THE GUT MICROBIOME

Exploring the Connection between Microbes, Diet, and Health

ANA MARIA R. MOISE

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This book is dedicated to my mother, Adriana.

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Introduction

Although we view ourselves as individuals, each person is in fact a superorganism that includes trillions of microbes. The human body functions alongside these microbes, forming an ecosystem. Increasingly, health researchers are appreciating that in order to understand health and disease, we must explore the interplay between humans and the microbes that inhabit their bodies.

The community of microbes that lives in the gastrointestinal tract, called the gut microbiota, is known as the forgotten organ because of its immense influence on many systems in the body. In fact, the combined genomes of these microbes, called the gut microbiome, provide a vast amount of genetic material that affects human health. Gut microbes regulate our natural immune responses and determine risk for developing chronic illness. For example, altered gut microbes are associated with obesity, type 2 diabetes, and heart disease, and may also have implications for neurological disorders like autism, multiple sclerosis, and Parkinson's disease. Cutting-edge research has even identified links between microbes and our mood and mental health. These connections between the microbiota and health outcomes raise the question of whether the microbiota can be manipulated to improve human health. Indeed, numerous lifestyle factors, such as diet, antibiotic use, sleep, and exercise, may influence health through mechanisms involving gut microbes.

This book will cover a variety of topics involving the gut microbiota, but there are a few overarching themes woven throughout the chapters. One of these themes is the role of gut microbes in many of the body's immune functions, including its ability to fight off infectious disease; the development of autoimmunity, where the immune system attacks healthy human cells; as well as the development of environmental allergies. Another theme that will appear throughout the book is dysbiosis, a state of imbalance in the composition of microbe populations, which can alter the interactions microbes have with their human host in ways that promote disease. This book focuses most deeply on the role of nutrition in maintaining balanced and healthy gut microbe populations, which can mitigate the development of disease.

The nexus of nutrition and research on the gut microbiota is an area replete with new discoveries and important but unsettled research questions. This book will often draw on recent and current research findings and also discuss current methods used to explore the microbiota and its effects on human health. This book reviews various cross-cultural comparative diet studies that provide insight into human gut microbiota populations around the world. Much of this book also references research using animal models, such as germ-free animals lacking normal gut microbes. Also, we will review recent clinical trials that identify potential health-modulating microorganisms.

The first section of the book discusses the influence of dietary shifts throughout the evolution of the human gut microbiota, showing how gut microbe populations in traditional societies are distinct from those in modern Western societies. Chapter 1 examines the history of our symbiotic relationship with microbes. We will explore how microbes were an integral part of human evolution by providing us with the flexibility to adapt to a variety of different diets. This chapter will also examine how the evolution of diet from hunter-gatherers to traditional farming societies, and finally to modern Western cultures, has influenced the types of microbes in our gut. Given that both hunter-gatherer and traditional farming societies lack the prevalence of chronic disease seen in Western cultures, we explore how this dietary impact on the gut microbiome may influence disease risk.

Next, chapter 2 will examine the different sections of the gastrointestinal tract and discuss how they serve as distinct habitats for specific types of microbes. This chapter introduces key members of the gut microbiome and describes the different kinds of interactions between these bacteria and their human host. We will also briefly describe the process by which gut microbiota populations develop early in life and how they change during later stages of life.

Following our introduction to important gut microbes, chapter 3 describes how diet can be used to manipulate the composition and function of the gut microbiota. The first section will review how diet determines the types of microbes present in the gut, and the second section will highlight which foods encourage the growth of beneficial microbes. Finally, the third section of this chapter explores the dietary components that promote the growth of health-inducing bacteria, as well as other dietary components that produce toxic substances as a result of interactions with gut bacteria.

The second half of the book contains individual chapters that highlight specific body systems (gastrointestinal, cardiovascular, nervous, immune, etc.) and their interactions with the gut microbiota. These chapters focus on the gut microbiota in the context of different diseases. Chapter 4 provides a foundation for understanding how microbes interact with the immune system in ways that either prevent or promote chronic disease. This chapter also explains how our immune system learns to tolerate the enormous number of microbes

living in the gut while simultaneously protecting us from infectious organisms. The complex interactions between microbes and immune cells are further outlined in the last four chapters of this book.

Chapter 5 explores how the body's immune response to resident gut microbes influences metabolism and contributes to obesity during states of dysbiosis. This chapter discusses the role these microbes have in regulating fat cells, hormone function, and even appetite. The last section examines how sleep and physical activity affect gut microbes in ways that help manage body weight and a healthy metabolism.

Next, we explore several kinds of gastrointestinal diseases in chapter 6. We will examine the types of dysbiosis seen with inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, as well as in functional bowel disorders like irritable bowel syndrome. In this chapter, we will also discuss dysbiosis related to the use of antibiotics and other medications.

Chapter 7 covers the effects of diet and gut microbiota on cardiovascular disease. We will discuss how gut microbes interact with different components of our diet in ways that affect the health of our heart and arteries. We will pay special attention to the effects of gut microbes on known risk factors for cardiovascular disease, such as high cholesterol and high blood pressure.

Finally, chapter 8 reveals how microbes communicate with the brain through the gut-brain axis. We will learn about the different biological pathways by which this communication can happen, and we will discuss the types of chemicals produced by both microbes and the body to facilitate this communication. The second half of this chapter explores the role of dysbiosis in neurodevelopmental conditions such as autism and neurodegenerative diseases like multiple sclerosis and Parkinson's disease.

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A Cultural Context for Human-Microbe Symbiosis

Since the Stone Age, human cultures have evolved from hunting and gathering bands to small-scale agricultural communities and eventually to larger societies that rely mainly on industrial agriculture. Through this cultural evolution, shifts in diet and lifestyle have led to changes in the human body. Along with these adaptations in human physiology, researchers have found that the composition of gut microbiota has changed as well.

Archaeological records give some information about the diet, food preparation practices, and physiology of our ancestors, but we have little direct view of the microbiota of early humans. Some indirect evidence may be available by observing contemporary cultures that live much as our ancestors did. Although few societies have escaped the influence of the Western diet, some cultures have sustained their way of life over thousands of years, living as modern-day hunter-gatherers or practicing traditional farming. Research comparing those cultures to modern Western populations is beginning to reveal how variations and imbalances in the gut microbiome are related to culture and lifestyle. For instance, comparing the gut flora of modern Western populations with that of traditional societies has revealed a link between diet and the composition of gut microbiota. Cultures that maintain traditional diets often have different gut microbes and greater microbial diversity than Western cultures. These findings suggest that the food we eat powerfully influences the types of bacteria growing in our gastrointestinal tracts.

While the links between diet and health have been long established, research on the role of the gut microbiota in human health and longevity offers new approaches for disease prevention and treatment. Again, cross-cultural comparative research can provide a valuable view: Cultures that maintain traditional diets and have more diverse microbiota also tend to have lower rates of common Western diseases such as diabetes, heart disease, and obesity. Given the importance of gut flora for human health, this chapter will

trace the history of the human diet as an avenue to explore the evolution of the gut microbiome.

In this chapter we will discuss how the modern Western diet differs from that of our ancestors. To better understand what our ancestors ate, we explore the diets of a few different modern-day hunter-gatherer societies that reflect what we ate during the Paleolithic Era. We discuss how large-scale agricultural technologies contribute to diet-induced shifts in gut microbiota. Specifically, we examine how over-processed foods that are common in the Western diet are depleting our gut microbes.

Evolution of the Human Diet: What Our Ancestors Ate and Why It Matters

Diet is a driving force in human evolution. Our omnivorous diet has given us flexibility to adapt to a wide range of environments. While this adaptability has likely supported many significant advancements in civilization, modern diet and lifestyle have also been accompanied by a significant increase in the incidence of chronic disease. The spread of Westernized dietary patterns has been associated with growing epidemics of obesity, heart disease, and diabetes. These diseases of civilization may be directly tied to the foods we eat.

Some health advocates suggest that eating a diet similar to that of our Paleolithic ancestors may be an effective way to prevent many of these modern Western diseases. The human diet has changed considerably under the influence of modern Westernization, and the importance of dietary evolution is raising many questions about the role food plays in the prevention or acceleration of disease. The transition into agriculture and animal husbandry that occurred about 10,000 years ago ignited a shift in our diet that may have been too rapid for human physiology and genetics to properly adapt. This mismatch of genetics and culture has been used as a primary example to demonstrate how diet is at the root of many chronic diseases. This idea of evolutionary discordance between modern industrialized diets and human evolution suggests that we remained genetically adapted for a diet of the Paleolithic era. Indeed, there is significant evidence that our bodies may not be well adapted to handle the highly processed, calorie-dense foods that have replaced a natural whole foods diet.

This focus on the gene-diet mismatch has overlooked a crucial component of human evolution: the gut microbiome. The microbial composition of the gastrointestinal tract is highly influenced by diet, and varies greatly between populations following a modern Western diet and those eating more traditional foods. Over the course of human evolution, the human genome has coevolved with culture in the context of relationships with other species, including the

microbes within our gut. In particular, the gut microbiome appears to play an important role in our adaptability to new environments. Understanding the changes in composition of gut microbes since Homo sapiens diverged from other species provides helpful insight into current health problems faced by the modern Western world.

Early Humans and the Foraging Diet

The fossil record shows the evolution of the body in response to variations in diet. For example, the current shape and size of our gastrointestinal tract, as well as the formation of our teeth, originate from dietary shifts that took place during the Stone Age. Adaptations in gut shape and size likely led to alterations in the gastrointestinal tract's internal environment, conditions that may have selected for different types of bacterial life within the gut. Dietary intake, physiological adaptations, and gut bacterial composition evolved simultaneously and depended on one another. Thus, the human microbiome coevolved with the human genome and with human cultures (including diet, agriculture, food preparation, etc.). Looking into the evolution of the human diet gives clues to how our gut flora has changed over time.

Prior to learning how to cultivate plants for food, humans survived by foraging, searching their habitat for wild food sources. This foraging diet was far from static and depended on the availability of foods, which varied across seasons, weather conditions, and local environments. In order to survive in these variable habitats, early hunter-gatherer populations had to develop flexibility in the foods they ate.

One of the first major dietary shifts in early human history occurred approximately 4.4 million years ago, when humans split from prehistoric apes. Modern humans evolved from earlier hominins, a group that includes extinct human species, our recent ancestors, as well as modern humans. This evolutionary split is reflected in a number of recognizable physical adaptations that point to changes in both diet and habitat. Archeologists have uncovered fossil evidence revealing that hominins gradually developed larger, thickly enameled teeth. These changes in tooth structure emerged during a time when hominins were learning to use their premolars on harder foods, and thus were likely linked to the introduction of new foods. This dental adaptation is indirect evidence for the introduction of starch-rich underground storage vegetables such as bulbs, corms, or tubers. Similar underground vegetables continue to play an important role in the diets of the few remaining modern-day hunter-gatherer societies.

With diverse foods of both plant and animal origin, subsequent hominins adapted to various habitats and eventually transitioned to more open

environments like savannas. As hominins were learning how to thrive in new habitats, technology also began to transform the human diet. The use of fire became one of the first technological advancements that propelled another major dietary shift for early humans. Again, archeologists noticed significant physiological changes from this period, although this time as a result of the dietary transition to cooked food. The introduction of softer cooked foods led to a reduction in tooth size. Over time, these new diets caused the gut to compartmentalize, improving nutrient absorption. As cooking increased digestibility of foods, humans were likely able to eat plants that might otherwise have been unpalatable. The growing diversity of plants within the ancient hunter-gatherer diet is particularly relevant for the microbiome. In modern times, a traditional diet with a greater variety of minimally processed plant foods is associated with more diverse gut flora than a Western diet.

Modern-Day Hunter-Gatherers: The Hadza of Tanzania

Archeological research provides only limited insight into the diet and gut microbial composition of early foraging humans. Fortunately, microbiologists and anthropologists are collaborating to study the last few surviving indigenous populations that practice foraging or other traditional dietary patterns. The hunter-gatherer diet is not universal, and in fact, nutritional patterns among current-day hunter-gatherer communities around the world are greatly varied based on the availability of plant and animal resources. The closer a population is to the equator, the more reliant they are on gathering. This is not surprising, considering the immense plant biodiversity found in these warmer habitats. Hunter-gatherer communities living in the coldest regions rely more on hunting. All foraging groups share one commonality: their mode of subsistence exerts only little control over their habitat and reproduction of resources. This is in contrast to food cultivation and farming that involves deliberate manipulation of the environment for subsistence.

One of the world's last remaining hunter-gatherer groups, the Hadza of northwest Tanzania, live in the same East African region inhabited by early hominins. They are an ideal example of an indigenous community whose traditional lifestyle has remained mostly unchanged despite the advancements of surrounding populations. While the Hadza are still considered a modern human population, their dietary resources are similar to those of our hominin ancestors. To investigate what the microbiome of our ancestors may have looked like, researchers examined the gut bacteria of the Hadza in the context of their traditional unprocessed diet. As expected, their gut microbiota proved to be reflective of their diet. The Hadza have great microbial richness and

higher biodiversity in their gastrointestinal tracts than their Western counterparts. Unraveling the specifics of their diet composition may provide answers to how this beneficial richness and diversity can be achieved by eating the right foods.

Approximately 200–300 Hadza living in small mobile camps are currently engaged in a foraging lifestyle. Similar to the resources of early hominins, unrefined foods that are native to the environment are central to the Hadza diet. Agricultural products from external sources are scarce, so the Hadza rely heavily on their local habitat. Although the Hadza do consume some meat, the bulk of their calories comes from plant foods. Their overall diet fluctuates based on seasonal availability of foods, yet their plant intake consistently ranges between 70–80% of total foods consumed. During the dry season, plant foods become somewhat less available, and the Hadza rely more on meat, with approximately 30% of total calories from hunting. The wet season leads to an abundance of plant matter, and meat consumption drops to less than 20% of the diet. Such ecological variability greatly changes the composition of the Hadza diet.

The Hadza forage for a variety of plant species, inducing several types of tubers, baobab, berries, and honey with bee larvae. Much like early hominins, they rely on underground storage organs such as tubers. This fallback food is available all year round, both in the rainy season and the dry season. Although several varieties of tubers are significant staple foods, fruit from the baobab tree contributes more calories to the overall Hadza diet than any other food. Aside from year-round fallback foods, the composition of the Hadza diet reflects seasonal availability of native foods. When the arid environment transforms during the wet season, the lush green landscape provides a variety of berries that becomes the dominant food. Similarly, when honey is plentiful it becomes the focal point of their foraging.

Hadza foods are sometimes processed, however minimally. While they consume some tubers raw, the Hadza use a flash-fire roasting method with other tubers, cooking them for a few minutes to increase their digestibility. Starch constitutes as much as 80% of the dry weight of tubers and other underground vegetables. In its raw form, this starch is not easily broken down to be used as energy in the body. As we will see later in this chapter, the body gets much of its energy from glucose, a simple sugar that can be derived from many carbohydrates. Starch is a complex carbohydrate and contains many units of glucose that are bonded together. Cooking breaks down these bonds between glucose units and increases the availability of energy-yielding glucose. Interestingly, the use of fire to cook starchy tubers dates back to ancient hunter-gathers and provides more evidence for the importance of carbohydrates among early foraging hominins.

SIDEBAR 1.1 Fire and Dietary Starch Led to Increased Hominin Brain Size

The body is able to digest starch with the help of the enzyme amylase. Chimpanzees, our oldest living relatives, have fewer copies of the amylase gene compared to humans, which indicates that the number of amylase genes increased during human evolution. Scientists previously hypothesized that the emergence of agriculture led to higher starch consumption and thus increased the need for amylase production. However, recent advances in biotechnology, which allow scientists to analyze the DNA of early European hunter-gatherers, show extra copies of the amylase gene in humans well before the beginning of agriculture.

Some evolutionary geneticists argue that the advent of cooking fueled the need for more amylase. Early foragers depended on starchy plants such as tubers, and as they began cooking, the digestibility of these carbohydrate-rich foods increased. Since amylase is more effective at breaking down cooked rather than raw starch, heating starchy foods quickly gave this enzyme a more significant role, and human DNA evolved to produce extra copies of the amylase gene. These findings are raising many questions about the composition of the hunter-gatherer diet and the importance of carbohydrates in human evolution.

The food processing techniques of the Hadza are far more rudimentary than those common in Western cultures. In addition to flash-fire cooking, another processing technique used by the Hadza is the grinding of baobab to make a coarse flour. When Hadza are out of camp, baobab is usually consumed raw and unprocessed, but once it is brought back to camp, the pulp and seeds can be pounded into a flour using a hammerstone. Whereas modern milling removes much of the fiber content to produce refined flours, the Hadza method of grinding baobab flour retains much of the dietary fiber. The combination of minimally processed foods and high intake of diverse whole plant foods provides a variety of dietary fibers for the Hadza. This in turn has implications for the gut microbiota, as many of these fibers are the primary food source for bacteria. In this way, the Hadza diet directly promotes microbial diversity within the gut.

The Hadza division of labor specifies particular roles for men and women. These gender roles affect overall diet and are reflected in the slight differences in gut microbiota composition between Hadza men and women. Although both genders share any foraged food with the whole group, daily food intake varies by gender. Men are more mobile foragers and travel farther away from camp, searching for fruit, wild game, and honey, depending on seasonal

availability. Women often remain closer to camp, spending 2–3 hours each day foraging in groups for tubers, baobab fruit, berries, and other plant foods. Women commonly dig together for tubers, gathering multiple species. During certain times of the year, Hadza women and men share more similar diets. For instance, at the height of berry season, women and men leave camp together, both collecting a variety of species.

SIDEBAR 1.2 Human Remains and Fossilized Evidence of the Ancient Gut Microbiome

The search for fossilized human remains has provided some limited evidence regarding the composition of our ancestors' microbiome. Preserved human specimens and byproducts from ancient human gut microbiota are difficult to find. At the time of death, the ecology of the human microbiome shifts rapidly to encourage soft tissue decomposition. Since the bacteria within the human body change quickly after death, only two types of human materials are preserved sufficiently to be used as evidence for the composition of ancient oral and intestinal microbiota.

The first of these materials, dental calculus, is a form of plaque created by oral microbes that is semi-fossilized at death and does not decompose. Comparisons of dental calculus bacteria in Neolithic and modern samples show shifts in types of oral bacteria that correspond to significant dietary changes associated with early agriculture practices as well as the industrial revolution.

Human coprolites are the second of these valuable archeological materials. Coprolites are a form of desiccated or mineralized fecal matter. Most coprolites are not recovered from individual human remains but rather from communal latrines. These fossilized human feces may therefore prove useful for characterizations of microbiomes at a population level. Within coprolites are also preserved evidence of bacterial, viral, and parasitic infections. In the future these materials might provide insights as to the prevalence, transmission, and evolution of infectious disease, as well as overall gastrointestinal health. Coprolites could also potentially provide some evidence regarding ancient practices of hygiene and sanitation.

Although human coprolites are quite rare, archeologists have discovered some samples from a few different time periods. Specifically, samples from Texas and Mexico provide some idea of pre-industrial human microbiomes. The older coprolites, from Texas, date from approximately 8,000 years ago and do not resemble modern-day human gut bacteria. However, the more recent 1,400-year-old Mexican coprolites showed bacteria somewhat more similar to those found in modern feces. These samples point to changes in gut microbes over time, with the development of modern agricultural practices.

On the whole, Hadza women generally collect plant foods, and they rely more heavily on tubers throughout the year. Women have overall higher plant consumption and therefore more fiber intake than men. This greater dietary fiber has led to differences in Hadza women's gut microbiome. The gut microbiota of female Hadza better supports digestion and energy extraction from fibrous plant foods. In this way, gut microbes have adapted to ensure that women's foraging is adequate to meet nutrient requirements during pregnancy and lactation.

The Yanomami: Amazonian Hunter-Gatherers

Another highly significant group in the quest to identify the hunter-gatherer gut microbiome are the Yanomami of Venezuela. Until the 1960s, the Yanomami had lived in isolation within the Amazon jungle for over 11,000 years. While there are approximately 35,000 Yanomami, one unmapped village was recently discovered deep in the rainforest. This uncontacted Yanomami group has had significantly less interaction with the Westernized world than the Hadza, and their lifestyle remains preserved without much outside influence.

The Yanomami offer particularly interesting insight into the dietary influences of the gut microbiome. The gut bacteria of the uncontacted Yanomami has the highest microbial diversity of any human group yet observed. Unfortunately, extensive research on diet composition of this group is lacking, but parallels between the diet of these uncontacted Amazonians and the Hadza point to higher fiber and plant diversity as the explanation for increased microbial diversity. The Yanomami were observed to eat wild bananas, plantain, and other fruit, as well as tubers such as cassava. They hunt mostly small animals, although animal sources likely do not make up the bulk of caloric intake. Considering the high biodiversity within the Amazon rainforest, it is expected that foraging groups within this environment rely heavily plants. Although further research on this indigenous group would reveal a more comprehensive understanding of how their diet influences gut microbiota, future studies should be done cautiously, with the protection of this unique village taking precedence.

High-Fat, Low-Carbohydrate Hunter-Gatherers

Thus far, we have taken an in-depth look at the gut microbiota composition of both traditional and hunter-gather societies, but these diets have been characterized by high carbohydrate intake. Other traditional diets, such as that of the Inuit of the Canadian Arctic, are low in carbohydrates and high in animal fat and protein. While the traditional Inuit diet is based on a hunger-gatherer lifestyle, Inuit individuals are now consuming a more Western diet. Many Inuit

are now eating store-bought food and in general adopting a more Western lifestyle.

One group of Canadian researchers compared the gut microbiota composition of urban-living individuals from Montreal to that of the Inuit. Surprisingly, they discovered that the two groups had very similar gut microbiomes. One interesting observation was the abundance of *Prevotella* species in both Montrealers and the Inuit. High numbers of *Prevotella* species are usually seen in traditional diets with high fiber intake. Researchers also noted that Montreal samples were enriched with *Faecalibacterium prausnitzii*, while some Inuit samples were enriched with the methanogen *Mehtanosphaera* (see chapter 2 for more information on these species).

Although traditional diets are usually associated with higher levels of microbial diversity, the Inuit microbiomes weren't much more diverse than those of the Montrealers. As the Inuit switch from their traditional hunter-gather diet to a Western one, the incidence of obesity among this indigenous group is increasing. The researchers theorize that lower level of microbe diversity may be driven by increasing body weights, as obesity tends to promote significant shifts in gut microbiota composition. They also speculate that lower diversity may reflect lower fiber intake. Inuit of Nunavut consume around 13–14 grams of fiber per day—significantly lower than other hunter-gatherer diets previously mentioned, which can contain over 100 grams of daily dietary fiber.

Transition into Agriculture: Early Farming to Industrial Food Production

The "First Agricultural Revolution" occurred in the final period of the Stone Age, during the Neolithic Era. This marks the historical beginning of human agriculture and animal husbandry. Archeologists find clear evidence that foraging societies began to cultivate wild foods approximately 10,000 years ago. Although this cultural change allowed humans to create settlements and become less nomadic, historical ecologists continue to debate the exact motivations for this shift. It might seem that hunter-gatherers saw farming as a more efficient means of subsistence, but these early agricultural practices were, in fact, very labor-intensive and time-consuming. Given that for thousands of years, early hominins were well sustained by foraging, why did hunter-gatherers begin to invest such time and effort into plant cultivation?

Most archeologists agree that human population density increased with the emergence of agriculture. However, the question remains as to whether population pressure led to food cultivation or cultivation caused population growth. The idea of population pressure suggests that foraging societies experienced nutritional stress due to insufficient food sources. Yet archeologists have failed to find evidence of significant nutritional stress in human skeletal remains

prior to food cultivation. In fact, hunter-gatherers were well-nourished and, in some instances, even healthier than the first farming societies. Furthermore, increased population density during the Neolithic era led humans to live in close proximity, which favored the transmission of infectious disease and ultimately led to the first waves of widespread human diseases.

Despite the lack of clearly defined motivations for this cultural shift, humans gradually learned to manipulate their surroundings and began to combine plant cultivation with foraging. Many of the foods found in early agriculture, such as tubers and root vegetables, are similar to wild foods consumed by foraging societies. Later we will see a modern indigenous culture that still practices this combined form of subsistence, both foraging for wild foods while also cultivating plants. However, this is rare, and most other rural cultures have transitioned more thoroughly into agriculture and rely only on cultivation and animal husbandry.

Scientific interest in these early forms of agriculture is growing as researchers trace the origins of modern Western diseases and investigate their relationship to the introduction of agriculture. Recent research reveals that some traditional farming practices support a diverse gut microbiota, similar to that of the foraging Hadza. Along with their increased gut microbial diversity, many of these populations have low incidence of chronic diseases and are typically free of obesity, diabetes, and heart disease.

Subsistence Agriculture

Some traditional cultures around the world still engage in subsistence agriculture, a practice that focuses on growing the food necessary to sustain the needs of a family or village. For example, many Yanomami have started to transition away from the hunter-gatherer lifestyle that is seen among the uncontacted Yanomami group discussed earlier. Most groups now use foraging only to supplement the diet in times of agricultural shortages such as crop failure. Within their gardens, Yanomami often grow plantains and cassava as main crops but also other plants such as banana, sweet potato, mango, and corn.

Although there are likely many dietary differences, some overlap between the entirely hunter-gatherer Yanomami and the forager-farmer Yanomami groups is seen with their consumption of various tubers and other underground vegetables, as well as their reliance on banana and plantain. Comparative gut microbial analysis between the two Yanomami lifestyles is lacking, although it is interesting to consider how this shift from hunting and gathering to farming might influence gut microbiota composition. Other cultures with similar subsistence agriculture practices reveal that patterns of gut microbial richness and diversity are common among traditional societies around the world.

In the rural village of Boulpon, in Burkina Faso, Africa, residents have escaped most Western influences and practice only subsistence agriculture. Unlike foraging African communities such as the Hadza, who reflect an early Paleolithic diet, the rural Burkina Faso diet is likely more comparable to a Neolithic African diet following the agricultural revolution. Interestingly, despite this inclusion of agricultural products in the traditional Burkina Faso diet, recent research has shown that this population has more microbial richness within their gut flora than those consuming a Western diet.

Traditional African cuisine tends to be predominately vegetarian. Grains, legumes, and vegetables are cultivated to produce a fiber-rich diet with high carbohydrate content. Intake of fat and animal protein is limited; only a small percentage of the rural Burkina Faso diet comprises meat and termites, particularly in the rainy season. Many of these plant foods, especially grains and certain legumes, were added to the human diet only following the introduction of agriculture.

Food processing remains minimal in rural sections of Burkina Faso. Similar to the foraging Hadza, who stone grind baobab fruit, in rural sections of Burkina Faso, millet and sorghum grain are stoneground by hand to make flour. These rudimentary stone-grinding methods do not remove any part of the grain and therefore maintain its fiber content. Given the minimal processing and high reliance on fiber-rich plant foods, residents of Boulpon and farming Yanomami groups share significant parallels with hunter-gatherer societies.

Many of the same health advocates who encourage a hunter-gatherer diet for prevention of disease claim that certain carbohydrates, particularly grains and legumes, contribute most to the epidemic of obesity and diabetes. However, considering that the gut microbiome plays a crucial role in these diseases (more on this in chapter 7), it is interesting to note that grains and legumes are prominent foods within the rural Burkina Faso diet, yet their gut microbiome is more similar to that of hunter-gatherer populations than to that of Western societies.

As researchers continue to explore the connection between diet, disease, and the gut microbiome, rural populations in Papua New Guinea have also been a point of interest, given that some of these groups have a low incidence of diabetes and obesity. Certain traditional groups in Papua New Guinea, living as horticulturalists with little access to Westernized foods, do not eat grains or sugar, yet their carbohydrate intake is quite high, consisting of 60–70% of total energy intake. Similar to foraging diets that rely on underground vegetables, tubers and corms such as yams and taro are staple carbohydrate foods in rural Papua New Guinea. As expected, the gut microbiome of rural Papua New Guineans showed microbial richness and diversity.

Anthropologists discovered one indigenous group on the island of Papua New Guinea that is free from heart disease, diabetes, and obesity. Despite their

high-carbohydrate diet, Kitavan Islanders have lower blood glucose and insulin levels, two makers indicating decreased risk for diabetes. (The next section of this chapter will detail the connection between blood glucose, insulin, and the development of diabetes.) Proponents of low-carbohydrate diets have overlooked the role of fiber, which beneficially alters the digestion of carbohydrates and also promotes certain healthy gut microbes that are linked to the prevention of diabetes and obesity. The carbohydrate-dense diet of Kitavan Islanders indicates that a plant-based fiber-rich diet is consistent with diverse gut microbiota and decreased risk of Western diseases.

SIDEBAR 1.3 Social Behavior and Sharing Gut Microbes

As we have seen with hunter-gatherer groups such as the Hadza, social behaviors can affect gut microbe populations. For instance, during the wet season, both Hadza men and women forage for plant foods. It would be interesting to see whether gender differences in gut microbiota composition are less obvious during the wet season, due to this shift toward similar social behavior. However, Hadza men generally tend to hunt more and eat fewer high-fiber tubers compared to Hadza women. These typical differences in social behavior are the source of the gender discrepancies in gut microbiota composition.

One team of researchers studying chimpanzees in a national park in Tanzania, to determine patterns of similarity within their gut microbiomes, found that the chimps' level of social interaction impacted their diversity of gut microbes. Similar to the Hadza, foraging encourages increased sociability, as chimps are working together as a group in the search for food. Foraging is more common during the wet season. During other times of the year, chimps are less social and tend to spend time in smaller groups.

Interestingly, when studying samples from both the wet season and other times of the year, the researchers discovered that the chimps have more microbial diversity during the wet season. However, there was no change in level abundance within gut microbiota. Given these observations, researchers continue to explore the extent to which social patterns can predict gut microbe diversity.

Other forms of social behavior in primates may shape gut microbiota composition. Contact through grooming also seems to influence bacterial populations. However, it is uncertain how these social behaviors impact gut microbes, because they do not seem to have any connection to diet. For this reason, researchers are working to determine whether certain behaviors may encourage social transmission of microbes.

Still, it is important to note that seasonal dietary changes are likely to play a major role in shaping diversity. Just as the Hadza's diet shifted between dry season and wet seasons, the chimpanzees' diet diversity increased during the wet season and likely contributed to more microbial diversity as well.

The Industrialized Western Diet

While foraging cultures have nearly vanished, and plant cultivation persisted through to modern times, the changes occurring during the most recent agricultural revolutions in human history were far more radical than early humans' transition from foraging to farming. The Industrial Revolution, which took place in United States during the eighteenth and nineteenth centuries, drastically changed dietary patterns in the Western world. Transportation, refrigeration, and preservation created an unprecedented abundance of food. Improved food production, coupled with reliable long-term storage techniques, led to a more reliable food supply.

The subsequent Green revolution occurred post-World War II and prompted a series of technological advancements in agricultural production worldwide. High-yield crops, especially varieties of cereal grains, were developed. The use of synthetic pesticides, herbicides, and fertilizers increased significantly. Many of these agricultural food initiatives were aimed at developing countries, with the idea that increased food production would solve global food shortages. Unfortunately, many decades later, food insecurity continues to be a serious problem. Additionally, nations affected by this agricultural revolution lost the diversity in their foods that was once found in more traditional forms of agriculture.

These new food production technologies supported monocultures, a type of agriculture that produces only one type of plant rather than rotating or simultaneously growing multiple crops. This practice expanded globally, and traditional societies transitioned away from subsistence farming. Industrial agriculture completely changed the common staple foods within these populations, and developing countries consequently saw a greater incidence of conditions associated with nutrient deficiencies. The worldwide expansion of the Westernized diet has brought chronic diseases like obesity, cardiovascular disease, and type 2 diabetes to countries that had once been virtually free of these conditions.

Fiber

Following the more recent advances in food technology, our modern food supply evolved further away from the unprocessed foods of our ancestors. The quality and quantity of fiber is one of the main differences between hunter-gatherer and modern-day diets. Dietary fiber, which is only found in plant foods, is not digestible by human enzymes. These plant components are either used as food by our gut bacteria or for easing of bowel movements by adding bulk to the stool. Fiber consumption during the Paleolithic era is estimated to be greater than 100 grams per day. Early human diets consisted of a wide

variety of fiber sources, from many different types of plants. The minimally processed plant foods found in traditional diets ensured that these foods remained whole and none of their fiber was removed.

The average American today is likely to consume only around 15 grams of daily fiber despite the Institute of Medicine's suggestion that 25–38 grams is necessary for health. In a typical Western diet, the tendency to rely on processed foods, such as added sugars, flour products, and refined oils, is one of the major factors that has contributed to replacement of fiber-rich whole foods with calorie-dense, low-fiber foods. Sources of dietary fiber in the Western diet are largely from cereal grains, which considerably limits the quality and diversity of fiber types. Modern milling of grains produces a refined flour that is typically much lower in fiber than the original whole grain. Even products labeled as "whole wheat" are a deceptively processed version of grains that are often quite low in fiber. Half a cup of cooked wheat berries (which are the whole version of the wheat grain) contains about 6 grams of fiber. The average slice of whole-wheat bread only has about 2–3 grams.

Reliance on primarily cereal grains for fiber not only limits overall variety; it also leaves most individuals with a diet lacking in resistant starch. This type of carbohydrate resists digestion in the small intestine and travels into the colon, where it serves as a food source for our gut bacteria. Traditional diets seen in rural parts of developing countries are simple, with grains and vegetables processed in ways that preserve higher quantities of the non-digestible carbohydrates that are a primary energy source for gut microbiota. The types of dietary carbohydrates found in the diet directly shape the composition of gut bacteria. Furthermore, highly processed carbohydrate foods that are typical in Western cultures encourage the production of detrimental microbes in the GI tract. The impact of carbohydrates and overall diet composition will be discussed in more detail in chapter 3.

Carbohydrates: What Are We Feeding Our Gut Bacteria?

The types of carbohydrates dominating our modern food supply may be the foundation of many diet-related chronic diseases. In popular culture, low-carbohydrate diets have been promoted as a weight loss plan that promises quick results. Considering our modern diet's strong reliance on carbohydrate-dense grain products, some nutrition specialists have started to question how we look at modern staple foods—the foods that are most common in the typical Westernized diet—in order to find potential links with growing rates of obesity and type 2 diabetes.

The modern Western diet is characterized by an abundance of processed foods and large portions. While this caloric excess is certainly a relevant factor in populations' substantial increase in body fat, the widely accepted idea of

"calories in, calories out" is far too simplistic and does not take into account that processed foods are often handled differently by the body, compared to whole foods. The exact interactions between types of macronutrients, such as carbohydrates, fats, and proteins, and our digestive process may provide a more substantial perspective that suggests not all calories are created equal. For the purposes of this chapter, we will focus mostly on carbohydrates.

In order to be used in the body as energy, all digestible carbohydrates are broken down into more simplified forms like glucose. Once glucose enters the bloodstream, it must find its way into cells, where it can be converted into energy. In a healthy person, a rise in blood glucose is met with an increase in the hormone insulin. This regulatory hormone, secreted by the pancreas, acts as a key that unlocks muscle and fat cells, allowing blood glucose to enter. While this mechanism is meant to keep our blood glucose levels balanced, a high rate of carbohydrate digestion can quickly throw this balance off.

The glycemic index indicates this rate of carbohydrate digestion and measures the expected rise in blood glucose associated with ingestion of a specific food. Simply put, the more processed the carbohydrate, the more easily it is digested and absorbed into the bloodstream. Refined sugars hardly require any digestion and therefore have the potential to cause a spike in blood glucose. Refined flours similarly are lacking in fiber and can be broken down more easily than intact whole grains. The more refined the carbohydrate, the higher its glycemic response.

A diet that is high in refined carbohydrates causes rapid spikes in blood glucose levels. Consistently elevated blood glucose can eventually cause cells to stop responding to insulin, which leads to a state known as insulin resistance. If blood glucose isn't able to enter cells properly due to insulin resistance, type 2 diabetes develops. Both elevated blood glucose and insulin are warning signs for this disease. While the glycemic effect of foods has been somewhat useful in showing that various carbohydrates cause different responses from our metabolism, insight into the ways macronutrients interact with gut bacteria during digestion provides a more in-depth perspective about the process that creates the metabolic imbalances seen with obesity and type 2 diabetes. Chapter 7 will elaborate on how gut microbes can lead to increased body weight and raise the risk of type 2 diabetes.

While the types of dietary macronutrients tend to differ greatly when comparing traditional and modern diets, variations in carbohydrates are particularly important, as these are a main food source for gut bacteria. The differences observed in bacterial composition among various populations appear to be directly tied to the types of carbohydrates we feed our bacteria. Some cultures with zero or low incidence of metabolic conditions or heart disease are now being studied in the context of their gut bacteria composition. The specific types of bacteria that compose the gut flora appear to be similar

among Western cultures yet differ greatly from the composition of gut bacteria among traditional and indigenous communities. Given the low incidence of metabolic disease within these traditional populations, the investigation of how various diets affect the types of bacteria in the gut may be able to explain the connections between foods and chronic Western diseases.

Conclusion

Despite their location around the world, traditional societies of foragers and subsistence farmers show many similarities in gut microbiota. Using data from Burkina Faso and Papua New Guinea, as well as the Hadza and Yanomami, comparative research is beginning to analyze the implications of traditional diets on health and disease. The universally carbohydrate-rich diets found among traditional societies in the context of their low rates of Western diseases is not only reframing our understanding of the root cause of these diseases but is also redefining the early human diet.

Chapters 3 and 4 will delve into how these traditional high-fiber diets encourage growth of specific gut microbiota. We will cover which types of bacteria are most prominent in the gut microbiota of Western cultures compared to the ones most abundant within the guts of traditional societies. Chapter 4 will reveal how fiber feeds certain bacteria and leads to a more diverse gut microbiota.

2

The Gastrointestinal Tract and Its Microorganisms

About 4 billion years ago, when the earth cooled and became habitable, the first known organisms emerged as simple singled-celled life-forms. These first microorganisms began evolving about 3 billion years before the first plants or animals. In fact, fossil evidence shows that the formation of microbial communities eventually set the stage for the evolution of more complex life-forms.

As life on earth evolved, microorganisms began to develop functional relationships with one another and formed complex communities. These communities likely created a foundation for the first multicellular organisms. Many evolutionary biologists propose that this joining together of single-celled microorganisms prompted the formation of multicellular life forms. It is fascinating to consider that complex organisms such as humans may have evolved from simple single-celled microorganisms.

These single-celled organisms are called prokaryotes, one of two major types of cells. Prokaryotic cells are simpler in structure compared to more evolved plant and animal cells, but they are also highly organized organisms. Prokaryotes, such as the bacteria and archaea living in the human gut, play an integral role in our internal and external environment.

As more complex organisms began to develop, prokaryotes formed into eukaryotes, the second major cell type. Eukaryotic cells have many internal structures that are lacking in prokaryotes. One of these complex internal structures is called the mitochondria, which functions as the "powerhouse" of eukaryotic cells. Some biologists argue that the existence of mitochondria provides evidence that the formation of eukaryotic cells resulted from the joining of two prokaryotic cells.

The evolutionary theory known as symbiogenesis suggests that the creation of eukaryotic cells occurred as a result of one prokaryote organism entering into another. The name of this theory is based on the symbiotic relationship that developed between two separate organisms. This chapter will discuss in

detail how the nature of symbiosis has shaped the close association between microbes and humans. Symbiosis is a typically beneficial interaction between different species, such as with humans and their microbes. However, as we will see, these interactions can often fluctuate depending on external environmental factors and may become detrimental to our health. In fact, our microbial populations can vary among individuals based on factors such as diet, antibiotic use, illness, and even delivery method at birth.

In this chapter, we will explore the differences between beneficial microbe populations and detrimental ones, and consider how various factors influence our relationship with gut microbiota. The first section of this chapter explains how bacteria are classified. We will cover important taxonomical classifications, classifications determined by the structure of bacteria, as well as classifications based on the types of interactions with their human host. This section will also explore laboratory methods that are used to determine such classifications and how technological advances have revolutionized the ability to identify different gut microbes. Following sections will compare the different types of microbial habitats in the GI tract and introduce some key members of resident gut microbes. The last section of this chapter describes the process of colonization, a period during which a mother passes along microbes to her offspring. We will also briefly discuss changes that occur in the gut microbiota due to aging.

Classification of Gut Bacteria

The gut microbiome contains a diverse population of microorganisms. Microbiologists estimate that there are approximately 500–1,000 species of bacteria in the human colon. Although this incredible diversity includes microbes such as archaea and fungi, this section specifically explores the classification of bacteria with the gastrointestinal tract. We will first focus on taxonomic classifications of gut microbiota.

Organisms are classified into groups based on shared characteristics using taxonomic rankings. Although broader taxonomic rankings such as phylum may be used for comparisons between different populations, smaller levels of classification may be more useful in identifying more specific variations from person to person. Not surprisingly, gut microbiota shows the most variation on the species level, the most specific division of classification. However, because diversity is so great on the species level, most research characterizing person-to-person variations is performed at the next level up, the genus level. Some of the most prominent genera of bacteria found with the gastrointestinal tract include *Bacteroides*, *Lactobacillus*, *Escherichia*, *Prevotella, Bifidobacterium, Eubacteria*, *Clostridium*, *Prevotella*, and *Fusobacterium*.

Recently, scientists have discovered that all humans can be classified based on which genus of bacteria dominates their gut microbiota. In fact, the human gut microbiota is generally dominated by either *Prevotella* or *Bacteroides*. These classifications are largely dependent on long-term dietary patterns and will be discussed in more detail in chapter 3 (see section on enterotypes).

While some research focuses on species- or genus-level differences in gut microbiota, using broader levels of classification is useful in grouping individuals and identifying patterns of gut microbial populations. Most broadly, at the phylum level, there are four main taxa found across human populations. The most predominant are Bacteroidetes and Firmicutes, which make up over 90% of total gut bacteria. Actinobacteria and Proteobacteria are also relevant phyla, though they only comprise between 1 and 5% of total gut bacteria.

Laboratory Methods Used to Characterize the Gut Microbiome

Until recently, the only technology used in laboratories to classify bacterial organisms was the culture method. This greatly limited which bacteria were identified, especially because many of the bacteria living within the human body exist only in a low-oxygen environment. These anaerobic bacteria lack protective mechanisms against the toxic effects of oxygen and typically do not thrive in open-air environments. Since culturing bacteria exposes them to oxygen, this method does not favor anaerobic organisms present in our gastrointestinal tract.

In the 1990s, technological advances allowed scientists to use molecular methods to determine which organisms make up the human microbiome. Molecular techniques provide new knowledge about bacteria that was not accessible through culture-based methods. This technology has significantly broadened the data on known anaerobic microbial organisms, particularly those living within the gut microbiota. Also, molecular techniques are providing some answers about the specific compositional differences of the gut microbiota in health and disease.

However, it is still important to remember that both culture-based and molecular methods have potential for bias, in that they may support identification of particular types of bacteria. Culture-based methods are clearly biased towards aerobic microbes that prefer to grow in oxygen-rich environments. On the other hand, some molecular techniques may favor bacteria that are more responsive to DNA amplification or other aspects of molecular techniques. Despite any bias in these molecular technologies, there is no doubt that these methods are extraordinarily valuable in detecting gut microbes. While each technique has its limitations, combining evidence from multiple methods provides more detailed understanding of gut microbiota composition.

As scientists no longer rely on culture methods and microscope-based technologies, analysis of the gut microbiota is now done primarily through next-generation sequencing technologies. Ribosomal RNA is one important molecule that can be used with these technologies in bacterial identification. Sequencing techniques specifically isolate the 16S RNA gene from bacterial DNA. This approach is particularly useful in identifying unknown species.

Polymerase chain reaction is another laboratory technique used to identify gut microbes. This method amplifies and makes copies of specific bacterial DNA sequences. It is particularly useful in studies that track changes in gut microbiota due to aging, disease, and antibiotic use. In general, this technique is best used in combination with other methods to provide a more in-depth look at the diversity and abundance of gut microbes. Identification methods based on polymerase chain reaction are limited, in that they fail to detect unknown species and can also be labor intensive.

Culture-independent techniques are used to characterize entire populations of microbes. Certain molecular techniques are also particularly effective in providing insight into metabolic functions of a bacterial population. Scientists are able to detect metabolites, which are products that result from microbial metabolism. Metabolomics is the study of metabolites that may be present in stool, urine, or blood. Metabolites produced by microbes can be present in any of these bodily fluids, and their measurement can indicate how these microbes are functioning within the body. Additionally, metaproteomics more specifically identifies proteins made by gut bacteria and provides a clearer picture of microbial metabolic functions.

Gram-Negative vs. Gram-Positive Bacteria

Gut bacteria are also classified based on their type of cell envelope, a protective structure that surrounds the organism. The two major types of cell envelopes are gram-negative and gram-positive. Of the four predominant phyla within the gut microbiota, Firmicutes and Actinobacteria are mostly gram-positive, whereas Bacteroidetes and Proteobacteria are mostly gram-negative.

To determine the type of cell envelope found on a specific bacterium, scientists use a technique called a gram stain that identifies the cell wall composition. With this test, gram-negative bacteria do not retain a stain after they are washed with alcohol, whereas gram-positive does become visibly stained. This is due to gram-positive bacteria's thick cell wall that absorbs the stain used during the test.

While gram-positive bacteria contain only a thick cell wall with no outer membrane, gram-negative bacteria have a cell envelope composed of two parts: the cell wall and an additional outer layer. This outer later is made of molecules called lipopolysaccharides (LPS). We will continue to discuss the

relevance of lipopolysaccharides in future chapters, especially as it relates to disease. As we will see, the outer membrane of gram-negative bacteria can be very toxic. This is in part due to characteristics of lipopolysaccharides that make them more resistant to the body's immune defenses. These detrimental health effects are surprisingly associated with gram-negative bacteria that normally live within the gut as a members of a healthy microbiota. However, in a balanced gut microbiota, gram-negative organisms reside in the gut without these toxic effects. In fact, many gram-negative bacteria have a variety of health benefits when inside the colon.

Classifying Types of Host-Microbe Interactions

In addition to taxonomical classification, gut microbes are also described in terms of their interactions with humans. As mentioned earlier, when two different organisms live closely together and form a long-term association, this type of relationship is defined as symbiosis. Symbiotic interactions can be further characterized as commensalistic, parasitic, or mutualistic.

Most of the microbes living in our gut are commensal organisms. Commensal relationships benefit one organism while leaving the other relatively unaffected. Earlier research on gut microbiota revealed a mostly commensal relationship between the human host and its gut microbes. In this case, the gut bacteria benefit humans by breaking down otherwise indigestible food molecules and produce substances that promote human health.

On the other hand, a pathogen is any microorganism that causes disease. While it might be easy to characterize microbes as good or bad for human health, in reality the relationships between microbes and their host is far more complex. In fact, some microbes can change their behavior based on environmental or genetic factors. Opportunistic microbes, for example, generally live in the GI tract as commensal organisms but can become pathogenic under the right circumstances. These microbes can activate immune response and produce an inflammatory state. Interestingly, some research suggests that diet and the overall state of gut health can determine the pathogenicity of opportunistic bacteria.

Unlike opportunistic bacteria, which are associated with acute infections, a pathobiont is a type of microbe that is associated with chronic inflammatory diseases. These microbes are not abundant but tend to coexist with commensal microorganisms. Although pathobionts also often display characteristics of commensal organisms**,** they have the potential to become pathogenic if given the opportunity.

Researchers also often make a distinction between transient flora and indigenous flora. Transient flora refers to microorganisms that only inhabit a particular environment under abnormal circumstances. Most pathogenic

microorganisms are transient. Indigenous flora refers to native microorganisms that colonize specific habitats within the gastrointestinal tract. These microbes have maintained a symbiotic relationship with humans for thousands of years and likely co-evolved with their human hosts. Interestingly, because of this long-term relationship, researchers are using certain indigenous microbes as markers to track human evolution. Furthermore, although indigenous bacteria can be distinguished from what is considered normal gut flora, these terms are often used interchangeably. Normal flora simply refers to microorganisms that are ubiquitous within a community or population of people.

Introduction to the Gastrointestinal Tract and Its Microorganisms

The gastrointestinal (GI) tract is a tubular organ that digests food, absorbs nutrients, and excretes waste. Macronutrients such as proteins, carbohydrates, and lipids must be broken down in the GI tract before they are available to the body. The absorption of micronutrients such as vitamins and minerals also occurs in the GI tract. Aside from these duties as part of the digestive system, the GI tract hosts the most diverse microbial populations in the human body. Interestingly, these gut microbes are an integral part of digestion, and they closely interact with both macronutrients and micronutrients in the body.

