

KEYWORDS: Diet, Intestinal microbiota

RESUMO

O efeito da alimentação na microbiota do tracto gastrointestinal humano tem sido discutido já há várias décadas. No entanto, avanços nas técnicas de microbiologia molecular têm expandido o conhecimento sobre o efeito da alimentação e das mudanças alimentares nesta comunidade de microrganismos. Tem-se tornado evidente que a alimentação influencia a composição da microbiota. Este artigo tem como objectivo apresentar uma revisão sobre o efeito da alimentação na microbiota fecal humana.

PALAVRAS-CHAVE: Alimentação, Microbiota intestinal

INTRODUCTION

Although the physiological role of the gastrointestinal (GI)-tract is to process and digest the food ingested, it offers several niches for colonization by a variety of microorganisms. The main metabolic function of the intestinal microbiota is the fermentation of non-digested dietary materials and endogenous substances such as mucus produced by intestinal epithelial cells (1). Fermentation is the process in which microorganisms break down dietary nutrients and other substrates under anaerobic conditions, to obtain energy for growth and maintenance of the cellular functions (2). Therefore, diet is of primary importance as a source of microorganisms and especially as a substrate for intestinal microbes (3). The undigested food materials not degraded and absorbed in the small intestine reach the large intestine, where they support the microbiota as a source of nutrients and energy.

Carbohydrates

Carbohydrates are organic molecules composed of carbon, oxygen, and hydrogen. Simple sugars or monosaccharides are seldom found free in nature and are typically linked into oligosaccharide and polysaccharide forms through glycosidic bonds (4). The human digestive capacity for carbohydrates is limited to only a few of the many possible oligosaccharide and polysaccharide configurations in the food supply. Most of the carbohydrates ingested by humans are starch polysaccharides, which are hydrolysed by α -amylase secreted by the salivary glands and pancreas. In addition, brush border enzymes of the intestinal epithelial cells cleave glycosidic bonds of the disaccharides sucrose, maltose, isomaltose and lactose into monosaccharides that are absorbed through the small intestine (5). Carbohydrates containing other linkages cannot be digested by human enzymes, and therefore reach the large intestine available to be fermented by resident bacteria. They are commonly classified as dietary fibres (6).

Fibres are the main substrates available to the bacteria in the human colon (7), and their fermentation produces

the primary source of energy in the large intestine. A large proportion of these carbohydrates is starch resistant to the activities of host amylases (resistant starch) (8). The solubility of dietary fibres that reach the large intestinal lumen is variable. Fibres such as structural plant cell wall components are primarily cellulose, hemicellulose, pectin and the non-polysaccharide lignin. Whereas pectin and some hemicelluloses, in addition to gums and mucilages, are soluble and readily fermented by the colonic microbiota, other hemicelluloses, lignin and cellulose are insoluble and much less fermentable (9). Therefore, carbohydrate polymers with different solubilities are likely to be digested at different rates and may affect the species composition of the microbiota along the intestinal tract (10). The carbohydrate digestibility follows a longitudinal gradient along the colon, reciprocal to the thickness of the intestinal mucus barrier, with greatest thickness in the sigmoid colon and rectum (11). In the proximal colon, the mucus layer is thin, the transit time of colonic content is faster, and bacteria are likely to target more soluble and rapidly digestible carbohydrates. By contrast, the distal colon has a much thicker mucous layer, transit time is slower, and the residual carbohydrates that fuel bacterial growth are likely to be less soluble and therefore take longer to degrade (10).

The insoluble carbohydrates, in particular plant cell wall components such as cellulose or resistant starch particles, are first decomposed by primary degraders capable of binding and digesting these polysaccharides (12). It is estimated that up to 70% of cellulose and hemicellulose present in the normal food material is fermented during passage through the large intestine. The bacteria involved include members of both Gram-positive Firmicutes and Gram-negative *Bacteroides* spp. (13). After initial degradation of these complex carbohydrates, more soluble polysaccharides are able to be digested by the secondary degraders (10). This process allows cross-feeding between primary degraders of complex substrates and other bacterial species, involving fermentation products such as

hydrogen and lactate, as well as partial degradation products (ex. cellobiose from cellulose) (14, 15). The secondary degraders metabolize the first set of products, forming others. Metabolic cross-feeding is therefore a central feature in anaerobic microbial communities.

