THE IMPACT OF THE MICROBIOTA ON HUMAN HEALTH AND DISEASE

Hannah Riches

Gold Crest Project Report
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Introduction

I began researching intestinal microflora after reading several articles about links between composition of microbiota and mental health. Since then I have discovered several common conditions that have been associated with a change in microorganisms in the gut, and I decided to begin my Crest Project on this because of how current and relevant of this topic is. It has been incredibly exciting following this area of research because of how new this area of science is, however I have found it difficult when comparing contrasting experiments and studies, as it is challenging knowing which source to trust. As a project, it has evolved immensely, as I discovered new and exciting fields to follow and learn more about. This means that the plan I constructed at the start of my Project has changed quite dramatically as my project progressed.

I got my information from many different places, the most common being the NCBI website (National Centre for Biotechnology Information), where I discovered that I had access to thousands of scientific articles and information. Prior to reading an article about Gut Health, I knew very little about this topic, which is partly why this Project has been so exciting, as all the information I have been reading about is new and relatively unheard of. To begin with, my aim was to gain an overall understanding of this topic, so that I would be able to establish an overall direction for my project to follow.

The intestinal microflora is a term that describes the collection of microorganisms that inhabit our digestive system. It is a positive health asset that crucially influences the structural and functional development of the mucosal immune system. The flora has a collective metabolic activity equal to a virtual organ within an organ, which has led to debates about whether it should be considered an organ because of its great importance to human health. Interactions between the host and the microbe occur primarily along mucosal surfaces, the largest of those being the human intestinal system. The diverse bacterial community is separated from the internal human tissue by only a layer of epithelial cells. Bacterial cells makes up 60% of faecal mass.¹

Original Plan

My first aim was to develop my own understanding of the topic I have chosen to follow for my Crest Project. I began by establishing interesting areas to research and explore and discovering the best ways to find information and whether to trust my sources, something that proved difficult due to the lack of articles available for crosschecking.

I first considered various areas of interest:

- Is the gut microbiome really the forgotten organ?
- What species of bacteria and other microorganisms colonise the human gut?
- Location of the different species of bacteria and other microorganisms in the human intestine.
- What techniques are used to identify and classify bacteria?
- When do bacteria first colonise the human gut?
- What is the effect of having no bacteria in the human gut?
- Are we as a society too clean, and what is the long term effect of this?
- What environmental factors can affect the structure and composition of intestinal flora?

• What chemical substances are synthesised by the gut microbiota and what is their importance in the human body?
• What is the difference between pathogenic and beneficial bacteria?
• What is the relationship between the gut microbiome and obesity?
• What is the effect of the composition of the microorganisms in the gut on mental health, and the chemistry behind it?
• What other medical conditions are affected by the gut microbiome?
• Nobel Prize 2005-The discovery of the bacterium Helicobacter pylori and its role in gastritis and peptic ulcer disease.
• How does the host’s immune system detect and eliminate pathogenic organisms, but not mutualistic ones?

Once I had gained a greater level of knowledge, I could begin to think about what I could achieve with my Crest Project, further than just expanding my own knowledge. Starting this project, I knew that completing some practical work would be very difficult, however the process of planning and developing ideas for experiments has been as useful to me as actually being able to carry out the experiments myself. A problem that I have discovered whilst researching this area, is the complexity of the information, meaning that it was very difficult to select what I understood to include in my project. 30 hours into my project, it is this that I decided to direct my project towards, as a great deal of this information and research is very relevant to everyday life. However, because of the format that it is explained in the scientific papers, it is very difficult for people without a background knowledge of science to relate to what is being discovered, and I believe that this is an area that I could develop in order to benefit others.

After my project is completed, I hope that I will have understood a great deal more about the relationship between our gut microbiota and our health, and that I will continue following the research and exploration into this fascinating topic. As well as this, I hope that by the end of my project I will have made this information more accessible to people who would not choose to read a science paper for fun, and that this enables them to positively influence their own health through a greater comprehension of the intestinal microbiome.

Background Research
In order to gain a general understanding of the gut microbiota, I watched many TED talks. I used these to begin researching various areas of interest, of which there was an endless supply. I found several thousands of scientific papers and articles on the general topic, and it has been a challenge locating the information that I understand and being able to put it into simpler terms to record in this report. It has also proved difficult knowing what point I no longer understood the information, and where to admit defeat.

Initial colonisation of the human gut—where it all begins
Infants rely on colonisation to develop their immune system and the gastrointestinal tract. The first few months of life can drastically affect the child’s likelihood of developing diseases such as obesity, food allergies and inflammatory bowel disease. The mode of delivery affects the initial composition of the microbes that first colonize a newly born child. For instance, children that are delivered by normal birth are colonised primarily by vaginal microbes. However, children delivered via caesarean section will develop an ecosystem more similar to that of skin. From then, diet during infancy affects how the microbiota develop, for example whether the baby feeds on breast milk or formula feeds.
Infants who are put on antibiotics during the first 6 months of life are more likely to develop obesity, as a result of the antibiotics disrupting the balance of the microbiome, resulting in dysbiosis.2

The earliest colonising bacteria, including *Escherichia*, and *Enterococcus*, establish an anaerobic environment shortly after birth. Consequently, this allows obligate anaerobes such as *Firmicutes*, *Clostridia*, Bacteroidetes, and in particular *Bifidobacteria* to successfully colonise the gut. This is an important genus, as *Bifidobacteria* develop into the largest group within the infant microbiome, and takes part in many reactions. From here on, the microbiome diversifies. These first species of bacteria are known as the ‘pioneer microbiome’, as it is the first exposure of the immune system to intestinal microflora and provides favourable conditions for colonisation, consequently allowing more microbes to colonise. Bacterial strains taken from adults are often found in their relatives too, showing a connected colonisation within people who grew up or even live together.

Contrary to what was previously believed, the intrauterine environment is not sterile. The infant’s first stool (meconium) is thought to reflect the intrauterine environment. There is a noticeable lack of research and knowledge surrounding the topic of intrauterine colonisation, and therefore a limit to what I am able to include here.

The vernix caseosa, the waxy skin coating of a foetus, is shed a few weeks before birth into the amniotic fluid, which surrounds the foetus. Shortly before birth, the foetus swallows amniotic fluid containing fragments of vernix caseosa, which is made of short chain fatty acids (SCFAs). These are the first probiotics, which are compounds that are indigestible by human enzymes, but provide a rich medium for the growth of bacteria. SCFAs are linked to prevention of nectrotising enterocolitis, because they alter the microbe composition, as well as increasing the cytokine interleukin. After birth, the mother’s breast milk contains viable bacteria from the maternal gut, increasing bacterial diversity. It is relatively unknown where in the mother’s body these bacteria originate from, however it has been suggested that some species may move via specific immune cells to the mammary glands.3

Colostrum, the first milk made by the mother, contains a high concentration of human milk oligosaccharides (HMOs), which cannot be digested by human enzymes, and therefore serve as probiotics to promote the growth of intestinal microbes. HMOs are known to selectively encourage the growth of commensal bacteria, while suppressing growth of pathogenic bacteria such as *E. coli* and *C. Perfringens*. Growth of *B. infantis* on HMOs instead of lactose results in the bacteria binding more frequently to intestinal epithelial cells. This provides anti-inflammatory effects, whilst creating a stronger barrier between the lumen of the gut and the bloodstream, which prevents pathogenic bacteria crossing the mucosal membrane.2

From birth, the gut microbiota has three important roles in the baby’s body: protection, metabolism, and tropic functions. The commensal microorganisms act as a barrier, preventing pathogenic microorganisms colonising the epithelium wall and causing infection. They also break down colostrum, breast milk and baby foods, and are important in vitamin synthesis and absorption of