In this section, we will discuss how microbe populations vary based on their location in the gut. Beginning with the mouth and ending with the colon, we will explore different parts of the gastrointestinal tract as distinct microbial habitats. We will identify characteristics that influence the types of microbial populations found within each of these sections. We will also review how anatomical features of the GI tract influence microbial populations.

Distinct Microbial Habitats in the GI Tract

During digestion, food travels through the lumen, the cavity or tubular structure that serves as a passageway for digesting materials. As food makes its way through the lumen, it encounters a few distinct microbial habitats. Although microorganisms are found in a variety of locations on the human body, the bacterial populations within the gastrointestinal tract are most numerous.

The gastrointestinal tract is divided into two sections: the upper GI, which contains the oral cavity, esophagus, and stomach, and lower GI, which is made up of the small and large intestines. Furthermore, different sections of the GI tract are also referred to based on their distance from the center of the body. For example, the proximal colon refers to the part of the colon closest to the center of the body, whereas the distal colon is the part farthest from the center.

SIDEBAR 2.1 The Oral Microbiome

The digestion of food involves a complex system of mechanical and chemical processes. Digestion begins in the mouth, where food is mechanically broken down by chewing. Chewing not only reduces the size of food particles but also stimulates the release of saliva, which contains its own bacteria. Bacteria within the mouth also interact with food as we chew.

The mouth itself has a rich microbiome that strongly influences the health of teeth and gums. Maintaining a balanced oral microbiome is not just important for oral health; it may have implications for overall health as well. Some detrimental bacteria that grow in the oral cavity may actually be linked to greater risk for developing cardiovascular disease. For instance, certain microbes in the mouth cause periodontitis, an infection that damages the tissue and bones surrounding the teeth. Interestingly, these oral microbes can enter blood circulation and contribute to plaque buildup along the blood vessels.

Unlike any other site on the body, the colon presents a unique environment with very distinct microbial populations. This portion of the GI tract provides the most hospitable habitat for a very diverse population of microorganisms. While the colon is an ideal environment for many microbes, the stomach and small intestine have significantly fewer bacterial species. This lack of diversity is mainly due to a low pH that makes the stomach and small intestine inhospitable for most microorganisms.

The structure and environment of the GI tract are two important factors that determine the type of microbial habitat. For example, pH levels vary through the GI tract and can determine which microbes thrive in these various sections. Acidity leads to less microbial diversity. Thus, the microbial populations in the stomach and small intestine are far less diverse than those within the large intestine. Yet, some acid-resistant commensal bacteria such as *Lactobacillus* and *Streptococcus* are able to live within the stomach. On the other hand, the alkaline environment of the large intestine is more habitable for a variety of microbes. The following section will explore the various microbial environments found within the GI tract and discuss some of the microbes found living in each of these distinct habitats.

Compared to other parts of the GI tract, researchers have gathered significant amounts of information about microbial communities within the large intestine. This is partly because such information is relatively easy to obtain, through fecal samples that help us better understand these bacterial populations. However, the microbiota of the stomach and small intestine is more difficult to profile. The next section explores these less-hospitable microbial environments.

The Stomach and Small Intestine

After food is chewed and swallowed, it travels down the esophagus, a tube-like structure that connects to the stomach. While food may interact somewhat with oral microbes, these interactions are short-lived as bacteria enter the highly acidic environment of the stomach. The low pH of the stomach is essential for chemical processes that break down macronutrients. This acidic environment is particularly important during the initial stages of digestion, to help activate stomach and pancreatic enzymes that help digest food. For example, large proteins must be chemically digested with the help of stomach acids.

A low pH not only supports the action of digestive enzymes; it also protects the stomach and small intestine from bacterial overgrowth. The presence of bacteria in these parts of the GI tract would otherwise create nutrient competition. Particularly within the small intestine, where most nutrients are absorbed, large numbers of bacteria would feed on those same nutrients and prevent them from being absorbed by the body. Interestingly, the acidity of stomach secretions not only protects the stomach and small intestine from bacterial overgrowth but also helps maintain balanced bacterial populations in the large intestine as well.

H. pylori: Friend or Foe?

There are very few microbes that inhabit the stomach, but one such microbe, *Helicobacter pylori*, has a very long-standing relationship with humans. In fact, many scientists are now classifying it as an indigenous microorganism. This gram-negative bacteria is very common and is present in approximately half the human population. Unless *H. pylori* is treated with antibiotics, this infection can remain established within the stomach for many years, if not a lifetime.

This bacterium prefers to live in the mucous layer of the stomach, but it can also survive in parts of the small intestine. *H. pylori* can be found within free-floating bacterial populations, as well as adhering to epithelial cells. It is important to note that the interactions between *H. pylori* and its host differ between strains. Microbiologists have observed that some strains act as commensals, while others are pathogenic.

For many years *H. pylori* has been classified as a harmful pathogenic bacteria. Infection with this microbe affects the physiology of the stomach. Many people who are infected with this bacterium experience gastritis (irritation of the stomach lining), but this consequence is usually asymptotic and often goes unnoticed. On the other hand, chronic *H. pylori* infection can cause very noticeable symptoms. Specifically, *H. pylori* is associated with the development

of ulcers and cancer within the stomach. It is also linked to certain nutritional deficiencies, such as low iron and vitamin B_{12}. There is no doubt that this bacterium can do great harm. However, more recent research suggests that its influence on human health is much more complex.

Surprisingly, this vilified bacteria appears to be protective against certain illnesses, such as asthma and acid reflux. One strain of *H. pylori* is shown to affect the secretion of stomach acid. Interestingly, strains that are more likely pathogenic may also be protective against chronic acid reflux, a condition known as gastroesophageal reflux disease (GERD). Interestingly, GERD is linked to asthma, and some researchers theorize that the disappearance of *H. pylori* may be connected to the increase in asthma prevalence. Furthermore, these strains are also protective against esophageal cancer.

This bacteria may also affect the body's metabolic functions. *H. pylori* influences hormones in the stomach (such as ghrelin and leptin) that regulate appetite and fat storage. Alteration of these hormones can change energy homeostasis and the accumulation of fat tissue in the body. These hormones will be discussed further in chapter 5.

Our evolutionary ancestors carried this bacteria, and until recent generations it was present in all humans. The long-standing relationship between humans and this microbe may soon become a thing of the past: *H. pylori* is rapidly diminishing from modern human populations. There is presently no conclusive cause for the reduction in *H. pylori* populations, but it may be due to the advancement of sanitation technologies and increased use of antibiotics.

Scientists are currently working to trace the history of the human relationship with *Helicobacter pylori*. Ötzi the Iceman, who lived approximately 5,300 years ago, gives us valuable knowledge about this particular bacteria that has been passed down through generations for thousands of years. Ötzi has been analyzed many times since his discovery back in 1991, but scientists only recently located the mummy's stomach. They found that not only was Ötzi's stomach full of food contents from his last meal, but it also contained *H. pylori*.

As an indigenous microorganism, *H. pylori* may hold some answers for researchers working to trace human migrations. It appears that *H. pylori* was brought to the Americas from northeast Asia about 13,000 years ago. This bacterium was found in an indigenous group living in remote areas of the Amazon rain forest. Another *H. pylori* strain was found among individuals living in South American cities. These *H. pylori* populations are traced back to Europe or Africa. Interestingly, the *H. pylori* in the indigenous group is genetically different from the type found with the South American cities. These findings reflect what is already known about the history of human migration patterns.

SIDEBAR 2.2 Microbial Samples from Stomach Lumen and Mucosa

The microbial populations living within the mucosa of the stomach are much more difficult to access compared to planktonic microorganisms. Planktonic bacteria within the lumen can be readily obtained through samples of stomach fluid. On the other hand, samples from the mucosa are typically obtained from invasive procedures such as endoscopy, which involves the insertion of a tube into the stomach. For this reason, most samples of mucosa-associated microbiota are collected from individuals undergoing endoscopy due to a preexisting stomach disease.

Microbes of the Small Intestine

The stomach changes the consistency of ingested food to form a fluid substance that promotes easier digestion. Next, this fluid leaves the stomach and enters the small intestine. This partially digested food is still quite acidic upon leaving the stomach. The pancreas produces alkaline substances rich in bicarbonate, to help neutralize the pH. Additionally, the lining of the GI tract contains glands that produce viscous, alkaline mucus-containing secretions that help neutralize the pH to protect the small intestine. Still, the pH of the small intestine remains low enough to support digestion and discourage bacterial overgrowth.

The proximal small intestine contains both strict anaerobes that thrive in low-oxygen habitats and facultative anaerobes, organisms that are able to survive with or without oxygen. Some bacteria that may be found in small intestine are *Bacteroides*, Enterobacteria, *Lactobacillus*, *Streptococcus*, and *Staphylococcus*.

There are various structural components in the small intestine that promote nutrient absorption while also providing a habit for a small number of microorganisms. The structure of the small intestine has a number of features that support its major role in nutrient absorption. It has an enormous surface area that maximizes the body's exposure to food passing through. Three unique structural components create this large surface area. First, the small intestine is lined with a thin tissue called the epithelium that contains many large folds and pits that slow down the movement of food. These folds along the epithelium not only dictate the movement of digesting food but expand the overall surface area of the small intestine. Second, this epithelial layer is also covered with fingerlike protrusions called villi. The villi contain hundreds of enterocytes, cells within the intestinal lining that help absorb nutrients. These enterocytes contain a third structural feature: microvilli. These are microscopic hairlike extensions found on the outer membrane of enterocytes. Digestion is completed within enterocytes with the assistance of microvilli.

The entire gastrointestinal tract is lined by a membrane called the mucosa. Intestinal mucosal cells produce enzymes that hydrolyze partially digested nutrients such as carbohydrates and protein. On the surface of this lining is a mucus layer that serves a protective role. For instance, epithelial cells in the small intestine can inhibit growth of bacterial populations by producing an antibacterial substance that makes the mucus lining resistant to bacterial penetration. Interestingly, animal studies show that *H. pylori* infections reduce gastric mucins, structural components of the mucus layer. This is one way *H. pylori* can overcome the body's natural protective mechanisms.

Dysbiosis—imbalance among the microbes—of the small intestine can occur in a variety of conditions. Researchers are working to obtain a more complete profile to determine which microbes are present in a healthy small intestine. One of the most common forms of dysbiosis within this organ is called small intestinal bacterial overgrowth (SIBO). Symptoms of SIBO include changes in bowel movement, nausea, and vomiting. Persistent cases can prevent the absorption of nutrients and lead to weight loss and malnutrition.

SIBO is a sign that the body's protective mechanisms are not properly functioning, creating changes in the natural environment of the small intestine that allow bacterial overgrowth. Normally, in a healthy small intestine, an acidic environment prevents most bacteria from taking up residence. Any underlying medical conditions that decrease production of stomach acid can subsequently create a more alkaline environment in the small intestine. Also, certain medications that are meant to lower stomach acid might promote SIBO (there is some controversy with this that will be discussed further in chapter 6). Specifically, a group of drugs used to treat acid reflux, called proton-pump inhibitors, are not recommended for long-term use because they may chronically increase pH in the GI tract. Unfortunately, a more alkaline environment can be a hospitable place for pathogenic bacteria and can also encourage overgrowth of commensal bacterial.

Transit time is an important factor that can either promote or discourage microbial growth. For instance, food substances stay in the colon longer, whereas they spend less time in the stomach. The quicker transit time through the stomach ensures that microbes are not easily able to grow in that part of the GI tract. The transit time for food through the small intestine is normally around 2 to 4 hours. It is important for food to move through this part of the GI tract at this rapid pace to prevent microbial colonization.

Interestingly, some foods also buffer the affects of acidic digestive secretions. Following meals, microbial populations between the proximal portion of the small intestine and the distal portion of the colon gradually increase. However, due to the various factors that make small intestine an undesirable habitat for microbes, these populations do not typically increase to a significant number.

The Large Intestine

While digestive enzymes are produced in mouth, stomach, pancreas, and small intestine, the body is still not able to digest all parts of our food. A portion of dietary carbohydrates and proteins escape absorption in the small intestine and find their way into the large intestine. This part of the digestive tract, which is also referred to as the colon, also provides an ideal environment for microbial growth. Within the large intestine, there are a few distinct environments where bacteria are found: the lumen, the epithelial mucosa, the mucus in intestinal crypts, and the surface of mucosal epithelial cells.

Biofilms and Planktonic Microbes

Gut microbes are also categorized based on where they live within the large intestine. For instance, planktonic microbes are free within the lumen, whereas mucosa-associated flora are attached to the mucosa along the epithelial layer. Bacteria are also able to form communities called biofilms that adhere to cells or to the surface of free-floating materials within the gut. Biofilms are colonies of bacteria that adhere to a surface and to each other. They are complex networks that can contain multiple species. Microbes living in the biofilm display different traits compared to their planktonic counterparts.

Aside from mucosa-associated organisms, most bacteria in the lower end of the colon are not free-floating but rather attached to food particles, forming biofilms on the surface of those particles. These biofilms, called particle-associated biofilms, are found on digestive residues. Specific types of food particles are more likely to encourage biofilm formation. For instance, wheat bran and resistant starch are associated with biofilms that contain groups of *Ruminococcus.* Biofilms that are attached to food particles are similar in composition to planktonic microbes, but they are metabolically very different. For example, particle-associated species have higher enzyme activity. Unfortunately, biofilms in the lower digestive tract are difficult to access, and much of our understanding about the functions and structure of these communities is based on observations of microbial biofilms within the mouth.

Biofilms support coordinated interactions between different species of microbes. As mentioned, the same species can act differently, depending on whether they are part of a biofilm or living as planktonic non-adherent microbes. Not only do biofilm species differ in their metabolic properties, their survival is also improved compared to planktonic organisms. Living in a biofilm community offers microbes an added level of protection against the host's defense mechanisms. For example, biofilms are more resistant to digestive acids and host-produced antimicrobial agents.

The microbial composition of particle-associated biofilms is surprisingly similar to that of planktonic communities. Both communities have high numbers of *Bacteroides* and *Bifidobacterium*. The digestive functions of biofilm communities differ in their types of preferred carbohydrate sources. Biofilm communities specialize more in digesting polysaccharides, whereas unattached microbes ferment oligosaccharides. These two types of carbohydrates will be discussed in more detail in chapter 3.

In addition to differences in carbohydrate preferences, the types of metabolites produced during digestion differs as well. This is not surprising, because metabolites are determined by the type of carbohydrate being digested. Biofilm communities efficiently produce the short-chain fatty acid acetate, and unattached communities produced higher levels of butyrate. These metabolites will also be discussed more extensively in the next chapter.

The Colonic Epithelium

Covering gut epithelial cells is a mucous membrane that protects the cells from exposure to luminal contents. This innermost layer of the gastrointestinal tract is the mucosa. The colonic epithelium and mucosa determine the interactions between the human body and the external environment. They function as an important barrier that protects the body against invasion of both pathogenic and commensal microorganisms. This intestinal barrier is designed to instigate protective immune mechanisms in response to any threats of intrusion by gut microbes. When this barrier malfunctions, bacteria are able to escape the bloodstream, and cause harm to other areas of the body.

Lesions in the intestinal epithelium leave the host vulnerable to microbial penetration and are therefore immediately repaired. The body helps maintain this barrier function by constantly renewing intestinal epithelial cells. The colon contains glands called colonic crypts, which facilitate the production of new epithelial cells.

In addition to this physical barrier, the intestinal epithelium provides a dynamic communication network that supports a balance between defensive responses and tolerance to microorganisms. Intestinal epithelial cells mediate communication between microorganisms and mucosal tissue. This communication allows mucosal tissue to produce different immune responses to commensal and pathogenic microbes. This balance must be finely tuned, because disruption causes undesirable immune reactions toward commensal microbes.

The epithelial lining contains specialized cells called goblet cells, that secrete mucus. Their name comes from their goblet-like shape, as the top of the cell is wider, like a cup, and the bottom is narrower, like a stem. The mucus produced by goblet cells is greatly important for both the human host and the gut

bacteria. The mucus acts as a lubricant, aiding in the movement and elimination of waste material from the large intestine. Goblet cell–produced mucus coats the epithelial surface, creating a gel-like film that functions as a barrier to prevent bacteria from leaving the gut. The presence of intestinal bacteria signals the goblet cells to reproduce. An increase in this type of cell allows for more mucus production and therefore protection of the epithelial surface.

The colon has two different mucus layers along the intestinal wall. The outer mucus layer is closest to the lumen and contains many microorganisms. The inner mucus layer has a different structure, which is more resistant to microorganisms. The inner mucus layer adheres tightly to the epithelium, but the outer mucus layer is loosely attached and can be removed from the mucosa.

Mucins can trap bacteria and eliminate them through intestinal peristalsis, the movement of luminal contents resulting from wave-like contractions along the smooth muscle of the GI tract. This helps balance microbial populations within the colon. A slow rate of intestinal movement can promote bacterial overgrowth. The rate of peristalsis is therefore very important in maintaining colonic health. If peristalsis is sluggish in the large intestine, adding fiber will increase bulk and soften the stool, to increase strength of intestinal movement. Fiber increases movement that propels food through the large intestine. This important nutrient is discussed more in the next chapter.

The Appendix

The appendix is a narrow tube attached to the upper part of the large intestine. The appendix contains many immune cells, called lymphocytes. When these lymphocytes are overactive and the appendix becomes inflamed, a condition called appendicitis occurs. Appendicitis can cause serious complications and lead to death if not treated early. Currently, the only treatment for appendicitis is the removal of this organ, a procedure known as an appendectomy.

Until recently, the medical community understood that the appendix has no known physiological functions. This understanding is supported by the lack of side effects following an appendectomy. Yet, a new theory suggests that the appendix may have an important function after all: it serves as a reservoir for commensal bacteria.

Researchers now believe that our commensal bacteria are stored in the appendix and can then be used to repopulate the colon after an infection has passed. The appendix, which is isolated from the other parts of the GI tract and has only a small opening, possesses an ideal structure and location that protects it from any influx of pathogenic bacteria. These features are useful in the case of infections that cause irregular bowel movements that might wipe out commensal flora.

Over 5% of people in industrialized societies will experience appendicitis in their lifetime. This condition is characterized by inflammation of the appendix and can be life-threatening. If left untreated, there is a 50% chance of mortality. The common treatment for appendicitis is appendectomy. This urgent surgery is necessary before the appendix ruptures, causing bacteria to leak out and contaminate the abdominal cavity.

Interestingly, appendicitis is rarely seen in developing countries, but is instead much more prevalent in industrialized countries. As developing nations become Westernized, the incidence of appendicitis often increases. This is demonstrated in both African and European cultures that have more recently adopted Western lifestyles.

Some biologists speculate that the appendix was more useful in the past, when humans were at higher risk for bacterial infections. Widespread infectious intestinal diseases are not common in developed nations, due to advances in sanitation technologies and medical practices. When these types of diseases were more common, commensal bacteria were at risk of being wiped out from the GI tract. Interestingly, appendices are not common in other mammals, and so it is possible that this organ is a unique protection mechanism that evolved in humans.

Upon examining tissue from healthy human appendices, researchers discovered that biofilms are also present in the lining of the appendix. While it is clear that the appendix can serve as a safe storage place for commensal gut bacteria, there are currently no experiments that provide conclusive evidence of its effects on infectious gastrointestinal diseases.

Bacteroidetes, Firmicutes, and Actinobacteria

The following sections briefly introduce some of the more important members of the gut microbial community. It highlights some key members of Bacteroidetes, Firmicutes, and Actinobacteria, three of the four major phyla found within the human gut. The fourth major phylum, Proteobacteria, contains members such as *Helicobacter pylori*, which was discussed above in the context of the stomach microbiome. Future chapters will describe in more detail how these microbes interact with the human body to promote health or disease.

This section pays special emphasis to Bacteroidetes and Firmicutes, as they are the two predominant phyla in the large intestine. Bacteroidetes can be fairly flexible about their surrounding environment due to a high level of adaptability towards various pH levels and nutrient availability. Members of Bacteroidetes are able to digest both protein and carbohydrates. This allows Bacteroidetes to colonize different parts of the GI tract. In fact, Bacteroidetes account for 10–20% of total bacteria within the stomach, the most acidic section of the GI tract.

Bacteroidetes and their human hosts have a generally mutualistic relationship. This phylum is involved in activating the immune system, aiding in digestion of food to produce byproducts that are beneficial to the host, and also helping to eliminating toxic substances from the body. Firmicutes also plays an important role in human metabolism. Species within this phylum also have numerous dynamic interactions with the host immune system. Finally, Actinobacteria is one of the main phyla acquired early in infancy, and we will specifically discuss commensal *Bifidobacteria* within this phylum.

Bacteroides

Bacteroides is a bacterial genus within the Bacteroidetes phylum. These gram-negative bacteria make up approximately 25% of gut microbial species. *Bacteroides* are also the most predominant anaerobes in the colon. Our long relationship with *Bacteroides* begins at birth, when these bacteria are passed along to the child from the mother's vaginal flora. This relationship between *Bacteroides* and their human host is usually mutualistic, but the types of interactions are quite diverse. Under certain circumstances, *Bacteroides* can display pathogenic behaviors.

Some *Bacteroides* species have a unique ability to utilize various nutrient sources depending on what is available. They are able to adapt to different nutrient sources because they have multiple genes for starch metabolism. *Bacteroides* also have a relatively large genome, which gives them a variety of possible genetic expressions that influence their interactions with the human host. Often depending on external factors, these bacteria can turn on specific genes that may switch interactions from commensal to pathogenic.

Bacteroides are bile-resistant and may cause disease. They can also become antibiotic-resistant. If large numbers of *Bacteroides* species leak out of the gut (typically as a complication from intestinal surgery), they can become pathogenic and cause abscesses formation. As we will see in future chapters, these gram-negative bacteria can also leak out of the gut as a result of various chronic diseases. Unfortunately, when gram-negative bacteria enter the bloodstream, they trigger the immune system to produce an inflammatory response that can lead to a number of health consequences.

Bacteroides thetaiotaomicron

As mentioned, some Bacteroidetes bacteria demonstrate flexible digestive capabilities, as they are able to utilize different nutrient sources based on what is available. *Bacteroides thetaiotaomicron* is one such species that produces different enzymes by sensing the available carbohydrates present in the gut lumen. This mutualistic bacterium, which is most concentrated in the lumen,

has a large variety of genes that specifically aid in metabolizing many different carbohydrates.

This bacterium can utilize both dietary carbohydrates as well as carbohydrate components that make up parts of the human tissue. For this reason, a diet high in refined carbohydrates, which deprives *B. thetaiotaomicron* of polysaccharides, can cause this bacterium to produce enzymes for digesting carbohydrates within intestinal mucosa. These carbohydrates within the mucosa are called glycans and may represent an important nutrient source for *Bacteroides thetaiotaomicron*. These bacteria even produce special enzymes that disable the host's defense mechanisms against microbial digestion of glycans. However, mucosa is frequently renewed, and so the gut sheds this mucus regularly. It may be advantageous to both the host and the microbe for *B. thetaiotaomicron* to assist in the removal of discarded mucus. Microbiologists believe that the digestive adaptability of *B. thetaiotaomicron* helps to maintain homeostasis in the gut by allowing microbiota to respond more efficiently to dietary changes without altering gut microbiota composition.

In mouse studies, these unique digestive capabilities of *Bacteroides thetaiotaomicron* appear to play an important role in prenatal gut development. When young mice are suckling, *B. thetaiotaomicron* in the gut produces enzymes capable of digesting host-derived polysaccharides as well as carbohydrates from mother's milk, such as monosaccharides and oligosaccharides (see chapter 3 for more information on carbohydrates). After weaning, when the mice have sufficient plant-derived polysaccharides present in the diet, *B.thetaiotaomicron* is signaled to expand its production of enzymes that support the digestion of these new carbohydrates. It would be interesting to see if the same sensing mechanisms that determine nutrient availability also help human infants adjust the metabolic activity of microbes during these types of dietary shifts.

Clostridia

Clostridia is a class of Firmicutes that is highly involved in the overall maintenance of gut homeostasis. Commensal *Clostridia* make up a large portion of total gut bacteria. They are early colonizers of the GI tract. Breastfeeding generally promotes *Clostridia* colonization in infants, and these populations are present within the first month after birth in breastfed infants.

Clostridia produce compounds through fermentation that keep colon cells healthy. Commensal species protect against inflammatory conditions within the GI tract, such as colitis, and also protect against colorectal cancer by inducing cell death for cancerous cells.

Clostridia also influence immune function in a number of significant ways, although researchers are still working to determine exactly how *Clostridia*

interacts with the immune system. Some propose that metabolites such as short-chain fatty acids and secondary bile acids produced by these bacteria are recognized by epithelial cells in the gut. Once epithelial cells detect these metabolites, they may send signals to other immune cells through a constant cross-talk that occurs between these two types of cells.

Faecalibacterium prausnitzii belongs to *Clostridium* cluster *IV*, which increase production of anti-inflammatory molecules. *F. prausnitzii* is the most prevalent microbial species in the gut and makes up over 5% of total bacterial population. *F. prausnitzii* belongs to the *Faecalibacterium* genus and is the only known species of gut bacteria within that genus. Chapter 6 will discuss how this bacterium can reduce inflammation in the gut and influence the development of chronic inflammatory conditions. In fact, we will see that *Clostridium* cluster *IV (*specifically *F. prausnitzii)* and *XIVa* are substantially less abundance in people with inflammatory bowel disease. Whether this is a cause or consequence of this chronic inflammatory disease is unknown.

Ten to 40% of total gut bacterial populations are made up of species within *Clostridium* cluster *XIVa* and *IV*. Animal studies indicate that *Clostridium* cluster *IV* and *XIVa* are found within mucosal folds in the GI tract. Similar structural folds that are also present within the small and large intestine of humans are likely provide a good habitat for these bacteria as well. *Clostridium* species living within the mucosa influence the structure and function of the intestine.

Not all members of *Clostridia* are commensal organisms. For example, *Clostridium difficile* is an opportunistic gram-positive bacterium within the Firmicutes phylum. *C. difficile* infections are potentially life-threatening. They are most often seen in hospitalized and elderly patients but are also common following prolonged antibiotic exposure. *C. difficile*'s interactions with its human host are complex. One study demonstrated that spores from *C. difficile* strains that are not known to produce a toxic effect can inhibit infection by those strains that do demonstrate toxic effects. This will be discussed further in chapters 4 and 6.

Clostridium butyricum

Clostridium butyricum is a gram-positive commensal bacteria. It is found in the infant gut not long after birth, indicating that it is one of the early colonizers in the developing gut microbiome. This bacterium is a commonly used probiotic in Asia. Through fermentation of dietary carbohydrates, *C. butyricum* produces metabolites (SCFAs) that are beneficial to human health.

In mice, one strain of *C. butyricum* mediated the activity of immune cells within the mucosa and encouraged the production of molecules that reduce inflammation (an immune reaction discussed in detail in chapter 4). The

observations from this study demonstrate that within these animals, this strain of *Clostridium buyricum* successfully prevented an inflammatory intestinal condition known as colitis. Interestingly, researchers also observed that this bacterium may help prevent *C. difficile* infection. In addition to observations made in animal studies, clinical studies demonstrate this strain's ability to prevent as well as treat antibiotic-associated diarrhea in children. Interestingly, it also helps maintain populations of *Bifidobacterium* species that might otherwise be reduced following antibiotic treatment.

As mentioned, *Clostridium* species are also implied in pathogenesis. Some strains of *Clostridium buyricum* are associated with certain illness in young infants, such as botulism (in infants) and necrotizing enterocolitis (mostly in preterm infants). Botulism is a type of severe food poisoning caused by bacteria. The *Clostridium buyricum* strains that cause botulism have toxic effects on the nervous system. *Clostridium buyricum* is similar to *Clostridium botulinum*, the primary bacterium that causes botulism, in that it also carries botulism-causing toxins.

Clostridium buyricum is also associated with necrotizing enterocolitis, a condition in preterm infants that causes GI bleeding, ulcers, and cell death within mucosa. While the pathogenesis of this disease is unknown, studies in preterm infants with necrotizing enterocolitis have identified this bacterium in both blood and stool cultures. Other studies show that *C. butyricum* can be passed along from contact with medical staff (if the bacterium is present on their hands), which indicates the possible need for preventive measures through appropriate cleaning and sanitizing before interaction with preterm infants.

Bifidobacterium

Bifidobacteria is an important genera of Actinobacteria within the gut microbiota that plays a significant role in health throughout the lifespan. These bacteria are passed through the mother's vaginal tract and are also present in breast milk. Interestingly, *Bifidobacterium* was recently detected in the placenta and amniotic fluid, indicating that this bacteria may in fact inoculate the child even before birth. While *Bacteroides* usually begins to appear in an infants' stool approximately ten days after birth, it is not the dominating genus of bacteria for breast-fed infants. Instead, infants who are breastfed have gut bacteria dominated by the *Bifidobacteria* genus. Breast-feeding helps colonize *Bifidobacterium breve*, *Bifidobacterium bifidum*, and *Bifidobacterium longum*. In fact, breast milk has special carbohydrates that feed these beneficial microbes and encourage their growth. While *Bifidobacterium* species are abundant in infants, these populations typically decrease somewhat in adulthood.

Bifidobacterium strains are commonly used in probiotic supplements, because they have a number of beneficial effects on gastrointestinal health.

There is a fair amount of evidence showing they may be effective in preventing pathogenic colonization, reducing certain GI symptoms associated with inflammatory bowel disease, alleviating constipation, and even reducing the effects of carcinogens on cells. Mouse studies show that strains of *Bifidobacterium longum* and *Bifidobacterium breve* protect DNA from cancer-causing substances. Other studies also suggest that using prebiotics in conjunction with bifidobacteria effectively protects colon cells from cancer-related mutations.

Archaea and Fungi

While bacteria represent the vast majority of microbes with the body, there are several species of archaea and fungi whose interactions with the human host is well documented by microbiologists. This section specifically highlights one archaeon and one fungus that play important roles in human health.

Archaea: Methanogens

The vast majority of research on the microbiome focuses on bacterial species. However, there are other types of microorganisms in the gut, including one group called archaea. These single-celled organisms are similar to bacteria in shape and size. Many of them prefer to live in harsh environments where most bacteria cannot thrive. Methanogens require hydrogen for their metabolic functions. These microbes use hydrogen to produce methane.

Only two archaeal species have been identified within the gut: *Methanobrevibacter smithii* and *Methanosphaera stadtmanae*. *Methanobrevibacter smithii* is present in more than 50% of the adult population, whereas *Methanosphaera stadtmanae* is found in only 30% of people. These two species of archaea belong to the methanogen group, and they live alongside bacteria in the human gut. Like most gut bacteria, methanogens are anaerobic microorganisms. Along with bacteria, methanogenic archaea are a part of the complex mucosal biofilm communities.

Methanogens in the gut improve digestion by enhancing the fermentation process and by improving the absorption of short-chain fatty acids. However, researchers believe that this increased efficiency in the fermentation process may contribute to weight gain, especially in situations of excess caloric intake. While the overall role of methanogens in the human body is largely unknown, there is some information on their contribution to fat accumulation. Furthermore, methanogens may be linked with certain GI dysfunctions, specifically given that increased levels of methane are associated with constipation and irritable bowel syndrome.

Archaea are able to metabolize certain gaseous byproducts of fermentation produced by gastrointestinal bacteria. Bacterial fermentation, which generates

mostly SCFAs, also forms other products, such as carbon dioxide and hydrogen. This hydrogen gas is eliminated by the body in a few different ways. It can enter into circulation and leave the body through respiration, be expelled as gas through the GI tract, or be used by gut microbiota. Archaea, as well as certain bacteria, are able to metabolize hydrogen in the colon.

Individuals that host these microorganisms are described as methanogenic. However, some individuals do not have significant populations of methanogens within their gut microbiota. These people are more likely to eliminate hydrogen through a process called sulfate reduction, with the help of sulfate-reducing bacteria such as *Desulfovibrio piger.* Either way, the elimination of excess hydrogen is important in reducing flatulence. The methanogenic process, as well as sulfate reduction of other microbes, assists in metabolizing hydrogen and reduces flatulence in humans.

A breath test can be used to determine the presence of methanogens in the gut, based on the amount of methane exhaled. Breath testing indicates that only about a third of healthy individuals excrete methane gas—but other studies, using fecal testing, showed that about 72% of the population are methane producers. Interestingly, the infant microbiome does not produce methane. This gas is detected only after the age of three, when microbial colonization is more advanced. In other words, the lack of methane gas produced by children under three indicates that methanogens are not early colonizers of the gut microbiome.

Although it is uncertain why some people have more significant quantities of this bacterium, there are some potential factors that influence its presence in the gut. For example, some evidence suggests that methanogens thrive better if food moves more slowly through the colon. Decreased transit time, as with constipation, increases methane production. In fact, some individuals see a direct reduction in methane when given laxatives to speed up bowel transit time. Researchers also propose that methane itself may have a regulatory effect on transit time.

Transit time and diet may significantly influence the presence of methanogens, but researchers have also discovered that mothers pass methane-producing microbes to their children. At first, researchers wondered whether genetics might play a role in how this microbe is passed down through generations. However, a twin study that tested the methane production rates of both fraternal and identical twins showed that genetics did not predict the acquisition of methanogens. Yet, it appears that methane-producers are passed along from the mother's microbiota. This indicates that the colonization of methanogens in the gut relies on transmission from the mother.

Methanobrevibacter smithii is the most abundant methanogen species in the human gut. Within a normal gut microbiota, methanogens can comprise up to 10% of total anaerobic microbes. This bacterium has a few important

roles in microbial fermentation. While *Methanobrevibacter smithii* is not saccharolytic, it does help other gut microbes in their digestion of carbohydrates. Specifically, *M. smithii* and *Bacteroides thetaiotaomicron* have a mutualistic relationship, in which these organisms interact to enhance their individual

SIDEBAR 2.3 Viruses

While viruses are not technically considered living organisms, some microbiologists group them as members of the gut microbiome. The composition of gut virus populations varies much more from person to person, as compared to gut bacterial populations. Just as we have both mutualistic and pathogenic bacteria, researchers have observed that viruses also have a wide variety of interactions with their human hosts. Although viruses are well recognized for their ability to cause acute disease, these microbiologists argue that the virome should be considered an important part of the gut microbiota.

Recent research demonstrates that viruses can signal host cells in similar ways to those seen with commensal bacteria. There is much less research on the intestinal virome at present, mostly due to the technological challenges it presents—specifically in identifying viruses within the gut.

Interestingly, a virus cannot only infect its host; some can even infect other gut microbes. Bacteria, fungi, and archaea are all susceptible to viral infections. As research into viral members of the gut microbiome continues to grow, it will be interesting to see how these viruses can influence the composition and function of other resident microbe populations through infection.

One current observation of interactions between a virus and gut bacteria involves norovirus. This virus can contribute to intestinal abnormalities that are characteristic of inflammatory bowel disease. Researchers note that human norovirus may be dependent on commensal bacteria in order to properly replicate. One group of researchers discovered that many common structural and functional changes in germ-free mice were reversed following intentional infection of norovirus in these mice. Interestingly, this virus also protected the mice from intestinal damage caused by antibiotic treatment.

Viruses that infect bacteria living in the human host are called bacteriophages. These viruses likely play a direct role in shaping the bacterial communities within the gut. Bacteriophages can live on mucosal surfaces along with microbes. Researchers have identified genes in gut bacteriophages that are beneficial for commensal bacteria. These genes can instruct bacteriophages to help bacterial populations maintain stability and resilience, which generally supports homeostasis of gut bacteria within the intestinal environment. Interestingly, other genes involved in metabolism of carbohydrates and protein are also detected in gut bacteriophages. Researchers believe this indicates that these viruses may influence human metabolism through interactions with gut bacteria.

metabolic processes. *M. Smithii* induces *B. thetaiotaomicron* enzymes for fructan digestion. *M. smithii* reduces the expression of *B. thetaiotaomicron* genes responsible for fermentation of other types of carbohydrates.

M. smithii changes its gene expression if *B. thetaiotaomicron* is present in the gut. This methanogen shifts its focus to fermentation of fructans in the gut when it interacts with *B. thetaiotaomicron*. Unfortunately, if gut microbes ferment higher levels of fructans, it increases the production of acetate, which can contribute to weight gain. (Acetate is a short-chain fatty acid discussed more in chapter 3.) *M. smithii* can produce glycans that serve as an important food sources for fermenting bacteria. This archeon can utilize metabolites produced by gut bacteria such as formate, an organic acid produced during bacterial fermentation. Also, the presence of *M. smithii* promotes the growth of both populations, another aspect of their mutualistic relationship.

Candida albicans: An Opportunistic Fungus

One study sampling gut microbiota of 98 healthy individuals identified three major genera within those samples. The most prevalent, *Saccharomyces*, was found in 98% of samples. *Candida* and *Cladosporium* were found in 57% and 42% of samples, respectively. Given that this book focuses mostly on the bacteria within the gut, this section will briefly discuss only one member of the fugal microbiome: *Candida albicans*.

Some commensal microorganisms that live in the gut can become problematic if their populations become too large. For example, *Candida albicans* is an opportunistic pathogen that lives in a variety of sites on the human body as a component of a healthy microbial community. However, overgrowth of this fungus causes a state of Candidiasis.

In some cases, colonization of this fungus produces inflammation, which may have detrimental effects on gut microbiota. In other cases, *C. albicans* lives in the stomach as a commensal organism, without producing inflammation. For this reason, researchers are now exploring the role of bacteria in determining which course this opportunistic fungus takes. It appears that reasons *C. albicans* shifts from displaying commensal characteristics to pathogenic ones are largely dependent on surrounding bacterial populations.

Ordinarily, a healthy and balanced gut microbiota will prevent the overgrowth of fungus, but dysbiosis related to antibiotic use can provide fungi like *C. albicans* with a perfect opportunity for colonization. In fact, studies in germ-free mice show that these animals are very susceptible to colonization of *C. albicans*, since they are lacking protective commensal microbes. Other animal studies show that altered gut microbiota composition is one of the primary requirements for *C. albicans* overgrowth.

SIDEBAR 2.4 Germ-Free Mice

Many researchers use a germ-free mouse model in microbiome research. However, in most cases, the diseases or conditions being studied are not naturally occurring in these animals. Scientists must create animal models that mimic these diseases. In this book, we will also frequently refer to the germ-free mouse model that is devoid of normal gut flora. Germ-free mice are born and raised in isolated, sterile environments, fed sterilized food, and given filtered air to breathe. Germ-free animals are useful as models for simplified microbiota populations. Researchers are able to create ecosystems consisting of a single species only, or specific combinations of species. These mouse models provide more control over different factors such diet and exposure to other microorganisms, allowing researchers to determine the exact mechanisms by which gut microbes influence physiology. Still, throughout this book it is important to ask: how is the information gleaned from animal models related to human disease?

Both yeast cells and bacterial cells compete for the same adhesion sites, so it is important to maintain populations of indigenous microbes to crowd out fungus. In humans, systemic *Candida* infections can be life-threatening for individuals whose immune system functions are suppressed (as in cases of chronic illness or the use of immune-suppressing medications). Understanding how this fungus enters the bloodstream may give insight into the mechanisms behind the progressing of systemic *Candida* infection.

When commensal microbes are not able to protect the host from mucosal penetration of *C. albicans,* this fungus can more readily colonize the stomach. Animal studies illustrate that after *C. albicans* populates the stomach, it may enter blood circulation. This can lead to dissemination to visceral organs. Under normal conditions (that is, while using no antibiotics), *C. albicans* only leaves the GI tract if it is present in large numbers.

One animal study demonstrates the effects of *C. albicans* colonization the GI tract of microbiota-depleted animals. In this study, hamsters received gastric injections inoculating them with *C. albicans*. Some of the hamsters were also given antibiotics to diminish commensal gut bacterial. These hamsters had high amounts of *C. albicans* in the gut. Additionally, in some of these animals, the fungus had disseminated to their visceral organs.

Some commensal gut microbes can directly protect against overgrowth of *C. albicans*. For instance, *Lactobacillus* species produce hydrogen peroxide, which inhibits growth of this fungus and reduces its ability to cause disease. *Lactobacillus* also prevents this fungus from adhering to stomach tissue; *C. albicans* is inclined to enter the epithelial layer of the stomach. Conversely,

one study demonstrates that following antibiotic treatment, *C. albicans* can inhibit the regrowth of *Lactobacillus* species within the stomach.

Additionally, *C. albicans* promotes the growth of *Enterococcus* species such as *Enterococcus faecalis* in the stomach following antibiotics. This bacterium can be problematic in hospitals, especially for critical care patients, due to its tendency to become antibiotic resistant. It can also bypass some of the body's natural protective mechanisms against pathogenic infection. For instance, *E. faecalis* can adhere to a mucus layer and survive in the epithelium despite changes in pH level throughout the GI tract. While it is clear that *C.albicans* can promote the growth of this bacterium, it may not promote these pathogenic characteristics in *E. faecalis*. In fact, some research indicates that the coexistence of *E. faecalis* and *C.albicans* encourages commensal characteristics instead of pathogenic ones.

Furthermore, researchers are now curious whether the survival of *C. albicans* and *Enterococcus* along the mucosa is affected by resident lactic acid bacteria, such as *Lactobacillus* species. Given the interactions observed between *Lactobacillus* and *C. albicans*, these researchers are interested to know how this may affect the mutually beneficial relationship between *E. faecalis* and *C.albicans*. However, at this time there is only some evidence showing an antagonistic relationship between lactic acid bacteria and these two opportunistic pathogens within the mucosal environment.

Baby's First Encounter with Microorganisms

When a child is born, it is instantly exposed to microorganisms from the mother's body as well as from the surrounding environment. This exposure is the start of a process known as microbial colonization. Humans are colonized by a succession of microbes during early childhood that eventually become the fully developed communities typically found in adults. Interestingly, researchers are discovering that the microbes that take up residence during this early period can affect an individual's health later in life. This section explores how both the fetal environment and birth method may determine the development of early gut microbiota populations.

Birth Method

Birth method is an important factor that influences the development of gut microbiota. As the infant leaves the womb, it is immediately exposed to new microorganisms. During vaginal birth, the child picks up the mother's bacteria from the birth canal. The baby's gut is then gradually colonized with those same microbes at this early stage in life. On the other hand, babies born through cesarean section are instead exposed to skin microbiota. Interestingly,

researchers hypothesize that these bacteria, which resemble populations typically observed on the skin, are transmitted by healthcare providers and previous hospital patients rather than by the mother's skin microbiome.

There is no doubt that C-sections are a life-saving procedure. In fact, infant mortality rates are higher in areas without access to C-sections. The Center for Disease Control reports that nearly 33% of all children born in the United States are delivered by C-section. Also, the rate of C-section increased by 60% from 1996 to 2009. Understanding the impacts of this procedure on gut microbiota development may encourage healthcare practitioners to adopt practices that encourage proper microbial colonization of these C-section newborns.

Given this disruption of the developing gut microbiome, microbiologist Maria Gloria Dominguez and her team are experimenting with a method to restore the microbiota of infants born through C-section. This technique involves the collection of microbe-rich vaginal fluid prior to delivery. This fluid is then applied to the newborn baby's skin in order to colonize the infant with these vaginal microorganisms. This practice may prove beneficial as long as the mother's microbial populations are healthy and in balance. Screening individuals for sexually transmitted infections as well as for dysbiosis are important measures prior to the use of this microbial restoration method. Vaginal microbes, just like gut microbes, are affected by pH. A healthy vaginal microbiome is dominated by *Lactobacillus*.

While this type of microbial restoration does successfully introduce some vaginal microbes to babies born by C-section, it is still uncertain whether this method has any direct health implications. Researchers are still exploring whether microbial colonization differences related to birth method really impact health outcomes. It does appear that these newborns initially have gut microbiomes that are similar to skin microbiome populations rather than those found in the birth canal. Yet, these populations eventually start to shift and resemble more normal gut microbiota populations.

In addition to the change in gut microbiota from the delivery method itself, antibiotics that are routinely given during C-section births may also alter the infant's microbiota. These antibiotics are, of course, protective for the mother, as women undergoing cesarean section are significantly more likely to develop an infection compared to women giving birth vaginally.

Although there is no conclusive evidence regarding how strongly birth method dictates the future health of a baby, exploring patterns in microbial population development is important in understanding potential effects on the gut microbiome. Yet, this can still affect the development of the immune system and may determine the risk of environmental or food allergies.

Delivery method at birth appears to have more of an effect on rate of development rather than on ultimate microbial composition. After the first year of life, little distinction can be made based on C-section or vaginal delivery. In

fact, the gut microbiota is developing until about age two, when the microbial composition becomes more stable. Although mode of delivery may affect microbiota composition at the beginning of life, the differences observed based on delivery method diminishes after the age of four months.

The Maternal Gut Microbiome

A mother's microbiome plays a role in her baby's early gut microbiome development. While medical literature characterizes the womb as a sterile environment, new research indicates that there are some live bacteria present. The womb likely does not have a plentiful bacterial population, and this mostly sterile environment may protect the growing fetus from any potential microbial threats by keeping these populations at bay. However, scientists have recently observed a small number of bacteria present in the environment around the fetus as well as in the fetus' intestines. This may indicate that a baby's first exposure to microbes may actually take place long before it passes through the birth canal.

While some bacterial DNA was discovered within the placenta, it is still unclear whether bacteria is definitely present on the fetus side of the placenta. More recently, researchers confirmed that small amounts of bacteria can be found within the amniotic fluid that surrounds the developing fetus. These bacteria are likely sourced from the mother's mouth or vagina and transported to the fetus through the bloodstream. Interestingly, the bacteria that are normally present in a healthy placenta share some surprising similarities to oral microbiota communities. This may indicate that microbes travel from the oral cavity to the placenta. The most abundant microbial phylum found within the placenta is Proteobacteria. Further research is needed to understand the nature of this bacteria in the fetal environment and determine whether it is commensal.

Meconium is the first stool produced by newborns. The materials excreted through meconium are indicative of what the fetus ingested while in utero. The presence of bacteria within meconium suggests that the fetus is not sterile but is exposed to microbes during pregnancy and not solely during birth. The bacterial composition of meconium is less diverse than populations detected within adult stool samples. Similar to the placenta, meconium is enriched with Proteobacteria. However, populations of Bacteroidetes are reduced in this early stool.

Microbiota Changes during Pregnancy

During pregnancy, the mother's gut microbiota goes through significant compositional changes. Between the first and third trimester, the populations of Actinobacteria and Proteobacteria increase. Researchers propose that these

changes may help the mother better absorb dietary fats and carbohydrates, as well as enhance immune system functions.

The mother's vaginal microbes are very important to the developing infant microbiome. Just like the gut microbiota, the vaginal microbiota undergoes significant changes during pregnancy. *Lactobacillus* species increase, and the overall diversity of microbes decreases. These lactic acid bacteria are usually present in the vagina, but their increased abundance during pregnancy helps lower the pH of the vagina more than normal. Interestingly, towards the end stage of pregnancy, the vaginal microbiota composition is again very similar to that of non-pregnant women.

The Aging Microbiome

While the gut microbiota is fairly stable during adulthood, the microbe populations at the beginning and end stages of life are much more variable. The microbiota of elderly individuals is characterized by higher numbers of Bacteroidetes. Younger adults typically have higher numbers of Firmicutes.

Researchers have also assessed gut microbiota composition in relation to individual degree of frailty, an age-associated state of increased vulnerability to injury and disease. They observed that frailty decreases the overall diversity of microbial populations. Individuals who were observed to be in a very frail state had significantly lower numbers of *Faecalibacterium prausnitzii*, as well as reduced populations of members from the *Bacteroides*, *Lactobacillus*, and *Prevotella* genera. These individuals instead showed elevated numbers of Enterobacteriaceae and *Ruminococcus*.

Residence location among elderly populations can significantly impact their gut microbiota composition. In general, community residents were found to have more diverse gut microbial populations compared with those who are hospitalized or in long-term care living facilities. As mentioned earlier in this chapter, elderly individuals may be more susceptible to *C. difficile* infection, particularly if they are living in long-term care or being treated with antibiotics.

