university of groningen

Chandan Raghunath
Masters Biomolecular Sciences
(S3956954)

Essay topic: The role of gut microbiome in health and disease

Supervisor: Prof. Dr. G.J.W. Euverink

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1.0 ABSTRACT

A new era of understanding the working mechanism of the human body with respect to health and disease has brought about a lot of insights towards trillions of prokaryotic and eukaryotic organisms constituting the gut microbiome and is being explored in order to better understand how the human body develops these gut microbiota and what their role is in maintaining homeostasis in the body. There is substantial evidence from recent years that maintaining a healthy gut microbiome is the key to tackling many diseases such as inflammatory bowel disorder, obesity, diabetes, asthma and even colorectal cancer. A fascinating connection between the gut and the brain known as the gut-brain axis is being studied and explains how mental health and behaviour is related to the gut microbiome and also helps recognise the interplay between the gut health and neurological disorders such as autism, Parkinson's disease and multiple sclerosis. Although there are a lot of factors (such as mode of delivery during birth, the living environment and exposure to allergens) determining the composition of the gut microbiome in an individual, the major factor is the type of diet that is followed. A high fiber diet is broken down in the gut by several types of bacterial species that releases metabolites such as short chain fatty acids (SCFAs) which plays a huge role in maintaining a healthy balance in the gut and in turn the whole body. The importance of gut microbiome is increasing and may be the key to a futuristic way to treat any disease.

Keywords: Gut microbiome, gut microbiota, gut-brain axis, short chain fatty acids.

2.0 INTRODUCTION

The human body is largely inhabited by a variety of microorganisms that are present in many regions either on or inside the body. These are collectively referred to as the human microbiota. The ones present in the gut [1] have been attracting importance of late. The gut microbiota itself consists of trillions of bacteria [2] and quadrillion viruses (virome). Although the term microbiota and microbiome can be used interchangeably, the former is the taxa of related organisms and the latter represents the collection of the genes encoded by the microbiota [3]. Conventionally, in order to identify the type of organisms in the gut, they had to be cultured and categorised from the fecal matter but advancements in the field of experimental biotechnology have paved way for the organisms to be sequenced using Next Generation Sequencing techniques such as 16s rRNA sequencing [4]. These gut microbes have a symbiotic function in the host related to energy harvesting and storage, fermenting and absorbing undigested carbohydrates and have importance in the immune system [5]. Hence, their categorisation and identification has become a crucial act. With the advent of transcriptomics, 16s rRNA sequencing and Whole Genome Sequencing, scientists have been able to successfully complete the Human Microbiome Project (HMP) [6] and Meta HIT (Metagenomics of the Human Intestinal Tract) [7] to map the organisms and their interactions in the human gut. It was found that there is less similarity between the bacterial taxa of two different individuals and there is different overall composition of the organisms but is found to express similar functionality [8]. Based on several factors, there are two major types of Enterotypes that develop in a human gut- *Bacteroides* and *Firmicutes* [9]. Apart from these, there are innumerable prokaryotes and viral organisms (Virome) as well. Currently focus is shifting on these organisms as their dynamic interactions with the prokaryotes and the human body is said to have important outcomes [10]. Imbalance in the gut microbiome is observed to cause a lot of discomfort and disease states also known as dysbiosis in humans [11]. The elucidation of these organisms may be the novel plan to tackle many diseases currently and in the future.

2.1 Development of gut microbes

A new born foetus has a completely sterile intestine with no growth of microorganisms [12]. Several factors affect the inoculation of the above mentioned microbes in the gut such as mode of delivery i.e., vaginal or cesarean section and environmental habitat [13]. It is said that vaginal microbiota thriving in *Lactobacilli* are proven to be much more beneficial to the baby than getting exposed to the environment or the skin surface microbiota rich in facultative anaerobes like *Clostridium* species when born though a C-section [14]. At first, aerotolerant microbes such as *Actinobacteria* and *Proteobacteria* start growing and progressively anaerobes start colonizing the gut [15]. Viruses may get transmitted maternally or picked up from the environment, although the exact mechanism is not well understood [16]. As the age progresses, the preliminary microorganisms are replaced by others. From the age of about two and a half years to old age (around 60) the microbiota composition in the gut remains the same, unless altered by external factors [17]. After the age of about 65, *Clostridium* species start colonising the gut along with the loss of certain microflora [18].

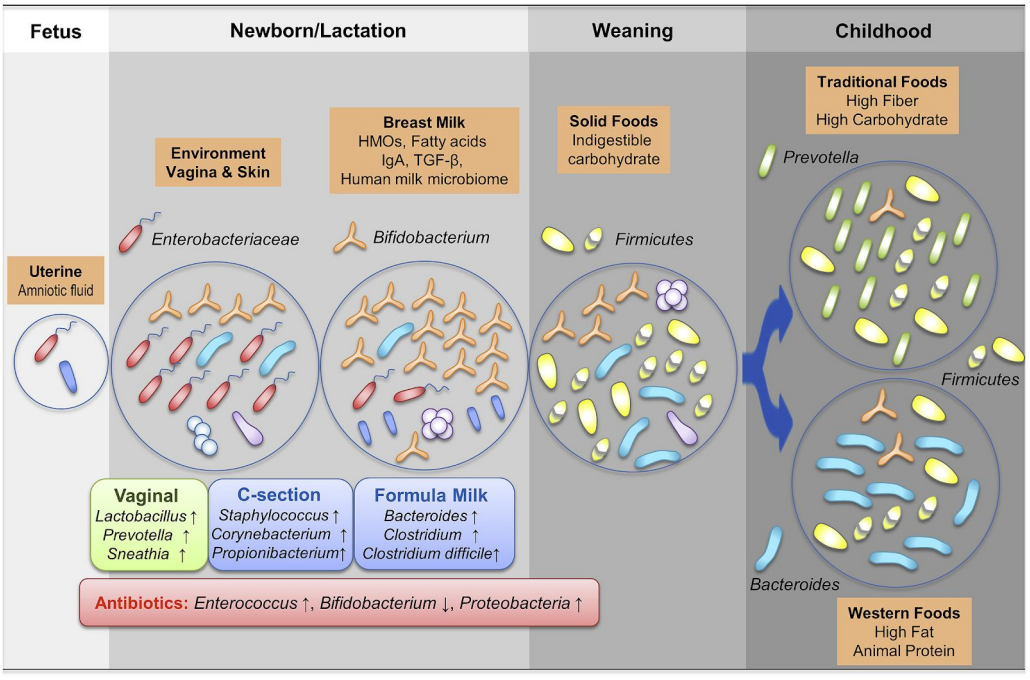

Figure 1: The growth progression of different types of microorganisms starting from the fetus to early childhood mainly determines the gut microbiota establishment in an individual. Since the fetus is sterile, there is no growth of any organisms. When the baby is born, the type of delivery (vaginal or C-section) marks the growth of the corresponding organisms in the above figure. A mother's breast milk promotes the growth of *Bifidobacterium*

species and when a newborn is fed special formula milk (with added beneficial compounds), it results in the development of several other useful bacteria, in turn promoting release of antibodies (IgA) and growth factor proteins (TGF-β). Use of antibiotics curbs the budding of beneficial bacteria and promotes the thriving of other harmful species in the gut. Once the child feeds on solid food nutrition rich in carbohydrates, there is enhanced growth of *Firmicutes* in the gut. As the age progresses, the type of diet (traditional or western) followed by an individual plays a huge role in composing the gut microflora.

Source: [12]

2.2 Factors affecting the growth of microbiota in the gut.

As discussed above, one of the primary factors aiding in the type of microbes growing in the gut is the mode of delivery. Other major factors include use of antibiotics, the type of diet followed by an individual, the environment in which they live and the occurrence of a disease [19]. Antibiotics, when used, kill a number of host bacteria as well apart from the pathogenic organisms rendering the gut bacteria to diminish in number and variety [20]. Another ill effect of the use of antibiotics is that the host microorganisms start developing resistance to them which may pass on to the harmful pathogens as well. Using less antibiotics decreases the possibility of growth of antibiotic resistant organisms [21].

Diet plays a huge role in the development of gut microflora [22]. Studies have shown that a change from a high fiber, low fat diet to a low fiber, high fat diet or vice versa can induce observable changes in the gut microbiome content within 24 hours [23]. Individuals with a high fiber diet live a healthier life owing to the specific types of beneficial microflora that grows in their gut [24]. The human genome takes years to change but the microbiome just days and keeping balance between them is a key aspect in living a healthy lifestyle. Infants being fed breast milk rich in fucosylated oligosaccharides are utilised mainly by *Bifidobacterium langum* and the gut becomes rich in Bacteroides species but less of *E.coli, Clostridium perfringens* growth occurs [25]. Formula milk rich in nutrients, when fed to infants, stimulates the growth of *E.coli, Clostridium difficile, Bacteroides fragilis* and *lactobacilli.* Undernourished kids have the growth of *Enterobacteriaceae* that proves to be harmful in the gut [26].

The living environmental surroundings and hygiene affect the growth of several microorganisms in the gut at an early stage and plays a role in development of either a healthy or disease states in the body [27]. Environmental stress like high altitude, cold

weather, heat stress, presence of pathogens and pollutants defines the shaping up of the gut microbiome [28].

