## INTRODUCTION

The human body is an amazing collection of specialized systems that perform a diverse number of biologically intricate and extremely complex functions. However, the human body does not consist of human cells alone, but is also composed of an overwhelming number of single celled microorganisms. The estimated numbers of microbes inhabiting a healthy human body is estimated to be 10 microbes for every human cell, with these microbes also contributing an estimated 8 million protein coding genes. This number is massive in comparison to the only 22,000 genes in the human genome[143]. The collection of all these microorganisms living on or in the human body is known as the microbiome. Every human has a unique microbiome, and no two microbiomes are the same. The microbiome is a part of everyday life and the collections of microorganisms that compose it are comparable to another organ. This is because the microbiome performs tasks such as nutrient digestion, vitamin synthesis, and even aiding development of the immune system. The microbiome is involved in preventing infection by constantly stimulating the immune system, preventing harmful pathogens through competition. Through coevolution with a diverse number of microorganisms, we have forged an alliance with our small friends, in which both host and guest benefit in a symbiotic, commensal relationship. We gain the benefits listed above, and possibly many others still unknown, while the microbes get nutrients and a sustainable habitat. Because of this coevolution, many of the developmental processes, as well as fundamental functions of the body, are dependent on a healthy microbiome. Our own healthy development or health is disturbed when our microbiome is thrown into chaos by antibiotics, stress, diet, or disease.

The microbes that compose our microbiome vary greatly, and can be found in a number of areas of the body. One of these areas is the gastrointestinal (GI) tract, where the collection of

all non-host organisms living there is commonly referred to as the gut microbiota. In many cases, the gut microbiota plays the largest role in affecting its host because the majority of all microorganisms in the body live there. Because the microbiota is involved in so many crucial processes throughout the body, it is no surprise that the disturbance of the gut microbiome is being increasingly linked to the causes of many diseases, including but not limited to diabetes, obesity, ulcerative colitis, and irritable bowel syndrome. Much of this research has provided focused evidence supporting a link between the gut microbiota and host through dietary molecules known as short chain fatty acids (SCFAs). SCFAs are molecules that fall into the category of fatty acids (FA). FAs contain a carboxylic acid chemical group with aiphatic tails of varying length. SCFAs include FAs with tails of 2-6 carbons, while physiologically relevant SCFAs include two carbon acetate, three carbon propionate, and four carbon butyrate. The major source of SCFAs in the body is the microbiota, and it has been shown that SCFAs themselves may have an influence on many different host cellular processes. The varying effects of SCFAs, which are produced by the gut microbiota, provide a link between disease states, including obesity, metabolic disorders, chronic inflammation, type 2 diabetes, and the microbiota itself. This review, critically analyzes current research focused on the effects of the microbiota's production of SCFAs on the human body's state of health.

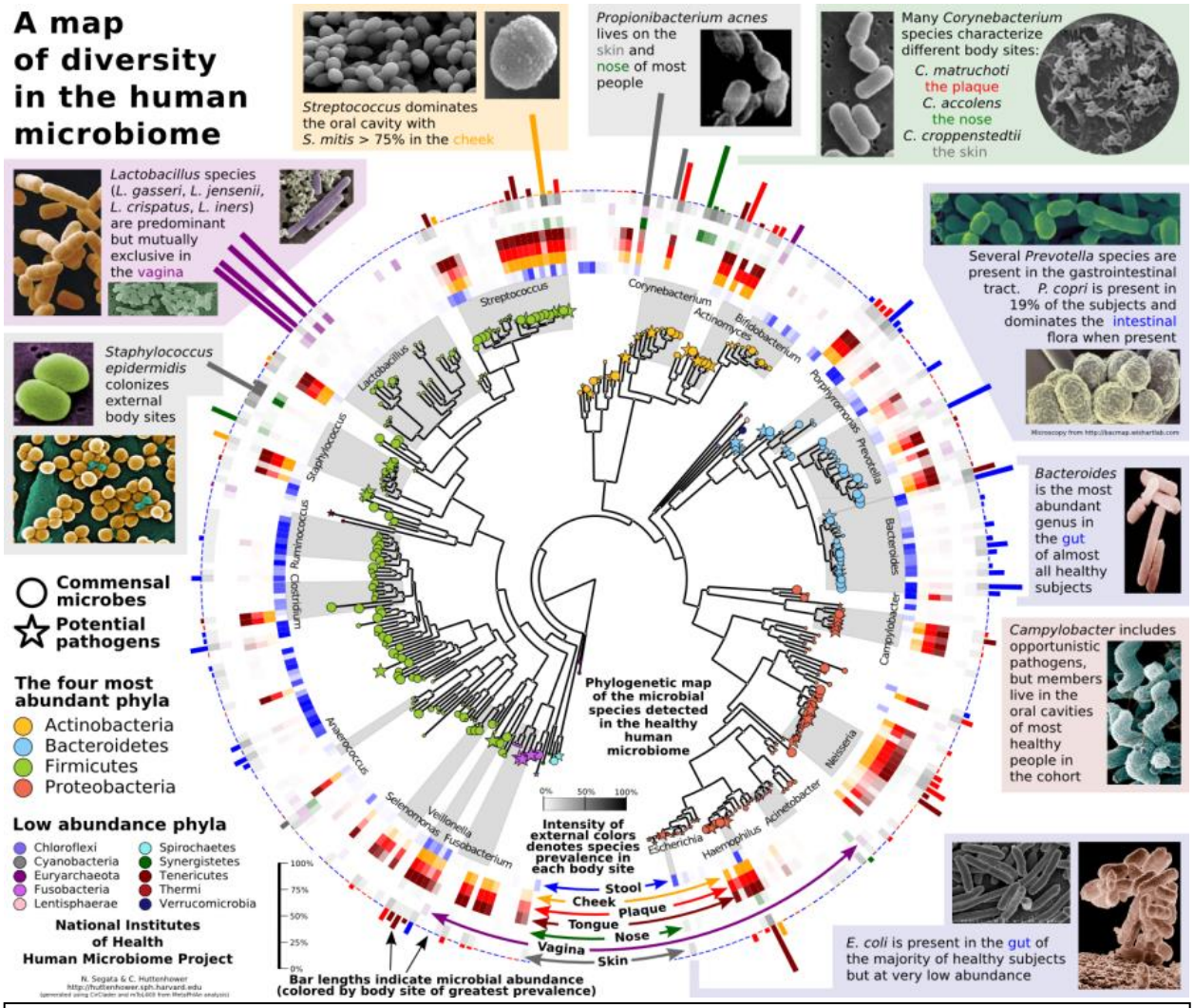



**Figure 1:** A map of the complex nature of the human microbiome including examples of potential pathogens, commensal microbes, and relative abundances of different phylum. (147)

## HISTORY OF THE MICROBIOTA

The importance of a healthy gut has been known by man for thousands of years. In fact, the manipulation of the gut has been used by ancient doctors and medicine men to treat patients for almost as long. For example, in ancient china it is described in the handbook of medicine that doctors would prescribe what was known as 'yellow soup' to patients experiencing intestinal or stomach discomfort. Yellow soup was made from the feces of infants, and when eaten was

reported to have curative effects[150]. In relation it is recorded that ancient Bedouin tribes of northern Africa would consume camel feces to cure diarrheal symptoms now known to be caused by bacterial dysentery. This practice was rediscovered by invading German soldiers during WWII[151]. The consumption of camel feces was adopted as a treatment for bacterial dysentery after German regiments suffered losses to diseases that caused diarrheal symptoms, but noticed that the locals had no such problems. These two stories show the importance of a healthy gut was obvious to our ancestors, but it wasn't known until recently the exact mechanisms by which these treatments worked. Now, due to advanced techniques that allow for analysis of the human gut, we know that these treatments were using manipulation of the gut microbiota to treat diseases caused by an imbalance in a healthy microbial composition.

The microbiota has been shown to be of great importance in influencing its hosts state, both by current research and ancient medicinal practices. Because of this, current research has focused on trying to find the mechanisms by which the gut microbiota influences the host. What has been found is that microbiota composition varies between healthy and diseased patients, and that this variation in gut microbiota is influenced heavily by the diet[152]. It has been proposed that the diet influences the gut microbial composition and the metabolites produced by the gut microbiota. Metabolites in turn influence the host.

## INTRODUCTION TO THE EFFECTS OF SCFAs AND THE MICROBIOTA

Recent research has provided a link between the production of various SCFAs by the microbiota and their effects on the host. SCFAs are produced through a process of fermentation of dietary components in the gut. There are three major categories that contribute as the major dietary energy source for fermentation. The first of these is carbohydrates, the second fats, and the third proteins and amino acids[1]. The larger, more complex, fiber rich dietary sources of

carbohydrates are indigestible to the human gut[2]. However, if aided by the microbiota, these indigestible macromolecules can be converted to smaller micronutrients, such as short chain fatty acids (SCFAs). The breakdown of fibers is accomplished by the process of fermentation carried out within a bacterial cell. Fermentation is a process made possible by gene products not available in the limited human genome[18]. Through fermentation, bacteria break down these large, fibrous macromolecules into many different SCFAs, which are then absorbed by endothelial cells[19]. Once absorbed, a select few of the SCFAs produced in the gut have a large role to play in the body and have effects on many different systems. Physiological effects of SCFAs include the mediation of hormone production, such as insulin and leptin[20,21,22], the stimulation or suppression of immunomodulatory cytokines and chemokines, modulation of metabolic anabolism or catabolism[20], and regulation of transcriptional factors, such as histone deacetylases[23]. Through the production of SCFAs by fermentation of dietary fibers, and the subsequent interaction of these SCFAs with cells, the gut microbiota is able to interact and influence its host.

**DIETARY FIBER**

The first source of energy, carbohydrates, is also the major source of calories in a majority of diets around the world. Carbohydrates come mainly from simple cereal grains, such as wheat, barley, rice, and rye, but can also be found in large, macromolecular structures such as in fibrous vegetables and whole grain food products[2]. Although there are other fermented substrates in the gut, these fibrous foods are the major source of fermented carbohydrates. This is because complex carbs are the most likely to be undigested by host processes, and will reach the gut with most of their structure intact[9]. Typical sources of dietary fiber include cellulose,

pectin, brans, and gums [9], as well as fructo-oligosaccharides, galacto-oligosaccharides, sorbitol, and xylitol[10].