Conclusion

We have learned how the body creates an inhospitable environment for microbes in the stomach and small intestine in order to limit any nutrient competition between the host and its microbes. This chapter provided a foundation of information about resident colonic microbes that will be useful in future chapters as we discuss their role in human health and disease. Now that we have introduced key members of the gut microbiome, the next chapter will focus on how these microbes interact with the diet.

3

Nutrition and the Gut Microbiota

Diet is a highly influential factor that shapes the gut microbiome. Commensal bacteria depend on host food intake for their own nutrition. In fact, the term "commensal bacteria" is based on the Latin word *commensalis*, meaning "together at the table." This is a particularly accurate description of the beneficial bacteria that reside in our gut, as they directly participate in the digestive process. In this way, the partnership between the human host and commensal bacteria is based on diet and shared resources.

Despite shifts in popular dietary trends, nutrition professionals generally agree that limiting processed foods is a primary component of an optimal diet. As we discussed in chapter 1, the standard Western diet can be devoid of complex carbohydrates that feed our commensal microbiota. These important nutrients are most often lacking due to modern processing techniques, which affect the structure of food, particularly in carbohydrates. A diet rich in highly processed carbohydrates reduces fiber content and unfortunately limits our commensal bacteria's food source.

This chapter will discuss how culture and diet can shape the gut microbiome. The first section will describe how gut microbiota populations differ in plant-based versus animal-based diets. This section will also explain the interactions between the gut microbiota and the three dietary macronutrients used by the body for energy: carbohydrates, protein, and fats. We will also explore in great detail the different types of carbohydrates and how they influence gut microbiota composition and function. The final section of this chapter introduces the various compounds produced by bacteria and discusses their effects on human health.

Culture, Diet, and Varying Gut Microbiota Populations

The type of bacteria that grow in the gut is largely determined by the food we eat. The food present in the gut is a large part of the ecosystem that forms within the lumen of the large intestine. In the animal kingdom, omnivores,

herbivores, and carnivores all have distinct gut microbial populations. However, the omnivorous human diet can vary based on cultural and geographic influences. This section discusses how diet composition can be used to characterize humans based on the overall profile of their gut microbiota.

Enterotypes

Researchers have now analyzed human gut microbiota from many countries around the world. Interestingly, patterns began to emerge based on diet rather than other factors such as age, gender, body weight, or nationality. Variations in diet produce different types of ecosystems in the gut and shape the composition of the gut microbiome. In fact, all human microbiomes can be classified into larger groups called enterotypes.

In 2011, Peer Bork and his colleagues proposed three classifications of enterotypes based on different genera of bacteria. Their observations suggested that people are either *Bacteroides*-dominant, *Prevotella*-dominant, or *Ruminococcus*-dominant. Then, in a subsequent study, it became evident that the *Ruminococcus* enterotype is less easily distinguished, and it was therefore fused with the *Bacteroides* enterotype. All humans thus are now considered to fall into two enterotype categories: the gut microbiota are dominated either by *Bacteroides* or by *Prevotella*.

Given that these classifications are often predictable based on diet, individuals can shift their enterotypes by changing their food intake. Unlike blood types, which are permanent classifications determined at birth, enterotypes are not as distinct or unchanging. Still, these classifications are generally based on long-term dietary patterns and appear to remain stable throughout life. For instance, a diet high in protein and animal fat is strongly associated with *Bacteroides*, whereas a carbohydrate-based diet will lead to a *Prevotella* enterotype. However, with major shifts in diet, it appears that these enterotypes can be altered.

Diet Rapidly Changes Gut Microbiota

These new understandings about the connection between diet and gut microbes left researchers wondering just how quickly diet can reshape gut microbiota populations. In fact, several studies now show that gut microbiota rapidly shift in response to changes in diet. One such study analyzed individuals consuming either a plant-based diet or an animal-based diet. Individuals on the animal-based diet ate meals and snacks comprising solely meat, dairy, and eggs. The plant-based diet was rich in grains, beans/legumes, fruits, and vegetables. One of the subjects in the study, who was a lifelong

vegetarian, showed significant changes after just one day on an animal-based diet.

Individuals who eat a mostly plant-based diet, such as vegans and vegetarians, have gut microbiota populations dominated by *Prevotella* and are more lacking in *Bacteroides*. On the other hand, omnivores had an abundance of *Bacteroides*. After four days consuming an animal-based diet, the vegetarian's *Prevotella*-to-*Bacteroides* ratio was inverted. This substantial dietary change caused the gut microbiota population to shift so that *Bacteroides* outnumbered *Prevotella*.

These types of rapid microbial population shifts reflect how our gut microbiota gives us great flexibility in our ability to digest different foods based on what is available. This advantageous evolutionary trait demonstrates the symbiotic relationship between humans and their gut microbes. However, while this flexibility is an important survival trait, it is still unknown how each enterotype informs long-term health patterns. Researchers are still working to determine whether certain microbial populations are more reflective of overall health and disease prevention. They are hoping to identify a "core" microbiome by specifying which taxa are shared by most healthy individuals. However, some researchers question whether these microbial community types are not a distinct group (such as defined by enterotypes) but rather a gradient.

The Gut Microbiome around the World

One way to explore the impact of diet on the gut microbiome is by comparing gut microbe populations in the context of varying dietary patterns around the world. This type of research is particularly valuable given that the modern Western diet has altered the composition of the human gut microbiota. As discussed in chapter 1, traditional diets comprise minimally processed foods that provide a variety of dietary compounds to nourish a diverse gut microbial population. The Western diet, which is typically high in fat and refined carbohydrates, decreases overall microbial diversity. Below, we identify some of the differences in gut microbe populations found in traditional and modern societies.

Given that the diet in industrialized cultures tends to be higher in fat and animal protein, these groups have a greater proportion of *Bacteroides* compared to traditional human populations. Traditional societies are generally characterized by an agrarian culture (higher in plant foods) that promotes a *Prevotella*-dominant gut microbiota. For instance, in chapter 1, we examined the case of a rural village in Burkina Faso. Gut microbiome researchers working in the village of Boulpon compared the gut microbes of this rural

traditional population to European samples. Specifically, they analyzed gut microbes from Italian children, who represent a more Western diet. On the other hand, children from the rural African group represented a more traditional diet.

The most relevant differences between the two cultures were in the proportions of four phyla. Actinobacteria and Bacteroidetes were more abundant in children from rural Burkina Faso. Also, Firmicutes and Proteobacteria phyla were more represented in the Italian children's microbiota. An increase in ratio between Firmicutes and Bacteroidetes may be indicative of a higher-calorie diet, as seen in Italian children. In addition to differences in these four phyla, researchers also noticed that three genera were exclusively present in these rural African children: *Prevotella, Treponema,* and *Xylanibacter*. They propose that the presence of these genera may be a consequence of a high-fiber diet. These microbial groups are likely more abundant in order to enhance energy extraction from plant fibers that are otherwise not digestible by humans.

Another cultural comparison of the gut microbiome involved children from Bangladesh and the United States. In general, investigators noticed that Bangladeshi children had more overall diversity in microbiota compared to U.S. children in the same age range. Bangladeshi children had microbiota that were clearly dominated by Firmicutes, representing 60% of total gut bacteria. Only about 20% of their gut microbiota was Bacteroidetes. On the other hand, U.S. children were shown to have microbiota evenly dominated by the two phyla: 46% of microbiota was Firmicutes, and 43% was Bacteroidetes. (The remaining 11% of bacteria belonged to the phyla Tenericutes, Proteobacteria, and Verrucomicrobia.) Further analysis at genus level showed even greater distinctions between Bangladeshi and U.S. children. Also, in Bangladeshi adults, a similar abundance of Firmicutes was seen in their microbiota, with about 50% of total gut bacteria belonging to Firmicutes. Bacteroidetes makes up about 20% of gut bacteria in Bangladeshi adults.

Finally, we will revisit the Hadza, a hunter-gatherer group that was introduced in chapter 1 as we discussed ways in which the gut microbiota of this group differs in composition when compared to Westernized populations. Overall, the Hadza gut microbiota is dominated by Firmicutes and Bacteroidetes, which is similar to most Western populations. However, Hadza gut microbiomes are clearly more abundant in Bacteroidetes. Also, compared to Italian individuals who consume a typical Western diet, the Hadza had a higher abundance of *Prevotella* as well as reduced *Bacteroides* species.

Researchers also observed an absence of Actinobacteria, particularly *Bifidobacterium* species, in the Hadza gut microbiome. They speculate that the lack of *Bifidobacterium* is linked to the low meat and dairy intake of this group. In a normal gut microbiome, *Bifidobacterium* makes up between 1 and 10% of total microbes. These researchers note that the Hadza are the only known

SIDEBAR 3.1 Childhood Malnutrition

Childhood malnutrition, a significant contributor to childhood mortality, is a condition in which inadequate calorie intake prevents normal growth. The United Nations International Children's Emergency Fund (UNICEF) program reports that nearly 50% of all deaths in children under the age of 5 occur due to childhood malnutrition. Many of these children have problems with brain development and proper immune system function.

Unfortunately, increasing caloric intake in malnourished children does not solve the problem, and they often have trouble recovering. Researchers are now using data collected from mouse studies to better understand why recovery is so difficult. They observed that a depleted gut microbiome may affect growth outcomes. Conventional mice with normal microbes, who were fed a diet with insufficient protein, showed fewer signs of malnourishment compared to germ-free mice. Mice who had normal gut microbes had larger, more developed bodies. Given that germ-free mice were most affected by this inadequate diet, researchers hypothesize that the lack of gut microbes made the animals more susceptible to growth problems related to malnutrition.

In addition to the information collected from mouse studies, data collected from human samples also points to an altered gut microbiome in cases of childhood malnutrition. One study examining Malawian children compares gut microbe populations of healthy and malnourished children. The investigators observed that malnourished children had poorly developed microbiomes. Interestingly, malnourished Malawian children had gut microbiota similar in composition to that of younger infants, indicating that their microbiomes remained immature.

Researchers also analyzed the breast milk of Malawian mothers and found that the presence of a specific carbohydrate that contains sialic acid led to healthier babies. Sialic acid is actually a type of monosaccharide found as a component of oligosaccharides within the breast milk. In addition to its role in central nervous system development, sialic acid promotes the growth of commensal microbes (specifically *Lactobacilli* and *Bifidobacteria*) and also prevents viruses and bacteria from attaching to epithelial cells. Interestingly, it has been difficult to replicate these benefits using infant formula. Since cow's milk does not have the same large amounts of sialic acid, dairy-based infant formulas do not similarly promote the growth of commensal microbes.

culture that lack *Bifidobacterium*. Yet, they do mention that groups such as vegans and Koreans who eat very little dairy and/or meat also have reduced populations of *Bifidobacterium*.

Another study investigates differences in gut microbe populations between this group and agrarian African groups, such as those seen in rural Burkina Faso. Compared to these farming societies, the Hadza have greater microbial

diversity. Agrarian groups did have Actinobacteria, which was lacking in the Hadza group. Also, both the African hunter-gathers and agrarian populations had an abundance of *Prevotella*, which is indicative of their high fiber intake.

Interestingly *Treponema* is found living as a commensal organism within the Hadza gut microbiota. This genus of bacteria contains members that are opportunistic pathogens when found in individuals living in industrialized populations. This bacterium is responsible for a few diseases, including yaws and syphilis. Yaws is rare skin infection that typically occurs in tropical locations with higher temperatures and humidity. Syphilis is a sexually transmitted infection that can either cause obvious symptoms such as sores and a rash, or remain latent without producing any overt symptoms. Increased *Treponema*, specifically seen in Hadza women, is likely a result of higher dietary fiber intake. Hadza women, whose diet includes more fiber-rich tubers than Hadza men, gain enhanced digestion of this dietary fiber with the help of *Treponema*.

Although researchers identify differences in gut microbial populations within various cultures, the health implications of these differences is still unknown. Further exploration of diet and its potential to change gut microbiota populations may provide a better picture of the development of chronic illness.

Carbohydrates: Nutrition for Host and Commensal Bacteria

To better understand how diet shapes the gut microbiota, this section provides a more in-depth description of macronutrients (fats, protein, and carbohydrates) and explains their various interactions with gut microbes. Bacteria can digest both carbohydrates and protein, but it appears that the former macronutrient is better suited for maintaining a balanced gut microbiome. There is a vast body of research covering the role of carbohydrates in microbial metabolism. In comparison, studies looking at microbial use of protein are more limited.

Dietary reference intakes (DRI), a set of values set by the United States Department of Agriculture (USDA), suggest that our diet should be primarily composed of carbohydrates. In fact, the USDA recommends that a range of 45–60% of total calories should come from carbohydrates, to meet essential nutrient needs while reducing the risk of chronic disease. This exceeds the recommended intake for the other two macronutrients: 10–35% of calories from protein, and 20–35% from fat. The following section will first discuss how carbohydrates differ and which types are more likely to support a healthy gut microbiota.

Gut Microbes Contribute to Digestion

Carbohydrates are a major energy source for all living organisms, including plants, animals, and many microbes. Most carbohydrates in the human diet come from plants, but other sources include dairy and honey. Carbohydrates

are sometimes described as either simple or complex. Although these terms are very general and potentially oversimplified, they do give some insight into how carbohydrates vary greatly based on their molecular structure.

The structure of carbohydrates is relevant to gut bacteria because of bacteria's crucial role in the digestion process. While the majority of digestion requires the activity of human enzymes to help break down dietary components, this process is also reliant on enzymes that are produced by gut microbes. Bacteria residing in the large intestine supply enzymes to aid in the digestion of carbohydrates and protein that remain intact after passing through the small intestine. The combined actions of human and microbial enzymes allow for the most complete digestion and maximize nutrient extraction from ingested food.

Carbohydrates that are not broken down by human enzymes in the small intestine are considered non-digestible carbohydrates. Whole plant foods provide a valuable source of non-digestible carbohydrates that feed our commensal gut microbes. When found in nature, carbohydrates are usually complex molecules. Yet modern technologies allow food manufacturers to easily process these natural carbohydrates into more refined products. In the diet, simple carbohydrates are found in these more processed foods, whereas complex carbohydrates are usually in foods that have maintained more of their whole form. Sugar and refined flour products are common examples of simple carbohydrates. Whole grains, beans/legumes, and many vegetables are good sources of complex carbohydrates. Unfortunately, since our commensal gut bacteria thrive on these more complex carbohydrates, their depletion in the Western diet may be starving our beneficial microbiota.

Classification of Carbohydrates

To better define carbohydrates and understand their accessibility to colonic microbiota, it is important to further explore their classification. Carbohydrates are also known as "saccharides," from the Greek word for "sugars," and they are categorized into four groups based on the number of sugar molecules they contain: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Monosaccharides are the most basic types of carbohydrate. These simple sugars are found within the diet as single molecules of glucose, fructose, or galactose.

Glucose is the most abundant monosaccharide found in plant foods. While glucose is a main energy source for human cells, this monosaccharide is mostly obtained indirectly from more complex carbohydrate sources. It is typically not found on its own but rather as a component of disaccharides and polysaccharides. Fructose is the sweetest-tasting monosaccharide. It is found in many fruits, certain vegetables, and honey. Galactose is made from lactose (a disaccharide found in dairy) during the digestive process.

Disaccharides are made of two monosaccharides. For instance, table sugar (also known as sucrose) contains one molecule of glucose and one of fructose. Lactose, a carbohydrate found in dairy, is made of one glucose unit and one galactose unit. Interestingly, fermented dairy products often contain less lactose than pure cow's milk. The process of dairy fermentation involves the addition of bacteria that are able to ferment lactose to form lactic acid (an acid byproduct from fermentation). Yogurt, certain cheeses, and other dairy products are made by adding specific strains of bacteria that ferment lactose. For individuals who are lactose intolerant, these dairy products may be more tolerable.

Less complex carbohydrates such as disaccharides don't require enzymatic activity in the mouth or stomach and are entirely digested within the small intestine. Disaccahridases are enzymes located within the microvilli of the intestinal mucosal cells, that break down these sugars—for instance, lactase, which breaks down lactose (the sugar in milk), and sucrase, which breaks down sucrose (common table sugar).

The disaccharide sucrose is a common form of refined sugar. Frequent consumption of added sugar has numerous detrimental health effects. Unfortunately, the average American gets about 15% of total calories from added sugar. Sucrose provides a relatively large amount of calories in small quantities and contains little nutrient value. Such added sugars contain no fiber that would otherwise promote a feeling of fullness. This often encourages individuals to eat excessive amounts of sugary foods. The lack of fiber also increases the rate of digestion and absorption into the bloodstream. These simple carbohydrates are rapidly digested and serve as quick energy sources. Yet frequent rapid increases in blood glucose, resulting from high sugar intake, may promote insulin resistance. Excessive sucrose or fructose consumption contributes to metabolic conditions such as type 2 diabetes and non-alcoholic fatty liver disease.

Added sugars are considered "empty calories," as they do not contain other nutrients such as vitamins or minerals. For the microbiota, fiber is a particularly important missing nutrient. When a significant percentage of the diet comprises added sugars and refined carbohydrates, dietary fiber intake is usually low.

Due to their easy-to-digest nature, monosaccharides and disaccharides are quickly absorbed in the small intestine and are not able to reach microbiota in the large intestine. On the other hand, the more complex carbohydrates described in the next section require additional enzyme activity to be properly broken down. Many of these larger carbohydrate molecules escape digestion in the small intestine and remain sufficiently intact in order to pass into the colon. Here, their digestion is completed with the help of the gut microbiota.

Oligosaccharides and Polysaccharides

Unless an individual is consuming large quantities of refined sugar, the majority of carbohydrates in the diet are not simple sugars but rather molecules containing multiple glucose units. In fact, most carbohydrates found in nature are polysaccharides (also known as glycans), which contain more than 20 monosaccharides. They are found in plants as both structural and storage components. For instance, cell walls are a structural component of plant cells. They contain carbohydrates like pectin, xylan, and cellulose. These cell wall components from plant foods within our diet are not digestible by human enzymes.

Polysaccharides are the most complex type of carbohydrate, given their large number of monosaccharide units. In general, the digestive process for carbohydrates varies based on the amount of effort it takes for the body to break down different types of carbohydrates. Given that polysaccharides are the most complex form of carbohydrate, they require multiple digestive steps to be broken down. Digestion of these carbohydrates begins in the mouth with the help of an enzyme in the saliva. Once the food is swallowed, enzymes continue to digest polysaccharides within the stomach. By the time carbohydrates reach the small intestine, the polysaccharides have been sufficiently broken down into smaller particles that can be readily absorbed by the body. However, certain polysaccharides cannot be broken down by human enzymes and escape digestion within the small intestine.

Oligosaccharides are smaller than polysaccharides and are made up of short chains of 3–10 monosaccharides. Two examples of oligosaccharides are fructooligosaccharides (made of multiple fructose molecules) and galactooligosaccharides (made of multiple galactose molecules). Humans lack the necessary enzymes to break the bonds between the monosaccharide molecules in oligosaccharides, but certain gut microbes are able to digest these carbohydrates. These carbohydrates are found in beans, peas, and whole grains. Also, oligosaccharides are abundant in human breast milk, to promote the growth of commensal microbes in the developing infant. Unfortunately, dairy-based infant formulas do not usually contain these beneficial carbohydrates and may lead to differences in gut microbiota development. Epidemiological studies demonstrate that mortality rates are significantly lower among breast-fed infants than among bottle-fed infants. This protective effect is partly due to the beneficial bacteria and oligosaccharides that are present in the mother's milk. As we will later see, these dietary components of breastmilk have a great impact of the developing infant.

Mucins (a type of glycan) within the gut mucosa also contain oligosaccharide side-chains that may be broken down by bacterial enzymes. Mucins are produced by goblet cells. They maintain the structural integrity of the intestinal epithelium. Although the completed digestion of host-derived mucins

usually requires a cooperative effort by multiple species, some bacteria are able to complete this process on their own. For instance, certain bacteria belonging to *Bifidobacteria*, *Bacteroides*, and *Ruminococcus* can produce all the appropriate enzymes for mucin degradation.

Fiber

Fiber is a non-digestible carbohydrate found in plants. Given that it is not absorbed by the small intestine, it is a good candidate for microbial fermentation. Dietary fiber is generally characterized by its water solubility and thus is grouped into two categories: soluble and insoluble. In addition to its usefulness in supporting our commensal gut microbes, fiber provides a number of other health benefits. For instance, soluble fiber delays gastric emptying by adding bulk to the stool. It also reduces absorption of cholesterol and decreases the rate at which glucose flows into blood. Insoluble fiber can have laxative effects and is beneficial in keeping bowel movements healthy and regular.

Both daily value (DV) and dietary reference intake (DRI) indicate how much total fiber individuals should have in their diet. The DV is 12 grams for every 1,000 calories consumed. However, little distinction is made between the different types of dietary fiber. The Nutrition Facts labels found on packaged foods calculate fiber intake percentage based on a recommendation of about 25 grams for the typical 2,000-calorie diet. Until the age of 50, adult men are recommended to consume 30 grams of fiber per day, and adult women should be getting 25 grams per day. After the age of 50, the recommended amounts decrease for men and women to 30 grams and 21 grams, respectively.

In terms of their role in feeding gut microbes, soluble fibers can be more fully metabolized by bacteria. Soluble fibers that are easily fermented by bacteria include beta-glucan, pectin, guar gum, wheat dextrin, and inulin. Although insoluble fibers are not as easily fermented, some of these fibers, such as certain pectins, are available for bacterial metabolism. Other insoluble fibers, such as cellulose, do not encourage growth of microbes because they are not easily fermented.

Some less-fermentable fibers, such as psyllium husk, can interact with other complex carbohydrates. Psyllium is shown to shift the fermentation of some resistant starches to the lower end of the colon. Resistant starch is the preferred substrate of *Bifidobacteria* and will be discussed in detail in the following section.

Although it is best to diversify the diet to provide a variety of non-digestible food sources for our microbes, direct fiber supplements can also

SIDEBAR 3.2 Blending, Juicing, and Grinding Food

Blending fruits and vegetables is a popular dietary trend. Individuals trying to maximize their fruit and vegetable intake may use juicing or blending as an efficient way to drink their nutrition. Green leafy vegetables can be particularly hard to incorporate regularly into the diet, and blending them with fruit increases their palatability.

However, blending breaks down the cell walls and fibers in fruits and vegetables, which allows for more efficient digestion and increases absorption of nutrients in these foods. Breaking down food in such a way prevents much of it from reaching the colon. For example, grains and nuts that are ground up are more readily digested by the human host and therefore don't travel as far as the large intestine. On the other hand, intact grains and nuts are able to also feed the gut microbiota in the colon

provide substantial benefits. For instance, healthy individuals who normally consume the average U.S. fiber intake showed significant shifts in microbiota populations after just a few weeks of eating a daily fiber bar. This bar, which contained 21 grams of fiber, decreased the Firmicutes population while encouraging growth of Bacteroidetes. This type of ratio between Bacteroidetes and Firmicutes (as further discussed in chapter 7) may be associated with healthy body weight.

Low-fiber diets can drastically reduce the diversity of microorganisms in the gut. Actually, a diet limited in non-digestible carbohydrates can essentially starve commensal microbes. Furthermore, mouse studies show that this lack of microbial diversity is passed down through generations. Pups who are born to mice fed a low-fiber diet inherited less diverse microbiomes. As generations went on, microbial diversity continues to decline. Not surprisingly, these lineages lacked the diversity of species seen in mice eating a high-fiber diet. Researchers also noticed that the disappearing species did not easily return, even after switching to a high-fiber diet.

While certain carbohydrate-digesting bacteria die off, others are equipped to break down the carbohydrate of mucin found in the mucosal lining of the gut. In general, it might be helpful for mucin-degrading bacteria to feed off of these host-derived carbohydrates to support renewal of the mucosal lining. However, it becomes problematic if these microbes rely too much on this nutrient source. It is possible that a low-fiber diet can have detrimental effects on the mucus layer, which serves as a protective barrier to keep toxins and bacteria from escaping the gut (more on this important function in chapter 4).

Resistant Starch

The most common polysaccharides in the human diet are starches. Starch, which is made up of only glucose molecules, is the primary form of stored energy within plants. During photosynthesis, plants create glucose and store it in the form of the more complex molecules of starch granules. This starch, along with certain plant fibers, can help promote the growth of beneficial bacteria. However, not all types of starch reach the microorganisms living in the large intestine. Only starches that are resistant to digestion in the small intestine are available to gut microbiota.

There are three types of starch, which are classified based on their rate of digestion. The first two, rapidly digestible starch and slowly digested starch, are broken down within 20–120 minutes of ingestion. These starches are not readily accessible to gut microbes, because they are well digested in the small intestine. On the other hand, resistant starch remains undigested after two hours and is able to escape intact from the small intestine and reach the colon. This last form of starch is a good fuel source for gut bacteria. Dietary sources include beans and legumes as well as unripe bananas.

There are three main types of resistant starch: physically inaccessible, resistant granules, and retrograded starch. A fourth type of chemically modified starch also exists, though this starch does not naturally occur in food and is instead used as an additive to processed foods.

Physically inaccessible starch is found in plant foods that that still have their cell wall. Good sources for physically inaccessible starch are legumes and partially milled grains. Another type of resistant starch is resistant granules, which are able to resist digestion because their structure prevents digestion by human enzymes. Resistant granules are found in raw potatoes and unripe bananas.

Starch granules are composed of two polymers: amylose and amylopectin. Amylose and amylopectin are found in cereal grains, potatoes, and legumes, as well as fruits and vegetables. Each plant food contains starch granules with amylose and amylopectin. These granules differ in size and shape, as well as amylose-to-amylopectin ratio. Amylopectin is a larger molecule compared to amylose, and is the more abundant type of starch in tubers and grains. These factors may lead to a more compact structure for some granules and influence the level of digestive resistance.

The last type of naturally occurring starch is retrograded starch. This is the most abundant of the resistant starches, because it is found in our most frequently consumed foods: bread and cereal grains. Resistant starch is formed through food processing by cooking the starchy food and then cooling it down. Cooking foods with starchy granules containing amylose and amylopectin causes these granules to absorb water, and thus the starch gelatinizes. This process also causes the cell wall to soften, which increases the digestibility

of the starch. However, some portion of this starch does remain resistant to digestive enzymes. Once granules swell up and amylose starts to leak out, the starch is fully in its gelatinized form. This gelatinized form of starch is more easily digested than resistant starch.

Once in this gelatinized form, the food can be cooled down to form resistant starch. Cooling allows amylose and amylopectin to recrystallize into a new compact structure. This process is called retrogradation. A retrograded starch contains amylose and amylopectin that are arranged into a more crystalline structure. Retrogradation decreases or inhibits the actions of digestive enzymes. Although this crystalline structure is non-digestible, it can be gelatinized by heat again to increase digestibly. A common example of retrograded starch is cooked and cooled potatoes.

Bacterial Metabolism

Bacteria digest macronutrients through the process of fermentation. Fermentation is an anaerobic process, since it must occur in the absence of oxygen. Bacterial fermentation of carbohydrates and proteins takes place in large intestine, although there are some fermenting microbes present in the junction between the small and large intestines as well. Bacteria that digest carbohydrates, or saccharides, are called saccharolytic. Protein-digesting bacteria are called proteolytic. The majority of saccharolytic bacteria reside in the proximal colon, where carbohydrates are most abundant. Fermentation of protein by proteolytic bacteria increases in the distal colon, where few carbohydrates are available.

As food leaves the small intestine, most available carbohydrates are fermented in the proximal colon. Since the greatest amount of undigested food is found in the proximal colon, this is the site of most bacteria fermentation. With a typical Western diet, approximately 30–60 grams of fermentable carbohydrates reach the colon to be metabolized by gut microbes. Furthermore, about 30 grams of bacteria are produced for every 100 grams of fermentable carbohydrates.

The symbiotic relationship between gut microbes and the human host allows for maximum utilization of dietary carbohydrates. Our commensal bacteria give us access to a wide variety of enzymes that are not produced by the human body. This section explains how certain fermentable carbohydrates support the growth of our symbiotic bacteria. We will also discuss the types of metabolites produced from both proteolytic and saccharolytic fermentation.

Prebiotics: Functional Foods for a Healthy Microbiome

Fiber and resistant starch are the last remaining indigestible components of the polysaccharide to enter the colon. Saccharolytic bacteria are able to metabolize carbohydrates and break these polysaccharides down through fermentation.

Although many carbohydrates are fermentable and can serve as food for microbes, ongoing research aims to determine exactly which of these provide beneficial effects to our gut microbiota. In fact, some carbohydrates specifically promote growth of commensal bacteria and therefore play a crucial role in maintaining homeostasis within the gut microbiota.

While probiotics are the live bacteria that contribute to a healthy gut, prebiotics are dietary components that feed our beneficial bacteria. Gibson and Roberfroid originally introduced the idea of prebiotics in 1995. They defined prebiotics as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health." Prebiotic foods not only increase select microbial populations, they can also inhibit the growth of pathogenic bacteria. Oligosaccharides, beta-glucan, and pectin all demonstrate the ability to inhibit proliferation of pathogenic bacteria by promoting growth of commensal bacteria that crowd out invading pathogens.

These naturally occurring compounds are scarcely found in highly processed carbohydrate foods. The Western diet is usually lacking in unprocessed plant foods that contain carbohydrate sources for gut microbiota. For instance, food processing usually removes much of the fiber content of plants and decreases the quantity of prebiotics that reach the colon (see chapter 1 for more details).

The most extensively studied prebiotics are the dietary fibers inulin and fructooligosaccharides. Fructooligosaccharides are oligosaccharides that form as a result of inulin being broken down. Inulin and fructooligosaccharides belong to a group called fructans that are polysaccharides made of fructose molecules. Fructans cannot be digested by human enzymes within the small intestine. They pass into the large intestine, where they interact with *Bifidobacteria* and other saccharolytic bacteria that can provide significant amounts of β-fructosidase, an enzyme that can break down these fructans.

Certain prebiotics, such as fructans, are considered "bifidogenic" because they promote the growth of *Bifidobacteria*. Inulin, fructooligosaccharides, and galactooligosaccharides are all bifidogenic prebiotics. Furthermore, these prebiotics provide an additional benefit by helping with the absorption of dietary minerals.

Supplementation with certain prebiotics may also benefit the body's metabolic functions. As mentioned, the ratio between Bacteroidetes to Firmicutes species differs greatly between traditional societies and modern Western populations. Both traditional agrarian and hunter-gatherer groups have increased abundance of Bacteroidetes, along with a decreased abundance of Firmicutes. This is reflective of the type of fermentable carbohydrates found within traditional diets. In fact, supplementation with resistant starch increases Bacteroidetes and Actinobacteria but causes Firmicutes populations to decrease.

Given that this abundance of Bacteroidetes is associated with lower body weight and healthy metabolic profiles, it is interesting to consider how prebiotic consumption may help maintain metabolic homeostasis.

Some prebiotics may also help reduce the effects of certain acute intestinal infections. One clinical study suggests that fructooligosaccharide (FOS) supplementation may help with traveller's diarrhea. In the study, 244 healthy individuals were given either 10 grams of fructooligosaccharides or placebo to determine the prebiotic's effect of risk and duration of traveller's diarrhea. The individuals who received the prebiotic supplement two weeks prior to their trip, and for the duration of the trip, had less severe diarrhea when traveling to medium- to high-risk locations. However, no significant preventative effect was seen among the prebiotic group. Although one study cannot provide definitive proof of the benefits of fructooligosaccharides in this type of GI condition, there is growing evidence that the body's response to infectious GI diseases is likely influenced by commensal microbes.

Adding certain prebiotics to the diet can produce a laxative effect. Since these prebiotics increase gut microbe populations, these additional microbes contribute to the total bulk of stool. Increasing bacterial mass through the use of prebiotics encourages peristalsis. Studies have demonstrated that supplementation of 15 grams of FOS or inulin has a mild laxative effect. This may be particularly important for individuals looking to improve symptoms of constipation.

As mentioned, xylan, a component of plant cell walls, promotes the growth of *Bifidobacteria*. Another cell wall component called arabinoxylan is a prebiotic within dietary fiber from certain whole grains such as rice and wheat that also enhances *Bifidobacteria* populations. Additionally, arabinoxylan can also protect against diet-induced changes in the bacterial community. For instance, excess amounts of dietary fat can change the composition of the gut microbiota. Supplementing with this prebiotic may help protect against these fat-induced changes. Other bifidogenic prebiotics are likely to have the same protective effects against a high-fat diet.

Short-Chain Fatty Acids

Bacterial fermentation in the proximal colon produces various compounds called metabolites. The compounds being fermented determine which types of metabolites are formed. For instance, the metabolites produced during saccharolytic fermentation differ from those produced during proteolytic fermentation. These metabolites have numerous effects on the human host.

The primary metabolites that result from bacterial fermentation of carbohydrates are short-chain fatty acids (SCFAs). Acetate, butyrate, and propionate are the main SCFAs created by gut microbiota. Other short-chain fatty acids

are also produced through bacterial fermentation, but at lower concentrations. These compounds have many important roles, including the maintenance of intestinal cells and immune system function. They also influence fuel availability within the body and regulate signaling related to energy balance.

SCFAs are used by the human host as well as other bacteria. About 10% of human caloric requirements can be acquired from SCFAs. This makes them a valuable energy source. Through the production of SCFAs, bacteria increase the efficiency of digestion by extracting the maximum caloric energy from food. These SCFAs are particularly useful to colon cells. In fact, approximately 95% of SCFAs are taken up by cells in the large intestine, as well as by cells found in the junction between the large and small intestines. The rate at which SCFAs are absorbed depends on their concentration within the colon. The amount of SFCAs decreases significantly as they travel from the proximal to the distal colon.

Butyrate is most readily absorbed by intestinal cells, while acetate and propionate more often enter circulation and travel to peripheral tissues. As much as 70% of acetate circulates to the liver, where it can be used to make cholesterol and other fats. Acetate is also used by other tissues, such as muscle and adipose tissue. Propionate and lactate, another acid formed as an end product of bacterial fermentation, can also be used by liver cells.

SIDEBAR 3.3 Measuring SCFA Production

Measuring the amount of SCFAs produced by bacteria in the colon is not an easy task. Acetate is typically the most abundant SCFA in fecal measurements. However, since these metabolites are readily absorbed and utilized, the remaining SCFAs excreted in fecal matter are not likely an accurate representation of SCFA production. These measurements are also not indicative of the amount of each SCFA within the large intestine, and so it is difficult to assess which SCFA is most abundant.

Given that fecal SCFA excretion is a poor measure of microbial SCFA in the intestine, many studies on SCFA production have been performed in vitro (that is, in a bacterial culture dish). Yet even these measurements are not likely to be a complete representation of SCFA production in the human gut. In vitro experiments have a very different microbial diversity, which significantly impacts SCFA profiles. Also, microbial byproducts tend to accumulate during in vitro experimentation, which may also alter SCFA measurements.

SCFAs are useful in studying variations in gut microbiota among different populations around the world. In addition to examining phylogenic diversity, metabolomics can be used to assess metabolite production (SCFA) of microbes. The types of metabolites present (including various SCFAs) can indicate the presence of certain bacteria with the GI tract.

In addition to their role as an energy source, SCFAs are particularly important in keeping the intestinal epithelium healthy. They keep potentially damaging immune responses (such as those characterized by chronic inflammation) at bay. SCFAs also inhibit growth of pathogenic bacteria by supporting the growth of commensal microbes.

SCFAs are crucial in maintaining a healthy structure in the GI tract. Depleting these fermentation metabolites by reducing dietary fiber can have implications for intestinal health. In animal studies, a lack of soluble fiber changed the structure of mouse intestines within just days. These animals developed thinner intestinal walls, and the intestine itself became shorter. Once the mice began consuming soluble fiber again, their gut structure changed back to its original form. Researchers hypothesize that this change in structure is due to the lower production of SCFAs seen in diets low in soluble fiber.

The lumen environment can shape the types of bacteria present, and therefore the types of metabolites produced. Specifically, changes in the pH level within the gut lumen can cause shifts in bacterial populations. Butyrate-producing bacteria tend to prefer a more acidic (pH 5.5) luminal environment, whereas propionate-producing bacteria prefer a slightly more alkaline environment (pH 6.5).

On the other hand, these metabolites can also affect the overall environment of the gut lumen. For instance, acids lower luminal pH in colon. This can cause changes in nutrient absorption and alter the growth rates of various microbial species. Higher production of SCFAs lowers the pH of the large intestine. This is a significant change in the environment of the lumen and affects the types of microorganisms that grow in the gut. High-fiber diets can therefore indirectly alter the pH of the lumen by supporting increased SCFA production. Oligosaccharides, for instance, increase overall SCFA production while promoting the growth of *Bifidobacteria* populations. Butyrate specifically reduces the pH in the intestinal lumen and encourages growth of more butyrate-producing bacteria that prefer this lower pH.

Butyrate

Butyrate is a short-chain fatty acid that plays a crucial role in maintaining general gut health. In comparison to other microbial fermentation byproducts, the benefits of butyrate are very well researched. Butyrate is most frequently a metabolite of Firmicutes, with commensal members of the *Clostridia* class being primary producers of butyrate.

Epithelial cells in the colon rely on butyrate as a primary energy source. Colon cells get between 60 and 70% of their energy from butyrate. Acetate and propionate do not serve as nourishment for colon cells in the same way as butyrate. Given that butyrate is highly interactive with colon cells, it is not

surprising that this SCFA also appears to regulate cell proliferation and gene expression within these cells.

Along with butyrate, propionate also increases cell proliferation within the colonic epithelium, specifically in the crypts where mucus production occurs. By enhancing mucus production, SCFAs help fortify the colon's protective mucous layer. Butyrate especially increases mucus production in the gut and therefore helps decrease intestinal permeability. Promoting a healthy mucosa protects against dysbiosis and invasion of pathogenic microorganisms.

Butyrate helps maintain structural components of the colonic epithelium. Colonic epithelial cells divide rapidly and therefore have a higher need for molecules that make up their structural components for the constant production of new cells. Cholesterol and phospholipids are two important fatty compounds used in the formation of epithelial cells. These fats can either be created from cholesterol and fats within the bloodstream or from butyrate produced by gut microbes. Colonic epithelial cells appear to have the capacity to synthesize their own fatty acids and cholesterol using this microbe-derived butyrate.

Increasing certain dietary carbohydrates is an effective way to increase microbial butyrate production. Overall, plant-based diets produce more SCFAs than animal-based diets. Plant-based diets are likely to have more carbohydrates that are accessible to colonic bacteria. Specifically, carbohydrates such as resistant starches support butyrate production. All three forms of naturally occurring resistant starch increase microbial production of butyrate.

While butyrate is most effectively produced by bacterial fermentation of resistant starch, other prebiotics, such as inulin and fructooligosaccharide, produce smaller quantities of butyrate. Inulin can increase production of propionate and butyrate while also decreasing acetate.

In addition to being produced by gut bacteria, butyrate can also be provided in the diet, from foods containing dairy fat. Cow's milk contains butyrate from bacterial fermentation that takes place within the bovine intestine. However, butyrate from dietary sources is handled differently by the human body. Dietary butyrate is treated like other fats and is absorbed in the small intestine. Unlike butyrate produced by human gut microbes, it does not reach the colon and therefore does not have the same beneficial effects as bacteria-produced butyrate.

Given that dietary butyrate is easily absorbed in the small intestine, therapeutic treatments aimed at rebuilding the epithelial layer must be protected from this first stage of digestion. Butyrate supplements can be administered in capsules to protect the SCFA from acidity within the stomach and small intestine. In addition to these special capsules, butyrate supplements can also safely reach the colon through rectal enemas.

Gas: A Side-Effect of Fermentation

Adding fermentable foods such as prebiotics to the diet has many health benefits but is also often accompanied by undesirable flatulence. Prebiotic fermentation produces gases such as carbon dioxide and hydrogen that can cause stomach cramps, bloating, and other symptoms of gastrointestinal discomfort. High-fiber foods such as beans and legumes are particularly noteworthy for their ability to cause flatulence. These side-effects are often mild but may deter many individuals from increasing dietary fiber or using prebiotic supplements, despite potential health benefits.

A number of gases are produce by microbes during fermentation, including methane, hydrogen, hydrogen sulfide, and carbon dioxide. Hydrogen, as well as some other gases, can be used by other bacteria, which reduces the output of gas from the intestine. Excess unused gases are then excreted.

Unfortunately, some studies show that even after four weeks of prebiotic supplementation, participants did not experience much adaptation to these prebiotics, and symptoms of gas and bloating continued. Researchers are seeking prebiotic carbohydrates that may excrete fewer gases and are therefore more tolerable. It appears that carbohydrate molecules with longer chain lengths may produce less hydrogen during fermentation. Generally, it also advisable to gradually increase dietary fiber sources, to reduce the severity of flatulence during the transition to a higher-fiber diet.

Certain digestive impairments can increase gas production in the colon. For instance, lactose intolerance impairs the digestion of lactose in the small intestine. Lactose intolerance results from an insufficient amount of lactase, an enzyme used in the digestion of lactose. With insufficient levels of lactase, lactose escapes digestion in the small intestine and enters the colon. Here, it is easily fermented by gut bacteria and unfortunately leads to excess excretion of hydrogen, carbon dioxide, and methane. Some of these gases are also excreted through the breath, as seen in the hydrogen-breath test which can be used to diagnose lactose intolerance.

Bloating and excessive flatulence can be caused by functional disorders that result in excess microbial fermentation. Treatments for these symptoms can vary from pharmaceutical intervention (such as medications that encourage easier passage of intestinal gas), reduction of dietary carbohydrates, and probiotics. Not surprisingly, the use of antibiotics has also proven to be effective, likely due to a reduction in fermentation by antibiotic-induced depletion of gut microbes. Given the detrimental impact of antibiotics on commensal bacteria, even low-dose treatments may cause dysbiosis if used repeatedly.

An alternative therapeutic treatment for excessive flatulence is the administration an enzyme called alpha-galactosidase. This is one enzyme produced only by bacteria, and is lacking in humans. Saccharolytic bacteria use this

enzyme to digest oligosaccharides in the colon. Oral supplementation of this enzyme would therefore aid in the digestion of oligosaccharides and promote absorption of these carbohydrates in the small intestine. For individuals using this enzyme supplement, it may be important to consider the potential implications of reducing the availability of these complex carbohydrates within the colon. Commensal gut microbes that rely on oligosaccharides for their own fermentation processes may be impacted if these carbohydrates are absorbed before reaching the intestine. As a long-term approach to treating flatulence, it may be more beneficial to consider the root causes of excess gas. For instance, if dysbiosis is contributing to excess gas, treating the underlying imbalance in gut microbiota may provide a more lasting relief from associated gastrointestinal discomfort. Chapter 6 of this book discusses in more detail the role of the gut microbiota in gastrointestinal disease.

Types of Fermenting Microbes

Given that different groups of microorganisms thrive at various pH levels in the gut lumen, the increasing pH in the distal part of the intestine creates a very distinct ecosystem. In this section of the gut, SCFA production decreases, which subsequently raises the pH to around 6.5. Butyrate-producing bacteria prefer the lower pH found in the proximal colon, and so their populations are very low in this higher pH zone. In the proximal colon, butyrate-producing bacteria make up about 20% of the total microbial population.

A number of beneficial species prefer the lower pH found in the proximal colon. For instance, *Faecalibacterium prausnitzii*, a commensal microbe belonging to the Firmicutes phylum, is more abundant in the proximal colon. Other non-butyrate-producing bacteria tend to dominate the distal colon. For instance, members of *Bacteroides* prefer the higher pH of the distal colon and are found in greater abundance in this part of the colon. These bacteria generally produce acetate and propionate.

Microbes from specific phyla such as Firmicutes, Bacteroidetes, Verrucomicrobium, and Actinobacteria are able to degrade carbohydrates. Bacteroidetes is a primary fermenter. Members of this phylum prefer to ferment polysaccharides like starch and xylan. Aside from saccharolytic bacteria, there are no other carbohydrate-metabolizing microorganisms in the gut. However, the archeon Methanobrevibacter helps enhance the digestive actions of certain saccharolytic bacteria and therefore increases production of SCFAs.

In addition to carbohydrate-fermenting capabilities, some bacteria within Bacteroidetes and Firmicutes phyla are able to ferment dietary protein as well. Specifically, *Bacteroides* in the Bacteroidetes phylum and *Clostridia* in the Firmicutes phylum are also proteolytic. The ability for a bacterium to be either saccharolytic or proteolytic is determined by the types of enzymes it is able to

produce. Bacteria produce a number of different enzymes, including polysaccharidases, proteases, peptidases, and glycosidases. Glycosidases and polysaccharidases are enzymes that aid in carbohydrate metabolism. Proteases and peptidases are enzymes that aid in protein metabolism.

The differences in enzyme availability not only determine a bacterium's preference for protein or carbohydrate; these enzymes also influence the specific type of carbohydrate it is able to metabolize. For example, Firmicutes plays a more important role in breaking down resistant starch, as compared to *Bacteroides* species. Studies show that individuals lacking *Ruminococcus bromii,* a member of Firmicutes, are not able to fully digest resistant starch as it passes through the bowel.

Metabolites produced during fermentation can be used by other gut microbes. Metabolic cross-feeding is a process that allows one group of microbes to utilize metabolites produced by other types of microbes. For instance, *Lactobacillus* and *Bifidobacteria* ferment inulin and fructooligosaccharide to produces the metabolites acetate and lactate. Other bacteria such as *Faecalibacterium*, *Eubacterium*, and *Roseburia* are then able to use acetate and lactate to produce butyrate.

Lactate produced through fermentation is also metabolized by other gut microbes. Lactate is mostly formed through bacterial fermentation of starch. On the other hand, polysaccharides from plant cell walls do not encourage lactate production. Once produced during fermentation, this acid is then used by some cross-feeding microbes, thus preventing accumulation in the gut lumen. Not all lactate is used in cross-feeding, as some is taken up by mucosa.

Certain gases can also be used in metabolic cross-feeding. During fermentation, Bacteroidetes produces carbon dioxide and hydrogen. Hydrogen is particularly important for the metabolic processes of archaea. Hydrogen is also formed when acetate is produced. The microbial community works together to limit hydrogen accumulation in the colon, as excess amounts of hydrogen in the gut can interfere with certain fermentation processes in primary fermenters.

Dietary Protein

The use of high-protein, low-carbohydrate diets for weight loss continues to grow in popularity. Dietary protein can promote a feeling of fullness and can also improve insulin sensitivity. While these metabolic benefits are undeniable, excessive protein intake can actually contribute to detrimental outcomes.

As mentioned, a low-carbohydrate diet can limit fiber intake and reduce the total fermentable carbohydrates that reach our commensal microbes. This type of diet also decreases the number of butyrate-producing bacteria, potentially affecting the integrity of the intestinal epithelium. Furthermore, proteolytic

fermentation produces toxic compounds that influence colonic homeostasis. Total protein intake should be considered in the context of potential adverse effects on gut microbiota and overall colon health.

High-protein diets can provide undigested protein for bacterial fermentation, but the metabolites produced are potentially harmful for colonic health. Individuals who consume carbohydrates containing resistant starch may protect the colon from some of these harmful components by promoting the growth of butyrate-producing bacteria. Butyrate supports the health of the protective epithelial wall and may thus reduce the negative effects of any toxic metabolites from proteolytic fermentation.

Although some SCFAs are produced as a result of protein fermentation, carbohydrates are by far the best substrate for SCFA production. Acetate is the most abundant SCFA byproduct of protein fermentation, followed by butyrate. In addition to SCFAs, a less substantial amount of branched-chain fatty acids (BCFAs) are also created by protein digestion. Unlike SCFAs such as butyrate, BCFAs do not stimulate the growth of more colon cells. A number of microbial species are able to produce BCFAs. Since BCFAs result primarily from animo acid digestion, these metabolites are a good measure of proteolytic fermentation.

The highest concentration of BCFAs is found at the farthest end of the colon. This indicates that protein digestion increases in this section of the colon. Given that many disease-promoting toxins found within the large intestine are produced in the distal parts, it is important to consider the role of protein fermentation in this area of the colon that may be producing pathogenic substances.

One study published in the *American Journal of Clinical Nutrition* observed the effects of a high-protein, low-carbohydrate diet in 17 obese subjects. The average weight loss was about 14 pounds, which translated to just under 6% of total body weight over a 28-day period. Researchers observed significant changes in the types of metabolites produced during bacteria fermentation associated with this type of macronutrient profile. The lack of fermentable carbohydrates in the study participants' diet reduced the concentration of butyrate by 50%. Additionally, higher protein intake increased metabolites associated with proteolytic fermentation. These individuals had increased concentrations of BCFAs.

