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How Diet, Exercise, and Probiotics Influence Diversity in Gut Microbiota

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Introduction

Microscopic organisms, or microbes, are found in considerable numbers in the distal gut of the mammalian digestive tract. Without exposure to a variety of microbes at a young age, humans can develop allergies and autoimmune, respiratory, and intestinal diseases, all of which can cause different types of cancer. To understand the complexity of the human intestinal gut and bacteria, it is essential to understand the physiology, anatomy, and evolutionary path of vertebrates. Vertebrate evolution and today’s modern lifestyle have influenced the microbe population living in our intestines (gut microbiota). Multiple studies have been conducted on different vertebrates with objectives to better understand how diet, exercise, and probiotics affect the microbiota in the gut.

Evolutionary impacts on the digestive system and gut microbiota

Microbes inhabit every corner of life. They define multiple symbiotic relationships both inside and outside the body cavity. Internally, the gut is of great importance for overall health, as it is a primary location of disease. The gut contains about 50% of the body’s immune cells, uses 40% of the body’s energy, and harbors ~10^{14} bacteria with between 800 and 40000 bacterial species (Rauch and Lynch 2011; Thomas and Ockhuizen 2011). Normal flora of the gut helps vertebrates in the fight against pathogens and with the ability to extract necessary amino acids and proteins from food sources during digestion.

Evolution has created slight variations of the intestinal anatomy and function that has impacted the host’s microbiota. Vertebrates have also evolved based on their diet and environmental conditions. An increase in plants using C4 carbon fixation and photosynthesis occurred around 1.8 million years ago, decreasing CO$_2$ in the atmosphere creating a spike in plant variation (Ley et al. 2008a). This shift in availability of leafy green plants led to the selection of high-crowned teeth and longer gut retention time for effective digestion (Ley et al. 2008a).

Herbivores, or animals that selectively feed on plants, are thought to have longer guts due to the difficulty in digesting cellulose and in gaining energy through the digestive process. All vertebrates lack the cellulase enzyme necessary to digest cellulose and yet many animals have evolved mechanisms to process and digest plant material. One common feature of all herbivores is the abundance of symbiotic microbes - which can degrade cellulose - in their guts. From a comparative perspective, herbivores have the greatest microbial diversity, which is to be expected, followed by omnivores, and then carnivores (Ley et al. 2008a). Lengthening the gut provides additional space, optimal living conditions, and longer retention times for bacteria to facilitate the fermentation process.
Depending on where the gut microbes reside, herbivores can be grouped into foregut and midgut fermenters, while omnivores can have simple guts or be hindgut fermenters. Examples for foregut fermenters include cattle and sheep, whereas horses, pigs, rabbits and rats are hindgut fermenters.

**Gut microbiota diversity**

A wide variety of microbes exist in the intestinal gut of vertebrates. The major group or phyla of bacteria found in the guts of herbivores, omnivores, and carnivores is referred to as Firmicutes (Ley et al. 2008a). Cattle are herbivores and contain a rumen, which is an anaerobic compartment that houses various phyla. The rumen also provides optimal conditions for the microbial breakdown of cellulose and conversion into energy products, such as glucose and fatty acids. In a study based on the rumen microbiota of cattle, Jami et al. (2012) discovered that Firmicutes was the dominant phyla, along with Bacteroidetes. Together, these microbes comprise approximately 93% of the bacterial community. Proteobacteria accounted for 5.5%, while Actinobacteria and Tenericutes together total just 1.5%. However, the ratios of the microbes were different between individual animals, demonstrating variability in the resident communities of microbes. Bacteroidetes readings varied dramatically, ranging from 26 to 70%. This variability provides indirect evidence of the potential importance of this particular phylum (Jami et al. 2012). The above experiment was performed using the same breed and herd; therefore, future studies should compare the complement of gut microbes in other breeds of cattle, on various diets, and under different environmental conditions (Jami et al. 2012). Additionally, future research needs to determine the role of the various microbes in the foregut or rumen.

