# INVESTIGATING THE INFLUENCE OF SEROTONIN AND FECAL SHORT CHAIN FATTY ACIDS (SCFAs) ON GUT-MICROBIOTA BRAIN AXIS IN HEALTHY SUBJECTS




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Degree of

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**In**

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# In the Name of Allah Who is the Most Gracious and the Most Merciful



## DECLARATION

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Tayyaba

**Tayyaba zaheer kayani**

# CERTIFICATE

The thesis entitled **Investigating influence of serotonin and short chain fatty acids (SCFA) on gut-microbiota brain axis in healthy subjects** submitted by **Ms. Tayyaba Zaheer Kayani** is accepted in its present form by the department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan. As satisfying the thesis requirement for the degree of Master of Philosophy in Microbiology.

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**Prof. Dr. Aamir Ali Shah**

**Date:** **13-September-2022**

# DEDICATION

EVERY CHALLENGING WORK NEEDS EFFORTS AS

WELL AS GUIDANCE OF

ELDERS ESPECIALLY WHO ARE VERY CLOSE TO

OUR HEART. MY HUMBLE EFFORTS I DEDICATE TO

MY SWEET AND LOVING

FATHER & MOTHER

WHOSE AFFECTION, LOVE, ENCOURAGEMENT AND

PRAYS OF DAY AND

NIGHT GIVE ME STRENGTH TO WORK HARD WITH

HONESTY ALONG WITH ALL RESPECTED AND

HARD WORKING

TEACHERS.

# Acknowledgement

The success and final outcome of this thesis required a lot of guidance and assistance from many people. I am extremely fortunate to have got all this along the completion of thesis. Whatever I have done is only due to such guidance and assistance and I would not forget to thank them.

The most important acknowledgement of gratitude I wish to express is to my mentor and guide **Dr. Muhammad Imran**, Associate Professor, Department of Microbiology, who has put his valuable experience and wisdom at my disposal. He provided critical advice in my research, calculations and suggested many important additions and improvements. It is generally enriching experience to me to work under authoritative guidance. It was only because of his keen interest and continuous supervision that gave my work this extent form.

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## Abstract

Human digestive tract is colonized by numerous symbiotic microorganisms that possess an ability to orchestrate host metabolic and physiological process. The relative abundance of between symbionts and pathobionts in gut has a profound impact in modulation of host health and disease. The human super organism resulting from complex gut microbiota interaction with host has a profound role in human neurophysiology. Microbial metabolites are usually bioactive compound that have ability to manipulate immune system, protect gut epithelial barrier and regulate host neuronal health. Diet and gut flora together have produced symbiotic which have been deemed effective in bidirectional communication between enteric and central nervous system. Dietary compounds that are indigestible reach unabsorbed to intestines where gut microbiota utilize them to gain energy and thereby producing end products that are highly beneficial for host. These gut microbiota derived metabolites either directly or indirectly effect the crosstalk between host gut and brain via several pathways like HPA, BBB and vagal nerve. SCFAs are low molecular weight that have ability to influence the communication between gut and CNS but has demonstrated a role as neuroprotective compound. Furthermore, it triggers the production of other important neuroactive compounds such as serotonin from EC cells which have been implicated in overall health of nervous system. Imbalance in serotonin levels is usually associated with a dysbiotic gut which leads to inflammation and thereby progression of psychiatric or neurodegenerative disease. Therefore, it extremely important to understand role of such compounds in maintain homeostasis in human body. Population samples from three distinct region with a different dietary pattern and lifestyle were assessed based on effect of these patterns on their SCFAs and serotonin concentrations. Total 35 serum and fecal samples of healthy individuals were analyzed for microbiology and serotonin respectively. Highest incidence of serum serotonin was found in Sindh region mainly due to higher consumption antioxidant rich food. A profound impact of dietary habits was discovered on serum serotonin levels. The subsequent impact of serotonin correlated with the brain health of these individuals. It was observed that dietary patterns influence the serum serotonin levels through microbiota modulated activity which was thereby is responsible for maintaining a homeostatic balance promoting a healthy brain activity in all subjects.

# Chapter #1

# Introduction

# 1. INTRODUCTION

Human gut is colonized by a plethora of microorganisms that have developed a symbiotic relationship and contribute directly or indirectly to health and wellbeing. The conglomerate of human cells and resident gut microflora is considered as human superorganism which is believed to have an ability to orchestrate the host metabolic and physical health (Mohajeri et al., 2018). Growing evidence suggests that the gut microbiota is an extraordinarily varied and highly populous organisms that plays an essential role in human health maintenance and disease causation. Host-microbiota interface generates an array of essential metabolites that behave as primary communicators between symbiotic microbiota and gut but also other human system as well. (Cresci et al., 2019). Gut microbiota structure and metabolic processes are influenced by a person's diet. Dietary habits can regulate gut microbial composition by controlling the quality or quantity of nutrients either by restricting or providing the necessary dietary constituents for the growth of certain microorganisms over others. In an indirect way diet impacts microbial composition by influencing host metabolic state or immune response or by disrupting protective functions of the intestinal barrier causing dysbiosis and thereby promoting inflammatory processes (Zmora et al., 2019). *Bacteroides* for example is known to cause inflammation and has been linked to an increased risk of metabolic syndrome whereas *Prevotella* have largely been associated with protective and anti-inflammatory role. Anaerobic bacteria like Firmicutes and Bacteroidetes work with *Bifidobacteria*, an oligosaccharide-fermenting species to break down indigestible carbohydrates into short-chain fatty acids (acetate, propionate, and butyrate). SCFAs boost digestive health through a variety of local actions, including maintaining gut barrier stability, mucus formation, and inflammatory protection, as well as lowering the risk of colon cancer (Silva et al., 2020). When it comes to immunological system, butyrate and propionate play important roles in maintaining immune homeostasis is regulated suppression, which induces regulatory T cells and Th17 cells in the body. Anti-inflammatory actions of butyrate may be due to the suppression of histone deacetylase (Andoh, 2016). Along with that Enteric microbiota alters gastrointestinal permeability, and motility influencing host emotional and physiological responses and stimulating enterochromaffin cells produce neurotransmitters. Enterochromaffin (EC) cells produce a large amount of peripheral serotonin which is influenced by the resident gut flora or its metabolites (Yano et al.,

2015). Microbial metabolic product such as SCFAs have been observed to impact serotonin production thereby affecting epithelial permeability of gut (Ge et al., 2018). Commensal bacteria, on the other hand, may create serotonin directly from luminal tryptophan. In numerous bacteria from the genera *Lactobacillus*, *Lactococcus*, and *Escherichia coli*, expression of tryptophan synthetase was shown to be sufficient to promote serotonin synthesis (O'Mahony et al., 2015). It plays avital role in regulation of metabolic homeostasis as well as cognitive function of an individual. Behavioral and mood alterations have been associated with an imbalance in serotonin thereby contributing to progression or development of psychological disorders (Matthes et al., 2018). Dietary modulation that results in profusion the probiotic gut bacteria involved in attaining normal serotonin concentrations have emerged as potential therapeutic intervention in such ailments (Sun et al., 2021). Recently, evidence has emerged showing that the gut–brain axis, or the bidirectional connection between the resident microorganisms of the GI tract and the brain, plays an important role in sustaining brain health, extending the advantages of human–microbe symbiosis. Much research has associated gut disorders as triggering factor for psychological disorders mainly stress, depression and anxiety. Gut communicates with brain via HPA axis, blood brain barrier and vagal nerve which makes it the second brain in human also referred as enteric nervous system (Safadi et al., 2021). As enteric system holds an abundance of microbial population, any Mental disease can be influenced by the bacteria in the digestive tract. New information regarding microbiome activity and relevance in the gut–brain connection has widened our understanding of this axis to include the "microbiota–gut–brain axis," stressing its role in controlling gut–brain communication (M Hasan Mohajeri et al., 2018). Dysbiosis (alteration of microbial structure and function) is linked to a variety of psychological and neurodegenerative diseases. Relationship between microbial dysregulation and pro-inflammatory gut condition are quite evident. Leaky gut is a term used to express increased permeability of gut epithelial barrier which is major consequence of inflammation. Bacteria and bacterial metabolites translocation across the mucosal membrane causes autointoxication, which adds to the persistent inflammation observed in many mental illnesses as Alzheimer's and Schizophrenia (Halverson et al., 2020). Over the years many diagnostic techniques like electroencephalography (EEG), Magnetic resonance imaging have been developed to understand the etiology of mental ailments whether psychiatric or neurocognitive. EEG however has taken a

superiority among other techniques specially in case of psychiatric disorder (Armitage et al., 2001). EEG represents the accretion of millions of nerve cells post-synaptic potentials usually called as EEG signals. EEG signals are often collected to indicate two types of brain activities i.e., spontaneous and event-related activities (Gui et al., 2010). The changes in brain wave patterns are associated with presence or absence of psychotic ailment. It has long been utilized in clinical settings to assess seizure disorders, although it is rarely employed in neuroscientific studies (Neto et al., 2019). Profiling of fecal microbiota along with understanding brain wave pattern in different mental conditions is crucial for understanding impact of diet and microbial composition in such ailments. Moreover, it allows to identify potential intervention agents either psychobiotics, microbial metabolites or dietary components that will help to prevent or reduce the extent of mental disorders (Ogawa et al., 2020).

## 1.1. AIMS OF STUDY

To investigate the influence of Serotonin and Fecal Short Chain Fatty Acids (SCFA) level on Gut- Microbiota Brain Axis in healthy subjects in three distinct population.

## 1.2. OBJECTIVES

- To check the impact of dietary habits profile on serum serotonin level.
- To determine correlation between Serum Serotonin and brain wavepattern in healthy subjects.
- To study level of Fecal Gram-positive and Gram-negative bacteria in healthysubjects.

# Chapter #2

# Literature Review

# 2. HUMAN GUT MICROBIOTA

The gut has become one of the most studied biogeographical niches present in humans, not only due to the diverse nature of microbiota dwelling in it but also the fact that some of them can be cultivated in laboratory settings. (Cryan et al., 2019). The Human Gastrointestinal tract (Anderson et al.) allows a substantial interaction between environmental factors, human surfaces, and metabolites. On average daily human food consumption is between 3 to 5 pounds of food which brings along a plethora of microorganisms. These environmental microorganisms interact with resident human microflora, which constitutes bacteria, viruses, and fungi termed a" Gut microbiota" (Thursby et al.). The symbiotic relationship between gut and microbiota has evolved and contributes to the host metabolic process to an extent where it is now considered as another major organ for the human body, sometimes also referred to as a superorganism (Wang et al., 2018). Human gut microbiota has grasped the attention of researchers over the past few decades due to its ability to regulate human metabolites as a therapeutic target and as the source of therapeutic drugs themselves. The importance of the gut microbiome has become more evident with the advancements in genetic tools and metagenomics. Understanding gut microbiota composition and characteristics have triggered the interest in understanding its potential link to disease or health conditions. (Cani, 2018).

## 2.1. SIGNIFICANCE OF GUT MICROBIOME:

Synergistic relationship of host and gut microbiota results in desensitization towards normal gut flora. It acts as a protective barrier against pathogenic microorganisms to which the gut is usually exposed (Sommer et al., 2013). Co-metabolism at the host-microbiota interface results in the production of several important molecules. Many of these molecules serve as key communicators and transmitters between the host and their symbiotic microflora (Nicholson et al., 2012). Short-chain fatty acids (SCFA) are produced during carbohydrate fermentation and are used by the host (Ramakrishna, 2013). Short-chain fatty acids (SCFA) are produced during carbohydrate fermentation and are used by the host (Ramakrishna, 2013). Butyrate, acetate, and propionate produced as a result of fermentation are critical regulators of host health as they promote the integrity of epithelial barrier by supplying energy to epithelial cells (Cresci et al., 2019). *Bifidobacterium*, along with lactic acid bacteria, poses an ability to synthesize

vitamins such as folate, riboflavin and vitamin K (LeBlanc et al., 2013). The enteric microbiome also affects the human brain directly and indirectly from alterations in gastrointestinal secretions, permeability, and motility. Neurotransmitters released by enterochromaffin cells under the influence or directly by gut bacteria impact multiple brain regions regulating the host emotional and physiological response (Aziz et al., 2013). Moreover, gut microbiota can also metabolize protein and carry out catalysis of amino acids leading to the synthesis of many important immunomodulators and signaling molecules such as histamine and GABA (Hollister et al., 2014).

## 2.2. COMPOSITION OF CORE GUT MICROBIOTA:

More than 35000 species of bacteria constitute the human gut microbiota, and non-redundant genes are suggested to be higher than 10 million based on total bacterial genes, the Metagenome of the human intestinal tract, and the human microbiome project (Jandhyala, 2015). The core gut microbiome greatly varies among individuals; however, bacterial phyla *Bacteriodetes* and *firmicutes* usually dominate, constituting approximately 95% of the gut microbiome within healthy individuals, followed by *Actinobacteria* and *Verrucomicrobia* as the second most abundant phyla. (Allaband et al., 2019). The biogeographical location in the gut influences the configuration of gut microbiota, causing diversity and abundance of bacteria to change significantly, i.e., from $10^1$/g to $10^{12}$/g as it transitions from the esophagus to distill portion of the gut. (Jandhyala et al., 2015). The esophageal portion of the gut is dominated by *streptococcus* genera, whereas the stomach microflora is inhabited by *Prevotella*, *Veillonella*, and *Rothia*, with a higher dominance of *Helicobacter* and *Streptococci* genera. Most Helicobacter members live commensally, promoting diversity; however, a significant diminished is observed as they conform to a pathogenic phenotype. More than 70% of the human microbiome resides in large intestines with a higher prevalence of *Firmicutes* and Bacteroidetes. A lower abundance of primary pathogenic species constituting *Campylobacter jejuni, Vibrio cholera, Salmonella enterica, E Coli, and Bacteroides fragilis* has also been reported. A healthy gut is usually associated with the abundance of specific genera,

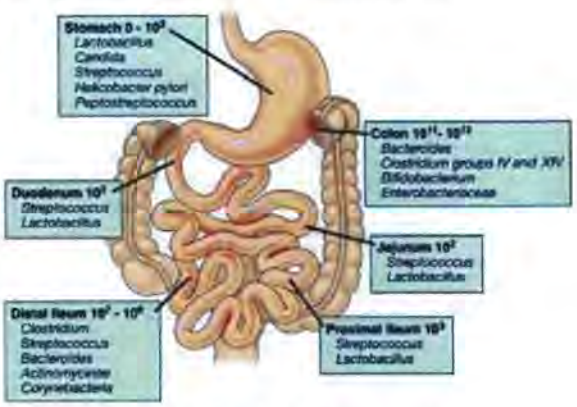


***Figure 1**: Distribution of core gut microbiota in digestive tract. (Sartor, 2008)*

including *Bacteroides, Ruminococcus, Prevotella, Lactobacillus, Clostridium, Enterococcus, and Enterobacteriaceae*. (Jandhyala et al., 2015).

## 2.3. FACTORS AFFECTING GUT MICROBIOTA:

Gut microbiota is shaped by the interaction of both host internal and external factors, including birth method, age, diet, antibiotic use, and host genetics.

### 2.3.1. AGE:

Long-term health effects are linked to the initial microbiota that takes possession of the human gut. Taxonomic diversity increases with age till adulthood; however, many researchers have reported a decreased diversity in old age resulting from elevated levels of cytokines and decreased variety of microbiota (Singhvi et al., 2020). Gut microbiota evolves and changes its composition over the entire period of human life under the influence of the host's internal conditions and environmental factors. Some researchers have reported microbiota establishment in the prenatal period supported by the isolation of microorganisms from fetal membranes, umbilical cords, amniotic fluid, and placenta. Placental microbiota is widely characterized among all and shows the presence of both gram-positive and gram-negative species in preterm and normal birth. The exact mechanism of microbe transfer into the intrauterine cavity, otherwise considered sterile, is still unestablished (Dunn et al., 2017). Newborns acquire the microbiota, mainly from the gut and vaginal microbiome of the mother at the time of birth, but the microbiome composition of neonates is significantly influenced by the delivery mode and gestational age at birth. It was reported that neonates delivered vaginally at full term had a relatively stable gut microbiota and showed a higher fecal abundance of *Actinobacteria* and lower abundance of *Firmicutes* in comparison to full-term neonates delivered via C-section in the first week of birth. However, the c-sectionally delivered neonates developed microbiota like the spontaneous ones within about 8 weeks. Compared to full-term neonates, preterm neonates had a higher number of *Proteobacteria* residing at the first week of birth (Anderson et al.). One of the studies reported a higher prevalence of *Actinobacteria, Bacilli, Clostridium*, and *Bacteroidetes* among children ranging from 1 to 5 years. Microbial communities in older children ages 3-4 years were rich in *Bifidobacterium* but had an overall decrease in diversity (Derrien et al., 2019). A gradual change in gut microbial was observed during age 1-5, showing overall stability of specific phyla where a few of them were still altering.

However, a higher number of butyrate-producing bacteria was reported in children of 5-6 years. It was observed that the gut microbiota is still established in the early years to form a core microbiota and diversity to reach adult-type composition (Cheng et al., 2016). Progressive changes occur in intestinal microbiota during the adolescent period, and an increase in anaerobic species, whereas a reduction is observed in the count of aerobic species.Genera of *Bifidobacterium* and *Clostridium* appeared to be significantly higher in adolescents in contrast to adults (McVey Neufeld et al., 2016).