One important thing to note about this particular study is that, while the participants got 29% of total daily calories from protein, they were also getting 66% of calories from dietary fat. Such high fat intake also has major implications for the gut microbiome, and we will discuss these in the following section. However, this study is useful in showing that a low-carbohydrate diet not only reduces SCFA production but also encourages proteolytic fermentation, which alters the composition of bacterial metabolites.

In addition to SCFAs and BCFAs, some of the metabolites produced during proteolytic fermentation include phenols, hydrogen sulfide, amines, and

ammonia. Phenols can irritate tissues in the colon. If produced in excess, these metabolites can have a destructive effect on mucous membranes within the gut. Proteolytic bacteria also produce hydrogen sulfide, a toxin that damages DNA and is implicated in the development of inflammatory bowel disease. This metabolite is mostly produced by sulfate-reducing bacteria (that is, bacteria that are able to convert sulfate to hydrogen sulfide). Interestingly, animal-based diets increase the abundance of bacterial sulfite reductases, enzymes that facilitate this conversion. This type of high-protein diet is also associated with enhanced activity of sulfate-reducing microbes. Furthermore, the production of hydrogen sulfide is linked to the growth of a pathogenic bacterium known as *Biophila wadsworthia*.

Another metabolite from proteolytic fermentation is an amine called histamine. This amine is formed by bacteria through the metabolism of histidine, an amino acid found in high-protein foods. Meat, poultry, and fish are sources containing the most histidine, though a number of plant foods have smaller quantities of this amino acid. Amines such as histamine are also found in the diet. Researchers are working to compare the health effects of amines produced by gut microbiota with those from dietary sources.

As bacteria break down amino acids within dietary protein, they also create ammonia, which can be reabsorbed by the colon. Ammonia from the gut is then taken to liver for production of urea, which can be excreted in urine. 25% of total urea is repurposed in this way. The liver's conversion of ammonia protects against accumulation of this microbe-derived metabolite, which can be toxic if not properly eliminated. Liver disease (cirrhosis) can limit this process. Excess ammonia may contribute to hepatic encephalopathy, a condition that impairs brain function as a result of toxin buildup. A low-protein diet is often recommended in cases of advanced liver disease, to protect against the accumulation of toxins, including ammonia produced through proteolytic fermentation.

In addition to dietary protein, microbes can also access protein sources directly from the host. For instance, digestive secretions and other circulating proteins, such as albumin in the blood, as well as tissues like collagen that are formed from protein, all provide fermentable materials for gut microbes.

Dietary protein comes from both animal and plant sources. However, most studies generally do not differentiate between the effects of different dietary protein sources on the gut microbiota. It is also important to note that many plant protein sources, such as beans and legumes, also contain non-digestible carbohydrates that feed commensal microbes. In fact, fiber intake has an impact on the genetic expression of gut microbes and influences their metabolic activity. A high-fiber diet lowers the activity of genes connected to protein metabolism, encouraging microbes to focus on carbohydrate metabolism.

While certain compounds produced by bacterial fermentation of dietary protein are suspected to be detrimental, their overall effects on human health

are not well understood. Many additional factors affect the ways these compounds interact with the body. For instance, the epithelium may be able to adapt to these substances. Intestinal mucosa may also help in detoxification. Of course, the concentration of these compounds within the lumen of the gut can also influence any negative health effects.

Dietary Fats

There is a significant body of research showing that a high-fat diet is detrimental for the gut microbiota. A wide variety of animal studies demonstrate that high fat intake can cause dysbiosis and lead to numerous other health complications resulting from changes in gut microbiota composition. In mouse studies, animals with normal gut microbiota composition experience significant changes after being switched to a high-fat diet. Both obese and normal-weight mice show an increase in the abundance of Firmicutes and Proteobacteria. Also, their populations of Bacteroidetes species subsequently decrease.

A high-fat diet during pregnancy may also have impact on future generations. Another study showed that feeding rats a high-fat diet of condensed milk (44%) and some corn oil (8%) while they were nursing alters their pups' gut microbiota. These pups have increased *Lactobacillus* and *Enterococcus* species in addition to depleted *Prevotella* and Bacteroidetes populations.

However, it is important to consider that many of these animal studies consist of diets with up to 60% of total energy coming from fat. It is rare for human diets to reflect such a high percentage of total fat. The ketogenic diet is one example of a very high-fat diet, consisting of about 70% of calories from fat while limiting carbohydrate intake to 5–10%. This type of diet may be used to control seizures in certain individuals with epilepsy. Although similar low-carbohydrate diets have gained some popularity for weight loss, only extreme cases will apply such high fat intakes.

A small number of studies address the impact of different types of fat on the gut microbiome. It appears that high intakes of omega-6 polyunsaturated fatty acid (specifically from safflower oil) reduces Bacteroidetes and increases Firmicutes. Omega-6 fats are abundant in vegetable oils, which are commonly found in the Western diet. On the other hand, omega-3 fatty acids may not have the same effects on composition, and they are also generally anti-inflammatory (compared to omega-6, which can be pro-inflammatory). Saturated fat found in butter, cheese, fatty meats, and coconut oil may also alter the gut microbiome if consumed in excess. Research shows that saturated fat intake increases the Bacteroidetes-to-Firmicutes ratio. More research is needed to understand the implications of these fat-induced changes in microbiota composition.

The implications of dysbiosis caused by a high-fat diet are discussed at length in chapter 5. This chapter focuses on the impact of gut microbiota on

obesity and metabolic imbalances. We will see that a high-fat, high-calorie diet can alter microbe composition in ways that lead to inflammation and fat accumulation.

The overall composition of macronutrients should be considered when exploring the impacts of diet on gut microbiota. Given the protective role of prebiotic carbohydrates, it may be possible that adding these to a high-fat diet may reduce some of the effects seen on microbiota composition. There is another important dietary factor that may affect overall health outcomes. We have already discussed how a diet low in soluble fiber can also promote inflammation in the gut and lead to weight gain. A high-fat, low-fiber diet is likely only to exacerbate these negative effects.

Bacterial Production of Vitamin K and B Vitamins

Some gut bacteria are able to produce important vitamins. Many of the vitamins necessary for human health are obtained from food, as the body is not able to produce them internally. Lack of overall variety or decreased intake of certain food groups can cause vitamin deficiencies that may have significant health consequences. Supporting vitamin-producing gut bacteria may prove to be a useful alternative to certain man-made supplements. However, it is important to note that, unlike vitamins found in food that are absorbed in small intestine, those produced by bacteria function within the large intestine. It is uncertain whether the location of these vitamins can potentially change their physiological role (at least compared to dietary vitamins).

Some *Bifidobacteria* strains, such as *Bifidobacterium bifidum* and *Bifidobacterium longum*, produce folate, a B vitamin that promotes cell growth and supports protein metabolism. Folate is also crucial for normal fetal growth and development. Maintaining healthy folate levels is particularly relevant for cells that line the gastrointestinal tract, because those cells are rapidly reproducing. Any cells with that are constantly dividing and reproducing are more dependent on folate. Folate supports the production of genetic material such as DNA and RNA within these cells as they grow and divide. Supplementing with *Bifidobacterium bifidum* and *Bifidobacterium longum* can increase folate levels in the stool. Some lactobacilli found in fermented foods are able to produce folate as well. Further research is needed, to understand how folate produced by bacteria is absorbed and how its functions differ from those of dietary folate absorbed within the small intestine.

Another important B vitamin produced by gut microbes is vitamin B_{12}. The human body is not able to produce B_{12}. Deficiencies can occur in individuals who consume a vegan diet, as this B vitamin is only found in meat and other animal products. There is currently no evidence that B_{12} produced by bacteria can prevent or correct these deficiencies. Some *Lactobacillus* species, such

as *Lactobacillus reuteri*, produce a B_{12}-like substance, but researchers are still working to determine whether it is indeed biologically active. It is interesting to note that B_{12} works in conjunction with folate, helping to transform folate into its active form. While these interactions are clearly observed with dietary B_{12} and folate, we do not yet know whether these two nutrients interact when produced by bacteria within the large intestine.

In addition to these two water-soluble B vitamins, gut bacteria are able to synthesize vitamin K. Specifically, *Bacteroides* as well as a few other genera, produce this vitamin. In certain individuals (likely depending on gut microbiota composition) bacteria supply up to 50% of the body's total vitamin K requirements. In fact, bacteria appear to play such an important role in vitamin K production that germ-free mice require vitamin K supplementation since they lacked gut bacteria to synthesize the vitamin.

While vitamin K is a general term that refers to a few compounds, there are two main forms of active vitamin K. Vitamin K_1 is found in plant sources such as spinach and broccoli. Vitamin K_2 comes from certain animal foods in the diet and is also formed by bacteria. The main storage organ for vitamin K is the liver. The liver is also able to recycle and reuse this vitamin. Damage to the liver (which is a major metabolic organ) can increase the risk of deficiency.

As mentioned, germ-free mice also developed vitamin K deficiency; thus it may be important to consider how less-severe forms of dysbiosis in humans

SIDEBAR 3.4 Drug Metabolism

Gut microbiota not only affect the metabolism of food; they also play a role in how drugs are broken down in the digestive tract. For example, over-the-counter medications such as nonsteroidal anti-inflammatory drugs (NSAIDs) may be directly affected by our gut bacteria. Aspirin and ibuprofen are NSAIDs commonly used to reduce pain and inflammation.

Unfortunately, long-term use of these drugs may change the composition of intestinal microbiota in ways that promote inflammation. A study in mice shows that depleted microbial populations following treatment with antibiotics caused the mice to process NSAIDs differently. Specifically, an enzyme within bacteria appears to increase absorption of the drug. With fewer gut bacteria (thus decreasing the quantity of this enzyme), less of the drug enters the bloodstream; the drug is instead eliminated from the body. Reducing populations of commensal bacteria therefore leads to less exposure to the drug. However, a lack of gut microbes (as seen with the germ-free mice) impairs the effectiveness of the drug. Researchers continue to explore these microbe-drug interactions as they consider how individual responses to drugs may vary depending on gut microbiota composition.

may also affect vitamin K levels in the body. Certain medications can inhibit vitamin K in the body. Most importantly, antibiotics can increase the risk of vitamin K deficiency, because they kill commensal bacteria, preventing them from producing vitamin K in the colon.

Food sources for vitamin K can be limited in a typical Western diet. Leafy green vegetables such as spinach, broccoli, and green cabbage are some of the best sources, but most individuals do not consume these regularly. Fermented foods that contain probiotic bacteria are also a good source of vitamin K.

Minerals

Emerging research shows that gut microbiota can influence the absorption of dietary minerals. Studies have demonstrated that certain prebiotics, such as galactooligosaccharides, fructooligosaccharides, and inulin, enhance the absorption of certain minerals such as calcium, iron, and magnesium. Additionally, it appears that mineral deficiencies in the body can alter the gut microbiota.

The use of specific prebiotics can alter gut microbiota composition in ways that promote increased mineral absorption. For example, studies demonstrate that dietary calcium absorption increases when taken in conjunction with prebiotic supplements. Proper absorption of calcium helps maintain good bone mineral density and is therefore important in preventing osteoporosis.

Most dietary calcium is absorbed in the small intestine and does not reach gut microbiota in the colon. However, some research suggests that fermentation can liberate some otherwise inaccessible calcium from plant foods. Microbial fermentation breaks down a plant compound called phytate. Phytates are found in a wide range of healthy foods such as whole grains, legumes, and nuts and seeds. However, given that phytates bind to minerals and block digestion in the small intestine, gut bacteria can be very useful in reducing this absorption-limiting factor.

In addition to liberating calcium by metabolizing phytates, another proposed mechanism suggests that short-chain fatty acid production may indirectly increase calcium absorption. As the intake of prebiotics increases SCFAs, these compounds lower the pH in the gut. Researchers propose that this lower pH enhances the accessibility of calcium found in the colon.

Whether one or both of these proposed mechanisms is responsible for boosting mineral absorption, it is still important to test these theories using different types of prebiotics. Unfortunately, studies demonstrating the exact effects of prebiotic supplementation on calcium absorption in humans are somewhat lacking. However, a couple of studies suggest that taking 40 grams of inulin per day for about a month can increase calcium absorption, while a smaller dose of 15 grams per day does not have the same effect.

While the gut microbiota affect the availability of dietary minerals, the reverse is also true: the availability of certain minerals in the body may also affect the gut microbiota. For example, a study from Cornell University recently showed that zinc deficiency alters gut microbe populations. Approximately 17% of the world's population is at risk for insufficient dietary zinc.

In this study researchers used chickens, birds that naturally consume an omnivorous diet, to determine the effects of an altered diet that contained inadequate zinc sources. When the chickens were fed a zinc-deficient diet, they subsequently displayed decreased gut microbial diversity. Lower diversity can decrease bacterial functions such as the production of SCFAs through fermentation. Given that these SCFAs play a role in the absorption of important minerals, a reduction in SCFAs can further exacerbate the deficiency. Furthermore, a zinc-deficient diet encourages the growth of bacterial groups that may compete with the host for available zinc. These changes gut microbiota have prompted researchers wonder if this zinc-microbiota connection may lead to novel ways to assess zinc status using fecal samples rather than blood samples.

Polyphenols

Plant foods also contain many various compounds called phytochemicals (*phyto-* means "plant"). While some phytochemicals are absorbed in the small intestine, others of them reach the colon, where they are metabolized by microbial enzymes. This process helps break down these beneficial plant chemicals to lower-weight molecules, thus increasing their availability in the body.

Within this very diverse classification of dietary compounds is one group known as polyphenols. Polyphenols have been extensively studied for their impact on human health. A diet rich in fruits and vegetables provides many kinds of beneficial polyphenols. Of these known polyphenols, only a few have been studied in the context of the gut microbiome. Researchers are now studying these interactions to better understand how microbes metabolize plant compounds. Interestingly, a number of studies show that gut microbes enhance the health-promoting properties of polyphenols.

Flavonoids are a subset of polyphenols that have received some attention within gut microbiome research. Flavonoids have many reported health benefits due to their antioxidant and anti-inflammatory effects. Antioxidants are compounds that protect cells with the body from the damaging effects of oxygen. It appears that the antioxidant effects of certain flavonoids are enhanced following interactions with the gut microbiota.

For instance, one type of flavonoid, called soy isoflavone, is a phytoestrogen that occurs naturally in the soy plant. Gut microbiota convert this flavonoid to another phytoestrogen, equol, and increase the antioxidant effects. It is important to note that there is much controversy regarding the health

effects of estrogen sources from plant foods. Specifically, some research points to endocrine-distrusting effects of phytoestrogens found in soy. While this research argues that these flavonoids interfere with normal hormone function, there is no conclusive evidence for these detrimental effects.

Given that the antioxidant effects of polyphenols can be enhanced by gut microbiota, phytoestrogens appear to play a beneficial role in human health. In fact, there is also evidence from population studies that soy-based phytoestrogens are associated with lower rates of breast cancer, cardiovascular disease, and diabetes. These findings mostly result from observations in Asian populations, where soy is consumed as a part of a traditional diet and where these chronic diseases do occur at much lower rates compared to Western populations. A few studies also investigate anti-inflammatory properties of soy flavonoids, but researchers have not yet explored how gut microbes modulate these beneficial properties. Since there is contradictory information on the health effects of phytoestrogens, it is interesting to consider the potential role of gut microbes in determining these effects.

More studies continue to compare the antioxidant capacity of substances produced by microbes to their metabolic precursors (that is, the original antioxidant compounds found in food). Another flavonoid, called quercetin, also functions as an antioxidant in the body. This flavonoid is found in various vegetables, fruits, and grains. Similarly to soy isoflavone, quercetin can be metabolized by gut microbiota. The microbial metabolite produced from quercetin had greater antioxidant activity compared to its precursor. Additionally, some microbial metabolites created from polyphenols found in citrus fruit limited the proliferation of prostate and colon cancer cells. Given the vast amount of research on polyphenols, it is important to examine how we may maximize their benefits by supporting commensal microbes and their metabolic interactions with these phytochemicals.

Conclusion

A diet lacking in whole, unprocessed plant foods diminishes the body's availability of vitamins, minerals, and other important plant nutrients. Many of the same foods that benefit our health in other ways also ensure the survival of these good bacteria. As we have learned, slow-digesting carbohydrates not only are important for maintaining balanced blood sugar levels but also provide non-digestible components that feed our resident colonic microbes.

In this chapter we have seen how our symbiotic relationship with commensal gut bacteria allows us to extract the maximum nutritional benefit from our food. Furthermore, we have explored how diet can be used to manipulate gut microbiota populations in ways that improve composition diversity and increase the abundance of probiotic species.

4

Immunity

When the germ theory of disease was first introduced, the scientific community was not yet willing to accept the idea that microorganisms cause disease. The idea that disease can be passed from person to person dates at least as far back as classical Greek times, yet scientists did not understand the mechanism of contagion until the late nineteenth century, when they began to speculate that microbes might be responsible for the transmission of disease.

A French scientist named Louis Pasteur was inspired by his understanding of the fermentation process and quickly became fascinated by the role of microorganisms in the development of infectious disease. Pasteur is most famous for his discovery of pasteurization, which is now commonly used to prevent bacterial contamination of food. Through his studies of fermentation and pasteurization, Pasteur realized that microbes do not spontaneously generate within the body (as was the current understanding at the time) but instead appear from external sources.

Pasteur eventually learned about the work of a German scientist named Robert Koch, who had identified the bacterial pathogen linked to anthrax infection. This led Pasteur to develop the first vaccine for anthrax. Although it was once believed that tiny organisms were unlikely to have such enormous effects, blood samples from animals that had died from anthrax were found to be full of bacteria. Not long after this discovery, the bacterium, which was named *Bacillus anthracis*, was transferred from diseased tissue to healthy mouse tissue in a laboratory. Pasteur was also able to identify specific viruses causing rabies, and he developed the first vaccine for this disease. These were some of the first treatments for diseases that directly targeted microorganisms and viruses. The research of Pasteur and Koch established credibility for germ theory and created the foundation for medical microbiology.

The acceptance of germ theory revolutionized medical practices and treatments for what is now understood as infectious disease. Microorganisms soon came to be recognized as being associated with illness, and eradicating them from the body became the standard of care. Vaccines and antibiotics are

life-saving medical interventions that have significantly decreased mortality rates related to infectious disease. The eradication of life-threatening diseases such as smallpox is owed to the use of vaccines. Antibiotics are equally important to our survival. However, treating bacterial infections with antibiotics has one major downside: these treatments also demolish beneficial gut microbiota.

Although our understanding of pathogenic microorganisms allows for the prevention of many serious, potentially life-threatening infections, other non-pathogenic microbes are now proving to lower the risk of certain diseases. This chapter discusses how overly sanitizing our environments and eradicating microorganisms through the use of antibiotics is changing our immune systems.

We will also examine the overall role of gut microbes in maintaining immune homeostasis. While the gastrointestinal tract is not typically viewed as a part of the immune system, the gut is arguably the largest immune organ in the body. Furthermore, the gut microbiota regulates many of the body's immune responses and has the potential to produce detrimental health outcomes in states of dysbiosis.

Microbes Influence the Development and Function of the Immune System

Growing knowledge of disease-causing microorganisms has created an excessively cautious attitude towards microbes. This fear of microbes is now used to market thousands of antimicrobial products that protect us from germs. In addition to hand sanitizers, antibacterial and antifungal substances are also added to toothpaste, children's toys, bedding, kitchen utensils, and many other consumer products. Unfortunately, this view of microbes as enemies of health perpetuates the overuse of antimicrobial products, as well as other health practices which may reduce our exposure to diverse microorganisms that were once naturally found in the environment.

Researchers are now finding that this diminished exposure to microorganisms may have detrimental health effects. In fact, our understanding of the human microbiome now shows a more complex relationship with the microbes in our environment.

The Hygiene Hypothesis: The Cost of Limiting Environmental Microbes

Altered microbial exposure is particularly relevant within a child's surrounding environment. Environmental changes that limit the diversity of microbes early in life are now implicated in the development of allergies and potentially other immunity-related diseases. In fact, the prevalence of allergies in Western

countries is quickly rising. Researchers believe that decreased microbial exposure is a causative factor in hypersensitive allergic reactions later in life. Recent research on the microbiome also now provides even more substantial evidence that our childhood environment and lifestyle can shape the microbe populations in our body. Early exposure to microorganisms is necessary for the development of a healthy immune system.

The hygiene hypothesis proposes a causal link between modern lifestyles and an increased risk of developing allergic diseases. This hypothesis suggests that exposure to both pathogens and symbiotic microorganisms promotes healthy immune function that can protect against the development of allergic response.

Scientists first applied the hygiene hypothesis to environmental allergies. Individuals whose immune systems act up around environmental allergens such as animal dander and pollen were suspected to have poorly developed immune responses from lack of exposure to these substances early in life. Recent research reveals that lifestyle factors such growing up on a farm, spending time outdoors, and having pets all help immune system development during childhood. In fact, urban living can significantly decrease the diversity of microorganisms that we are exposed to. As demonstrated in a comparison between Swedish children living in an urban setting and Pakistani children growing up in a rural setting, individuals living in rural settings are exposed to a much broader range of microbes.

A 2014 study published in the journal *Pediatrics* looked at microbial exposure based on method of washing dishes and the risk of allergy development. More than a thousand Swedish children participated in this questionnaire-based study to determine how often their families washed dishes by hand or by using a machine dishwasher. This study showed that children living in families who used machine dishwashing are more likely to have conditions such as eczema and allergic asthma. Using a dishwasher is more effective at killing microorganisms compared to hand washing and may therefore decrease exposure to various microbes. While dishwashing method is one lifestyle factor that affects level of diversity in microbial exposure, children in machine-using families may also be increasing their risk of developing associated allergic conditions through other lifestyle choices. For instance, children fed fermented foods or who ate produce directly from local farms also had a lower risk of developing allergies. These results of the study are in line with the ideas proposed in the hygiene hypothesis.

Variations in environmental microbial exposure may also explain why some traditional populations, such the group from rural Burkina Faso, have unique gut microbial populations. As mentioned, children living in the village of Boulpon, Burkina Faso, have significantly different gut microbes compared to children living in an urban European settings. Also, food allergies

are rare in Burkina Faso, a trait that is likely related to exposure to a large variety of microorganisms found in this rural setting. Similarly, children in Pakistan who are colonized by a more diverse group of commensal microbes appear to be better equipped to deal with antigens found in food compared to Swedish children. This observation has helped scientists further develop the hygiene hypothesis by understanding the role of gut microbiota in regulating the immune response.

Some scientists also propose that the hygiene hypothesis applies to reduced exposure to pathogens during childhood as well. While exposure to infectious disease is not likely connected to increased prevalence of environmental or food allergies, some theorize that limiting this exposure has compromised the immune system's ability to regulate its responses to the outside environment.

Although we may consider the broader implications of reduced microbial exposure through the perspective of the hygiene hypothesis, hygiene itself is often very protective against infections. Both personal hygiene and advancements in sanitation practices offer a great deal of protection against pathogenic organisms. Sanitation technologies provide clean drinking water and facilitate proper sewage treatment. Personal hygiene such as proper hand-washing habits also decreases risk of microbial infections and therefore reduces the spread of infectious disease. Instead of eliminating these protective hygiene habits, it may benefit our developing immune system to diversify our exposure to microbes by spending more time in natural environments and introducing healthy gut microbes through fermented foods.

The Gut Barrier and Intestinal Permeability

The gastrointestinal tract is constantly exposed to external substances, making it one of the most vulnerable internal organs. Yet, the body uses this vulnerability to its advantage by collecting information in the gut that is then communicated to the immune system. Gut microbiota act as the "middlemen" in this situation, as they help facilitate communication between the gut lumen and the rest of the body. In fact, our commensal gut microorganisms are key players in regulating the immune system.

Since gut microbes have the ability to activate certain immune responses, it is important to limit their contact with the systemic immune system. The structure of the intestine helps create a physical barrier that keeps gut microbes securely in the lumen. This important physical barrier, known as the intestinal epithelium, is part of the immune system's multi-layered defense against pathogens. The gut is also constantly exposed to many external substances, and this structural component protects the rest of the body from this exposure. The epithelial lining acts as a barrier to keep both gut microbes and toxins separate from other tissues.

Along the intestinal epithelium, there are two mucus layers: the inner and outer layer. The outer layer is closest to the lumen and contains significant numbers of bacteria. The inner mucus layer is meant to remain relatively free of microbes. In fact, this inner mucus layer is naturally resistant to bacterial colonization and serves as a key component of the gut barrier.

A certain degree of intestinal permeability is expected in a healthy GI tract, to allow the passage of nutrients into the bloodstream. However, if mechanisms that regulate the level of permeability fail, this intestinal barrier is compromised, and the gut becomes "leaky." Until recently, the concept of a "leaky gut" was mostly propagated by alternative health practitioners, who claim that many chronic diseases are linked to leakiness in the intestine that allows substances to enter the bloodstream and over-activate the immune system. Interestingly, research on the gut microbiota has led major scientific institutions to evaluate the evidence for this theory.

There are two routes by which bacteria can leak through the protective gut epithelium. First, the transcellular route allows bacteria to enter directly between cells. The second route is through the disruption of tight junctions. Tight junctions are the areas between epithelial cells that act as a sort of glue that holds these cells together. Tight junctions eliminate any space that might otherwise exist between those cells to prevent gut bacteria and other luminal contents from escaping.

Gut microbes play a significant role in maintaining tight junctions. Bacteria can increase activity of zonulin, a protein that regulates intestinal permeability. Zonulin can rapidly open intestinal tight junctions. Bacteria may be involved in the over-activation of this protein, thus causing dysfunction of tight junctions. This type of dysfunction is seen in celiac disease and type 1 diabetes, due to elevated levels of zonulin. Further, certain molecules produced by the immune system (cytokines) can also influence zonulin and promote increased permeability through the actions of this protein.

Butyrate-producing bacteria are also crucial in supporting healthy gut epithelium function. Butyrate increases the production of proteins used in the formation of tight junctions between epithelial cells. Maintaining strong tight junctions prevents foreign compounds from entering the bloodstream and aggravating the immune system.

The gut barrier can be disrupted by many factors. For example, while commensal bacteria produce substances that support the health of the epithelial lining, some pathogenic bacteria cause infectious diseases that increase gut permeability. Cholera is a bacterial infection transmitted through contaminated food or water. This infection affects the small intestine and increases intestinal permeability.

In addition to pathogenic bacteria, other types of dysbiosis in the gut can lead to intestinal permeability. Aside from dysbiosis related to diet and other

lifestyle factors, intestinal barrier function can be compromised due to dysbiosis caused by physical or psychological stress. For instance, severe gastrointestinal injury, surgery, and chronic illness are all physical stressors that increase intestinal permeability. Certain types of psychological stress may affect the health of the gut barrier. It is much easier to document the effects of psychological stress in animals, so much of our understanding about this connection between mental stress and the integrity of the gut barrier comes from observations in animals. In fact, research animals tend to experience stress from handling and during transportation. Since this stress decreases intestinal barrier function, these animals are often given a 1- to 2-week recovery period, to ensure that gut function normalizes following this contact with humans.

Inflammation

Increased intestinal permeability is now well-recognized in the scientific community as research continues to reveal that gut microbes have a major regulatory effect on the body's inflammatory response. If the intestine becomes too permeable, numerous gut bacteria are able to penetrate the gut wall. When microbes escape from the gut and enter the bloodstream, they trigger an inflammatory immune response. Inflammation is part of the body's second line of defense against invasive substances when physical barrier defenses fail. These same bacteria that play a mutualistic role as part of the gut microbiota greatly contribute to inflammation when outside of the gut lumen.

Inflammation is part of a complex defensive response that protects against infection. When inflammation is triggered, the immune system increases blood flow and sends special inflammatory cells to the site of cellular damage. Inflammation is meant to remove any harmful substance as well as any injured or dead tissue. When toxins or foreign substances leak into the bloodstream, localized inflammation can help prevent them from spreading. This is particularly useful when the occasional resident microbe passes through the gut barrier.

Inflammation can be problematic if the immune system becomes overactivated. In fact, chronic inflammation is a sign of disease. Increased intestinal permeability can create an inflammatory cycle that compromises the immune system and eventually leads to disease. Intestinal permeability is one of the major causes of low-grade systemic inflammation, a type of inflammation that perpetuates throughout the body.

Throughout this book, we will continue to make references to substances and microbes that either promote or inhibit inflammation. Substances that fight inflammation are called anti-inflammatory, whereas substances that promote inflammation are called pro-inflammatory. The body itself creates both types of molecules. Bacteria also have components that can either instigate this type of immune response or help suppress it.

SIDEBAR 4.1 Fighting Inflammation with Food

With the rise of chronic disease now understood to be strongly connected to systemic inflammation, researchers are exploring ways in which diet and lifestyle can improve health by reducing inflammation. Diets rich in anti-inflammatory foods are often recommended by nutritionists for conditions such as autoimmune diseases, which are characterized by systemic inflammation. As we will see in future chapters, microbiome research now shows that many other chronic conditions are accompanied by inflammation.

Many of the same dietary components that benefit the gut microbiome also help reduce systemic inflammation. For example, one of the best ways to support our commensal gut microbes is by providing them a diet rich in complex carbohydrates. High-fiber diets help reduce inflammation by providing food for beneficial bacteria but also because high-fiber foods tend to be rich in additional anti-inflammatory components. Fiber also lowers C-reactive protein, which is a substance made in the liver that indicates levels of inflammation within the body.

A number of other foods have anti-inflammatory properties. Herbs and spices also have significant anti-inflammatory properties. Turmeric, a plant that is in the same family as ginger, is commonly used in Asian cuisine. The spice is made from the underground portion of the plant called the rhizome and contains an active ingredient called curcumin.

Yet another important component of an anti-inflammatory diet is healthy fat. The types of dietary fats consumed determine the health of gut microbes. As we will discuss in chapter 5, excess intake of saturated fats promotes dysbiosis and intestinal permeability. Yet, another type of dietary fat, called an omega-3, is known for both supporting commensal microbes and for fighting inflammation. These oils, found in nuts, seeds, and fatty fish, are now packaged as supplements that may help reduce the use of traditional anti-inflammatory medications.

Innate and Adaptive Immunity

There are two branches of the immune system that work together to protect the body from disease: the innate and adaptive immune systems. The innate immune system, the body's first line of defense against microbial infection, responds immediately to potential pathogens and works to eliminate them. The second branch, the adaptive immune system, is more sophisticated, in that it can learn to target specific antigens. The effects of the adaptive immune system take place less immediately but provide the body with long-term protection. Although these two branches facilitate different immune functions, they work together to remove pathogens. For example, the adaptive immune system is activated after the innate immune recognizes a familiar pathogen or familiar molecules associated with a pathogen.

The gut microbiota interacts with both of these immune strategies. In fact, gut microbes are in constant communication with immune cells in the intestine, which helps shape the immune system's response to different types of microbes (that is, pathogens and commensals). The adaptive immune system uses a number of specialized cells to recognize both commensal and pathogenic microbes. These immune cells work in tandem to tolerate beneficial microbes while attacking harmful ones. In a healthy individual, commensal microbes do not trigger hypersensitive reactions within the innate immune system. A balanced adaptive immune response maintains a tolerance to both human cells and resident microbes and the metabolites they produce. The ability to maintain tolerance to host cells is an important regulatory function. The following section details these interactions between gut microbes and immune cells, and explains how this delicate balance is maintained.

The Innate Immune System: First Defense Against Pathogens

Increased permeability can lead to antigens entering into the mucosa. An antigen is any foreign substance that triggers an immune response. Most commonly, antigens are proteins or polysaccharides. In the case of microorganisms, the immune system can sense certain components of the bacteria themselves as well as protein or polysaccharide components of their metabolites. For example, the immune system can recognize the outer membrane of gram-negative bacteria as antigens.

When bacteria penetrate the epithelial layer, bacteria-associated antigens enter the lamina propria, a layer of tissue beneath the epithelium that is part of the mucosa. The lamina propria contains specialized immune cells called phagocytes, which help to initiate inflammation and inactivate foreign microbes. These cells use a process called phagocytosis to trap the escaped microbes and remove them from the body. Phagocytes are able to attach to microbes using special receptors that bind to specific microbial cellular components not found on human cells. Once they have attached to the microbe, the phagocytes are able to engulf and destroy it. Although several different types of phagocytes are involved in innate immunity, the specific type of phagocytes found within the lamina propria are called macrophages, known for their uniquely large size.

Although macrophages are located throughout the body, the intestine has the largest amount of tissue macrophages. Their location within the lamina propria is ideal for capturing microbes that have escaped the lumen and crossed the gut barrier. Intestinal macrophages are in close proximity with gut microbiota, but they are also released into the intestine.

Certain pathogenic microbes have their own defense mechanisms that inhibit the action of phagocytic cells. Some have a coated outer layer that

mimics substances found naturally within the human body, thus disguising themselves and evading removal by phagocytes. Other pathogens such as *Mycobacterium tuberculosis*, which causes tuberculosis (an infectious disease that usually affects the lungs), evades removal by the innate immune system because it is able to remain safely hidden within a phagocyte while still resisting destruction by that immune cell. This microbe is able to thrive and grow within the phagocyte and thus uses the host's natural defenses to its advantage.

Some commensal gut microbes enhance the actions of phagocytes. For instance, lactic acid bacteria (such as those used in the fermentation of vegetables and dairy products), have beneficial effects on the host's immune function. These bacteria influence innate immune responses by enhancing phagocyte activity. Studies specifically show enhanced activity within the lungs and peritoneum (a lining that covers organs in the abdominal cavity). Certain strains are better at enhancing phagocyte function. *Lactobacillus casei* has shown to be more effective in supporting macrophage function when compared with *Lactobacillus acidophilus*. Some lactic acid bacteria also influence the adaptive immune system. One animal study shows that feeding lactic acid bacteria to mice can improve the specific immune response to intestinal pathogens. When these mice were infected with *Salmonella,* antibody and mucosal responses were increased.

Neutrophils

In addition to macrophages within the intestine, the immune system also calls upon phagocytes from the bloodstream. For instance, neutrophils are one of the first phagocytic cells to be brought in at the start of the inflammatory response. Neutrophils are one of the most abundant phagocytes. They are innate immune cells that respond to components on the surface of pathogens. Neutrophils are highly mobile and can travel across the epithelial lining to enter the gut lumen, where they act as antimicrobials.

The presence of gut microbes appears to help regulate neutrophils. Researchers have observed that fewer antimicrobial genes are expressed within the intestinal epithelial cells of germ-free mice. The lack of microbes in these animals prevents normal immune regulation. Also, germ-free mice have altered neutrophil function. Interestingly, the presence of normal gut flora promotes the production of these antimicrobial cells, which facilitate innate immune functions.

Toll-Like Receptors

Certain cells in the innate immune system create pattern-recognition receptors. The main purpose of these receptors is to recognize various molecules associated with microbes. Pattern-recognition receptors are present on various

types of host cells. They are found on certain phagocytes such as neutrophils and macrophages, and they facilitate the elimination of problematic microbes.

Pattern-recognition receptors sense microbial DNA or microbial structural components. Lipopolysaccharides, flagellins, lipoproteins, and peptidoglycan are all examples of structural components that are recognized by pattern-recognition receptors and produce a pro-inflammatory response. Some pattern-recognition receptors can even bind directly to pathogens in order to mark them for removal. Once these receptors sense an invading microorganism, they send signals that stimulate the production of pro-inflammatory molecules.

One group of pattern-recognition receptors that are used frequently for microbial surveillance are known as toll-like receptors (TLRs). TLRs recognize both commensal and pathogenic microbes. They collect information on resident microbes as well as on microbes that are just passing through. After these receptors sense the environment for microbes, the information collected by the TLRs is then used to determine various immune functions. The ability of TLRs to identify resident bacteria helps to support intestinal homeostasis and prevents maladaptive immune reactions.

Microbiologists continue to explore how TLRs are able to differentiate between molecular patterns observed in pathogens versus those that are present in commensals. Many of these molecular patterns that can either form structural components of microbes or represent metabolites produced by microbes are very similar in their makeup. Given that many of these molecular patterns are found on both commensals and pathogens, it is unclear how the innate immune system can distinguish between these two groups. One proposed explanation is that commensals may be more inclined to remain within the gut lumen, whereas pathogens make more effort to penetrate the gut epithelial barrier.

There are many different kinds of toll-like receptors, each of which recognizes specific microbial components. Also, these different TLRs may be present in areas that make them most effective so that they are best able to fight pathogens while remaining less reactive towards commensal microbes. For instance, TLR4, which specializes in recognizing lipopolysaccharides (LPS), is found within intestinal epithelial cells instead of on the surface of these cells. This makes it less likely that commensal microbes will interact with TLR4 and trigger an inflammatory response. If there were an abundance of TLR4 on the surface of epithelial cells, lipopolysaccharides belonging to gram-negative commensal bacteria would instigate a continuous inflammatory response. Similarly, macrophages located in relatively close proximity to luminal bacteria have fewer TLR4.

While TLR4 recognizes LPS from gram-negative bacteria, TLR2 can be activated by components of gram-positive bacteria such as lipoproteins and peptidoglycan. Yet another kind of toll-like receptor, called TLR5, is responsible

for recognizing bacterial flagellin. Flagellin is a cylindrically-shaped protein that helps forms a protruding appendage called the flagellum, found on many opportunistic pathogens. *Helicobacter pylori*, for instance, has a flagellum.

When TLR5 senses flagellum-containing bacteria, it activates an inflammatory response. However, many commensal bacteria are also equipped with this appendage. Flagellin belonging to commensal organisms also interacts with TLR5. Researchers believe this interaction may somehow help to support mucosal immune homeostasis.

One study involving TLR5-deficient mice demonstrated some interesting behavior in flagellin-containing commensal microbes. When researchers bred mice without this specific flagellin-recognizing receptor, they found that bacteria that are not ordinarily pathogenic (that is, pathobionts) more easily entered the mucosa and lingered there long enough to promote inflammation. This was a surprising outcome, as researchers were expecting the opposite effect. They assumed that the absence of TLR5 would reduce inflammation because the body had lost its ability to sense the bacterial flagellin and therefore did not have a reason to instigate an inflammatory response. However, the resulting inflammation was actually due to the presence of increased lipopolysaccharides. When the pathobionts lingered in the mucosa layer, this caused the LPS to trigger TLR4.

This study also demonstrated some other differences between TLR5 and TLR4. The activation of TLR5 by bacteria is an immediate response that occurs the moment flagellin is detected. On the other hand, the TLR4 response to lipopolysaccharides takes longer but may produce a consistent state of chronic inflammation.

These experiments also caused researchers to wonder whether administering flagellin could activate the TLR5 response to eliminate some of these pathobionts. If TLR5 is activated by flagellin given as a sort of vaccine, it decreases levels of flagellin-containing bacteria. Furthermore, the administration of flagellin teaches the immune system to permanently suppress these specific microbial populations. Researchers wonder whether administering flagellin may therefore have therapeutic potential for chronic inflammatory diseases.

Researchers were intrigued to discover that the gut microbiota of TLR5-deficient mice began to display traits that are seen in metabolic disease and inflammatory bowel diseases. These diseases are associated with chronic inflammation and, in the case of the TLR5-deficient mice, are likely related to bacteria moving closer to the gut epithelium and triggering inflammation. The researchers also observed that flagellin-containing bacterial populations grew in the TLR5-deficient mice. Lipopolysaccharide-containing bacteria also increased and then triggered TLR4 to produce further inflammation.

Although the TLR5-deficient mice were genetically altered to lack this particular receptor, some humans are also born without TLR5. In fact,

approximately 1 in every 200 people is genetically lacking TLR5. This condition is rare, but it can lead to systemic inflammation. TLR5-deficient humans can develop metabolic imbalances related to this chronic inflammatory state. They may develop type 2 diabetes or obesity. Unfortunately, the lack of TLR5 receptor is rarely identified as the cause of these outcomes. (Chapter 5 discusses the impact of inflammation on metabolic function.)

Dendritic Cells

Dendritic cells facilitate communication between the innate and adaptive immune systems. They are highly involved in immune activation and also help with maintaining tolerance to commensal microbes. Dendritic cells assist with surveillance of gut microbiota and inform other immune cells of existing pathogens. In order to distinguish between good microbes and harmful ones, dendritic cells use their various toll-like receptors to identify surrounding microbes. In fact, dendritic cells have a large number of TLRs.

Dendritic cells are found in various tissues throughout the body, but their location within the laminal propria allows them to protect the entire gut epithelium. Within the lamina propria, dendritic cells can be triggered by microbial antigens to produce pro-inflammatory molecules. Dendritic cells are also phagocytes that can engulf microbes; they can carry microbial antigens and present them to cells within the adaptive immune system. This is why dendritic cells are in a group of cells called antigen-presenting cells.

Several types of immune cells can function as antigen-presenting cells. Macrophages, lymphocytes, epithelial cells, and dendritic cells can all process antigen material and present it to T cells. Just like other phagocytes, dendritic cells engulf microbes that penetrate the gut barrier. Yet dendritic cells are uniquely able to transport live microbes and present them to adaptive immune cells. Microbes can live for several days inside of dendritic cells. Dendritic cells containing live microbes remain within the mucosa.

The gut microbiota influences the development of antigen-presenting cells particularly because these cells are also in close proximity with gut microbes. Specifically, microbial metabolites such as SCFAs interact directly with dendritic cells. Butyrate and propionate limit the formation of new dendritic cells within the bone marrow. In fact, animal studies show that high-fiber diets, which produce larger quantities of SCFAs, protect against certain allergic responses but reduce the production of dendritic cells.

There are different subtypes of dendritic cells found within the gut. Researchers continue to explore whether these subtypes vary in their functions, especially given that dendritic cells are important in determining whether a microbe is tolerated or attacked. It is uncertain whether specific subtypes are specialized based on their ability to tolerate or instigate an immune response.

Another possibility is that all subtypes maintain a degree of plasticity, in which their functions are determined by cues within the gut environment.

Lymphocytes: T cells and B cells

A group of white blood cells called lymphocytes are mainly responsible for carrying out adaptive immune responses. Lymphocytes are the most abundant type of cell found in lymph, the fluid that circulates through the lymphatic system. Lymphocytes can travel through the blood, as well as lymph, and are concentrated in lymphoid tissues within the GI tract. In fact, the immune system within the GI tract is also called gut-associated lymphoid tissue. The two main types of lymphocytes, T lymphocytes (also called T cells) and B lymphocytes (also called B cells), are both key players in adaptive immunity.

While all lymphocytes originate with the bone marrow, some of them travel to the thymus, a lymphoid organ located near the heart, where they become T cells ("T" is for thymus). The lymphocytes that remain in the bone marrow are called B cells. Although it might make sense to assume that the "B" stands for bone marrow, in fact these cells were named after a lymphoid organ known as the bursa of Fabricus, which is found in birds, not humans.

T cells are a type of blood cell that plays a crucial role in the body's immune system. These specialized cells help defend the body against invaders such as pathogenic bacteria. As T cells develop, they learn to differentiate between the body's cells and outside substances. They also learn to differentiate between commensal microbes and detrimental ones. Part of this learning process happens in the gut, where T cells are exposed to commensal bacteria. These immune cells learn to tolerate our beneficial bacteria and react aggressively only to pathogens. However, certain disease states cause T cells to lose their tolerance and start attacking commensal bacteria.

Once T cells are activated, they can consequently activate B cells. An activated B cell can make multiple copies of itself. In this way, a specialized B cell can multiply and spread throughout the body, ready to produce antibodies if it senses the antigen with which it is associated.

Because T cells have such specialized roles, they form a main component of adaptive immunity. There are four subtypes, which must remain in balance in order to maintain health: T helper 1, T helper 2, T helper 17, and T regulatory cells. T helper 1 cells protect against microbial infection within cells. The over-activation of T helper 1 cells is associated with chronic inflammation. T helper 2 cells provide defense against parasitic infections. The over-activation of T helper 2 cells is associated with allergic diseases. T regulatory cells regulate immune tolerance, and T helper 17 cells help maintain mucosal integrity. Uncontrolled reactions involving T helper 1, T helper 17, and T regulatory cells are generally associated with autoimmunity.

T cells release cytokines that regulate the activity of other immune cells. A cytokine is a protein produced by various immune cells that facilitates communication between cells. Cytokines can be either pro- or anti-inflammatory. One important anti-inflammatory cytokine is called interleukin-10, or IL-10. This cytokine is produced not only by T cells but also by B cells, macrophages, and dendritic cells. Certain bacteria, such as *Lactococcus lactis*, are able to produce IL-10 as well.

IL-10 has a number of anti-inflammatory actions. First, IL-10 inhibits the production of pro-inflammatory cytokines by leukocytes and phagocytes. Second, IL-10 modulates the immune response by preventing the antigen-presenting functions of dendritic cells. Third, it interacts with TLRs to inhibit inflammation facilitated by these cell receptors.

T Regulatory Cells

T regulatory cells are a primary producer of anti-inflammatory IL-10 cytokine. The production of this anti-inflammatory molecule is in part supported by commensal bacterial. *Bacteroides fragilis*, for example, creates a substance called polysaccharide A that helps reduce inflammation in a number of ways. Polysaccharide A not only stimulates the actions of anti-inflammatory IL-10 but also decreases the production of the pro-inflammatory interleukin-17 (IL-17).

T regulatory cells play a critical role in helping the body maintain tolerance to its own cells as well as its resident microbes. The activation of toll-like receptors reduces the activity of T regulatory cells. Microbiologists propose that TLR signaling must be kept in balance so that excessive TLR activation does not induce harmful immune responses. Over-activation of TLR can lead to suppression of the beneficial anti-inflammatory effects of T regulatory cells, while simultaneously inducing the pro-inflammatory effects of T cells. This ultimately reduces the body's tolerance to commensals, which is otherwise controlled by T regulatory cells.

The gut microbiota not only regulates the activation of T regulatory cells; they also influence the number of these activated immune cells. Commensal bacteria in the *Clostridium* genus are among the most effective at increasing the production of T regulatory cells. Researchers demonstrate that mice inoculated with commensal *Clostridium* species are far less reactive than standard mice when their allergic responses are tested. *Bacteroides fragilis* also interacts with T regulatory cells to reduce mucosal inflammation.

In addition to gut microbes directly interacting with T regulatory cells, microbial metabolites such as SCFAs also significantly affect the actions of T regulatory cells. SCFAs not only increase the number of these T cells within the large intestine but also enhance their regulatory effects.

Given that T regulatory cells function to suppress the immune system and help inhibit inflammation, they play a major role in reducing a number of diseases associated with chronic inflammation. Research shows that animals lacking T regulatory cells are more susceptible to developing allergies and autoimmune diseases.

T Helper Cells

One of the main jobs of T helper cells is to warn other immune cells about immune threats. T helper cells are responsive to antigen-presenting cells. Once a T helper cell recognizes an antigen, additional identical versions of that helper T cell are created. This network of activated T helper cells produces a variety of cytokines that stimulate other lymphocytes. Pro-inflammatory cytokines produced by activated T helper cells are very effective in activating B cells. T helper cells that have not yet been activated by an antigen are called naive T helper cells. Interestingly, certain commensal gut microbes, such as *Bacteroides fragilis*, induce the conversion of naive T cells to T regulatory cells.

Antigens stimulate the production of B cells. These B cells then secrete specific antibodies that bind to the antigens with which they are associated. Antibodies are molecules produced by immune cells in response to specific antigens. By binding to these specific microbial antigens, antibodies from activated B cells mark these microbes to facilitate phagocytosis. Antibodies are then able to neutralize any problematic microbes. Pathogens bound to antibodies are prevented from attaching to receptors on host cells.

B cells and T cells have thousands of antigen receptors. Despite this large number, the receptors on each single cell are actually identical, allowing them to be specific to just one antigen. In this way, antibodies contribute to the more specialized immune reactions of the adaptive immune system that are lacking in the more generalized responses of the innate immune system.

Interestingly, the body naturally produces T cells and antibodies that are specific to commensal microbes. This challenges the notion that an immune response against our friendly microbes is always associated with a disease state. However, if these finely tuned regulatory responses fail, pathology can occur.