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ions. Trophic functions include growth and differentiation of epithelial cells in the intestines, and the maintenance of the immune system. The infant gut is fully colonised at around 3 years of age, where immune homeostasis is achieved. If, for any reason, the infant gut has not been fully colonised by this point, this could lead to dysbiosis, a microbial imbalance in the gut.4

The species of microorganisms which colonise the human gut
The term ‘microorganism’ does not only refer to the billions of bacteria that colonise the human gut, but also to the Archaea and Eukarya. Intestinal microflora contains over 400 bacterial species, of which more are obligate anaerobes than facultative anaerobes (bacteria which are able to change between respiring aerobically and anaerobically depending on the concentration of oxygen available) by a factor of 1000.5

Bacteria that can survive using sporulation have been discovered in human faecal samples. These bacteria form spores in order to be transferred between two individuals, which only germinated in the presence of bile salts. It is estimated that these spore forming species make up 30% of total intestinal microflora (Trevor Lawley’s Study 2016). Bacteroidetes and Firmicutes are the main bacteria involved in the metabolism of undigested food.6

Communication between the gut and the brain
It is now known that the gut and the brain communicate in both directions by three pathways: immunological, endocrine, and neural pathways.7 The role of the gut brain axis is to control gut functions, as well as linking emotional and cognitive centres of the brain with intestinal functions, activation of the immune system, permeability of the intestines, enteric reflex and entero-endocrine signalling. This network includes the central nervous system, autonomic nervous system, enteric nervous system and the hypothalamic pituitary adrenal axis.

There has been recent evidence to suggest that enteric microbiota effect the gut brain axis, by interacting with intestinal cells and the enteric nervous system (ENS) as well as the central nervous system (CNS) using neuroendocrine and metabolic pathways. The first evidence that brought about this discovery was the observation of the significant improvement in patients with hepatic encephalopathy, after taking antibiotics. More recently, research has shown that the microbiota can influence anxiety and depression, as well as affecting dysbiosis in people with autism. It has been found that in irritable bowel syndrome; the gut-brain axis is disrupted, resulting in several important changes in the gut. For example, differences occur in movement and secretion of the intestines,

visceral hypersensitivity (pain of the inner organs) can be caused, and changes may occur in the cells of the immune systems and entero-endocrine cells.

Studies conducted on germ-free animals have shown that the colonisation of the gut by bacteria is crucial for the development and maturation of the enteric and central nervous system. If colonisation does not occur, i.e. the animal is germ free, the way that neurotransmitters work in both nervous systems changes, as well as changes to gut sensory-motor functions. These can include delayed gastric emptying and the time food remains in the digestive system, as well as enlargement of the cecum. Gene expression of enzymes involved in the synthesis and transport of neurotransmitters is reduced in germ-free animals. Following colonisation of the gut, i.e. when the animals return to a non-sterile environment, these abnormalities return to normal.

It has also been found that germ free mice have an increased stress response, with higher levels of cortisol and ACTH (produced in response to stress and involved in the hypothalamic pituitary adrenal axis). This change can only be reversed if the mouse is young when it is introduced into a non-sterile environment, suggesting that there is a critical period where the plasticity of the brain is sensitive to input from the microbiota. Interestingly, memory dysfunction has been found in germ free animals, thought to be due to the different expression of brain-derived neurotrophic factor (BDNF), the main factor involved in memory. This molecule is located in the hippocampus and cerebral cortex, and it regulates various brain activities, cognitive functions, and muscle repair, regeneration and differentiation.

A diverse microbiota also helps modulate the serotonergic system, as an increase in serotonin and changed levels or related metabolites has been observed in the limbic system of germ-free animals. Through changing the composition of the gut microbiota by using probiotics and prebiotics, scientists have identified that certain microbiota can alter the neurochemistry of the brain. In particular, certain species can produce molecules that act as neurotransmitters, such as GABA, serotonin, melatonin, histamine and acetylcholine.

Communication also occurs in the other direction. The brain can change the permeability of the intestines, which means bacterial antigens can go through the epithelium and result in an immune response in the mucosa. Stress may be a factor contributing to this.

This communication between the microbiota and brain is thought to involve the vagus nerve, which relays signals from the intestinal lumen to the central nervous system. The previous behavioural and neurochemical effects of altered microbiota composition were not seen in vagotomised mice (mice with parts of the vagus nerve severed). This suggests that the vagus nerve is the primary pathway through which the gut microbiota and the brain communicate.

Distribution of microorganisms along digestive system

There are many more microorganisms in the lower bowel than there are in the stomach and upper intestine, and they vary between existing loose in the lumen and attached to the mucosa. The upper gastrointestinal tract contains fewer microflora; 10^4 organisms/ml of intestinal secretions, most of which are from the oropharynx (area including the mouth, throat and soft palate). As these organisms mostly come from ingested food, the majority of microbes pass out of the gut with each meal. It is unusual for the upper intestine to be colonised, however this occurs during infections of *Vibrio Cholerae* and *Escherichia Coli*. In contrast, the large intestine usually contains microflora in concentrations of 10^{11} /g of stool. Overall, microorganisms outnumber our human cells roughly 10 to 1. Bacteria occupy the lumen, overlie the epithelial cells and stick to the mucosa. It is unusual for
bacteria to pass through the mucosal membrane, and this would occur with pathogens such as *Shigella*, *Salmonella* and *Campylobacter* and can result in serious infections.3

The composition of bacteria along the gastrointestinal tract varies depending on the individual. Hydrochloric acid in the stomach kills most microorganisms ingested with food, and bile contributes to controlling the populations of bacteria along the digestive tract due to its antibacterial properties. Peristalsis prevents colonisation of the upper intestine because of the constant movement. The microorganisms themselves also control the colonisation of other species, by secreting antibacterial substances. This contributes to protecting the host against pathogenic microorganisms.3

**Probiotics**

**History**

Humans have known about the beneficial effects of lactic acid fermentation for thousands of years—drinking sour milk was even mentioned in the Bible. In Ancient Egypt ‘leben raib’, a type of sour milk, was drunk, and people in the Balkans drank a similar formula called ‘jahurt’. However, the science of these effects on human health were not fully understood until Elie Metchnikoff, a Russian scientist, began researching the connection between immunology and raw foods (therefore containing bacteria). He went on to win the Noble Prize in medicine for this in 1907, by recognising that humans were altering their immune system by consuming large volumes of raw food (kefir, sauerkraut, pickles, sour milk), which introduced proliferating lactic acid bacteria into their intestines. 8

**Benefits**

Arguably, their most important function is to ensure that there is little space on the epithelial walls of the lumen, so that pathogenic bacteria cannot bind. They are often prescribed following large periods of antibiotics doses, in order to recolonise the gut with beneficial bacteria without allowing pathogenic bacteria to outcompete the few surviving strains of commensal bacteria. This is a common problem following long or strong courses of antibiotics, as species such as *Clostridium Difficile* can quickly colonise the gut. Probiotics also have a positive effect on the treatment of food allergies because of their relationship with the immune system. The mechanisms for this are relatively unknown, but it is related to how gut bacteria enable the immune system to distinguish harmful cells or toxins from beneficial ones. There is evidence to suggest that probiotics can reduce the instances of dental cavities caused by acid-producing bacteria in the mouth, particularly in children. Many probiotic organisms are producers of B group vitamins, increase immune system efficiency, enhance vitamin absorption, and stimulate generation of amino and organic acids.