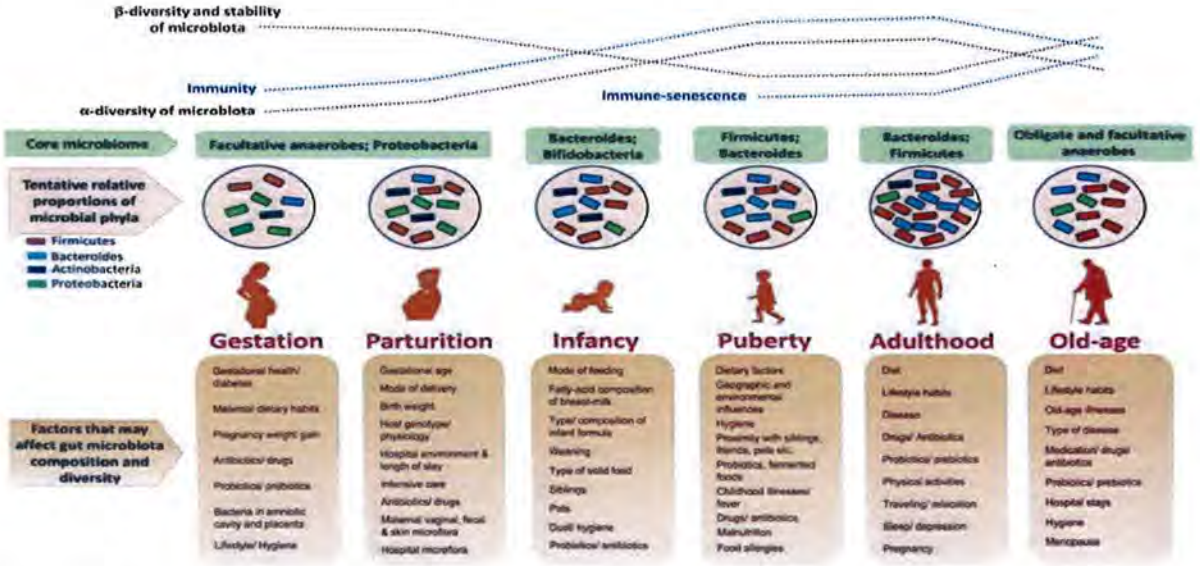


***Figure 2**:Age related change in gut microbiome and factors influences these changes. (Nagpal et al., 2018)*

The gut microbiome in adults is surprisingly stable, extremely diverse, and highly adapted. There is a predominance of phyla *Firmicutes, Bacteroidetes, Proteobacteria*, Actinobacteria, and *Verrucomicrobia*. (Donaldson et al., 2016) A comparison of changes in gut microbiota composition with age revealed an abundance of genus *Clostridiaceae, Bateriodetes, Bifidobacterium* along with the higher number of *Peptoniphilus* and *Megmonas* in the elderly. (Odamaki et al., 2016). The significant variance was observed among raito of *Bacteriodetes* and *firmicutes*. Butyrate-producing genera, *Proteobacteria*, and *Fecalibacterium* showed high variability in the elderly when compared to younger subjects. (O'Toole et al., 2012)

### 2.3.2. DIET:

Diet has proven itself one of the most important regulators of gut microbiota either by elevating useful or depleting harmful microbiota-derived metabolite levels (Gomaa,

2020). Changes in dietary habits result in compositional changes in the gut's microbial population. Complex microbial communities may be shaped by a wide range of food ingredients consisting of both macronutrients and micronutrients (Dixit et al.). A lack of dietary fiber in the diet correlates with an increased breakdown of the mucus barrier in the colon, increasing the susceptibility to pathogens. As a result, one of the healthiest dietary practices is consuming more fiber. the best approaches to keep a healthy gut microbiota in balance. (Barber et al., 2020). It has long been established that a vegetarian diet is associated with better health, a more diverse gut microbiota, and a higher proportion of *Firmicutes* and *Bacteroidetes* (Ray et al., 2018). Diet rich in meat, sugars, and dairy products results in a reduction of *firmicutes* and a higher number of *Bacteroidetes* resulting in lower levels of butyrate and acetate in blood (Cai et al., 2021). High protein diets usually cause excessive production of L-carotene, leading towards cardiovascular disorders due to its conversion into trimethylamine (Mitchell et al.), which is then oxidized by flavin-containing monooxygenase (FMO) to trimethylamine N-oxide (TMAO) (Mitchell et al., 2019). An abundance of *Alistipes, Bacteroides*, and *Bilophila*, all of which are highly tolerant to bile, is observed in such diets. (Forouhi et al., 2018)

### 2.3.3. LIFESTYLE:

Exercise, alcohol consumption, smoking, stress and sleep patterns are non-nutritional components of an individual's lifestyle that play a vital role in shaping the gut microbial community (Conlon et al., 2015). Long term physical activity positively impacts gut microbial population and regulates host energy levels. GIT's permeability, fecal transient duration as well as redox and inflammatory responses can be regulated by exercise (Sohail et al., 2019). Athletes have a higher diversity and have an advantage for growth lactate utilizing bacteria. High physical activity is associated with the abundance of beneficial bacterial species including *Prevotella, Akkermansia, Veillonella* (Marttinen et al., 2020). Animal models have been assessed for identifying predominant species in gut associated with exercise. *Bifidobacterium* and *Lactobacilli* species are positively corelated with exercise along with a higher *Bacteroidetes* to *Firmicute* ratio (Sohail et al., 2019). Sleep is physiological process essential not only for metal recovery but also for maintance of other physiological process. Adequate sleep duration is positively associated with an increased Alpha diversity of gut microbiota (Grosicki et al., 2020). *Ruminococcus* and *Blautida* are in abundance in

individuals that have regular sleep along with *Verrucomicrobia* and *Lentisphaerae*(Anderson et al., 2017). A disturbed circadian rhythm is correlated with reduction in *Lactococcus, lactobacillus, Proteobacteria, Ruminococcus and Dorea*.and elevated population of *Fusobacteria, Paraprevotella and Fusobacterials* (Carasso et al., 2021). Alcohol abuse is consistent with gut microbiota dysbiosis with a higher representation of *Proteobacteria* and *Fusobacteria* in comparison to *Bacteroidetes* and *lactobacillus* species. Overuse not only promotes the growth of pathogenic genera but also diminishes gastric motility (Meroni et al., 2019). Immunosuppression and bacterial colonization as consequence of subjection to cigarette and bacteria in cigarette can significantly alter the gut microbial population. Biofilm formation by pathogenic bacteria is enhanced with exposure to smoke and a reduction in Ruminococcus and lactobacilli species in cecum (Huang, 2019). Immunosuppression and bacterial colonization as consequence of subjection to cigarette and bacteria in cigarette can significantly alter the gut microbial population. Biofilm formation by pathogenic bacteria is enhanced with exposure to smoke and a reduction in Ruminococcus and lactobacilli species in cecum (Huang, 2019). Divergence in diversity of gut microbial population is associated with the geographical location of individuals. People belonging to same ethnic group and geographical region tend to have similar gut microbial composition. Urban population has a low diversity as compared to rural inhabitants as consequence of higher exposure to preservatives and processed foods (Gupta et al., 2017).

### 2.3.4. MEDICATIONS:

Broad spectrum antibiotics have capability to orchestrate the human gut diversity. Long lasting effects of antibiotic administration have been observed as they exert a selective pressure in gut. An example of such selective pressure is vancomycin administered for Gram positive population has a negative impact on mutualist gram negatives (Becattini et al., 2016). Misuse of antibiotic have serious implication on gut microbiome as it not only manipulates the diversity but also results in development of antibiotic resistance in bacterial communities (Iizumi et al., 2017). Drugs including anti-psychotics, anti-inflammatory and antihistamines also have significant effects of gut community. Most of these commercially available drugs inhibit at least one species of beneficial gut bacteria thereby inducing gut dysbiosis (Schmidt et al., 2018). Probiotics and prebiotics positively impact gut microbiota by improving the gut health and overall immunity.

Gut associated diseases can be managed and treated using probiotics as they have both dietary and immunomodulatory capabilities (Seon-Kyun et al., 2019). They ability to enhance micronutrient absorption and elevate production of vitamins, folate and short chain fatty acids significantly improves the gut conditions. Gut infections such as *Clostridium difficile* infection have been treated with appropriate administration of probiotics (Hajela et al., 2015).

### 2.3.5. HOST IMMUNITY:

Host and indigenous microbiota exhibit a symbiotic association where these commensal bacteria contribute significantly to development and regulation of host immune system. Symbiotic microbiota acts a host defense barrier by competing with pathogenic microbes(Wang et al., 2018). SCFAs are major product of bacterial carbohydrate fermentation which are important growth regulators of hematopoietic and non-hematopoietic cells (Rooks et al., 2016). Adaptive immunity retains the memory of complex cluster of commensal microbiotas. Perhaps host's adaptive immune system can also influence gut microbial composition. In case of immune deficits gut microbiota responses are regulated to counteract the consequences (Zhang et al., 2015). Microbial metabolites can modulate the host's susceptibility in autoimmune disorders. A few gut microbes have ability to metabolize polyphenols which would otherwise reach the colon unabsorbed. The lightweight phenolic products have anti oxidative and anti-inflammatory properties and can sustain in blood for longer period (Sarrías et al., 2017).

## 2.4. Dysbiosis:

Contribution of resident gut microflora contributes to development and maintance of human health are on forefront. It's not only a pivotal regulator of host digestive system but also modules host immune system through its metabolites. Therefore, this cross talk between host and enteric microflora must be kept at homeostasis (Belizário et al., 2018). However, changes in host micro or macro environment can lead to an imbalance in gut microbial composition termed as "gut dysbiosis". Relative proportion of pathogenic and symbiotic microbiota is considered integral for disease development. Disruption in gut community occurs when pathobionts outnumber the symbionts leading to disturbance in normal enteric functions. Shifts in enteric microflora are consequence of a compels interplay of multiple factors including change in dietary patterns, alcohol abuse, misuse of antibiotics, smoking, disturbed circadian cycle etc. (Dixit et al., 2021).

Dysbiosis is powerful inducer of metabolic ailments, allergies as well as mental disorders by reduction in diversity or by causing elevation in pathogenic population (Carding et al., 2015).

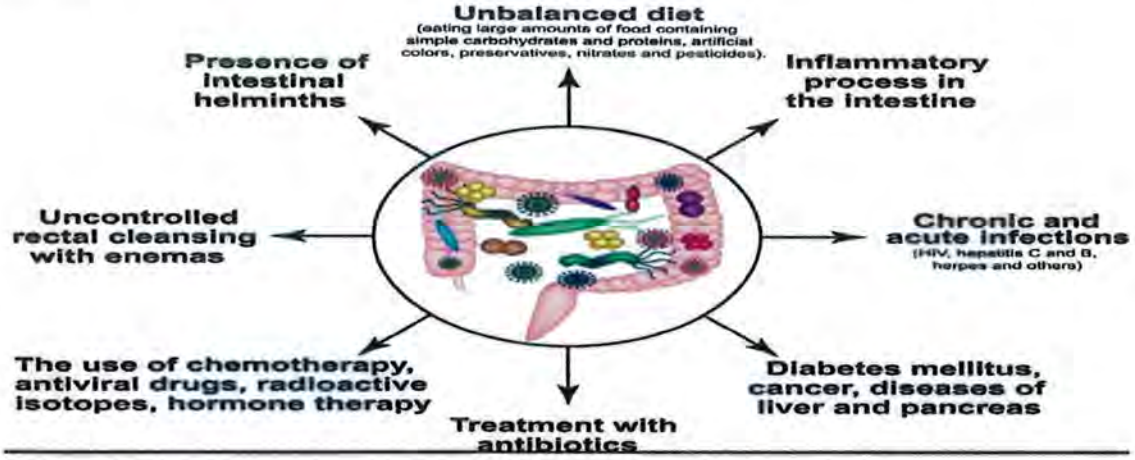


*Figure 3:Factors leading to dysbiosis.*

## 2.5. GUT BRAIN AXIS

Gut hormones and metabolites established a communication pathway between gut and brain via circulatory system. Besides an extensive set of neural networks is present in gut known as enteric nervous system thus there exists a pivotal influence of gut on nervous system. "Gut-brain axis" is a term that represents a bidirectional interaction between enteric system and central nervous system via both endocrinal and neural pathways (Romijn et al., 2008). The gut neuronal system is somewhat autonomous division comprising of neuroglial circuits that can regulate motor functions, transport and secretion in mucosa and localized blood flow together with immunomodulation. A significant number of enteric nerve cells of myenteric eric plexus are engaged in motor activity together with sensory neurons contained in submucosal plexus (Costa et al., 2000). Conferring to the specific functional parameter associated, each plexus constitutes a unique set of nerves cells. Spinal thoracolumbar, spinal lumbosacral, HPA axis and vagal nerve a pathway are essential communicators between enteric system and nervous system (Furness et al., 2014). The central nervous system behaves mostly as a reception center in bidirectional communication as most of vagal nerve fibers are afferent in nature. Thus, establishing the fact that Enteric nervous system holds a capacity to modulate neural responses (Rao et al., 2016).

### 2.5.1. GUT-MICROBIOTA BRAIN AXIS:

Enteric system is considered as third division of central nervous system and sometimes referred as second brain primarily because of modulation that gut regulates though an extensive supply of neurons and endocrine cells located in it. Microflora that inhabits gut possess an ability to manipulate the behavioral patterns and physiological activities of the central nervous system. these modulations are orchestrated metabolites such as short-chain fatty acids, neurotransmitters, or precursors of neurotransmitters produced by gut microbiota (Wang et al., 2016). A dysbiosis in gut is usually associated with alteration in brain function leading to a profound change in an individual's mood and cognitive patterns. A plethora of evidence have suggested a comorbidity between enteric dysfunction psychological disorders (Arneth, 2018). Disturbance in gut microbiome can trigger or enhance the development of synucleinopathies. Researchers have that gut microbiota-derived SCFAs directly influence depression-associated neuronal dynamics. Their administration was positively correlated with a reduction in depressive behavior. Human eating patterns and appetite can be regulated by the alteration in gut microbiome through the Delta cells. By dint of their endocrinal role, they produce home glucagon-like peptide-1 (GLP-1) which was downregulated in dysbiosis gut (Morais et al., 2021). Identification of imbalance in gut-associated with a neurogenerative or psychiatric disorder can make gut microflora a powerful tool in managing such disorders.

## 2.6. GUT MICROBIOTA AS REGULATOR OF BRAIN HEALTH:

Investigations have consistently proved that gut microflora are powerful regulators of human brain health. Members of resident flora have been established as indicators of health and illness and play a vital role in development of human brain. Mainly because commensal bacterial population in gut releases a large array of metabolite which can act as immunomodulators. Immune system is an important regulator of gut brain link which can be simulated by signaling molecule released by gut bacteria. These molecules include cytokines such as interleukin-1β which has a proinflammatory property which either influence the brain via blood brain barrier (BBB) or are transported through vagal nerve (Sherwin et al., 2016). Different gut bacterial species have been reported to impact a certain type of neuronal clusters such *lactobacillus* species demonstrated the ability to influence the nerve cells in hippocampal region of CNS. Another gut bacteria called as *Lactococcus lactis* was found as an auditory brain nerve cell modulator (Mohajeri et al., 2018).The beneficial

impact of microbiota on brain lead to the upgradation of concept of "prebiotics" (live bacteria that have beneficial impact on gut microflora) to "pshycobiotics" which are prebiotics that impart a advantageous impact on both gut and brain in individuals suffering from psychotic ailments (Zagórska et al., 2020). Recent investigation has revelated a huge pshycobiotics potential in *Bifidobacterium*, *Streptococcus* and *Lactobacillus* species. *Lactobacillus caesi* was reported to elevate the mood in elderly and *Bifidobacterium longum* was associated with improved cognitive activity and reduction in stress level (Zagórska, 2020).