Dr. Erica and her group conducted a study with a tribal hunter community in Tanzania who have a very high fiber rich diet and they were shown to have lots of beneficial microorganisms in the gut leading to a healthier lifestyle. This group can be considered as a proxy/control group associating with people living 10000 years ago as they use only traditional high fiber food, live in a microorganism rich environment without the use of any external antibiotics as compared to the modern population who live a completely opposite lifestyle. The microbiota identified in people living an urban life had much lower variety and number. The microbiota is said to have passed on from generations and the ones following the same dietary inputs such as the hunters have been conserving certain endangered species of microbiota that seems to have been lost in the people living in cities. Changing the diet to a high fiber one did not bring about recovery of these endangered microbiota [29].

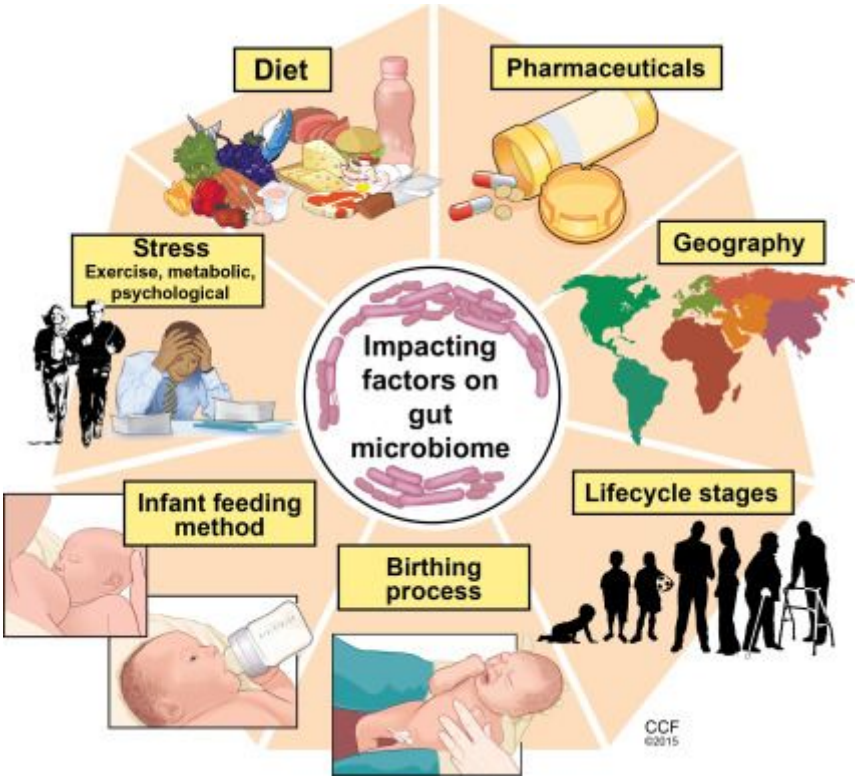

Figure 2: There are several factors apart from the mode of delivery, type of milk feeding, diet and use of antibiotics as mentioned in Fig1 that influence the types of microorganisms flourishing in the gut such as the geographical location of a person. Microorganisms found in one part of the world may not be the same as in another part and hence abundance of different species of microorganisms is seen in the gut of different individuals. Stress, be it mental or physical is shown to control the development of a healthy microbiome

(discussed later in the essay) . The lifestyle followed by a person (hygiene, aging) also impacts the composition of the gut microbiota.

Source: https://www.sciencedirect.com/topics/medicine-and-dentistry/gut-microbiome

2.3 Growth and survival of gut microbiota

The growth of microbiota generally depends upon the chemical, nutritional and immunological characteristics of the gut. The small intestine is slightly acidic and rich in antimicrobials and thus facilitates rapidly growing facultative anaerobes that attach to the mucosal epithelial cells to survive. In the small intestine of mice, *Lactobacillaceae* is abundant [30]. The colon however has densely populated bacteria consisting of anaerobes such as *Prevotellaceae, Lachnospiraceae* and *Rikenellaceae* that can utilise undigested carbohydrates from the small intestine [31]. Mucus layer is rich in *Firmicutes* such as *Clostridium spp* and is different in composition as compared to fecal/lumen microbiota [32]. For experiments related to gut microbiota, carefully selected sampling is necessary. Core microbiota generally consists of *Bacteroides, Prevotella* and *Ruminococcus* species and although there are differences in composition in two individuals, their functionalities in the body will be similar [33].

The microbiota works symbiotically to enhance the host's immune system. The short chain fatty acids (SCFAs) produced by the microbiota plays a major role in the formation of a dynamic intestinal barrier consisting of three important distinctions- the physical layer with epithelial and mucous membrane, the biochemical layer with important enzymes and antimicrobial proteins and the immunological layer with antibodies, IgA and other immune cells [34]. The microbiota that are symbiotically beneficial to the host only survive and other ones are removed or phased out through generations. Meta-transcriptomic studies have been used to map ideal microbiota in a host and they metabolise simple sugars available in the small intestine. Microbiota in the colon require carbohydrates to survive. These nutrients are known as Microbiota Accessible Carbohydrates (MACs). Lower availability of MACs in the diet results in lower diversity of microbiome in the gut. In order to restore the lost microflora, bacterial taxa missing can be administered to the gut along with a high MAC diet [35]. Diversification of the microbiome may result due to mutations or gene transfer through

generations. Addition of new species as a result of social interaction to the microbiota of an individual results in an increase of variety of organisms in the microbiome [36].

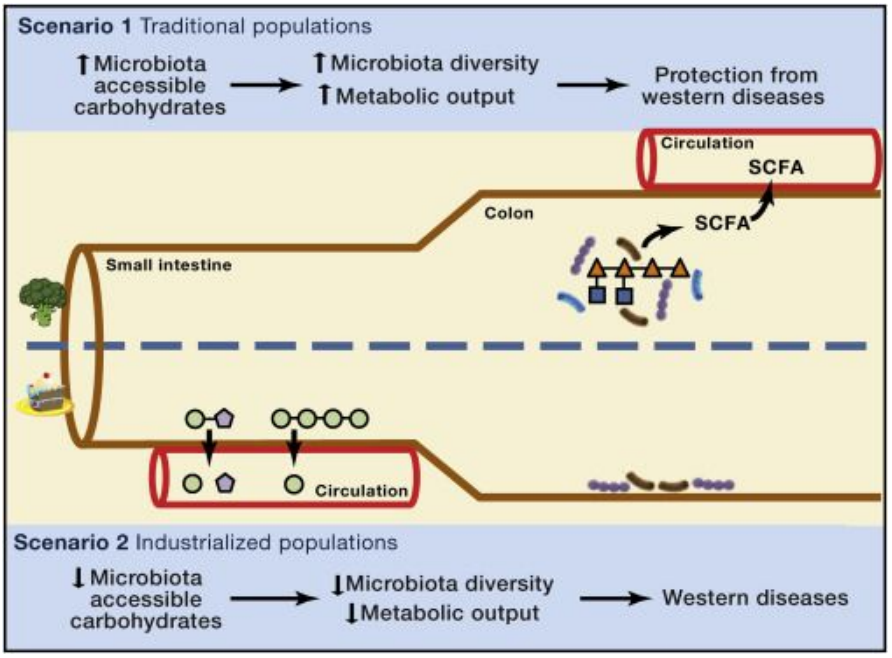

Figure 3: Comparing consequences of different populations consuming a high and a low MAC diet. A high MAC diet influences the microbiota to break down carbohydrates and release SCFAs into circulation thereby promoting healthy microorganisms to thrive in the gut that play a big role in protecting hosts against various diseases. Calorie utilization is also high in these individuals. When a diet involving low MACs is consumed, apart from lower calorie utilization, there is an increase in the number of unnecessary microorganisms that compromise the intestinal integrity, mucosal barrier depletion and eventually leading to the onset of western diseases.

Source: [37]

3.0 PREBIOTICS AND PROBIOTICS

Prebiotics are substrates used by the host microbiota that may have an outcome of a health benefit [38]. These prebiotic substances are fermented by a certain bacterial species that when administered to a host, aids in the development of their gut bacteria [39]. Prebiotics such as Inulin fructans help in growth of certain species of microorganisms depending on the individual. When infants whose parents have a history of atopy were given breast milk supplemented with galacto oligosaccharides and long chain inulin, they were observed to have reduced atopic dermatitis and having long term effects of reduced allergies and increased immunity due to changes in the gut microbiota [40]. Prebiotics help increase satiety

and this may be a new way to treat obesity. It increases Glucagon Like Peptide 1 (GLP) and Peptide YY concentrations in the plasma gut which is a regulator of energy uptake in overweight children [41]. Prebiotics induce growth of several species of bacteria in the gut that help in digesting dietary fiber and produce SCFAs that have their own beneficial role in the body including immunity boosting, proper bowel movement and maintaining intestinal barrier integrity [42].

Probiotics are living, viable microorganisms that when administered to an individual at certain concentrations, may carry health benefits by acting in relation with the gut microbiome [43]. They aid in maintaining or regrowth of deceased gut microbiota due to factors such as dysbiosis or use of antibiotics [44]. Immunological pathways are enhanced to function better, increasing production of **β**-defensin, IgA and cytokines [45]. The rigidity of intestinal mucosal barrier and tight junctions increases due to probiotic induced mucin production which in turn helps in enhancing the body's immunity [46].