**Prebiotics**

Prebiotics are ‘selectively fermented ingredients that result in specific changes in the composition and/or the activity of the gastrointestinal microbiota thus conferring benefits upon host health.’9 They include substances such as dietary fibres and carbohydrate polymers, which are not digested by human enzymes when they are ingested, meaning they are broken down by bacteria in the gastrointestinal tract. The type and quantity of prebiotic can therefore influence the composition of

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the gut bacteria in the human gut. Shifting from a vegetarian diet to a meat-based diet dramatically changes the bacterial diversity in the gut, as the quantity of indigestible material ingested decreases. Studies on humans have shown that greater fibre intake is associated with increased diversity of the bacteria in the gastrointestinal tract. Associations have been observed between low-fibre diets and chronic diseases such as obesity, cardiovascular disease, type 2 diabetes and colon cancer.

The definition of prebiotics have changed since they were originally defined in 1995 as ‘a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health.’ In 2004 this was updated in order to include three things: resistance to gastric acidity and hydrolysis by mammalian enzymes and gastrointestinal absorption, fermented by intestinal microbiota, and selectively stimulate the growth and/or activity of intestinal bacteria associated with health and well-being. As more research is completed on the gastrointestinal microbiome, it is likely that this definition will continue being updated.

Bifidobacteria and Lactobacilli were originally the main beneficial bacteria that were targeted by growth encouraging prebiotics. Bacteria are able to produce a wider variety of enzymes that can break down glycosidic linkages, which are found in substances like starch. This allows us to change our diet in order to modulate the microbiota, because growth of these organisms depends on substrate availability. 10

The Hygiene Hypothesis

The hygiene hypothesis was first introduced in the late 1980s by David P. Strachan, a professor of epidemiology (the branch of medicine that deals with the incidence, distribution, and possible control of diseases and other factors relating to health). He found that children in larger households had fewer instances of hay fever because they are exposed to germs by older siblings. This finding led to further research, which uncovered that a lack of early childhood exposure to unclean conditions can increase the individual’s susceptibility to various diseases. It is also suggested that the hypothesis could be extended from the link with allergies to a link with autoimmune diseases such as type 1 diabetes and multiple sclerosis. 11

For example, in the late 1990s, Dr. Erika von Mutius, a health researcher, compared the rates of allergies and asthma in East Germany and West Germany. Her initial hypothesis was that East German children, who grew up in dirtier and generally less beneficial conditions, would have more allergies and increased cases of asthma than the West. However, her research found the opposite: children in the polluted areas of East Germany had lower allergic reactions and fewer cases of asthma than children in West Germany, which was much cleaner.

Research on a much larger scale has found that children in developing areas of the world are less likely to develop allergies and asthma compared with children in the developed world. Despite this, scientific evidence for this is relatively limited to animal trials and a few clinical trials.6

11 Okada H. (2010) The ‘hygiene hypothesis’ for autoimmune and allergic diseases; an update
The Epidemiology of Allergic and Autoimmune Diseases

Asthma, allergic rhinitis and atopic dermatitis have all significantly increased in industrialised countries, with the prevalence of atopic dermatitis doubling in the last 30 years. Autoimmune diseases have increased with a similar pace, for example type one diabetes now occurs much earlier in life, causing significant problems, especially in Finland. Cases of inflammatory bowel diseases (IBDs), including Crohn’s disease, ulcerative colitis and primary biliary cirrhosis, are also rising. It is also evident that frequency of infectious disease has decreased, due to better water sanitation, pasteurisation of milk and dairy products, vaccination against common infections and the widespread use of antibiotics. Parasitic diseases have decreased significantly in developed countries, and it is in these countries where a rapid increase of allergic and autoimmune diseases has been observed.  

Data regarding geographical distribution of such diseases is fascinating- the arrangement of allergic and immune disease is a mirror image of that of different infectious diseases. Genetics could explain this to a certain extent; however the incidence of diabetes in Finland is six times that of the Karelian republic of Russia, which is directly next to Finland, clearly demonstrating that there must be another influential factor. Migration studies also disprove the idea that this pattern can be explained by genetics, as it was found that offspring of immigrants from one country with a low incidence of allergic and autoimmune diseases quickly acquire the same incidence as the country they are living in shortly after their parents permanently moved there. This proves that environmental factors must play a fundamental part in the development of these diseases. One theory is that modern hygiene standards reduce the frequency of contact with pathogens, which cause the immune system to be overactive and respond to compounds that are not allergens.

New research has discovered the benefits of having pets around the house during childhood because they cause a change in the composition of microbiota, which results in a lower risk of the development of allergies and obesity later in life. The University of Alberta led the study, which recorded the exposure of dogs, cats and other similar animals to children prenatally and up to 3 months following birth. Children who had been exposed to a pet had significant changes in gut microbiota- increases in two beneficial bacteria; Ruminococcus and Oscillospira. These two bacteria have been associated with decreased risk of both developments of allergies and obesity in later life. Dog exposure in the first year of a child’s life resulted in a 13% reduced risk of asthma in late childhood.  

Canadian Healthy Infant Longitudinal Development Study

CHILD cohort included mothers during pregnancy between 2009 and 2012, who were asked to report on pet ownership during their pregnancy and up to three months afterwards. Infant gut microbiota were collected using faecal samples at the average age of 3.3 months old, and these were analysed using 16S rRNA sequencing. Categories of a) Pet exposure only during pregnancy and b) Pet exposure both pre and post-natally were compared to no pet exposure under different birth scenarios.

13 Tun H.M. (2017) Exposure to household furry pets influences the gut microbiota of infants at 3-4 months following various birth scenarios
8% of mothers were exposed in pregnancy alone (a) and 46.8% were exposed both before and after birth (b). In both situations, pet exposure enriched the proportion of Ruminococcus and Oscillospira. All correlations were independent of maternal asthma/allergies, siblings, breastfeeding and other home conditions.

Atopic Diseases

Changes in intestinal microbiota composition as a result of changes in lifestyle or environment may be partly responsible for the increasing development of atopic diseases, in particular those in childhood.14

Atopic diseases are diseases that are brought about by a problem with the immune system. The primary cause for this is the development of a certain immunoglobulin (IgE), which is sensitive to allergens that are usually harmless. Examples of this include atopic dermatitis (eczema), allergic rhinitis, asthma and food allergies.

Atopic dermatitis is a relapsing inflammatory disease of the skin. It is the most common skin disease in children (about 10-20% of children in US and Western Europe have eczema).

Allergic rhinitis is caused by exposure to an allergen, which triggers allergic inflammation in the nose and throat. A commonly known example is hay fever, but also includes allergies to animal fur.

Asthma is a disorder where airway obstruction occurs, and the lungs are much more sensitive to allergens and irritants, occasionally resulting in chronic airway inflammation.

Food allergies are the most common in children, often nut and egg allergies. Occasionally these allergies disappear as the child grows up, but others do not.

There is an interesting link between these atopic diseases. It was found that about 75% of children with atopic dermatitis will develop allergic rhinitis and more than 50% will develop asthma. There are two factors that play an important part in whether a child develops an atopic disease: genetics and the environment.

The hygiene hypothesis suggests that early microbial exposure may enhance postnatal development of the immune system, helping to protect against allergic diseases. Normal development brings about a change from T helper 2, more common in foetuses, to more mature T helper 1 cells. An excess of T helper 2 cells in response to allergens is a clear indicator of allergic diseases. One study found that there was a stronger link between broad-spectrum antibiotics and atopic disease that there was for narrow-spectrum antibiotics.15

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15 Jedrychowski W. (2011) Wheezing and asthma may be enhanced by broad spectrum antibiotics used in early childhood. Concept and results of Pharmcoepidemiology Study
KOALA Birth cohort study 2006

This was the first large-scale prospective study comparing the composition of gut microbiota to prevalence of atopic diseases. The faeces of 957 infants aged 1 month were analysed, and information on atopic symptoms was gathered. Total and specific IgE were measured in venous blood samples taken when the infant reached 2 years of age. At the same time this blood sample was taken, a clinical diagnosis of atopic dermatitis (eczema) was made.