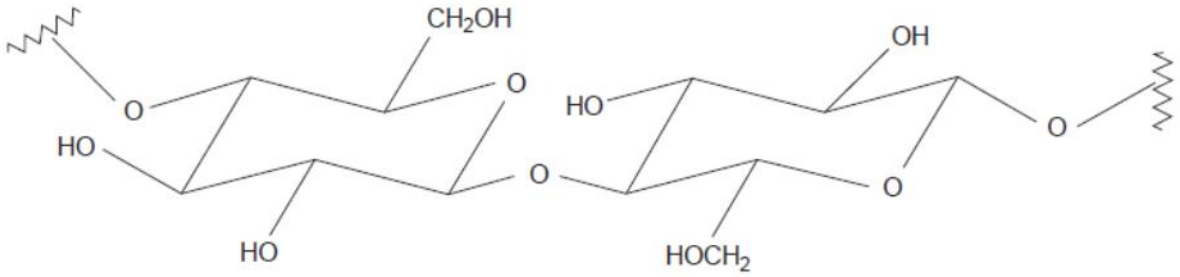



**Figure 2:** Example of the repeating unit of a carbohydrate polymer. Two glucose monomers are bonded by the presence of a glycosidic linkage. (148)

**PROTEINS AND AMINO ACIDS**

Although not a carbohydrate, proteins and amino acids are also an important source of fermentation substrates in addition to dietary fiber. The metabolism of various peptides by microorganisms can lead to the production of toxic byproducts in the gut. According to some research, the presence of a healthy gut microbiota can help in the reduction of these toxins, as the microbes remove toxic products from the gut for incorporation into their cellular components[9].

**SCFAs and FATTY ACIDS**

SCFAs fall under the biochemical category of fatty acids, as their name describes. A fatty acid is defined as a carboxylic acid with the presence of an aliphatic tail of varying length[24]. A SCFA is a fatty acid with a typical tail length of 6 or less carbon atoms, a medium chain fatty acid has 6-12 carbons, and a long chain fatty acid has 12 or more carbons in its tail[25]. All FAs have varied effects on the body. However, when studying interactions between the microbiota and host, SCFAs are the most important of the three categories of fatty acids. This is because the

microbiota is the major source of SCFAs found within the host, and the dynamic nature of the microbiota causes ever changing amounts of SCFAs to be produced[22]. When a change in SCFA concentrations occurs, the effects that the host experiences change as well.

## TYPES OF SCFAs

There are many fatty acids found within the body but it is the SCFAs that are important in the majority of studies of host:microbiota interactions. The fatty acids that are characterized as SCFAs have tails with only 1 to 5 carbons. These include 1 carbon formic acid (IUPAC name – methanoic), 2 carbon acetic acid (IUPUAC name – ethanoic), 3 carbon propionic acid (IUPAC name – propanoic), 4 carbon isobbutyric acid (IUPAC name – 2-methylpropanoic, 4 carbon butyric acid (IUPAC name – butanoic), 5 carbon isovaleic acid (IUPAC name – 3-methylbutanoic), 5 carbon valeric acid (IUPAC name – peentanoic), and 5 carbon 2-methylabbutanoic acid[25]. Two molecules similar to SCFAs and with important physiological roles in the body but not defined as SCFAs include succinate and acetoacetate[26]. All of these SCFAs have pKas ranging from 3.6 to 4.7, with a decreasing trend in water solubility as the number of carbons in the aliphatic tails increases. For example, 5 carbon valeric acid is less soluble than 1 carbon formic acid. Low size and high solubility in aqueous solutions are important in the functions of SCFAs.

## CONVERSION OF DIETARY FIBER TO SCFAs BY THE GUT MICROBIOTA

The first step in the interaction between the body and the gut microbiota is the conversion of indigestible dietary fibers to SCFAs. As mentioned, this is accomplished through the process of fermentation. Indigestible fibers, or dietary fibers, consist of carbohydrate polymers. They are composed of glucose monomers bonded by β- and α-glycosidic linkages[3, 10,39]. In many

organisms the only complex carbohydrates that can be broken down to a large extent, without aid from bacterial fermenters, are those carbohydrate polymers containing α-1,3-glycosidic linkages[2,10]. The major enzyme category involved in the breakdown of these digestible carbs is the α-amylase family, present mostly in the saliva and small intestines[5, 10]. However, the presence of β-linkage systems, and unfamiliar a-linkages, makes dietary fiber mostly indigestible by human α-amylase enzymes[4,39]. This is because human α-amylases enzymes lack the needed specificity to cleave specific linkages between sugar monomers within complex, fibrous carbohydrates. Therefore these fibers are not absorbed and the energy of these fibrous, carbohydrate sources goes through the body untapped.

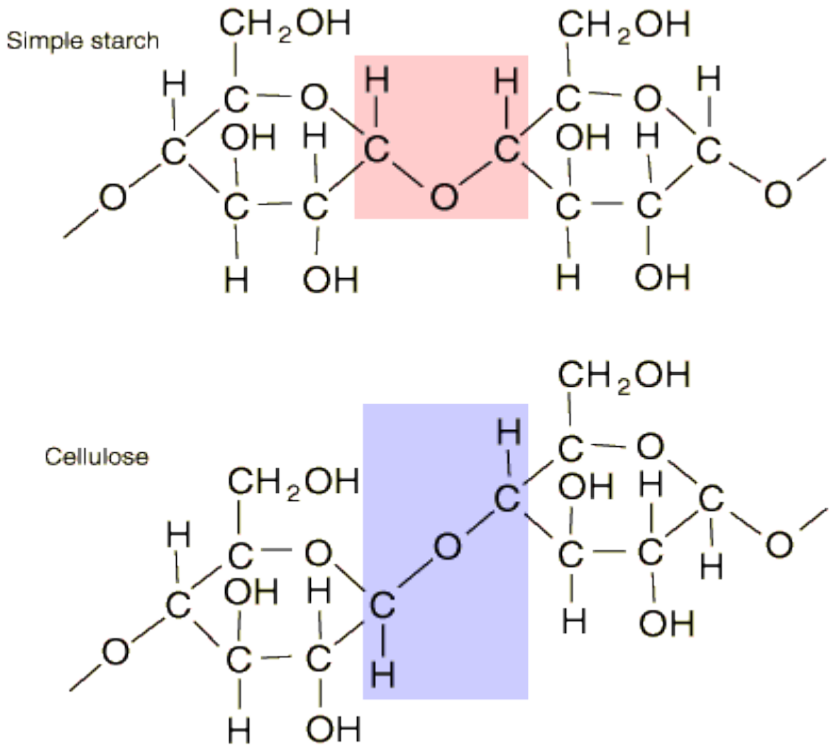



**Figure 3:** Example of a simple starch and a cellulose molecule. The simple starch is linked by an a-glycosidic bond and can be hydrolyzed by a-amylases, while the cellulose molecule is linked by a B-glycosidic bond and can be hydrolyzed by B-amylases. (149)

In the case of the microbiota however, bacterial genes coding for specific sets of enzymes allow for fermentation processes to break down the large fibers into smaller micronutrients. One key group of enzymes that makes digestion of various β-linkages possible is known widely as β-amylases[10]. These enzymes are able to break the various β-glycosidic linkages that make up indigestible, non-crystalline fibers such as cellulose, pectin, and starch[5]. Also, many bacterial families contain α-amylase enzymes not found in the human gene repertoire. For example, in *Firmicutes* there is an overexpression of 1,4-α-glucanohydrolases that allow for digestion of 1,4- and 1,6-α-linkages[39].

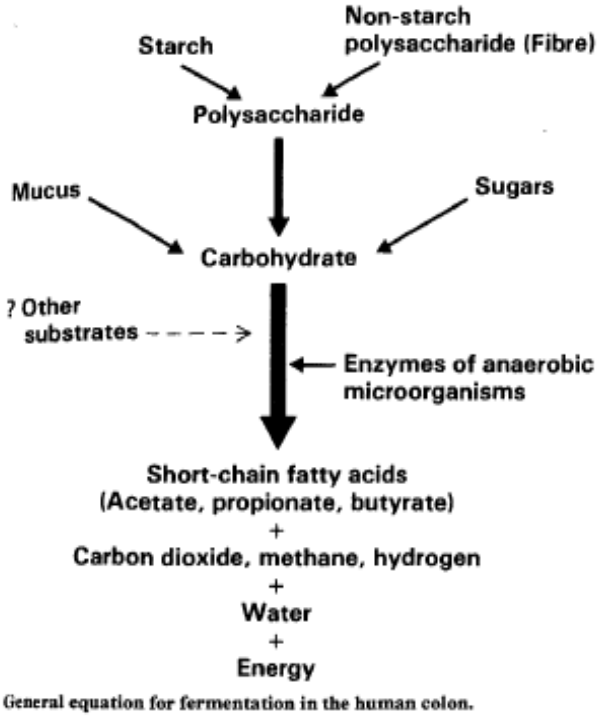



**Figure 4:** The general aerobic fermentation pathway in gut bacteria. Polysaccharides are converted into carbohydrate polymers and ultimately into various metabolites, some of which are released into the gut lumen. (148)

The process of fermentation within bacterial cells is complex and varies from species to species[11,12], with different classes specializing in the metabolism of different fibers ranging in complexity. In some species that are able to breakdown intact plant cell walls for example, there is a cellobiose phosphorylase complex located within the bacterial membrane[17]. This complex allows for the phosphorolytic cleavage of cellobiose and cello-oligosaccharides found in the structures of plant cell walls. Cleavage of these complex, fibrous carbohydrates results in the glucose and glucose-1-phosphate being taken into the cell as the complex cellulobiose is broken down in the enzyme

complex. This product is then ready to be used in a number of metabolic pathways to meet the current demands of the cell. Bacteria containing this form of cellobiose degradation are not typically found within the human gut, but can be found in the GI tracts of ruminants that digest larger, more complex fibers in their diets. For example, *Ruminococcus flavefaciens* is often found within ruminants[16].

Regarding other, non-ruminant organisms such as humans, the digestion of complex, crystalline oligosaccharides found in intact plant cell walls is not usually possible. This is because of the physiological limitations of a non-ruminant, quick moving, single chambered, gastrointestinal (GI) tract [43, 44]. Although intact plant cell walls of a highly complex nature are not digestible in non-ruminants, there are many dietary fiber sources of a slightly less complex nature that are. In general, the fiber macromolecules are broken down by bacterial β- and α-amylases, or other bacterial enzyme complexes. Once digested, theses fibers become single monomeric glucose or fructose molecules within the bacterial cell. After the constituent molecules of the fibrous carbohydrates are obtained, the fermentation process now continues by further catabolism of these simple sugars, until finally the desired end product is obtained. The end product of fermentation is dependent on the classes of bacteria metabolizing the sugars, and the fiber substrates being metabolized. In a majority of cases, the end product is a SCFA and ethanol, plus energy in the form of adenosine triphosphate (ATP) to be utilized by the bacterial cell. All of the end products are used or released by the bacteria into the gut lumen where they are absorbed by the host to be used as fuel or act as messenger molecules[7]. In general, fermentation rates are regulated by the rate at which complex polymers are depolymerized[9], showing the importance of unique, bacterial amylases in the fermentation pathway.