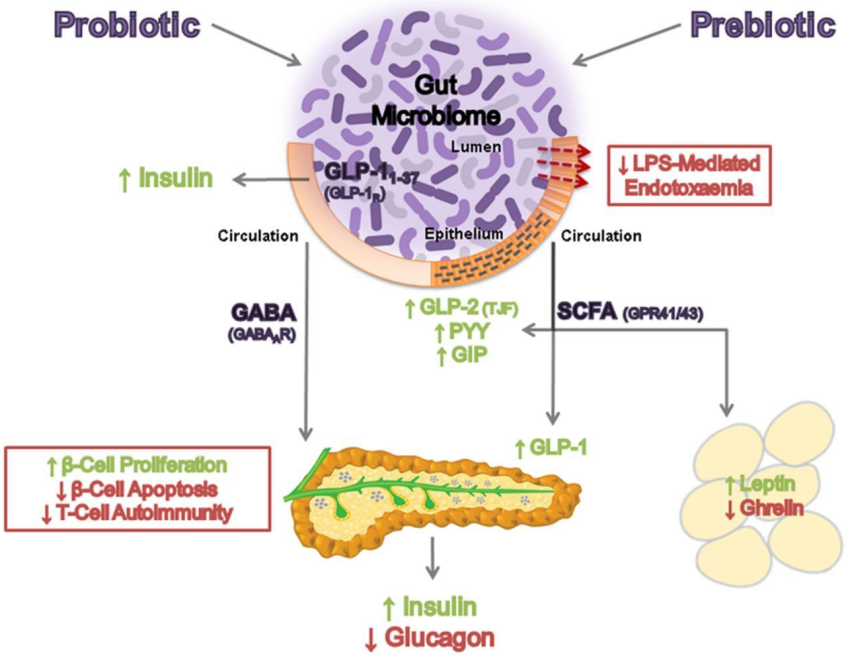

Figure 4: This shows the positive effects of pre and probiotics on the gut microbiome in regulation of hormones, compounds and certain processes related to diabetes. Green texts indicate upregulation and red show downregulation.

Source: [47]

4.0 ROLE OF GUT MICROBIOME IN HEALTH AND DISEASE.

The microbiome releases certain Short Chain Fatty Acids (SCFA) such as acetate, propionate and butyrate in the intestine as a result of fermenting food rich in fiber content that plays a huge role in boosting immunity of the body [48] along with many other important functions which have been elucidated further in this essay. The gut harbours several bacterial species and helps mainly in maintaining the thickness of the mucous membrane between the gut and the upper intestine, preventing contamination of the intestine with host bacteria and also boosts the production of IgA [49]. These bacterial surfaces have TLRs (Toll Like Receptors) as a result of a special property known as Microbe Associated Molecular Patterns (MAMPs) that helps the host recognize the microbiota [50].

SCFAs help integrate the intestinal mucosa which comprises of two distinct layers, the inner sterile impermeable layer and an outer permeable layer. The outer layer is composed of O-glycan that is used by certain commensal bacteria as binding sites. The mucous layer and mucin glycosylation majorly shapes the gut microbiome [51]. Starving the microbiota that is, not having a high fiber or MAC diet results in the thinning of mucous membrane and the bacteria may start eating the cell wall and encroach the intestine resulting in these microbes entering the bloodstream causing sepsis and over expression of inflammatory markers [52,53]. Imbalance in the functioning of microbiota that maybe caused by external factors such as high fat diet or extensive use of antibiotics results in a state known as dysbiosis that is responsible for the host to suffer with a number of complications ranging from Irritable Bowel Syndrome, Crohn's disease, Obesity, Cardiovascular disturbances, autoimmune diseases to also neurological disorders like Autism and Parkinson's [54,55]. Hence, it is important to map the microbiota present in the gut and their loss in variety during a disease state or change in diet or use of antibiotics in order to better understand their mechanism of action and treat diseases. There are successful cases of diseases being treated with a process called Fecal Microbiota Transfer (FMT) where the microbiota from a healthy individual is administered to a person in diseased state. However, the mode of transplantation, compatibility and procedures are to be carefully orchestrated [56].

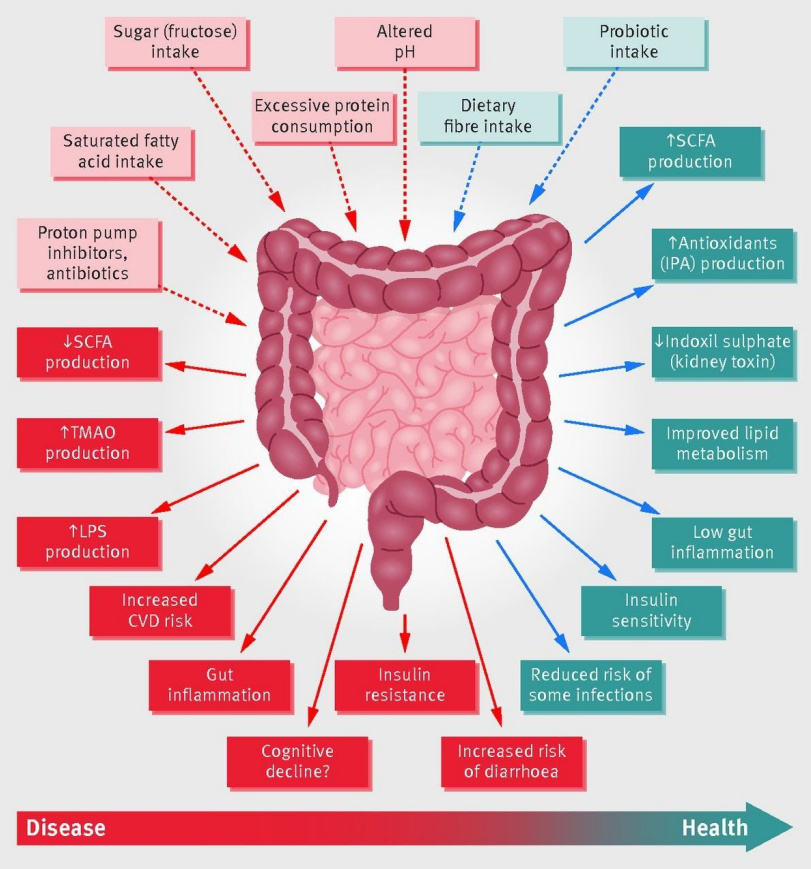

Figure 5: Factors such as high sugar, protein, saturated fatty acid, proton pump inhibitors and antibiotic intake leads to imbalance in normal functions of the gut microbiome resulting in a diseased state. Following a healthy diet supplemented with probiotics enhances the functionality of the gut microbiota in turn maintaining a healthy lifestyle in any individual.
Source: [3]

5.0 TYPES OF BENEFICIAL COMPOUNDS METABOLISED IN THE GUT [57]

SCFA (acetate, propionate, butyrate, valerate, hexanoate):

Gram negative *Bacteroidetes* species and gram positive *Firmicutes* (belonging to the *Ruminococcaceae* and *Lachnospiraceae* family) in the gut are a few organisms that produce SCFAs during the fermentation of dietary carbohydrates. *Bifidobacterium* species also produce certain SCFA compounds [120]. SCFAs activate G-Protein coupled receptors present on epithelial cells, macrophages, dendritic cells and mast cells and act as an energy source, helps in maintaining energy homeostasis by interacting with Glucagon Like Peptide-1, mediating immune responses, producing anorectic hormones that are responsible for maintaining a good appetite, tumor suppression, central and peripheral nervous system

modulation by activating dendritic cells [58,59]. Transcriptome analysis of the microbiota during the production of butyrate revealed differential expression of genes involved in fatty acid oxidation, electron transport chain and oxidative stress pathways [60]. Butyrate has an important role in maintaining the intestinal barrier integrity, through it's reaction with histone deacetylase inhibitor, by producing mucin, antimicrobial peptides and tight junction proteins. This indirectly influences the reduction of oxidative stress in the gut [61]. SCFAs are involved in adjusting glucose tolerance and insulin sensitivity and have a function in intestinal gluconeogenesis, lipogenesis and fasting induced adipose factor suppression [62].