The presence of *Escherichia coli* was associated on average with a higher risk of developing eczema, and infants who were colonised with *Clostridium difficile* were also associated with a higher risk of eczema. These results clearly show that differences in gut microbiota often come before the development of atopy, i.e. development of diseases such as eczema. Genetic susceptibility is involved in the development of atopic diseases, however the increase in frequency of these conditions has been far too rapid for there to have been any shift in genetic makeup. This is why the hygiene hypothesis was suggested as the only possible explanation. Some scientists have now developed this theory, to include the idea that the disturbance of the compositions of gastrointestinal microbiota due to changes in lifestyle and environment have also disrupted important mechanisms in the body involved with the development of the immune system.

The study also found that *C. Difficile* is associated with a higher risk of atopic eczema, including increased sensitisation (higher levels of Immunoglobulin E in the bloodstream). Furthermore, allergic children had much higher levels of i-caproic acid in their stools compared to children who were not allergic. This short chain fatty acid is thought to indicate the presence of *C. Difficile* in the intestines. Interestingly, when scientists used IgG serology (a test which is able to test for levels of Immunoglobulin G, the most common type of antibody found in the circulation), they found increased IgG specific to *C. Difficile* in sensitised wheezy infants compared with non-sensitised and non-wheezy children.

Their study surprised several other scientists, because unlike many other studies, they did not find a negative correlation between allergies and Bifidobacteria. However, they believe that this may have been because their study group were all of a very young age, where colonisation of Bifidobacteria is very common. Consequently, there was a lack of contrast in terms of composition of Bifidobacteria.

Increased levels of *E. coli* and *C. Difficile* is important, because it results in decreased levels of beneficial bacterium. This may result in reduced induction of T regulatory cells by these beneficial bacteria, leading to immune dysregulation. Without optimal levels of immune regulation, a person may develop Th1 (examples include Crohn’s disease and autoimmunity) or Th2 (for example atopic diseases) mediated inflammatory disorders. It is also thought that *E. coli* and *C. Difficile* have a direct effect on the production of cytokines by antigen presenting cells, consequently affecting the production of T cells. Another possible hypothesis is that *E. coli* and *C. difficile* increase the permeability of the intestine walls, which could allow the penetration of harmless antigens, resulting in sensitisation. Toxins A and B produced by *C. Difficile* have been proven to compromise the cells of the wall of the intestines. Increased intestinal permeability has been observed in patients with several atopic diseases, including food allergies, eczema and asthma, in comparison to healthy people.

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The findings of this study strengthen the previous ideas about a causal relationship between gut microbiota and development of atopic diseases. This is important, because it suggests that there are potential benefits of using probiotics to treat and prevent these diseases. It is now known that there are several factors that can influence the composition of the gut microbiota, including mode of delivery, antibiotic treatment, hospitalisation and formula feeding.

Recent research has uncovered the link between the human biota and the pathogenesis of atopic diseases. There are clear differences in the lung microbiomes of healthy and asthmatic patients. Developed countries have seen a sharp increase in the numbers of cases of atopic diseases, despite being exposed to far fewer and less diverse microorganisms.

Firstly, the gut microbiome plays a fundamental role in forming the host’s immunity, and it does this by balancing the activities off Th-1 cells and Th-2 cells. There is plenty of knowledge on the intestinal microbiota, however at present the lung microbiota is much less explored. There are several factors that are associated with increased chances of developing asthma and allergies, including breastfeeding, introduction of solid foods, use of antibiotics by either mother or infant, and birth by caesarean section. For example, a recent study on infants aged 1 month found that birth by caesarean section is directly linked to later development of asthma at 6 years of age, and colonisation by \textit{Clostridium difficile}. On top of this, children born by caesarean section have twice the risk of developing egg and milk allergies.\textsuperscript{9}

\textbf{Study of association between gut microbiota and food sensitivity}\textsuperscript{17} revealed that among 166 infants, 7.2\% displayed allergic reactions to one or more food allergens at 1 year of age, which correlated with increased lower gut microbiota richness and elevated \textit{Enterobacteriaceae:Bacterioidaceae} ratio. This suggests that the species that initially colonise the gut may contribute to development of atopic diseases.

\textbf{Symbiosis of Intestinal Microflora}

The normal gut microbiota has a specific function in host nutrient metabolism, xenobiotic and drug metabolism, maintenance of structural integrity of the gut mucosal barrier, immunomodulation, and protection against pathogens.

In total, over 1000 different bacterial species have been found in the human gut, with around 160 in any one individual.\textsuperscript{18} The human gut microbiome is thought to be about 3 million genes, which is 150 times larger than the human genome. These species have great importance in terms of the digestion of our food- many microorganisms secrete enzymes that we cannot make, simply because our genes cannot code for them. For example, the microbiota supplies essential nutrients, synthesises vitamin K, aids in the digestion of cellulose and promotes angiogenesis and enteric nerve function. Research has shown that there is most likely a connection between the gut microbiota and the aetiology of conditions like Inflammatory Bowel Disease, Irritable Bowel Syndrome, colon cancer and antibiotic-related diarrhoea. Studies that are more recent have revealed a possible link with obesity and diabetes. \textit{Bacteroidetes} and \textit{Firmicutes} are the main bacteria in the gut to metabolise undigested

\textsuperscript{17} Azad M.B. (2016) \textit{Infant gut microbiota and food sensitisation: associations in the first year of life}

\textsuperscript{18} Zhang Y. (2015) \textit{Impacts of Gut Bacteria on human health and diseases}
Metabolism of nutrients
The gut microbiota mainly uses undigested carbohydrates from the diet of the host as their energy source, resulting in the formation of Short Chain Fatty Acids, which are good sources of energy for the host. Another important function of the gut microbiota is to synthesise Vitamin K and some components of Vitamin B.\(^{19}\)

Short Chain Fatty Acids\(^{20}\)
Butyrate, the third most common SCFA, is considered the most important in terms of human health. It serves as the main food source for colonocytes, and is believed to have anti-cancer activity via its ability to cause apoptosis of colon cancer cells. There is also evidence that butyrate can initiate gluconeogenesis (metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as lactate, glycerol, and glucogenic amino acids). Propionate, the second most common SCFA, does similar things in that it is also a food source for colon epithelial cells; however, it is thought to be important in satiety signalling. This is very complicated, but is related to the molecule’s interaction with gut receptors, which can then signal to the brain when a person is full. The conversion of propionate to glucose via gluconeogenesis encourages energy homeostasis, which consequently reduces the production of hepatic glucose (glucose produced from stores in the liver), which prevents against adiposity. Acetate, the most common SCFA, is an essential cofactor for the growth of other bacteria. For example, *Faecalibacterium prausnitzii* is not able to grow in culture without presence of acetate.\(^{21}\)

Preventing against infection
Commensal bacteria prevent the invasion of pathogenic bacteria because they compete for nutrients and attachment sites on the mucosal surface, known as colonisation resistance. In addition, the growth and survival of pathogenic bacteria is inhibited because the pH of the intestine drops due to production of lactate and short chain fatty acids by commensal bacteria. Some beneficial microorganisms produce toxic or carcinogenic metabolites, which can kill or inhibit the growth of pathogenic bacteria. As well as preventing the actual cause of disease, intestinal bacteria have been found to develop and strengthen the immune system. Gut bacteria in many animals stimulate antibody diversification in GALT (gut associated lymphoid tissue) after birth, and it is assumed that the same thing occurs in humans.\(^{5}\)

Studies of animals bred under sterile conditions showed that these animals developed morphological, structural and functional abnormalities. The majority of animals showed reduced

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\(^{21}\) Rowland I. (2017) Gut microbiota functions: metabolism of nutrients and other food components
vascularity, digestive enzyme activity, muscle wall thickness, cytokine production, immunoglobulin levels, and fewer intra-epithelial lymphocytes. A similar study revealed that butyrate, a short chain fatty acid produced by commensal microorganisms, might enable extrathymic (outside the thymus gland) production of T regulatory cells.5

Maintaining normal gut function
Gut bacteria benefit the host in many different ways, including regulating gut motility, producing vitamins, transforming bile acids and steroids, metabolizing xenobiotic substances, absorbing minerals, and activating and destroying toxins, genotoxins and mutagens. In the proximal region of the colon, bacteria produce SCFA’s, which act as sources of energy for colon cells in the mucosa. These organic acids help to reduce pathogenic bacteria by lowering the pH in the colon. Oxalibacterium formigenes is a species of bacteria in the proximal colon which regulates the balance of oxalic acid, which is a toxic metabolite of glyoxylic or ascorbic acid, as well as reducing the formation of kidney stones.