In the general non-ruminant gut, there is often a complex system of cross feeding between bacterial species[11, 12], as described in Figure 5. Many organisms gain metabolic substrates from the products released by other bacterial residents of the gut[11, 12]. Also, this cross-feeding allows many organisms to feed on the waste products of other organisms, causing removal of these waste products from the environment. This in turn thermodynamically promotes the quicker metabolism of organisms higher in these cross-feeding pathways [12, 15]. A few bacterial classes shown to be found in the GI tract of non-ruminants include *Firmicutes*, *Flavobacteria*, and *Bacteroidetes*, all of which contribute to the breakdown of pectins, xylans, and other plant polysaccharides[13,14]. All of these cross-feeding interactions allow for complex microbiota:microbiota interactions. However, this area of microbiota research is not well studied, as the focus of many projects is geared toward the discovery of host:microbiota interaction pathways.

**DESCRIPTIONS OF SCFAs PRODUCED BY FERMENTATION IN THE GUT**

Within the category of SCFAs, there are a few important SCFAs produced through bacterial fermentation in the gut[144]. These SCFAs have varying physiological effects on the body, and include acetate, propionate, and butyrate. Most studies to date have focused on these three SCFAs and their effects on the body's physiological state. Acetic acid is a molecule found largely in vinegar, and it is also produced within the gut by multiple bacterial species. Acetic acid is a major player in carbohydrate and fat metabolism through the tricarboxylic acid (TCA) cycle and other catabolic and anabolic processes. This is because it is often found bound to CoA in the form of acetyl-CoA[27], an intermediate substrate used in many metabolic pathways. The 3 carbon propionic acid is produced via bacterial fermentation in the gut and plays a role in the catabolism of odd chain fatty acids[28]. Also, a disease state resulting in the buildup of propionic

acid is inducible by a mutation in an essential enzyme in odd chain fatty acid oxidation, propionyl-CoA carboxylase[29]. The final SCFA found in high concentration in the body and produced by bacterial fermentation is the 4 carbon butyrate. Naturally occurring in plant and animal fats, butyrate is a colorless liquid and is produced through anaerobic fermentation in the GI tract.

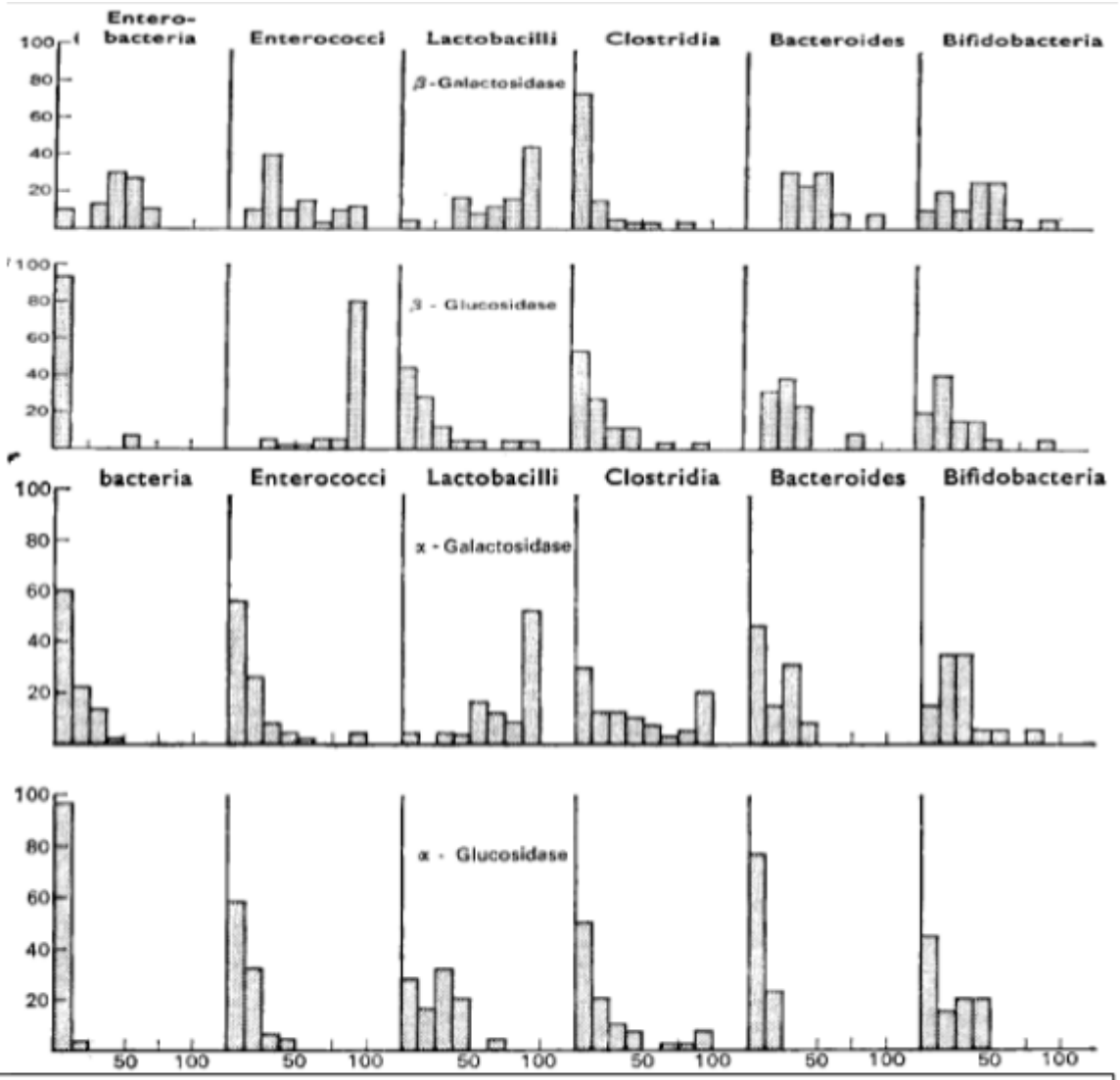



**Figure 3**: Relative enzyme activities in various bacterial phylum located in the gut. Enzymes included are B-galactosidase, -glucosidase, and –glucuronidase, a-galactosidase, and –glucosidase. Relative enzyme abundance correlates with ability of phyla to digest certain carbohydrates (55)

**SCFAs NOT PRODUCED IN GUT FERMENTATION**

The other few SCFAs not mentioned above do not come mainly from bacterial gut fermenetation, but are found throughout other sources. The physiological effects of these SCFAs is not completely understood, but some do play a role in different disease pathologies [30, 31]. The smallest of these SCFAs (1 carbon formic acid) plays very little role in the body in relation to the microbiota, as it is not present in detectable physiologic amounts unless a diseased state of methanol poisoning is detected[30]. The other few SCFAs include isovaleric acid, valeric acid, and 2-methylbutanoic acid. Isovaleric acid is found in plants and essential oils, and implicated as the poisionous buildup product resulting from the improper metabolism of leucine due to an autosomal mutation[31]. Valeric acid is found in perennial flowering plants, and 2-methylbutanoic acid is found in animal fats, but neither fatty acid is well studied.

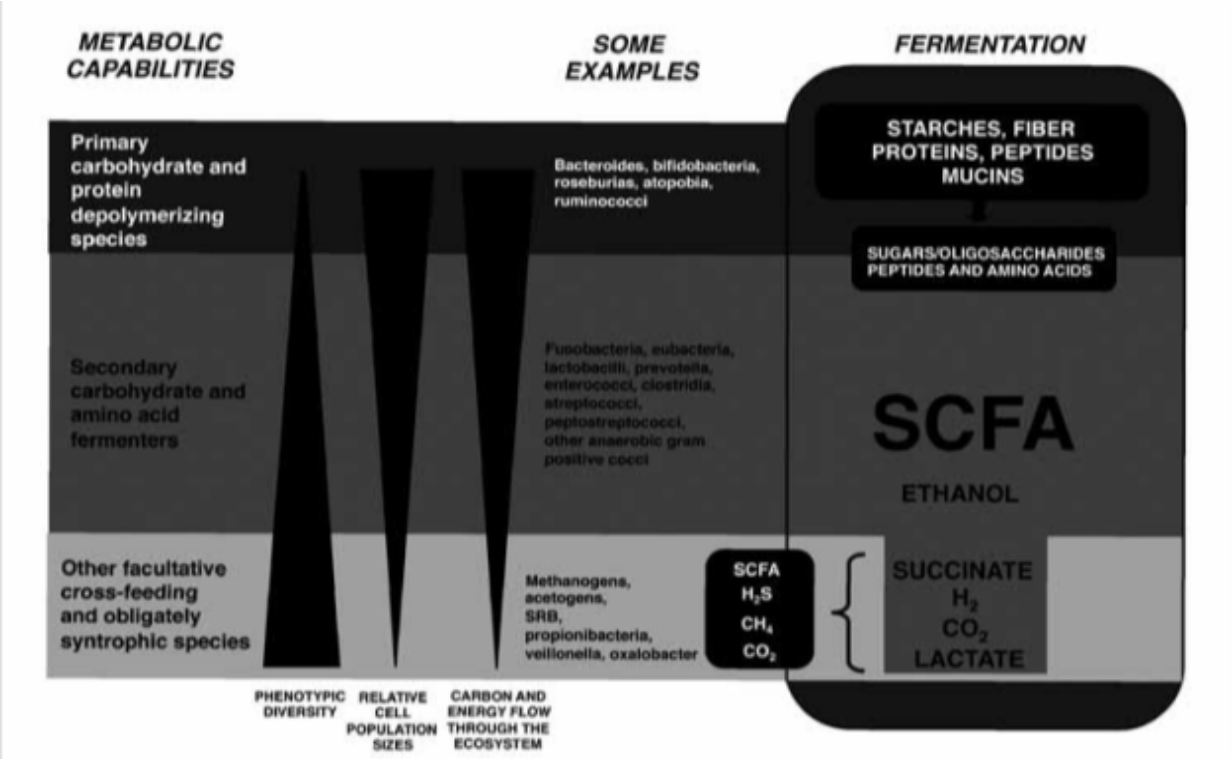



**Figure 5**: Cross-feeding among bacterial phyla in the gut allows for the digestion of complex, dietary carbohydrate polymers. Primary depolymerizing species break down starches, fiber, proteins, and mucins. Secondary fermenters create SCFAs from the products of primary depolymerizers. All other species convert remaining metabolites into basic molecules that are excreted from the cell and either absorbed by the host or used by other species higher in the depolymerizing chain. (9)

**SOURCES OF SCFAs IN THE BODY**

As previously mentioned, SCFAs are produced as the result of bacterial fermentation of various nutrient sources. These sources include mostly indigestible or undigested carbohydrates that reach the distal small intestine and colon in the forms of starches, non-starch polysaccharides, and non-digestible oligosaccharides[32]. Some proteins are also used as fermentation substrates once they have been broken down into their constituent amino acids. The SCFAs produced by fermentation are used by endothelial colon cells as fuel, making only small amounts present in circulation. This is especially true in the case of butyrate, as it is used up as a major fuel source for endothelial cells before it can reach systemic circulation[33].

An alternate source for the production of SCFAs in the body is the oxidation of LCFAs during times of starvation through fatty acid oxidation pathways[28]. Also, SCFAs can be generated in times of energy abundance through fatty acid synthesis by the use of amino acid and glucose based anabolic pathways[28, 34]. This is evident in times of fasting when fatty acid oxidation raises the levels of Ac-CoA and ketone bodies present in circulation.