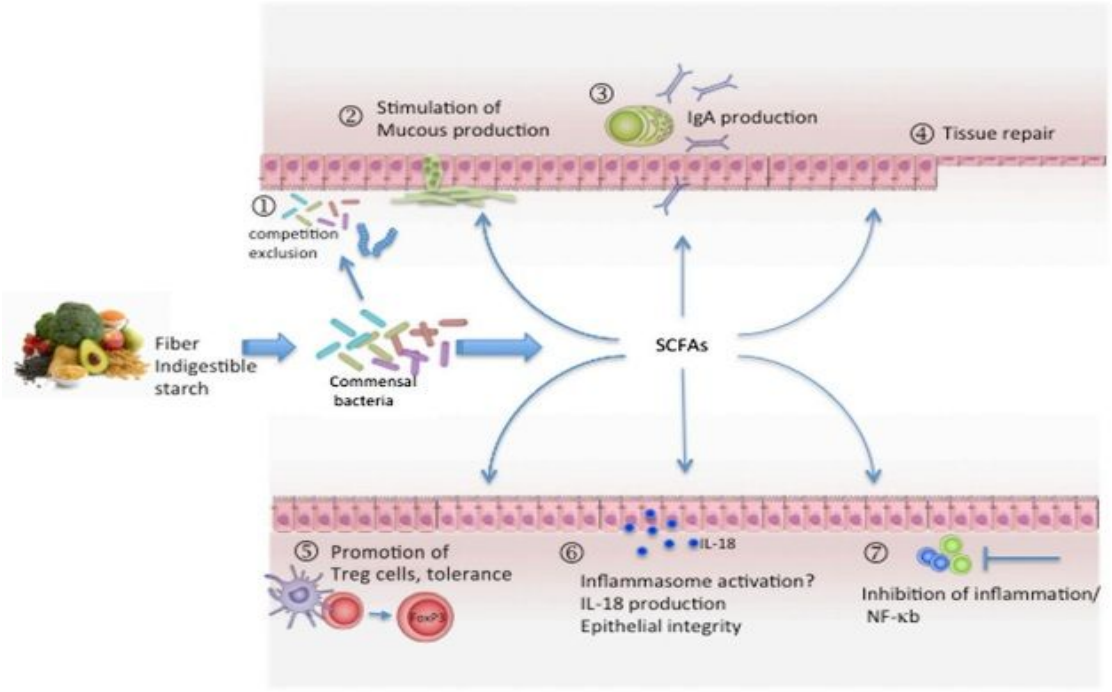

Figure 6: Dietary starch and undigested carbohydrates are utilized by bacterial species and broken down to SCFAs. These compounds diffuse throughout the intestine, maintain the intestinal barrier integrity and play a major role in the functions shown in the figure.

Source: https://cristivlad.com/dietary-fiber-scfas-and-mechanisms-of-gut-homeostasis/

Indole derivatives (indole, indoxyl sulfate, indole-3-propionic acid {IPA}):

The tryptophanase enzyme encoded by the tnaA gene facilitates the gut bacteria in the conversion of tryptophan to indole and its derivatives. Although there is no circumstantial evidence about which group of microorganisms in the gut are responsible for the production of these groups of compounds, tests have been carried out using *E.Coli* as a host for production of indole derivatives [121]. IPA is a powerful antioxidant and possesses neuro and

cytoprotective properties against oxidotoxins. Indole derivatives controls intestinal barrier function through a xenobiotic sensor, Pregnane X Receptor (PXR) reducing intestinal inflammation by downregulating cytokines, TNF-**α** and upregulating mRNA coding for tight junction proteins. This manages intestinal permeability and mucosal integrity. Indoxyl sulfate can be used as a biomarker since it is found in people with an impaired excretion system [63,63,65].

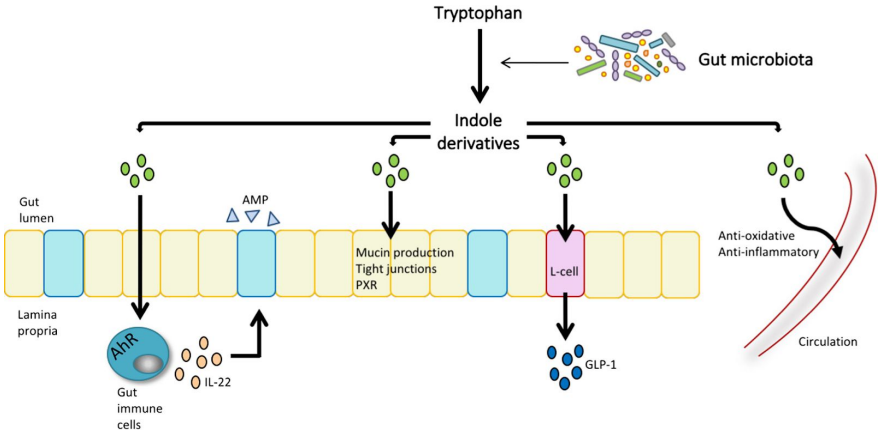

Figure 7: Indole derivatives produced from tryptophan by the gut microbiota have several functions. They act as ligands for aryl hydrocarbon receptor (AhR) which promotes interleukin-22 (IL-22) production in turn maintaining mucosal barrier immunity by inducing antimicrobial proteins (AMP). Some compounds play a role in decreasing intestinal barrier permeability as mentioned above through its interaction with PXR. They also induce release of glucagon like peptide-1 (GLP-1) in enteroendocrine L-cells and modulates appetite, insulin production and gastric release. Indole derivatives enter the blood circulation providing anti-oxidative and anti-inflammatory properties to certain organs like the liver.

Source: [66]

Choline metabolites (Choline, trimethylamine N-oxide {TMAO}):

An experiment conducted by Kymberleigh A., *et al* helped identify certain species of microorganisms in the gut that are capable of metabolising dietary choline to TMAO. Although low levels of choline is responsible for maintaining intestinal barrier function, accumulation of large amounts of choline has a connection with several metabolic disorders in the body and hence need to be converted to useful and non harmful compounds such as TMAO. TMAO levels could be used as biomarkers and have been linked with a couple of diseases such as cardiovascular distress and colorectal cancer. The stains of bacteria cultured from the gut microbiome of a human that are capable of metabolising choline include

Anaerococcus hydrogenalis, *Clostridium asparagiforme*, *Clostridium hathewayi*, *Clostridium sporogenes*, *Escherichia fergusonii*, *Proteus penneri*, *Providencia rettgeri*, and *Edwardsiella tarda*. When these strains were introduced in the gut of germ free mice, TMAO levels were seen to rise under the addition of choline [122]. Choline and TMAO have a function in lipid metabolism and glucose homeostasis. They are also involved in non alcoholic fatty liver disease and cardiovascular disease progression [67,68].

Bile acid metabolites (Deoxycholic acid, lithocholic acid):

Bile salt hydrolases are a group of enzymes present in certain gut microbiota (specifically Firmicutes such as *Bacteroides*, *Clostridium*, *Lactobacillus*, and *Bifidobacterium*) that react with bile acids to metabolise them into deoxycholic acid. Other organisms belonging to *Eubacterium* and *Clostridium* genera have been identified that aid in the formation of lithocholic acid from its precursors. Improper metabolism of bile acids and bile acid cross talk in the intestine have been related to a state of dysbiosis leading to several gastrointestinal, metabolic and inflammatory disorders, including those associated with aging-related decline [123]. These metabolites also activate the nuclear receptor and cell signalling pathway in the host in order to control bile acid, cholesterol, glucose, lipid and energy metabolism. They act as antimicrobials as well [69,70].

Phenolic derivatives (Phenyl acetic acid, equol, urolithine, enterolactone, enterodiol, phenyl derivatives of propionic acid and valeric acid):

Phenolic compounds in the diet have several advantages such as their antimicrobial effects. Phenolic compounds are broken down by the gut microbiota, specifically organisms belonging to the *Clostridium* and *Eubacteria* genera. These metabolites have properties that moderates gut health, acts against oxidative stress, estrogen regulator and prevents platelet aggregation. Urolithin has cancer chemoprotective effects as well [71,72,73].

Vitamins (B1 Thiamine, B2 Riboflavin, B3 Niacin, B5 Pantothenic acid, B6 Pyridoxine, B7 Biotin, B11-B9 Folate, B12 Cobalamin, K2 Menaquinone):

Although vitamins are not a product of gut microbiota action, the presence of vitamins induces the growth or survival of several beneficial strains of bacteria such as *Clostridium,*

Bacteroides, Lactobacillus and *Ruminococcus species*. Growth of these organisms facilitate advantageous functions of the gut microbiome in maintaining a healthy lifestyle [22]. Vitamins play a very important role in energy production, red blood cell formation and are coenzymes and cofactors for many biochemical reactions in the body [74].

Polyamines (putrescine, spermidine, spermine):

Dietary polyamines are known to be broken down to putrescine and spermidine in the gut by *Bacteroides* spp. and *Fusobacterium* spp. The complex biochemical pathway in metabolising polyamines has been demonstrated in *E.coli* experimentally but there are many species of gut microbiota that are involved in the process in reality. *Enterococcus faecalis* and *Bifidobacterium* spp are known to aid the production of polyamine metabolites by creating a favourable environment [124]. Polyamine metabolism disturbances may result in formation of tumours. They control the production of tight junction proteins such as occludin and E-cadherin. It enhances the immune system of the host. It also prohibits pro-inflammatory M1 macrophage activation [75,76,77].