Gut bacteria are also involved in breaking down many natural compounds, allowing the metabolites of the compounds to benefit the host. One example is lignans, (found in flaxseed, vegetables, fruit), which are involved in protecting the host against cardiovascular diseases, osteoporosis, and various cancers. In a similar way, gut bacteria also take part in the metabolism of Isoflavones, which are structurally similar to oestrogen. These compounds are also involved with protecting the host against cardiovascular diseases, osteoporosis, and various cancers.16

Biochemical Processes of Intestinal Microflora
Specialised gut bacteria perform reductive reactions such as methanogenesis, acetogenesis, nitrate reduction and sulphate reduction.

Methanogenesis
Methanogenesis is the formation of methane by microbes called methanogens. These organisms are no longer considered prokaryotes, and are now compared to eukaryotes.22 Methanogenesis is the final step in the biological decomposition of biomass in the absence of oxygen. About 70% of biologically released methane comes from the conversion of methyl group of acetate (a short chain fatty acid) to methane, however methanogenesis is the production of methane from H₂ and H₂O. This is a symbiotic process because the methanogens use the energy from the reaction to generate a chemical gradient for ATP synthesis, whilst reducing the gas molar volume in the intestine (4H₂ produces 1H₂O). Methanogenesis is a complex chemical pathway which involves enzymes with novel cofactor and metal requirements- for example nickel is crucial for this particular pathway to work. Scientists have suggested that methanogens may play a role in the progression of several diseases in humans, however, this is not certain.23

Acetogenesis
Acetogenesis is the process through which acetate is produced from CO₂, and H₂ (an electron source) by anaerobic bacteria via the Wood-Ljungdahl pathway. The acetate generated by the microbial

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22 Chang C. et al. (2014) Methanogens, Methane and Gastrointestinal Motility
23 Chaudhary P.P et al. (2018) Methanogens in humans: potentially beneficial or harmful to health
metabolism is a beneficial nutrient for other microorganisms and the host. As well as this, acetogens deplete the levels of hydrogen gas that builds up due to the natural biodegradation of organic compounds. Increasing levels of hydrogen inhibits biodegradation by creating unfavourable thermodynamic equilibrium; therefore because acetogens deplete levels of hydrogen in the gut, they enhance biodegradation. Because acetogens have to compete with methanogens, they are forced to resort to many other metabolic pathways, which include the breakdown of many natural compounds such as cellulose, lignin, alcohols, organic acids, aldehydes, etc.24

Nitrate Reduction
Recent evidence has shown that the first breakdown of inorganic nitrates occurs in the mouth, where bacteria use enzymes to reduce the nitrate to nitrite. This is then reduced to NO under acidic conditions in the stomach by non-enzymatic disproportionation, however it can also occur in different tissues under hypoxic or ischemic conditions. The nitrate may have originated either from the diet or from endogenous synthesis. A few enteric bacteria are able to reduce nitrate to ammonia with a catalyst, via nitrite during dissimilatory respiration.25 This connection between nitrate reduction and the gut bacteria could be another way that the microbiome links diet and health.

Parkinson’s and the Gut
Parkinson’s disease is the second most common neurodegenerative disease, with symptoms including tremors and difficulty walking. The connection between the microbiome and Parkinson’s disease was first noted in 2003, when Heiko Braak, a German neuroanatomist observed that Lewy bodies appeared in both the brain and the gut. This team of scientists proposed that this link was due to an unknown pathogen, which somehow travelled through the vagus nerve from the gut to the brain.26 It was noticed that some people with Parkinson’s disease experienced constipation and pain in the gut around 10 years before tremors appeared. In patients with the disease, a small protein called alpha-synuclein aggregates in the endings of neurons forming ‘Lewy bodies’, having a significant effect on brain function. These toxic alpha-synuclein fibres are usually soluble, and so do not build up in healthy neurons.

How does α-Synuclein work?
It is a presynaptic neuronal protein27, which causes the disruption of homeostasis in cells, and the death of neurons. It is also believed that α-Synuclein can affect synaptic function, although it is not known how. Genome studies were conducted in order to establish whether genes had an effect on Parkinson’s, and it was discovered that SNCA was involved, the gene that codes for α-Synuclein. To explore this further, another study found that, following an autosomal-dominant pattern,

triplications on the SNCA gene occurred within families, which resulted in three times the concentration of α-Synuclein. Duplications of this gene also occurred, and the nature of the mutation (triplication or duplication) affected the onset and severity, so in cases where the SNCA gene had triplicated, onset of Parkinson’s was earlier and advanced more rapidly than in duplication cases. This connection was observed in both rare familial cases and sporadic instances of Parkinson’s.

Following many autopsies of Parkinson’s patients, scientists suggested that the disease began in the olfactory bulb and dorsal vagal nucleus, with α-Synuclein building up in other cortical regions. It was evident that the abnormal deposition of α-Synuclein contributed to the development of Parkinson’s disease. However, α-Synuclein is abundant in the nervous system, and its main function is the control of neuro-transmitter release, which is necessary for normal brain function. There are similarities between Parkinson’s and Alzheimer’s, including the fact that α-Synuclein can form β-sheet structures under certain circumstances. This is important because β-amyloid has a similar structure, and this is the protein which is involved in the most common neurodegenerative disease: Alzheimer’s. As α-Synuclein intermediates form, collections of these called protofibrils become insoluble, group together, and coalesce into fibrils. This process, called α-Synuclein aggregation, forms clumps called Lewy Bodies, which can be neurotoxic.

The substantia nigra is a small area near the centre of the brain in which dopaminergic neurons are involved in movement and reward. Aging of the substantia nigra correlates with increased concentrations of α-Synuclein, which causes further degeneration of the dopaminergic neurons in the substantia nigra. The effect of overexpression of α-Synuclein was discovered much more recently (2009-10), and included the loss of presynaptic proteins, decrease in release of neurotransmitters, and enlargement of synaptic vesicles. These events usually preceded synaptic and neuritic degeneration, however it is still unknown as to at what point concentrations of α-Synuclein change from essential for brain function to neurotoxic. 28

How does gut bacteria affect Parkinson’s?
Recently a team of scientists from Californian Institute of Technology conducted a study looking at genetically modified, genetically identical mice, engineered to overproduce α-Synuclein. They were raised either in normal cages, or in a sterile, germ-free environment. The mice raised in germ-free cages were found to have lower levels of α-Synuclein, as well as fewer motor deficits. Non-sterile mice developed Parkinson’s symptoms, and had higher levels of α-Synuclein in their brain and gut. However, following this, the gut bacteria from humans with Parkinson’s disease was transplanted into the previously sterile mice. Rapidly these mice developed similar symptoms to the non-sterile mice. Mice with faecal transplants from humans without Parkinson’s displayed no symptoms of Parkinson’s.