Because there are multiple sources of SCFAs in the body, each SCFA is described as exogenous or endogenous. This is done in order to describe whether or not the SCFA was made by host or microbiota processes. All SCFAs produced in the gut are referred to as exogenous, and all SCFAs produced through cellular pathways are referred to as endogenous[35]. There has only been a small amount of research in the areas of SCFA production within the body, and more research into this area is needed to better understand the effects of endogenous SCFA production. For this review it is more important to note the production of exogenous SCFAs by bacterial fermenters.

**DIFFERENT BACTERIA PRODUCE DIFFERENT TYPES OF SCFAs DEPENDING ON DIETARY INTAKE**

As mentioned above there are varying types of SCFAs produced by bacterial fermentation in the gut. The major three SCFAs that remain the focus of a majority of studies are acetate, butyrate, and propionate. It has been shown that the type and amount of SCFAs produced in the gut is dependent on the microbiota composition and the dietary intake, both of which exist in a dynamic nature[9]. Because of this dynamic, co-dependent nature, SCFA production varies between individuals. For example, according to MacFarlane et al. the end products of fermentation are dependent on the rates at which complex carbohydrate polymers can be degraded into constituent molecules that the bacteria can further process[36]. Also as described by MacFarlane et al, the catabolites supplied to bacteria in the diet can cause expression of catabolite regulatory mechanisms among the different bacteria[37]. This means that the bacteria are able to selectively ferment certain substrates depending on the environment. The production of reduced fermentation products (i.e. lactate, succinate, and butyrate) stems from the need to maintain a redox balance during fermentation. ATP generation is then linked to the formation of more oxidized compounds further down the fermentation pathway[38].

As described in Fig 6, some studies have shown that different bacterial species produce different end product SCFAs . For example, groups within the *Bacteroides, Bifidobacteria, Lactobacilli, Clostridia,* and *Enterobacteria* typically produce acetate as an end product during fermentation. Propionate production is favored by members of *Bacteroides* and *Clostridia*, while Butyrate is favored by *Faecalibacteria*. The production of Lactate is favored by a large number of groups, including *Bifidobacteria, Bacteroides, Enterococci,* and *Faecalibacteria*, just to name a few. A more detailed list of FAs favored by certain bacterial groups is found in Fig 6.

Although capable of producing a larger number of FAs than those listed, these are the typical FAs favored by various fermentative bacteria.

Another group has compiled data as to the effects of various carbohydrates on the presence of SCFAs in the gut. These carbohydrates are widely known as prebiotics, and are defined as dietary components (carbohydrates, vitamins, fats) that induce a compositional or metabolic change in the microbiota. This diet dependent change in SCFAs produced indicates a change in the fermenting products of the gut bacteria as well. For example, Flint and Bayer have shown that SCFA ratios can be mediated by introducing various types of prebiotic sources[145]. In agreement with Flint and Bayer, Abbeele et al have also provided support for the prebiotic meditated microbiota metabolic changes[46]. In their work, introduction of long chain arabinoxylans (LC-AX) and inulin (IN) to humanized rat models was shown to select for propionate and butyrate SCFAs. AX alone has also been shown to increase propionate levels in vitro[45,46], as well as in vivo [47], while IN typically increases butyrate levels[46]. An increase in concentrations of these two SCFAs was linked to increased levels of SCFA producing bacterial groups[46].

As described in other studies, the increases described above are explained by the effects of the dietary pre-biotic fibers on the relative populations of specific groups of bacteria. For example, butyrate production is favored by Roseburia intestinalis[48], Eubacterium rectale[49], and Anaerostipes caccae[50]. In contrast, some studies report no increase in propionate producing bacteria to explain the increase in propionate production upon introduction of AX51.

| Acetate | Propionate |
|---|---|
| Bacteroides | Bacteroides |
| Bifidobacteria | Propionibacteria |
| Eubacteria | Veillonella |
| Lactobacilli | Clostridia |
| Clostridia | Prevotella |
| Ruminococci | Porphyromonas |
| Peptostreptococci | Megasphaera |
| Propionibacteria | |
| Veillonella | |
| Fusobacteria | |
| Butyrivibrio | |
| Peptococci | |
| Streptococci | |
| Enterobacteria | |
| Atopobium | |
| Enterococci | |

| Butyrate | Lactate[a] |
|---|---|
| Roseburia | Bifidobacteria (L) |
| Faecalibacteria | Bacteroides (D) |
| Clostridia | Peptostreptococci (L) |
| Eubacteria | Lactobacilli (D/L) |
| Fusobacteria | Eubacteria (L) |
| Peptostreptococci | Ruminococci (L) |
| Butyrivibrio | Fusobacteria (L) |
| Peptococci | Enterococci (L) |
| | Clostridia (L) |
| | Peptococci (L) |
| | Streptococci (L) |
| | Enterobacteria (L) |
| | Faecalibacteria (D) |
| | Atopobium (L) |

**Figure 6**: Different bacteria produce different types of SCFAs during fermentation. (9)

**SCFAs, THE MICROBIOTA, AND DISEASE**

Much of the research relating to the microbiota has been aimed at understanding how the microbiota is involved in the pathogenesis or treatment of disease through production of SCFAs. This is because in a majority of cases, it has been shown that the microbiota interacts with the body mainly through its metabolites, although specific mechanisms by which this occurs are still under research. These metabolites, such as SCFAs in particular, are released from gut bacteria and interact with endothelial colon cells before being absorbed into systemic circulation, where they then interact with the body further. It has also often times been shown that there are strong differences of the microbiota in healthy and diseased patients. For example, research has shown for quite some time that microbial composition of the microbiota varies between obese and lean, diabetic and non-diabetic, and other healthy and diseased states[61, 124]. Until recently however, even though there are clear differences in the microbiota compositions between individuals, it is not known whether the microbiota composition is the reason for the change, or if the body's state of health is influencing the microbiota instead.

Current research reported on in this review shows that different types of SCFAs produced by fermentation in the gut have different effects on the body. Also, the types of SCFAs produced in the gut are dependent on the ratios of microbial species in the gut. This allows the conclusion to be drawn that the microbiota influences the body's state of well-being by producing varying types of SCFAs that affect the body in different ways. However, this is only one way in which the microbiota may influence the body. More research is needed to determine what other ways the microbiota influences the body. However SCFAs, and their effects on the body in relation to the microbiota, is the focus of this review.

Cellular receptors are important in the body for the recognition of SCFAs. A majority of the receptors that respond to SCFAs are in the category of G-protein coupled receptors (GPCR)[64]. These receptors are defined by the presence of a 7-transmembrane portion, linked to an internal multimeric G-protein[146]. Generally, the 7-TM portion binds an external ligand, which triggers a conformational change in the internal G-protein. This conformation change causes the inactive G-protein to release bound guanosine di-phosphate (GDP) and to bind guanosine tri-phosphate (GTP). By releasing the inhibitory GDP and binding the activating GTP ligand, the G-protein sheds its B and y subunits. Once this occurs, the α-subunit is able to continue signal transduction through a varying number of steps.

For example, in some pathways the activated α-subunit goes on to activate adenylate cyclase. Adenylate cyclase is an enzyme involved in producing the messenger molecule cyclic amp (cAMP) from ATP. cAMP is then used to initiate a large number of cellular processes. GPCRs are among the most important receptors in a cell. This fact is supported by the large part of the genome dedicated to GPCRs[83].

**SPECIFIC GPCRs for SCFAs**

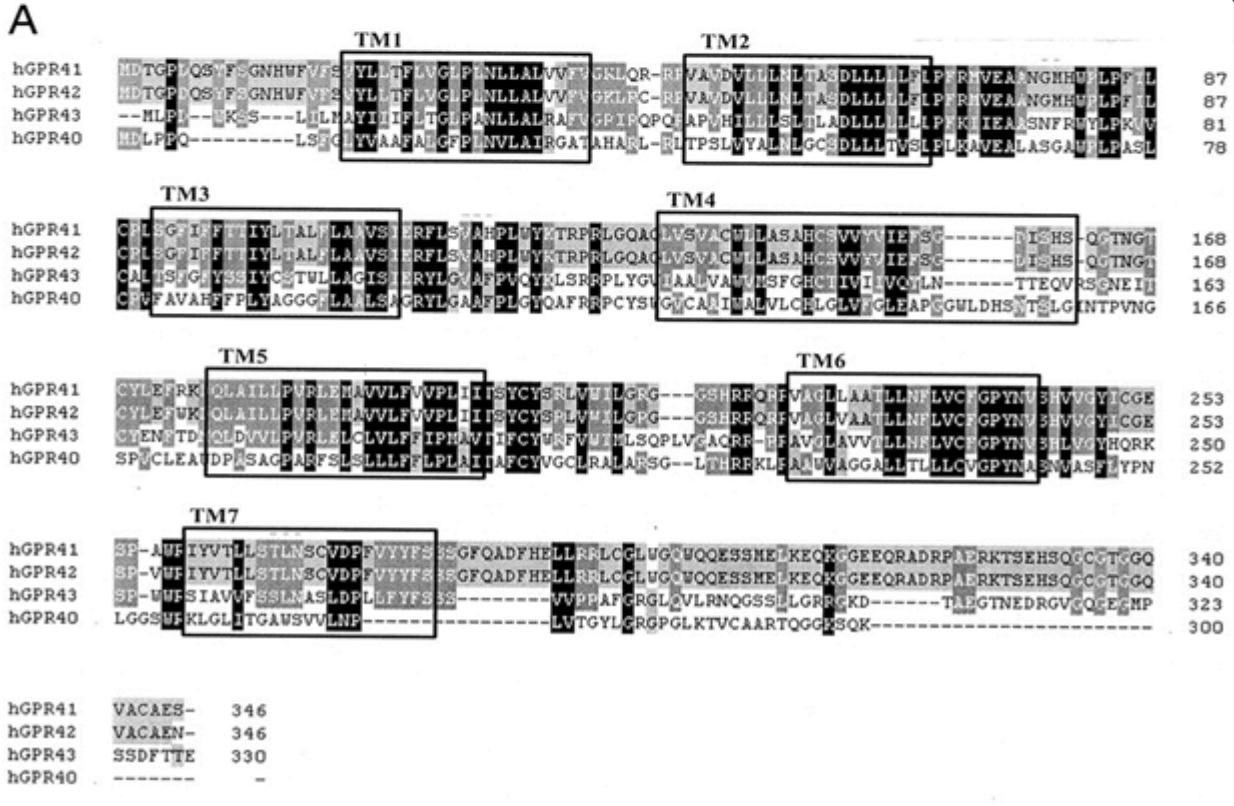
The cellular GPCRs that respond to FAs include GPCR120, 119, 84 and the GPCR40 family, containing GPCR40-43. GPCR 41 and 43 are specific for SCFAs with 6 or less carbons including acetate, propionate, butyrate and lactate[79]. GPCR120 and 40 have high affinity for long and medium chain fatty acids with 6-12 or more carbons[80,81,82], while GPCR119 is activated by only long chain fatty acids. GPCR84 is only specific for medium-chain fatty acids with between 6 and 12 carbons.