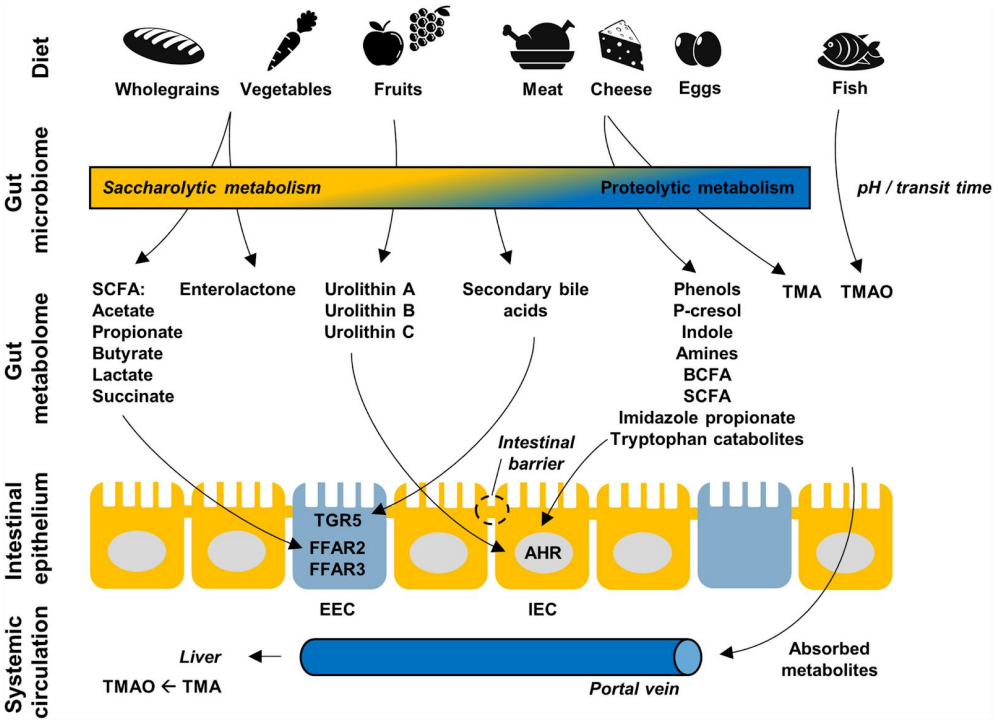

Figure 8: Dietary sources of the above mentioned compounds are metabolised in the gut by several bacterial species and depend on the pH, time of transit in the gut and metabolism rates. As a result, various beneficial metabolites are produced and play their respective roles in different parts of the body. They enter the intestinal

epithelial cells (IEC) and enteroendocrine cells (EEC) reacting with G- protein coupled receptors (TGR5, FFAR 2/3) and aryl hydrocarbon receptor (AHR). These play a role in maintaining homeostasis between the gut and body. Certain metabolites enter the liver where they are further broken down or converted to products such as TMA and TMAO (the features are explained before under choline metabolites).

Source: [78]

6.0 GERM FREE MICE EXPERIMENTS

Mice that are grown in a completely sterile environment since its birth do not contain any type of host microbiota and are used as a standard or a control in gut microbiome testing experiments such as fecal microbiota transplant [79] where the organisms found in the feces of one mouse, be it healthy or diseased, is administered to another healthy or diseased mouse to test how they react to it. The growth of fresh gut microbiome can be observed, the chronological development of the microflora and loss of microflora during dysbiosis, antibody administration or environmental and dietary stress can all be mapped in order to better understand the importance of gut microbiome in health and disease [80].

Figure 9: Mice grown in a completely sterile environment

Source:

https://www.enidnews.com/news/omrf-opens-state-s-first-germ-free-mouse-research-facility/article_9fc27fec-b042-11e7-8806-ab356739304e.html

7.0 GUT-BRAIN AXIS

The gut-brain axis also known as hypothalamic pituitary adrenal axis has functions of immune system regulation and consists of beneficial bacterial metabolites, hormones and neurotransmitters. It overlooks and combines functions of the gut and connects the emotional and cognitive center of the brain to the intestinal lumen. It acts as a neuro-immuno-endocrine regulator and plays a role in immune system activation, enteric reflex and entero-endocrine signalling. The gut brain axis is a dual directional pathway acting back and forth from the intestinal lumen to the central nervous system (CNS-brain and spinal cord), autonomous nervous system (ANS), enteric nervous system (ENS) and hypothalamic pituitary adrenal (HPA) axis [81,82]. When germ free mice are treated with healthy gut microflora, they showed better stress responses, increased motor activity and lower anxiety levels as compared to the control. In germ free mice, there is no microbiota colonisation and certain observations like altered neurotransmitter expression, gastric problems and improper digestion was made along with reduced expression of enzymes that aid in production of neurotransmitters and muscle contraction proteins. These abnormalities were normalised upon colonisation of the gut by microflora [83,84]. This shows a strong relation between the brain and gut microbiome. SCFAs influence the Blood Brain Barrier (BBB) through production of tight junction proteins like claudin-5 and occludin, thereby strengthening the intestinal barrier and preventing diffusion of unnecessary molecules into the blood. MAMPs produced by the gut microbiome has major neuroimmune functions and acts by releasing cytokines TNF-**α**, IL-6 and IL-1β. These cross the BBB in turn activating microglia and neurons which are responsible for altering neurological functions such as mood or behaviour. These are confirmed by functional MRI monitoring brain activity [85].

Since the concept of gut brain axis is bidirectional we look at the connections from the gut to the brain first. Germ free animals commonly have a memory impairment due to the disruption in expression of brain derived neurotrophic factor (BDNF) that also affects serotonin levels in the host. Administering *Lactobacillus rhamnosus* TB-1 to any host produces variations in gamma-aminobutyric acid (GABA) which is a neurotransmitter in the brain [86]. With the use of probiotics, reduced stress responses, anxiety and depression could be observed in subjects. Conversely, using antimicrobials elevated abnormal behaviour [87].

The vagus nerve is said to be a major connection between the lumen and CNS signals [88]. The microbiome brain interaction has a close correlation with the intestinal barrier integrity. *Lactobacillus helveticus* and *Bifidobacterium longum* when ingested by the host, restoration of tight junction barrier in the gut, strengthening of HPA axis and ANS functions can be seen [89]. This could be confirmed through measurements of plasma cortisol and catecholamine levels. Probiotics also avoided perturbations in hippocampal neurogenesis and expression in the genes responsible for synaptic plasticity in the hypothalamus. The gut microbiota's relation with the gut brain axis affects the Calcium dependent Potassium channels through the afferent sensory nerves and was detected by the addition of *Lactobacillus reuteri* causing changes in the pain receptors [86]. SCFAs mentioned above are also responsible for production of mucosal serotonin and enhancing memory functions through the sympathetic nervous system [90].

The connection from the other side i.e., brain to gut is equally important in understanding how the two way mechanism works. When an individual is exposed to conditions of external stress, the composition of the gut microbiome changes drastically. Host-enteric microbiome signalling, intestinal environment perturbations through the ANS and HPA is seen through neuroendocrine outputs and also affects the emotional motor system [91]. Certain bacteria in the gut have neurotransmitter receptors or binding sites and hence any release of a neurotransmitter in the body directly affects microbiota reactions [92]. The brain also handles the release of certain acids and compounds in the intestine, simultaneously having a hold on the intestinal barrier rigidity and permeability [93]. Many neurological diseases such as ill effects of Alzheimer's have been treated using certain gut microbes that helps improve the mental state of the patient. There is experimental evidence which shows that understanding the connection between the gut microbiome and the brain is the key to treat and alleviate the symptoms of a lot of neurological disorders such as autism, ADHD and Parkinson's [94].

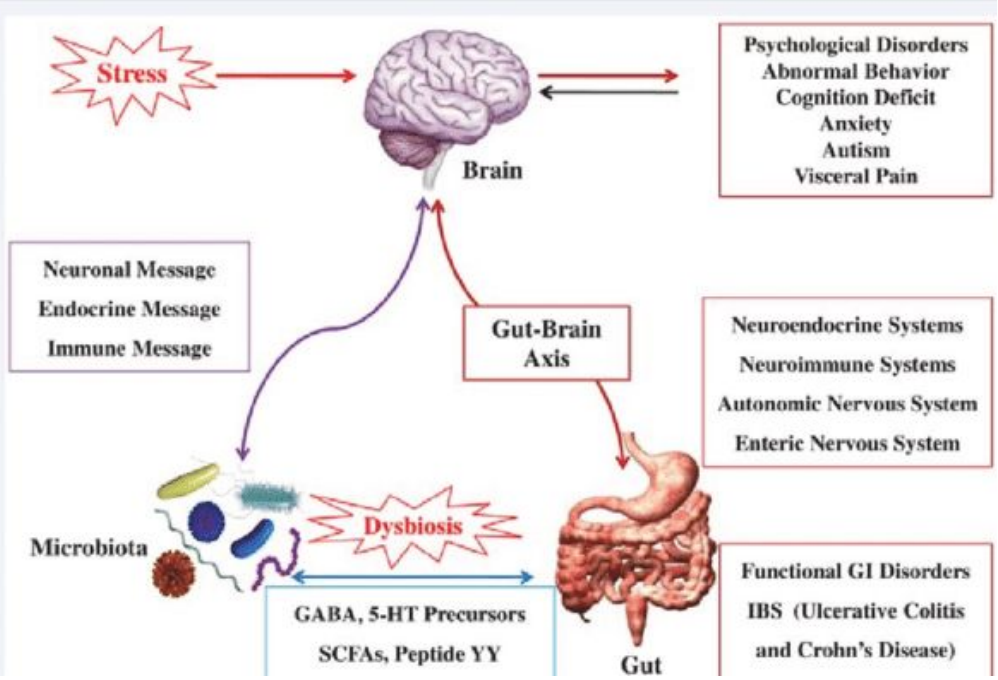

Figure 10: This shows a bidirectional action of the gut-brain axis. The gut interacts with the brain through a direct reaction with mucosal cells (endocrine message), immune cells (immune message) and neural endings (neuronal message) and influences the development of the brain. When there is external stress induced on the brain, it results in gut dysbiosis leading to gastrointestinal (GI) disorders, in turn compromising the intestinal barrier functions. Improper barrier integrity may cause certain metabolites from the gut to diffuse to the brain leading to abnormal behaviour, anxiety and other brain related disorders.