This study is of huge importance, as if scientists were able to identify and isolate the strains bacteria causing this, there may be new treatment opportunities for sufferers of Parkinson’s.

At the University of Edinburgh, Dr Maria Doitsidou and her team are using genetically engineered microscopic worms to investigate how different bacteria affect dopamine-producing cells. This could mean that specific species of bacteria are identified as having protective effects on these dopamine-producing cells, therefore reducing the symptoms of Parkinson’s. These beneficial species could then be encouraged to thrive in the human digestive system with probiotics or prebiotics.

28 Stefanis L. (2012) α-Synuclein in Parkinson’s Disease
The Vagus nerve

The vagus nerve is a collection of fibres that begin in the brain and connects major organs including the gut. It is thought that it could be the route of pathological causes of Parkinson’s from the gut to the brain, and studies\(^29\) have shown that vagotomy patients have a lower risk of developing the disease (halved after 20 years). It has been shown in rodent studies that α-Synuclein fibres can travel from the gastrointestinal tract along the vagus nerve to the brain, where they accumulate and result in degeneration of dopaminergic neurons. Furthermore, proteins produced by bacteria in the gut may cause aggregation of α-Synuclein into Lewy bodies.

The study that observed the effect of vagotomy on subsequent risk of Parkinson’s was published in 2015. They saw that risk of Parkinson’s was significantly reduced following a full truncal vagotomy, where one of the branches of the vagus nerve is severed. However, patients who underwent a selective vagotomy, where the vagus nerve is cut much further down so only some parts of the gastrointestinal tract are disconnected, did not experience the same reduced risk of Parkinson’s. These results suggest that the vagus nerve is critically involved in the pathogenesis of Parkinson’s disease.

The gut-brain axis, in this case via the vagus nerve, is believed to be partly responsible for causing various other Parkinson’s symptoms. This communication pathway is bidirectional, so both the brain may influence the composition of the microbiota and the gut may influence the activities of the brain.\(^30\)

Potential Treatment Options

Given it is now known that composition of gut bacteria affects in some way the pathogenesis of Parkinson’s disease, perhaps therapies targeting the microbiota could improve symptoms of patients, or even delay development of the disease. One that has been proposed is faecal microbiota transplantation (FMT), with the aim of restoring normal symbiosis. Despite there being limited evidence regarding the effectiveness of FMT for Parkinson’s disease in humans, it has been observed that FMT in mice has had a neuroprotective effect. Professor Thomas Borody showed this in Sydney, through suppression of neuroinflammation and reduction in the signalling pathway between the gut and the brain.

Another suggestion is the use of antibiotic therapy, to prevent the formation of α-Synuclein clumps (lewy bodies) and neuroinflammation. As an altered microbiota composition has been observed in patients with Parkinson’s disease, taking probiotics to encourage a healthy gut microbiota may improve symptoms. Currently *Lactobacilli, Enterococci and Bifidobacteria* are used as probiotics, which have been shown to strengthen the epithelial layer of the intestine, prevent the barrier being disrupted, stimulating homeostasis of mucosal immune system and preventing growth of pathogenic bacteria.\(^31\)

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\(^29\) Svensson E. (2015) *Vagotomy and subsequent risk of Parkinson’s Disease*

\(^30\) Felice V.D. (2016) *Microbiota-gut-brain signalling in Parkinson’s disease: Implications for non-motor symptoms*

\(^31\) Perez-Pardo P. (2017) *The gut-brain axis in Parkinson’s disease: Possibilities for food-based therapies*
Nutrition-based interventions such as phospholipid membrane precursors have also been suggested to combine with traditional Parkinson’s treatments, because current treatments do not address the intestinal/digestive symptoms associated with Parkinson’s. Dietary changes may also improve gastrointestinal symptoms.

**Obesity and the gut**

Recent studies have shown that the intestinal microbiome is interwoven with our general health. Specific changes in the composition of the human microbiota have been directly linked to obesity-related metabolic disorders, and studies have shown that the gastrointestinal organisms can affect the way humans digest and absorb the energy and nutrition from our food. It is thought that they do this by both altering the amount of energy that is used from the diet and influences genes that regulate energy expenditure and storage.

The composition of the intestinal microbiota is not permanent, and so altering diet can result in drastic changes. From a medical point of view, this suggests that it could be possible to stimulate weight loss just by changing the proportions of different organisms in the gut. This would mean either consuming probiotics, prebiotics, or even faecal transplants in order to forcibly introduce a new microbiota composition.32

Microbes are heavily involved in the energy extraction from the food ingested. The enzymes secreted by a human cannot digest the majority of plant polysaccharides and complex carbohydrates, and so the bacteria break these down to short chain fatty acids (SCFA’s). Differences in these compounds have been detected in lean and obese mice; obese mice have higher levels of butyrate and acetate in the ceca, and less energy in their faeces in comparison to lean mice. The link between obesity and the composition of the microbiota was first suggested following a study comparing normal and germ-free mice. It was found that mice raised under normal conditions have a 40% higher body fat content than the germ-free mice that were raised in a sterile environment, and therefore had no microorganisms in their gut. When a section of the microbiota in the normal mice was transplanted into the germ-free mice, there was a 60% increase in body fat during the two weeks following. Throughout the study both groups of mice consumed the same volume and type of food.

When scientists investigated why this was the case, they discovered that the transplanted microorganisms increased the amount of energy released from plant carbohydrates, as well as changing the mice genes that control the amount of energy which is deposited in adipocytes, cells which store energy. One such genetic change was the production of fasting-induced adipocyte factor (FIAF), which is a lipoprotein lipase inhibitor. The suppression of this chemical is crucial for the microbiota-induced deposition of triglycerides in the fat storing cells, adipocytes. This indicates that the composition and richness of the gut microbiota may influence how much energy is extracted from the food that humans consume, and therefore their adiposity.

The majority of these studies have not yet been carried out in humans, however transplantation studies using identical human twins have shown that germ-free mice inoculated with microorganisms from either obese or human twins adopt the characteristics of the donor. The mice that received the microbiota from the obese human twin developed an increase in adiposity;

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however the mice that received microbiota from the lean twin remained lean. When these two groups of mice were reintroduced to each other, the progress of increased adiposity halted, suggesting that the mice previously colonised with microbiota from the obese human twin had been newly colonised by bacteria transmitted by the mice colonised with microbiota from the lean twin. However, the mice with a lean microbiota were not successfully colonised by the microbes from the obese twin. This is very important, because it suggests that the transmissibility of intestinal microbes and the adiposity phenotype were closely linked, and that the lean phenotype is dominant. Of course, these findings are only based on observations of rodents and humans are very different both physically and metabolically.33

**Effect of use of antibiotics on development of obesity**

It is now widely believed that the increasing prescription and use of antibiotics is contributing to the obesity epidemic. In a recent study, mice whose mothers were treated with penicillin both before the pups’ birth and during weaning displayed clear differences in body composition in adulthood. These included elevated fat mass, increased hepatic expression of genes involved in adipogenesis, reduced mineral content in bones, and increased bone surface area.

Gastric bypass surgery is a common treatment for obesity. Following the procedure in mice, scientists observed that it had resulted in a rapid change in the composition of the microbiome, which was not because of the weight loss due to following the diet.29

**Human studies**

The same association has been seen in humans. In overweight or obese humans, low bacterial diversity correlates with higher adiposity and dyslipidaemia, impaired glucose homeostasis and higher low-grade inflammation. When experts collected faecal samples from obese and lean humans and analysed the different strains of bacteria, they found that obese individuals had more *Firmicutes*, and almost 90% less *Bacteroidetes* than lean individuals. More importantly, when the obese humans began a low fat/carbohydrate diet for a year, as well as losing 25% of their body weight, the proportion (in their colon) of *Bacteroidetes* rose, and *Firmicutes* decreased.