**The evolution of human nutrition and gut microbiota**

Lipids, proteins, and carbohydrates are chemical compounds collected from the environment to be converted into cellular energy. Humans use fermentation to catabolize carbohydrates and gather energy, but the digestive tract does not contain a rumen. According to Ley and colleagues (2008b), the spread of C4 plants on grasslands created the first major impact on human diet and behavior. The second event came during the Pleistocene age, where larger quantities of meat encompassed the diet. This brought on the “hunter-gatherer” culture, leading to the third major change in diet with the adoption of agriculture, preservation, and domestication of animals. Ley et al. (2008b) also posits that these critical diet changes have left an imprint on the human genome.

Researchers categorize human populations according to their histories, lifestyles, and diets. Human relatives contain more similar gut microbes than...
unrelated humans who live in the same habitat. Similarly microbial communities in the human gut are more comparable to one another than other primate species’. In a study done by Ley et al. (2008a; 2008b), fecal samples from domesticated and wild primate species (Bonobos, Chimpanzees, Orangutans, and Gorillas) indicated a close relationship of human fecal microbiotas to other primates’ relative to non-primates’. Of all the primates, Bonobos’ had the most similar fecal microbiota compared with humans (Ley et al. 2008b). Initially it was thought that, due to humans’ modernized synthetic diet, human gut microbes would differ more from other primates’. Interestingly, human gut microbe diversity was not drastically different from select omnivorous primates’ used in the study. This may be an effect of the loss in environmental biodiversity. The biodiversity hypothesis states “reduced contact of people with natural environmental features and biodiversity may adversely affect the human commensal microbiota and its immunomodulatory capacity,” (Hanski et al. 2012). Researchers predict that by 2050 two-thirds of the global human population will be living near little green space and will have limited contact with nature and biodiversity, resulting in an increase in illness (Hanski et al. 2012).

**Gut microbes and human disease**

Human diseases directly correlated to the intestinal gut include obesity, irritable bowel syndrome (IBS), colon cancer, and inflammatory bowel disease (IBD) (Rauch and Lynch 2011; Thomas and Ockhuizen 2011; Ley et al. 2008b, Hold 2014; Allen et al. 2015). The gut also affects upper respiratory tract infections (URTI), Type I diabetes mellitus, rheumatoid arthritis, advancement of HIV infections, cardiovascular disease, allergies, and asthma (Rauch and Lynch 2011; Thomas and Ockhuizen 2011; Ley et al. 2008b; Hanski et al. 2012; Gleeson et al. 2012; Clarke et al. 2014; Hold 2014; Ray 2014; Allen et al. 2015). Many of these diseases or disorders are caused by a loss in microbiota diversity or when one bacterial species overwhelms the microbial community, called dysbiosis (Rauch and Lynch 2011). With the loss of bacterial variability, key energy and digestive requirements are lacking for lipids, proteins, and carbohydrates.

To maintain bacterial variance in the gut, one must eat a healthy diet and stay active. Like all living species, microorganisms can only survive in specific conditions. Once their environment is altered, they struggle for survival. Most gastrointestinal pathogens (microbes causing disease) are temperature specific, which is why mammalian temperatures rise naturally during fever to kill off pathogens. Ectotherms, that cannot regulate their own body temperature, therefore they migrate towards warmer areas to raise their internal temperature and kill off pathogenic microbes. A study by Matthew Kluger (1979) concluded that the prevention of an elevated temperature during sickness is harmful. This provides
evidence that vertebrates have evolved physiological and behavioral mechanisms to raise internal temperatures and offset homeostasis to fight disease. For example, *Escherichia coli* causes many gastrointestinal diseases and has an optimal temperature of 37°C. With even a slight increase in temperature, *E. coli* is unable to replicate and dies off.

**Diet diversity, health status, drugs, and gut microbes**

Body temperature is not the only environmental factor for maintaining microbiota diversity. Diet and nutrition have substantial effects on the gut microbiota and the overall health of an individual or vertebrate. Various microbes use different mechanisms to collect energy from lipids, proteins, and carbohydrates. A recent study revealed that children raised on a ‘Western’ diet, or high-fat, low-fiber diet, contain a drastically less diverse fecal microbiota compared to children in a rural village in Burkina Faso, Africa, where the children are fed plant based, high fiber diets (Rauch and Lynch 2011; Thomas and Ockhuizen 2011). Bacteroides and Firmicutes were the two dominant phyla found in the African children’s fecal samples (Rauch and Lynch 2011). Loss of diversity in microbiota has been correlated to health concerns such as obesity. Obese individuals tend to have altered levels of specific bacterial groups with a loss in diversity being the consistent outcome (Gleeson et al. 2012; Hold 2014).