Source: [95]

8.0 SPECIFIC DISEASES AND THEIR ASSOCIATION WITH GUT MICROBIOME

8.1 Obesity:

The intestinal microbiota is known to play an important role in terms of energy usage, energy expenditure and storage derived from the diet by regulating the appropriate genes responsible in the body of the host. Experiments conducted with mice models indicate varying levels of SCFAs in lean and obese subjects. Obese mice had decreased levels of butyrate and acetate, higher *Firmicutes* and lower *Bacteroides* number and increased gene expressions in an individual for polysaccharide degradation. The above findings and differences were seen to be similar in case of obese and lean humans [96]. A confirmatory test where microbiota from an overweight mouse was transferred onto a germ free mouse showed an increase in body fat

in the germ free mouse, thereby implying there was a high calorie release from the diet and activated genes affecting adipocyte deposition [97]. Lean mice are seen to have increased skeletal muscle levels of protein kinase, acetyl CoA carboxylase and carnitine palmitoyl transferase that play a role in degrading the chemicals released as a result of a high calorie rich diet. One of the possible ways to treat obesity would be to use prebiotics and probiotics. Prebiotics such as inulin stimulate growth of beneficial gut bacteria such as *Lactobacilli* and *Bifidobacteria* that are responsible for modulating the levels of GLP-1 in the gut which is a signalling protein involved in connecting the food intake to the satiety center in the brain. Probiotic use is advantageous when multi strain bacteria are used. They are said to protect against fat accumulation and metabolic disruption in obesity caused due to a particular dietary intake. Some useful probiotic bacterial strains include *Lactobacillus gasseri*, *Lactobacillus rhamnosus*, *Lactobacillus maltodextrin*. Yeast such as *Saccharomyces boulardii* is said to increase the rate of metabolism in digesting food to convert it into energy in the body. Synbiotics is the use of pre and probiotics simultaneously such that the prebiotics act as substrates for the probiotic organisms in the gastrointestinal tract and obtain maximum efficacy [98].

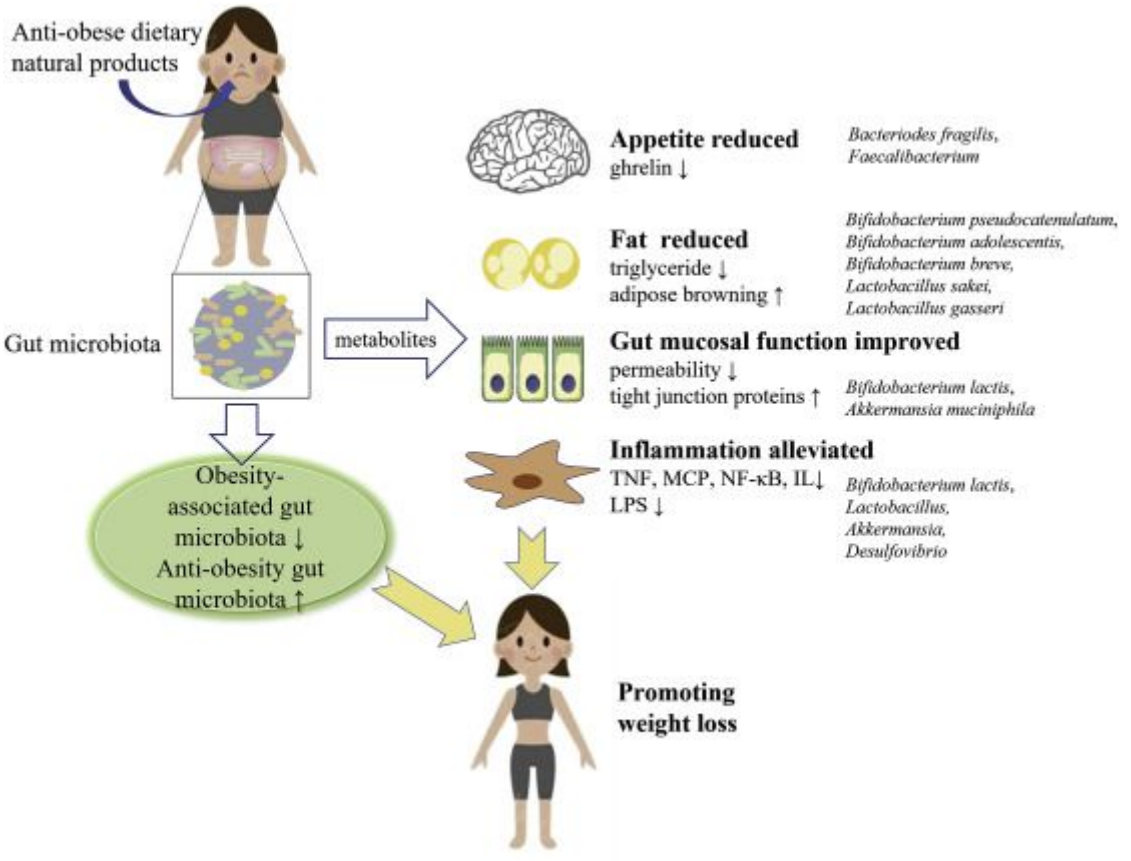

Figure 11: Under the influence of obesity, following a diet with natural anti-obesity products alters the gut microbiota thereby reducing appetite, fat deposition, improving intestinal barrier integrity and decreasing inflammation reactions by reducing expressions of TNF, LPS and IL. Several bacterial species involved in these processes are enhanced in number upon change in the type of diet followed by an individual hence assisting weight loss.

Source: [99]

8.2 Inflammatory Bowel Disease, Crohn's Disease and Ulcerative Disease:

This disease is characterised by chronic inflammation of the terminal ileum in the intestinal tract caused due to an auto-inflammatory response to the gut microbiota in a genetically vulnerable host. Gut microbes producing SCFAs help build a strong intestinal barrier with the production of mucins that in turn play a major role in keeping the gut bacteria in their place. Certain harmful bacterial species like *Oscillospira* degrade mucins thereby increasing intestinal permeability in turn causing IBD or endotoxemia [100]. Use of antibiotics or consumption of a high fat, low fiber diet causes depletion in healthy gut microbiota and promotes possible attack and growth of pathogenic organisms. Patients with IBD or Colitis have decreased amounts of tryptophan derived indole derivatives which disrupts the regular

homeostasis in the gut leading to a high IL-22 expression *in vivo* and this leads to inflammation. *Faecalibacterium prausnitzii* is a biomarker for a healthy gut and plays a role in maintaining immunity of the host as well as having anti-inflammatory properties and loss or alterations in the abundance of this organism results in inflammatory disorders such as IBD [101]. *Saccharomyces cerevisiae* and *Lactobacillus spp* have anti inflammatory properties that when inculcated in mice showed reduced levels of TNF-**α** expression and thus helps in reduction of chronic inflammation. Use of such targeted therapeutics along with use of synbiotics and fecal microbiota transplant in the gut will provide useful insights in tackling these inflammatory disorders [102,103].

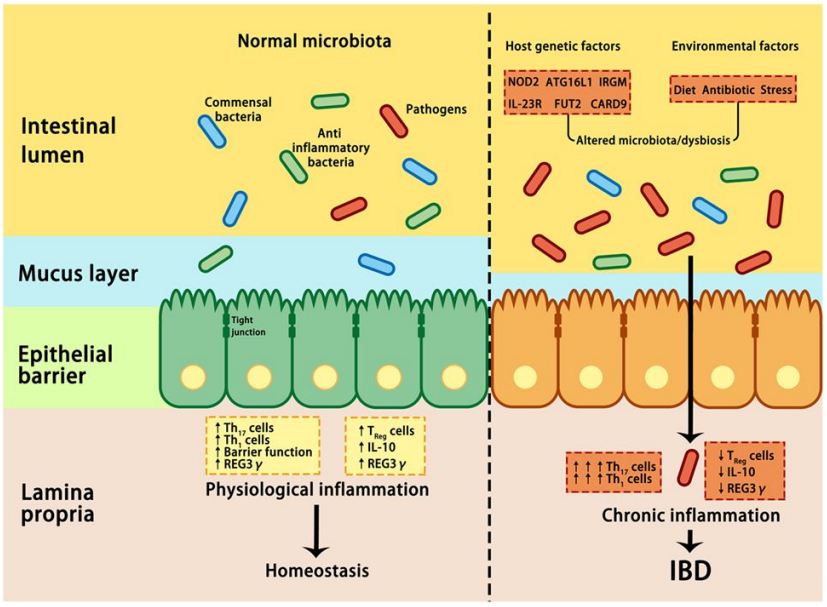

Figure 11: The left side indicates a healthy gut microbiome action, where several commensal bacteria helps maintain homeostasis and intestinal barrier integrity by enhancing anti-inflammatory properties of immune cells. The right side indicates a state of dysbiosis occurring due to external factors such as dietary changes and environmental stress. This compromises the barrier integrity and allows pathogens to enter the gut and induce inflammatory response. Th (T helper cell), T_{reg}(T regulatory cell), REG3 (antimicrobial protein), IL-10 (interleukin).