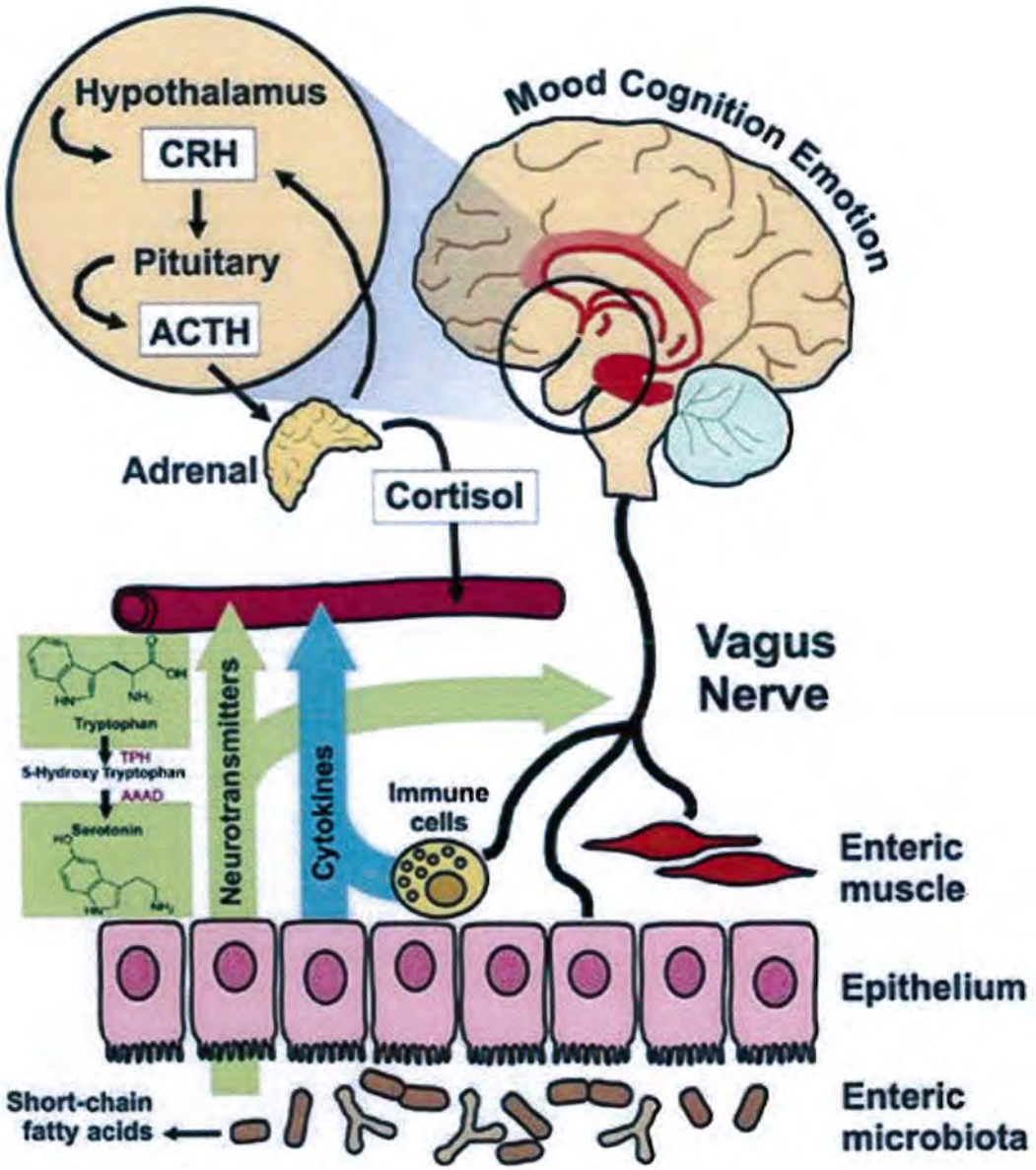


***Figure 4:*** *Mechanisms by which gut microbiota regulates host's cognitive health.(Grenham et al., 2011)*

## 2.7. THE SHIFT IN MICROBIOTA AND GUT DYSBIOSIS:

An increase in the relative abundance of morbific bacteria to beneficent bacteria lead to development of dysbiosis in gut. There by altering the bidirectional communication between enteric system and central nervous system. Multiple studies on germ free mice have reported an elevated stress level in comparison to one with normal flora. Such an HPA stress was ascribed to a reduced level of brain derived neurotrophic factor (BDNF) in both cortex and hippocampus. *Bifidobacterium infantis* was considered effective in alleviating the stress Via HPA (Tognini, 2017). Neurodegenerative disorders are becoming a huge burden to public health Alzheimer's and Parkinson's being the majors. Studies have discovered elevated expression of *Escherichia* and *shigella* generally associated with gut inflammation and accumulation of amyloid in brain of neurodegenerative patients. AD has also been reported to be consistent with profusion of *Bacteroidetes* and a reduction in *Bifidobacterium* and *Firmicute (Rinninella et al., 2019)*. Neuroinflammation in PD is associated with an overabundance of genus Catabacter and Akkermanisa which have ability to degrade mucus. A significant reduction in *Fecalibacterium* and *Roseburia* linked to a decreased level of short chain fatty acids was detected was observed in PD (Nishiwaki et al., 2020). Emotional fluctuations and psychological disorders can also be a consequence of an imbalanced microflora. Depression stands as one of the major psychiatric disorders worldwide. Human studies have indicated an altered gut microbiota composition in fecal material in Major depressive disorder (MDD) (Cheung et al., 2019). Multiple reports have suggested a lower representation of *Bifidobacterium* and an over expression of *Prevotella* and *Bacteriodetes* in MDD. *Bifidobacterium* has an anti-inflammatory role and carries a gene for GABA production therefore reduction in its level can lead to progression of MDD (Guo et al., 2019). Autism spectrum disorder (ASD) is a collection behavioral and neurological disturbances which has been linked with gut dysbiosis. A study revealed found elevated level of neurotoxin associated with a higher abundance of *clostridium* promoting regressive ASD. ASD was also corelated with an over expression of *Bacteroidetes* and *Desulfovibrio* consistent with the fact that they cause inflammation of GI tract. Commensal communities with probiotic properties were found to be reduced in such individuals. Thus, gut dysbiosis was found to a contributor to mental and neurogenerative disorder and reestablishing the resident flora a can ease the symptoms and severity of disorders (Hughes et al., 2018).

## 2.8. DIET AND GUT MICROBIOME:

Interplay of diverse dietary components can play a vital role in shaping resident gut community. Diet can modulate both functional and compositional changes in microflora and metabolites that they in gut (Leeming et al., 2019). The dietary components are precursor molecules for gut microbial population that are necessary to maintain a healthy gut. Therefore, Changes in dietary patterns exerts selective pressure on gut microbial community, rapidly excluding taxonomic units of microbiota (Heiman et al., 2016). The differences in the makeup of the intestinal microbiota between vegans and omnivores have been extensively studied. Researchers have compared \ the microbiota of omnivores, vegetarians and vegans revealing a somewhat different diet-induced microbiome (Tomova et al., 2019). Dietary patterns are usually associated with culture and geographical regions therefore individuals residing similar location or belonging to same culture have similar dietary patterns and relatively like microbial diversity in comparison to other groups (Chen et al., 2014).

### 2.8.1. INFLUENCE OF DIETARY PATTERNS ON GUT MICROBIOME AND GUT BRAIN COMMUNICATION:

Dietary habits have a profound impact on gut associated microbiome as taxonomically diverse bacterial population utilizes different type of substrates. Inclination toward a specific nutritional routine will provide a limited type or quantity of dietary components there by promoting growth of a set of microbiotas that can metabolize them (Graf et al., 2015). In general, dietary patterns are classified as either as vegetarian diet and omnivorous diet with only distinction that one involves consumption of meat and other doesn't (Matijašić et al., 2014). Microbiota metabolize the dietary constituents producing molecules as by product that are precursors of neuroactive compounds or themselves have neuro-modulatory function.

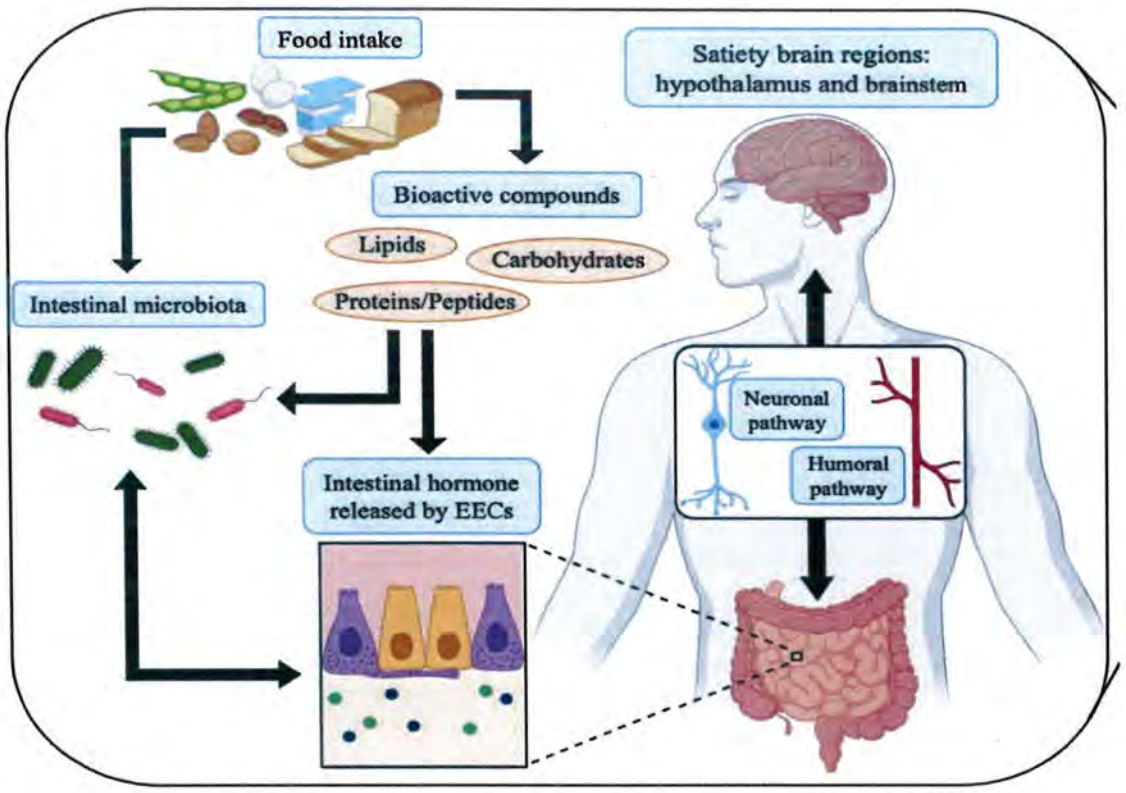


***Figure 5:****Role of Diet in maintaining healthy Gut-Microbiota-Brain Axis (Pizarroso et al., 2021)*

### 2.8.2. INFLUENCE OF CARBOHYDRATE ON GUT MICROBIOME:

A major fraction of dietary intake consists of carbohydrates that influence the taxonomic composition as well as metabolic process of gut microflora. Digestible and indigestible carbs make up most dietary carbohydrates. Enzymes degrade digestible carbs to provide us with energy (Rasnik K Singh et al., 2017). Microbial population of colon is dominated by polysaccharide-degrading bacteria that produce SCFAs utilizing starch, glycogen and fibers. *Bifidobacterium spp., Prevotella spp., Akkermansia muciniphila, Clostridium spp., and Bacteroidetes* have all increased in abundance in diets with high carb content (Sonnenburg et al., 2016). Carbohydrates present in plants such as hemicelluloses are examples of resistant carbohydrate compounds. *Bacteroides sp. or Ruminococcus sp.* are involved in the degradation of cellulose, which leads in the synthesis of SCFAs (Rajilić-Stojanović et al., 2013). Simple sugars or artificial sweeteners in the form of carbs increased risk of glucose intolerance due to an increase

in *Bacteroides* genus and *Clostridiales* order bacterial population (Suez et al., 2014). Glucose reduction can be directly sensed by brain nerve cells which is positive corelated with mental obstruction and low willpower in adults (Marty et al., 2007). Diets high in sugar content have been reported to impair the chemo sensing activity of brain. Furthermore such diets can cause pathological changes in brain circuits involved in reward related behaviors (Lennerz et al., 2018). Even though carbs are not considered necessary for humans but the balance between simple sugars and non-digestible carbohydrates appear to have a vital role in maintaining good physical and mental health (Ezra-Nevo et al., 2020).

### 2.8.3. INFLUENCE OF PROTEIN ON GUT MICROBIOME:

The exogenous proteins which governed by the dietary habits based on kind and quantity of protein intake has been a major contributor to microflora development (Hayes, 2018). The impact of protein in shaping gut microbiome is associated to the extent it is available in intestinal region as it is partly metabolized but not absorbed in prior gut regions (Ma et al., 2019). Typically, on an average 6-18gr protein reaches the colon on daily however it might differ based on individual or cultural eating habits (Yao et al., 2016). When it comes to protein absorption in the large intestine, plant-based vs animal-based diets have a significant impact on the amount that is absorbed depending on the overall quantity of protein consumed. Protein consumption was found to have a substantial correlation with the degree of muscle mass (Wielen et al., 2017). Protein fermenters residing in gut not only ferment it but also utilize the nitrogenous end products as precursors of their metabolites. Protein excess, even highly digestible animal proteins, may saturate digestive capacity, increasing protein putrefaction (Peled et al., 2021). moreover, reduced digestive efficiency increases protein substrate availability for bacterial breakdown in the large intestine. Toxic nitrogenous and sulphurous compounds are produced during protein fermentation by gut bacteria (O'keefe et al., 2016). A meat-based diet has been linked to higher *Alistipes, Bacteriodetes and Bilophila* whereas decreased population *Bifidobacterium adolescentis* due to high protein content (R. K. Singh et al., 2017). A wide variety of gut bacteria may ferment amino acids to varying degrees. Most the gut's proteolytic species are members of the *Bacteroides* genus in addition Microbial species Propionibacterium, Streptococcus, Fusobacterium, Lactobacillus, and Clostridium

were documented for their ability in the proteolysis of food products. Thus, a high protein diet results in profusion of these species (Yadav et al., 2018).

### 2.8.4. INFLUENCE OF FAT AND LIPIDS ON GUT MICROBIOME:

A large portion of our daily calorie intake comes from fat which can be both plants based including olive oil, flex seeds etc. and animal based including dairy and meat products (Leone et al., 2015). High fat plant-based diet including low starch food items have been associated with health by improving producing metabolites neuroprotective in nature (Ludwig, 2019). furthermore, scientific investigations have shown that ω-3 fatty acids help preventing excess weight and inflammation by influencing the bacteria in the intestines (Kaliannan et al., 2016). Studies in animal models have revealed that gut microbial composition was changed in stressed mice by supplementation with eicosapentyl/docosahexyl intervention (EPA/DHA). Showing that n-3 polyunsaturated fats and gut microorganisms may play a significant role in expanding our knowledge of diseases of mood and cognitive performance, such as clinical depression (Pusceddu et al., 2015). The health benefits of some fats outweigh the risks, but this is not true for all fats. People's health and illness status is not affected by how much fat they consume, but rather by the quality of the fats (Nettleton et al., 2017). High saturated fat intake often derived from fried and processed meat or dairy food products has been associated with elevated LDL also called as bad cholesterol is positively corelated with vascular disease and cognitive decline, among other things (Zimmerman et al., 2021). A higher level of *Actinobacteria* and *Bacteroides* were related with a longer-term high-fat diet, whereas lower levels of Firmicutes and Proteobacteria were associated with a lower diet (Wu et al., 2011). *Enterobacteriaceae*, a kind of Gram-negative bacteria, can be promoted by high-fat meals to raise intestine LPS levels. High level of LPS contributes to intestinal dysbiosis, which results in decreased protection of the gut barrier and an increased localized inflammation response (Barber et al., 2021).

## 2.9. SHORT-CHAIN FATTY ACIDS

The holobiont subsequently formed by complex cross communication between resident enteric microflora and human produces vast variety of biomolecules that have ability to regulate host physiology (Gallausiaux et al., 2021). Gut bacteria involved in metabolism of non-digestible dietary fibers are of particular importance in gut due their ability to produce a range of products that play a dynamic role in host health. Short

chain fatty acids (SCFAs) are bioactive end products of bacterial fermentation consisting of carbon chain of up to 6 carbon atoms. Several receptors have been recognized to bind SCFAs throughout entire human body indicating their role in regulation multiple human activities. SCFAs regulate homeostatic balance in intestinal regions by three well studied G-protein coupled receptors (GCPRs). However, the exact function associated on various cell types still fully characterized (van der Hee et al., 2021). Substrate type, source, chemical structure, amount, solubility, molecular weight along with micro-environmental condition, composition of microflora and transit time of intestine are agents that influence concentrations of SCFAs in gut (García et al., 2013).

### 2.9.1. BENEFITS OF SHORT CHAIN FATTY ACIDS:

Various health benefits are assorted with SCFAs as they take part in intestinal integrity furthermore, they also possess anti-inflammatory properties. Moreover, SCFAs that cross blood brain barriers can impact positively cognitive and behavioral patterns of individuals (Silva et al., 2020). It is estimated that about 60% to 70% of the energy demand for colonic epithelial cells is fulfilled by SCFAs. Butyrate acts as the primary source of power followed by propionate and the acetate (Brahe et al., 2013). Butyrate is principal regulator of homeostasis and integrity at colonic and intestinal epithelial barrier. Increasing evidence are indicating SCAFs as potential preventive and therapeutic agents for metabolic disorders (Kasubuchi et al., 2015). Animal studies have reported suppression in diet induced obesity and protection from developing resistance towards insulin by administration of butyrate, propionate and acetate (Lin et al., 2012). "Oxidative eustress" is an expression for advantages attributed to redox signaling. In vitro studies have suggested protective role of SCFAs by Nrf2 signaling to reduce oxidative stress on blood brain barrier (Bosch et al., 2021). Butyric acid possesses an anti-inflammatory role due to ability to upregulate production of prostaglandin E2 (PGE2). Inflammation can be reduced production of PGE2 which downregulates release of TNFα and IL-1β by the macrophage and lowers signaling by T cell receptors (Allaband et al., 2019).

The beneficial role attributed SCFAs has made SCFAs producers as the potential probiotic organisms. *Bifidobacterium* are when used as probiotics puts a constrain on growth of enteric pathogens by production of acetate (Fukuda et al., 2011).

Due to propionate's ability to block the TLRspecific pathway, it's possible that this me tabolite might shield the BBB from the harmful effects of Gram-negative bacteria LPS (Hoyles et al., 2018).