Many of the immune cells discussed so far are effective in clearing out pathogens that travel freely through the body. However, in some cases, microbes can find their way inside cells, and a different type of T cell is called to action. Cytotoxic T cells specialize in the removal of microbe-infected cells. Individuals with inflammatory bowel disease have higher numbers of cytotoxic T cells in the lamina propria and epithelium of the intestine. The presence of these cells is higher in areas of active inflammation and likely contributes the state of disease.

Immunoglobulins

Antibodies are also known as immunoglobulins. B cells produce an antibody known as immunoglobulin A (IgA) that is specific to antigens associated with commensal microbes. Dendritic cells that are loaded with commensal microbes stimulate IgA production. IgA therefore assists the immune system in recognizing and tolerating resident gut microbes. Due to its association with these commensal microbes, IgA is a non-inflammatory antibody.

IgA is a major antibody produced within the gut, and it is generally found along the mucous membrane. IgA works to prevent pathogens from entering the mucosa. IgA within the intestine also limits the contact between commensal microbes and the lamina propria. This antibody is therefore a natural mechanism that prevents the over-activation of the innate immune system by resident gut microbes. Since IgA is regularly interacting with the gut microbiota, these microbes are able to regulate the production of IgA. In fact, researchers observe that germ-free mice have depleted levels of IgA, resulting from their lack of commensal microbes.

Another important antibody is immunoglobulin E (IgE). IgE protects against parasitic infection. However, in industrialized cultures, excess production of IgE is associated with allergies. Interestingly, in pre-industrial cultures, higher levels of IgE are normally present. This is likely due to the increased prevalence of parasitic infections in these pre-industrial cultures.

SIDEBAR 4.2 The Appendix and Gut Immune Function

Secretory IgA and mucin both facilitate the growth of biofilms along the intestinal epithelium. As we learned in chapter 2, the appendix is a safe house for commensal microbes in the case of enteric infection. The appendix contains biofilms of commensal microbes that can be used to repopulate the gut if an illness depletes the gut microbiota. Furthermore, gut-associated lymphoid tissue is abundant in the appendix, indicating a potential role of the appendix in gut immune function.

Researchers also suggest that the appendix, which houses biofilms of mutualistic resident microbes, likely sheds this biofilm on a regular basis. This is based on the understanding that biofilms along the gut epithelium have a rapid turnover and are constantly shedding. For this reason, researchers propose that biofilms along the epithelial surface of the appendix also shed and serve to inoculate other sections of the GI tract by releasing parts of this biofilm.

Gut-Associated Lymphoid Tissue

As we have seen with lymphocytes, the lymphatic system plays a significant role in the gut's immune function. In fact, gut-associated lymphoid tissue makes up over 70% of the total immune cells within the body. This lymphoid tissue is important for removing harmful microbes. Antigens that enter the body through the gut epithelium are carried into lymphoid organs, and the lymphatic system helps rid the body of waste.

Another important component of gut-associated lymphoid tissue is Peyer's patches. Located within the mucosa of the small intestine, Peyer's patches play a crucial role in protecting against GI infections. Peyer's patches contain dendritic cells that produce high amounts of IL-10, an anti-inflammatory cytokine. Once presented with antigens, T cells and B cells can be activated within Peyer's patches.

Although Peyer's patches are the primary locations where mucosal immunity is induced, recent research indicates that this lymphoid tissue may not always be necessary to induce immunity.

Certain lymphoid structures are altered in germ-free mice. For example, these animals tend to have smaller Peyer's patches. This indicates that normal gut flora is need for the development of gut-associated lymphoid tissue. Just like other components of the immune system, gut-associated lymphoid tissue is not fully developed at birth. Infants are born with an underdeveloped immune system that allows microbiota to colonize without producing an automatic immune response. Unfortunately, this also leaves young children at higher risk of infection as their immune system matures.

Certain lymphoid structures become active in the intestine after colonization of commensal microbes at birth. Once a child is born, commensal microbes help train the developing immune system. As we learned earlier in this chapter, exposure to microorganisms during the first years of life is crucial for the developing immune system, and much of this early exposure is dependent on the transfer of the mother's microbes.

In mouse studies, translocation of bacteria from the gut is increased during pregnancy and lactation. These studies also show that dendritic cells within the mother's milk carry bacteria from her body that help inform the mouse pups' immune response to commensal organisms. These methods of early exposure provide helpful information that shapes the developing adaptive immune system.

Natural Antimicrobial Agents

Antimicrobial peptides are proteins produced by epithelial cells that limit contact of resident microbes with the surface of epithelial cells. Two main types of antimicrobial peptides in the GI tract are alpha-defensins and beta-defensins. Within the small intestine, alpha-defensins are produced by Paneth cells.

Paneth cells, one of the main cell types found within the small intestine, are stimulated by bacteria to produce alpha-defensins. These cells can sense both gram-negative and gram-positive bacteria, as well as any antigens associated with these microbes. By secreting alpha-defensins into the lumen, Paneth cells protect against pathogens but also work to keep other microbes away from the intestinal barrier. Beta-defensins, on the other hand, are found throughout the entire GI tract. Researchers propose that this second antimicrobial peptide helps regulate gut microbe populations.

Commensal microbes also produce antibacterial substances that inhibit their own species. *E. coli* makes antimicrobial molecules called bacteriocins that can limit the growth if its own species or similar species. In this way, commensal strains of *E. coli* can produce bacteriocins that inhibit pathogenic strains of *E. coli*. The commensal variety protects against colonization by the pathogenic variety. In this way, commensals offer direct prevention of infection.

Of course, maintaining healthy gut flora also helps to crowd out pathogenic organisms. Commensals compete with pathogens for similar resources within the gut, and an abundance of beneficial microbes will limit nutrients for pathogens.

Mast Cells

While mast cells make up only 2–3% of total cells within the lamina propria, they play an important role in immune function primarily by controlling gut

SIDEBAR 4.3 A Helpful Virus

While this chapter has focused on the role of bacteria in human immunity, other microorganisms in the intestinal flora also play into this complex symbiotic network. Viruses, for instance, appear to have a surprising role in maintaining homeostasis within the GI tract. (As a side note, the classification of viruses remains controversial. Some microbiologists maintain that they are nonliving and should not be classified as microorganisms.)

Typically recognized for their harmful effects, intestinal viruses can actually protect against pathogenic bacteria. In fact, certain viruses that infect bacteria or archaea tend to be permanent residents within the intestine. Studies on germ-free mice revealed that exposure to a specific non-pathogenic virus reversed defects of the immune system typically seen in these mice.

As mentioned earlier in this chapter, germ-free mice have poorly developed lymphoid structure and function, in addition to structural defects within their GI tract. The virus repaired defects in the mucous membrane as well as the function of Paneth cells. Researchers speculate that this virus can actually take the place of gut microbiota and strengthen immune function. The role of intestinal viruses in humans is still, however, unknown.

permeability and smooth muscle peristalsis. Like T cells and B cells, mast cells also come from the bone marrow.

Mast cells are responsible for releasing chemical signals that instigate an inflammatory response when tissue damage is detected. Mast cells store a chemical called histamine. Histamine dilates nearby capillaries and increases their permeability, in order to increase blood flow to the site of inflammation. This increased blood flow is responsible for the heat, redness, and swelling produced by localized inflammation.

Gut microbiota play a role in the activation of mucosal mast cells. One study in rats showed that only four days of antibiotics caused a decrease in the abundance of gut flora, which subsequently reduced the numbers of mast cells within the intestinal lymph. Interestingly, these researchers observed that antibiotic treatment also protected against intestinal permeability by reducing the body's ability to absorb dietary fats. Excessive fat intake has a detrimental effect on gut microbiota populations and induces intestinal permeability. The rats in this study were protected from the effects of a high-fat diet due to the antibiotic-induced reduction of gut microbes. (In chapter 5, we will discuss the role of a high-fat diet in promoting inflammation and reduced gut barrier function.)

Additionally, dietary fats activate mucosal mast cells, although researchers are still exploring possible mechanisms by which this occurs. This research also demonstrates that depleting gut microbes reduces the activation of mucosal mast cells in response to dietary fats. Researchers of this study speculate that the activation of mucosal mast cells resulting from dietary fat consumption is related to the effects of fat on the gut barrier. Furthermore, they note that certain structural components of bacteria (such as lipopolysaccharides) are absorbed in conjunction with dietary fats. These bacterial components can have toxic effects that also activate mucosal mast cells.

Gut Microbes and Immune Dysfunction

In the final section of this chapter, we will discuss conditions that result due to abnormal interactions between gut microbes and the immune system. We will see how immune responses can be misdirected at human cells or commensal microbes. In addition, we will explore how different gut microbes influence inflammatory diseases that can affect the lungs.

Autoimmunity

In order to keep our immune responses in check, the body must maintain a state of self-tolerance. That is to say, the immune system must learn react towards invading microbes while learning to tolerate host cells or host commensal microbes. When this self-tolerance is lost, the immune system attacks

SIDEBAR 4.4 Modern Hygiene and Immune Hyper-Reactivity

One group of researchers propose that the human body evolved to have certain environmental pressures that help balance the immune response. Our post-industrial culture has eliminated many of these environmental factors, with modern sanitation and medical practices, but this may have thrown off our finely tuned immune responses.

These researchers point to a specific evolutionary relationship between symbiotic worms, called helminths, and their host. While these worms are not part of the gut microbiome, they serve as another useful example of how modern sanitation practices can perpetuate a state of hyper-responsiveness within the immune system. These worms have likely co-evolved with humans for millions of years and helped shape the structure and function of our current immune system. The presence of these worms actually helps regulate immune response, by inducing a suppressant effect that prevents hyper-reactivity.

Just like with commensal microbes, this intestinal resident decreases the immune response, to protect itself from destruction by host immunity. So researchers experimented with helminth colonization for individuals with immune disorders. Surprisingly, these worms reduced the patients' overactive immune responses and slowed the progression of their immune-associated disorders.

healthy human tissue. A hypersensitive immune system may also attack benign substances such as food, pollen, and harmless microbes.

Type 1 diabetes is a type of autoimmune disorder in which the immune system attacks cells in the pancreas and inhibits the production of insulin. This form of diabetes is very different from type 2 diabetes, which is instead caused by insulin resistance rather than lack of insulin itself. The gut microbiota composition of individuals with type 1 diabetes differs from that of healthy individuals. Interestingly, the microbiota composition seen in type 1 diabetes is also distinct from those with other kinds of autoimmune disorders. Specifically, research demonstrates that individuals with type 1 diabetes may have a higher numbers of Firmicutes species and fewer Proteobacteria species.

Asthma and Lung Infections

Since the gut microbiota is so closely involved in regulating the body's immune function, it also modulates immune responses in other mucosal tissues, such as those found in the lungs. A number of researchers have observed that microbes within the nasal cavity of mice are soon found within the gut, implying that

microbes that may find their way into our nose can quickly travel down into the GI tract, where they can interact with the enteric immune system. This also means that all pathogenic microbes entering the airways interact with microbiota. In fact, researchers believe that commensal microbes regulate the body's defenses against pathogenic infections, maintaining appropriate inflammatory responses within the lungs. Researchers propose that TLRs in the intestine trigger lung immune responses.

Asthma is one condition which affects the lungs that is linked to microbe-immune interactions. Asthma is a chronic inflammatory condition that is characterized by structural changes of the airways. These altered airways constrict breathing, and the airways become hyper-responsive. This condition is associated with a higher production of inflammatory molecules called leukotrienes, which are produced by mast cells with the help of a specialized enzyme. The bacterial metabolite conjugated linoleic acid (CLA) decreases the action of this enzyme. CLA levels in the body are only increased by certain commensal bacteria. For example, *Bifidobacterium* and *Lactobacillus* are able to synthesize CLA. As a side note, CLA also converts to DHA and EPA, two omega-3s which act as anti-inflammatory molecules in the body.

Interestingly, depleting the gut microbiota with antibiotics early in life may cause immune dysfunctions such as those linked to asthma. The use of broad-spectrum antibiotics in childhood may be associated with asthma in later years. This is likely due to the consequences of antibiotic-related dysbiosis during the crucial developmental period of the immune system.

SIDEBAR 4.5 Critical Illness

Critically ill patients may have less-stable microbial populations. These individuals are vulnerable to microbiota changes on multiple levels. First, critical illness can alter the reproductive rates of commensal bacterial species, thus influencing overall composition of bacterial populations. Next, these individuals are more likely to have new bacteria moving into the gut, while losing current microbial members. For example, bacteria from the mouth can travel down to the GI tract, where it can be introduced into resident gut microbes. Unfortunately, crucially ill patients have noticeable shifts in oral microbial communities, and these shifts can determine which types of bacteria are transported to the gut. As illness progresses, for instance, the number of proteobacteria in the mouth increases.

Additionally, one of the main factors that regulate the rate at which bacteria are eliminated from the gut is digestive transit time, the amount of time it takes for food and waste to move through the gut. Transit time is also generally slowed in critical illness, thus causing shifts in normal gut flora.

In addition, infectious diseases also play a role in the development of allergies. For example, *Helicobacter pylori*, a microbe typically characterized as pathogenic, may actually be protective against the development of asthma and allergies.

Asthma and allergic reactions are associated with over-responsive T helper 2 cells. The presence of *H. pylori* in the stomach activates mostly T helper 1 cells. Some researchers theorize that this activation of T helper 1 cells helps to balance out allergic T helper 2 responses. *H. pylori* infection is also associated with elevated numbers of T regulatory cells within the stomach as well as in circulation. Higher levels of T regulatory cells may further decrease the risk of developing allergic diseases. These are two proposed mechanisms by which *H. pylori* may protect against hypersensitive immune reactions that are characteristic of allergic diseases.

Antibiotics, Commensal Microbes, and Gut Health

Antibiotics are a type of medication that inhibits bacterial growth; they can be used to treat and prevent bacterial infections. The overuse and improper use of antibiotics contributes to the proliferation of antibiotic-resistant bacteria, which are very difficult to eradicate. Antibiotic resistance occurs in bacteria that have mutated to reduce the effectiveness of pharmaceuticals aimed at curing the bacterial infections. When prescribed antibiotics, it is important to complete the entire course of the medication so that it can effectively eliminate the pathogen while decreasing the possibility of it breeding resistant bacteria. Due to the increase in antibiotic-resistant bacterial strains, some physicians are beginning to prescribe antibiotics more cautiously.

Another consequence of frequent antibiotic use affects the gut's commensal microorganisms. Unfortunately, antibiotics do not target individual bacteria but instead work by eliminating a large population of our beneficial bacteria as well. Some antibiotics affect the gut microbiota more harshly. For example, Ciprofloxacin had the longest-lasting effect. This antibiotic specifically reduced types of bacteria that produce the short-chain fatty acid butyrate. Amoxicillin is much kinder to commensal bacteria and showed no significant effect on the gut microbiome. A number of studies confirm that just one week of antibiotics can change the gut microbiome for up to a year.

Frequent antibiotic use can increase the abundance of antibiotic-resistant genes within the gut microbiome. Either gut microbes can acquire these resistant genes through genetic mutation following antibiotic exposure, or it can be acquired from another bacterium containing those genes.

Infants can also acquire resistant microbes from their mother's microbiome. Interestingly, antibiotic-resistant genes have even been found among indigenous people living isolation. Researchers studying the Yanomami, an

Amazonian hunter-gatherer group in Venezuela, found these resistant genes in this group of people despite their lack of exposure to antibiotics. Microbiologists now wonder whether a certain amount of these antibiotic-resistant genes occur naturally in the human microbiome. As mentioned earlier in this chapter, microbes have evolved their own defense mechanisms against naturally occurring antibiotic substances. Some researchers propose that the use of therapeutic antibiotics may have caused the rapid increase in resistant strains due to these microbial mechanisms.

Overexposure to antibiotics is a concern not only because of the growing number of antibiotic-resistant microbes but also because of the disruption antibiotics cause to microbiome homeostasis. The gut microbiome is exposed to antibiotics directly, through medical use, and also indirectly, from their use in the farming industry. Dysbiosis caused by antibiotics leaves the microbiota more susceptible to pathogenic invasion.

In addition to altered gut microbiota composition, antibiotics also disrupt the interactions between gut microbes and the innate and adaptive immune systems. Once dysbiosis occurs, altered gut microbiota composition results in a very different set of microbes being presented to toll-like receptors. The new microbial patterns recognized by these receptors are communicated to other immune cells. This updated information about the altered gut microbiota populations can then influence lymphoid tissue function and even alter the action of important immune cells such as neutrophils and T cells. In fact, animal studies provide evidence that antibiotic use can inhibit the innate immune system's ability to fight pathogenic gram-negative bacteria. In another animal study, using the antibiotic amoxicillin to induce dysbiosis, the antibody immunoglobulin G was decreased, showing changes in adaptive immunity. One specific antibiotic, vancomycin, also caused alteration in adaptive immune cells. This antibiotic reduced T regulatory cell numbers and impaired T helper 17 function.

Although the gut microbiome is somewhat resilient and can try to return to its original composition following antibiotic treatment, the original composition is often not fully restored for months or even years. Repeated antibiotic treatments make this restoration even more difficult.

Aside from complications later in life, dysbiosis in the infant gut microbiome from antibiotic use may also put the child at risk of more immediate infection. For instance, in the case of premature infants, an inflammatory disease called necrotizing enterocolitis may be linked to a lower abundance of *Bifidobacterium*. Infants born prematurely also tend to be exposed to more antibiotics.

Some antibiotics can also harm the epithelial barrier. Metronidazole, a medication used to treat less-severe cases of *Clostridium difficile* infections, can actually alter the expression of certain genes within the mucin layer. This

thins the gut's protective barrier and promotes intestinal permeability. This is yet another way in which an antibiotic can influence innate immunity.

Conclusion

The body's overall health and degree of disease resistance is dependent on optimal interactions between gut microbes and the immune system. As we have observed, gut microbiota can dictate the immune system's sensitivity and regulate inflammatory responses throughout the entire body.

Gut microbes train the immune system to recognize, respond, and adapt to commensal microbes. In this way, they build immunologic tolerance to these resident microbes and learn to differentiate between these familiar organisms and pathogenic invaders.

5

Obesity and Metabolic Syndrome

As the modern Western lifestyle continues to spread worldwide, traditional diets are rapidly disappearing. The standard modern diet is largely to blame for growing obesity rates, which, according to the World Health Organization, have more than doubled since 1980. This obesity epidemic is putting many populations at higher risk for developing associated conditions such as heart disease, stroke, type 2 diabetes, and certain cancers. Even developing countries with previously low incidences of these modern diseases are seeing a rise due to the abundance of processed, calorie-dense foods.

Along with the ever-growing obesity epidemic, it is not a coincidence that the prevalence of type 2 diabetes has simultaneously increased. In fact, obesity is a risk factor for the development of type 2 diabetes. Unmanaged diabetes can cause damage to blood vessels, thus increasing an individual's risk of heart disease and stroke.

Just as diet may increase risk for obesity, it is one of the most significant factors in the development of type 2 diabetes. The American Diabetes Association explains that while genetic predisposition is a risk factor for all types of diabetes, the disease must be activated by environmental factors. Yet researchers have long tried to understand why some individuals appear more predisposed to these effects of the modern lifestyle.

In 1962, the proposed "thrifty genome" hypothesis suggested that humans maintained traits that favor energy conservation as a protective mechanism in times of food shortage. This hypothesis argues that the body adapted during paleolithic times to conserve energy by increasing body weight when food resources were abundant. While this type of energy efficiency was protective when food became less available, such a genetic adaptation may be problematic in the context of a modern diet containing an abundance of calorie-dense foods. This hypothesis provides a possible explanation for a genetic predisposition to insulin resistance and weight gain even though, in today's environment, these "thrifty genes" increase risk for type 2 diabetes and obesity.

The effects of environmental factors such as overall food abundance on these predispositions is not well defined. However, new understandings suggest that our genetics may in fact be influenced by diet in ways that lead to obesity, although through a different avenue than the one proposed by the thrifty genome hypothesis. In fact, researchers are now finding that genes can change their expression based on their interactions with gut microbe populations, which are heavily influenced by diet.

Recent evidence suggests that our gut microbiota play a crucial role in regulating the body's energy balance and weight gain. For example, gut microbes can increase the calories we extract from food, promote body fat storage, and affect feelings of fullness. Our gut microbes also influence the immune system in ways that may lead to obesity and associated diseases such as type 2 diabetes and non-alcoholic fatty liver disease.

In the following sections, we will explore how diet influences the composition and functions of bacterial populations in ways that promote or prevent metabolic diseases. We will also discuss how bacteria aids digestion and regulates fat storage in the body. In addition, the relationship between gut microbiota and the immune system (as detailed in chapter 4) will explain the role of inflammation in the development of obesity. In fact, an unhealthy diet can influence microbes to produce an inflammatory immune response that contributes to weight gain and the development of diabetes.

Metabolism and Energy Homeostasis

The body's use of energy from food is regulated by a sophisticated set of mechanisms that make up the human metabolism, the physical and chemical processes that sustain life. Macronutrients such as fat, protein, and carbohydrates provide energy for the body's various metabolic needs. Obesity and type 2 diabetes are both conditions associated with imbalances in the body's metabolism.

The body's metabolic processes help create a balance of energy known as energy homeostasis, by regulating how the body uses calories from food. Disruptions in energy homeostasis can signal the body to gain or lose weight. When energy intake from food is higher than energy expenditure, this is called a positive energy balance, and results in weight gain. In addition to surplus dietary calories, weight gain may also be caused by a disrupted metabolic state that leads the body to store excess energy. However, new evidence suggests that weight gain and obesity may not be solely explained by nutritional habits or decreased physical activity. Gut microbiota also greatly influences energy homeostasis in a variety of ways. The interactions between the host and gut microbiota shed some new light on the mechanisms behind the development of the metabolic conditions associated with obesity.

The maintenance of energy homeostasis involves the action of various signaling molecules to communicate information about the energy needs of

human cells. Hormones in the endocrine system and neurotransmitters from the nervous system send signals throughout the body that regulate energy use and storage. The human gut microbiota also communicates with these hormones and neurotransmitters, providing the body information about dietary intake. In these ways, our gut bacteria can influence two very powerful body systems that determine metabolic patterns and, ultimately, our state of health.

In fact, obesity and type 2 diabetes are part of what is called metabolic syndrome, a group of interrelated conditions that exist simultaneously and are associated with the development of heart disease. It is also important to note that one of the underlying causes of metabolic syndrome is insulin resistance, a condition that develops with type 2 diabetes and is significantly associated with gut microbiota activity. Insulin is one of main metabolic hormones that have an integral relationship with the gut microbiota particularly through the inflammatory processes mentioned in chapter 4. Later sections of this chapter will delve into the mechanisms by which gut microbes influence insulin, other metabolic hormones, and brain chemicals in ways that inform the body's use of energy. First, we will discuss the original observations that led researchers to investigate the relationship between differing gut microbiota populations and the development of obesity and metabolic disease.

The Energy Harvest Hypothesis

Some of the first observed effects of the gut microbiota on weight gain were seen in mouse studies comparing germ-free mice and wild-type mice. Germ-free mice, as opposed to wild-type mice with normal microbiota, have no gut bacteria. Researchers noticed that these mice, lacking gut microbes, had 40% less body fat even though they consumed 30% more calories than mice with normal gut microbes. This observation suggests that the absence of gut bacteria prevents weight gain.

When the germ-free mice received microbes transplanted from the large intestine of wild-type mice, their body fat increased by 60%, and they also developed insulin resistance. These physiological changes occurred within two weeks, even in the context of a lower calorie intake. These observations raised questions regarding gut microbiota's association with weight gain and disturbed blood sugar regulation in the presence of excess food intake.

Given this observation in germ-free mice, a few mechanisms have been proposed to explain how microbes may have fattening effects and predispose certain individuals towards obesity. The "energy harvest" hypothesis is one mechanism that attempts to explain how gut microbes help the body utilize energy (and therefore harvest extra calories) from indigestible carbohydrates.

Gut microbiota increase energy extraction from food through the digestion of resistant starches and other dietary fibers. Microbes ferment food particles, which are otherwise not broken down by human enzymes during digestion in

the small intestine. Short-chain fatty acids (SCFAs) are created as a byproduct of this fermentation process and serve as an additional energy source for the human host. With the help of gut microbes, humans are able to achieve maximum energy extraction from food. This idea is the basis of the energy harvest hypothesis.

Furthermore, this hypothesis suggests that higher intake of dietary fiber implies extra available calories and therefore weight gain. With increased energy extraction, higher levels of glucose and insulin (a hormone that helps the body metabolize glucose) are present in the blood. An increased concentration of these two substances promotes lipogenesis, the conversion of carbohydrates (which is to say, glucose) into fat. Lipogenesis is one way the body conserves any excess energy from calories and therefore leads to fat storage. However, not all carbohydrates are digested at the same rate. Dietary fiber can actually increase digestion time and prevent too much glucose from being released into the bloodstream at once. This promotes more stable glucose and insulin levels. Given that fiber can have these beneficial metabolic effects, it appears unlikely that the energy harvest hypothesis explains the primary mechanisms by which gut microbiota lead to weight gain.

The energy harvest hypothesis also conflicts with evidence from numerous studies showing that a diet high in fiber protects against obesity. Although a small percentage of total energy may be extracted from the fiber, diets high in fiber typically have an overall lower energy density. Additionally, fiber promotes satiety, or the feeling of fullness, and can help individuals reduce overall caloric intake. Many vegetables are not only high in fiber but also less calorie-dense compared to other foods. Therefore, they are useful in decreasing total caloric intake and preventing weight gain.

Considering the health benefits associated with dietary fiber, scientists are proposing alternative ideas to the energy harvest hypothesis that may better explain the connections between gut microbiota composition and weight gain. In fact, it appears that certain types of bacteria are protective against obesity. Using data from both human populations and rodent models, researchers are now working to identify what types of gut microbial populations are associated with increased risk of developing these metabolic diseases. Additionally, they have pinpointed a few mechanisms by which gut microbes affect the body's overall energy regulation through specific host-microbe communication. In following sections we will delve into the specific ways gut microbes regulate weight gain and influence the body's metabolic functions.

Differing Microbiota Populations and Risk for Obesity

As discussed in chapter 1, some rural and traditional populations are virtually free of obesity and other metabolic diseases that are becoming more prevalent

in Western societies. Research on these traditional societies raises questions regarding potential connections between gut microbiota composition and the development of obesity. With these questions in mind, scientists analyzed gut microbial populations in animals and confirmed significant differences in the composition of microbiota within genetically obese mice versus lean mice.

At first, observations indicated that obesity is related to altered gut microbial composition. In particular, the ratio between Firmicutes and Bacteroidetes appears to shift in an obese state. In one study, obese rodents had a 50% reduction in Bacteroidetes, along with an abundance of Firmicutes. It is still unknown whether a shift in these two major gut phyla is significant. Researchers are also now collecting data from human populations and comparing microbiota composition in obese and normal-weight individuals. Yet, there is still no general consensus on any clear associations between human obesity and specific gut microbiota profiles. While some human studies revealed similar ratios of Bacteroidetes to Firmicutes in obese humans as were seen in obese mice, other studies reported conflicting results.

The emergence of conflicting data may be in part due to the different methodologies used to classify bacterial taxa. Bacteroidetes and Firmicutes are both phylum-level classifications, with a large variety of species identified within these groups. Given that these species each have unique functions, better understanding of the different functions of individual species may create a clearer picture about how microbes influence obesity. For instance, the Bacteroidetes and Firmicutes phylums are general classifications that include both pathogenic and commensal species. *Clostridium botulinum* and *Faecalibacterium prausnitzii* are both classified under the phylum Firmicutes. *Faecalibacterium prausnitzii* is a beneficial microbe that produces butyrate, whereas *Clostridium botulinum* is a pathogenic microbe that produces a toxic substance that can damage nerve tissue. These species-level discrepancies indicate that a clearer characterization of gut microbiota in obesity may be achieved by looking at microbial functions and behavior at the species level.

In addition to functional variation among different species, the inconclusive data may also be due to confounding factors such as age and diet, the latter having significant influence over gut microbiota composition. Specific diets may be adopted by participants in gut microbiome studies. If calorie intake is reduced or the overall composition of macronutrients (fats, protein, and carbohydrates) changes, this causes a shift in gut microbial populations.

One small study involved 12 obese individuals placed on an energy-restricted diet that was either reduced in fat or reduced in carbohydrate content. After 52 weeks of dieting, the subjects lost weight and showed a change in microbial composition such that Bacteroidetes increased and Firmicutes decreased. The weight loss appears to create a Bacteroidetes-to-Firmicutes ratio that mirrors observations seen in lean mice. However, the diet used to

induce this weight loss may have changed the macronutrient composition of the subjects' normal diet.

Considering that weight loss is usually directly connected with significant dietary changes, altered food intake alone becomes an important variable when measuring the effect of weight loss on the gut microbiota. Therefore, it is difficult to conclude that weight loss is the main factor that alters gut microbiota composition when these shifts in microbial populations can be directly connected to changes in diet composition.

In addition to understanding patterns of gut microbiota in obese individuals, researchers are working to determine how the developing gut microbiome during childhood affects risk for obesity later in life. In fact, differences in gut microbiota populations can begin very early in life. Infants in their first year can begin to develop patterns in gut bacteria that predict overweight or obese body types later on in childhood. Normal-weight children often have a greater number of *Bifidobacteria* (a member of Actinobacteria), whereas children who became overweight had higher populations of *Staphylococcus aureus* (a member of Firmicutes) in infancy. Again, this appears to be evidence that the ratio between Bacteroidetes and Firmicutes is somehow tied to the development of obesity.

SIDEBAR 5.1 Antibiotics and Obesity

Regular antibiotic use in children can cause increased weight gain. Routine use of these medications in childhood can have lasting consequences that are carried into adulthood. A study published in the *International Journal of Obesity* used medical records from pediatric patients to determine correlations between the number of antibiotic prescriptions and elevated weights. Children who had taken seven or more courses of antibiotics weighted on average 3 pounds more than children who had never taken antibiotics. While this number may seem insignificant, the weight gain continued more rapidly in adult years.

Children may be exposed to antibiotics even before birth. In fact, children's weight gain patterns may also be affected by their mother's use of antibiotics during pregnancy. Given that a child's developing gut microbiome is influenced by maternal microbiota, it is possible that dysbiosis related to antibiotic use can also be transmitted from mother to offspring.

Furthermore, in recent years, there has been growing concern over use of antibiotics in raising livestock. It is common practice in modern livestock production to treat animals with a low dose of antibiotics to increase their growth rate. This practice began many decades ago, as livestock farmers struggled to meet food demands. With the growing problem of antibiotic resistance, many advocates are urging livestock farms to discontinue use of antibiotics. Reducing exposure to these environmental antibiotics is likely to be important in keeping commensal microbes balanced.

The implications of the Bacteroidetes-to-Firmicutes ratio for obesity are still inconclusive. In general, larger and more comprehensive studies are needed to confirm the connection between altered gut microbial populations and obesity. As researchers study fluctuations in these populations, they continue to also explore how dietary patterns determine which microbes reside in the gut. In fact, diet is not only one of the most significant factors that influences the composition of these microbes, it is also an important factor in determining microbial interactions with human hosts.

High-Fat Diet and Metabolic Endotoxemia

The types of bacteria found within the gut are largely determined by diet, and major shifts in diversity may put the host at risk of developing metabolic syndrome. In fact, metabolic syndrome is correlated with a less diverse microbiota. As researchers explored dietary factors that decrease microbial diversity, they discovered that a high-fat diet is linked to microbial dysbiosis.

Although it is well-known that a high-fat diet can lead to obesity due to the calorie density of fatty foods, recent research reveals new and distinct mechanisms by which dietary fat is linked to metabolic syndrome. In fact, excessive fat intake is shown to cause detrimental interactions between the gut microbiota and the immune system. Surprisingly, these interactions, which result in an inflammatory response, contribute to the development of obesity and metabolic syndrome.

While a high fat intake may not be the only contributing factor to this inflammatory state, researchers observe that in animal models, this type of diet substantially influences the immune system in ways that eventually manifest in metabolic syndrome. This research supports the understanding that obesity is a physical state involving complex metabolic imbalances and cannot be defined simply as excessive weight gain. Given that obesity is characterized by a state of low-grade inflammation throughout the entire body, it is important to consider how a high fat intake can play a role in this inflammatory process.

As discussed in chapter 4, the immune system uses inflammation as a protective reaction against foreign or toxic compounds within the body. In the case of obesity, the compounds triggering this inflammation are, in fact, coming from the gut microbiota. In a healthy person, commensal gut microbes are well tolerated by the immune system as long as they remain within the gut. However, if gut bacteria begin to leak out of the colon, the immune system is triggered to remove the escaped microbes from the body. With a high-fat diet, gut microbes are more likely to permeate through the protective lining of the colon. In fact, a specific component of gram-negative bacteria are found in elevated quantities in the blood of obese subjects. These molecules are called lipopolysaccharides or endotoxins.

Lipopolysaccharides are part of the outer layer of gram-negative bacteria. These gram-negative bacteria are a normal component of a healthy and balanced gut microbiota population. However, lipopolysaccharides can enter the surrounding environment due to certain dietary factors and dysfunction of the intestinal lining. In order to prevent the buildup of endotoxins, the immune system has specific cells equipped with proteins called toll-like receptors (TLRs) that recognize invading microorganisms. Certain TLRs detect lipopolysaccharides in the blood and identify them as a potential threat. This triggers an inflammatory response to eliminate the endotoxins.

A rapid immune response is often very useful for recognizing disease-promoting microorganisms. Yet consistently elevated levels of endotoxins in the blood can lead this natural immune response to produce a chronic condition of low-grade systemic inflammation known as metabolic endotoxemia. Systemic inflammation is not isolated in certain tissues but instead can affect many areas of the body. While low levels of lipopolysaccharides in the blood are normal in healthy individuals and don't promote systemic inflammation, a constant influx of lipopolysaccharides is linked to this chronic inflammatory state that is associated with metabolic diseases such as obesity and type 2 diabetes. In one animal study, injecting mice with lipopolysaccharides caused an increase in fasting blood glucose and insulin levels in the blood—two markers for the development of diabetes.

Although there are a number of factors that can increase lipopolysaccharides in the blood, a high-fat diet has direct effects on gut microbiota and can cause these endotoxins to escape from the gut. In numerous studies, high fat intake increases lipopolysaccharides in the blood. There are different mechanisms by which a high-fat diet can potentially increase lipopolysaccharide levels. For example, it is possible that excess dietary fat compromises the protective lining of the gut, making it more permeable and allowing bacteria to travel into the bloodstream. As we learned in chapter 4, the gut epithelium serves as a protective barrier that keeps bacteria inside the gut lumen. Given that diet determines which bacteria reside within the gut, altering macronutrient balance with a high-fat diet can lead to shifts in gut microbiota populations. If these shifts decrease the abundance of microbes that normally play a role in strengthening the gut epithelium, this may explain how a high-fat diet promotes intestinal permeability and the influx of endotoxins to the bloodstream.

Another hypothesized mechanism suggests that lipopolysaccharides may attach to the same carrier molecules that are used to transport dietary fat out of the gut. Our bodies produce different transport molecules for certain dietary nutrients. These specialized transport molecules carry nutrients from the gastrointestinal tract to various tissues throughout the body. Because lipopolysaccharides are made of fats and carbohydrates, they interact with some of these transport molecules. For example, the fat component of these

SIDEBAR 5.2 Non-Caloric Artificial Sweeteners

Non-caloric artificial sweeteners are popular as food additives due to their perceived health benefits. They are generally recommended over caloric sugars, to prevent weight gain and development of type 2 diabetes. However, even prior to their approval by the United States Food and Drug Administration (FDA), studies revealed conflicting evidence regarding the benefits of artificial sweeteners. Some studies indicate that these sugar substitutes may actually be associated with weight gain and increased risk of type 2 diabetes.

In 2014, research performed on mice showed concerning evidence regarding commercial formulations of popular non-caloric artificial sweeteners such as sucralose, saccharin, and aspartame. Each of these commercial formulations contains approximately 5% artificial sweetener and 95% glucose. Within one week, mice consuming these commercial sweeteners showed significant development of glucose intolerance compared to mice consuming glucose or table sugar (sucrose). Saccharin had the most profound effect of the three commercial sweeteners.

Although these first experiments were performed by feeding mice commercial saccharin, a subsequent experiment used doses of pure saccharin that corresponded to the FDA's acceptable daily intake for humans (5 milligrams per kilogram of body weight) and modified this dose to mouse weights. These mice, who were also fed a high-fat diet (about 60% of calories coming from fat), developed glucose intolerance as early as five weeks from starting this diet. This high-fat diet was used to create an "obesity setup" and shows that consumption of non-caloric artificial sweetener corresponds to glucose intolerance in both lean and obese mice.

Most non-caloric artificial sweeteners are not digested by humans and pass directly into the lower gastrointestinal tract, where they interact directly with gut microbiota. To investigate these interactions, researchers gave antibiotics to both lean mice and mice fed the high-fat diet. This four-week antibiotic regimen wiped out the mice's commensal gut microbiota, and the researchers observed no differences in glucose tolerance between mice consuming the artificial sweeteners and regular sugar. Furthermore, germ-free mice (those raised in a strict germ-free environment) who were given a fecal transplant, receiving microbiota from mice consuming commercial saccharin, also developed glucose intolerance within several days of fecal transfer. These results suggest that gut microbiota may dictate the interactions between non-caloric artificial sweeteners and the body.

endotoxins allows them to be transported on the same carriers used for dietary fat. Production of these specific carriers, called chylomicrons, is increased in response to dietary fat. Just as chylomicrons attach to dietary fats, they also have an affinity for lipopolysaccharides. Since excess dietary fat leads to an

abundance of chylomicrons, a greater number of lipopolysaccharides can be carried out of the gut when fat consumption is high.

Although researchers are not certain whether one or both of these methods causes endotoxins to enter blood circulation, several studies now confirm that

SIDEBAR 5.3 Emulsifiers

Artificial additives are common in many processed food products. Although they are generally regulated to assure safely for human consumption, some of these additives disrupt gut bacteria even if they are labeled as safe. One such group of disruptive additives are emulsifiers. Food manufacturers use emulsifiers in many common products to improve texture and also to lengthen shelf life. Emulsifiers work by helping to stabilize fat and liquid mixtures, which otherwise separate over time (as with oil and water). Foods such as ice cream, mayonnaise, and margarine can maintain their smooth texture because of the stabilizing effects of added emulsifiers.

Consuming emulsifiers can increase the amount of lipopolysaccharides escaping through the gut wall. Researchers used animal models to examine the effects of polysorbate 80 and other emulsifiers on gut microbiota. They originally wondered whether these food additives weaken the mucus layer, allowing bacteria to pass through. However, studies on germ-free mice revealed no changes in the mucosal layer when they were fed emulsifiers. So researchers then explored how emulsifiers may be causing changes in the microbiota itself.

Using animal models, researchers tested emulsifiers in quantities lower than those approved by the Food and Drug Administration, as well as quantities that reflect amounts an individual might ingest if their diet is high in processed foods. Mice with normal immune systems developed low-grade inflammation that quickly led to metabolic disease. In addition to becoming obese, their appetites increased, they had higher blood sugar levels, and they developed insulin resistance. Emulsifiers inhibit satiety, or the feeling of fullness, which can contribute to overeating. Researchers argue that the most likely mechanism explaining this increase in appetite is the development of resistance to leptin and insulin, associated with low-grade inflammation. If regular consumption of emulsifiers increases lipopolysaccharide levels, these additives may be responsible for indirectly inhabiting the normal appetite-regulating functions of these two hormones.

Diets high in processed food can lead to increased consumption of emulsifiers. There are also many health foods that appear minimally processed yet contain emulsifiers. For instance, these additives are common in low-fat dairy as well as gluten-free products. Much research on emulsifiers and gut microbiota focuses on man-made emulsifiers, yet natural emulsifiers such as lecithin, carrageenan, and various gums (such as guar gum and xanthan gum) are also increasingly used in food production.

a high-fat diet increases the level of lipopolysaccharides and thereby promotes inflammation. Yet, it is still important to consider that while a high-fat diet can significantly influence the gut microbiota, the metabolic effects vary based on the type of dietary fats consumed. For instance, omega-3 fatty acids found in fish and certain seeds, such as flax and chia, are generally recognized for their anti-inflammatory effects.

One study published in the journal *Cell Metabolism* compared the effects of either fish oil rich in omega-3 or saturated fat on gut microbial composition. Mice were fed either fish oil or lard as a part of their diet. Lard, an animal fat that contains about 40% saturated fat, caused metabolic disease in mice after just 11 weeks. The mice who were fed fish oil showed positive health effects. Moreover, when the gut bacteria from the mice who were fed fish oil were transferred to mice who were then fed a high-lard diet, those newly introduced gut microbes had a protective effect against the detrimental metabolic effects of a diet high in saturated fat.

Adipose Tissue

As we have learned, diet and gut microbiota can be linked to an inflammatory state that promotes fat storage. To delve more deeply into the processes by which systemic inflammation eventually contributes to obesity, it is valuable to understand how fat tissue functions in the body. Gut microbes interact with body fat in various ways that either promote or protect against metabolic imbalances and weight gain. In addition to the inflammatory process, gut microbiota produces various molecules that influence how fat tissue functions.

Fat is stored in the body in the form of adipose tissue, a collection of fat cells linked by connective tissue. While adipose tissue serves as an important energy reserve, it is also a metabolically active organ that directly influences energy use and storage. There are two main forms of adipose tissue: white adipose tissue and brown adipose tissue. These tissues vary greatly in appearance and function, yet both play crucial roles in maintaining energy homeostasis and managing body weight.

White Adipose Tissue

White adipose tissue is the most abundant type of fat tissue in the body. It is capable of expanding to store fat as an energy reserve. Each white adipose cell contains a single droplet of triglyceride, a form of stored fat that can be released during fasting periods. White adipose cells increase in size and volume as more fat is stored within the cell. While a certain amount of body fat is healthy and protects against potential food shortages, accumulating large quantities of white adipose tissue has numerous health consequences.

As white adipose tissue expands due to excessive caloric intake or other metabolic imbalances, it can build up underneath the skin and between organs like the intestines, liver, and stomach. Fat tissue that accumulates in the abdominal cavity and surrounds the internal organs is called visceral adipose tissue. An excessive amount of this body fat is associated with metabolic imbalances such as those seen in type 2 diabetes. This is due to the role of white adipose tissue in the production of important metabolic hormones. In fact, white adipose tissue regulates both the process of fat storage as well as insulin sensitivity.

Chronic systemic inflammation, as seen with metabolic endotoxemia, alters the normal regulatory functions of adipose tissue. Given that white adipose fat is hormonally active, over-expansion can promote the development of insulin resistance, a hallmark of type 2 diabetes. This type of dysfunction in adipose tissue contributes significantly to metabolic syndrome. Later in this chapter, we will also examine how improperly stored fats can lead to accumulation in the liver and cause inflammation and liver disease.

Leptin

The over-expansion of white adipose tissue is associated with weight gain and has a number of other physiological consequences, including increased production of a hormone called leptin as well as death of adipose cells. These two changes in adipose cell function contribute to the immune responses that connect inflammation with obesity. For instance, if an adipose cell dies as a consequence of expansion, specialized immune cells called macrophages infiltrate the adipose tissue to remove the dead or dying cells. Adipose cell death occurs rarely in lean individuals but is a common symptom of obesity. Similarly, increased leptin production also causes macrophages to infiltrate adipose tissue. This process, known as macrophage infiltration, is characteristic of the inflammatory immune response to changes in adipose cells during obesity.

Increased leptin production and adipose cell death are two ways in which the expanding adipose tissue can create an inflammatory cascade that causes one immune response to trigger another. For example, increased macrophage infiltration of adipose cells leads to the production of other pro-inflammatory molecules, called cytokines. These molecules signal other cells in the body, particularly in the immune system, to continue a cascade of inflammatory responses. These secondary responses then lead to the production of more cytokines and eventually can manifest in cellular dysfunction.

As mentioned, another example of cellular dysfunction relates to the increased cellular production of leptin. In healthy individuals, white adipose tissue secretes leptin to inhibit appetite and signals feelings of fullness in the brain. However, obesity and its associated state of low-grade inflammation is

unfortunately associated with leptin resistance, and these adipose cells are not able to properly use this hormone to limit appetite. The relationship between gut microbiota and leptin function is discussed further in the next section of the chapter.

These are among several factors that demonstrate how gut microbiota may produce a state of chronic inflammation and alter the hormone function of white adipose tissue. That is to say, low-grade inflammation related to metabolic endotoxemia can promote a state of insulin and leptin resistance in adipose tissue. These hormones, which regulate energy use/storage and appetite, respectively, can become dysfunctional if the gut microbiota is out of balance.

Brown Adipose Tissue

In addition to its effects on white adipose tissue, the gut microbiota also influences how brown adipose tissue functions. Until recently, brown adipose tissue was not believed to play a significant role in adult metabolism. This type of body fat was thought to be present mostly in infants, functioning as a protective adaptation to help them maintain body heat. Only small amounts of brown fat are found in adults, and therefore its contribution to energy expenditure and metabolic processes was overlooked. However, researchers have now identified functional brown adipose tissue in adults.

Although after puberty the amount of brown adipose tissue and its activity decreases, this tissue remains present in adults within a few regions of the body. The most concentrated areas of brown adipose tissue are in the neck region, above the collarbone, around the spinal cord, as well as around the aorta. Just as in children, this brown fat in adults can produce heat to regulate body temperature. This function of brown fat is called thermogenesis. Recent research reveals that brown adipose tissue not only plays a role in regulating body temperature but also influences weight gain and fat metabolism. In fact, brown adipose cells attribute their darker color to an abundance of mitochondria, a structure known as the powerhouse of the cell due to its ability to produce energy.

While brown fat activity is generally stimulated by exposure to extremely cold conditions or by exercise, the gut microbiota can also activate this type of adipose tissue to a certain extent. When brown adipose tissue is stimulated, it has a significant impact on overall energy balance. In fact, researchers show that activating brown adipose tissue through cold exposure is beneficial for regulating body weight in mice. This is because activation increases uptake of glucose and lipids within brown adipose tissue. The higher mitochondria concentration of brown fat increases its ability to utilize glucose and lipids. Increasing the function of brown fat may therefore prevent elevated blood glucose levels as well as lipid accumulation in the blood. These are positive effects

that decrease risk for diabetes and heart disease (which is linked to fat buildup along the arteries). On the other hand, if brown fat becomes dysfunctional, the body responds by expending less energy.

Factors That Regulate Fat Storage

As we have learned, gut microbes regulate the expansion of adipose tissue and directly affect factors related to weight gain such as appetite. For instance, a substance produced by microbes, called conjugated linoleic acid (CLA), may have positive effects on both brown and white adipose tissue. CLA is a type of fat created by bacteria through the conversion of linoleic acid and alpha-linolenic acid (ALA), two dietary fats found in many foods such nuts, seeds, and vegetable oils.

There is some evidence that CLA may encourage lower body fat. In fact, some supplement companies have marketed CLA for weight loss despite inconclusive and contradictory results from studies testing the effectiveness of CLA supplementation. Yet, researchers are now studying CLA-producing bacteria to observe whether these probiotic strains induce any beneficial changes in fat tissue. One study showed that supplementation with the bacterium *L. paracasei* may increase nerve activity in fat cells in two beneficial ways. CLA produced by *L. paracasei* appears to increase a process known as lipolysis in white adipose tissue. Lipolysis is the breakdown of stored fat. This process therefore decreases triglycerides within adipose tissue and prevents over-expansion. CLA may also increase thermogenesis in brown adipose tissue. Animal studies demonstrate that administration of this bacterium decreases triglycerides in the bloodstream and leads to overall lower body fat.

In addition to bacteria-derived CLA, gut microbiota communicate with adipose tissue by interacting with a protein called fasting-induced adipose factor (Fiaf). Fiaf is produced by cells within the intestine, skeletal muscle, and adipose tissue. A primary function of this protein is to inhibit the enzyme lipoprotein lipase, which controls the amount of fat stored in adipose cells.

Some gut microbes may have the ability to suppress Fiaf. Germ-free mice, for instance, have higher levels intestinal Fiaf, which decreases triglyceride storage and lowers body weight. In another animal study, mice raised without the gene encoded for this protein had only a 10% body fat increase, compared to a 50% body fat gain for normal mice born with the Fiaf gene. Both groups consumed a high-fat, high-carbohydrate diet, yet lack of Fiaf made the genetically altered mice resistant to weight gain.