Today, doctors and researchers suggest that the biodiversity in the gut could become the new biomarker for human health status (Rauch and Lynch 2011; Thomas and Ockhuizen 2011; Hanks et al. 2012; Gleeson et al. 2012; Clarke et al. 2014; Hold 2014; Ray 2014; Allen et al. 2015). The bacteriological ecosystem, consisting of three enterotypes (Bacteroides, Prevotella, and Ruminococcus) has been found to be independent of the host’s ethnicity and country (Thomas and Ockhuizen 2011). Identification of an individual’s enterotype may help identify potential health risks, allowing doctors to inform patients and take preventive measures (Thomas and Ockhuizen 2011). Thomas et al. wrote about a Professor Haslberger from Austria who emphasizes how genetic factors, aging, environment, pharmacological and chemical therapies, and diet are continuously impacting the microorganism community. Haslberger’s studies have shown a less diverse microbiota and lower levels of *Clostridium* cluster IV (Ruminococcaceae) in the elderly population. Chemotherapy combined with antibiotic treatment decreases overall bacterial count, as expected. Genera that are negatively affected by these therapies and treatments include *Clostridium* clusters XIVa and IV, levels of *Faecalibacterium* spp., and Proteobacter. However, *Enterococcus faecium* increases numbers as resulted from the chemotherapy and antibiotic treatment. Understanding the impact of diet, nutrition, and pharmaceuticals is fundamental to maintaining a healthy microbiota.
**Effects of exercise on gut microbe diversity**

Physical exercise has also been linked to an increase in gut biodiversity (Clarke et al. 2014; Hold 2014; Ray 2014; Allen et al. 2015). In 2014, Clarke and colleagues published the first study directly correlating the effects of exercise and diet on the gut microbiota (Clarke et al. 2014; Hold 2014; Ray 2014). These researchers predicted that athletes would have a more diverse microbiota than their non-athletic counterparts. They studied a professional all male rugby team during the preseason training in a regulated environment. Given that extremely rigorous exercise is often associated with extreme diets, the findings were correlated with diet as well as exercise. As hypothesized, athletes demonstrated significantly higher gut microbiota diversity compared with controls of the same age, body mass index (BMI) and gender. Fecal microbiota collected from the athletes contained 22 distinct phyla in the gut compared to 11 phyla and nine phyla in the low and high BMI control groups. Compared to controls, athletes also showed lower inflammation and improved metabolic markers.

However, the dietary intake of the rugby athletes and control groups were drastically different. The rugby players consumed a greater amount of calories, protein, fat, and carbohydrates per day than both control groups. Due to a higher caloric and energy intake gut diversity was positively correlated with protein intake and plasma creatine kinase levels (an enzyme marker of extreme exercise released from muscle into the blood). This study demonstrates that both diet and exercise can alter microbiota diversity. To confirm that exercise has a direct effect on the gut microbial community, independent of diet, and a causative role in altering their diversity, future studies are required. As research in the diet and exercise variables continue, others are looking into the effects of introducing microbes into the body for beneficial effects (i.e., probiotics).

**Dietary probiotics, health, and exercise**

Medical doctors use probiotic (live microbe) therapy to increase the intestinal gut microbe community and boost the immune system. Researchers have been experimenting with the effectiveness of various bacterial strains and their effect on athletes’ health and performance. In order to successfully validate probiotic health claims, one should follow recommended guidelines including; the selection of an appropriate target population and comparison group, a reasonable duration period, precise identification of the test microorganism, the determination of biomarkers that are exhibitive of disease reduction and health, and appropriate safety assessments (Rauch and Lynch 2011). Athletes are a good group to study and observe evidence for health benefits (Thomas and Ockhuizen 2011; Gleeson et al. 2012; Clarke et al. 2014; Hold 2014; Ray 2014; Allen et al. 2015). For example, marathon runners are prone to illnesses, particularly in the
upper respiratory tract. Beyond the physical and energetic stresses of exercise, exercise also increases psychological stress, and may lead to a deficient diet, time zone traveling, disrupted sleep habits, exposure to unusual environments, and greater exposures to pathogens (Thomas and Ockhuizen 2011; Gleeson et al. 2012; Clarke et al. 2014; Hold 2014; Ray 2014). During endurance training, respiration is of great importance for aerobic energy production. However, increasing lung ventilation increases the potential for exposure to pathogens (virus and bacteria) in the respiratory system.