### 2.9.2. ROLE OF SHORT CHAIN FATTY ACID IN MAINTAIN BRAIN HEALTH:

Short chain fatty acids (SCFAs) play a significant role in modulation of gut brain axis. A diverse microbial population resides in gut which has demonstrated an ability to regulate the crosstalk between enteric system and central nervous system. Lack of bacterial colonizing in gut is consistent with alteration in blood brain barrier thereby influencing neurological activities. Gut dysbiosis has been associated with development of neurodegenerative and neuropsychiatric ailments and SCFAs have been observed to alleviate the symptoms in such disorders (Cenit et al., 2017). Presence butyrate producers are correlated shown to enhance learning associated regions of brain, moreover they possess an ability to promote ontogenesis of nerve cell (Matt et al., 2018). Apart from neurogenesis, Cerebral edema associated with cerebral ischemic stroke was relieved by increasing butyrate production.it was also observed to decrease cerebral infraction in rat models rendering commensal butyrate producer a possible therapeutic intervention (R. Chen et al., 2019). Numerous mental health issues have been related to inflammation and an enhanced immunological response, as seen by raised cytokine concentrations. SCFAs can upregulate serotonin from enterochromaffin cells (EC) which can uplift the mood and help in patients with MDD (Meyyappan et al., 2020). Evidence suggests a key role of propionate in reducing gut permeability along with having anti-inflammatory properties. As a result of the direct stimulation of enteric–CNS connections, propionate has been associated to reduced stress behaviors and enhanced reward system activity along with promoting glucogenic pathway in gut (Hoyles et al., 2018).

### 2.9.3. PRODUCTION OF SHORT CHAIN FATTY ACID IN GUT:

Bacteria dwelling in colon are majorly responsible for synthesis of short chain fatty acids. Two molecules of acetyl-CoA are combined, to synthesis acetoacetyl-CoA, which is then reduced to butyryl-CoA via glycolysis. To complete butyrate synthesis, two distinct routes have been discovered. One of which involves butyryl-CoA and acetate CoA transferase whereas the other pathway involves phosphatidylinositol 3-

transferase and butyrate kinase. Dietary fibers can undergo two different pathways to produce acetate. Either it can be synthesized using pyruvate molecules utilizing acetyl CoA or via Wood Ljungdahl pathway which split two ways to create acetate. It can produce acetate either by reduction of formate by carbon dioxide or by producing carbon monoxide pathway resulting from reduction of carbon monoxide (Mirzaei et al., 2022). Three distinct pathways are utilized by gut bacteria to produce propionate. Using succinate pathway under the action of enzyme methylmalonyl-CoA. Acryloyl-CoA activity on lactate via acrylate pathway also results in production of propionate. Propanediol pathway metabolizes fucose and lactate via two separate pathways (Reichardt et al., 2014).

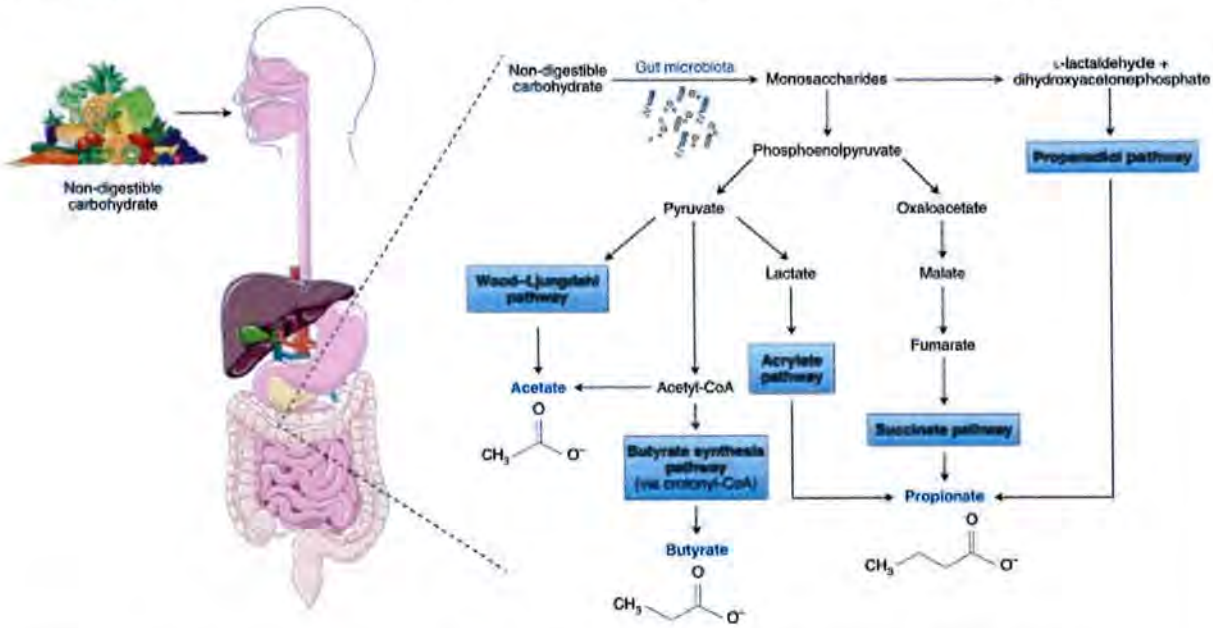


***Figure 6:*** *Pathways for production of SCFAs non-digestible carbohydrates in gut. (Frampton et al., 2020; Ríos-Covián et al., 2016)*

### 2.9.4. MICROFLORA PRODUCING SHORT CHAIN FATTY ACIDS:

Enteric microflora consists of numerous bacteria with ability to synthesize SCFAs. *Faecalibaterium prausnitzii* belonging *Ruminococcaceae,* many *Eubacterium strains* and few *Roseburia* members are obligate anaerobes with ability to produce butyrate through butyryl-CoA and acetate CoA transferase pathway. In existence of riboflavin along reduced level of oxygen enhances the growth of bacteria (Morrison et al., 2016). Hemicellulose and starch are major precursors utilized butyrate producing bacteria however it has been observed that utilization of pectin compound along with inulin derivatives is also common. Furthermore, some firmicute species such as *Coprococcus*

*eutactus, Subdoligranulum variabile* follow a somewhat different pathway utilizing butyrate kinase to produce butyrate (Zhernakova et al., 2016). Succinate pathway is carried out two of Bacteroidetes species namely *Bacteroidetes thetaiotaomicron, Bacteroidetes vulgatus* to synthesize propinoate while Acrylate pathway is reported to be utilized by *Coprococcus catus* (van der Hee et al., 2021). *Akkermansia muciniphila* along with three other enteric species namely *Prevotella, Ruminococcus* and *Bifidobacterium* through acetyl-CoA pathway utilizing pyruvate (Nogal et al., 2021). Wood Ljungdahl pathway is carried ut by *Clostridium* and *Streptococcus* species along with *Blautia hydrogenotrophica* to synthesize acetate in gut (Koh et al., 2016).

## 2.10. SEROTONIN

Serotonin is an inhibitory neurotransmitter produced from both CNS and enteric system also present in smooth muscles and platelets. Serotonin was named after its first source "serum" and is activity to modulate vasoconstriction. Chemical structure of serotonin was laid out in 1950 and was called as 5-hydroxy tryptamine (Shad, 2017). The Serotonin plays a profound role in modulation of sinoatrial and atrioventricular node thereby controlling the heart rate {Ayala, 2019) The Serotonin plays a profound role in modulation of sinoatrial and atrioventricular node thereby controlling the heart rate (Ayala, 2019). 5-HT receptors are found in majority of bone cells including osteocytes, bone marrow stem cells etc. and activation of them regulates the actions of bone cell. 5-HT signaling in bone cells has been tested in vitro with inconsistent results reported. It has been suggested that 5-HT stimulates bone production pathways directly, whereas others have discovered that 5-HT inhibits bone development (Bliziotes, 2010). Due to their close vicinity in the digestive tract, enterochromaffin cells and immune cells might influence each other's activity. Serotonin uptake and 5-HT receptor expression in these cells show that 5-HT has an important function in developing innate immunity (Shajib et al., 2015). Research have reported Endocrine enterochromaffin cells exude serotonin that activates mucosal nerve ends of the intrinsic primary afferent neurons of the enteric nervous system, which coordinates the secretory and motor processes of the digestive system. Moreover, serotonin stimulates the extrinsic primary afferent neurons in the brainstem that project to the vomiting center to release their vagal afferent nerve end (Keszthelyi et al., 2009).

### 2.10.1. PRODUCTION OF SEROTONIN IN GUT

Fetus receives serotonin at early stages from maternal blood which found to play avital role neurological development. Peripheral serotonin contributes to 90% serotonin existing in human body where only 1% of it is synthesized by mesenteric nerve cell and rest is released by enterochromaffin cells (EC). Biosynthesis of 5-HT is carried out in human gut from hydroxylation of a precursor known as tryptophan. Tryptophan hydrolase 1 (TPH1) in EC cells along with tryptophane hydrolase 2 (TPH2) in mesenteric nerve cells are gut produced enzymes that carry out hydroxylation (Margolis et al., 2016).

### 2.10.2. SEROTONIN AND GUT BRAIN AXIS:

Early life serotonin production regulates development and mitigation of brain cell. However, it attains a diverse role afterwards as neurotransmitter thereby modulating maturation of neural circuits of CNS responsible for circadian rhythm, concentration and behavioral patterns (Goeden et al., 2016). Increase in serotonin uptake was reported to positively associated with behavioral modulation especially motivation (Meyniel et al., 2016). An enterochromaffin cell releases serotonin into the bloodstream in response to gastrointestinal or pharmacological stimulus, resulting in 5-HT3 receptors on the vagal afferent terminal to be activated. GI vagal afferent neurons have 5-HT3 receptors on their soma that can be triggered by 5-HT circulated throughout the body. Additionally, 5-HT3 receptors may be found in the central terminals of vagal afferents, which boost glutamatergic synaptic transmission to second-order neurons in the brainstem's nucleus tractus solitarius. Thus, it appears that interactions between the vagal nerve and serotonin systems in the gastrointestinal tract and the brain play a significant role in the treatment of mental illnesses (Breit et al., 2018).

### 2.10.3. MICROBIAL MODULATION OF SEROTONIN SYNTHESIS:

An extensive portion peripheral serotonin is product by EC cells. However extensive number of animal and human studies have demonstrated role of resident gut bacteria in modulation serotonin levels by a metabolite-cell component dependent pathway (Yano et al., 2015). It has been suggested that the generation of serotonin regulates the gut permeability, and its production is influenced by microbiota-derived metabolites, such as bile acids and short-chain fatty acids (Ge et al., 2018). There are various distinct kinds of 5-HT and SCFA receptors that may be expressed by EC cells, allowing them

to directly sense diverse microbe-derived compounds. Serotonin synthesis and secretion in EC cells may be induced by the SCFAs (Xie et al., 2020). In contrast, commensal bacteria may utilize luminal tryptophan directly to produce serotonin. The expression of tryptophan synthetase has been found to be sufficient to induce the production of serotonin in several bacteria belonging to the genera, *Lactobacillus, Klebsiella Streptococcus, Lactococcus, and Escherichia coli* (O'Mahony et al., 2015).

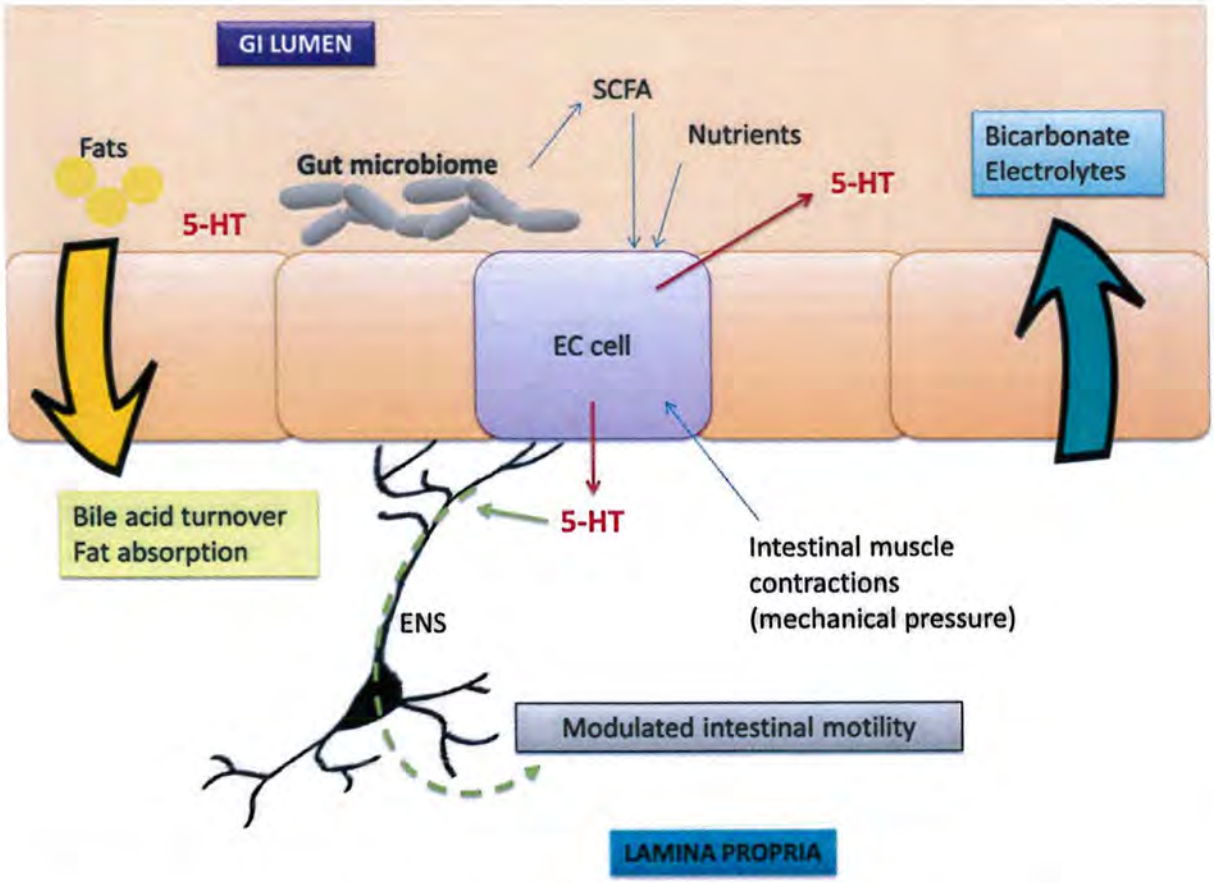


***Figure** 7:Modulation of 5-HT by gut microbiota and its impact on gut motility. (Martin et al., 2017)*

### 2.10.4. GUT DYSBIOSIS, SEROTONIN AND PSYCHOLOGICAL DISORDERS:

Numerous mental and neurological conditions have been linked to a dysfunction in a key component of the serotonin production system, which regulates nearly every aspect of brain activity. Nearly for a decade research are focused on pinpoint role of serotonin in initiation and progression of neuro-psychiatric disorders. Serotonin as assumed a role of a stress regulator via Hypothalmic-pitutary axis which considered important in

neuropathy related to stress disorders (Brummelte et al., 2017). Many gastrointestinal and extraintestinal illnesses, including those of the neurological system, can be caused by dysbiosis, which is caused when the resident microbiota is disrupted (Lange et al., 2016). High level of peripheral serotonin is associated with increased gut permeability causing subsequent release of microbial metabolites and inflammatory cytokines to enter blood. Anxiety, despair, and memory loss may result from their release (Szőke et al., 2020). Stress is potential agent that causes depression by damaging pathways for serotonin production in gut. Moreover, it disturbs circadian rhythm thereby impacting mood regulating brain sections leading to development of anxiety (Daut et al., 2019). Down regulation of TPH 1 is associated with obesity, gastric inflammation, cancer and diabetes plummeting the enteric serotonin synthesis. As a result, decreasing levels of serotonin in the host may contribute to the development of mental disturbances (Matthes et al., 2018).