**FFA sensing GPCRs and their cognate ligands**

| GPCR | Ligands |
|---|---|
| GPR40 | Long chain fatty acids (C12-C16) |
| GPR41 | Short chain fatty acids (C3-C5) formate, acetate, propionate, butyrate and pentanonate[89] |
| GPR43 | Short chain fatty acids (C2-C3) formate, acetate, preferentially propionate, butyrate and pentanonate[89] |
| GPR120 | Long chain fatty acids (C14-1C8) Omega 3 fatty acids, EPA, DHA, palmitoleic acid, α-linolenic acid (ALA) |
| GPR119 | Oleoylethanolamine and *N*-oleoyldopamine |
| GPR84 | Medium chain fatty acids, Capric acid (C10:0), undecanoic acid (C11:0), and lauric acid (C12:0)[62] |

**Figure 7A**: Different GPCRs have higher affinities for some fatty acids over others. For example, GPCR41 has high affinity for 3-5 carbon fatty acids. **7B**: The amino acid sequence for the GPCRs 40-43. (24,61)

A



## LOCATIONS OF SPECIFIC SCFA RECEPTORS

FA specific GPCRs are located on various cell types throughout the body. These include macrophages, adipocytes, pancreatic β-cells, and endothelial cells in the GI tract. The class of receptor present varies depending on the cell type. For example, GPCR41 is highly expressed in immune cells, such as neutrophils and monocytes, while it is also observed in adipose tissues[84], the distal colon[85,86], and heart and skeletal muscle[83,87]. GPCR43 is expressed in similar tissues included adipose, spleen, and immune tissues[83]. Also, it has recently been discovered that pancreatic beta cells express both GPCR41, and 43[88,89,90].

## SCFA ACETATE

One of three major SCFAs linked to the microbiota: host interaction scheme is acetate. Acetate has been shown to induce many different effects upon the body including modulation of the immune system, colonic function, and adipogenesis. Acetate has also been linked to carcinogenesis in some studies through its effects on the immune system.

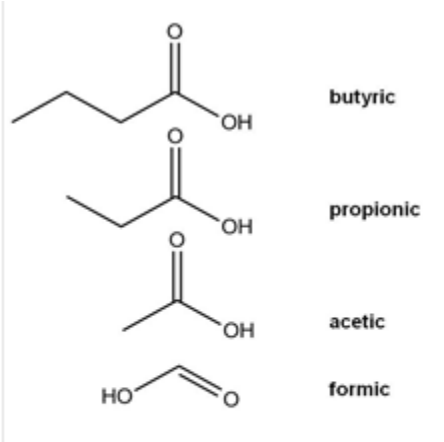



**Figure 8**: Examples of 4 SCFAs that are the focus of a majority of research.(144)

In relation to the immune system for example, acetate interacts with GPCR43 and 41 on immune cells[62,63], but is a more potent agonist to GPCR43[65]. Also, acetate binds GPCR43 of leucocytes[64]. Binding of acetate to GPCR43 has been shown to initiate inositol 1,4,5-triphosphate formation, raise intracellular $Ca^{2+}$ levels, activate extracellular signal-related kinases (ERK ½) pathways, and inhibit cAMP accumulation in cells[65]. These responses have been linked to the exposure of human neutrophils to chemo-attractants. Inositol triphosphate (IP3) and diacylglycerol (DAG) are formed from cleavage of

phosphatidylinositol 4,5-bisphospahte (PIP2) upon acetate binding. IP3 triggers increases in intracellular levels of Ca2+ by activation of phospholipase C (PLC)[66,67,68]. Increased Ca2+ levels have been linked to neutrophil exposure to chemo-attractants, such as LPS[66,67,68]. Activation of ERK kinases by mitogen activated phosphokinases (MAP-kinases) has been shown to be linked to production of interleukin (IL)-8 pro-inflammatory cytokines in neutrophils[69]. All of these responses contribute to inflammation and neutrophil activation in the gut in response to acetate.

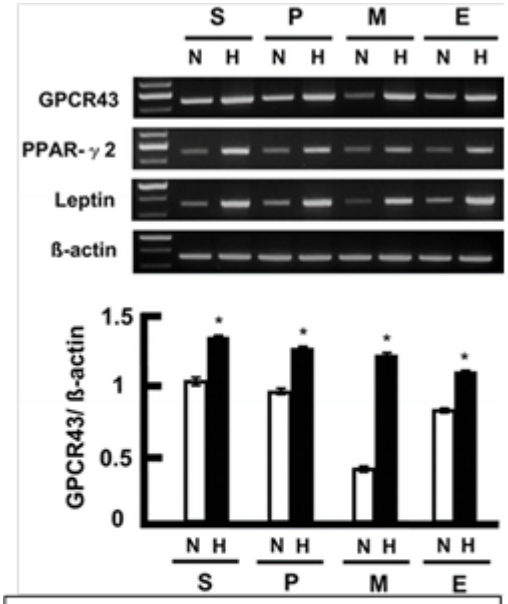



**Figure 9**: GPCR43 activity was upregulated in mice fed a high fat (H) diet vs. a low fat (N) diet. Levels of GPCR43, PPAR-γ2, Leptin, and B-actin were all increased in mice fed H diets (75)

In addition, acetate reduces the release of LPS-stimulated tumor necrosis factor-α from neutrophils[70]. Tumor necrosis factor (TNF)-α is a pro-inflammatory cytokine produced mainly by macrophages, but also produced by many types of immune cells during the acute immune response[70]. Inhibition of its release by acetate supports the conclusion that acetate has anti-inflammatory effects. Also, acetate is involved in the inhibition of TNF-α mediated activation of the NF-kβ pathway[62]. This also contributes to prevention of inflammation as a result of inhibition of the inflammatory effects elicited by the nuclear factor (NF)-kβ pathway, a pathway involving the NF-kβ transcriptional activator of inflammatory genes being released from its inhibitor co-factor, I-kβ[72,73]. Also, acetate was shown to inhibit release of IL-6, a 26 kDa glycoprotein with pro-inflammatory effects [64,74].

Acetate has also been shown to effect colonic function by modulating the frequency of longitudinal muscle contractions in colonic smooth muscle[75]. This was shown to reduce the transit time of food boluses and increase digestion[75]. It was shown that acetate facilitated decreased frequency of spontaneous colon contractions by inhibiting the enteric nervous system through interactions with nicotinic and 5-hydroxytryptamin-3 (5-HT-3) receptors in colonic nerves[75]. The interactions of acetate with these two receptors is supported by studies showing that enteric neurons in the myenteric plexus (involved in colonic peristalsis) are stimulated to release acetylcholine by high levels of 5-HT in the colon stimulated by introduction of SCFAs[125,126].

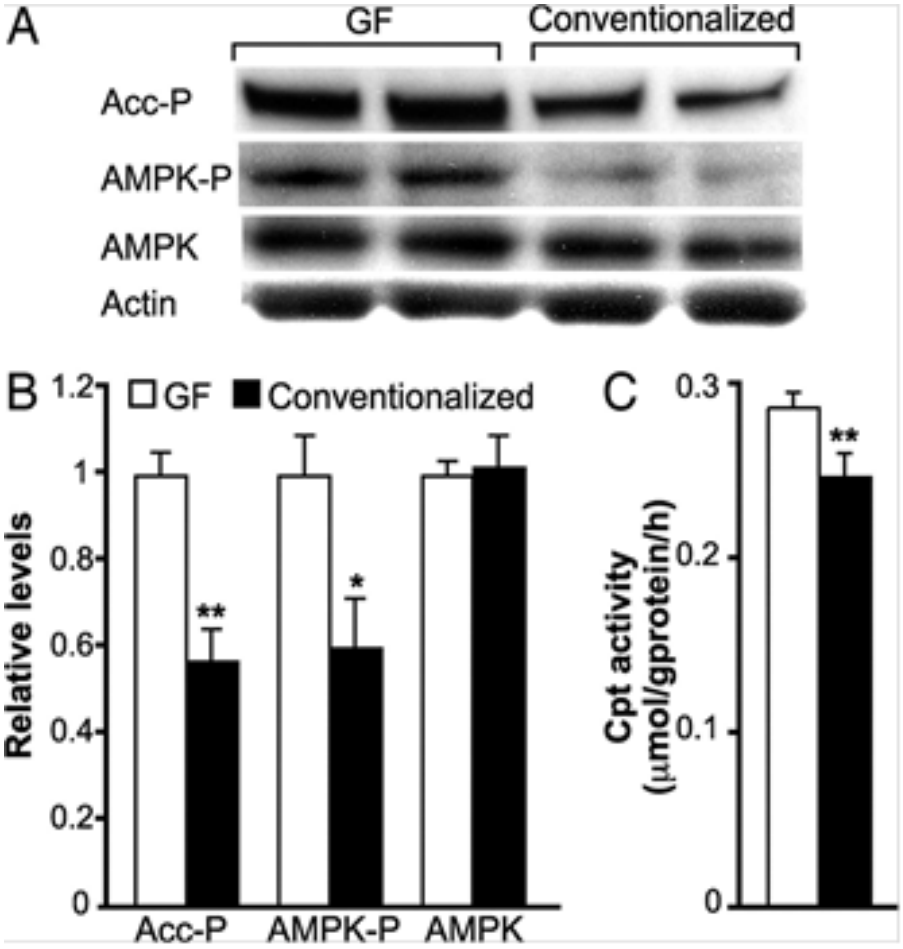



**Figure 10**: AMPK mRNA and enzyme concentrations are increased in GF vs. conventionalized mice.(55)

Acetate has been linked to the proliferation of normal crypt cells in the colon[91]. In addition, this increased proliferation may lead to increased resistance to leaky gut and inflammation in the colon due to a healthy number of new

endothelial cells that strengthen the gut barrier and prevent migration of bacteria from the gut lumen to systemic circulation[91].