End-Products Of Carbohydrate Fermentation

Carbohydrate fermentation in the large intestine results in the production of short chain fatty acids (SCFA), mainly butyrate, acetate and propionate (16), and a number of other metabolites such as lactate, pyruvate, ethanol and succinate (17). SCFA are the principal aqueous solutes in colonic contents, and their concentration in feces can exceed 100 mM. It has been estimated that 90% of the SCFA are absorbed across the intestinal wall (18). The degree to which fibre is metabolized by colonic bacteria and the products of fermentation depends on the specific dietary substrates. High-fibre diets generally increase fecal bulking, transit rate and SCFA production along the large intestine. Butyrate is absorbed by the intestinal mucosa where it is the main energy source for colonocytes (16), providing up to 70% of their requirements (19). When deprived of butyrate, colonocytes undergo autophagy (20). Butyrate has anti-inflammatory and anti-carcinogenic effects (21). Two important groups of butyrate-producing bacteria are found within the phylum Firmicutes: *Eubacterium rectale* and *Roseburia* spp., comprising 5–10% of the total microbiota, and *Faecalibacterium prausnitzii*. Acetate and propionate are absorbed into the blood circulation and utilized by other organs (22). Propionate is transported to the liver, where it has a role in glucogenesis, lipogenesis and protein synthesis (23). Genera within the Family Veillonellaceae include propionate-producing bacteria such as *Megasphaera*, *Veillonella*, *Megamonas* and *Selenomonas* (24). Acetate is transported to the peripheral tissues via blood circulation, and is a substrate for lipid and cholesterol synthesis (23). In addition, acetate is utilized by resident bacteria of the colon, in particular the butyrate-producing bacteria (25). Many colonic bacteria produce lactate as a fermentation end product. However, only low levels of lactate are usually detected in feces of healthy individuals (26), since it serves as a substrate for lactate-utilizing bacteria such as *Eubacterium hallii* and *Anaerostipes caccae* (27) and sulphate-reducing bacteria. Up to 20% of butyrate formation is estimated to be derived from lactate (28). Since many metabolic properties are shared among the microbiota community, it is difficult to link the capacity of producing specific SCFA to phylogenetic information. Carbohydrate fermentation is enhanced by mechanisms that decrease the volume of colonic gas, involving utilization of hydrogen which is formed by many of the anaerobic bacteria inhabiting the colon. Hydrogen is consumed by methanogens, acetogens and sulphate-reducing bacteria, which convert this gas to methane, acetate or hydrogen sulphide, respectively, depending on the types of microorganisms present (29). The concentration of methane is extremely variable, and like hydrogen sulphide, is not present in all individuals (30).

Proteins

Dietary protein sources make up at least half of the protein material that reaches the large intestine every day, while the remaining proteins are produced

endogenously. Dietary proteins undergo structural changes during ingestion, digestion and absorption. The digestibility of proteins is affected by the type of protein and its state of processing before ingestion. Proteins and their hydrolytic products are largely hydrophilic and, unlike fats, do not require bile acids for solubilisation (31). Ingested proteins are first hydrolysed by proteinases such as pepsin, trypsin and chymotrypsin in the small intestine to produce peptides of various lengths. The peptides produced are further digested by brush-border peptidases at the surface of the epithelial cells to amino acids, while some oligopeptides remain unhydrolysed. Peptides are therefore present at different stages of the digestion and may exert a variety of functions in the GI-tract (32).