## 2.11. EEG (ELECTROENCEPHALOGRAPHY):

EEG is considered to be a window to the mind. It involves the recoding of the electrical activities or the changes in the electrical potential in brain by placing electrodes on the scalp. (Nunez and Srinivasan, 2006) Hans Berger was the first one to discover the phenomena to study the neurological activities in 1970. Before this there was no other method to study the complex activities in the brain. Since then, it has grasped the interest of various scientists and engineers. (Shipton, 1975). Anatomically the brain has been divided in three major portions i.e. The cerebrum, Cerebellum and the brain stem. Cerebrum has lateral hemisphere with a complex surficial layer known as cerebral cortex. This Cortex plays key role in the activities in the central nervous system. Cerebrum coordinates the initiative of movement, sensation made consciously, switching in the mood, emotions or behavior. Where cerebrum is responsible for all the voluntary movements and balancing body. Brain stem controls the heart regulations respiration etc. (Abiri et al., 2019) The electrical signal in the cerebral activity has a dominant impact on EEG due to its position. The active neurons generate electrical signals. EEG records the electrical activity through cortical surface when the local neurons are excited. The flow of current is majorly observed while synaptic excitation. The electric potential generated is due to the creation of electrical dipole during the synaptic and postsynaptic activities between soma and the apical dendrites. The electrical signals are usually generated by the movement of ions (Na+. K+, Ca+ and Cl-

) across the cell membrane and are being recorded in the form of synapses in EEG. EEG provides an impressive opportunity to study the normal and abnormal brain activity quantitatively. (Lotte et al., 2018)

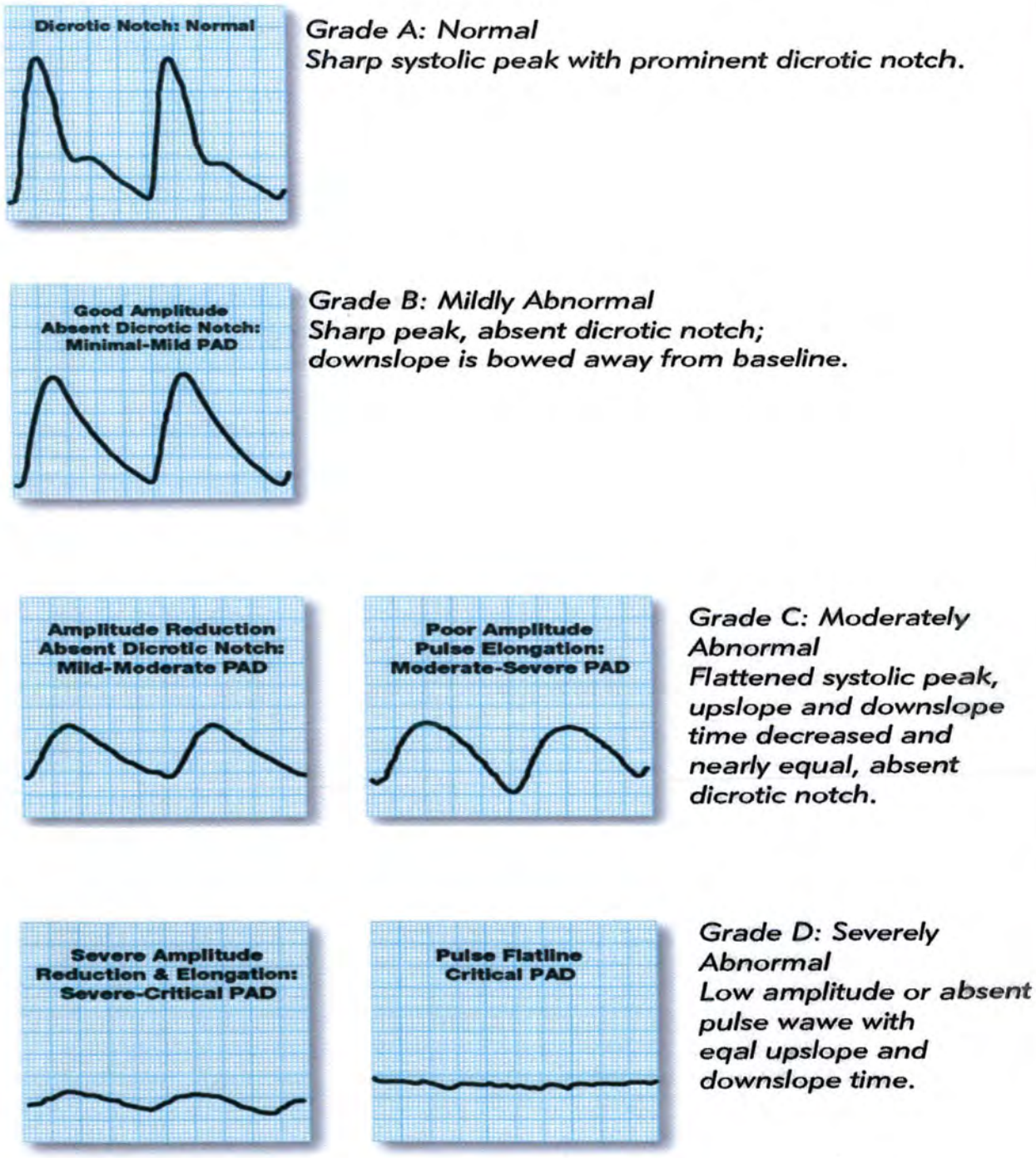


***Figure 8:*** *Normal and abnormal characteristics of wave forms generated from EEG.(Davies, 2014 #230)*

### 2.11.1. WAVE FORMS IN EEG:

The EEG is recorded in the form of wave. The waveform can be classified on the basis of location, frequency, morphology, symmetry and reactivity. However, the frequency is the most frequent variable to characterize EEG by applying Greek numerals, Delta; theta; alpha sigma; and beta. Whereas there are certain wave forms such as infra slow oscillations and high frequency oscillations are also clinically important. (Hosseini, Hosseini and Ahi, 2021)

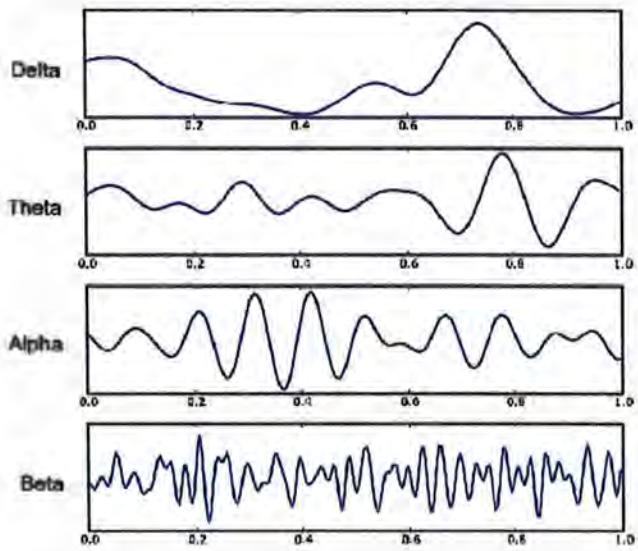


***Figure 9:*** *Types of wave form generated by brain neural activity. (Anwar, 2018 #229)*

### 2.11.2. MUSE 2

It is a portable EEG headband brain sensor device. MUSE is the brain sensing headband, is an electroencephalography (EEG) technology. EEG is considered to be the most effective and non-injurious technique for documenting the brain activity. MUSE is a digital enterprise for analyzing the thoughts. Typically, the analysis of the thinking process is being done by using index cards, sketch books or by CAD diagrams. MUSE 2 provides a digital workspace for the analysis of the brain activity. MUSE is widely used by the clinical researchers in the domain of neurological sciences to study brain health, abnormal and normal brain activities. (2022) Muse electrode placement id based on 10-20 EEG system where two electrodes are present in frontal and two are located on temporoparietal region with a reference sensor Fpz.

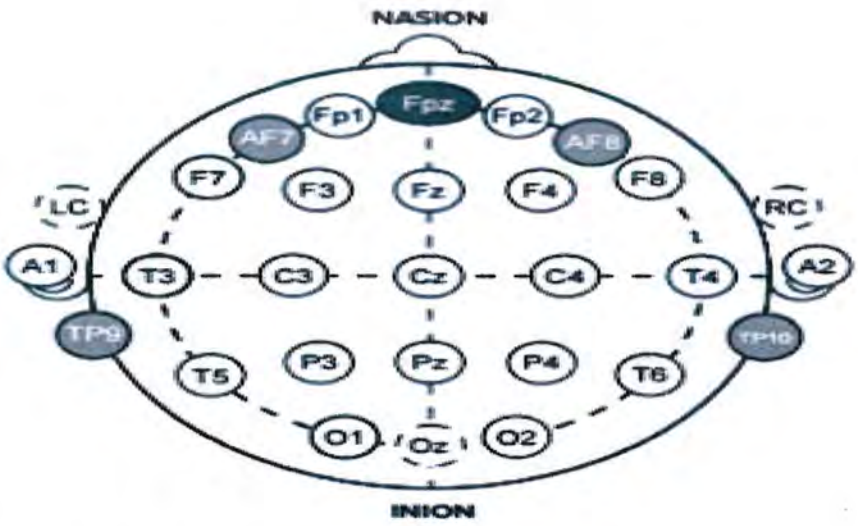


***Figure 10:*** *Electrode placement of MUSE 2.(Teo, 2018 #228)*

### 2.11.3. TECHNICAL SPECIFICATIONS

It is a flexible and easy to use device with two channels on either side which explore the lateral hemisphere of the brain. The Muse 2 device is being tested with the Brain Vision ACTi CHAMP system and g.TEC g. USBamp system. Muse 2 head band consist of five Forehead sensors (1), 5 LEDs (2), power button(3), Charging ports (4), two smart sense conductive rubber ear sensor (5). (2022). (2022).

# Chapter 3

# Material and Methods

# 3. MATERIALS AND METHODS

## 3.1. SELECTION OF HEALTHY SUBJECTS:

### 3.1.1. DEMOGRAPHIC DATA:

Demographic data acquired from questionnaire-based survey was used for subject recruitment in the study. A population set between age group of 18-55 was studied. Individuals with metabolic or gut disorder such as diabetes irritable bowel syndrome were excluded as well as individuals who have utilized antibiotics in past year also eliminated.

### 3.1.2. MENTAL HEALTH EVALUATION:

To assess the mental health of subjects a questionnaire was requested to be filled. Based on the scores individuals with high score were excluded as high score. A high score in Beck Depression Inventory version 6 was associated with depression therefore individuals with low were only selected. The questionnaire consists of twenty-nine multiple choice questions and specific scores are associated with each option provided. The total score of participants reflects the metal state whether depression level is high low or no depression at all(Beck et al., 1961).

### 3.1.3. DIETARY PATTERN ASSESSMENT:

Selected individuals were then asked to provide a thorough information on their daily eating patterns. The primary objective of the questionnaire was to compile a list of food and beverage item consumed in the region. Each category of food was listed separately fruits, grains, and meat. Frequency of certain food intake was also collected using same. Diet is major regulatory agent of human health therefore observing and assessing dietary pattern can revel plenty of information about their health condition. We may obtain a sense of a person's health state by looking at their food, which plays a vital role in developing their gut flora.

### 3.1.4. COLLECTION OF FECAL SAMPLES:

Fecal were collected from the subjects in separate collection container to avoid any contamination from external sources. Samples were transferred to glycerol vials than stored in nitrogen shipper. Glycerol acts a transfer medium and low temperature prevents the death of microbes as well as well as decrease in metabolites present in them. After wards samples were stored at -80°C. Microbiota and metabolite

composition in fecal samples reflects the gut health of an indiviual and is a noninvasive procedure for such assessments.

### 3.1.5. GUT MICROBIOLOGICAL ANALYSIS:

Aseptic conditions were maintained while processing fecal sample to avoid any contamination. Serial dilution of samples was performed, and 100 microliter of each dilution was spread on two different media i.e., De Man Rogosa and Sharpe agar (MRSA) and Eosin Methylene Blue agar (Tomova et al.) for gram-positive and gram-negative bacteria respectively. The inoculums were then incubated in anerobic incubation chamber for 72 hours at 37°C. Relative abundances these population play an important role in gut health and disease therefore it was along with evaluation of diversity in normal healthy individuals was conducted based on macroscopic and microscopic properties.

## 3.2. FECAL SHORT CHAIN FATTY ACID EXTRACTION:

### 3.2.1. CHEMICALS:

Chemicals utilized were propionic acid (99%), n-butyric acid (99%), acetic acid (100%) and n-valeric acid (99%). For internal standard, 2-Ethylbutyric acid (Sigma-Aldrich) is used. Standards employed for analysis were gas chromatographic grade and were used as collected. Formic acid (98-100%) and HCl (30%) of analytical grade was used. All processing was done using ultra-pure water.

### 3.2.2. PREPARATION OF STANDARDS' STOCK SOLUTION:

Internal standard stock solution was prepared by using 2-ethyl butyrate solution containing 12% formic acid.PH of solution was adjusted by use of 5% hydrochloric acid. Dilution was prepared for every acid to develop a standard curve.

All standard stock solution were stored at -20°C.

### 3.2.3. SAMPLES PREPARATION:

Samples previously stored at -80 °C were utilized for analysis. 500 µl ultra-purified water was used to suspend 100µl of stool sample. Suspended samples were than vortexed for 3minutesand than kept for 5 minutes at room temperature. Fecal suspension's pH was adjusted at 2-3 by addition of 50µl of 5mol/L hydrochloric acid solution. Samples were than centrifuged at 3698rpm and 4°C. then 10µl of internal standard solution was added to 90 µl supernatant. Prepared samples were than stored at -20°C for further analysis by GC-FID. For analysis 1µl was injected in GC.

## 3.3. SERUM SEROTONIN ANALYSIS:

### 3.3.1. Serum collection and processing:

Serum samples were collected and stored in plasma separator tube (PST) and were kept at room temperature for two hours. The samples were then poured into Eppendorf's and centrifuged at 1200 rpm for 10min. the blood cells settle down and serum is collected and stored at -80°C for further processing.

### 3.3.2. Serotonin analysis:

Quantification of serum serotonin level was conducted using Biovision Serotonin ELISA Kit. It is a competitive ELISA kit that used to detect serotonin levels in serum at 450nm absorbance. The procedure followed to conduct analysis was same as supplied with the kit.

## 3.4. Electroencephalography (EEG):

EEG of subjects was carried out on spot using a portable headband called MUSE 2 in a resting eyes closed state. It is supplied with four electrodes that measure the electrical signals in brain cortex through scalp. Mind monitor app was used to record EEG data which was then further assed using EEG. It allows to remove artifacts such as jaw clenches and blinks along with removal of bad channels and sections of EEG caused due to the noise. The data was referenced to an average reference and preprocessed using a basic FIR filter which removes any frequency above 40 or below 0.5 Hz as they correspond to movement. The power spectrum was then plotted at each frequency. Graphs were than analyzed to observe coherence between two corresponding regions of hemispheres as well as intensity of different wave forms in different regions of brain.

# Chapter 4

# Results

# 4. RESULTS

## 4.1 DIETARY PATTERN

### 4.1.1 DAIRY PRODUCTS:

There is a large proportion of the population that consumes dairy products. #% of population in Islamabad reported milk as part of their daily food intake followed by yogurt, ice-cream and butter at 60%, 35% and 27.5% respectively, whereas cheese consumption was lowest at 15%. In Sindh a 90% of population had a daily consumption of yogurt followed by homemade butter (60%) and Cheese (15%). However, no intake of ice-cream was reported by subjects. A large set of population (95%) had a daily intake of milk along with yogurt intake by 87% of population in Hunza. Cheese and ice-cream lowest consumed dairy foods. Foods made from milk and fermented milk products are good for the stomach because they include a wide range of nutrients along with Probiotic-Lactic Acid Bacteria, which offer several advantages for maintenance gut integrity.

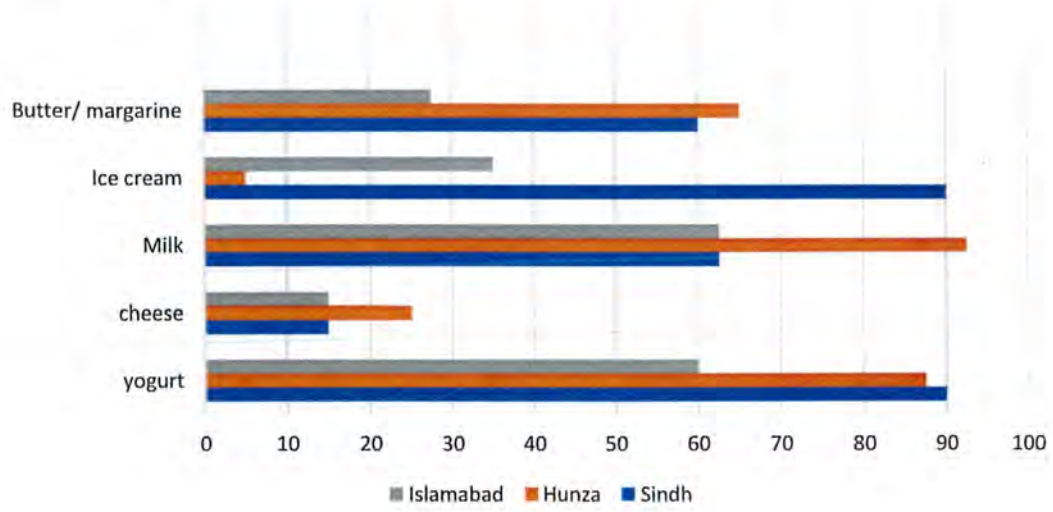


***Figure 11**:Graph showing variation in daily consumption of various dairy product in subjects.*

In comparison between three different populations a higher consumption of milk (95%) in Hunza whereas in Sindh a higher intake of yogurt (90%) was observed. Ice-cream was consumed by higher set of population in Islamabad as compared to other population.

### 4.1.1.1 FATS:

Fats either in form of animal or plant source whether its oil or ghee is commonly used among all population. However, subjects from Hunza set had highest consumption of animal-based fat 72% of individuals whereas a lower use of plant-based fat was observed on daily basis. In contrast fat utilization from vegetable source was 97% much higher than any other population in Islamabad.