When microbes suppress Fiaf, lipoprotein lipase activity increases and promotes triglyceride accumulation in adipose tissue. A low level of circulating lipoprotein lipase thus increases fat storage and weight gain. Given that Fiaf

prevents excessive fat accumulation in adipose tissue, further research may help us understand more about why microbes may suppress this beneficial protein.

The Endocannabinoid System

Another important metabolism-regulating system in the body involves a different group of g-protein coupled receptors. The endocannabinoid system has receptors in several metabolically active tissues within the body, including adipose tissue and the gastrointestinal tract. Consequently, the placement of these receptors allows gut bacteria to influence metabolic functions within adipose tissue through the endocannabinoid system. Some research suggests that through these communication pathways, gut bacteria are able to influence appetite and weight gain.

Endocannabinoid Receptors

Scientists currently have identified two major endocannabinoid receptors: cannabinoid receptor type 1 (CB_1) and cannabinoid receptor type 2 (CB_2). Most research currently points to CB_1 receptors as key modulators of metabolic function. These receptors receive signals from chemicals produced in the body, called endocannabinoids. In contrast with phytocannabinoids found in certain plants, endocannabinoids are compounds produced by the body. When these substances activate cannabinoid receptors, they are able to influence many important physiological functions, including the maintenance of energy homeostasis. In fact, the endocannabinoid system regulates metabolism and energy storage in multiple ways.

CB_1 receptors are the most abundant in the body. They are found within adipose tissue as well as other metabolic organs such as the liver. They are also expressed within the epithelial lining of the gastrointestinal tract. Interestingly, CB_1 receptors are often altered in obesity. In obese individuals, the genes containing information that enables the production of these cellular receptors are expressed differently. That is to say, the gene expression, or the process by which this information is used to create cannabinoid receptors, is abnormal in an obese state.

In order to test these receptors' degree of metabolic influence, researchers blocked specific cannabinoid receptors in animals fed a high-energy diet. They observed that these animals were protected against the development of obesity as well as the associated systemic inflammation. On the other hand, obese animals displayed endocannabinoid overactivity within both fat tissue and in the GI tract. This overactivity can be related to either changes in expression of the receptors or increased endocannabinoids in the body.

Endocannabinoid System Regulates Intestinal Permeability

An overactive endocannabinoid system appears to induce intestinal permeability and therefore increase levels of microbe-derived lipopolysaccharides in the blood. The pro-inflammatory effects of excessive circulating lipopolysaccharides are strongly associated with the development of metabolic disease, as mentioned in previous sections of this chapter. Lipopolysaccharides from gut microbes also stimulate the production of endocannabinoids. Thus, elevated levels of these endotoxins increase the presence of endocannabinoids in the body. This creates a loop in which both compounds increase the presence of the other in a way that promotes chronic inflammation.

Additionally, the endocannabinoid system regulates systemic inflammation through its control of the gut barrier. For instance, researchers demonstrated that blocking CB_1 receptors changed the distribution of proteins that form tight junctions between epithelial cells. Preventing the overstimulation of this system improves the formation of tight junctions and therefore strengthens the intestinal barrier. An overactive endocannabinoid system eventually leads to increased gut permeability, a state that allows endotoxins to escape the colon.

Endocannabinoid System Regulates Adipose Function

The endocannabinoid system also affects the metabolic functions of adipose tissue. When CB_1 receptors are activated in white adipose tissue, they begin to store energy in the form of triglycerides. Additionally, this activation also stimulates the production of more adipose cells. Conversely, when researchers inhibit these CB_1 receptors, the use of stored fat for energy production increases. Blocking the activity of CB_1 receptors further enables cellular energy production by stimulating the growth of pre-existing mitochondria. Thermogenesis, another process that uses stored triglycerides, is also enabled by the inhibition of these receptors. This is due to the increased conversion of white adipose cells to mitochondria-rich brown adipose cells. While these observations were made only *in vitro* (based on cells or tissue isolated from the body), researchers are now looking at CB_1 antagonists that block receptor activation as a treatment for obesity and metabolic disease.

Obesity typically leads to higher levels of endocannabinoids in the blood as well as within adipose tissue. These levels normally fluctuate based on food intake, and both obese as well as healthy-weight individuals have increased circulating endocannabinoids following a meal. Yet, these levels decrease in normal-weight individuals and remain elevated in obese individuals. This discrepancy led researchers to hypothesize that the dysregulation of endocannabinoids in obesity may encourage excessive food intake by overstimulating the appetite. In fact, animal studies confirm that inhibiting CB_1 receptor leads to a significant decrease in appetite.

The activation of CB_1 receptors within other tissues, such as the liver and the gastrointestinal tract, influences important metabolic hormones and promotes body fat accumulation. For instance, activating CB_1 receptors in the liver favors the conversion of energy to stored fat, and dysregulation of these receptors can lead to a disease state caused by excessive accumulation of fat in the liver.

Ghrelin

The activation of CB_1 receptors within the GI tract stimulates the production of another appetite-regulating hormone, ghrelin. The secretion of ghrelin usually occurs as a response to an empty stomach. Once the body senses the presence of food within the stomach, ghrelin is no longer needed, and production of the hormone in the GI tract stops. Yet, an overactive endocannabinoid system may increase ghrelin, overstimulate appetite, and lead to weight gain.

In addition to ghrelin, the hormone leptin also has a complex relationship with the endocannabinoid system. The presence of leptin reduces levels of endocannabinoids within the brain. When leptin levels are low, endocannabinoid levels in the brain increase and stimulate appetite. As mentioned in the previous section of this chapter, obese individuals often have increased leptin levels in the blood. In addition, leptin resistance, which often occurs in obesity, changes CB_1 receptors in a way that promotes excessive hunger. These observations have prompted researchers to continue to explore the connection between leptin and weight gain in relation to the endocannabinoid system.

SIDEBAR 5.4 *Helicobacter Pylori* and Appetite

The bacterium *H. pylori* has a long-standing history with its human host but is now frequently eradicated to prevent the development of peptic ulcers. (For more information, see chapter 2.) While *H. pylori* is known for its pathogenic effects, it also may have some beneficial role in regulating food intake. In fact, decreasing levels of *H. pylori* in the stomach appear to be associated with increased appetite.

H. pylori can control levels of ghrelin, an important appetite-suppressing hormone. Ghrelin is a fast-acting hormone (compared to leptin, which controls long-term hunger) that is released when the stomach is empty, signaling us to eat. As the stomach fills up with food, it stretches, and ghrelin production stops. Interestingly, individuals whose *H. pylori* populations were completely eradicated had significantly higher levels of ghrelin circulating in their bloodstream. Despite this observed correlation between bacteria and this appetite-regulating hormone, it is not conclusive that *H. pylori* is directly protective against obesity.

SCFA as Signaling Molecules

In previous sections, we have learned how the gut microbiota can disturb metabolic processes and increase fat storage. However, bacteria can also signal the body in ways that inhibit weight gain. Specifically, certain microbes produce molecules called short-chain fatty acids (SCFAs) that reduce systemic inflammation and also suppress appetite.

SCFAs are created as a byproduct of microbial fermentation. As discussed in chapter 3, food particles that have escaped digestion in the small intestine will reach the colon, where they are metabolized by microbes through the process of fermentation. In the case of undigested carbohydrates, fermentation produces SCFAs, which have various functions within the body. (For more information on SCFAs, see chapter 3.)

SCFAs and Appetite

Short-chain fatty acids are not only a valuable energy source; they also directly influence the hormones that regulate weight gain. For instance, SCFAs can activate receptors found on cell membranes that regulate appetite. Propionate, acetate, and, to some extent, butyrate can signal these receptors, which are located in the small and large intestine as well as in adipose tissue. When the receptors are activated, two appetite-suppressing substances are secreted: the hormone leptin and peptide YY.

Leptin produced by adipose cells decreases appetite and thus works to regulate the body's long-term energy balance. In addition to inhibiting excess food intake, leptin controls the amount of stored body fat can help to maintain healthy levels of total body fat. On the other hand, individuals with lower body fat release less leptin. This lower production of leptin then increases appetite and may promote the storage of more body fat if needed. Unfortunately, leptin receptors in the brain may become defective in overweight individuals. Obesity is associated with leptin resistance, a state in which cells are no longer responsive to the effects of this hormone.

Obese individuals with leptin resistance have higher levels of leptin circulating in the blood, much in the same way that insulin resistance causes higher levels of circulating insulin. In this situation, leptin loses its ability to suppress hunger, which perpetuates a cycle of overeating and further weight gain. Interestingly, some studies indicate that even individuals who are considered to be healthy but consume a standard Westernized diet may have significantly higher leptin levels than those with a non-Westernized diet.

The other appetite-suppressing substance produced in response to microbe-derived SCFAs is peptide YY. In general, this peptide is secreted in the small intestine and colon in response to food intake. Yet, researchers observed that foods containing resistant starch are able to stimulate the secretion of peptide

YY in a more prolonged manner throughout the day. Adding resistant starch to a meal provides more fermentative substance for microbes and therefore increases SCFA production.

Furthermore, the addition of fermentable carbohydrates to a high-fat diet can prevent weight gain due to the production of appetite-suppressing substances, including peptide YY. One study performed in rats showed that fermentable carbohydrates helped increase satiety and lowered overall energy intake when they were fed a high-fat diet.

Aside from appetite regulation, peptide YY also benefits the digestive process by promoting nutrient absorption. For instance, peptide YY decreases gut motility and slows down food's rate of passage through the intestine. This effect increases the amount of time food interacts with the absorptive surfaces of the GI tract, making the digestion more efficient and thorough.

SCFAs and Fat Metabolism

In addition to signaling the production of substances that aid in lowering appetite, SCFA also influence how the body metabolizes fat. For instance, SCFAs promote fat oxidation which reduces the amount of free fatty acids in the bloodstream. Increased fat oxidation thus reduces the amount of fat stored in adipose tissue. SCFAs also influence lipolysis within adipose tissue (though lipolysis in liver is not affected by these microbial products). The creation of new fatty acids is also regulated by SCFAs. In general, SCFAs help create a balance between this fatty acid synthesis and lipolysis in a way that reduces overall body fat.

Anti-inflammatory Activity of SCFAs

Short-chain fatty acids not only influence appetite and weight regulation; they also have a crucial role in preventing the state of low-grade inflammation typically associated with obesity and type 2 diabetes. For instance, butyrate, one of the major SCFAs produced through bacterial fermentation, has anti-inflammatory effects. Within the gut epithelial wall, butyrate protects against gut permeability by preserving the structural integrity of the colon's epithelial wall. This SCFA increases the production of proteins used to create the tight junctions between epithelial cells. Additionally, butyrate promotes the growth of new epithelial cells while also preventing death of living epithelial cells. Maintaining strong tight junctions prevents foreign compounds from entering the bloodstream and aggravating the immune system. The importance of this protective mechanism was seen earlier in the case of lipopolysaccharides. Tight junctions prevent these endotoxins from leaving the gut and triggering an inflammatory response. Given that this type of inflammation can lead to

obesity, the production of butyrate by gut microbes may play a role in protecting the body from excessive weight gain.

Butyrate's anti-inflammatory capabilities are also protective against insulin resistance, which otherwise may develop in individuals with low-grade chronic inflammation. One study involving 18 men with metabolic syndrome showed that increasing butyrate-producing bacteria within the gut improved their insulin sensitivity in just a few weeks.

Gut Microbes and the Liver in Metabolic Function

The liver is an accessory organ of the digestive system. It plays a significant role in the metabolism of carbohydrate, fat, and protein. Following digestion, nutrients leave the intestine through the portal vein. This blood vessel is a direct connection between the liver and the gut. Once this nutrient-rich blood reaches the liver, toxic substances are filtered out and nutrients are metabolized before the blood travels to other body sites. Due to this connection via the portal vein, the liver also has a close relationship with the gut microbiome. Specifically, gut microbes influence the amount of fat stored in liver cells, by the same mechanisms that they influence fat storage in adipose cells.

Nonalcoholic Fatty Liver Disease

Excess fat storage in the liver is often seen in obese individuals. Increased fat accumulation in the liver produces a condition called nonalcoholic fatty liver disease (NAFLD). NAFLD is the primary cause of chronic liver disease in Western populations. This condition, caused by a buildup of triglycerides (a stored form of fat), may be associated with inflammation resulting from elevated endotoxins. In fact, the liver is likely the first organ affected by increased circulating endotoxins.

In cases of intestinal permeability, lipopolysaccharides escape from the gut and travel to the liver through the portal vein. These endotoxins are recognized by immune receptors on liver cells. These pattern-recognition receptors are mediators for the interactions between the human host and its intestinal microbiota. They provide surveillance by recognizing microbial metabolites that originate within the gut. Some of these receptors are programmed to recognize lipopolysaccharides.

Scientists working to test the relationship between microbe-derived lipopolysaccharides and fatty liver used mutant mice with genetically deleted endotoxin receptors. Without these receptors, the mice do not experience the same deleterious effects from a high-fat diet or when receiving direct injections of lipopolysaccharides. These genetically altered mice also did not develop fatty liver. Furthermore, the absence of these endotoxin receptors prevented

macrophage infiltration in adipose tissue. Limiting all of these inflammatory factors inhibits the development of liver disease. On the other hand, normal rodents with intact endotoxin receptors had elevated liver cytokine production (a marker of inflammation) following just two weeks of a high-fat diet.

In order to better understand the mechanisms between microbiota and liver disease, researchers also investigated a process called liver fibrosis, a condition seen in chronic liver disease. As a result of chronic inflammation, the liver begins to form scar tissue called fibrosis, which causes liver cell death. Interestingly, when this condition was modeled in mice, the researchers noticed that mice lacking cell receptors for lipopolysaccharides did not develop significant amounts of fibrosis. In a second experiment, with mice whose gut microbiota was depleted using antibiotics, liver fibrosis was again significantly reduced. This led researchers to believe that there is a connection between gut microbiota and fibrosis.

Given the liver's crucial role in metabolism, it is not surprising that obesity may also increase the risk of developing liver cancer. Liver cancer is now the second leading cause of cancer mortality. A 2007 meta-analysis of 11 research studies found that obese individuals' risk for liver cancer was 89% higher than for people of normal weight. One risk factor for liver cancer is chronic liver inflammation caused by the effects of circulating lipopolysaccharides. Gut microbiota influence the growth of cancerous liver cells through dysbiosis and increased intestinal permeability.

Unfortunately, as liver disease progresses, the normal filtering processes usually performed by this organ are greatly compromised. For this reason, the liver is less able to clear out endotoxins from the body. Individuals with chronic liver disease have elevated endotoxin levels that perpetuate a cycle of systemic inflammation. Preventing gut microbial dysbiosis and intestinal permeability protects the metabolic functions and health of the liver.

Sleep, Exercise, and Other Lifestyle Factors

While human metabolism and the health of our gut bacteria is highly influenced by eating patterns, other lifestyle factors also shape metabolic and microbial functions. For example, both sleep and exercise influence gut microbiota composition. We will also review the effects of weight loss surgery, specifically Roux-en-Y gastric bypass, on gut microbiota composition. In addition, this section also discusses how metabolic changes in pregnancy are similar to those seen in obesity.

Sleep

Sleep patterns play a role in both weight regulation and preventing dysbiosis. Gut microbiota fluctuate in their composition and in their functions, based

on circadian rhythm, a cycle based on a daily 24-hour interval. Interestingly, a disrupted circadian rhythm may lead to gut microbial dysbiosis.

Both host and microbial metabolic functions are impacted by the 24-hour light/dark cycle. This daily light cycle mirrors internal biological cycles controlled by the circadian rhythm. Most living organisms, including humans and bacteria, have circadian rhythms that govern their biological functions. The circadian rhythm dictates numerous physiological activities, including sleep, digestion, and hormone production. Given that these cycles regulate and balance metabolic functions in humans and bacteria, chronically disrupted circadian rhythms can lead to metabolic dysfunction and gut microbial dysbiosis.

Irregular sleep patterns that interrupt normal circadian rhythm increase the risk for developing obesity and diabetes. Sleep disturbances also may occur as a side effect of obesity. Specifically, obese individuals are at high risk for developing sleep apnea, a disorder characterized by pauses in breath or shallow breathing during sleep. Much research confirms that individuals whose sleep patterns frequently shift, as due to chronic jet lag or working night shifts, tend to have metabolic imbalances and increased weight gain. Interestingly, it appears that disturbed sleep patterns also significantly affect the gut microbiota. One study shows that transplanting gut microbes from chronically jet-lagged individuals into the gastrointestinal tracts of healthy mice caused the animals to develop metabolic dysregulation and weight gain.

Knowing that gut microbiota play a critical role in gastrointestinal activities and metabolic function, this connection between disrupted circadian rhythms and dysbiosis may better explain why disturbed sleep leads to weight gain. For this reason, researchers are interested in how changes in host behavior (in this case, sleep patterns and time of feeding) may affect the structure of the gut microbiome over a 24-hour period. They observe that gut microbiota naturally fluctuates throughout the day. In fact, about 20% of the total gut microbe population oscillates based on daily patterns. Most of these microbes are fermentative types. The other 80% of gut microbiota are relatively stable during the 24-hour light/dark cycle.

Specifically, researchers observed that members of the Bacteroidetes phylum fluctuate most based on circadian rhythm. They also noticed that these normal cycles are decreased with a high-fat diet. However, this may be due to a high-fat diet lowering overall Bacteroidetes populations.

Disturbed sleep patterns also influence eating behavior, as in the case of shift workers who have altered eating schedules. Additionally, decreased sleep quality may influence food choices. Individuals with a disrupted circadian rhythm tend to choose calorie-dense, high-fat foods. A diet high in fat can then perpetuate a state of dysbiosis. Unfortunately, the intestinal permeability often associated with dysbiosis may be worsened by disrupted diurnal cycles.

Results from animal studies conclude that as little as three months of disturbed circadian rhythm promotes increased intestinal permeability.

Just as the body's sleep patterns are regulated by environmental cues of light and darkness, our internal circadian clock also regulates other physiological functions. The activity of the intestine, for example, is regulated by circadian rhythms that dictate metabolic functions and nutrient absorption. Some research indicates that toll-like receptors in the small intestine may also follow a diurnal cycle. These receptors are used by microbiota to communicate with intestinal cells and help maintain the normal patterns in various gastrointestinal functions.

Physical Activity

The benefits of exercise on metabolic function and weight maintenance are well established; increased physical activity helps offset some of the excess calories from food. Exercise also protects against hormone imbalances that can lead to chronic diseases like diabetes and obesity. In addition to these beneficial effects, exercise appears to play a role in the developing gut microbiome early in life.

Although the gut microbiome is malleable throughout the lifespan, it is most easily shaped during early childhood. A 2016 review published in *Immunology and Cell Biology* used rats to investigate the impact of exercise on the developing gut microbiota populations. They found that increased physical activity at a young age supported the growth of beneficial microorganisms. This represents a growing body of work on the "plasticity" of the microbiome. Researchers plan to test ways to influence this plasticity and induce changes in microbial populations that are more resistant.

While the research examining the impact of exercise on gut microbiota is fairly limited, one study published in *Gut* analyzed the composition of gut microbes in athletes. The researchers predicted that extreme athletics is a significant lifestyle factor that influences the gut microbiome. This group worked with a professional rugby team consisting of 40 male Irish athletes to complete a food frequency questionnaire. The participants' calorie intake was, not surprisingly, higher than average. It is important to note that protein made up about 22% of total caloric intake, a bit higher than the control groups, whose diets were only about 15–16% protein.

The researchers discovered that these extreme athletes had more diversity within their gut microbiota populations. This increased diversity may be related to both dietary and physical activity factors, but it may be difficult to determine how much of this difference can be attributed to exercise alone. One genus-level difference is the significantly greater numbers of *Akkermansia*.

Certain members of this genus (specifically *Akkermansia muciniphilla*) are correlated with lower body weight and healthy metabolic function.

Given that extreme physical activity in humans is usually associated with dietary changes such as increased caloric intake and differences in macronutrient profile, animal studies may do a better job of controlling for such differences in diet. One mouse study shows that physical activity can increase *Lactobacillus*. Another study confirmed a substantial increase in *Lactobacillus* genus when rats were made to exercise. Interestingly, when exercised animals are given a high-fat diet, the increased physical activity seems to protect against the detrimental effects of excess fat intake on gut microbiota composition.

When diabetic mice increased physical activity, they did not experience all the same benefits as healthy mice. For instance, when healthy mice exercise, *Bifidobacteria* increases, but this effect was not present in the diabetic group. This may be related to underlying gut microbiota disturbances linked to diabetes.

Interestingly, Fredrik Bäckhed and colleagues proposed the existence of a muscle-microbiota axis that explains the benefits of exercise for the gut microbiome. They suggest a number of possible mechanisms by which our gut microbes interact with muscle tissue. First, they observe that an enzyme (AMPK) which regulates energy homeostasis in the body is 40% higher in the muscle of germ-free mice. This enzyme helps muscle tissue use glucose and fats. Additionally, these researchers note that germ-free mice display greater locomotor activity, giving further evidence for a connection between microbiota and muscle function.

Exercise may influence microbial metabolites, at least according to animal studies. Animal models show that running increases butyrate levels. SCFAs can activate AMPK within muscle tissue. As mentioned previously in this chapter, SCFAs also increase levels of peptide YY, which not only increases satiety but also enhances the utilization of glucose by muscle.

TLRs are also a part of the muscle-microbiota axis. Gut microbes can also activate TLRs within the muscle. Both TLR4 and TLR5 are present in the muscles and can be activated by lipopolysaccharides and flagellin, respectively. Just as in other body tissues, the activation of these receptors triggers the production of pro-inflammatory cytokines within the muscle. However, exercise appears to suppress the activation of these receptors (at least with TLR4), which improves metabolic functions such as insulin sensitivity.

Altered Metabolism during Pregnancy

During pregnancy, the body experiences many metabolic changes. Not only do women begin to store more fat in preparation for the higher energy demands of pregnancy and lactation, they also experience significant changes in the blood glucose levels and insulin sensitivity. A consistent state of elevated

glucose in the blood ensures that the mother is able to provide enough energy to the developing fetus. In this way, increased weight and insulin desensitization are direct mechanisms used by the body to preserve energy, to ensure growth of the fetus. Interestingly, many of these hormone changes in pregnancy are similar to metabolic changes seen in obesity as well.

In a normal, health pregnancy, an increase in body fat occurs, along with a loss of insulin sensitivity. Outside the context of pregnancy, these metabolic changes are associated with abnormal weight gain and detrimental health outcomes. However, with regard to the growing fetus, increased body fat is beneficial for the mother and also prepares her for increased energy needs associated with lactation. Yet, metabolic complications can arrive during pregnancy. For instance, decreased insulin sensitivity and elevated blood glucose levels can lead to a condition known as gestational diabetes.

Over the first three trimesters, not only does women's blood glucose increase, but leptin, insulin, and cholesterol all significantly increase. One study showed significant changes in gut microbial composition during pregnancy, specifically seen with major shifts between the first trimester and third trimester. By the third trimester, in most pregnancies, Proteobacteria and Actinobacteria populations became more abundant. Researchers are currently proposing that these shifts may be driving these metabolic changes.

Although diet change is often the first factor considered when studying shifts in gut microbiota populations, the pregnant women in this study reported that their diet remained relatively consistent during the course of pregnancy. Researchers then suggested that the metabolic changes likely occurred due to hormonal or immune shifts.

Women with gestational diabetes are observed to have the least microbial richness during the first trimester, though their microbiota composition is similar to non-pregnant controls. (In this study, children born to women with gestational diabetes were not observed to have negatively altered gut microbiota.) Regardless of pregnancy weight and health status, phylogenic differences between individuals are greatest in first trimester. However, by the third trimester, these phylogenic differences disappear.

Gastric Bypass

Gastric bypass is a procedure sometimes recommended in cases of morbid obesity, to surgically alter the function of the stomach and small intestine. This surgery decreases the volume of food the GI tract is able to handle. Due to these drastic digestive changes, gastric bypass surgery leads to rapid weight loss. This procedure can even improve metabolic function by enhancing the metabolism of glucose, a function that is often compromised in obesity due to hormone dysregulation and other metabolic imbalances.

One type of gastric surgery, called a Roux-en-Y gastric bypass, appears to also cause shifts in gut microbiota populations. Researchers examined whether these changes in microbiota contribute to metabolic benefits resulting from the surgery. First, it is important to consider whether these changes are driven by the surgery or are perhaps caused by weight loss and lower caloric intake following the surgery—both factors that change gut microbiota composition.

In a mouse model, researchers observed significant changes just one week after the gastric bypass. They believe that changes within small intestine mucosal populations are more directly tied to the weight loss induced by the surgery. Other changes occurred downstream of the surgery, in the section between the small and large intestine, as well as within the colon itself. However, the most significant shifts in microbiota composition are seen in the distal part of the GI tract. Specifically, researchers observed an increased abundance of Verrucomicrobia following Roux-en-Y gastric bypass. Interestingly, the researchers note that members of the *Akkermansia* genus (which are part of the Verrucomicrobia phylum) can utilize mucus as an energy source during periods of caloric restriction. This may be a particularly adaptive trait during periods of low calorie intake meant to promote weight loss.

On the other hand, species in the *Escherichia* genus are the most enriched following bypass surgery in mice. This genus contains both commensal and pathogenic members. Some *Escherichia* pathogenic species are actually shown to contribute to metabolic syndrome and weight gain. However, researchers theorize that the species of *Escherichia* that increase following surgery are likely to be commensal strains such as those found to lower inflammation in the GI tract.

The mouse model also shows that relative SCFA production is also reduced following gastric bypass. SCFAs contribute to total energy, and decreased production may therefore lower adiposity. The changes in gut microbiota following surgery also impact the types of SCFAs produced within the colon. While propionate production increased, the amount of acetate was lower compared with animals without gastric bypass. The researchers propose that lower acetate levels may contribute to weight loss. Acetate can be used in fat cells or taken to other tissues to be turned into fat (a process called lipogenesis). Propionate also has beneficial properties in weight loss. This short-chain fatty acid reduces the amount of acetate that gets turned into fat within the liver and adipose tissue.

Conclusion

As we have seen, the gut microbiota improves the efficiency of digestion. There is debate regarding the effects of this improved digestion on human health. On one hand, gut microbes use dietary components to produce metabolites that reduce inflammation and support overall health. On the other hand, this increased level of digestive efficiency may become problematic in the context of excessive calorie intake.

6

The Gut Microbiota and Gastrointestinal Diseases

Dysbiosis changes the gut environment and can alter its function. This happens in large part due to gut inflammation that is associated with certain types of dysbiosis. Inflammation within the gastrointestinal tract can contribute to a number of chronic diseases, including ulcerative colitis, Crohn's disease, and cancer.

In this chapter, we will explore how immunity and dysbiosis play a role in chronic intestinal diseases. We will also discuss dietary interventions and the use of probiotics in the treatment of these diseases. After discussing inflammatory bowel diseases and colorectal cancer, we will review the effects of dysbiosis in the small intestine. In the last section of the chapter, we will discuss two pathogenic bacteria that infect the gastrointestinal tract.

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a subset of gastrointestinal conditions that are defined by chronic inflammation in the small intestine and colon. The two most common inflammatory bowel diseases are Crohn's disease and ulcerative colitis. Crohn's disease can impact any area within the gastrointestinal tract, whereas ulcerative colitis affects the colon and rectum. Much of the inflammation in ulcerative colitis takes place within the mucosa and submucosa. Typically, the presentation of symptoms first occurs in the distal colon and eventually progresses to the proximal colon. Individuals with IBD have disrupted bowel movements and usually experience pain and bloating related to the inflammatory state of the intestine.

Inflammatory bowel diseases are often described as autoimmune diseases, but recent research shows that these diseases are not caused by the body's lack of tolerance to its own cells. Instead, both diseases are associated with misguided immune responses that are directed at our commensal bacteria or tissues within the gastrointestinal tract. Researchers believe that this is likely

caused by a breakdown in the mucosal barrier, which is a symptom of IBD. Without this protective barrier, commensal microbes interact more easily with intestinal immune cells. This causes loss of immune tolerance to commensal microbes and produces a hyperactive inflammatory response.

Although there are other risk factors for these diseases, such as genetics, age, and ethnicity, researchers are also beginning to explore how certain members of the gut microbiota interact with the immune system in ways that may perpetuate inflammation in IBD.

Microbial Dysbiosis in IBD

IBD patients have overall lower gut microbial diversity. Most commonly, dysbiosis in IBD is characterized by an increase in Proteobacteria, along with a reduction in Firmicutes. Both Firmicutes and Bacteroidetes are more depleted in IBD patients compared to healthy adults. However, it is difficult to pinpoint a definitive microbiota profile in IBD, as the microbial composition in these individuals tends to shift depending on states of disease activity.

Crohn's disease patients have a lower abundance and diversity of species within the Firmicutes phylum. Specifically, *Faecalibacterium prausnitzii*, which is a predominant member of the Firmicutes phylum, is often reduced in these individuals. Researchers believe that diminished populations of *F. prausnitzii* cause a reduction in beneficial SCFAs. For instance, butyrate production appears to be lower in IBD due to lower populations of this butyrate-producing microbe. Further, the decreased abundance of *F. prausnitzii* can lessen the effectiveness of surgical treatment of Crohn's. When this species is diminished, the recurrence of Crohn's within the ileum (a portion of the small intestine) is increased post-surgery. *F. prausnitzii* has anti-inflammatory effects.

Researchers are also investigating the role of *F. prausnitzii* in promoting the secretion of antimicrobial proteins by Paneth cells. These specialized cells within the ileum produce proteins called defensins, to protect against dysbiosis. The presence of commensal microbes is important in stimulating the secretion of defensins. Unfortunately, Paneth cells are abnormal in certain Crohn's patients. Not only do the number and size of Paneth cells appear altered in Crohn's, but their function is also impaired.

According to a study that collected samples from nine ulcerative colitis patients and ten healthy individuals, the abundance of *Bifidobacteria* was significantly reduced in IBD samples. Specifically, individuals with ulcerative colitis had altered *Bifidobacteria* populations within their mucosal biofilm communities. Given that *Bifidobacteria* are important in regulating host immunity, it is possible that these missing microbes leave the gut more vulnerable to chronic inflammation.

Researchers are still working to determine whether dysbiosis causes IBD or whether these imbalances in microbial populations result instead from

inflammation following disease development. Interestingly, it appears that relatives of patients with Crohn's disease tested positive for some form of dysbiosis despite not having the disease themselves. This raises the possibility that microbial dysbiosis is related to genetic factors or familial factors, such as microbes inherited from the mother at birth. Yet these non-IBD relatives had a different kind of dysbiosis compared to their relatives with IBD.

While no specific pathogenic microorganism is yet identified as the cause of IBD, some research points to certain bacteria that are likely associated with the disease. In ulcerative colitis, *Fusobacterium varium* is found in areas where gut tissue is inflamed. This bacterium attaches to these areas of inflammation. It can also find its way into the mucosa if there is an ulcer present. Interestingly, *F. varium* is saccharolytic and produces butyrate, which is generally considered a beneficial metabolite of bacterial fermentation. Despite this seemingly beneficial contribution, researchers are considering that it might be pathogenic in the case of ulcerative colitis.

Excessive hydrogen sulfide levels, a metabolite of sulfate-reducing bacteria, has also been implicated in IBD. The gut is typically able to remove naturally occurring hydrogen sulfide levels, but its accumulation can have toxic effects that harms epithelial cells. One group of researchers hypothesized that *Desulfovibrio*, a common genus of sulfate-reducing gut bacteria, may be elevated in IBD individuals. However, when researchers compared levels of sulfate-reducing bacteria in ulcerative colitis patients to healthy individuals, they were surprised to find similar population sizes in these two groups. This observation led them to wonder whether the possible involvement of sulfide in ulcerative colitis may be related to problems in the host's detoxification systems that lead to buildup of this toxic compound. Interestingly, the mucosa plays an important role in the detoxification of hydrogen sulfide. It is possible that the altered mucosa typically seen in IBD may result in decreased mucosal detoxification.

SIDEBAR 6.1 The Appendix and Colitis

Surgical removal of the appendix appears to have no long-term side effects. In fact, some interesting benefits can result from an appendectomy. One study demonstrates that removal of the appendix can alleviate symptoms in 90% of individuals with ulcerative proctitis (a type of ulcerative colitis in which the lining of the rectum becomes inflamed and leads to the formation of ulcers).

This observation has led researchers to wonder what role the appendix might have in ulcerative colitis. Some theorize that the appendix may contribute to colitis by housing microbes to which the immune system has become hypersensitive. They also question the role of gut-associated lymphoid tissue, which is prominent within the appendix. This tissue may be a driving force in mediating immune responses during this inflammatory bowel condition.

Altered Immune Function in IBD

In addition to changes in gut microbiota composition, IBD is also associated with changes in the colonic mucosa. Specifically, ulcerative colitis affects the colonic mucosa and causes the boundaries between the mucosa and luminal environment to become less clear. Luminal microbes can therefore more easily penetrate the mucosal layer and adhere directly to the mucosa. In fact, a few studies show that IBD patients have more microbes within the mucosa compared to healthy individuals. As we have learned, excess microbes within the mucosa only perpetuates GI inflammation.

T cells are a significant driving force in inflammatory bowel disease. Dysbiosis in IBD may inhibit the normal interactions between gut microbes and T cells. For instance, certain commensal bacteria induce T regulatory cells to help them reduce inflammation. In IBD, T cells may actually malfunction to produce excess inflammation. One of the mechanisms by which this happens is through the production of pro-inflammatory cytokines.

Until recently, most research focused on two prominent pro-inflammatory cytokines involved in IBD: tumour necrosis factor and interferon-γ. New research now implicates another cytokine, interleukin-17, in the development of IBD. This cytokine also promotes inflammation and is produced by T helper 17 cells. (This kind of T helper cell functions mostly to recognize pathogens.)

There are also strong genetic components to the development of IBD that can affect gut immune function. For example, genes associated with T cell immunity are often altered in IBD as well. In addition, genes associated with bacterial sensing can become mutated in IBD individuals. This inhibits the body's ability to tolerate commensal microbes and maintain intestinal homeostasis.

In IBD, the immune system targets certain commensal microbes. Researchers have observed that characteristically, many of these commensals have the potential to switch to a pathogenic mode. *Clostridium*, *Helicobacter*, and *Enterococcus* species are some examples of these microbes targeted during IBD. Currently, *E. coli* is the only microbe that appears to be significantly associated with Crohn's disease. However, it is still unknown why any of these species cause inflammatory reaction in IBD when they live as harmless commensals within healthy individuals.

Another study, using an animal model for experimental IBD, demonstrates the effects of *Bacteroides fragilis* in modulating immune functions within the gut. When germ-free mice are colonized by this symbiotic bacterium, they are protected against the induction of IBD. *B. fragilis* plays a crucial role in determining host immune responses. It helps maintain a balance in T helper cells and supports the development of lymphoid structures within the gut. In addition, germ-free mice that are colonized with *B. fragilis* are able to correct deficiencies in T cells with the help of this bacterium.

Researchers attribute much of the protective effects of *Bacteriodes fragilis* to the production of polysaccharide A. Polysaccharide A induces IL-10, which fights inflammation in the gut. In fact, germ-free mice who are colonized with a mutant strain of *B. fragilis* that lacks the ability to produce polysaccharide A are not protected against IBD.

Treatments for IBD

Given that microbes play a significant role in IBD, antibiotics are used to treat this disease. Specifically, antibiotics such as ciprofloxacin can be effective in preventing recurrence following surgical treatments for Crohn's disease.

Physicians also commonly treat IBD patients with anti-inflammatory medications. Unfortunately, common medications used to reduce inflammation in IBD also have significant effects on microbiota composition. For instance, mesalazine, which is used in both ulcerative colitis and Crohn's disease, can decrease total bacterial populations by 50%. While antibiotics and anti-inflammatory medications can both contribute to dysbiosis, it is uncertain whether these treatments cause further harm due to resulting changes in gut microbiota.

Fecal microbiota transplantation is also a potential future treatment that may offer some benefits. This treatment may help restore healthy microbes by transferring the gut microbiota of a healthy individual to the IBD individual. However, it appears that not all patients with IBD respond to this type of microbiota transplantation, and researchers are still working to determine which individuals are likely to be most responsive to this new intervention.

Another microbiota-targeted treatment option involves the use of probiotics. Although many probiotics exist on the market, few of them are as well researched as VSL#3, which studies show to be effective in treating ulcerative colitis. Clinical trials show that VSL#3 helps reduce symptoms as well as recurrence rates. Interestingly, VSL#3 also showed a higher remission rate compared to common treatments such as mesalazine and steroids. Researchers are still working to determine how this probiotic benefits ulcerative colitis patients, but it appears to improve immune function by increasing the amount of T regulatory cells within the intestine. VSL#3 is a freeze-dried probiotic that helps improve the stability and shelf life of the probiotic bacteria. This probiotic supplement is a combination of several species, including *Bifidobacterium infantis, B. breve, B. longum, Lactobacillus bulgaricus, L. casei, L. plantarum, L. acidophilus, and Streptococcus thermophilus*.

Faecalibacterium prausnitzii, whose population are reduced in some Crohn's disease patients, also shows some beneficial effects as a probiotic when used in animal models of intestinal inflammation. This probiotic supplement increased the production of IL-10, which attenuated the inflammatory effects

of tumor necrosis factor—a pro-inflammatory cytokine—by decreasing its production.

Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) is a functional disorder that affects the large intestine. IBS is more common in women and causes gas, bloating, and altered bowel habit (constipation or diarrhea). Interestingly, IBS is a diagnosis of exclusion, meaning that diagnosis is established through a process of elimination where all other causes for gastrointestinal distress are ruled out. Also, there are no conclusive tests or specific measurable substances in the body that determine whether one has IBS. Instead, this condition is characterized by its symptoms. Individuals with IBS often experience gastrointestinal discomfort but lack any other diagnosable pathology.

Gut Microbes Implicated in IBS

The cause of IBS is largely unknown, but gut microbiota are providing more insight into this disorder. For instance, IBS is related to changes in the luminal environment that may directly impact gut flora. Researchers are still working to determine whether these changes occur as a result of the disease or whether perhaps the development of IBS follows certain changes in gut microbiota composition. Some studies have suggested a link between IBS and dysbiosis, but researchers have not identified any specific patterns in microbiota that help determine IBS risk.

Interestingly, the ways in which gut microbiota composition is altered often corresponds with certain predominant symptoms experienced by the IBS patient. For instance, in patients whose predominant IBS symptom is constipation, the microorganism *Methanobrevibacter smithii* might be elevated. This microbe produces methane, which is linked to slow bowel transit time. For this reason, an overabundance of *M. smithii* may contribute to constipation in IBS. On the other hand, IBS patients whose bowel pattern is predominately diarrhea might show reduced *Faecalibacterium* species, at least in comparison with other IBS patients.

In some cases, environmental exposure to infectious bacteria can be a direct cause of IBS. One interesting case study from Ontario, Canada, documented an outbreak of *Escherichia coli* and *Campylobacter jejune*. After the town's drinking water supply was contaminated following a flood, the prevalence of post-infectious IBS increased among town residents. While bacterial contamination may be more likely to cause acute infections, this particular outbreak is a good example of the potential long-term effects of certain bacterial infections.

Treatments for IBS typically aim to address symptoms rather than addressing any underlying gastrointestinal dysfunction. As researchers learn more

about possible connections between gut microbiota and IBS, new probiotic treatments are likely to become more prevalent. Specifically, there is promising research on this using *Lactobacillus* and *Bifidobacterium* species to reduce severity of IBS symptoms. One strain of *Bifidobacterium infantis*, for instance, helps alleviate symptoms of pain, reduces bloating, and improves bowel movements. Researchers believe this bacterium is beneficial due to its immune-modulating effects in the gut.

Fermentable Carbohydrates in IBS

Gut microbes may also contribute to IBS symptoms in their digestion of dietary carbohydrates. In general, the role of diet is not conclusive, yet many individuals with IBS report that their symptoms are often triggered by food. These reported symptoms are usually associated with dietary carbohydrates rather than other macronutrients such as protein and fat. While there is no conclusive data on a direct association between diet and IBD, certain diets that reduce or eliminate fermentable carbohydrates appear to help alleviate IBS symptoms. The next section will focus on the low-FODMAP diet, which eliminates these typically beneficial foods.

Sensitivity to dietary carbohydrates may indicate a food intolerance related to gut dysbiosis. Food intolerances or food sensitivities are different than food allergies. Unlike a food allergy which is an immune-mediated reaction, food sensitivities are not allergic reactions. Likewise, food intolerances, such as those seen with dietary carbohydrates in IBS, are not immune-mediated. However, some health professionals do question whether other types of food allergies may prompt symptoms of IBS. There is ongoing research to assess the possible role of food intolerances and food allergies in IBD.

IBS is also associated with changes in the intestinal mucosa. These changes can directly affect immune function. Just as with IBD, the breakdown of the mucosal barrier can increase the immune system's interactions with gut microbes. In this case, immune cells become over-activated and can increase markers of systemic inflammation, such as higher levels of pro-inflammatory cytokines in blood circulation. Furthermore, these alterations in GI immune cells also decrease the mucosa's ability to respond appropriately to pathogens.

Interestingly, the progression of IBS correlates with dysbiosis of mucosa-associated microbes rather than disturbances of luminal populations. Studies using culture-based techniques have analyzed luminal microbes of the small intestine, whereas mucosa-associated populations are analyzed using culture-independent molecular methods. While analysis of these various studies may indicate that IBS is more likely associated with dysbiosis of mucosal populations, no studies have compared both luminal and mucosa-associated microbiota within the same individuals with IBS.

Gut-Brain Connection in IBS

While chapter 8 will discuss the gut-brain axis in more detail, it is important to note that some of the gut dysfunction that occurs in IBS is related to this gut-brain connection. For example, mood appears to be strongly connected to the persistence of IBS. Mood disorders such as anxiety and depression can be triggers for IBS and may worsen symptoms.

The gut-brain connection plays a role in the abnormal bowel patterns that are characteristic of IBS. Specifically, individuals with IBS have higher postprandial serotonin levels following a meal. Higher levels of serotonin in the gut (which will be explained more thoroughly in chapter 8) are associated with altered stomach emptying and bowel transit time. Serotonin is an important brain chemical, but it also plans an important role in gut motility, as well as in the perception of pain.

SIDEBAR 6.2 Stool Consistency

Paying attention to the quality of an individual's stool can be useful in assessing GI health. The consistency of stool is usually noticed only in the case of constipation or diarrhea. One tool, called the Bristol Stool Chart, offers descriptive classifications to help identify and categorize different types of human feces. Identifying the type of stool can provide information about the length of time it takes food matter to move through the colon. For instance, type 1 describes a very constipated individual whose stools are separate hard lumps. Types 3 and 4 are considered normal, with well-formed stools. Types 5 through 7 represent soft stools.

Using the Bristol Stool Chart, researchers analyzed stool consistencies to see whether different types of stool relate to the state of health of the gut microbiota. They found that higher scores—meaning individuals with loose stools—had lower species diversity within their guts. Loose stool was also associated with the *Prevotella*-dominant enterotype. Harder stool samples, on the other hand, corresponded with the *Ruminococcus/Bacteroides*–dominant enterotype.

The length of time it takes food matter to travel through the colon is an important factor in gut bacteria's habitat. Quicker transit time may be indicative of diarrhea. However, a quicker transit time is also observed in the *Prevotella* enterotype, the typical enterotype of rural agrarian societies. Given the high intake of insoluble fiber, these mostly plant-based diets are usually associated with increased water content in the stool. Thus, it is uncertain whether the type of stool results from fiber intake or from changes in transit time.

On the other hand, constipation is associated with slower transmit time. Interestingly, increased methane production is seen in individuals with constipation. Further, low Bristol types have increased abundance of methane-producing *Methanobrevibacter*.

The Low-FODMAP Diet

In cases of gastrointestinal distress, nutrition professionals may recommend elimination diets to determine which foods contribute to symptoms. The goal of these elimination diets is to test individual foods by avoiding them for a period of a few weeks and reintroducing them one-by-one to test which specific foods cause symptoms to return. Elimination diets are typically used to determine food sensitivities or intolerances. Unlike food allergies, which can be determined conclusively by blood work, there are no conclusive laboratory tests for food intolerances. Some individuals who experience recurring symptoms such as bloating, abdominal pain, or altered bowel movements may be sensitive to fermentable carbohydrates. Both IBS and IBD can be linked to GI distress related to bacterial fermentation of carbohydrates. The low-FODMAP diet is a specialized elimination diet sometimes recommended for IBS and IDB, although it is sometimes used as a therapeutic approach for other bowel disorders with similar symptoms.

FODMAP stands for "fermentable oligosaccharides, disaccharides, monosaccharides, and polyols." These are all groups of carbohydrates that induce bacterial fermentation in the gut. (See chapter 3 for more information on fermentable carbohydrates.) A low-FODMAP diet reduces or eliminates any foods that fall into any of these carbohydrate categories.

Fructose is best absorbed when consumed with at least equal amounts of glucose. The presence of glucose improves fructose transportation in the body. If fructose is not absorbed in the small intestine, it may reach colonic bacteria for fermentation. One type of FODMAP are fructans, which contain multiple units of fructose molecules. Long-chain fructans are especially problematic for many individuals with IBS. These carbohydrates tend to increase bloating and occurrence of diarrhea.

On the other hand, galactooligosaccharides—a different fermentable carbohydrate—can be used as a prebiotic in IBS patients. In fact, galactooligosaccharides can help alleviate symptoms of pain and constipation. Researchers believe that the benefits of this fermentable carbohydrate are a direct result of their enhancing populations of probiotic bacteria. Galactooligosaccharides, specifically, increase populations of *Bifidobacteria*, which improve gut health in IBS.

The disaccharide lactose is also included in a low-FODMAP diet for individuals who don't absorb this sugar properly. This carbohydrate is usually digested with the aid of the enzyme lactase. If an individual lacks sufficient amounts of lactase, unabsorbed lactose travels into the large intestine. This lactose malabsorption can lead to increased flatulence and bloating following bacterial fermentation. Given that lactose is not considered a prebiotic, it is unlikely that removing lactose sources will significantly reduce beneficial gut microbes.

SIDEBAR 6.3 Celiac Disease

Children with celiac disease have different gut microbial populations. The presence of inflammation in the gut will naturally shift these populations, but some changes in the gut were actually observed even before the onset of the disease. For instance, family history and genetics can predispose certain individuals to this autoimmune condition. Infants born with these predisposed risk factors have reduced populations of *Bifidobacteria*. Interestingly, these bacterial populations do not replenish even after following a gluten-free diet. Frequent antibiotic use may set the stage for celiac disease, as it wipes out protective *Bifidobacteria*.

In celiac patients, gluten can cause substantial damage to the intestinal mucosa. Gliadin is a type of protein found in wheat and other grains. Gliadin is the primary toxic component in gluten. These proteins trigger the immune system and cause an inflammatory response. *Bifidobacteria* can reshape the structure of these proteins and reduce this hyperimmune response.

While a low-FODMAP diet can help manage symptoms, a diet deficient in fermentable carbohydrates can have potential long-term effects on colon health if the diet is maintained indefinitely. As we see with galactooligosaccharides, FODMAP carbohydrates are important in promoting the growth of probiotic gut microbes. A low-FODMAP diet is devoid of bifidogenic prebiotics and can reduce populations of *Bifidobacteria* species. This diet can also reduce populations of butyrate-producing bacteria, depriving the gut of this anti-inflammatory SCFA. Furthermore, a low-FODMAP diet decreases certain mucus-degrading microorganisms such as *A. muciniphila* and *R. gnats*. These microbes help remove old mucus as the mucosa continuously renews. *A. muciniphila* is also a butyrate-producing bacteria.

For individuals looking to receive symptoms of IBS or IDB, a low-FODMAP diet can offer significant reductions in pain and bloating due to excess bacterial fermentation. It is advisable that these individuals work with a nutrition professional to determine how to use this elimination diet without further compromising gut health. Ideally, it is best to find long-term dietary solutions that reduce symptoms while encouraging growth of probiotic microbes.

Colorectal Cancer

Colorectal cancer develops in the colon and rectum. It is the third most common cancer for both men and women in the United States. Genetics may play a role in the development of this disease, but research indicates that only about 25% of individuals with colorectal cancer have a family history of the disease. In fact, diet and lifestyle are more significant contribution factors.