Whereas carbohydrate fermentation mainly occurs in the proximal part of the colon, the protein fermentation takes place in the distal colon (1). As the digesta moves through the distal colon, carbohydrate availability decreases and protein and amino acids become the main bacterial energy source (33). Once carbohydrate sources have been used up in the proximal colon, most microorganisms switch to protein fermentation to salvage energy (34). The predominant proteolytic species identified in the human large intestine are *Bacteroides* spp. and *Propionibacterium* spp., present at 10^{11} – 10^{12} and 10^8 – 10^{10} CFU per g of dry feces, respectively (35). Other proteolytic species belong to the genera *Clostridium*, *Fusobacterium*, *Streptococcus* and *Bacillus*. The *Bacteroides* enterotype has recently been associated with animal protein and saturated fats intake, suggesting that the high meat consumption characterizing the western diet modulates this bacterial group (36).

Although proteins provide a less significant energy source in the large intestine, their importance lies mainly in the effects they have on the intermediary metabolism of the host (37). Whereas carbohydrate fermentation mainly leads to perceived health-promoting metabolites, anaerobic degradation of proteins yields toxic metabolites, e.g. sulphur-containing compounds such as ammonia, as well as phenolic and indolic compounds. The fact that protein is a major constituent of meat products and that protein fermentation metabolites such as ammonia and phenolic compounds have been found to be potentially carcinogenic, suggests a possible relation between meat intake, protein fermentation and colon cancer (38). Therefore, the impact of protein fermentation on intestinal health has become particularly relevant nowadays when widespread application of high protein diets for weight loss and body weight management have gained popularity.

Fats

Fats are composed of fatty acids, i.e. carboxylic acids with hydrocarbon chains, which are classified as saturated (no double-bonds) or unsaturated (4). Naturally occurring fats are mixtures of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), with one predominating type in most foods. Dietary fats are essential for the digestion, absorption, and transport of fat-soluble vitamins and fat-soluble phytochemicals such as carotenoids and lycopenes (39). In addition, dietary fat slows gastric emptying, depresses gastric secretions, and stimulates biliary and pancreatic flow.

The absorption of fat in the small intestine is generally efficient, although fractions of dietary fat may escape into the colon depending on the amount ingested (40). Therefore, high intake of dietary fat may increase the quantities of fat and bile acids that reach the large intestine. Long chain fatty acids are not absorbable by this organ and undergo a series of bacterial modifications (41). It has been suggested that the gut microbiota metabolize dietary fats (e.g. by producing diacylglycerols from polyunsaturated fats), convert primary bile acids into secondary bile acids and impact on the entero-hepatic circulation of bile acids and fat absorption from the small intestine (42).

Only few human studies have investigated the effect of high-fat diets on the fecal microbiota composition. Individuals in a low-carbohydrate/high-fat diet had significant lower fecal bifidobacterial numbers, concentrations of butyrate and total SCFA, defecation frequency and fecal excretion as compared with high carbohydrate/low-fat diet (43). Mice models are frequently used to understand the role of the intestinal microbiota in obesity, since these animals can be housed under controlled conditions and fed specific controlled diets such as diets rich in fat. The administration of a high-fat diet to both wild-type and *RELM β* knockout mice, resistant to fat-induced obesity, increased the relative proportions of the phyla Proteobacteria, Firmicutes, and Actinobacteria in the feces, whereas the levels of *Bacteroidetes* decreased in both mice (44). Another study that compared genetically induced obese mice fed a low-fat diet with wild-type mice fed either a low-fat or high-fat diet observed compositional changes in the fecal microbiota, primarily as consequence of the high-fat diet rather than of genetically induced obesity (45). These results indicated that the fat content in the diet itself rather than the obese state of the host induced the changes in the microbiota composition.