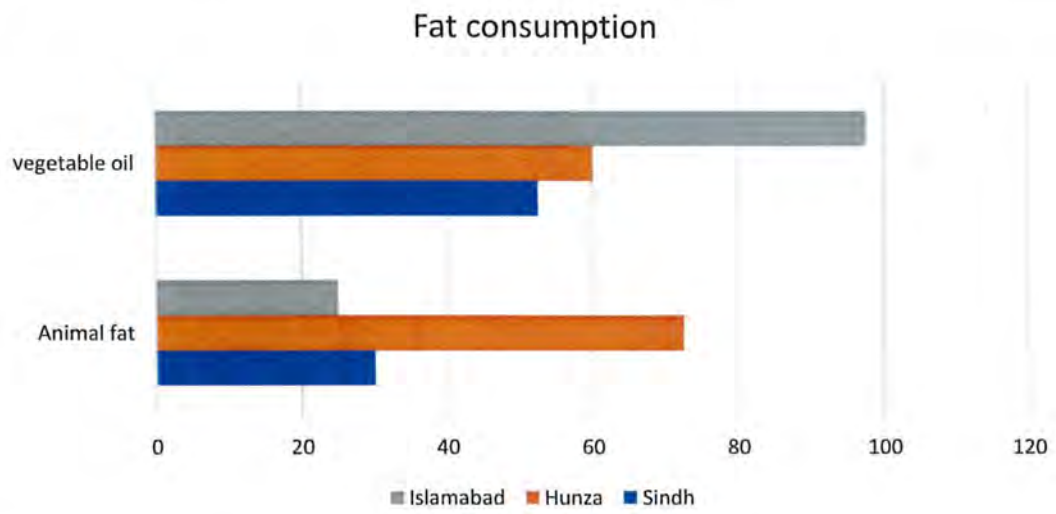


***Figure 12**:Graph showing variation in daily consumption of various Fat product in subjects.*

A very low daily animal fat consumption i.e., 25% and a little higher vegetable oil consumption was observed. Subjects from Sindh reported vegetable oil consumption higher than animal fats however its fat consumption was lowest among three groups. Types of fat consumed impact the gut epithelial permeability as well as provide specific precursor that can be utilized by gut microbiota to produce neuroactive active compounds that play major role in cognitive development and maintance.

### 4.1.1.2 CEREALS:

Daily consumption of cereals in the wheat was reported by all the subjects. Followed by rice at 65%,62% and 37% in Islamabad, Hunza and Sindh respectively. Highest use of corn among three population was reported in Hunza at 15% whereas pasta consumption was only observed in subjects from Islamabad region at 30% among

total cereal intakes.

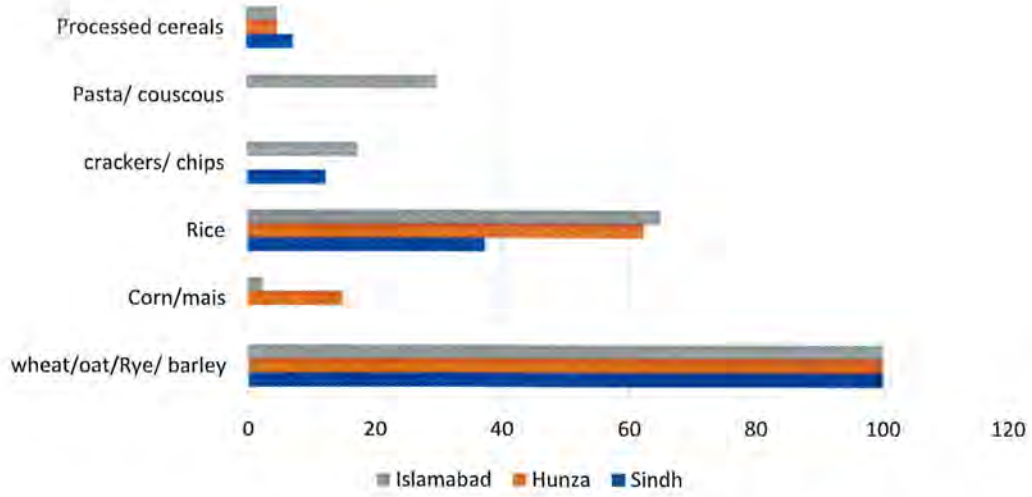


***Figure 13**:Graph showing variation in daily consumption of cereals in subjects.*

Utilization of whole grain cereals and meals provide with a variety of carbohydrates some of which are indigestible and reach the colon. They are then fermented by colonic resident bacteria to form useful molecules such as SCFAs and neurotransmitters that help to create homeostasis in gut and brain axis and other systems of host.

#### 4.1.1.3 FRUITS:

Bananas, apples, orange, lime, and mango were common among all three population with a varying daily intake based on season and availability. Consumption of coconut, grapes, cherries plums and apricots were common among subjects from Hunza and Islamabad with none of these consumed by Sindh subset. Only subjects from Islamabad showed utilization of strawberries. Berries are a rich source of polyphenols which associated with a neuroprotective role. Tomatoes in both raw and cooked form were utilized on daily basis by all three populations. These are prebiotic in nature to their high acidity yield from presence of citric acid. Use of tomatoes provide suitable conditions in gut for the growth of lactic acid bacteria which have been indicted positively with healthy gut. Other fruits constitute of useful vitamins and minerals along with carbohydrates which improves the metabolism and have antioxidant effects on gut.

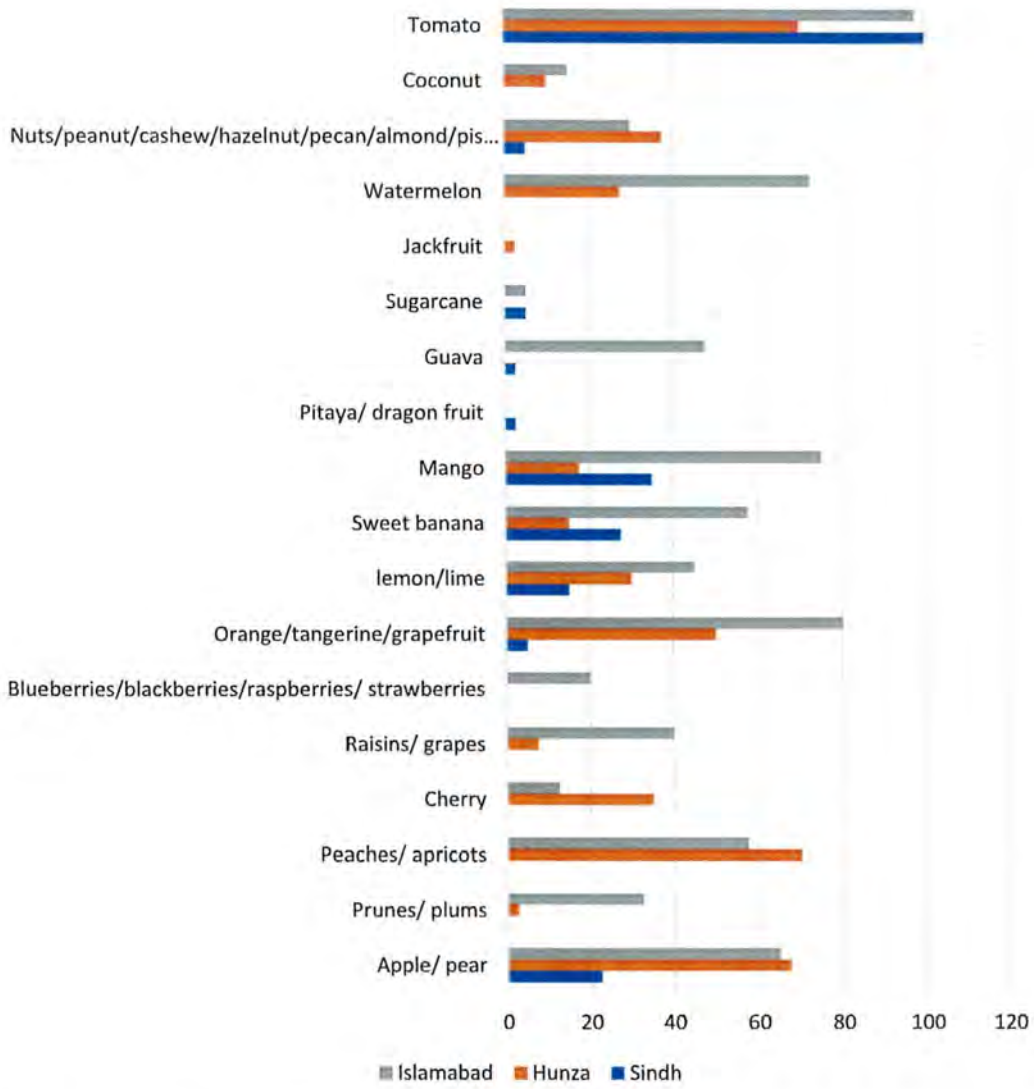


***Figure 14:****Graph showing variation in daily consumption of various fruits in subjects.*

### 4.1.1.4 VEGETABLES:

Consumption of garlic, onions, ginger carrots and potatoes were highest among all three populations. Daily consumption of onion was highest in Islamabad at 100%followed by Sindh at 95% where population used it and least in Hunza at 92.5%. Onions are nutrient packed vegetable with vitamin C along with folate that play role immune regulation, and nerve cell functioning respectively. Use of cucumber was observed in all population highest in Islamabad and lowed in Sindh subjects on daily basis. Along with that lettuce, cauliflower, brinjal and lady finger was high in Islamabad among three population. Vegetables are rich in carbohydrates that are metabolized by bacteria to produce bioactive compounds that positively impact human health.

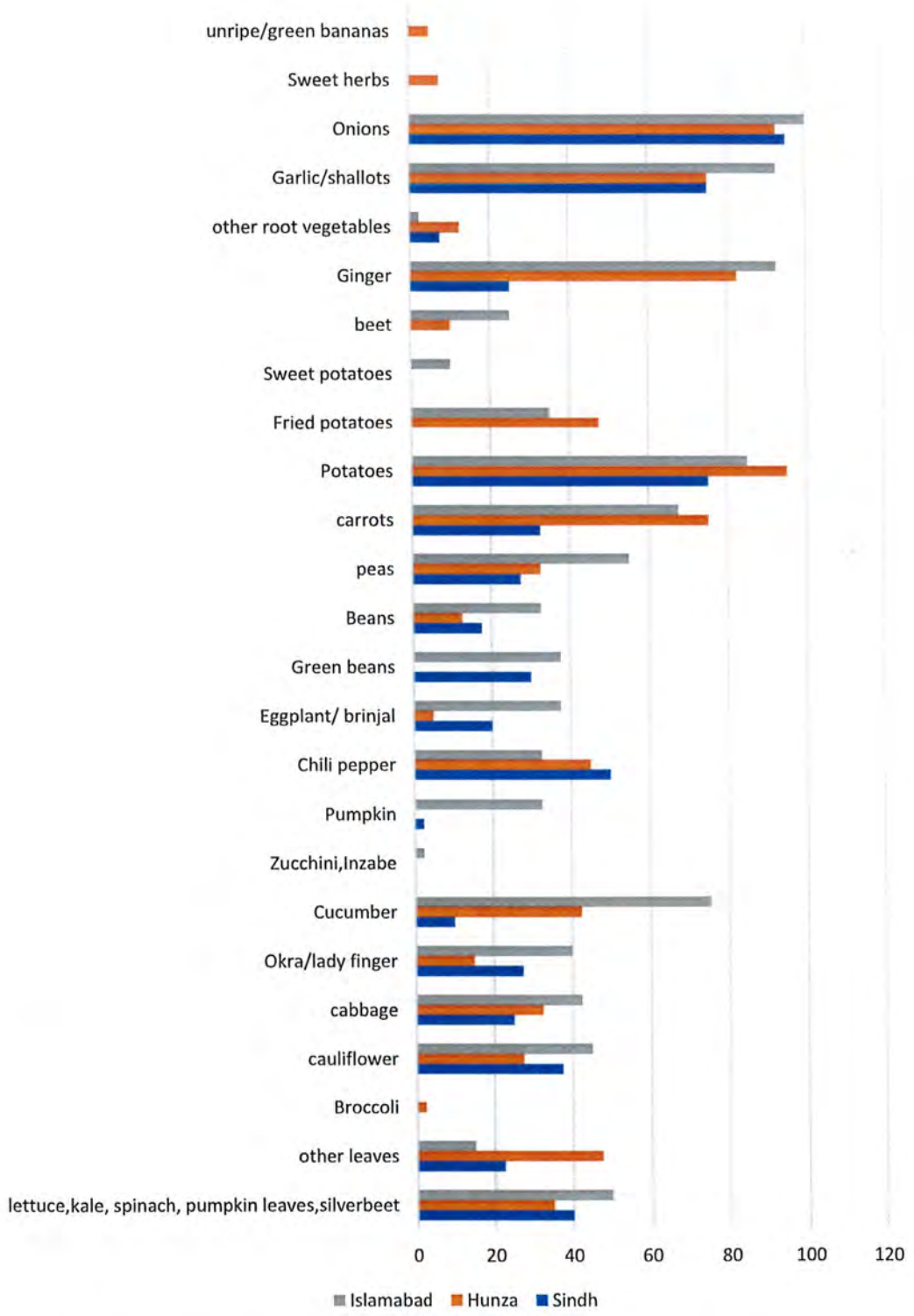


***Figure 15**:Graph showing variation in daily consumption of various vegetable in subjects.*

### 4.1.1.5 MEAT AND EGGS:

Egg consumption was highest in Islamabad where 75% of population utilized it on daily bases followed by Hunza subjects at 40% and least in Sindh where 20% subjects were utilizing it. Eggs are a rich source of protein, iron and vitamin B12 moreover it is easier to digest as compared to other high protein foods.

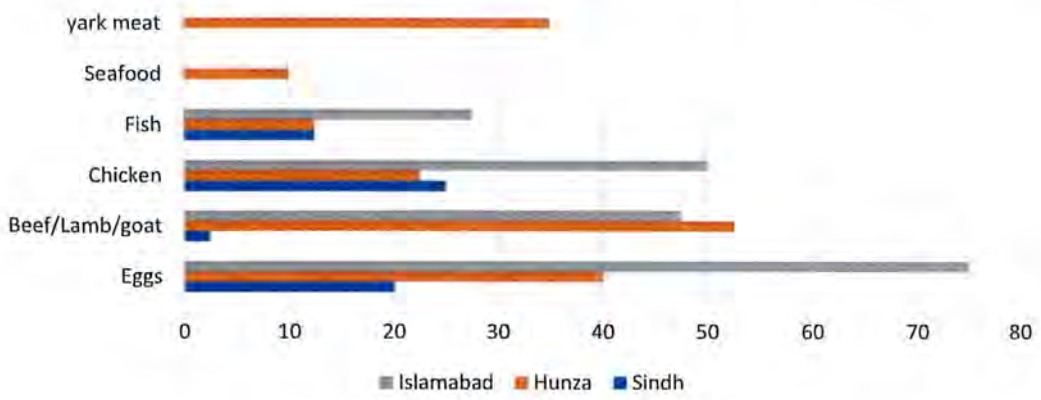


***Figure 16**:Graph showing variation in daily consumption of various Meat in subjects.*

Beef and lamb consumption was higher in both Hunza, and Islamabad set but very low in Sindh. Chicken consumption was high in subjects from Islamabad whereas yark meat was only consumed in Hunza but not on daily basis. Most of subjects utilized meat once or twice a week. Beef is containing bioactive peptides which have anti-inflammatory and immunomodulatory role however their content is decreased after cooking. Daily meat consumption is detrimental because it changes the intestinal flora and raises toxin levels in the blood owing to by-products of protein breakdown by gut bacteria. It has a bad impact on mental health as well.

### 4.1.1.6 SWEET AND BAKED GOODS:

Utilization of jam, honey, cake was common among all three populations whereas no use of baked goods was observed in subjects from Sindh. Highest used at 52% of cakes and brownies was observed in Isla mabad subjects followed by participants from Hunza at 20% and even lower in Sindh subjects at 5%.

Although higher population utilized jam in Hunza, but it was homemade whereas jam consumed by Islamabad population was processed and carried preservatives. Generally, baked goods are unhealthy however a lot them have high content of prebiotic fibers aid in the maintenance of a healthy gut microbiota. Prebiotic fibers like inulin derived from

baked goods encourage the formation of beneficial bacteria in the stomach.

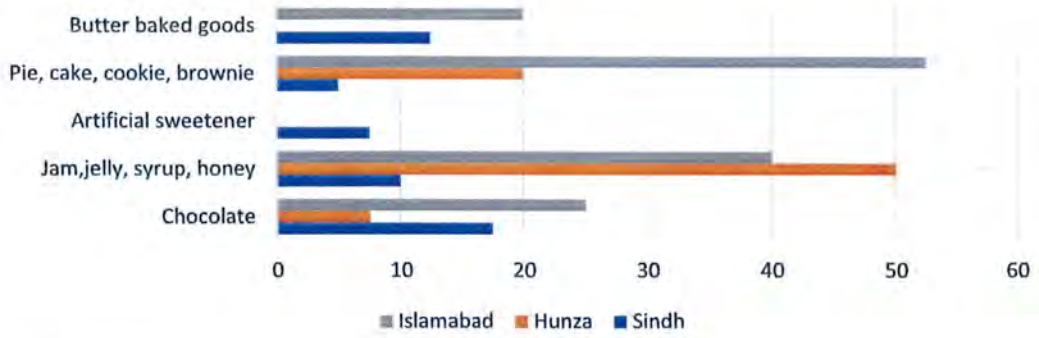


***Figure 17**:Graph showing variation in daily consumption of various sweet and baked goods in subjects.*

## 4.2 Fecal Microbiology:

Fecal microbiology was performed on two different Medias i.e. MRS for Gram positive microbiota and EMB for Gram negative bacteria. CFU count showed a higher gram-positive count on MRS in contrast to gram negative count Hunza and Sindh group.