Source: [104]

8.3 Atopic asthma:

An individual's exposure to the environment, antibiotics, pets, mode of delivery during birth and the type of diet plays a major role in the development of respiratory disorders. Exposure to good amounts of environmental microbes induces growth of a healthy gut microbiome

which in turn builds the host's immunity. Dust from pets, especially dogs, are rich in *Lactobacillus johnsonii* and when administered to mice, prevents respiratory pathway allergy by decreasing the levels of IL-4, IL-5, IL-13, IL-17 and increasing concentrations of T-reg. A high fiber diet when fed to mice, increases their SCFA concentration and consecutively reduces allergies in mice [105].

8.4 Colorectal Cancer (CRC):

The environment of a dysfunctional gut microbiome largely defines favourability of tumour growth [125]. Intestinal mucosal barrier dysfunction induces inflammatory response at old age and along with genetic vulnerability, proves a risk of developing CRC. Once the tumour progresses to the lumen, the intestinal barrier gets damaged, allowing microbiota and harmful substances infiltration into the tissues, thereby creating an inflammatory response and finally bringing about changes in the gut microbiome. The gut microbiota along with CRC cells, feed symbiotically on the host [57]. 16s rRNA sequencing performed on the microbiota of CRC patients showed an increase in the number of *Bacteroides fragilis.* Toxic strains of *B. fragilis* (BFT) produces an endotoxin and the bft gene is seen to be over expressed in individuals with CRC. When tumour prone mice are induced with enterotoxigenic strains of *B. fragilis* (ETBF) tumour formation is seen to be triggered and the ETBF seems to catalyze tight junction proteins such as E-cadherin. Loss of these proteins activates T-cell dependent nuclear signalling in epithelial cells which in turn stimulates the oncogene c-Myc thereby inducing cell proliferation. BFT along with IL-17 stimulates myeloid differentiation into monocyclic myeloid derived suppressor cells that upregulates vascular endothelial growth factor mediated tumour growth and downregulates anti tumour activity. *Fusobacterium nucleatum* cells seem to be high in number in patients with CRC and this affects Fad A adherin that promotes bacteria to damage tight junction proteins and upregulating c-Myc and cyclin D1 oncogenes. ETBF and *F. nucleatum* act as molecular signatures that can be utilised as a biomarker for identifying or predicting CRC in hosts. Antibodies that can target these organisms or their signalling pathways could help prevent tumour growth [106,107]. Butyrate producing bacterial strains like *Faecalibacterium* and *Roseburia* is tested to inhibit tumour growth. Butyrate also acts as a protector of oxidative stress induced by hydrogen peroxide. The exact mechanism of action and therapeutic use of butyrate must be studied in detail if administered to patients. Use of probiotic strains producing lactic acid is shown to reduce the

risk of tumour growth in hosts [108,109]. Non endotoxin producing *Bacteroides fragilis* can be administered to high risk individuals in order for them to develop immunity against the toxic strain [57]. There is also circumstantial evidence from recent studies that a strain of *E.coli* inhabiting the gut produces certain toxins that promote the possibility of tumour growth in the bowel. Although in some individuals these strains of *E.coli* are non toxic or dormant, several external factors may increase the chances of tumour growth. This could be prevented by killing these strains of bacteria using specific antibiotics. These strains of *E.coli* could also be used as a biomarker for early detection of CRC [126].

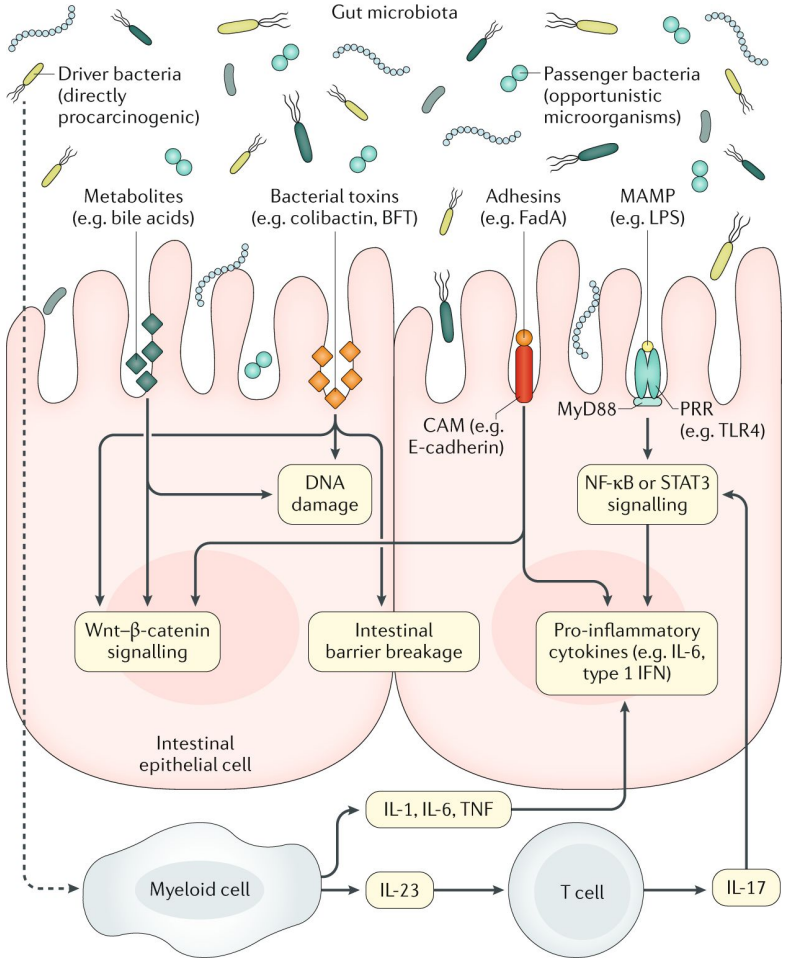

Figure 12: A possibility of attack of carcinogenic bacteria or release of toxins by some bacterial species could lead to the progression of tumour growth in the bowel. This disrupts the intestinal barrier and their proteins thereby resulting in damage of DNA, impaired cell signalling and eventually uncontrolled growth of cells leading to CRC. BFT, Bacteroides fragilis toxin; CAM, cell adhesion molecule; FadA, Fusobacterium adhesin A; IFN, interferon; LPS, lipopolysaccharide; MAMP, microbe-associated molecular pattern; NF-κB, nuclear factor-κB; PRR, pattern recognition receptor; STAT3, signal transducer and activator of transcription 3; TLR4, Toll-like receptor 4.

Source: [110]

8.5 Type Ⅱ diabetes mellitus:

It is seen that the number of SCFA producing organisms in the gut microbiome of a patient with type Ⅱ diabetes is seen to be significantly reduced. SCFA modulating its interaction with GLP-1 improves insulin sensitivity and controls satiety resulting in body weight loss. Decrease in the concentrations of SCFAs, downregulates the interaction with GLP-1 thereby increasing low grade inflammation resulting in the diabetes condition. This is majorly caused due to frequent use of antibiotics, following a high fat western diet and exposure to harmful microbes [111]. Upon examination of the fecal microbes of patients with type Ⅱ diabetes, it was seen that there was a decrease in the number of butyrate producing microorganisms, *Firmicutes* and *Clostridia* but prevalence of *Lactobacillus spp.* This implies a relation between plasma glucose levels and these gut microbiota. The specific gut microbiota can be used as a biomarker for type Ⅱ diabetes. Patients with type Ⅱ diabetes have pathogenic organisms in their gut including *Bacteroides caccae*, *Clostridium spp* and *E.coli*. Bile acid gets metabolised in the gut and activates Farnesoid X Receptor (FXR) and G-protein coupled receptor and this FXR downregulates the expression of certain sugar producing enzymatic reactions. As a confirmatory test, the knockout of FXR gene in mice resulted in weight gain, hyperglycemia and higher glucose tolerance. G-protein coupled receptor produces GLP-1 that enhances hepatic and pancreatic function in the host. Increase in intestinal permeability or damage to the intestinal barrier results in pancreatic β cell damage and also an increase in toxic antigens entering the bloodstream [112].

Treatment procedures that can be looked at involves administration of certain species of microorganisms such as *Bifidobacterium animalis* subsp *lactis* 420 to patients. This is proven to reverse low grade inflammatory responses in the gut. Fecal Microbiota Transplant from lean normal subjects to patients with diabetes resulted in better insulin sensitivity and an increased number of butyrate producing bacteria. Use of probiotics in combination with other diabetes medicines shows reduced fasting plasma glucose and improved oxidative status in type Ⅱ diabetes patients by increasing presence of antioxidants [113]. *Lactobacillus acidophilus* and *Lactobacillus casei* when administered to mice subjects resulted in reduced pancreatic damage and helped produce increased insulin sensitizing hormones. Probiotic yoghurt improves symptoms such as glucose intolerance, hyperglycemia and oxidative stress [114].