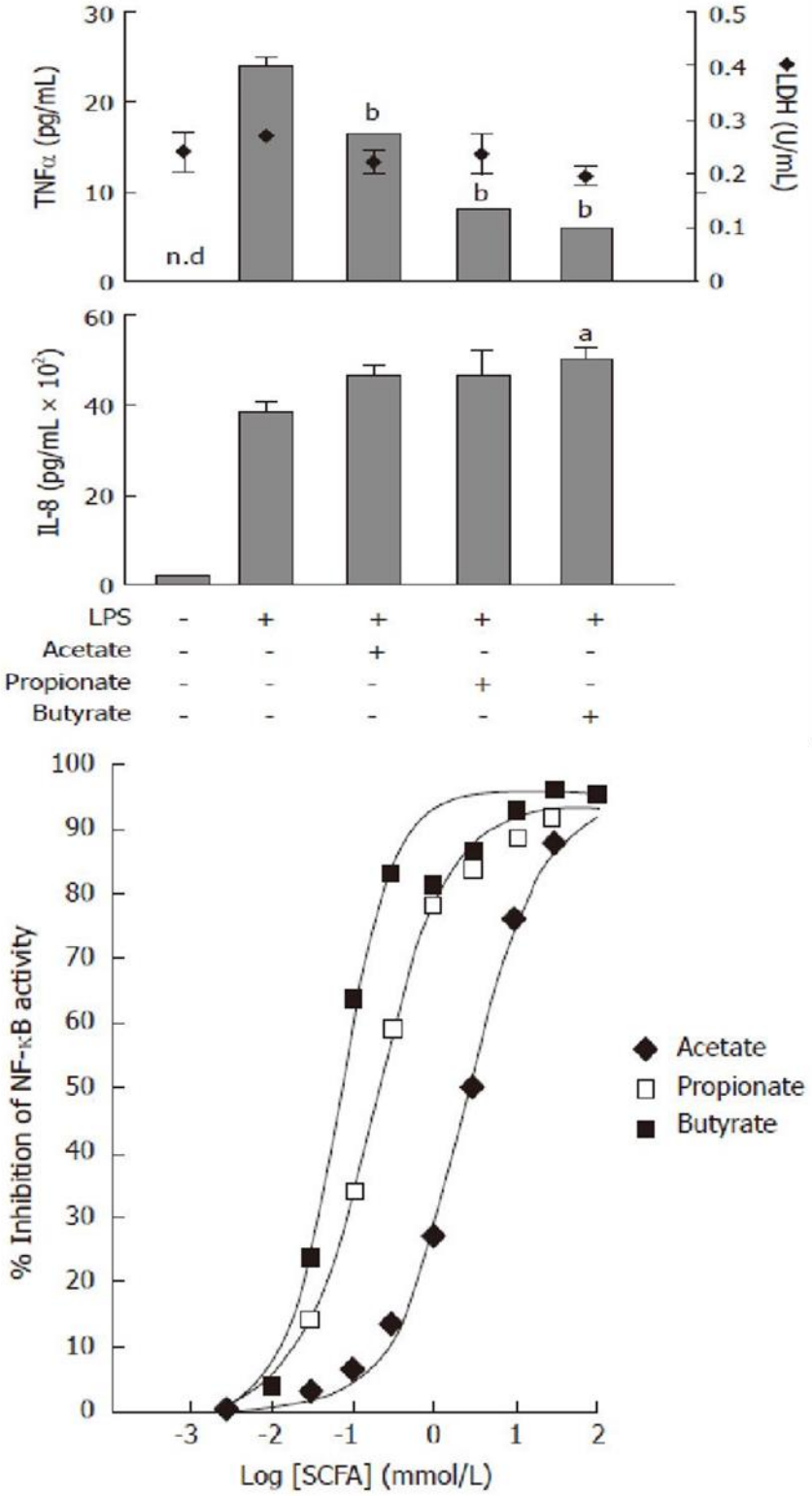



**Figure 10A**: The concentration of LPS induced TNF-a (pg/ml) was decreased in mice fed a SCFA supplemented diet. **B**: The concentration of LPS induced IL-8 (pg/ml x $10^2$) was increased in mice fed a SCFA supplemented diet. **C**: % inhibition of NF-kB transcriptional activator by addition of 3 different (61)

Acetate has also been shown to stimulate adipogenesis by its interactions with GPCR43[84]. Although the exact mechanisms of this action are not understood, expression of GPCR43 mRNA in adipocyte tissues was stimulated by addition of acetate and other SCFAs in mice models[84]. This up regulation of GPCR43 genes is hypothesized to allow for relief of repression on nearby

lipogenic genes, resulting in increased lipid synthesis and storage[84].

**SCFA PROPIONATE**

Propionate and its effects on the body are another primary focus of many research articles. Propionate has been linked with modulatory effects on many bodily systems. These effects include immune-modulatory, carcinogenic, metabolic, endocronological, neurological, and functional effects.

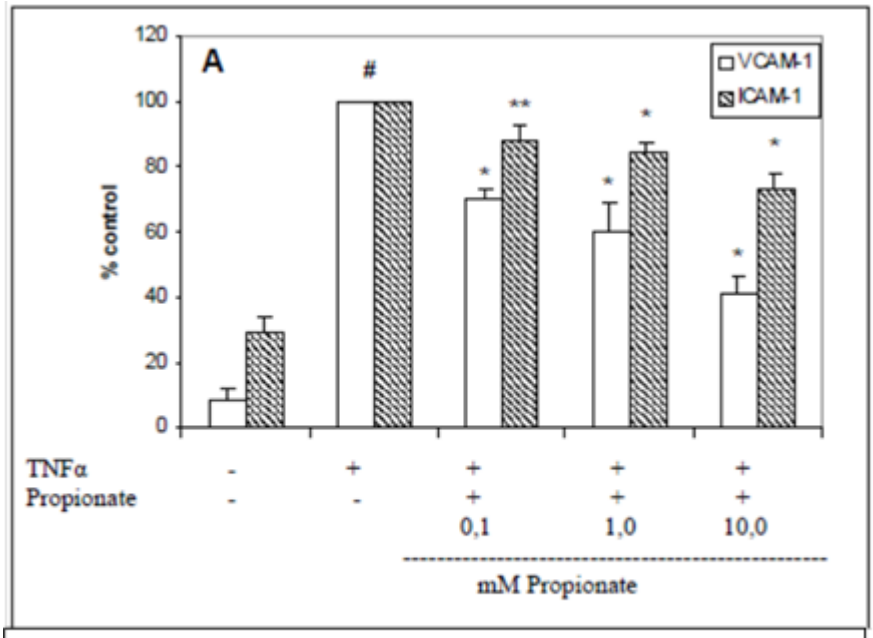



**Figure 11**: TNF-a induced expression of VCAM-1 and ICAM-1 was reduced in mice fed a diet supplemented with propionate. (78)

Like acetate, propionate has been shown as an agonist for both GPCR41 and GPCR43[62, 63] found on immune and adipose cells, although propionate elicits stronger effects at lower concentrations than acetate[65]. As described above, this stimulation triggers increases in cellular IP3 and $Ca^{2+}$ concentrations, and activates ERK ½ pathways while inhibiting cAMP degradation[65,66,67,68]. Many of these intracellular responses also occur in the case of immune cell interaction with homing chemokines. Therefore, it has also been shown that propionate elicits a similar effect as do chemokines released during the innate immune response, as both chemokines and SCFAs are recognized by GPCRs[127].

However, propionate has been shown to inhibit the effects of LPS-stimulated TNF-α production in neutrophils[70], indicating an anti-inflammatory effect of propionate similar to that

of acetate's[70, 129]. This is because the inflammatory effects of TNF-α activating NF-kβ and production of IL-1, as described in the acetate section above, are inhibited by propionate inhibition of activation of NF-kβ, a transcriptional activator factor that stimulates inflammatory cytokine production[62, 70].

In addition to this, propionate was shown to inhibit the induction of cytokine-induced adhesion molecules expressed on endothelial cells[78]. Adhesion molecules are crucial in the inflammatory response of homing neutrophils[78]. Adhesion molecules inhibited included vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1)[78]. Both VCAM-1 and ICAM-1 expression on endothelial cells is triggered by vascular endothelial growth factor (VEGF) [130]. VEGF is stimulated by NF-kβ, which is stimulated by TNF-α, evidence which supports a link between propionate and inflammation reduction by inhibition of TNF-α[130].

A separate study supported the anti-inflammatory effects of propionate through use of propionate metabolites produced during metabolism of propionate itself. These metabolites inhibited cyclooxygenase[76], leading to a reduction in proinflammatory eicosanoids produced in inflammatory responses[77]. The cyclooxygenase enzyme converts arachindonic acid to endoperoxides, creating prostaglandins, thromboxanes and prostacyclin[131]. Also supporting the anti-inflammatory effects of propionate is the fact that cyclooxygenase is a known target for many non-steroidal, anti-inflammatory drugs[131].

Inhibin, another pro-inflammatory cytokine was shown to be reduced in adipose tissues by addition of propionate[18]. Inhibins are heterodimeric proteins with an 18 Kd α- and 14 Kd β-subunit. Inhibins have been shown to be involved in immunosuppression when coupled with TGF-β[132]. Propionate also functions to reduce proliferation of activated lymphocytes [92,93]. The

immunosuppressive effects of propionate were first hypothesized by observing recurrent infections in patients suffering propionic acidaemia, a disease characterized by accumulation of propionate[133]. Also, the effects of propionate on immunosuppression have been shown to be concentration dependent in vitro[134].

In addition to the effects propionate has on the immune system, it also acts on various metabolic pathways in-host. Cholesterol levels in blood were shown to be reduced in rats and pigs when their diets were supplemented with propionate[94,95]. However, no observed decrease in cholesterol synthesis was observed. Cholesterol concentrations were merely reapportionad to different tissues. For example, in pigs cholesterol was reconcentrated in back fat instead of hepatic circulation due to reduced cholesterol transport from peripheral tissues to the liver[94]. In rats, cholesterol was concentrated in the liver instead of in the blood[95]. Also, decreased plasma cholesterol concentrations were observed when cholesterol synthesis was shown to be inhibited in in vitro rat hepatocytes[96]. Another study found that propionate lowers blood glucose levels and increases triglyceride concentrations in the gut while decreasing high-density-lipoprotein concentrations [97].

These effects were compounded with inhibiting effects on lipolysis in adipocytes through propionate interactions with GPCR43[135]. Propionate was shown to induce activation of GPR43 by use of GPR43 knockout mice. This in turn led to decreased levels of plasma FFAs[135]. Adipose tissue was also affected by propionate by the upregulation of peroxisomal proliferator activated receptor (PPAR-γ2) [84]. PPAR-γ is a member of the peroxisome proliferator activated receptor class, a member of the nuclear receptor family. PPAR-γ is activated by ligand binding and triggers adipocyte differentiation and promotion of lipid biosynthesis. In conjunction with other studies, free fatty acids (FFAs) have been shown to be ligands of PPAR-γ[84].

In addition to its metabolic targets, propionate affects the neuroendocrine system as well. Propionate was shown to increase leptin production by stimulation of GPR41[92, 101,102]. Leptin, a hormone involved in feelings of satiety in humans and other mammals, has been correlated with obesity in mice and human models. This is a probable cause for the studies that found that leptin increases satiety[100], and reduces food intake in subjects[94, 95].

Another way in which propionate interacts in the body is by effects on colonic function. Propionate increases frequencies of contractions in longitudinal, colonic smooth muscle[75], thereby decreasing food transit time (Figure 12). Also, propionate has neurological effects. It was shown that propionate infusion increases brain phospholipid and acylcarnitine levels in rats[103]. This observation is in line with studies showing high levels of acylcarnitines linked to high rates of beta-oxidation[136]. Propionate also has been linked to states of neuroinflammation and oxidative stress in certain brain regions after intra-ventricular infusion[104].