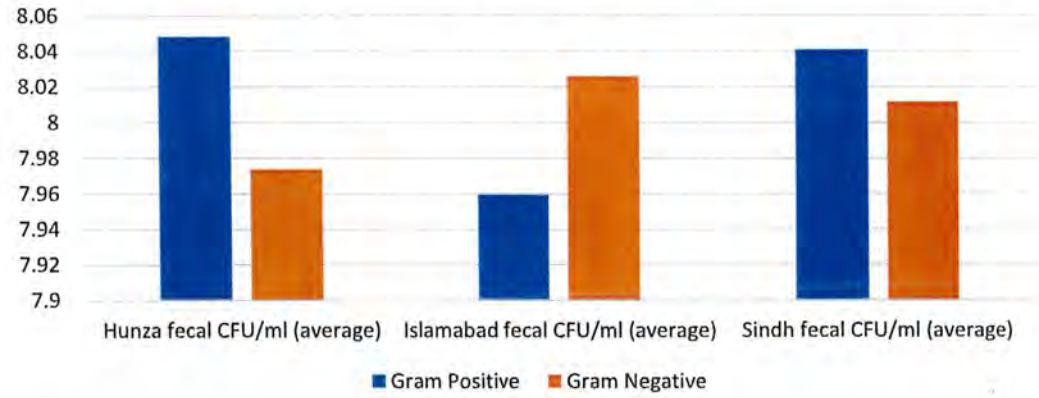


***Figure 18**: Graph showing CFU count of gram positive and gram negative in fecal samples of three populations.*

Fecal gram-positive count was higher in subjects from Hunza at $1.12\times10^8$ in contrast to fecal count of gram negative i.e., 9. 4×107. Islamabad sample showed lowest gram positive count at $9.1\times10^7$ in comparison to gram positive count at $1.06\times10^8$ . Small difference in gram positive and gram-negative abundance was found in subjects from Sindh at $1.1\times10^8$ and $1.03\times10^8$ respectively.

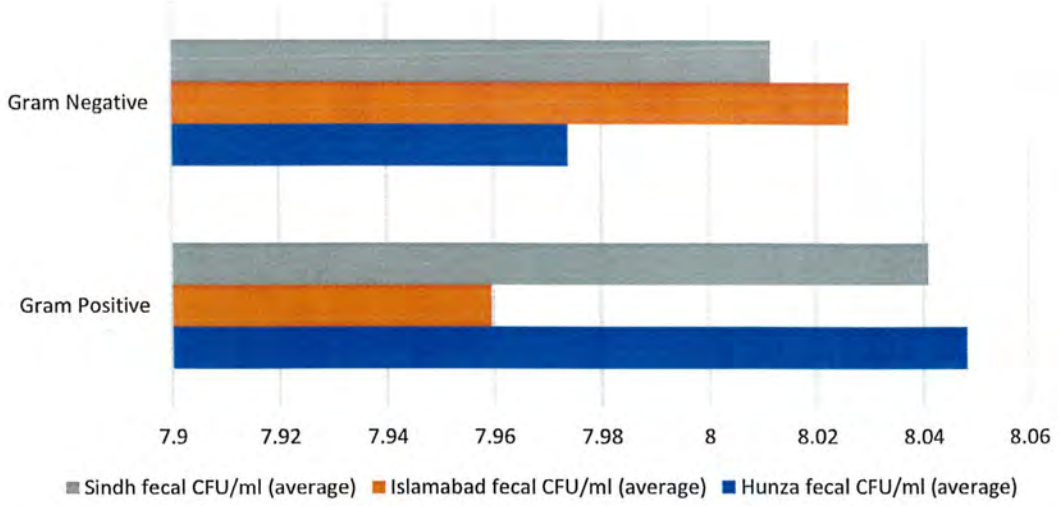


***Figure 19**: Difference in relative abundance of gram negative and gram positive bacteria among three different groups.*

Among three population, subjects from Islamabad show lowest gram-positive count whereas highest count was observed in participants from Hunza. Higher number of lactobacilli were observed in both Hunza and Sindh subjects' due consumption of fermented and plant-based foods respectively.

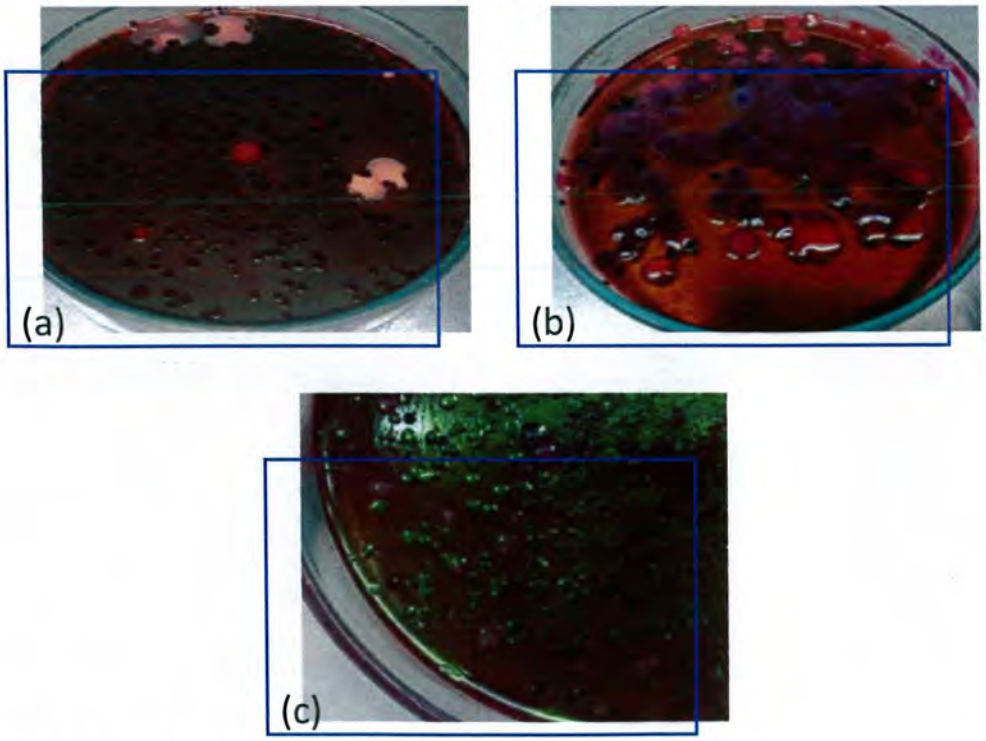


**Figure 20**:Fecal Gram-negative lactose fermenters on EMB agar (a) Sindh, (b) Islamabad and (C) Hunza

Round dark purple colonies of E. coli with green sheen were observed in all three population. Presence of purple color in high lactose fermentation capacity whereas sheen represents producing of acid on EMB agar. Among three different population mucous producing gram negatives were in high diversity in stools samples of subjects from Islamabad. Dark purple centered mucoid colonies represent the presence of Enterobacter which is a lactose fermenter as well as acid producer. Light pink color of mucous represents low fermentation of lactose which were found both in Islamabad and Sindh sample. Gram negative diversity was quite low in Hunza samples mainly due to utilization of homecooked organic food as well as utilized of a water source which carries least number of pathogens.

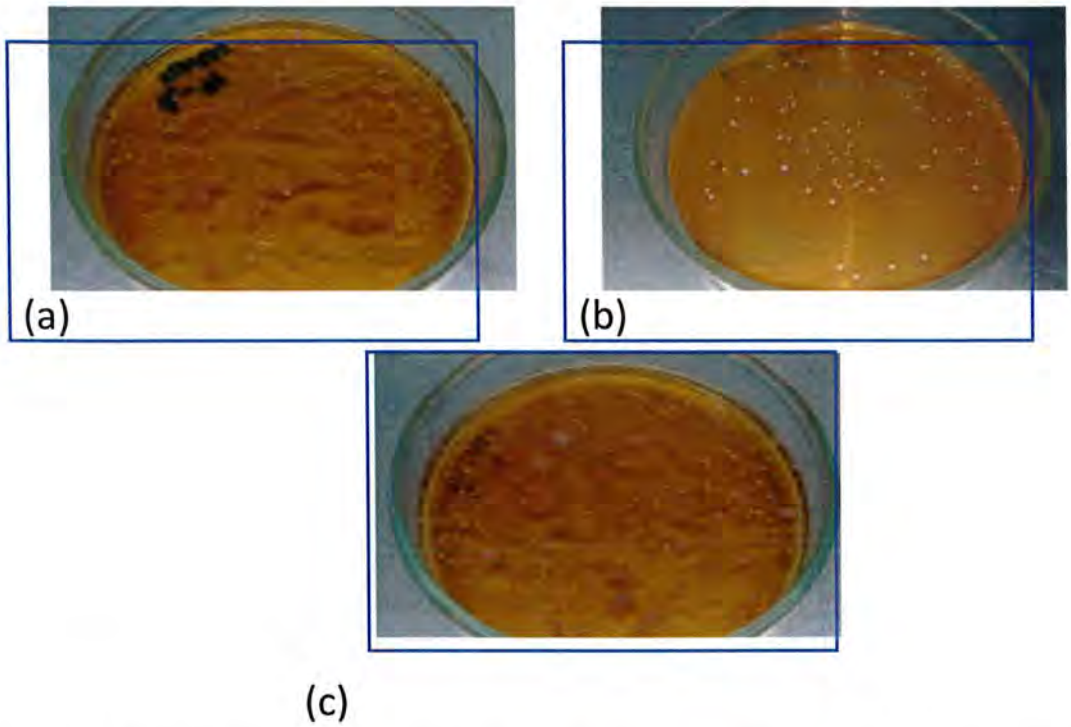


***Figure 21**:Fecal Gram-positive lactic acid bacteria on MRS agar (a) Sindh, (b) Islamabad and (C) Hunza*

Lactobacilli colonies were observed on MRS highest diversity was observed in Hunza samples with appearance of translucent, pink, creamy light pink along with creamy white colonies in different samples mainly due to higher consumption of fermented foods which promote growth of lactic acid bacteria. Sindh samples were observed to have same kind of diversity due to consumption of a plant-based diet. Lower lactobacillus diversity was observed in Islamabad sample, but their number were still high.

## 4.1. LEVEL OF SEROTONIN IN SERUM SAMPLES:

Highest serum serotonin levels were observed in Sindh mainly due to consumption of vegetables, fish and eggs that have antioxidants among many nutrients which may alter brain serotonergic state and improve mood. Subjects from Hunza revealed slightly lowered levels of serotonin than Sindh but are still accurate for brain function and mood regulation due use of tryptophan rich food.


***Figure 22:****Serum serotonin level in Three distinct populations.*

Lowest levels of serum serotonin were observed in Islamabad region mainly due to consumption of industrial diet. However, all the population had normal serotonin levels consistent with their BDI scores.

## 4.2. EEG Determinants of Brain electromagnetism:

EEG results of subjects were obtained using MUSE 2, a brain wave measuring device that consists of four electrodes located at frontal and temporoparietal regions of brain. Data obtained was first observed in Mind Monitor app and graphs were observed for absolute and relative values. The collected data was then processed in EEGLAB where eyeblinks and artifacts were removed, and noise variance was evaluated. Power spectrum analysis of waves showed a higher potential of alpha waves in all of four brain regions as compared to beta and delta as the subjects were in resting and closed eye state.

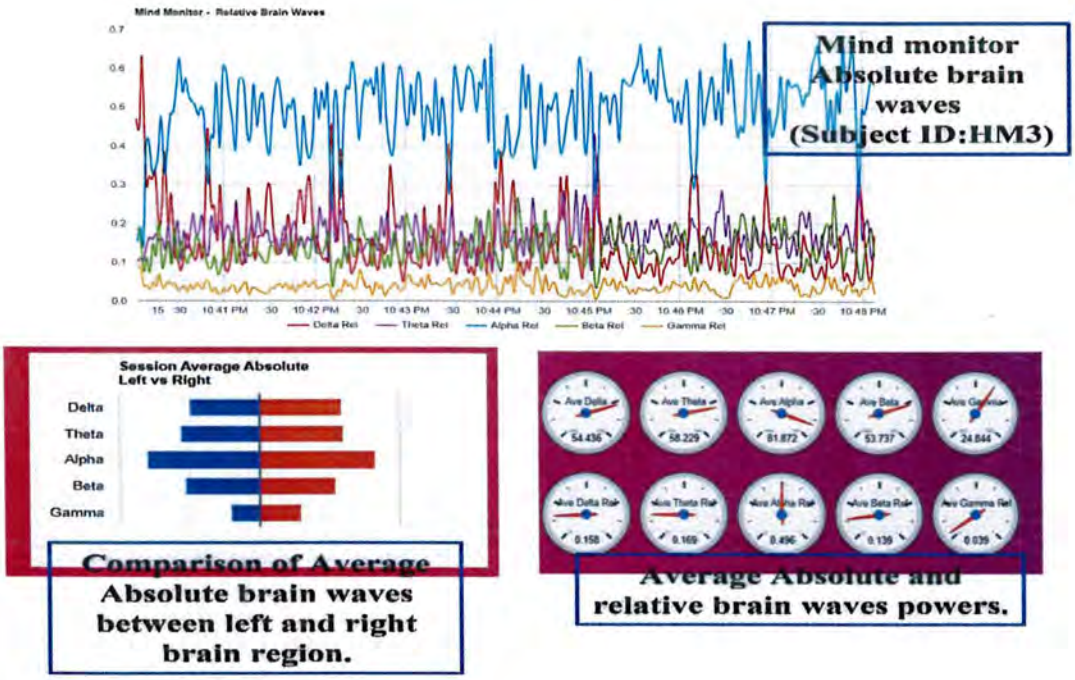


***Figure 23:*** *EEG analysis of subject HM3*

Intensity of different wave forms was unique among individual belonging to same population. Higher power of alpha wave forms was detected in individual from Hunza population where beta power varied among subjects because each of them were in different attention state. Whereas lower delta waves observed in all subjects showing that all of them had heathy brain activity as high prevalence of delta wave forms associated with depression. Many factors play avital role such high alpha wave forms one which is utilization of organic diet rich in protein, polyunsaturated fat and vitamins.

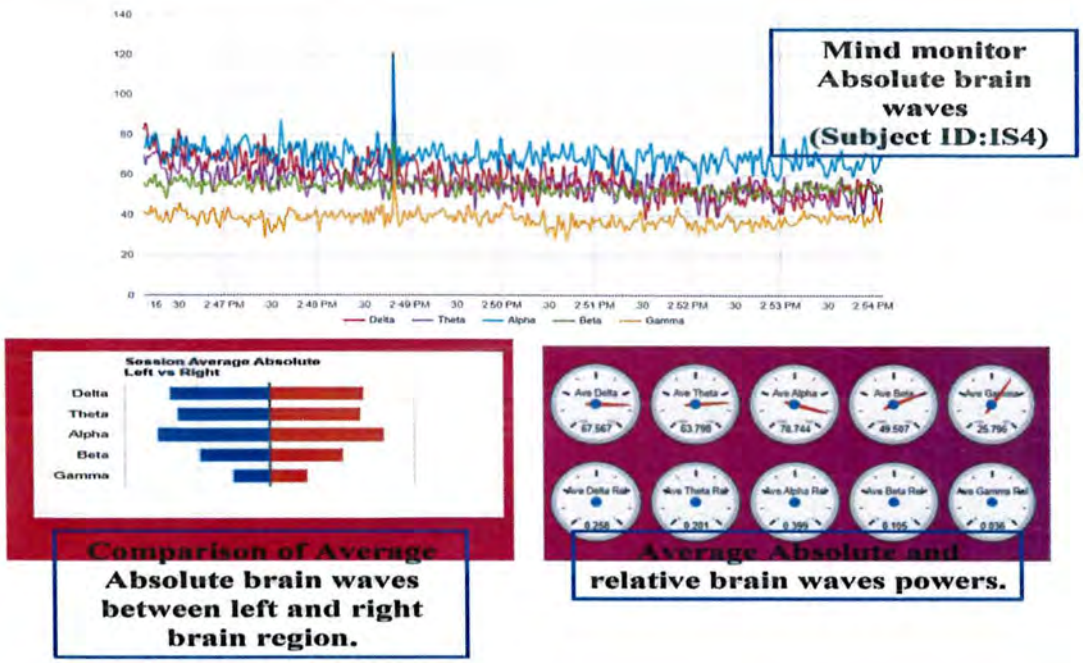


***Figure 24:****EEG analysis of subject IS4*

Two hemispheres of the brain were analyzed which showed a coherence between left and right cerebral hemispheres. All the subjects showed nearly equal prevalence of brain waves between two regions of brain.