While it is clear that diet influences gut microbiota, researchers are still looking to find a specific gut microbiota profile that is commonly seen in colon cancer. They have pinpointed certain commensal bacteria that become overpopulated within cancerous colon tissue. Commensals such as *Roseburia*, *Faecalibacterium*, and *Fusobacterium* are found in this tissue, but it is uncertain whether they are at all implicated in the disease itself. However, researchers believe that higher populations of one specific species, *Fusobacterium nucleatum*, may contribute to tumor growth. Despite these observations in cancerous colon tissue, it is unclear what changes occur in laminal microbes during colorectal cancer. It is also interesting to note that even within the same individual, there are differences in microbial composition of healthy tissue and cancerous tissue.

Diet and Colorectal Cancer

Our modern Western diets have long been implicated in colorectal cancer. Diets high in red meats such as beef, lamb, and pork, as well as processed meats like hot dogs or lunch meat, are well researched in terms of their contribution to this disease. Also, cooking meat and fish at high temperatures, as with grilling or frying, creates cancer-inducing compounds that interact with gut microbiota in ways that are detrimental to colon health.

Dietary fiber intake, which is often low in most Americans, can protect against colorectal cancer. In fact, switching from a traditional high-fiber diet to an American diet has been shown to increase the risk for colon cancer in African and Japanese individuals. Traditional African and Japanese diets are generally lower in processed foods, which allows them to have higher fiber content. When these groups switch to an American diet, animal protein and saturated fat intake often increase significantly. Given that low fiber intake depletes beneficial gut microbiota, researchers are exploring how these effects may contribute to cancer in the gut.

Inflammation

Immune function also affects the development of colorectal cancer, because inflammation influences the health of colon cells. The existence of other inflammatory bowel diseases can increase the risk of colorectal cancer. Chronic inflammation seen in Crohn's disease and ulcerative colitis makes individuals with these conditions five times more likely to develop colorectal cancer. As mentioned earlier in this chapter, individuals with IBD often have impaired gut barrier function, which results in inflammation.

Researchers also observe that TLR function influences inflammation-induced colorectal cancer. TLRs help inform immune cells how to respond to cancerous cells. When TLRs are over-activated, this leads to a poor immune response to tumors. With chronic inflammation, these TLR cells promote tumor growth.

As colon cancer progresses, tight junctions lose their ability to limit intestinal permeability. This disease also causes changes in mucins, which contributes further to a malfunctioning gut barrier. Once epithelial cells are no longer protected from contact with colonic microbes, inflammation increases.

Additionally, researchers discovered that they may be able to measure the body's immune response to a certain gut microbe as part of colorectal screening. Infection with this microbe, *Streptococcus bovis*, is associated with colorectal cancer, though not all individuals with this type of cancer have increased *S. bovis* populations. Still, researchers wonder whether measuring the body's level of inflammatory markers (specifically, IgG) in response to *S. bovis* might be part of an early screening for colorectal cancer.

Bacterial Metabolites That Increase Cancer Risk

Certain bacteria produce toxins and metabolites that contribute to colorectal cancer. Given that the types of microbial metabolites produced in the gut are often determined by gut microbiota composition, dysbiosis can lead to a buildup of detrimental metabolites. Most of these bacteria are a part of normal gut microbiota, but they can cause harm under certain circumstances.

One symbiotic bacterium that may contribute to the growth of cancer cells in the colon is *Bacteroides fragilis.* Most strains of *B. fragilis* support gut health and are a part of normal human gut flora. However, one strain, called enterotoxigenic *B. fragilis* (the prefix "entero-" refers to the intestine) creates a toxin that can increase tumor growth. This toxin harms epithelial cells by damaging a tumor-suppressing protein within those cells. This effect also leads to inflammation and diarrhea.

Hydrogen sulfide is also implicated in colorectal cancer. This metabolite, produced by sulfate-reducing bacteria, can create an imbalance between cells' natural processes of growth and development. As we have seen in chapter 3, increased dietary protein can promote the formation of problematic waste products.

One way in which high animal protein intake may contribute to colorectal cancer is due to the production of hydrogen sulfide. Sulfate-reducing microbes metabolize dietary protein to produce hydrogen sulfide. This metabolite causes DNA damage and also inhibits some of the beneficial effects of butyrate in colonic epithelial cells. Some dietary proteins, such as those containing aromatic amino acids (including histidine, which was discussed in chapter 3) are fermented by *Bacteroides* and Firmicutes species to produce nitrogen products that cause DNA mutations. These nitrogen products are typically more elevated in individuals with a high-protein diet.

Excess dietary fat may also contribute to colorectal cancer. For example, the liver produces a fluid called bile in order to properly digest fats. Microbes within the colon convert bile into secondary bile acids. Concentrations of secondary bile acids are often higher in individuals with colorectal cancer. These

bacterial metabolites may damage DNA and promote tumor growth. One secondary bile acid, known as deoxycholic acid, harms the mucosa and promotes the creation of chemicals called reactive oxygen species. Under normal conditions, these reactive oxygen species are properly eliminated to prevent cell damage. However, if they accumulate, they begin to damage DNA and can, in the long term, increase tumor growth.

Another DNA-damaging compound formed by bacteria is acetaldehyde, which is created from alcohol. Excessive alcohol consumption can increase the production of this carcinogen. Acetaldehyde attaches to DNA to produce carcinogenic effects. It also decreases folate in the colon; folate is a B vitamin that protects against colon cancer.

While excess animal protein and alcohol can encourage gut microbiota to produce carcinogenic metabolites, other dietary habits, such as high fiber intake, can encourage protective metabolites. Fiber and other prebiotic carbohydrates encourage the production of butyrate. This short-chain fatty acid not only reduces inflammation in the gut but also inhibits the growth of tumor cells. In addition, short-chain fatty acids help maintain a low pH level in the gut lumen, which prevents growth of pathogenic populations and also protects the gut from absorbing too many carcinogenic substances. These factors can directly reduce risk of colorectal cancer. Unfortunately, the typical Western diet is generally lacking in dietary fiber and fails to provide the microbiota with the appropriate carbohydrates for producing sufficient butyrate.

SIDEBAR 6.4 Barrett's Esophagus

Barrett's esophagus is a condition in which the tissue that lines the lower esophagus becomes abnormal. This condition significantly increases the risk of esophageal cancer. Acid reflux can greatly contribute to the development of this condition.

Microbiologists observe changes in the microbial populations within the distal esophagus of people with Barrett's esophagus. These populations are larger and more diverse compared to those belonging to healthy individuals. Interestingly, many of these microbes are responsible for converting nitrate to nitrite. Nitrate is a compound found in dark leafy greens such as spinach and lettuce, as well as other vegetables. Once bacteria convert nitrate to nitrite, it can then be turned into nitric oxide, a molecule that helps lower blood pressure and is beneficial for heart health.

However, Barrett's esophagus causes the population of nitrate-converting microbes to increase and produce excess amounts of nitric oxide. These higher nitric oxide levels have detrimental health effects. Researchers propose that high quantities of this molecule contribute to cell mutations that promote the progression of esophageal cancer in individuals with Barrett's esophagus.

Small Intestinal Bacterial Overgrowth

Although much research on gut microbiota dysbiosis focuses on imbalances within the colon, dysbiosis in the small intestine also has significant health implications. Since microbe populations within the small intestine are meant to remain significantly smaller than those of the large intestine, when these populations become too large, it is known as small intestinal bacterial overgrowth (SIBO).

Although antibiotics are the standard treatment for SIBO, some trials have shown possible benefits of certain probiotics. For instance, *Lactobacillus* may improve diarrhea associated with SIBO and is shown to reduce hydrogen breath levels. Still, other conflicting results showed few benefits.

Proton Pump Inhibitors

Small intestinal bacterial overgrowth can be a side effect of certain medications. For example, proton pump inhibitors are a type of medication often prescribed for gastroesophageal reflux disease (GERD). These medications suppress the production of stomach acid. If stomach acid escapes into the esophagus, it can have damaging effects. If left untreated, GERD can cause ulcers along the esophagus. Proton pump inhibitors are used to treat and prevent ulcers. Since these medications suppress acid production, some researchers have hypothesized that this increases the pH of the small intestine and encourages an overgrowth of bacteria. However, this side effect is only relevant for long-term use of the medication.

Aside from the use of proton pump inhibitors, the production of stomach acid can also decrease with age or from *Helicobacter pylori* infection. Regardless of the cause, decreased stomach acid raises the pH level and can encourage SIBO.

Accuracy of Diagnosis Tests for SIBO

A 2013 meta-analysis found that proton pump inhibitors are associated with SIBO risk. Previous studies have yielded conflicting results about this association. However, the use of different tests to diagnose SIBO is likely the cause of such discrepancies. The most common test for SIBO is the glucose hydrogen breath test. Studies that have used this breath test found no significant association. Yet the authors of this meta-analysis argue that the breath test is not sufficiently accurate for determining all cases of SIBO.

Other diagnostic tests for SIBO, such as culture tests, also may not definitively determine the presence of dysbiosis. These tests typically use samples from the proximal small intestine and therefore do not pick up overgrowth that may happen in the distal region. This may potentially create a false negative result.

While these two tests fail to give a complete picture of microbial populations in the small intestine, another test, known as aspirate cultures, offers greater accuracy in identifying SIBO. Aspirate cultures are considered the gold standard in SIBO diagnosis, but unfortunately the test involves drawing fluid from the small intestine. This test is nearly 100% accurate, but due to its invasive nature, it is rarely used. On the other hand, the less sensitive breath test is much more common because of its affordability and ease of use in a clinical setting.

Complications from SIBO

The effects of SIBO include weight loss, diarrhea, and malabsorption of nutrients. Severe cases may have significant complications as a result of vitamin and mineral malabsorption. Fat-soluble vitamins such as vitamins A, D, and E, as well as vitamin B_{12} and iron, are most vulnerable to poor absorption in cases of SIBO. Given that bacteria metabolize bile salts, excess bacterial growth leads to an increase in production of bile acids. These extra bile acids inhibit absorption of fats.

As SIBO progresses, it can result in an inflammatory response. For instance, in elderly patients, this type of bacterial overgrowth is more often associated with damage to the intestinal mucosa. These individuals have thinning mucosa as well as injury to the villi and crypts of the intestine. Both these factors promote an inflammatory immune response. Interestingly, GI disturbances in these individuals were reversed following antibiotic treatment.

Decreased Gut Motility and SIBO

Decreased mobility in the small intestine can also promote bacterial overgrowth. The proper movement of food through the gastrointestinal tract prevents buildup of debris or bacteria. Any underlying conditions that slow stomach emptying can encourage small intestine bacterial overgrowth. One condition that can severely delay stomach emptying is gastroparesis. Gastroparesis means "paralysis of the stomach," and the condition causes symptoms such as nausea, vomiting, bloating, and a feeling of fullness after even small amounts of food. Individuals with gastropareisis are more likely to develop bacterial overgrowth in both the stomach and the small intestine.

Another common condition that leads to altered gut motility is hypothyroidism. This endocrine disorder is caused by an underactive thyroid that does not produce sufficient thyroid hormone. A malfunctioning thyroid gland can have major metabolic implications, such as weight gain. Hypothyroidism often causes decreased GI motility and constipation. Individuals with hypothyroidism are more likely to develop SIBO.

Intestinal Infections

Bacterial infections in the gut are typically treated with antibiotics. However, with antibiotic resistance on the rise, it is important to consider whether improving overall gut health may reduce the risk of developing such bacterial infections.

The body has certain innate defense mechanisms that protect against pathogenic bacteria. As we discussed in chapter 4, the innate immune system provides nonspecific defenses that can be generally directed at most bacteria, while adaptive immune defenses attack specific bacteria. Researchers propose that it may be possible to use the body's nonspecific defenses to prevent infections in the gut. Using these defenses may help eliminate the pathogen before it leaves the lumen and has a chance to enter into the mucosa and bloodstream (thus traveling to and infecting other body tissues).

Some of these nonspecific defenses include maintaining healthy populations of commensal microbes, preserving healthy mucosa, and supporting proper motility of the intestine. These defenses can be strengthened through dietary interventions. For instance, providing gut microbiota with fermentable carbohydrates supports mucosal health through production of compounds such as butyrate. Additionally, researchers find that adding probiotic foods, such as yogurt and fermented vegetables, that also contain lactic acid, may improve the body's ability to resist certain pathogenic infections.

Salmonella

Salmonella is a genus of bacteria in the family *Enterobacteriaceae*. This family also contains other pathogenic bacteria such as *E. coli*. *Salmonella* infection can be caused by eating undercooked meat or eggs. Unfortunately, the number of antibiotic-resistant *Salmonella* species is growing. Further, antibiotics are usually only given if this pathogen has traveled into other parts of the body to cause systemic inflammation. Otherwise, if the *Salmonella* infection remains localized within the intestine, antibiotics can do more harm than good. Treating these less complicated cases with antibiotics often prolongs the infection and increases the chances of relapse. It also increases the risk of carrying this bacteria in an asymptotic way and passing it on to other individuals. On the other hand, probiotic foods have been found to reduce risk of *Salmonella* infection and should be considered as a possible preventative treatment.

Clostridium difficile

Clostridium difficile is a species of bacteria in the *Clostridium* genus. *C. difficile* infection is typically acquired during hospitalization; however, the numbers

of community-acquired cases are increasing. Symptoms from community-acquired infection are typically milder than in cases acquired by hospitalization. For hospitalized individuals, the duration of hospitalization determines the risk of infection. One study showed that half of patients who are hospitalized for over a month developed *C. difficile* infection. Long-term residence at other care facilities, such as nursing homes, can also increase risk of *C. difficile*. Elderly extended-care facilities may have even higher incidences due to risk associated with advanced age. In fact, *C. difficile* is often spread in healthcare settings because it can live on any surface within these facilities and is also transferred from the hands of healthcare providers to vulnerable individuals. These observations emphasize the importance of proper sanitation methods in the prevention of *C. difficile* transference.

Although some patients are at higher risk due to age or hospitalization, one of the most important causes of *C. difficile* infection is antibiotic use. As expected, antibiotics increase risk because they often lead to dysbiosis. Antibiotics reduce commensal microbe populations, killing beneficial bacteria in the gut. This eliminates pathogenic bacteria's competition, making it easier for pathogens to reproduce in the gut. Another risk factor is decreased stomach acid, which can result naturally from age or as a side effect of proton pump inhibitor use. Stomach acid is normally protective against intestinal pathogens; thus, its diminished production creates a more hospitable environment for opportunistic bacteria.

C. difficile causes colitis, or inflammation in the colon. This bacterium releases toxins that cause inflammation and cell death of intestinal cells. Given a direct effect on intestinal epithelial cells, this type of infection can mimic clinical symptoms seen in inflammatory bowel disease. One of the main symptoms is watery diarrhea. Some individuals may experience fever or abdominal cramps. This similarity to IBD symptoms may cause problems with diagnosis in patients with pre-existing IBD. Unfortunately, these patients are already at increased risk of infection.

C. difficile is particularly problematic because of the risk of recurrence. This bacterium is persistent, in part due to its ability to assume a dormant form that leads to recurrent infections. Also, *C. difficile* forms spores that are very difficult to eradicate. In fact, they are so persistent that they often linger within the GI tract for months or even years. While only about a fifth of all initial infections become recurrent, each subsequent infection substantially increases risk of future recurrence.

The formation of antibiotic resistance further perpetuates the problem of recurrent infection. Once *C. difficile* infection is detected, patients are usually asked to discontinue any current antibiotic plan. This alone may resolve infection in some cases, but it is usually advised that patients begin another antibiotic protocol that is directed at *C. difficile*.

SIDEBAR 6.5 Fecal Transplants for *Clostridium Difficile* Infection

Fecal transplants may sound unsettling, but for individuals suffering from recurrent *Clostridium difficile* infection, this medical treatment may be a life-saving maneuver. Fecal microbiota transplant are currently recommended after multiple rounds of antibiotics fail to eliminate the pathogen. A growing number of clinical trials show the effectiveness of fecal transplantation in the treatment of *C. difficile*. The transplanted material is typically administered by enema, using an instrument called a colonoscope.

Researchers are now testing to see whether fecal microbiota transplants can also be administered in capsule form. It appears that this improved administration method is very effective. In the first clinical trial for this pill, one dose of 30 capsules cured 70% of *C. difficile*–infected individuals. A second dose effectively cured 94% of infected individuals. On the other hand, colonoscopy cures about 90% of patients but is far more invasive. Furthermore, researchers are working on a capsule that likely will contain no human feces at all. These capsules would hold similar microorganisms but eliminate some of the risks (and discomfort) associated with swallowing feces-containing pills.

Conclusion

We have learned that both ulcerative colitis and Crohn's disease are mediated by the immune system. Gut microbes are involved in the development of these diseases and also contribute to the progression of the diseases, by triggering the hypersensitive immune system. We have also learned that symptoms of both IBS and IDB can be somewhat alleviated with certain dietary interactions such as a low-FODMAP diet. Finally, we learned that certain medications, such as proton pump inhibitors and antibiotics, can cause dysbiosis. In the case of antibiotics, dysbiosis can lead to both depleted commensal microbe populations as well as the development of antibiotic-resistant bacteria.

7

Cardiovascular Disease

Cardiovascular disease is the worldwide leading cause of death for both women and men. This disease, affecting the heart and blood vessels, develops due to a process called atherosclerosis. Atherosclerosis is a condition in which the arteries harden, limiting blood flow in the body. When arteries are not able to properly transport blood to the heart or other organs, there is an increased risk for heart attack, stroke, and circulation disorders affecting the peripheral blood vessels such those within the arms and legs.

Early research on heart disease explored the hypothesis that the narrowing of arteries was due to a buildup of calcium. However, scientific understanding of atherosclerosis has evolved significantly over the past several decades. More recently, in the 1980s, large population studies pointed to a link between elevated cholesterol levels in the blood and the risk of developing high blood pressure. Elevated blood pressure, as we will see later in this chapter, is a risk factor for cardiovascular disease. This research instigated further investigation that pointed to excess cholesterol in the body as a contributor to atherosclerosis.

The current understanding is that dietary fat and cholesterol promote the accumulation of a substance called plaque along the inner lining of the artery. The buildup of plaque, which contains both cholesterol and triglycerides, causes the hardening and narrowing of the arteries. While excess dietary fat and cholesterol may contribute to atherosclerosis, it seems that certain individuals with normal cholesterol levels still develop cardiovascular disease. In fact, even with the use of cholesterol-reducing medications such as statins, the risk of heart attack is reduced by about 36%. Heart attacks can result from blockage that occurs at sites other than just the narrowest arteries. These findings indicate that there is another contributing factor or factors in the progression of atherosclerosis.

Population studies reveal two major contributors to arterial inflammation: oxidized cholesterol and metabolic syndrome. Metabolic syndrome refers to a group of risk factors for developing cardiovascular disease. This chapter investigates these major contributors. For example, we will discuss how

inflammation plays a role in the development of heart disease, and how gut microbes regulate this immune-mediated mechanism. Furthermore, we will explore how microbes interact with diet in ways that influence cardiovascular disease risk.

Do Bacteria Contribute to Clogged Arteries?

Given the role of diet in cardiovascular disease, cardiologists began recommending a low-fat, low-cholesterol diet to help their patients reduce risk factors for atherosclerosis. In the effort to find a heart-healthy diet, red meat and egg consumption declined in the United States. Interestingly, these foods are again gaining popularity in heart disease research. It now appears that our gut microbes interact with certain compounds in red meat and eggs in ways that contribute to atherosclerosis.

Aside from saturated fat and cholesterol, red meat and eggs contain two other nutrients that are now implicated in cardiovascular disease. Specifically, the gut microbiota interacts with choline and L-carnitine in ways that increase risk. Gut microbiota convert these nutrients to a compound called trimethylamine (TMA). In the liver, TMA is then converted by enzymes into trimethylene N-oxide, or TMAO, a substance that appears to promote atherosclerosis. Research demonstrates that TMAO is associated with increased plaque in the aorta, a main artery that transports blood from the heart to other parts of the body.

It is uncertain exactly how TMAO contributes to atherosclerosis. A few possible mechanisms include increased formation of cholesterol or reduced clearance of cholesterol from the body. Mice given choline and L-carnitine had a 30% reduction in reverse cholesterol transport, the process by which cholesterol moves from tissues in the body back to the liver. However, this effect was only observed in mice with normal gut flora and not in germ-free mice.

L-Carnitine and Choline

L-carnitine is the active form of the compound carnitine. This biological active nutrient is synthesized in the body and is necessary for energy production. This compound is found in muscles, where it is available to help those tissues metabolize dietary fat to produce energy. Carnitine also serves as a transporter for certain types of fats (long-chain fatty acids) into the mitochondria, the powerhouses of cells.

The liver and kidneys produce carnitine from two different amino acids, lysine and methionine. However, carnitine is also found in animal products and is particularly concentrated in red meat. Although most dietary carnitine is absorbed in the small intestine, a portion of it reaches the large intestine, where it can interact with gut bacteria. Aside from cases of genetic disorders or

health conditions that reduce carnitine absorption, deficiency is not common. A diet low in carnitine typically has little effect on the body's total carnitine, as the kidneys conserve it efficiently.

Choline is a B vitamin with various important physiological functions, including the transportation of fats and the formation of neurotransmitters. This B vitamin is involved in the synthesis of lipoproteins, which are required to transport dietary fats. Choline promotes brain development in the growing fetus, and a deficiency may alter the structure and function of the brain's memory center.

Since the body is not able to produce sufficient amounts of choline to meet metabolic needs on its own, this nutrient must be acquired from dietary sources. The adequate intake (AI) for choline is 425 mg/day in women and 550 mg/day in men. The reason why women need less dietary choline than men is because estrogen stimulates an enzyme that is responsible for the production of choline by the liver. Eggs are a concentrated source of choline, containing 147 mg of choline per egg. Plant foods such as beans and grains also contain choline. For instance, one cup of soybeans contains about 216 mg of choline, and one cup of cooked quinoa contains 70 mg of choline.

Additionally, choline is a main structural component of cell membranes, as it helps form phosphatidylcholine, a type of fat abundant in cell membranes. Phosphatidylcholine is a source of choline found in meat and fish. Phosphatidylcholine is also known as lecithin. Lecithin is a common food additive used as an emulsifying agent that improves texture. Some individuals use lecithin supplements in an attempt to lower cholesterol, but there is no evidence that supplementation has this effect.

Interestingly, choline helps to lower levels of homocysteine, a protein in the blood that is linked to increased risk of cardiovascular disease. Homocysteine primarily comes from meat in the diet. Excess homocysteine levels are associated with factors that contribute to heart disease, such as inflammation and increased triglycerides. Given that elevated homocysteine levels may be linked to cardiovascular disease risk, it appears that choline can play a protective role by decreasing levels of this amino acid in the blood.

Choline is also used to provide another homocysteine-lowering compound. For instance, betaine is produced from choline in the liver and kidneys. Betaine is also found in foods like wheat bran, spinach, beets, and shrimp. Research shows that supplementation with betaine decreases homocysteine levels. Also, dietary choline and betaine both reduced inflammatory markers.

Another study conducted an "L-carnitine challenge" to determine if this compound was processed differently by the body following antibiotic use. One group of subjects was given antibiotics, and therefore had suppressed gut microbiota, while the second group had did not receive antibiotics. The antibiotic group exhibited inhibition of nearly all TMAO production.

Although TMAO may be implicated in heart disease, reduction of these nutrients may not be recommended, due to their important roles in the body. Instead, inhibiting the conversation of TMA to TMAO following ingestion of carnitine and choline-rich foods may be a more promising route.

New Heart Disease Treatments Target Gut Microbiome

Until now, medications for heart disease have targeted human cells. The newly discovered link between the gut microbiota and heart disease has inspired the development of a new medication, the first of its kind. It is the first treatment for heart disease that targets the gut microbiota, aiming to block the synthesis of TMA. Reducing the liver's supply of TMA decreases the formation of atherosclerosis-promoting TMAO.

Originally, researchers worked to block the conversation of TMA to TMAO by inhibiting the enzymes that support this conversion. Unfortunately, preventing the activity of these enzymes led to liver damage, as well as an unhealthy accumulation of TMA in the body. Researchers then changed their focus to targeting gut microbes to prevent the formation of TMA. Researches isolated compounds called DMB, found naturally in extra-virgin olive oil and balsamic vinegar. They observe that when DMB was given to mice, it lowered TMAO levels and prevented plaque buildup in the arteries. This medication also successfully blocked the TMAO conversation process without harming gut microbes.

Additionally, researchers notice that certain methanogens may have the potential to reduce TMAO. These archaea reduce TMA and may therefore reduce its availability for TMAO production. Specifically, a strain of *Methanomassiliicoccus luminyensis* can lower TMA levels. This gut resident tends to be more common in older adults.

Cholesterol and Triglycerides: Gut Microbes and Blood Lipids

As mentioned, fats play a large role in determining risk for heart disease. Certain dietary fats, such as saturated fat, found in foods like red meat, butter, and cheese, can promote atherosclerosis. This is because the fats in our diet determine the types of fats we have in our bloodstream.

When measuring an individual's risk for heart disease, physicians often perform a test called a lipid profile, to check levels of fatty substances in the blood. Two major blood lipids are associated with heart disease: cholesterol and triglycerides. Cholesterol is type of fat molecule that functions as a structural component of cell membranes and many hormones. Triglycerides are the primary type of storage fat in the body. Elevated levels of either cholesterol or triglycerides may be indicative of increased risk of cardiovascular disease.

In some individuals, dietary cholesterol can increase total cholesterol levels in the body. Interestingly, carbohydrates play a substantial role in determining an individual's lipid profile. A high-calorie diet, particularly one containing large quantities of refined carbohydrates, can increase triglycerides. In addition to these obvious dietary influences, researchers are interested to learn how the gut microbiota may influence fats found in our blood and body tissues.

Gut Bacteria and Lipid Composition of Body Tissues

Choosing appropriate dietary fats can prevent abnormal fat accumulation in both the bloodstream and adipose tissue. Other dietary fats, like omega-3 fatty acids, may actually protect against heart disease. Omega-3 fats are abundant in fatty fish, nuts, seeds, and certain algae. Omega-3 fats continue to gain popularity, not only due to their contribution to heart health but also because of their anti-inflammatory affects. As we know, systemic inflammation is associated with dysbiosis of the gut microbiota.

One species of gut bacteria, *Bifidobacterium breve*, helps increase the levels of omega-3 fats within adipose tissue. Researchers found that using a combination of *B. breve* and a-linolenic acid (ALA), a plant-based omega-3 fat, improves the lipid composition of several different body tissues. Specifically, animals receiving this combination had higher concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in their adipose tissue and liver. DHA and EPA are omega-3 fats that have many health benefits, including anti-inflammatory properties. As we extensively discussed in chapter 5, both adipose tissue and the liver are metabolically active organs that regulate energy homeostasis and fat storage. Inflammation of these tissues may contribute to weight gain, which is a risk factor for cardiovascular disease.

It is also interesting to note that ALA supplementation alone did not have the same effects on lipid composition of tissues. This indicates that *B. breve* mediates these compositional changes and is primarily responsible for this increase in EPA and DHA. Dietary sources of ALA are primarily nuts and seeds. On the other hand, dietary sources of EPA and EHA are animal based, and primarily found in fatty fish (though they can also be taken as algae supplements). Vegans and vegetarians may be deficient in these two omega-3s. At the same time, vegans and vegetarians are more likely to have a diet rich in bifidogenic carbohydrates (see chapter 3 for information on carbohydrates that promote growth of *Bifidobacteria*).

In 2015, an article published in *Circulation Research*, the official journal of the American Heart Association, provided further evidence for the role of the gut microbiome in blood lipids. This large-scale study looked at 893 individuals to determine how specific bacteria may be associated with levels of triglycerides and cholesterol.

This study explored the effect of gut microbiota on two types of cholesterol: high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol. LDL and HDL refer to the type of carrier molecule to which the cholesterol is attached. LDL cholesterol is attached to low-density lipoproteins; this type of cholesterol is associated with increased risk of heart disease. On the other hand, HDL cholesterol is attached to high-density lipoproteins, which are generally protective against heart disease. Although the gut microbiota appeared to have little effect on either LDL cholesterol or total cholesterol, it seems that certain bacteria do appear to help maintain normal levels of HDL cholesterol and triglycerides.

Some research also investigates the potential influence of lactic acid bacteria on blood cholesterol levels. Although research has not yet identified a definitive mechanism, some studies demonstrate that these bacteria can assimilate cholesterol and prevent its accumulation. One study shows that smokers who were given a *Lactobacillus plantarum* strain lowered their elevated LDL cholesterol levels by 12%, but these results were not found to be statistically significant.

Another study used a *Lactobacillus fermentum* strain to significantly reduce total blood cholesterol levels in mice whose diet was supplemented with added cholesterol. These researchers suggest that *Lactobacillus* increases the production of short-chain fatty acids through the fermentation of dietary fiber. These metabolites are then able to reduce the production of cholesterol in the liver. Also, it is possible that SCFAs may help facilitate the uptake of cholesterol by intestinal cells, thus reducing cholesterol levels in the blood. However, this data remains inconclusive, because a clinical trial using the same probiotic found only a small effect on blood cholesterol levels.

LPS, TLRs, and Inflammation in the Arteries

Similar to the lining found along the gastrointestinal tract, blood vessels are also lined with specialized cells that are vulnerable to damage caused by inflammation. This lining, called the endothelium, is made of up endothelial cells, which provide a barrier between the lumen of the blood vessel and the surrounding tissue.

This endothelium is also susceptible to the damaging effects of chronic inflammation. One cause of inflammation along the endothelium is interaction with gram-negative bacteria. Chapters 4 and 5 examine how gut permeability causes these bacteria to escape the gut and enter blood circulation. A couple of studies using animal models of hypertension show that these animals have fewer tight junction proteins. This alteration in tight junctions increases gut permeability and allows these lipopolysaccharide-containing (LPS) gram-negative bacteria to escape from the gut and produce an inflammatory immune

SIDEBAR 7.1 Obesity and Cardiovascular Disease

It is important to remember that obesity is a risk factor for cardiovascular disease. Chapter 5 discussed the direct associations between altered gut microbiota composition and obesity. Inflammation caused by gut microbiota dysbiosis and intestinal permeability can change fat cells in ways that promote chronic disease. As mentioned, one of these changes involves the hormone leptin, which is now being explored for its role in cardiovascular disease.

Chronic inflammation is associated with increased leptin and may result in leptin resistance. Higher levels of leptin may predict a risk for serious cardiovascular events. Furthermore, cardiac workload is generally higher in obese individuals, which tends to promote high blood pressure.

response. Given that systemic inflammation is also associated with cardiovascular disease, researchers now wonder whether levels of LPS could indicate risk for this disease as well.

Toll-like receptor 4, the cell receptor that recognizes bacterial lipopolysaccharide, is not normally expressed on endothelial cells. However, specific factors activate the expression of these receptors on endothelial cells. For instance, changes in blood flow, such as from high blood pressure, can also increase endothelial TLR4 expression.

Another important factor that induces TLR4 expression is oxidized LDL, one of the main components of plaque along blood vessels. When LDL becomes damaged due to interactions with oxygen, it turns into oxidized LDL. LDL cholesterol also oxidizes in the presence of inflammation. Therefore, it may be possible that systemic inflammation creates a positive feedback loop that perpetuates the formation of oxidized cholesterol, which then leads to more inflammation by inducing the expression of TLR4.

Dietary factors can also trigger expression of TLR4. Saturated fat and dietary components called advanced glycation end products (AGEs) both activate TLR4 receptors. AGEs are produced in certain foods that have been cooked at high temperatures. They also form in the body when sugars combine with fats or protein, through a process called glycation. Foods that are high in both fat and protein are more likely to create AGEs from high-temperature cooking such as grilling and frying. For instance, red meat is particularly vulnerable to the formation of AGEs. It is also interesting to note that increased AGEs in the body can damage cells and contribute greatly to aging.

Increasing TLR4 within endothelial cells through any of these means may promote inflammation. Higher numbers of TLR4 encourage the binding of LPS from any gram-negative bacteria. The binding of TLR4 receptors to LPS stimulates the release of pro-inflammatory cytokines that contribute to

atherosrerlosis. This inflammation not only encourages the buildup of plaque but can also cause the plaque to dislodge, causing potential blockage and resulting in heart attack or stroke.

Endothelial cells are directly affected by inflammation resulting from the TLR4 response to circulating LPS. As endotoxins, LPS trigger reactive oxygen species that damage endothelial cells. LPS also promote increased cell death within endothelial tissue. These endotoxins, in a way, change the fate of endothelial cells, causing them to die more rapidly by inhibiting the expression of a molecule on the surface of those cells that promotes survival. Not surprisingly, increased endothelial cell death is a characteristic of atherosclerosis.

Mice that have been genetically altered to be deficient in TLR4 are greatly protected against some of the effects of a high-fat diet. For instance, when these mutant mice were given high-fat feed, they did not develop the expected insulin resistance or experience higher levels of systemic inflammation.

Due to their possible interaction with endothelial linings of blood vessels, LPS levels in the body may also provide some indication of cardiovascular risk. One study that highlights data on the correlations between metabolic syndrome LPS-induced inflammation sampled LPS levels of 516 individuals. The researchers observed that levels of LPS generally ranged between 6 and

SIDEBAR 7.2 New Test for Heart Disease?

Cholesterol levels are often the primary indicator used to assess risk for developing cardiovascular disease. Elevated LDL levels typically indicate higher risk for heart attack. However, this test has considerable limitations, given that only about 50% of individuals who suffer a heart attack also have elevated LDL cholesterol. This leaves physicians wondering whether incorporating other tests might provide a way to detect increased risk for heart disease in those 50% of cases that are not associated with increased LDL.

In fact, researchers observe that inflammation may be an even better predictor of cardiovascular disease than cholesterol levels. They are able to measure inflammation by detecting levels of C-reactive protein, which increases in response to inflammation. Researchers are now suggesting that when used together, C-reactive protein and LDL cholesterol levels might identify a broader group of high-risk individuals.

Interestingly, C-reactive protein levels can also be used to measure the anti-inflammatory effects of a high-fiber diet. Studies show that dietary fiber intake reduces C-reactive protein levels in the blood. As we saw in chapter 3, one way that fiber reduces inflammation is by promoting the growth of beneficial gut microbes. Given that certain types of dietary fiber can also reduce LDL cholesterol, individuals who are looking to reduce risk of cardiovascular disease may benefit from increasing dietary fiber.

209 picograms per milliliter (pg/ml—a picogram is one trillionth of a gram). Individuals whose LPS levels tested above 50 pg/ml had three times greater risk of early atherosclerosis.

In addition to TLR4, changes in TLR5 expression may be linked to hypertension. Mutant mice who lack toll-like receptor 5 develop numerous characteristics of metabolic disease, including hypertension. These mice also showed altered gut microbial composition. Interestingly, when gut microbes from these TLR5-deficient mice were transferred to wild-type mice, the wild mice developed the same symptoms of metabolic syndrome. Researchers believe these findings support the theory that the gut microbiota influences blood pressure, but they are still exploring the role of TLR5 in this interaction. In general, animal studies have demonstrated that the activation of toll-like receptors is associated with higher arterial blood pressure. However, these immune-mediated causes of hypertension are not well understood.

Hypertension

High blood pressure, also known as hypertension, is a common condition in which the force of blood exerts too much pressure on artery walls, causing health problems such as damaged blood vessels and hardening arteries. There are two types of hypertension: essential and secondary hypertension. Essential hypertension describes 90% of cases, whereas secondary hypertension is less common and caused by another existing medical condition. Essential hypertension tends to develop later in life and is linked to both genetic and environmental causes. The most likely environmental factors are diet, obesity, alcohol consumption, chronic stress, and inactivity.

Blood pressure is written as two numbers that represent systolic (upper number) and diastolic (lower number). For instance, a normal blood pressure reading is 120/80, measured in millimeters of mercury (mmHg). Systolic blood pressure measures the amount pressure within the arteries when the heart beats. Diastolic blood pressure measures of the amount of pressure within the arteries between heartbeats. When systolic blood pressure and diastolic blood pressure are consistently above 140 mmHh and 90 mmHg, respectively, an individual is considered to be hypertensive.

Sodium Sensitivity and Gut Microbes

In additional to fats, another dietary factor that contributes to hypertension is salt. Sodium, an element found in salt, actually helps regulate blood pressure. However, excess sodium can contribute to hypertension. Some individuals are salt sensitive, meaning that their blood pressure increases more than 5–10% following sodium intake. Interestingly, the gut and its microbes can

also influence this sodium-mediated effect on hypertension. Certain gut hormones (such as gastrin and glucagon-like peptide-1) help regulate sodium. In addition, certain short-chain fatty acids produced by gut bacteria also have this regulatory effect on sodium.

To delve more deeply into this potential connection between gut microbes and high blood pressure, researchers compared gut microbiota of salt-sensitive rats to that of salt-resistant rats (those whose blood pressure was not affected by sodium intake). The gut microbiota of the sat-sensitive rats differed from that of the salt-resistant rats. Bacteria from the Bacteroidetes phylum and the *Veillonellaceae* family (part of the Firmicutes phylum) were more abundant in the guts of the salt-sensitive rats. Despite these observed differences in composition, the researchers did not see any changes in blood pressure when the salt-sensitive rats were given antibiotics to deplete their gut microbes. So it appears that these observed composition differences may not be mediating hypertension in these cases. Furthermore, the researchers were surprised to see that transferring gut microbes from salt-resistant rats to salt-sensitive ones actually worsened the hypertensive state of the latter group.

Another study in rats found significant differences in microbiota populations within hypertensive rats compared to normal rats. Rats with high blood pressure had a Firmicutes-to-Bacteroidetes ratio that was five times greater than that for normal rats. In addition, *Bifidobacteria* was reduced among the rats with high blood pressure, and the overall richness of their microbiota populations was lower. These researchers also note that the production of metabolites shifts due to hypertension related-changes in microbiota composition, specifically in terms of short-chain fatty acid production. For instance, the hypertensive rats had more lactate-producing bacteria, whereas non-hypertensive rats had more butyrate-producing bacteria. Although the implications of these shifts in microbial populations are unknown, it is possible that diminished populations of symbiotic microbes such as *Bifidobacteria* and butyrate-producing species may contribute to detrimental health outcomes, as seen in other chronic health conditions.

A third study explored a model of hypertension that was caused by a combination of a high-fat diet along with an experimental model of obstructive sleep apnea. This sleep disorder (discussed in more detail in chapter 5) is present in about half of hypertensive individuals. In this model, a high-fat diet is used to produce an obese state, which is also often associated with hypertension. While using obesity and sleep apnea to induce a state of hypertension in rats, researchers administered antibiotics to these rodents and noticed that they were able to prevent the hypertensive state. Interestingly, a transfer of microbiota from the rats with the sleep apnea-and-obesity-induced hypertension caused increased blood pressure in the control group within 1 to 2 weeks. This model of hypertension was also associated with an increase

in lactate-producing bacteria, along with a decrease in butyrate-producing bacteria.

SCFAs and Probiotics

Short-chain fatty acids (SCFAs) regulate blood pressure by activating certain cell receptors. Some of these receptors are located in the kidneys, where they interact with SCFAs to produce substances that help regulate blood pressure in the body. In addition, animal studies also show that the SCFA propionate may temporarily dilate blood vessels to improve circulation. When mice were given propionate, their blood pressure decreased by 20 mmHg, but this change lasted only a couple of minutes.

Although these cell receptors may have a role in hypertension, depletion of microbes via antibiotics increased blood pressure in mutant mice who lack these particular receptors, but not in wild-type mice. This indicates that gut microbes are key players in regulating blood pressure through these cell receptors.

Among the most common types of medication used in the treatment of hypertension are ACE inhibitors. This type of pharmaceutical drug inhibits an enzyme called angiotensin-converting enzyme (ACE), which naturally causes blood vessels to constrict and thus increases blood pressure. ACE inhibitors prevent the actions of this enzyme and thus help dilate and widen arteries. Interestingly, some probiotic bacteria may have similar abilities to reduce ACE. Fermented dairy products containing the bacteria *Saccharomyces cerevisiae* and *Lactobacillus helveticus* decreased blood pressure in rats by 20 mmHg. Another pair of probiotics, *Lactobacillus plantarum* and *L. paracasei*, also decreased systolic and diastolic blood pressure in hypertensive rats.

The benefits of *L. helveticus* were reproduced in a human study as well. This study, conducted in Japan, gave fermented milk containing this probiotic

SIDEBAR 7.3 Inflammation-Reducing Microbe Protects Arteries

Some gut microbes may protect the heart from the detrimental effects of a Western diet. For example, research in mice shows that administration of *Akkermansia muciniphila* reduces the development of atherosclerosis. This gut microbe is able to reduce inflammation throughout the whole body but, also, specifically at sites where atherosclerosis is developing. These mice showed reduced inflammatory markers, such as lower levels of macrophages and pro-inflammatory cytokines. Researchers believe that the main mechanisms by which this microbe protects against atherosclerosis is by improving the gut barrier, thus reducing LPS-induced inflammation within the arteries.

bacteria to 36 elderly individuals with high blood pressure. Those who consumed 95 mL of the fermented milk drink daily for two months showed a significant decrease in systolic and diastolic blood pressure when compared to the placebo group. A second study, using the same probiotic for only one month, showed a decrease of 5.0 mmHg in diastolic blood pressure and 11.2 mmHg in systolic blood pressure.

Dietary Nitrates and Endothelial Function

Most of this chapter discusses the interactions between the lower gastrointestinal microbiome and heart health. Yet the microbes in the upper GI tract have some surprising effects on heart health. Oral bacteria play a significant role in the conversion of nitrate to nitric oxide, an important compound for maintaining healthy blood vessels. Nitric oxide protects endothelial cells and causes blood vessels to dilate, helping improve blood flow.

In order for dietary nitrates to be converted into nitric oxide, the nitrates must be reduced to nitrite. Bacteria in the mouth help reduce nitrate to nitrite so it can then be converted into nitric oxide. Dietary nitrates are found in a number of vegetables, particularly in leafy green vegetables like spinach, collard greens, and dandelion greens. The amount of nitrates in vegetables is also dependent on the amount of nitrates in the soil. Adding nitrogen-containing fertilizer can improve the nitrate levels of foods.

A diet high in unprocessed foods, with a variety of complex carbohydrate sources, is not only beneficial for gut microbiome health, it is also the main components of diet recommended by the National Institute for Health for treating hypertension. For instance, the NIH recommends the "DASH" diet, which stands for "Dietary Approaches to Stop Hypertension"—high in fruits and vegetables. This diet not only increases nitrate sources but also provides symbiotic microbes with a higher quantity of prebiotic carbohydrates.

In one 4-week clinical trial, health volunteers drank 250 mL of dietary nitrates in the form of beetroot juice, to test the effects on their blood pressure. Not only was their blood pressure reduced, but endothelial function improved. The nitrates also caused a reduction in arterial stiffness. This clinical trials shows promise as researchers continue to explore the protective effects of nitric oxide.

Conclusion

Although there is still some controversy within the medical and research communities about the roles of saturated fat and dietary cholesterol in cardiovascular disease, there may now be yet another reason to limit intake of

red meat and eggs. We have discussed two other components found in these foods (phosphatidylcholine and L-carnitine) that interact with gut microbes in ways that contribute to the buildup of plaque along the arteries. We have also learned how microbes influence blood pressure as well as blood lipids, all of which are important factors in the development of cardiovascular disease.

8

Microbes and the Gut-Brain Axis

So far, we have discussed how gut microbes influence digestion, immunity, metabolism, and overall gut health. The role of these microbes in human health is clearly far-reaching, so it may not be surprising that gut microbes also influence brain function. Gut microbes communicate with the brain through a connective network called the gut-brain axis. Through this communication, our gut microbes may in fact be driving some of the brain's functions and influencing both mood and behavior.

The central nervous system, which consists of the brain and the spinal cord, is the primary control center of the body. It is increasingly clear that the functions of the central nervous system are often influenced by the regulatory effects of the gut microbiome. In fact, the gut itself is sometimes referred to as the "second brain," working in conjunction with the central nervous system to regulate our mood and overall neurological health.

This chapter explores the various pathways by which gut microbes communicate with the brain. We will examine how the body's stress response works and discuss the effectiveness of probiotics and prebiotics in improving this response. The final section of this chapter focuses on neurodevelopment and neurodegeneration. For instance, autism spectrum disorder—a disease that affects neurodevelopment—is associated with dysbiosis. We will also discuss neurodegenerative diseases such as Parkinson's disease and multiple sclerosis.

Communication between Gut Microbes and the Nervous System

The human host and its gut microbiota share two-way communication that allows the brain to influence what happens in the gut and also gives the gut and its microbes influence over brain function.

The enteric nervous system is a part of the body's nervous system that is found within the gastrointestinal tract. It controls many gut functions, such

as movement of food through the GI tract, blood circulation, the release of digestive secretions, and immune functions. In addition to these functions, the enteric nervous system facilities communication between the brain and gut microbes. The enteric nervous system contains neurons, or nerve cells, that can transmit information through electrical and chemical signals. Microbes are able to communicate with the host body through these neurons by producing neurochemicals that are similar to those produced by the human nervous system.

Many important neurochemicals originate in the gut. These neurochemicals may be produced by microbes or by humans through the influence of microbes. Some of these neurochemicals are relevant in maintaining the body's proper gut function.

Not only do gut microbes rely on the enteric nervous system to interact with other parts of the body, the nervous system actually relies on gut microbes for its own development. In fact, gut microbiota are crucial in the development of both the enteric and the central nervous systems. Researchers noticed this especially in germ-free mice, whose nervous system developed abnormally due to the absence of normal gut flora. These animals have dysfunctional neurotransmitters, which alters gastrointestinal function. Researchers are now wondering how less severe states of dysbiosis in humans may influence the development of the central nervous system.

In addition, intestinal permeability may affect the degree of interaction between microbial signaling molecules and the central nervous system. For example, in cases of systemic inflammation, where intestinal permeability increases, luminal bacterial may come into contact more easily with epithelial cells.

Many studies focus on the impact of the nervous system on luminal bacteria, but less is known about the effects on bacteria within the intestinal biofilm. Microbes located in biofilm may be less influenced by changes in gut environment, though they are likely more involved in gut-brain communication with the host.

Communication in Bacteria

Both prokaryotic and eukaryotic cells use hormones and hormone-like substances for cellular communication. Mammalian signaling molecules share structural similarities with those produced by prokaryotic cells. Within the human body, signaling molecules are produced by both host cells and microbial cells. Since these substances are similar in structure and function, they are able to support inter-kingdom communication between human and microbial cells. For instance, eukaryotic cells (such as those found in humans) use hormones to communicate with one another. Likewise, prokaryotes (such as bacteria) also interact using hormone-like substances.

Bacteria use quorum sensing to communicate with each other. This method of communication allows bacteria to determine their gene expression based on changes in their bacterial cell-population density. Quorum sensing can therefore regulate functions and behaviors of bacteria based on information about their own population. Depending on signals they receive from other bacteria and the host, bacterial gene expression can shift in ways that influence the structure and function of bacterial populations. Given that quorum sensing determines bacterial functions, it also impacts the types of bacterial metabolites produced.

In order to facilitate interactions through quorum sensing, both gram-positive and gram-negative bacteria produce signaling molecules called autoinducers. These autoinducers are similar to hormones produced in the human body. Due to this similarity, autoinducers can be used by bacteria to interact with human cells. For instance, autoinducers produced by microbes can bind to cells in the human body through noradrenaline receptors.

Noradrenaline is a neurochemical that is produced as a result of stress (i.e., as part of the fight-or-flight response) and to induce wakefulness after sleep. While autoinducers can bind to noradrenaline receptors, noradrenaline itself can interact with gut microbes and can influence their growth and behavior. For example, certain Proteobacteria species are responsive to noradrenaline. Noradrenaline can stimulate growth within several types of pathogenic microorganisms. For example, infectious species such as *E. coli* may display greater virulence when this stress hormone is elevated. Currently, more research is needed to determine the interactions between noradrenaline and nonpathogenic bacteria.

Quorum sensing can also determine whether certain bacteria will become pathogenic. Certain opportunistic microbes live in the gut without producing symptoms of infection until they are presented with the right circumstances. In some instances, bacteria can use autoinducers to create the ideal circumstances for infection. As a way to decrease the host's ability to eliminate the pathogen, bacteria may produce autoinducers that inhibit gut secretions that otherwise protect against infections. Microbial quorum sensing is also influenced by signals from other bacteria as well as the host; therefore, signals from either can regulate microbial genetic expression. This creates a complex communication network that determines pathogenicity based on microbial populations and the state of human health.