At the age of 6-12 months, varying composition of microbiota is thought to be able to indicate an increased risk of the child being obese at 7 years of age.29

**The Human Microbiome Project**34

This was a huge project than began in 2008, with the overall aim of characterising the human microbiota in order to understand how the microbiota impacts human health and disease. Scientists first identified microbes from various areas of the body of 300 healthy volunteers. 16S rRNA sequencing was then used to identify the various species of microorganism at the different body sites. Metagenomic whole genome shotgun (WGS) sequencing was also used to understand the functions and pathways of the microorganisms living inside humans. As a result of this project, more

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33 Davis C. D. (2016) *The Gut Microbiome and its Role in Obesity*

34 NIH Human Microbiome Project [https://hmpdacc.org/hmp/](https://hmpdacc.org/hmp/) (Accessed 24/02/19)
than 14 terabytes of data were collected on the microbiota, available for scientists all around the world to access.

As well as the Healthy cohort, samples were taken from a Disease cohort, aimed at studying associations between the composition of the microbiota and specific health conditions. As seen in my other research, there have been many hypothesised connections between various conditions, and this project was crucial in investigating these links.

There were several smaller projects within the Disease cohort, investigating:

- Evaluation of the cutaneous microbiome in psoriasis (Martin J Blaser)
- The Vaginal Microbiome: Disease, Genetics and the Environment (Cynthia Nau Cornelissen, Lindon J Eaves, Jerome Frank Strauss, Gregory A Buck)
- Diet, Genetic factors, and the Gut Microbiome in Crohn’s Disease (Frederic D Bushman, James D Lewis, Gary D Wu)
- The role of the Gut Microbiota in Ulcerative Colitis (Eugene B Chang, Folker Meyer, Thomas M Schmidt, Mitchell L Sogin, James M Tiedje, Vincent B Young)
- Urethral Microbiome of Adolescent Males (Dennis J Fortenberry)
- The Thrifty Microbiome: The Role of the Gut Microbiota in Obesity in the Amish (Alan R Shuldiner, Claire M Fraser)
- Metagenomic Analysis of the Structure and Function of the Human Gut Microbiota in Crohn’s Disease (Claire M Fraser)
- Effect of Crohn’s Disease Risk Alleles on Enteric Microbiota (Ellen Li)
- Metagenomic study of the human skin microbiome associated with acne (Huiying Li)
- Foregut microbiome in development of oesophageal adenocarcinoma (Karen E Nelson, Zhihend Pei)
- The Microbial Ecology of Bacterial Vaginosis: A Fine Scale Resolution Metagenomic (Jacques Ravel)
- Skin Microbiome in Disease states: Atopic Dermatitis and Immunodeficiency (Julia Segre)
- The human virome in children and its relationship to febrile illness (Gregory A Storch)
- The Neonatal Microbiome and Necrotising Enterocolitis (Phillip I Tarr)
- The Human Microbiome in Paediatric Abdominal Pain and Intestinal Inflammation (James Versalovic)
Own Work

Visit to Reading University

Approximately 10 hours into my project, I decided to do some research on the experts in this field, as this was a completely new area of science for me. I contacted Professor Glenn Gibson, who is a Professor of Food Microbiology at Reading University, and he kindly arranged for me to visit him and his labs at the University. We talked through various possible areas of research around the topic of intestinal microflora, including Obesity, IBS, Autism, and various autoimmune diseases, of which I included in my further research. Due to the complications involved with conducting experiments on humans, in their labs there are many gut models, where experiments are conducted and observed *in vitro*. It was very interesting to learn about the importance of controlling every factor, from pH to temperature to composition of nutrient medium.

In the photo, it is possible to see the separate chambers, which represent different sections of the intestines. Some models were split into stomach, small intestine, ascending colon, transverse colon, and descending colon, others into stomach, duodenum, jejunum and ileum, depending on which area of the intestine was being examined. I found it incredibly interesting hearing from both the professors and PhD students about the effects of changing various factors in the gut models.

Professor Gibson talked me through some of his findings, and I greatly enjoyed speaking to him about a topic we were both fascinated by.

Practical Work

Questionnaire

I started this project with high hopes of being able to complete at least some practical work, however unfortunately it has not been possible. Initially, in connection with researching about the correlation with antibiotic consumption and incidence of atopic disease, I thought about conducting a questionnaire. The questions I came up with were:

- Has child has taken antibiotics from the ages of 0-12 months, and (if known) approximately when and for how long?
- Was child breastfed, and if so for what duration?
- Does child have diagnosed asthma?
- Does child have allergic rhinitis (hay fever or sensitivity to animal fur)?
- Does child have atopic dermatitis?
- Does child have any food allergies (please specify)?

However, I soon discovered that there were several difficulties with asking these questions, one of which being that not many people could remember if their child had been prescribed antibiotics. Diagnosis for many of these diseases is often not clear, and so this would make my results quite unreliable. Furthermore, for any connections that I could discover, I would have no way of knowing whether they were correlation or causation. Eventually I decided that the potential information
gained would not justify the work involved with conducting the questionnaire, and so decided against completing it.

**Effects of different combinations of prebiotics and probiotics**

Prebiotics and probiotics are readily available, and so I wanted to understand the effects on human health following ingestion of different combinations of the two. However, the majority of beneficial effects I discovered would be difficult to measure and quantify, for example increased immunomodulation. Initially I thought that it would be relatively simple to carry out this experiment, as I could distribute different combination of prebiotics and probiotics to each of my friends, who are all relatively similar. However, I realised that it would be incredibly difficult to control the rest of their activities, such as diet and exercise, which would of course effect the dependent variables I would be attempting to record. Furthermore, these variables would all be incredibly subjective, such as ‘have you experienced better digestion’ or ‘Has your mood improved since taking these supplements’. Due to all of this, I decided against going through with the experiment, as any results I managed to collect would not be very reliable.

**Effect of different probiotics on the growth of different species of bacteria which are beneficial in the human gut**

I noticed that there are many different varieties of probiotics available, and wanted to investigate whether different combination of probiotics affect the growth of various species of bacteria in the gut which are beneficial to human health. I emailed Professor Gibson to ask for his help in setting up this experiment and he advised I carry it out anaerobically in Hungate tubes with a gas phase. He also suggested that I use OD (Optical density) or Viable counts to measure growth, using a general-purpose growth medium such as MRS (De Man, Rogosa and Sharpe agar). After speaking with the lab technician in the Biology department, it became clear that this method would not be possible. Firstly, I did not have access to several of the equipment Dr Gibson specified in his advice, which would have made it rather difficult to successfully carry out the experiment, and obtain valid results. Furthermore, due to health and safety it was not possible for me to grow anaerobic bacteria in the school labs, due to the possibility of growing a pathogenic organism.

It was very disappointing not to be allowed to conduct any of these practical experiments, but it is understandable that there regulations that need to be followed. Because I would have been handling bacteria grown in conditions similar to the human gastrointestinal tract, the possibility of culturing unwanted pathogenic bacteria was too high a risk. However, I was still able to learn a lot from speaking to Professor Gibson about theoretical methods that I could have used to investigate the questions above.

**Creating Leaflets containing the simplified information**

During my time researching, I noticed that despite the information being incredibly interesting, it was often explained in such a way that was too complicated for the public to understand. Furthermore, much of the research was applicable to the majority of people, for example, the effects of caesarean section on the developing immune system of a new-born baby, or how there may be possible treatment opportunities in the future for diseases like Parkinson’s and Obesity involving the gut microbiota. In my case, I did not have the knowledge nor the equipment to be
seriously contributing to this field of research, and so I had to decide what direction my project was to take.