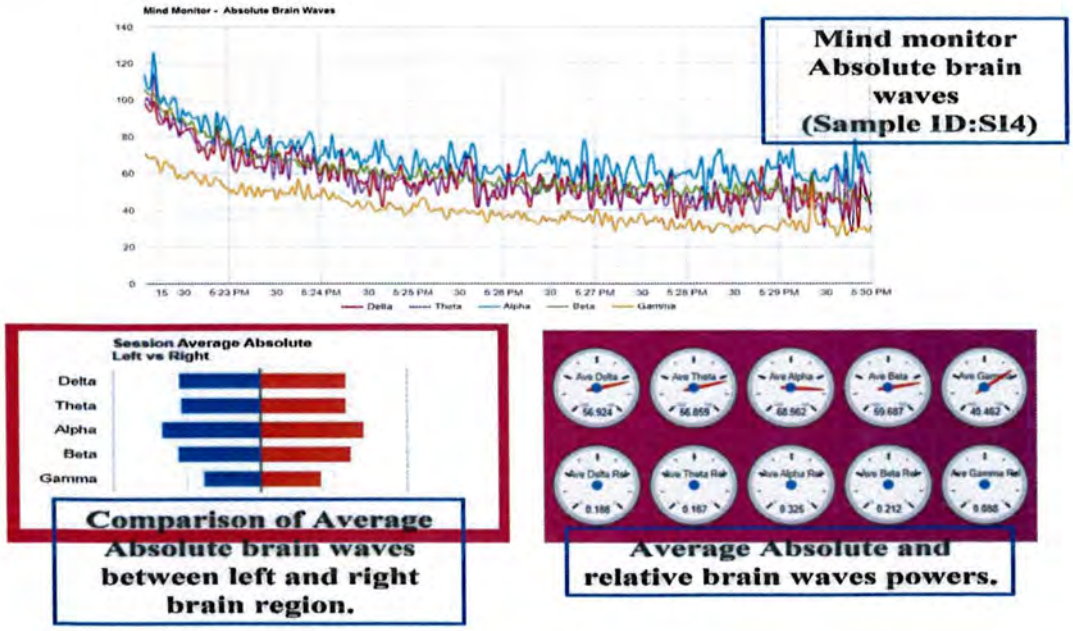


***Figure 25:****EEG analysis of subject SI4*

A major difference observed on performing Preprocessing of EEG data in EEGLAB. FFT analysis on each wave band showed prevalence of individual frequency which allows in depth analysis of EEG.

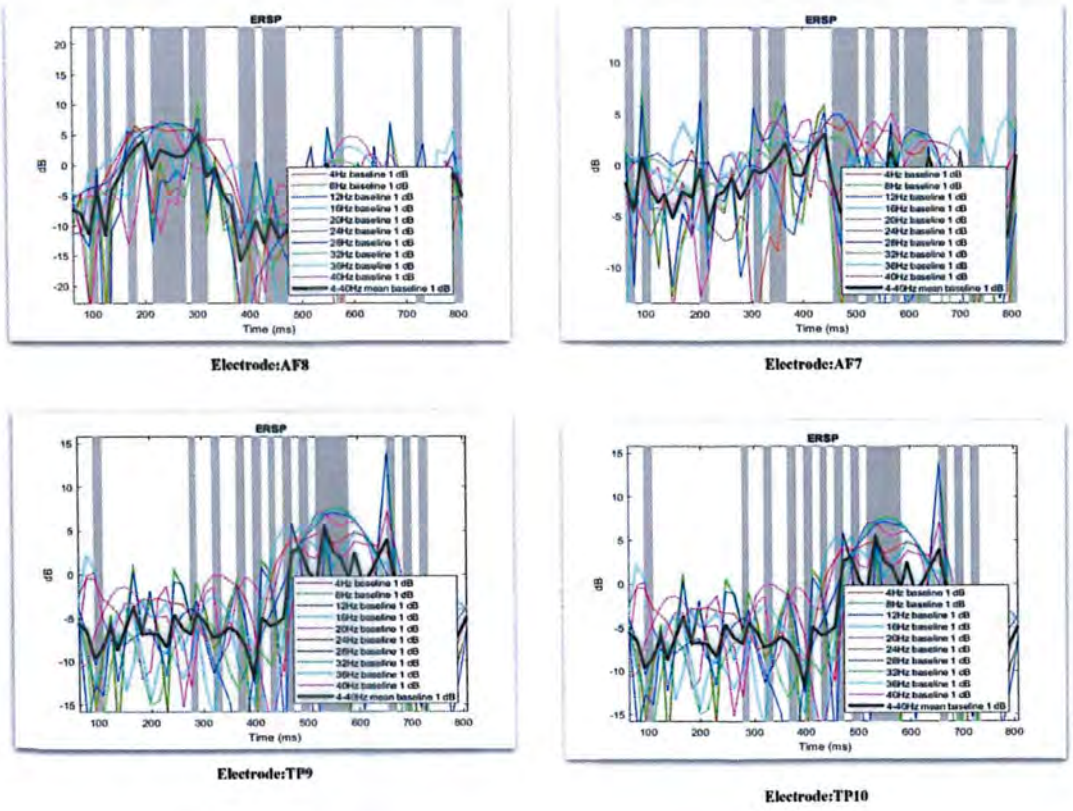


***Figure 26:****FFT analysis of Subject.*

All the subjects were found have normal EEG wave patterns showing absence of any kind of depression or depression like conditions.

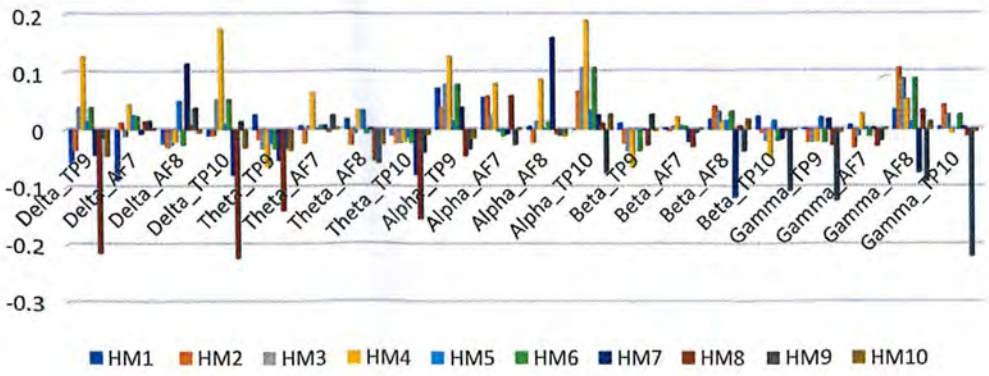


***Figure 27****:Relative presence of alpha, delta ,theta, gamma and beta in Subjects from Hunza at in eyes closed resting state.*

EEG analysis of waveforms in Islamabad revealed a higher alpha waveform than delta wave intensity however their delta was observed to be much higher than other two population and alpha was lower as well than rest of two groups.

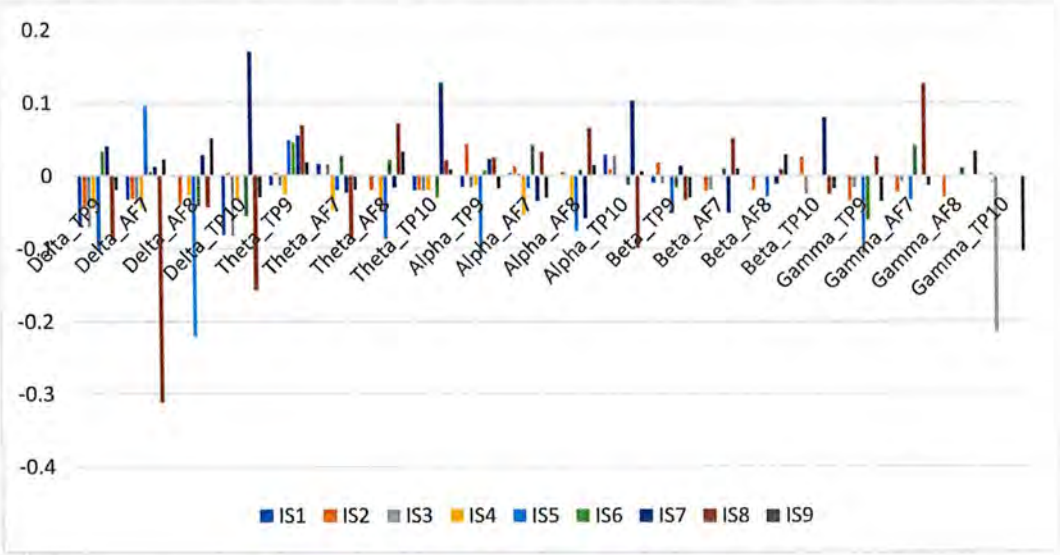


***Figure 28**:Relative presence of alpha, delta ,theta, gamma and beta in Subjects from Islamabad at in eyes closed resting state.*

Such a brain activity was primarily due to different lifestyle and eating habits where subjects in Islamabad revealed consumption of an industrial diet. However, over all EEG patterns were observed to be normal in all individuals.

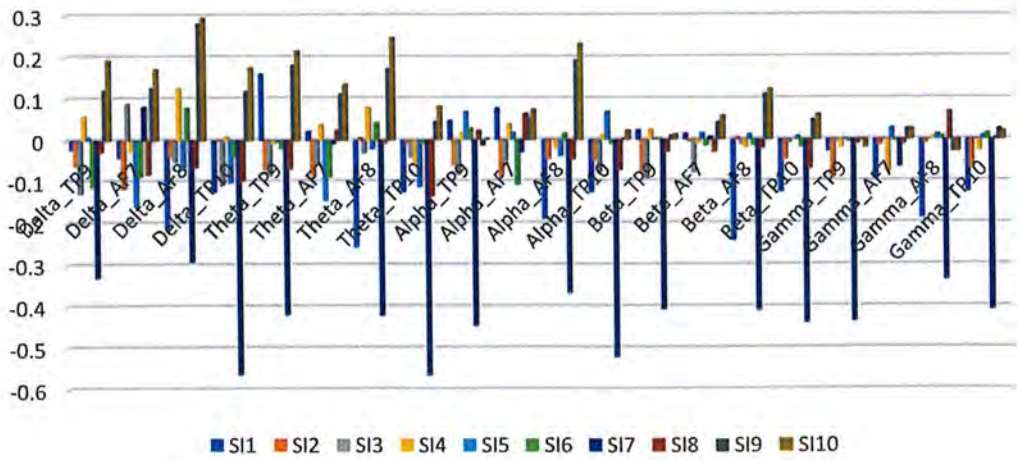


***Figure 29:**Relative presence of alpha, delta ,theta, gamma and beta in Subjects from Sindh at in eyes closed resting state.*

All subjects from Sindh showed a normal EEG wave pattern with a very low delta. However, alpha was lower than Hunza population but still higher than subjects from Islamabad. Some of the subjects showed higher alpha forms than others.

Primarily due to difference in eating habits active lifestyle. Altitude, weather changes might also be responsible for variation in EEG patterns of individuals.

# Chapter 5

# Discussion

# DISCUSSION

Symbiotic relationship between gut microbiota and gut has been linked to the host's cognitive and physical health. Complex interplay of intrinsic and extrinsic factors regulates the gut microbial diversity and stability. Diet has proved itself as a powerful agent in development and modeling of enteric microbial populations. Therefore, demographic diversity that govern dietary habits of individuals result in might lead to skewed findings. Demographic data was therefore collected beforehand in order to choose an unbiased research group and reduce the potential biasness in outcome. Subject of study were first evaluated for a psychological health using Beck Depression Inventory (BDI) scale 2 (Beck et al., 1961). Individuals experiencing anxiety or depression were excluded from the research, and only those with a low score were selected as subjects. To assess the dietary habits of subjects their dietary profile was taken using a questionnaire. The dietary patterns were assed for frequency of food intake in three different populations. Distinct dietary intake was observed in three population where diet in Sindh resembled a Mediterranean diet with consumption of plant-based foods where vegetables constituted approximately 37%, fruits at 13% and cereals at 8% along with 3% of vegetable oil. A very low consumption of meat products at 3.6% and animal fat at 1.8% of total food intake. Most meat products consumed were eggs and chicken both of which are high in protein and unsaturated fat content usually preferred for a healthy gut. Such a diet in high fiber usually high promotes the SCFA content such as butyrate, propionate in gut which are potential metabolites that have ability to regulate a gut epithelial permeability but can also regulate production of other neuroactive compounds such as serotonin that directly involved in maintance of healthy brain function (Soret et al., 2010). Increasing poly unsaturated fat content of diet which majorly consists of Omega 3 and omega 6 are polyunsaturated fatty acids produces endocannabinoids that is influences brain neuroplasticity, synaptic activity as well as endocrine system positively (Tosti et al., 2017). Subjects from Hunza population a reported a higher meat and fat consumption constituting about 5% and 3% of total dietary intake. That not only produce however, but the cooking methods were also different, and diet was very low in processed and fried foods. Fermented dairy foods prepared locally were a major part of daily food 17% of total dietary consumption. Such a dietary pattern supports the profusion of probiotic gut bacteria such as lactic acid bacteria and butyrate producing bacteria which is known to improve gut barrier

integrity (Soret et al., 2010). Milk and fermented milk product intake are much greater, which improves the nutritional profile of the subjects. They enhance the production of crucial metabolites in gut and cause reduction in pathobiont population creating a healthy gut environment (Veiga et al., 2014). A higher population in Islamabad reported intake of balance fat, protein and carbohydrate. Their diet included processed foods and fried food in higher proportion than other population. However, the subjects under study showed a heathy intake of fat necessary for energy requirement therefore based on previous studies it was concluded their gut health would not be affected.

Microbiological analysis by culture-based method using two differential media MRS and EMB showed no significant difference of diversity among three population. Lactobacillus plate count was higher than coliform count in all subjects. However, abundance lactobacilli count was observed higher in Hunza population as compared to other populations. it is associated with elevated SCFAs production linked healthy brain activity and decreased delta waves as compared to alpha waves. Use of processed foods was observed to be associated with decreased lactobacillus count in the population when compared to rest of two populations(Le Roy et al., 2015) .

The EEG was evaluated using the International ten-twenty system and using power spectrum analysis. The EEG wave patterns were discovered to be normal. The EEG was recorded in both a calm and a hyperventilated condition, which is used to assess the severity of brain abnormalities. Because there are several artefacts in the EEG, such as jaw clenching, heart rhythms, ambient sounds, eye blink, and so on, which lead to misinterpretation of brain wave pattern were filtered using Brainstorm software to avoid noise in the interpretation. Most research subjects had coherence between the temporoparietal and frontal lobes of brain. However potential of EEG waves was observed to be different in three regions primary due time of at which EEG was recorded or difference in environmental conditions. Similar wave potentials were observed in Hunza and Sindh region with alpha quite higher than delta in closed eye resting state whereas a lower difference in alpha and delta potential was observed in subjects from Islamabad(Soret et al., 2010).

# CONCLUSION

Diet is a powerful modulator individual's physical and psychological well-being by virtue of human gut microbiome. Selection of healthy and balanced diet promote establishment of appropriate balance of Gram-positive and Gram-negative microbiota and interact positively with the host, i.e., commensal or symbiotically with it. Many dietary components act as precursor molecule for synthesis of serotonin. A variation in serum serotonin level among different populations and within population is representation of the fact that the host's serotonin level are significantly affected by the dietary patterns. It can be directly or indirectly altered the complex bidirectional interplay. There are several mental and metabolic problems that can be improved by altering the microbiota in the digestive tract there by affecting the synthesis of essential metabolites such as serotonin. Moreover, metabolite concentration are indicators of guts over all physiology. Therefore, Gut health can be predicted by keeping an eye on neurophysiology. That's why food, gut microbiome, and mental health are all connected in some way.

## RECOMMENDATIONS:

A larger sample size must be considered for a deeper insight.

Recruiting subjects from same population or having analogues dietary pattern should be preferred to avoid chances of gathering biased data stemming from food preferences at individual level.

In comparison to conventional techniques, whole genome sequencing of fecal can reveal detailed and plausible knowledge about gut microbiome composition.

Interventional studies conducted over longer period should preferred to study neurotransmitter and hormonal impacts as they are regulator of circadian rhythms. Moreover, a detail analysis of lifestyle is preferred to understand it impact on such metabolites.

Electroencephalography should be tailored to the research and its interpretation should be made easier by customizing the technique and work plan. Importing data for statistical analysis necessitates the use of cutting-edge software tools.

Likewise, advised is the use of oddball tasks to perform EEG in an active state

A urine analysis and salivary microbiology is also suggested.

## FUTURE DIRECTIONS:

Neuropsychiatric and neurodegenerative disorder affects a large set of individuals throughout world. Therapeutical interventions are short term and unreliable due to poorly understood disease pathologies. Gut microbiome has shown its long-term, simple but highly effective impact not only in cure but prevention in many psychological ailments like depression, autism spectrum disorder. A deeper understanding on gut microbiome role in neurodegenerative disorder like Dementia, Parkinson Disease etc. can provide a potential therapeutic target. EEG devices have been very effective in assessment of behavioral and mental disorders in last few years. Furthermore, advancement in technology have led to portable EEG devices helping in real time monitoring of neurological heath. Soon this rapid technological progression coupled with increased understanding of gut microbiota brain interaction individuals will be found donning devices that will not only recognize EEG patterns but will also interpret them. Moreover, foreseeing possible susceptibility to development or progression of mental disorders. Near future will observe these noninvasive devices predicting bacterial prevalence form wave forms and suggesting correct dietary and lifestyle changes to improve psychological and physical health.

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