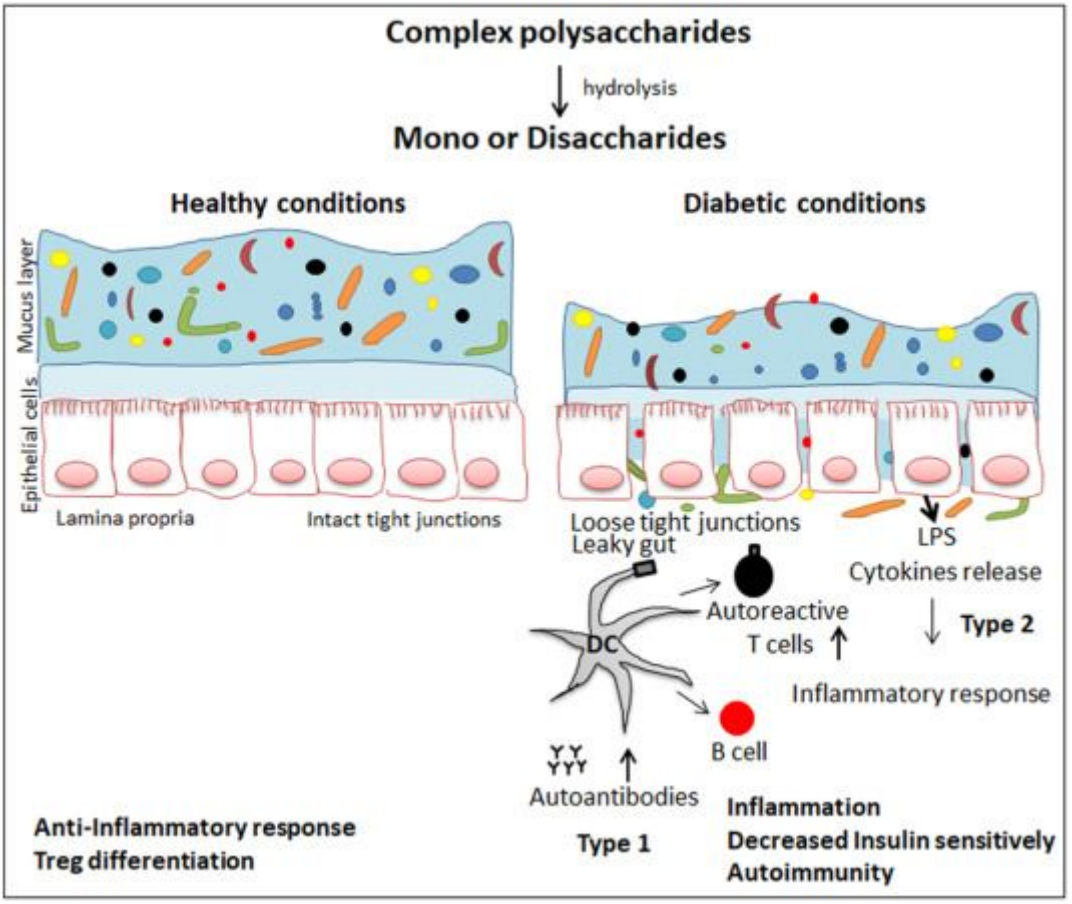

Figure 13: Dietary intake affects healthy and dysfunctional gut. Intestinal barrier is rigid in healthy conditions but compromised in unhealthy dietary conditions leading to a diabetic condition. In type 1 diabetes, the gut microbiota enter the bloodstream initiating an autoimmune response. In type 2 diabetes, hydrolysis of carbohydrates caused dysbiosis leading to inflammatory response which caused insulin sensitivity to decrease. Source: [115]

8.6 Autism Spectrum Disorder (ASD):

Although there is no specific cause of this disease, there is a relation between ASD and dysbiosis of gut microbiota through the gut-brain axis. Patients with ASD have shown symptoms of gastrointestinal disorders. The possible cause for this might be hindered MET signalling that is responsible for brain development, gastrointestinal health and immune response regulation. Gastrointestinal disturbances in ASD patients causes them to inflict self damage and treating this dysbiosis may help in soothing the harmful symptoms of ASD [116,117]. Patients with ASD show an increase in the number of *Clostridium, Bacteroides, Lactobacillus* and *Desulfovibrio* species and decrease in *Bifidobacteria* numbers in the gut microbiota implying the relation between ASD and gut microbiome dysbiosis. Treatment

options should look at targeting the gut microbiota so that it alleviates symptoms of ASD. Probiotic *Bifidobacterium* is said to have a counteractive neurotoxic effect in ASD patients showing an improvement in behavioural abnormalities. The exact working mechanism if the treatment options is yet to be elucidated but targeting the gut microbiota is the starting point [118].

8.7 Parkinson's disease (PD):

This is a common neurological disorder mostly developing in elderly people. Although the exact cause is not known, the compound α-Synuclein is said to induce the disease state when present in the mucosal and submucosal nerve fibers and ganglia. It has been observed that α-Synuclein gets transported from the gut to the brain via the vagus nerve. A vagotomy performed in mice showed a prevention of α-Synuclein translocation upward to the brain and curbed neurodegeneration and behavioural deficits proving that there is a direct link between PD and the gut brain axis. There are several bacteria seen to produce amyloid-like proteins such as α-Synuclein in older rats. More studies need to be performed to elucidate the connections between gut microbiota to motor and non motor symptoms in Parkinson's disease. When germ free mice underwent fecal microbiota transplant from patients with PD, symptoms of the disease such as motor deficits, tremors along with neuroinflammation was observed. When antibiotics were used in this condition, the symptoms seemed to be alleviated. In humans with PD there were decreased concentrations of SCFA in the plasma as compared to a healthy person. The drug levodopa used for treating this disease induces gut microbial decarboxylases in rat subjects causing attenuation of the drug in the plasma. FMT, use of probiotics and specific strains of bacteria in patients or subjects with PD could be an option as a treatment to reduce motor dysfunctions and treat other behavioural symptoms [119].

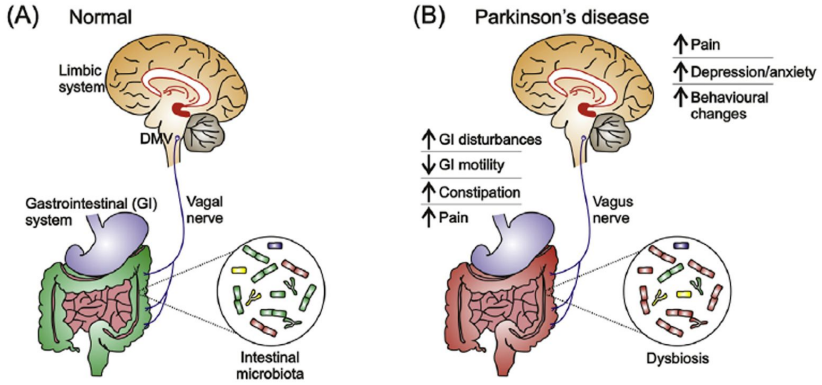

Figure 14: Normal condition in the body as shown in (A) results due to a healthy gut with the gut-brain interaction through the vagus (here referred to as vagal) nerve working as expected. A disturbance in the gut microbiome results in shifts in composition of organisms inhabiting the gut leading to a state of dysbiosis causing improper gut-brain interaction resulting in gastrointestinal (GI) disorders and in turn, behavioural changes as shown in (B) that is Parkinson's disease.

Source: https://www.gutmicrobiotaforhealth.com/new-insights-role-gut-microbiota-parkinsons-disease/

9.0 CONCLUSIONS AND FUTURE PERSPECTIVES

The human gut microbiome is gaining immense importance currently and is seen as no less than an organ by itself. Characterising the microflora in the gut is one of the primary steps that needs to be taken in order to better understand the working dynamics of the gut microbiome. A large population of prokaryotic organisms have been identified and mapped showing that there are mainly three enterotypes growing in the gut, depending upon not only the age, body mass index or gender [127] but also on the diet and lifestyle followed by an individual. Mapping and establishing a healthy gut microbiota is one of the first tasks to be completed in order to understand perturbations in them occurring due to external factors such as stress causing dysbiosis. A variety of eukaryotes including viruses also inhabit the gut and play a huge role in the intricate interactions between the host and gut but these organisms are yet to be identified and studied in detail [128]. Inter-individual differences in the composition of gut microbiome in different populations is seen to be less that 8 to 10% but of course depends majorly on their lifestyle [129]. With the advent of futuristic technologies such as next generation sequencing techniques, it has been simpler to identify and characterise the types of microorganisms growing in the gut. Identification of biomarkers of a healthy gut and a gut under dysbiosis can be easily done nowadays. Techniques such as FMT that helps

restore gut health post dysbiosis are still in its infancy but provide satisfying results in diseases such as IBD, colitis and Parkinson's. Building up on the existing knowledge base with respect to the gut microbiome and structuring the capabilities of using these microbiota to tackle diseases haunting humanity for a long time, could be the futuristic method of developing modern personalised medicine.

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