Dietary fat types also have distinct effects on the fecal microbiota. The habitual intake of different types of fats correlated with the fecal microbiota composition of monozygotic twins (46). Higher MUFA consumption was associated with lower bifidobacterial numbers. In addition, the increased ingestion of (n-3) PUFA had a positive association with the numbers of bacteria within the *Lactobacillus* group. In the same study, co-twins with the same SFA intake had very similar *Bacteroides* spp. profiles, significantly different from the twin-pairs having distinct SFA intake, suggesting that the intake of SFA affects the diversity of *Bacteroides* spp. by targeting specific strains within the same group. The *Bacteroides* enterotype was found to be highly associated with the consumption of fat, in particular with SFA and MUFA, in a study with healthy volunteers (36). These observations suggest that the consumption of fat and animal-derived products, typical of the Western diet, is associated with increased *Bacteroides* spp. prevalence in the human gut microbiota. In addition, a recent study identified *Bacteroides* spp., *Bilophila wadsworthia* and *Alistipes putredinis* as the most abundant taxon in individuals having an animal-based diet higher in fat as compared to a plant-based diet (47). The reason why the microbiota changes in response to high-fat diets is still not clear, and the relationships between changes in the microbiota and disease development remain to be elucidated. Different types of dietary fat have distinct effects on the fecal microbiota

composition, suggesting that a balanced diet with regard to fat consumption is critical not only for the host's health but also for the gut microbiota.

Polyphenols

Polyphenols are regular components of foods, being the most abundant flavonoids in the human diet (48). The main dietary sources of polyphenols are fruits, beverages such as coffee, tea and wine, chocolate, and to a lesser extent, vegetables, cereals, and legume seeds (49). Besides providing colour and flavour to fruits and vegetables, polyphenols influence health as a consequence of their antioxidant and antimicrobial properties, free-radical scavenging activity (50), and protective effect against cardiovascular disease, cancer and other degenerative conditions (51). Although flavonoids and their glycosides can be absorbed through the GI-tract (52), their intestinal absorption is usually slow, incomplete, and thus highly variable. Most flavonoids are glycosylated in food, which influences absorption through the intestinal barrier (49). Unabsorbed dietary phenolics and their metabolites, in addition to their direct beneficial effect on the human tissues, exert significant effects on the intestinal environment by modulation of the microbiota (50). Tea phenolics (e.g. epicatechin, catechin, gallic acid and caffeic acid) significantly repress certain bacteria such as *Clostridium perfringens* and *Clostridium difficile* and members of the *Bacteroides* spp., whereas members of bifidobacteria, *Lactobacillus* spp. and non-pathogenic *Clostridium* spp. were relatively unaffected (48). Moreover, the consumption of red wine polyphenols increased the numbers of *Enterococcus*, *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Eggerthella lenta* and *E. rectale*-group in the fecal microbiota of adult man (53). Knowledge about the impact of polyphenols on the composition and activity of the intestinal microbes is poor, however, recently developed technologies will certainly lead to a better understanding of the interactions between polyphenols and the microbiota.

CRITICAL ANALYSIS

It has become evident that diet has a considerable effect on the fecal microbiota. However, diet has been changing worldwide as a result of alterations in lifestyle, agricultural practices and population growth. Several features common to a Western lifestyle, including low levels of physical activity and consumption of processed foods, are known risk factors for development of diseases such as colorectal cancer and obesity. Diet induced changes in the microbiota composition may contribute to the development of these diseases. Therefore, a more comprehensive understanding of diet-related diseases will benefit from elucidating interactions between nutritional and microbial dynamics. There have been a growing number of studies relating dietary intake with the modulation of the intestinal microbiota. In addition, studies on the effect of controlled diets (e.g. high-protein or high-fibre diets) on the fecal microbiota have been performed in humans. Future studies considering other nutrients and food ingredients, in particular components of processed foods, may contribute to a better understanding of the impact of diet on the large intestinal microbiota.

CONCLUSIONS

Different substrates have distinct effects in the

composition of the large intestinal microbiota. Since these microorganisms live in symbiosis with the human host in a healthy environment, the study of the dietary effects on the composition and functionality of the intestinal microbiota is a topic of major importance in the current days.

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