Numerous microorganisms have been analyzed and scientists are discovering that not all species of microbes from the same genus have identical benefits, but varying genera have similar effects in the gut. Gleeson et al. studied various strain-specific Lactobacillus and their abilities to enhance health, particularly in endurance athletes (Thomas and Ockhuizen 2011; Gleeson et al. 2012). In particular, Lactobacillus fermentum has been shown to reduce the length and severity of upper respiratory tract infections (URTIs) in endurance athletes. This bacterial strain enhances the athletes’ immune system and response to antigens by increasing mucosal immunity (Rauch and Lynch 2011; Thomas and Ockhuizen 2011). In a similar study, endurance athletes were monitored over four months during spring training to see whether they were less likely to contract a URTI while taking a probiotic supplemental pill of the L. salivarius bacterium (Thomas and Ockhuizen 2011; Gleeson et al. 2012). Due to repetitious and strenuous workout regimens, athletes tend to have weakened immune systems causing higher rates of URTI. Each athlete was randomly placed into one of two groups: the control or placebo group and the probiotic group. Levels of white blood cells, immunoglobulin IgA, lysozymes, T-cells, B-cells, and natural killer cells were all evaluated and compared amongst the placebo and probiotic groups. Dr. Gleeson and his fellow researchers hypothesized that the probiotic supplement would help compensate for and boost the immune system, causing a reduction in the occurrence and severity in URTI. Unfortunately, results of the probiotic and placebo were almost identical and the proportion of athletes who experience one or more URTI was not significantly different than controls.

A study on healthy elite male Australian distance runners with Lactobacillus fermentum VRI-003 showed a correlation between runners with the bacterial strain and better health (Rauch and Lynch 2011; Thomas and Ockhuizen 2011). The runners on probiotics experienced URTIs with a shorter duration period and less intensity. Another probiotic study used Lactobacillus casei Shirota under double-blind, and placebo-controlled conditions with select endurance athletes receiving the bacteria over four months in the winter season (Thomas and Ockhuizen 2011). Results showed a concentration increase in salivary IgA with Lactobacillus casei Shirota. With this increase IgA benefited small numbers of
subjects distressed with gastrointestinal symptoms or those experiencing one or more weeks with URTI symptoms, along with a few subjects with URTI occurrences. Finally, another study by Gleeson was looking at species *Lactobacillus casei* DN-114001. This particular strain was monitored in army cadets during just three weeks of combat training. This strain of bacteria showed that it improved immune system maintenance with salivary IgA levels (Thomas and Ockhuizen 2011). One concern with this study is that its duration was too short to determine any effects on URTIs. Lastly, unlike *L. salivarius*, *Lactobacillus rhamnosus* GG did not improve the health of the athletes (Thomas and Ockhuizen 2011).

**Conclusion**

Collectively, these results help scientists, athletes, doctors, and civilians understand the effects of diet, exercise, and particular *Lactobacillus* probiotic strains on the gut microbiota. In the future, one may selectively determine which probiotic is most beneficial for both athletes and non-athletes according to a particular situation, circumstance, or context. Future studies on diet should focus on finding the best ratio of gut microorganisms and what their required environmental conditions should be. Having a better understanding of the gut microorganisms and their requirements will help construct an optimal diet for human health and performance. Research focused on exercise should determine what types of exercise and exercise characteristics (intensity and duration), for males and females, are most influential on the microbiota. Ultimately, more than one *Lactobacillus* probiotic strain must be analyzed to see if combinations of various strains are more beneficial than a single strain.

**References**