As I discovered incredible new things about the intestinal microbiota, I had conversations with various family members, teachers and friends, all who expressed the same amazement as I did. It was partly this that led to the idea of my project making all of this information accessible to the people it is most relevant to. For example, my Grandad, who suffers from Parkinson’s, was intrigued when I explained the various connections that are being discovered between the composition of gut bacteria and the development of Parkinson’s. It is very hard having these conversations, as Parkinson’s is one of the many conditions that still does not have a definite cure. However, my Grandad keenly follows the current research, and regularly discusses information he does not understand with me.

Furthermore, I believe that perhaps there are women who do not understand the full benefit of breastfeeding their newborn for a certain amount of time, or people who demand antibiotics from GP’s without fully understanding their effects on the composition of their gut microbiota, and therefore the possible impacts on their health.

Because of this, I decided that condensing all information I had worked so hard to decipher was something that was useful and that could benefit this area of science. Clearly, I am not qualified or knowledgeable enough to publish these leaflets; however, this could be something I help develop in the future. Even during the year I have been completing my Project, I have seen more simplified articles and papers, which is fantastic because it means that more people will be able to access and read about some of the information I have included in my leaflets.

Feedback on the leaflets

To get some feedback on the leaflets that I had created, I printed a few out and asked friends and family from non-scientific backgrounds what they thought of them. One thing that several people mentioned was that there were not enough pictures in either of the leaflets, and too much text, which could overwhelm people reading them. I found this quite difficult when writing the leaflets, because if I added more photos there was much less space for the text. This meant that there was less text available to explain the key ideas, some of which needed a significant amount of explanation in order to be understandable. To improve this, perhaps the leaflets need to be booklets instead, so that there is more space to include both the necessary text as well as enough pictures and diagrams.

The general opinion was that the content itself was well explained and people could understand the science behind it, and wanted to read more about it. However, some people said they would have wanted a leaflet about on ‘General Health and the Gut Microbiota’, because it would be applicable and relevant to everyone. This is definitely something that I would have completed if I had had more time, and will perhaps work on in the future.

Conclusion

I began my project by asking whether the bacteria in our gut are actually important to our lives and health, and have ended it in complete amazement at the magnitude of functions it actually has. An article I read very early on asked: ‘Is the gut microbiota really the forgotten organ’, and I believe that in some aspects it is more than an organ, because of the sheer quantity of processes it is involved in. I am excited to follow this area of research in the future, and I believe that it is a topic that will
become a much greater focus for scientific researchers. I am also looking forward to finding out how many more diseases the microbiota plays a part in, and following the potential treatments for conditions that may arise because of these discoveries.

I hope that I will see more ‘easy to read’ articles educating the public on these hugely relevant areas of research that are similar to the leaflets I created.

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<thead>
<tr>
<th><strong>Glossary</strong></th>
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<tr>
<td>Adiposity</td>
<td>A condition of being severely overweight or obese</td>
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<td>Aetiology</td>
<td>The cause, set of causes, or manner of causes of a disease or condition</td>
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<tr>
<td>Allergic rhinitis</td>
<td>A type of inflammation in the nose which occurs when the immune system overreacts to allergens in the air.</td>
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<tr>
<td>Angiogenesis</td>
<td>The formation of new blood vessels from existing vessels.</td>
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<tr>
<td>Aptosis</td>
<td>The death of cells that occurs as a normal and controlled part of an organism's growth or development.</td>
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<tr>
<td>Atopic dermatitis</td>
<td>A condition that makes the skin red and itchy, also known as eczema.</td>
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<tr>
<td>Autoimmune disease</td>
<td>A disease in which the body produces antibodies that attack its own tissues, leading to the deterioration and in some cases destruction of such tissues.</td>
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<tr>
<td>Colonocytes</td>
<td>An epithelial cell of the colon.</td>
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<td>Colostrum</td>
<td>The first milk produced by the mammary glands of a mammal following the birth of a newborn.</td>
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<tr>
<td>Co-metabolism</td>
<td>Simultaneous degradation of two compounds in which the degradation of the second compound depends of the present of the first.</td>
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<tr>
<td>Cross-feeding</td>
<td>The phenomenon that one species lives of the products of another species.</td>
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<tr>
<td>Cytokine</td>
<td>Any number of substances that are secreted by certain cells of the immune system and have an effect on other cells.</td>
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<tr>
<td>Endogenous synthesis</td>
<td>Process synthesised within the organism or system.</td>
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<tr>
<td>Enteric</td>
<td>Relating to or occurring in the intestines</td>
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<tr>
<td>Epidemiology</td>
<td>The branch of medicine which deals with the incidence, distribution, and possible control of diseases and other factors relating to health.</td>
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<td>Term</td>
<td>Definition</td>
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<tr>
<td>GALT</td>
<td>Gut-associated lymphoid tissue</td>
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<tr>
<td>Genotoxin</td>
<td>A chemical agent that damages the genetic information within a cell causing mutations, which may lead to cancer.</td>
</tr>
<tr>
<td>Gluconeogenesis</td>
<td>Metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as lactate, glycerol, and glucogenic amino acids.</td>
</tr>
<tr>
<td>HMOs</td>
<td>Human Milk Oligosaccharides- compounds found in human milk.</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>A deficiency of oxygen reaching the tissues of the body</td>
</tr>
<tr>
<td>I-caproic acid</td>
<td>The carboxylic acid derived from hexane with the chemical formula CH3(CH2)4COOH.</td>
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<tr>
<td>Intra-epithelial lymphocyte</td>
<td>Lymphocytes found in the epithelial layer of mammalian mucosal linings, such as the gastrointestinal (GI) tract and reproductive tract.</td>
</tr>
<tr>
<td>Intrapartum antibiotic prophylaxis</td>
<td>Antibiotics administered to pregnant women between onset of labour and delivery, when their babies are at risk of early-onset neonatal infection.</td>
</tr>
<tr>
<td>Intrauterine environment</td>
<td>The conditions within the uterus.</td>
</tr>
<tr>
<td>Immunomodulator</td>
<td>Modification of the immune system or the functioning of the immune system by the action of an immunomodulator.</td>
</tr>
<tr>
<td>Meconium</td>
<td>The dark green substance forming the first faeces of a newborn infant.</td>
</tr>
<tr>
<td>Microbiome</td>
<td>The combined genetic material of the microorganisms in a particular environment.</td>
</tr>
<tr>
<td>Mutagen</td>
<td>An agent, which causes genetic mutation.</td>
</tr>
<tr>
<td>Necrotising enterocolitis</td>
<td>A medical condition that often occurs in newborn babies, where a portion of the bowel dies.</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>The part of the pharynx that lies between the soft palate and the hyoid bone, includes the back of the tongue, soft palate, walls of the throat and tonsils.</td>
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<td>Term</td>
<td>Definition</td>
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<tr>
<td>Pioneer Microbiome</td>
<td>The first combination of microorganisms that colonise the human gut.</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>A non-digestible food ingredient that promotes the growth of beneficial microorganisms in the intestines.</td>
</tr>
<tr>
<td>Probiotics</td>
<td>A microorganism introduced into the body for its beneficial qualities.</td>
</tr>
<tr>
<td>SCFA’s</td>
<td>Short Chain Fatty Acids</td>
</tr>
<tr>
<td>T regulatory cells</td>
<td>A subpopulation of T cells that modulated the immune system, maintain tolerance to self-antigens and prevent autoimmune disease.</td>
</tr>
<tr>
<td>Vernix Caseosa</td>
<td>Greasy deposit covering the skin of a baby at birth.</td>
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<tr>
<td>Xenobiotic</td>
<td>A chemical that is found within an organism that is not naturally produced or expected to be present within an organism.</td>
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**Appendices**

- Parkinson’s leaflet
- Baby Leaflet
<table>
<thead>
<tr>
<th>Date</th>
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<td>Outline of plan</td>
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