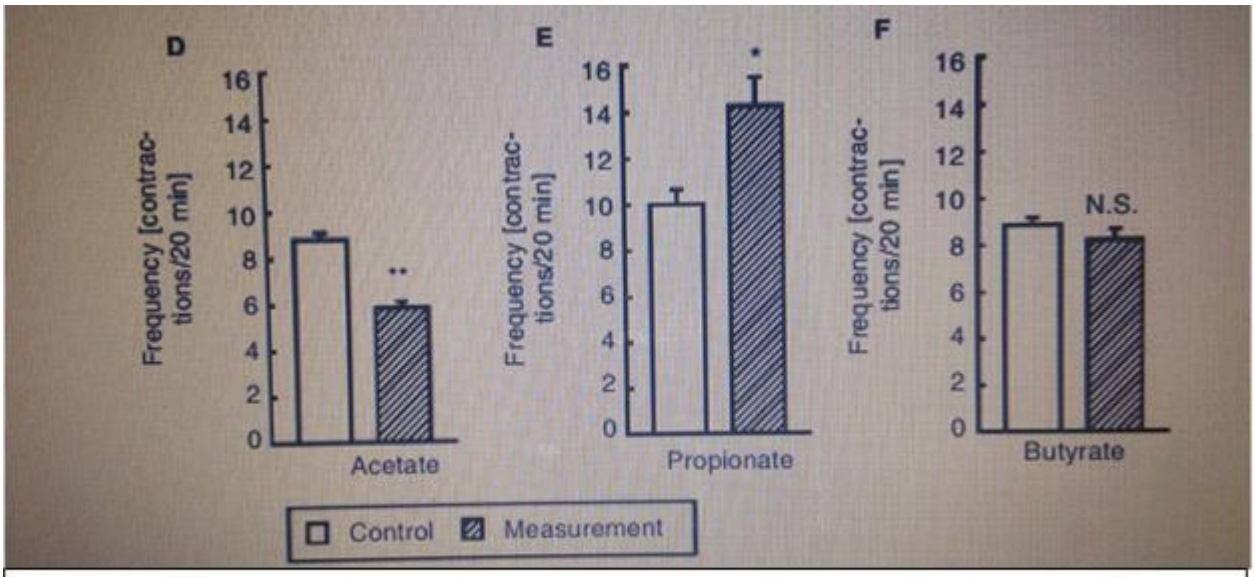



**Figure 12**: Effects of acetate **(D)**, propionate **(E)**, and butyrate **(F)** supplementation on the frequency of colonic smooth muscle contractions in the gut. (75)

**SCFA BUTYRATE**

The third major SCFA contributor to the host:microbiota interaction is butyrate. Produced in the colon through fermentation processes, butyrate effects many areas of the body similarly to acetate and propionate. Affected areas include the immune system and oxidative stress, gut barrier function, colon function, and insulin sensitivity.

Butyrate is the major fuel source for endothelial cells, and is found in small amounts in systemic circulation because a majority is used before being transported transluminally[137]. Butyrate, along with other SCFAs, has been shown to have anti-inflammatory effects. For example, butyrate prevents inflammation by activation of PPAR, and reduction in expression of IL-8 genes [105], while also suppressing IL-12 genes, and increasing IL-10 production in monocytes[109]. Butyrate also inhibits NF-kβ activation[106,107], while suppressing TNF-α in macrophages and monocytes by regulating messenger mRNA degradation[108]. IL-8 and IL12 are standard inflammatory cytokines produced in the immune response, while IL-10 is involved in regulation of NF-kβ, T-helper type 1 (Th1) cytokine production, and janus kinase-signal transduction activator of transcription (JAK-STAT) signaling pathways.

Another interesting way in which butyrate interacts with the body to decrease inflammation is by inducing apoptosis of activated and non-activated neutrophils[110]. The apoptotic effects of butyrate and other SCFAs are hypothesized to be a result of the histone deacetylase inhibitory effects of butyrate[138]. Butyrate was also shown to reduce neutrophils and lymphocytes invading the distal colon of ulcerative colitis patients[107].

In addition, butyrate reduces oxidative stress effects by protecting colonocytes from oxidative peroxide-induced DNA damage[111]. Also, butyrate increases glutathione levels[112], regulates fatty acid metabolism, electron transport, and oxidative stress pathways[113, 114].

Butyrate is also involved in barrier functions in the GI tract, specifically the colon. Butyrate is involved in the activation of mucin-associated genes (MUC1-4) in colon epithelial goblet cells[115]. Mucin genes regulate secretion of high molecular weight proteins known as mucins, which compose a majority of the mucus layer in the gut. The mucus layer is important in gut immunity[115]. In mice, butyrate enemas were shown to increase colonic expression of MUC1-4 genes, while reducing mucus layer thickness[116]. Butyrate also regulates tight junction zonulin and occludin genes[117]. Zonulin is involved in the modulation of tight junctions in the gut, and its dis-regulation has been linked to leaky gut syndrome[139]. Occludin has also been linked to the function of tight junction permeability[140]. The effects of butyrate have been shown to be concentration dependent, with high concentrations impairing proper function of intestinal barriers[118]. Butyrate dependent tight junction impairment has also been shown to contribute to the trans-locational potential of bacteria from the lumen to circulation[119].

In relation to the endocrine system, addition of oligofructose to the diet has been shown to increase satiety. This is most likely a result of conversion of oligofructose supplements into SCFA through bacterial fermentation[120]. In addition, butyrate has been shown to increase or decrease expression of certain peptides involved in appetite regulation, contributing to satiety. These include peptide YY (PYY) and proglucagon[120], as well as leptin[121]. Another factor that may contribute to butyrate's effect on promoting satiety is its function to reduce colonic smooth muscle contraction[123] and reduce transit time in the colon, as a result of interaction with enteric, colon neurons[122]. This would in turn increase feelings of fullness.

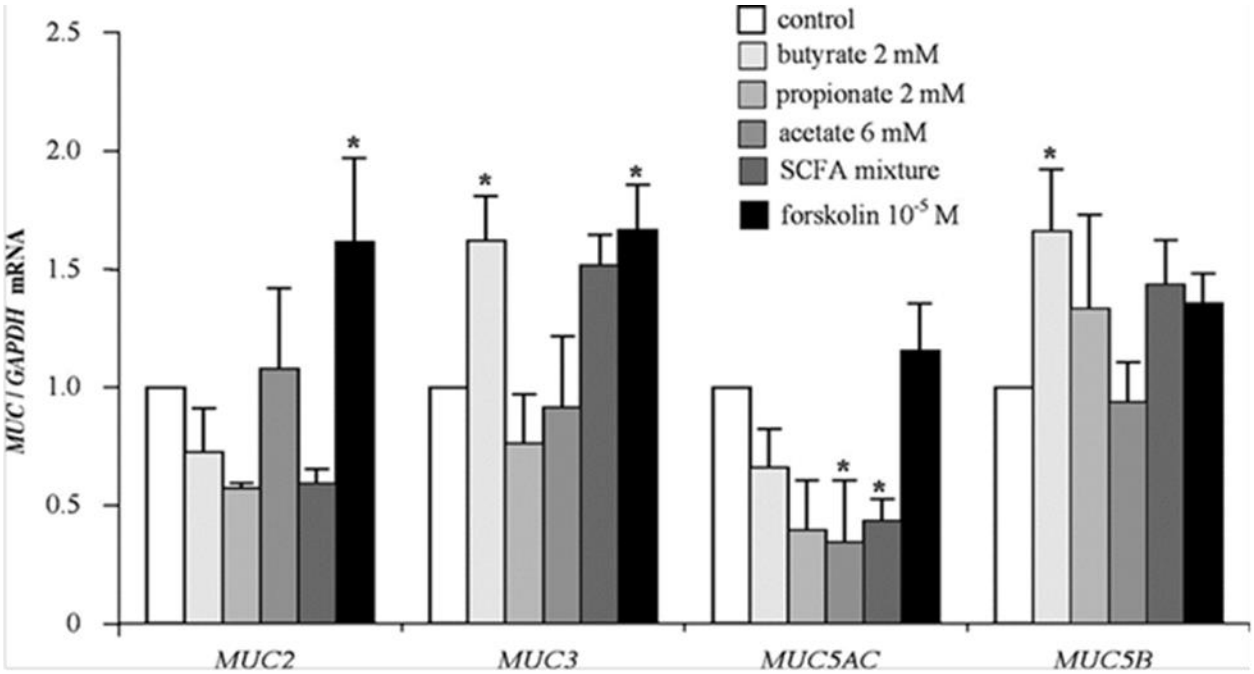
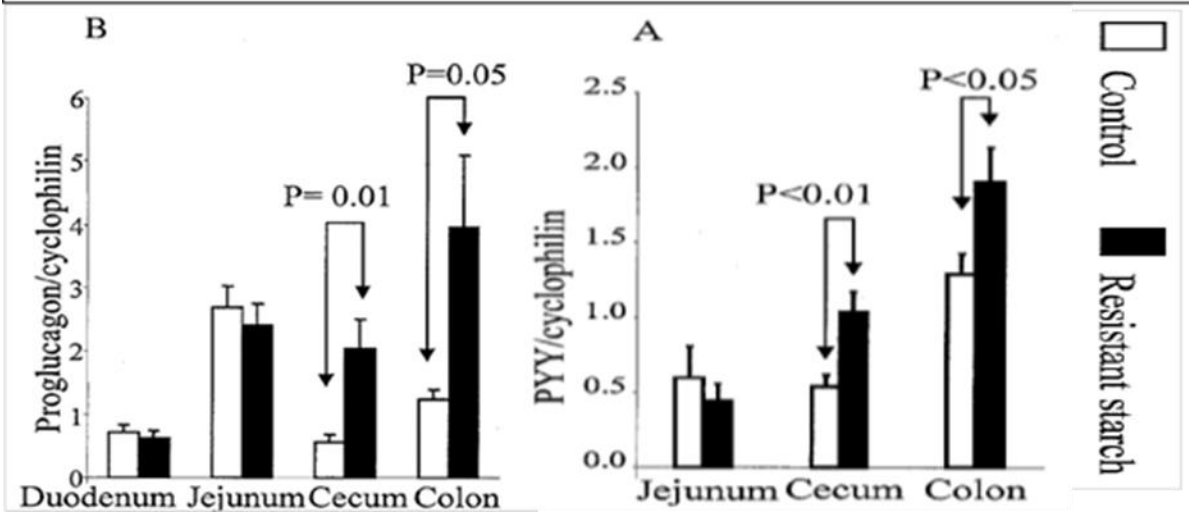



**Figure 13 (above)**: The effects of the addition of various SCFAs on MUC gene expression. (115)

**Figure 14 (below)**: The effects of a resistant starch supplement diet on the expression of **(A)** PYY and **(B)** proglucagon in mice models. (120)



**MICROBIOTA AND DISEASE**

The various effects of SCFAs on multiple bodily systems have been systematically linked to the pathogenesis and treatment of certain diseases. Most studies focus on the effects of SCFAs in relation to the development of obesity, diabetes, and general inflammation in the gut.

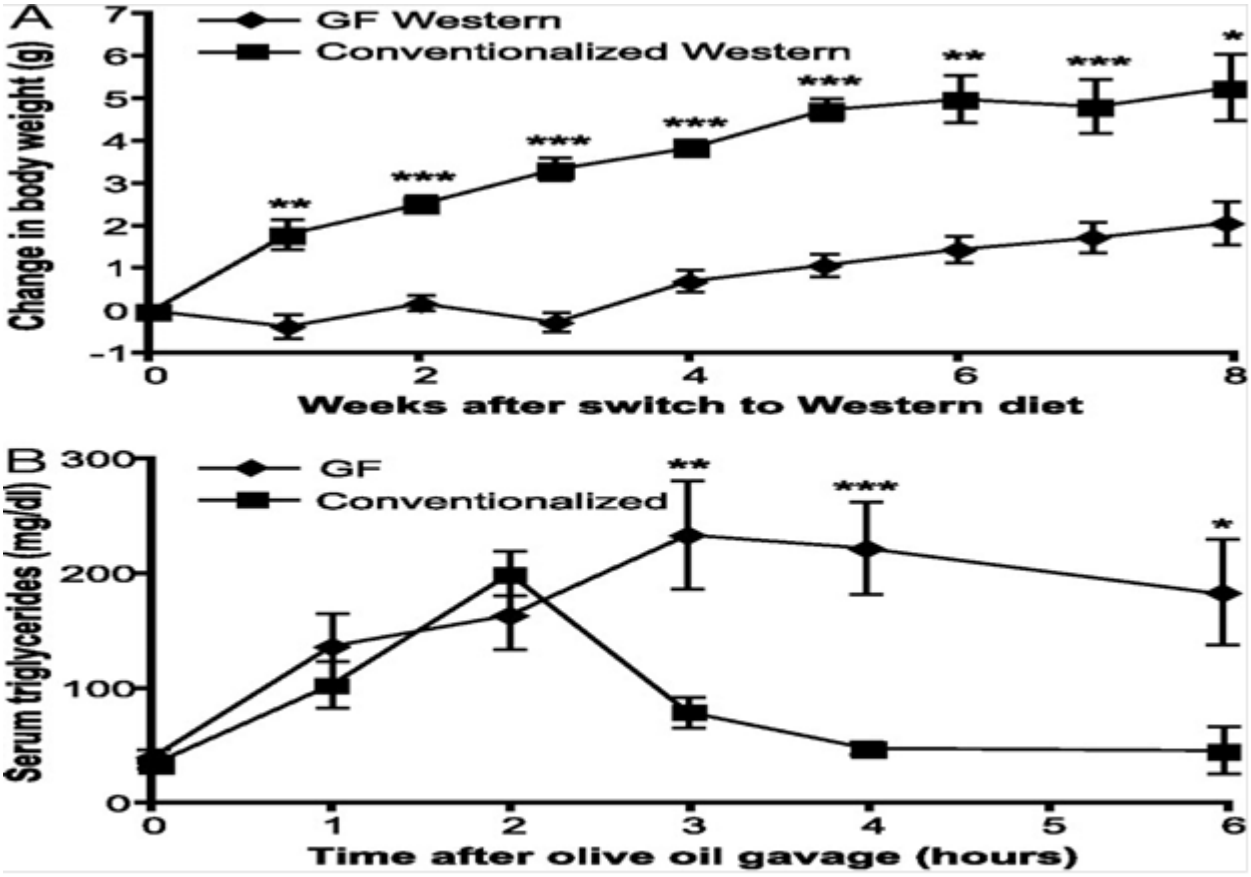



**Figure 14**: The effects of a western (high-fat) diet on (A) body weight (g) and (B) triglyceride levels of GF and conventionalized mice. (55)

For example, the microbiota has been linked to obesity. Obesity is already an established, major cause of health problems in western nations. But obesity is also a growing concern for the rest of the developing world, as rates of incidence rise rapidly due to high calorie diets becoming more readily available, and a more western diet being adopted[53]. Obesity is defined as an over deposition of lipid molecules within fat cells, as well as an overabundance of

production of new adipose cells[141]. Obesity has been linked to the pathogenesis of many other diseases including type 2 diabetes and heart disease[142]. Recently, research has provided a link between the gut microbiota and the development of obesity.

Backhed and Gordon et. al. were the first to discover this possible link when their lab showed that transplantation of microbiota from obese mice into germ-free (GF) mice caused the previously normal weight, GF mice to become obese, with an increase in body fat content of about 60% in just 14 days[54]. There were two possible hypothesis formed to explain the weight gain. The first was that the microbiota is able to facilitate improved digestion, and therefore increase the energy available to the host. If this facilitated digestion made the energy balance positive, this would lead to storage of the excess energy in the form of triglyceride deposits. This hypothesis was referred to as the energy harvest hypothesis[55].

The second hypothesis involved the microbiota mediated expression of certain signaling molecules. It has been shown that conventionalization of GF mice has a suppressive effect on the expression of fasting induced adipocyte factor (Fiaf) [55]. Fiaf belongs to the fibrinogen/angiopoietin like protein family and is expressed in adipose tissue and liver cells during times of fasting[58]. Fiaf is transcriptionally regulated by peroxisome proliferateor activated receptor α (PPAR-α)[58,59]. During times of fasting, Fiaf is released and acts upon lipoprotein lipase (LPL) to inhibit its function[58]. LPL is involved in fatty acid metabolism as a regulator of fatty acid release from triglyceride deposits in adipocytes, muscle and heart. LPL functions to cleave triglycerides in serum to allow them to be taken up by adipocytes and integrated into trigylcerides. LPL concentrations in various tissues have also been linked to obesity[60]. High levels of LPL in muscle and low levels in adipose tissue is associated with

obesity resistance. The increase in triglyceride storage triggered by LPL action is countered by Fiaf, resulting in prevention of weight gain by suppressing adipocyte growth and proliferation.

The expression of Fiaf was shown to be suppressed upon introduction of a normal mouse microbiota into GF mice by conventionalization, ultimately leading to weight gain[55]. Weight gain was then linked to Fiaf suppression by the microbiota. Weight gain was a result of increased deposition of triglycerides in adipocytes[55,58]. When suppressed by the microbiota, Fiaf is not present to suppress LPL, causing increased triglyceride cleavage and lipogenesis, all leading to weight gain.

In conjunction with the actions of the microbiota to suppress Fiaf, Gordon et al. also showed that the microbiota increases lipogenesis in the liver by providing large amounts of substrate SCFAs. This was shown by an increase in levels of carbohydrate response element binding protein (ChREBP) and sterol response element binding protein (SREBP-1) in the liver upon conventionalization of GF mice[55]. Both ChREBP and SREBP-1 are involved in dietary induced lipogenesis in the liver, and their expression has been shown to be triggered by the increased fatty acid and gluco se serum levels61.

In addition to obesity, inflammation and the microbiota have been linked. As described in the section above, production of many classes of inflammatory molecules and transcriptional factors are suppressed by various SCFAs, SCFAs that are produced by the microbiota. Also as described above, high fiber diets lead to reductions in inflammation as a result of the increased production of SCFAs by microbial colon fermentation.

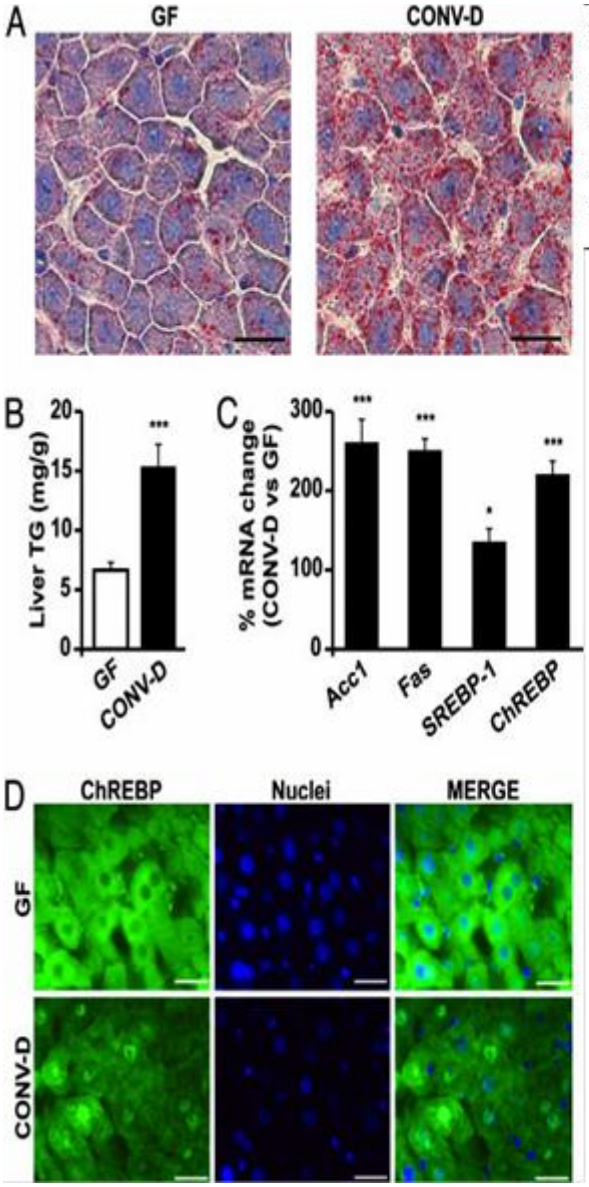



**Figure 14 B**: mRNA and amino acid sequence of the rat Fiaf gene and protein. Lowercase letters are non-coding regions and uppercase are coding segments. (C) Introns and exons of the Fiaf gene. (55)

## Conclusion

The human body is a complex organism composed of cells of human and non-human origin. These two groups work in harmony to promote health and survival of both the host and its residents. Because of this, the human body must be considered a super organism. The microbiota aids the host by lending its genes to the process of digestion of dietary components that the host cannot digest. The host in turn supplies the microbiota with necessary nutrients and a safe home. While this occurs, the host and microbiota are modulating each other. The products that the microbiota creates, specifically SCFAs have been shown as a major influencer of host physiology and should be studied further, in addition to the search for other metabolites that may have additional impacts. The host in turn modulates the microbiota through the immune system and dietary intake. Understanding how the host:microbiota interactions effect the host, but also the determinants that shape the microbiota, should be considered an important area of research in the future. If the intricate interactions can be deciphered, many medical treatments could emerge from the study of the microbiota. For example, the effects of microbial SCFAs include modulation of inflammation. Inflammation is an important factor in the pathogenesis of many diseases. The introduction of certain prebiotics or probiotics to the diet could possibly benefit the host by increasing the presence of beneficial SCFA producing bacteria. It will be many years before the emergence of approved methods of microbiota manipulation, but the importance the microbiota plays in the health of its host is made clear by the many effects of SCFAs on the body.

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