Additionally, autoinducers induce other behaviors within pathogenic microbes that determine the size of their populations. For instance, researchers observe that within a specific type of *E. coli*, an autoinducer binds to a bacterial membrane to signal certain genes that activate traits of virulence and motility. Bacteria's level of virulence determines their ability to overcome the host's natural defenses against infection. One gene, activated by

autoinducers, is responsible for the development of the flagellum, a protruding organ used for movement by bacteria.

Both physical and psychological stress can increase virulence traits in pathogenic bacteria. Stress can trigger the release of signaling molecules into the gut lumen, where they interact with gut bacteria. Bacteria have receptors for these molecules and use similar signaling mechanisms for intracellular communication (such as with adrenaline and noradrenaline).

Finally, it is important to note that microbiota-generated metabolites, such as short-chain fatty acids, also influence signaling pathways. These metabolites can have beneficial effects on gut-brain neural circuits. For example, serotonin, an important neurotransmitter, is produced by gut microbes using various metabolites including short-chain fatty acids.

The Autonomic Nervous System

The autonomic nervous system (ANS) facilitates communication between the central nervous system and internal organs. The ANS governs our stress response and plays an important role in a number of gut functions, including motility and the production of acid. It also regulates the secretion of mucus, which can impact the health of the intestinal mucosal layer. (As we've seen in other chapters, decreased mucus production can lead to intestinal permeability.) Given that this mucosal lining is an important habitat for intestinal commensal microbes, ANS functions directly impact the gut's microbial ecosystem.

The autonomic nervous system has two branches, which are both important in gut-brain communication: the parasympathetic nervous system and the sympathetic nervous system. The sympathetic nervous system is associated with our fight-or-flight response during stressful situations. When the brain perceives any immediate threat of injury or harm, the sympathetic nervous system triggers a number of physiological responses to this type of stress. For instance, it speeds up heart rate and increases blood flow to muscles, preparing the body for intense action in response to external stress.

As a part of the fight-or-flight response, the sympathetic nervous system also inhibits intestinal motility. Specifically, the sympathetic nervous system activates the production of adrenaline and noradrenaline, two important hormones involved in the stress response. These work as chemical messengers that influence the activity of internal organs in response to stress or perceived stress.

The parasympathetic nervous system controls the body's unconscious actions. Typically, these two branches of the autonomic nervous system are not simultaneously activated. When the sympathetic nervous system is activated, the parasympathetic is suppressed, and vice versa. Maintaining a balance between the actions of sympathetic and parasympathetic nervous system is important.

SIDEBAR 8.1 Motility

As mentioned in chapter 6, maintaining gut motility is very important in the prevention of microbial dysbiosis. Our body facilitates this movement through migrating motor complexes controlled by the parasympathetic nervous system. These motor complexes are electrical waves that pass through the stomach and intestines during a fasting state, such as between meals. They are also the cause of stomach rumbling as a result of hunger.

This movement through the GI tract facilities transportation of bacteria from the small intestine to the large intestine. The flow of GI materials also inhibits bacteria from moving from the large intestine back to the terminal ileum, the most distal part of the small intestine. If this protection mechanism fails, bacterial populations in the small intestine increase beyond normal levels, causing small intestinal bacterial overgrowth (SIBO).

Impairment of the parasympathetic nervous system may result in abnormal motor complexes. These abnormalities are associated with functional bowel disorders such as chronic constipation or chronic diarrhea. For example, giant migrating contractions are reduced in slow-transit constipation, whereas they are increased in diarrhea related to irritable bowel syndrome (IBS).

Certain microbes may interact with the parasympathetic nervous system to influence gut motility. Commensal organisms such as *Bifidobacterium bifidum* and *Lactobacillus acidophilus* can promote gut motility. Other bacteria, such as *Escherichia* species, can inhibit motility.

Diabetes is also related to alterations in gut motility. Diabetes causes impairments of the enteric nervous system, which can lead to dysfunctions in gut motility. During the first stages of diabetes, altered motility may, in fact, increase the rate of stomach emptying. However, progression of diabetes is associated with slower rates of stomach emptying and even the development of gastroparesis, a chronic condition in which stomach emptying malfunctions.

Animal models of type 1 diabetes reveal decreased numbers of neurons throughout the GI tract. While researchers are uncertain why diabetes causes degeneration in the enteric nervous system, it is possible that high blood sugar levels increase cell death, affecting neural cells. The effects of altered motility on gut microbiota are well documented. These changes in gut microbiota can interfere with normal neurotransmission and can have a direct effect on gastric motility, through signals to the central nervous system.

Vagus Nerve

One specific nerve within the parasympathetic nervous system, and one of the most direct pathways of communication between gut microbiota and the brain, is the vagus nerve. Vagus means "wandering," as this nerve wanders from the brain to many major organs, including those within the gastrointestinal tract.

Although this nerve is referred to in the singular, it is actually a group of twelve paired nerves.

The central nervous system receives information about the state of the gut lumen through the vagus nerve. For instance, this nerve transmits information about nutrients in the gut, as well as messages from various microbial metabolites.

Vagal tone indicates the "activity" of the vagus nerve and can measure vulnerability to stress. The brain controls the activity of neurons that send impulses from the central nervous system to visceral organs through the vagus nerve. In the presence of greater vagal tone, the body is able to promote a resting state in which its energy reserves are conserved. On the other hand, when vagal tone is diminished, this promotes more rapid energy expenditure and helps the body respond to immediate environmental stressors.

Dysfunction of the vagus nerve is tied to autoimmunity and neurodegenerative conditions like Parkinson's disease (more about this later in the chapter).

Stress and the HPA Axis

The hypothalamus is a part of the brain that regulates the autonomic nervous system as well as certain endocrine glands. These glands and the hypothalamus make up the hypothalamic-pituitary-adrenal (HPA) axis, another primary pathway of communication between gut microbes and the brain. The HPA axis regulates how the body responds to various stressors.

Along with the hypothalamus, the pituitary gland and adrenal glands communicate with each other using various hormones. The pituitary gland is an endocrine gland that produces a hormone called corticotropin. This hormone plays an important role in the HPA axis and the body's response to stress. Its primary role is to stimulate the adrenal glands (two other endocrine glands) into producing cortisol.

The hypothalamus produces corticotropin-releasing hormone, which triggers the pituitary gland to signal the adrenal glands to produce the hormone cortisol in response to stress. This includes stress as a result of systemic inflammation related to increased intestinal permeability. Cortisol, which suppresses the immune system, is the reason why stress can directly affect immune function.

Interestingly, germ-free mice have higher levels of corticotropin-releasing factor, which increases circulating cortisol in these animals. In addition to increased cortisol levels, germ-free mice have an altered HPA axis, which explains these differences in their stress-responses. Researchers repeatedly observe that germ-free mice who are exposed to external stress have an

exaggerated physiological stress response. They also observe that in mice with normal gut microbiota, exposure to stress can change the composition of these microbe populations. These two observations lead researchers to wonder how gut microbiota promotes a normal stress response and whether manipulation of the gut microbiota can in turn influence how we respond to various daily stressors. Is it possible that the gut microbiota can change stress-related behaviors?

As they continue to explore this question, researchers notice that the presence of normal gut microbes is a critical component to the formation of the body's natural stress responses. Interestingly, despite elevated cortisol levels, germ-free mice lacking normal gut microbes demonstrate less anxiety-like behavior, compared to control mice, when exposed to stressful stimuli. These behaviors are corrected if the germ-free mice are colonized with normal intestinal flora by a certain age. This observation leads researchers to hypothesize that gut microbiota significantly influence the development of these neural responses during a young animal's life. In fact, a number of animal studies show that gut microbiota help program the HPA axis.

On the other hand, certain gut microbes can promote anxiety-like behaviors in mice. Researchers identified a couple of species (*Campylobacter jejuni* and *Citrobacter rodentium*) that induce anxiety-like behaviors in mice within just a few hours after introduction.

During the early period of life, both the stress response and gut microbe populations are developing. For this reason, exposure to harsh stressors during this period can shape the adult stress response. To test the impact of early life stress on microbiota composition, researchers exposed rat pups to stress by separating them from their mothers for a few hours each day. The pups that experienced maternal separation developed increased anxiety, as well as functional bowel disorders. Surprisingly, the young rats' gut-brain axis was altered in such a way that caused any stress exposure to signal the autonomic nervous system and increase gut motility. The stress-related boost in gut motility therefore increased fecal output.

Changes in the HPA axis can also prevent the body's normal suppression of the immune system. Studies in rats show that consistent exposure to stress changes the rat's gut microbiota populations in ways that encourage inflammation. In fact, researchers noticed that in addition to altered gut microbes, chronic stress increases pro-inflammatory cytokines. Given our understanding about the effects of inflammation on gut health, it is no surprise that stress and its tendency to increase pro-inflammatory molecules can disturb the gut epithelium and increase intestinal permeability. Furthermore, other studies find that social stress can promote psychological states that increase translocation of gut microbiota.

SIDEBAR 8.2 Alcoholism Alters Microbes and Mood

Alcoholism may also have detrimental effects on the gut microbiota that are associated with increased anxiety and depression. Specifically, elevated lipopolysaccharides and increased intestinal permeability are associated with these distinct psychological characteristics in individuals with alcohol dependence.

Chronic alcoholics who also have more intestinal permeability showed more signs of depression and anxiety. Compared to other chronic alcoholics, those with increased permeability also showed alterations in gut microbiota composition. Specifically, they had lower populations of *Bifidobacteria* and *Faecalibacterium prausnitzii*. These observations led researchers to hypothesize that excessive alcohol consumption may deplete beneficial bacteria in certain individuals, leading to dysbiosis and increased permeability.

In other studies that examined the impact of stress on adult gut microbiota, researchers found that animals who are exposed to chronic physical stress in adulthood also displayed compositional changes. Specifically, they had lower populations of *Bacteroides* species and an abundance of *Clostridium* species compared to animals who had not been exposed to this type of stress.

Given these abnormal stress responses in germ-free mice and the stress-induced changes in microbiota, researchers wondered how gut microbes were able to modulate the body's reaction to stress. They hypothesized that gut microbiota interact directly with the HPA axis. While researchers can clearly observe changes in microbiota and inflammatory biomarkers, identifying the specific behaviors associated with an altered HPA axis response has been somewhat more challenging. For instance, when researchers placed germ-free mice through anxiety assessment tests, they expected to see an increase in anxiety-like behavior. Instead, the mice displayed lower anxiety-like behavior, and they showed a significant increase in exploratory behaviors.

Despite these interesting observations in animal studies, these observations are not easily replicated in human studies. After all, we may see some parallels, but humans are obviously quite different from rats. Humans have the ability to develop different types of adaptive qualities that can determine our psychological and physiological response to stressful stimuli. Researchers are working to identify which variables to take into account when trying to understand the implications of these observations in animal studies. It is possible that in humans, the microbiota can influence susceptibility to abnormal stress responses, although researchers do not fully understand how microbes may directly affect mood in humans.

A probiotic supplement consisting of specific strains of *Lactobacillus helveticus* and *Bifidobacterium longum* has been found to significantly influence stress responses. One human clinical trial found that the combination of these two strains reduced both physiological distress and cortisol levels. We also observe that stress and anxiety are connected to irritable bowel disease (IBD). This indicates the involvement of the gut-brain connection, as gut function appears interrelated with mood and psychological state. Also, researchers notice that stress during pregnancy appears to cause changes in toddlers' microbiomes.

Probiotics and Prebiotics for Mood Regulation

Many microbiome researchers are skeptical of studies that claim therapeutic benefits of prebiotics and probiotics for mood regulation. For instance, one study tested a commercial prebiotic supplement that was administered to individuals who were then asked to collect their own saliva samples upon waking up in the morning. Researchers used these samples to determine levels of cortisol in the saliva. The supplement, which contained galactooligosaccharides, decreased the amount of early morning cortisol, a stress hormone, suggesting anti-anxiety effects. The body naturally experiences a surge of cortisol within the first 20–30 minutes of waking, as a means to prepare for potential stress it may face during the day. These researchers speculate that prebiotic galactooligosaccharides can be used to alter this stress response. While this decrease in cortisol is an interesting observation, critics question the conclusion that these prebiotics might have similar benefits to certain anti-anxiety and antidepressant medications.

For consumers, it is important to be cautious about bias in these types of clinical trials. Some studies are, in large part, funded by supplement companies looking to promote their products. This is true not only of prebiotics but also for probiotic supplements that claim to help with anxiety and depression. That said, researching the potential effects of probiotic bacteria on overall mental health is important. Currently, a number of strains show promise in this field, and further exploration of their effects may provide better understanding about different aspects of the gut-brain axis.

For example, probiotic studies show that *Bifidobacterium longum* and *Lactobacillus helveticus* can influence cortisol levels and have some effect on HPA activity. When taken together, these two probiotics may effectively lower this stress hormone in humans. Another lactic acid bacterium, *Lactobacillus farciminis*, helps reduce gut permeability induced by stress. In rat studies, this bacterium attenuates the stress response by influencing the HPA axis. *L. farciminis* prevents increased permeability and limits excessive amounts of LPS

from entering circulation through portal blood. These actions reduce the effect of acute stress on HPA activity.

Probiotic beverages containing *Lactobacillus casei* also show potential effectiveness in treating anxiety and depression. One study found that three weeks of consuming this drink improved mood in elderly individuals with depression. However, another group, with chronic fatigue syndrome, did not notice any improvement in depression. They did, however, observe a decrease in anxiety.

The effectiveness of probiotic treatments for mood dysregulation in humans is difficult to assess. In fact, many factors limit the ability to measure the direct effects of these probiotics. It is difficult to measure baseline mood, and studies may also differ in their assessment of mood improvement following the treatment. Additionally, the composition of gut microbiota prior to treatment may also affect results.

Diet and Mood

In animal studies, diet has also been shown to influence behavior. For instance, a long-term high-fat diet can produce adverse health behaviors in rats. These animals are more likely to display anxiety-like or depressive-like behavior. As mentioned in previous chapters, a high-fat diet can drastically alter gut microbiota composition, which may in turn influence behavior in these animals.

One group of researchers observed that carbohydrate malabsorption may be an early sign of mental depression. The study conducted by this group tested the correlation between the malabsorption of two disaccharides, lactose and fructose, and higher scores on a depression questionnaire. Participants of the study, who all reported GI discomfort related to gas and bloating, were given 50 grams of lactose and 50 gram of fructose with 7 days between the two carbohydrates. That is the equivalent amount of the lactose found in about five cups of milk and the amount of fructose found in a little less than a liter of soda.

Malabsorption was measured through a breath test, which detects higher levels of hydrogen, produced by gut microbes through the fermentation of these carbohydrates. When these disaccharides are not absorbed before they reach the large intestine, they become available to gut microbes for their own digestive process. While the study was not able to establish any direct cause-effect relationship between carbohydrate malabsorption and depression, the researchers believe that fructose malabsorption may have some role depression. The study did not show an association with lactose malabsorption, at least not when consumed on its own. Researchers noted that lactose malabsorption did exacerbate the effects of fructose malabsorption on early signs of depression. However, these associations were observed only in female study participants and not males.

SIDEBAR 8.3 Sleep, Cortisol, and Immunity

Our sleep-wake cycles, which are regulated by circadian rhythm, are also connected to the gut-brain axis. We have previously discussed how disrupting our sleep schedule can negatively affect our gut bacteria. Chronic jet lag and sleep apnea (interrupted breathing during sleep) alter sleep patterns and cause changes in both diversity and composition of gut microbiome.

Bacteria produce peptides that stimulate immune cells in the gut to produce cytokines. These cytokines induce non–rapid eye movement sleep. Cortisol inhibits the production of these cytokines, and cortisol is released based on a circadian rhythm. Cytokines thus also follow a circadian rhythm, and the highest levels in the blood are found around midnight. Researchers speculate that the microbe-derived cytokines facilitate transitions between different stages in the sleep cycle. In this way, the level of exposure to cytokines may determine sleep patterns. Excessive production of pro-inflammatory cytokines is linked to altered sleep.

GABA

Gamma-amino butyrate, or GABA, is the primary inhibitory neurotransmitter in the central nervous system. One of its main roles is to inhibit the activity or excitability of nerve cells. Since this neurotransmitter reduces neuron activity, impaired GABA function is linked to overexcited neurons and is associated with a number of psychological disorders. In fact, when this neurotransmitter is depleted, it may lead to alterations in mood, such as depression, anxiety, post-traumatic stress disorder, and even chronic pain. In fact, common anti-anxiety medications such as Xanax and Ativan (a class of medications called benzodiazepines) target the same receptors as GABA and enhance its effects.

Although GABA is mostly produced in the brain, this neurotransmitter can be produced by certain gut bacteria. Also, while some bacteria produce GABA, others actually consume it and use it to stimulate their own growth. For instance, a number of *Lactobacillus* species and *Bifidobacterium* species produce GABA, whereas *Lactobacillus brevis* converts monosodium glutamate (MSG) to GABA. Other *Bifidobacterium* are capable of this same conversion, though they are less efficient in this process.

MSG is part of an amino acid called glutamate that naturally occurs in a number of foods, including cheese, tomatoes, processed soy products, and certain seaweeds. MSG is also commonly used by the food industry as a favor enhancer. Glutamate provides a naturally occurring "umami" taste that can enhance the savory flavor of cooked foods. Umami is considered one of five

main tastes found in food. Although there is some question around the safety of MSG added to foods, research indicates that the chemical structure of naturally occurring MSG in glutamate is the same as that of MSG food additives. Yet, many individuals complain of headaches and general malaise following consumption of MSG in processed foods. It may be interesting to consider the effects of MSG in the body in the context of microbial metabolism. Is it possible that the microbial conversion of MSG to GABA may influence its effect on the brain?

Other gut bacteria are also able to influence the production of GABA. One probiotic bacterium, *Lactobacillus rhamnosus*, uses the gut-brain axis to influence responses toward stressful stimuli. Mice given *L. rhamnosus* had both decreased anxiety-like behavior and increased levels of GABA during stressful situations. It seems likely that this bacterial strain may ease anxiety by enhancing GABA function. Also with this probiotic, the stress response of the mice produced lower levels of the hormone corticosterone.

Although GABA clearly interacts with gut microbes in a number of ways, researchers are still curious how it may specifically interact with GABA in the central nervous system. These observations with *L. rhamnosus* (which came from mouse studies), revealed that the effects of this probiotic strain rely on the vagus nerve. When mice underwent a procedure called a vagotomy, in which the vagus nerve is severed, the beneficial effects did not take place. Without a functioning vagus nerve, *L. rhamnosus* did not reduce corticosterone during the stress response. The vagotomy also inhibited the bacteria's effects on depression- and anxiety-like behaviors. The ineffectiveness of *L. rhamnosus* in mice with defective vagus nerves is a good demonstration of how gut microbes use this nerve to communicate with the brain and influence mood and behavior.

Serotonin

Serotonin is a neurotransmitter that plays a key role in gut-brain communication. It is generally responsible for keeping our mood balanced. Levels of this hormone within the body determine whether we experience emotions such as happiness, depression, or anxiety. Current research provides insight into how gut microbes influence serotonin in the gut and therefore has significant effects on behavior and brain function. In addition, serotonin also has a number of important functions in the gut, such as activating gut motility.

One of the first observations connecting gut microbes and serotonin involved experiments from the 1960s, when researchers discovered that germ-free mice have decreased gut motility. These researchers suggested that lower levels of serotonin in these animals may be the cause of this change in motility. Additionally, gut mobility increases in these germ-free mice when they are inoculated with normal gut microbiota.

Germ-free mice, in fact, have altered levels of serotonin compared to conventional mice. Given this observation, scientists more recently began exploring how gut microbes regulate serotonin in the gut. About 90% of the body's serotonin is found within the gastrointestinal tract. This neurotransmitter is produced by enterochromaffin cells, which are located along the epithelial lining of the gastrointestinal tract. Researchers believe that one way microbes can influence serotonin levels is though their interactions with enterochromaffin cells.

Enterochromaffin cells can also help transmit messages from microbial signaling molecules. Due to their location within the mucosa, these cells are easily accessible to luminal microbiota. Enterochromaffin cells use an amino acid called tryptophan to make serotonin. Changes in gut microbiota can alter the availability of tryptophan, which is required by the body in order to synthesize serotonin. Tryptophan is an essential amino acid (a building block of protein), meaning it is not produced by the body and must be supplied by the diet. Tryptophan is also transported to the CNS, another location where it is used in the production of serotonin, but the vast majority of serotonin is produced in the gut. Interestingly, germ-free mice have higher levels of tryptophan in their bloodstream, though they are shown to have depleted levels of serotonin.

Some bacteria also use tryptophan to meet their own growth requirements. For this reason, the host may actually be competing with its gut microbes over available tryptophan. Bacteria may have enzymes that either work to produce tryptophan or use it for the production of other compounds (such as indole or serotonin). For instance, the probiotic strain *Bifidobacterium infantis* can promote the production of tryptophan, which the body uses the create serotonin.

Bacteria can also influence serotonin produce via their metabolites. Some evidence suggests that certain bacterially produced short-chain fatty acids may influence the production of serotonin by enterochromaffin cells. Acetate and butyrate, as well as other bacterial metabolites like secondary bile acids, are most likely to interact with these cells and influence the rate of serotonin production.

Not all microbes have such effects on serotonin. Some members of the normal gut microbiota can more directly assist in the production of serotonin. Researchers are now looking to identify different groups of bacteria or specific strains that may affect this hormone. Spore-forming bacteria, specifically *Clostridia*, appear to best induce the gut to produce serotonin. Also, some members of *Enterococcus* and *Streptococcus*, two genera belonging to the Firmicutes phylum, are capable of increasing serotonin levels.

Finally, it is important to mention that the serotonergic system, which determines the production and function of serotonin, is not fully established at birth. In fact, some parts of this system only finish developing during the

teenage years. It may not be a coincidence that the body's serotonergic system develops slowly during the first part of life, considering the gut microbiota is also still developing during the first few years or life. These early formative years provide a window of opportunity for microbiota to influence the serotonergic system.

Despite these observed connections between microbes and serotonin production, more research is needed to understand the effects this might have on various tissues in the body whose functions rely on serotonin.

Gut Microbes and Autism

In addition to the many ways microbes influence mood through the gut-brain axis, they also appear to have a significant role in determining the progression of neurological disorders. One quickly growing subset of gut microbiome research involves its implications in the development of autism. Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects behavior and cognitive function. This diagnosis is typically made in early childhood. Interestingly, the gut microbiome is very malleable during these earliest years of life. This is also a vulnerable time for brain development. Given that both the brain and the gut microbiota are developing during this time, many researchers are exploring the role of gut microbiota in this neurodevelopmental disorder.

Over the past few decades, the incidence of autism in the United States has continued to increase. Currently, about 1 in every 45 American children is diagnosed with autism spectrum disorder. According to the Centers for Disease Control, boys are 4.5 times more likely to have autism. About 1 in 42 boys will have this diagnosis, compared to 1 in 189 girls.

Autism Diagnosis

There are no diagnostic tests that clearly identify autism. This is due to the lack of any clear biomarkers, or measurable substances that can be identified as an indicator of the disease. Instead, a diagnosis is usually made based on observed characteristics. Symptoms of autism include cognitive and behavioral problems, as well as impaired language and communication. The problem with diagnosing based on behavioral abnormalities simply is that there are many causes and conditions linked to similar behavioral problems.

Although the cause of autism is unclear, there is a general consensus that combinations of environmental and genetic factors play a role in its development. Researchers are now exploring how the gut microbiota may affect any of these risk factors. Currently, they are examining changes in gut microbiota composition and their potential associations with autism-like behaviors. While researchers are hopeful that the gut microbiota may provide potential insight

into the physiological characteristics of autism, they are still far from knowing whether the gut microbiota might provide biomarkers for autism diagnosis.

In addition to behavioral symptoms, children with autism frequently experience gastrointestinal problems—although only a subset of autistic individuals have these symptoms, and it is still unclear just how prevalent gut problems are for this population. To that end, researchers are working to determine whether GI disorders are more prevalent with autism, as compared to other disorders or within the general population. The most common GI symptoms reported in children with autism are abdominal pain, abnormal bowel movements (diarrhea and constipation), and bloating/gas. The severity of these GI issues can often be associated with the degree of behavioral abnormalities such as irritability, hyperactivity, and social withdrawal. Yet, it is still unclear whether these GI symptoms occur first and then cause changes to the gut microbiota or, perhaps, the changes in microbiota are causing these GI symptoms.

Dysbiosis in Autism

Currently one of the strongest connections between autism and gut microbiota are the changes in composition and diversity of microbial populations. Researchers have observed states of dysbiosis in autism, though no specific taxa are known to be responsible for causing the condition. Further, the abnormalities in gut microbiota composition that are observed in autism could be also related to other factors such as altered diet. Many children with autism are on restricted diets or taking medications (particularly antibiotics, which tend to be used more frequently among this population) that can change the composition of the gut microbiota. So it is difficult to assess whether observed changes are inherently present during disease development or whether perhaps there are other factors related to this condition that cause dysbiosis to occur.

Bacteroidetes populations tend to be larger in autistic children, making up about 50% of total microbiota. On the other hand, gut microbiota in non-autistic children is only about 30% Bacteroidetes, and instead shows more abundant populations of Firmicutes. These children had over 60% Firmicutes in their stool samples, whereas in autistic children, Firmicutes accounted for just under 40% of gut microbes. From these observations, researchers conclude that autism spectrum disorder is associated with a decreased Bacteroidetes-to-Firmicutes ratio.

A number of important saccharolytic bacteria are found to be lower in autistic individuals, and so metabolites from carbohydrate fermentation are reduced. In fact, the severity of ASD is also connected to lower SCFA production, due to the decreased abundance of saccharolytic bacteria. More research is needed in order to understand the implication of these changes in metabolite production. For instance, if butyrate-producing bacteria are reduced in autism, this may affect overall intestinal permeability.

Although SCFA produced by bacteria are generally recognized for their health benefits, some researchers discovered a potential downside to certain SCFAs in autism. Propionate, for instance, may have neurotoxic effects that may contribute to the development of autism. The reason for this increase in propionate production among some autistic children is still unknown. It is possible that the increase of Clostridial species often seen in autism may cause excessive fermentation that results in higher levels of propionate.

Animal Model for Autism

The specific ways in which the gut microbiota are involved in autism remain unclear, but there is good evidence that some microbes can influence social behavior. Just like most other conditions, the vast majority of this evidence is sourced from research on the done using animal models. However, this research works with animal models that display autism-like behaviors, given that it is not possible for animals to be specifically characterized as autistic. Scientists must therefore look at environmental or genetic factors that can be translated to an animal model so as to mimic how these factors may contribute to autism-like behaviors.

The Maternal Microbiome and Autism Risk

Researchers are also considering the state of the maternal microbiome during infant colonization. As we discussed in chapter 2, gut microbiota are passed down to offspring from the mother. Environmental or genetic factors may shape the maternal microbiome. Stress during pregnancy, antibiotic use, and even the mother's age can cause changes in the mother's gut microbiota.

Researchers are exploring whether any of these factors influence the maternal microbiome in ways that may be associated with autism. They are considering the effects of exposure to various stressors during pregnancy. For instance, there is some evidence that maternal infection during pregnancy may increase risk of autism. While this factor may not be a primary cause of autism, it appears that it may exacerbate the effects of other environmental or genetic factors in the development of the condition.

If altered microbiota are passed on to offspring at birth, these imbalances may have some effects on the infant's developing gut-brain axis. However there is currently no conclusive evidence that mode of delivery increases risk of developing ASD.

Other factors relating to maternal environment may also be relevant to the risk of autism in their offspring. For example, obesity can be related to alterations in microbe composition. If a mother is obese during pregnancy and consumes a high-fat diet, the offspring is more likely to have altered gut microbiota. In mice, these types of diet-induced changes in gut microbe populations

are linked to behavioral deficits that affect sociability. Yet when these mice where given gut microbes from healthy control mice, these deficits improved completely, and the mice displayed normal social behavior. This is another clear example of how gut microbes affect behavior in mice—but, again, leaves researchers wondering whether parallels can be drawn with human behavior, particularly with the behavioral deficits seen in autistic individuals.

Microbiota-Related Treatments for Autism

There are relatively few studies on gut microbiota manipulation as a treatment for autism-associated behaviors. Some researchers have explored whether altering gut microbiota populations with the use of antibiotics may improve behavior. For instance, one clinical trial showed that antibiotics (specifically vancomycin, an antibiotic typically used to treat intestinal infections) did somewhat improve behavioral issues in autistic individuals. However, the investigators noticed that this was a transient effect. Once the trial participants were taken off the antibiotic, the abnormal behavior returned.

Also it is important to consider that *Clostridium* species often form spores that are resistant to antibiotics. Given that this class of bacteria is more abundant in people with autism, the vancomycin treatment may simply cause these microbes to hibernate until the antibiotic is no longer present. Antibiotic treatments are likely not a viable long-term solution to behavioral issues, given the concern for promoting dysbiosis.

There is also considerable interest in exploring probiotic treatments to improve autism symptoms. Much of the probiotic-medicated behavioral changes are observed in animal studies. For example, *Bacteriodes fragilis*, when used as a probiotic treatment in mice, corrects autism-like behaviors. Although no clinical trials indicate similar effects in humans, it is good to note that populations of *Bifidobacterium* are depleted in autism. Another probiotic bacterium, *Lactobacillus reuteri*, restores altered social behavior seen in germ-free animals. However, the effects of this strain in humans are uncertain, and researchers caution against its use in ASD individuals at this time.

Diet and Autism

Despite conclusive data about the role of diet in autism, many parents have turned to elimination diets to help with gastrointestinal distress associated with this condition. For instance, many parents with autistic children may try to eliminate wheat and dairy. They argue that specific proteins in those foods cause digestive problems for their children. Furthermore, some of these parents have attempted to eliminate these offending proteins in hopes of also improving autism-associated behaviors. Yet, studies examining gluten and casein—the potentially problematic proteins in wheat and dairy, respectively—do not

indicate any significant evidence that these foods are poorly digested by autistic individuals or contribute to behaviors in autism.

Despite the lack of evidence that protein in wheat and dairy present digestive issue for autistic individuals, there has been new research on the role of dietary carbohydrates in GI discomfort. One group of scientists observed that some autistic individuals may be lacking in certain enzymes, called disaccharidases, which the body normally uses to digest disaccharides. Without this enzyme, disaccharides such as lactose and sucrose (table sugar) are improperly digested in the small intestine and may cause GI discomfort. So it is possible that parents who remove dairy from their autistic child's diet may notice improvements if there are digestive issues resulting from the carbohydrate components (i.e., lactose) of those dairy products. Furthermore, given the observation that the abundance of saccharolytic bacteria is decreased in autism, this further inhibits carbohydrate metabolism.

Immune Function and Inflammatory Markers in the Brain

One hypothesis proposes that the development of autism may be linked to increased intestinal permeability that leads to inflammation in the brain. In addition, there are a number of dysfunctions within the immune system in autism, indicating further possible dysregulation of inflammatory response. Inflammatory markers such as increased cytokines are found in the brains of deceased autistic individuals. These inflammatory markers were increased within the cerebrospinal fluid and in regions of the cerebral cortex. Also, deficiencies of certain T cells are observed in ASD. Specifically, the numbers of T regulatory cells and certain T helper cells can be lower in autistic individuals.

Furthermore, these individuals also had increased numbers of specialized macrophage cells in the brain and spinal cord. They are an important part of immune defense in the central nervous system. This is interesting, given that gut microbiota play a role in the proper function of these macrophages. In germ-free mice, these immune cells are altered and consequently produce an abnormal immune response during exposure to bacteria or a virus. These abnormal responses can be corrected if the mice are given SCFA supplements or a fecal transplant from healthy mice.

Neurodegenerative Diseases

As discussed in chapter 4, gut microbes interact directly with the immune system and can regulate many of its functions. Abnormal functions of the immune system contribute to the development of neurological disorders.

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease that affects the brain and spinal cord. This disease is characterized by a dysfunctional immune response that attacks parts of the nervous system and prevents its proper communication with the rest of the body. As the disease progresses, individuals with MS experience a severe decline in neurological function. The disease is progressive and affects motor function, causing loss of coordination and speech.

This neurodegenerative disease causes a part of neurons called the myelin sheath to deteriorate. Myelin is a fatty substance that covers and insulates nerve cells. The myelin sheath serves as an electrical insulator. Multiple sclerosis is also characterized by inflammation of the nervous system. Researchers are now exploring how gut microbes may play a role in the development of immune dysregulation and persistence of inflammation in MS.

As with other autoimmune diseases, MS is associated with over-activation of the immune system. Interestingly, individuals with MS also have a breakdown of the blood-brain barrier. This barrier typically protects the brain from invading pathogens. Researchers are now working to determine how inflammation and immune dysregulation play a role in the development of MS. Furthermore, they are working to determine whether gut microbes interact with the immune system to influence MS symptoms.

Researchers working to identify the mechanisms behind MS use an animal model of the disease that mimics some of the characteristic of MS. Researchers developed a disease in mice, called autoimmune encephalomyelitis, that mimics brain inflammation and causes damage to the myelin sheath.

Using this model, researchers have noticed that specific *Clostridia* strains and *Bacteroides fragilis* can activate T regulatory cells to suppress MS-like symptoms in mice. Then, if the activity of these T cells is blocked, *B. fragilis* is no longer able to protect against symptoms of MS. This indicates that interactions between T regulatory cells and commensal gut microbes may play a role in MS-like symptoms.

After this observation was made in mice, researchers analyzed gut microbiota populations in MS patients. There were distinct differences between the gut microbiota of MS patients, compared to healthy individuals. Specifically, a number of Bacteroidetes species and *Clostridium* species were much less abundant. These bacteria are known to have anti-inflammatory effects.

Researchers working with autoimmune encephalomyelitis animals recently discovered a potential therapeutic probiotic mixture of *Lactobacillus* species. Administration of this mixture reduces the number of inflammatory cells and suppressed the disease in the animal model.

SIDEBAR 8.4 Cigarettes, Coffee, and MS

Findings of research into multiple sclerosis reveal a protective role of cigarette smoking and ingestion of caffeine-containing beverages such as tea and coffee. Although there is no conclusive understanding for these interesting effects, some scientists point to the interactions between gut microbiota and the brain as an explanation. One group suggests that smoking cigarettes and drinking coffee alters microbiota composition in ways that reduce inflammation in the gut. For instance, drinking coffee can boost *Bifidobacteria* populations. However, it goes without saying that while coffee may have some general benefits, smoking should be considered in the context of its many health detriments.

Parkinson's Disease

Parkinson's disease is another neurodegenerative disorder that affects the central nervous system. A major complication with Parkinson's disease is impaired movement. Typically, the first observed motor problems are related to general slowness of movement and trouble walking. However, at the beginning stages of this disease, gastrointestinal symptoms associated with the disease may appear long before more overt Parkinson's symptoms.

Dysfunction in the GI tract is seen in over 80% of Parkinson's disease patients. Researchers hypothesize that alterations in the brain-gut axis may not only cause problems along the digestive tract but may also contribute directly to the development of Parkinson's. Issues with GI motility in Parkinson's disease result from damage to the vagus nerve and enteric nervous system. This disease causes GI motility dysfunction that leads to delayed stomach emptying and constipation. Due to these motility issues, small intestinal bacterial overgrowth (SIBO) occurs more frequently in individuals with Parkinson's disease.

These individuals are also more likely to develop ulcers and *Helicobacter pylori* infection. In fact, there is some evidence that *H. pylori* infection is associated with an increased risk for Parkinson's. Research shows that individuals who are prescribed medications for *H. pylori* infection for five or more years were at a 45% greater risk for developing the disease.

Interestingly, these issues are often observed long before individuals are diagnosed with Parkinson's. Given that these GI conditions are seen much sooner than other symptoms relating to the central nervous system, specific changes in gut microbes might provide earlier signals for this degenerative disease.

Interestingly, researchers who are working to identify specific changes in gut microbiota during Parkinson's disease noticed a lack of certain bacteria that are involved in protecting the intestinal mucosal lining. *Prevotellaceae*, a family of bacteria that is reduced in Parkinson's individuals, contains key

species that support mucosal integrity. Decreased populations of these bacteria limit the production of mucin and lead to intestinal permeability.

Differences in gut microbial composition also exist among Parkinson's patients themselves. For example, some differences occur depending on the individual's predominant symptoms of Parkinson's disease. Individuals whose posture and gait were unstable as a result of the disease have greater *Enterobacteriaceae* populations, compared to those whose predominant symptom was tremoring.

In addition to colonic dysbiosis, a group of researchers found that small intestinal bacterial overgrowth may also occur in up to 50% of Parkinson's subjects. When these patients were treated with antibiotics, some motor function problems were alleviated. Specifically, a symptom known as motor fluctuation improved. These motor fluctuations are movement problems that commonly occur following long-term use of certain Parkinson's medications. As the medication wears off, patients experience decreased control of motor skills. Researchers conclude that SIBO can exacerbate problems with motor function in Parkinson's patients.

SIDEBAR 8.5 Restless Legs Syndrome

Restless legs syndrome (RLS) is a neurological disorder that creates abnormal sensations, causing an uncontrollable urge to move the legs. These symptoms are generally present when a person is at rest. Individuals with this condition often have difficulty sleeping. Although the severity of these symptoms can vary from person to person, this condition affects around 10% of the U.S. population. The cause of this condition is unknown, but researchers have pointed to genetics, as well as the disruption of the brain's dopamine pathways.

Restless legs syndrome is associated with dysfunction of the gastrointestinal tract. Specifically, individuals with this condition are more likely to be diagnosed with irritable bowel syndrome and small intestinal bacterial overgrowth (SIBO). Other GI diseases, such as Crohn's disease and celiac disease, are also both associated with restless legs syndrome. Researchers believe that these GI disorders predispose individuals to restless legs syndrome, possibly due to the chronic inflammation associated with these diseases. One study reveals that 82% of participants with RLS had IBS. This is a considerably high percentage, given that only about 4% of the general population controls had this GI disorder.

Immune-mediated GI disorders (such as celiac disease and Crohn's disease) may have detrimental effects on central and peripheral nerves as a result of the inflammatory immune response that characterizes these disorders. While it is unknown whether the gut microbiota play a role in RLS, dysbiosis is associated with these GI disorders, which may increase the risk for RLS. Interestingly, restless legs syndrome is alleviated when SIBO is treated with antibiotics. This observation may encourage more research to better understand the possible link between dysbiosis (whether in the small intestine or in other parts of the GI tract) and RLS.

Cirrhosis and Brain Inflammation

Gut bacteria convert urea in the intestine to ammonia, a toxic compound that can cause brain inflammation. The liver helps detoxify the body from excess ammonia and prevents this toxin from entering circulation. However, an unhealthy liver may have a buildup of scar tissue, called cirrhosis. This condition prevents the liver from properly filtering toxins like ammonia. Unfortunately, ammonia is a neurotoxin and can impair brain function if it is continuously present in the bloodstream. Ammonia also damages the blood-brain barrier, a protective barrier that prevents unwanted substances from entering the brain. As a complication of cirrhosis, the build up of ammonia and other toxins in the brain causes a condition called hepatic encephalopathy. This condition is associated with cognitive decline, and individuals with this condition often experience memory loss, confusion, and irritability.

In cirrhosis, altered gut microbiota composition is found in individuals with cognitive dysfunction. Interestingly, these individuals noticed improvements in cognitive function when treated with a combination of probiotics and prebiotics. For instance, *Bifidobacterium longum* and fructooligosaccharide supplements taken together can lower the amount of ammonia in the bloodstream and improve brain function. Also, individuals with acute or chronic liver disease have disruptions in GABA and serotonin. Given that changes in the function of these two neurotransmitters may have implications on mood, it would be interesting to see how prebiotics and probiotics may influence them.

Lactic Acid and Cognitive Function

High levels of lactic acid, a metabolite of bacterial fermentation, are associated with memory loss. Excessive lactic acid production can be a complication of certain weight-loss surgeries that remove part of the small intestine. Although these operations were popular in the 1960s and 1970s for morbid obesity, there are many complications associated with these procedures, including abnormal speech, vision, and other neurological functions and so they are no longer performed, despite their effectiveness in promoting weight loss.

With this type of weight-loss procedure, a portion of the small intestine is surgically bypassed. This substantially affects digestion so that carbohydrates are not properly absorbed and instead pass into the large intestine. This increases bacterial fermentation in the cecum and colon, which in turn can increase the accumulation of acids such as lactic acid. Interestingly, when this surgical procedure was reversed, the negative neurological side effects improve.

Researchers are working to understand how elevated lactic acid from bacterial fermentation affects cognitive function. They propose that high lactic

acid levels could promote gut permeability and lead to systemic inflammation that eventually interferes with normal brain function. Other proposed mechanisms include the direct action of lactic acid on either the enteric nervous system or the brain.

Conclusion

There are four suggested pathways of communication between gut and brain: (1) the vagus nerve; (2) the immune system; (3) neurochemicals or other signaling molecules made by gut microbes; and (4) neurochemicals produced by the body through interactions with microbes. However, while human cell signaling is well documented, researchers are only beginning to characterize their microbial analogues. A large portion of the research on the gut-brain-microbiota axis relies on animal studies. Yet, there are concerns about drawing parallels between observations seen in these animal models and similar psychological states seen in humans.

The connections between dysbiosis and autism may offer more insight into this disease, whose cause has not yet been identified. For autistic individuals, addressing underlying dysbiosis with diet and probiotics has the potential to alleviate gastrointestinal side effects of the disorder. As for neurodegenerative diseases like Parkinson's and multiple sclerosis, understanding dysbiosis as a potential underlying risk factor may offer clinicians a window of early intervention.

Glossary

Adaptive immunity: a part of the immune system that can respond to specific microbes

Adrenal glands: endocrine glands that produce adrenaline and cortisol

Adrenaline: also known as epinephrine, a hormone and neurotransmitter that is responsible for the fight-or-flight response

Advanced glycation end-products: a harmful compound that is formed from fats or proteins that interact with sugars in the body and can contribute to aging

Anaerobe: an organism that lives in an oxygen-free environment

Antibody: also known as immunoglobulin; a protein produced in response to a specific antigen

Antigen: any substance that produces an immune response

Antioxidant: a substance that protects against damage caused by oxygen

Atherosclerosis: a condition in which plaque accumulates along an artery, causing it to harden and become narrower

Autoimmune encephalomyelitis: a type of animal model that creates brain inflammation that is used in multiple sclerosis research

Autoinducer: a molecule produced by bacteria that, based on its level of concentration, is able to determine genetic expression of these bacteria

Autonomic nervous system: a part of the peripheral nervous system that controls many involuntary bodily functions such as digestion, heart rate, and acute stress reactions

B cell: a lymphocyte that produces antibodies

Biofilm: a community of microorganisms that adheres to a surface and to each other

Biomarker: a measurable substance that can be used as an indicator of disease or other physiological conditions

Blood lipids: fatty substances in the blood, such as cholesterol and triglycerides, that indicate risk for cardiovascular disease

Branched-chain fatty acids: a byproduct of bacteria, mostly from the fermentation of dietary protein

Butyrate: a short-chain fatty acid produced during bacterial fermentation

Carbohydrate: a molecule found in foods and other living matter, usually in the form of sugar, starches, and cellulose

Carnitine: a compound that is concentrated in meat, which interacts with gut microbiota

Celiac disease: an autoimmune disease that causes hypersensitivity to gluten and leads to damage of the small intestine

Cholesterol: a fat-like compound made by the body but also found in foods such as eggs, fatty meats, and full-fat dairy products

Choline: a water-soluble vitamin that is produced by the liver but also obtained from a variety of dietary sources, including eggs, meat, wheat germ, and peanuts

Chylomicron: a particle that transports dietary fat in the body

Colonic crypts: glands found along the lining of the large intestine that help renew mucus

Commensal: a type of symbiotic relationship in which one species benefits while the other is unaffected

Conjugated linoleic acid (CLA): a type of fat that can be produced by bacteria and which may have beneficial effects on adipose tissue

Corticotropin: a hormone produced by the pituitary gland that functions as part of the body's stress response

Crohn's disease: an inflammatory bowel disease that leads to ulcers and causes abdominal discomfort, diarrhea, malnutrition, and weight loss

Cytokines: small proteins produced by various immune cells that function as cell-signaling molecules

Dendritic cell: a cell that processes antigens and presents them to T cells in the adaptive immune system

Disaccharidases: enzymes that break down disaccharides

Disaccharide: a sugar containing two monosaccharides; examples include sucrose and lactose

Distal: anatomical locations that are far from the center of the body

Dysbiosis: a state of microbial imbalance, particularly in reference to the composition and function of microbe populations

Endocannabinoid system: a signaling system made of up a group of cell membrane receptors that are involved in the regulation of appetite and energy metabolism

Endothelium: a layer of tissue that lines the blood vessels and heart

Endotoxin: also known as lipopolysaccharide; a molecule found as the outer part of gram-negative bacteria

Energy homeostasis: the process by which the body balances energy intake and expenditure

Enteric nervous system: a part of the nervous system that controls gastrointestinal functions

Enterochromaffin cell: a neuroendocrine cell within the epithelium that makes serotonin

Enterotype: a classification of humans based on their gut microbiome

Enzyme: a protein that facilities a specific chemical reaction within the body

Epithelium: a layer of tissue that lines body cavities, such as the intestine

Esophagus: a tubular organ that connects the throat to the stomach

Eukaryote: a multicellular organism, such as animals and most fungi, which is distinct from single-celled organisms like bacteria and archaea

Fasting-induced adipose factor (Fiaf): a protein that inhibits lipoprotein lipase, an enzyme that controls fat storage in adipose tissue

Flavonoid: a compound found in plant foods such as fruits and vegetables, which interacts with gut microbiota to enhance the health benefits of these foods

Fructan: a carbohydrate made of multiple fructose molecules that is fermentable by gut microbes

Gamma-aminobutyric acid (GABA): a neurotransmitter that inhibits excitability of neurons

Germ-free mice: mice that are raised in sterile conditions and lack normal gut microbes

Glucose: a simple carbohydrate (monosaccharide) that serves as the body's main source of fuel

Goblet cells: a type of epithelial cells that produce mucin, which is a component of the mucosa

Gram-negative bacteria: bacteria characterized by a thin cell wall and outer layer called a lipopolysaccharide; the thin cell wall of these bacteria is unable to retain the initial pigment in a Gram stain test

Gram-positive bacteria: bacteria characterized by a thick cell wall with no outer layer; the thick cell wall of these bacteria species retains pigment in a Gram stain test

Gut-associated lymphoid tissue: a part of the lymphatic system that contains important immune cells such as lymphocytes

Gut-brain axis: the pathways of two-way communication between the gastrointestinal tract and the nervous system

Gut microbiome: gastrointestinal microorganisms and their genome

Gut microbiota: the population of microorganisms living in the gut

Gut motility: movement within the gastrointestinal tract that supports the transit of digestive material

High-density lipoprotein (HDL) cholesterol: a type of cholesterol that is considered protective against cardiovascular disease

Homeostasis: the body's tendency to seek balance in its physiological functions and regulate its internal environment

Homocysteine: an amino acid that occurs as a byproduct of protein digestion and can be a risk factor in heart disease if levels become elevated

HPA-axis: a communication network between the brain and the endocrine system that is facilitated by the hypothalamus, the pituitary gland, and the adrenal glands

Hypertension: a condition in which blood places too much pressure on artery walls, to the point of causing health problems such as cardiovascular disease

Hypothalamus: a part of the brain that regulates the autonomic nervous system and the pituitary gland

Inflammation: an immune response that helps eliminate invading substances such as microbes

Innate immunity: the body's nonspecific defense mechanism against foreign microbes

Insoluble fiber: a type of dietary fiber that does not absorb water and is not easily fermented by bacteria

Insulin: a metabolic hormone produced by the pancreas that facilitates the use of glucose by cells for energy

Insulin resistance: a condition in which cells are not responsive to insulin, leading to high levels of glucose in the blood

Intestinal permeability: a characteristic of the intestine that controls the materials that move through cells in the gut barrier

Irritable bowel syndrome: a functional bowel disorder that causes abdominal pain, gas, bloating, and abnormal bowel movements

Lamina propria: a part of the intestinal mucosa that lies beneath the epithelium

Lecithin: a fatty substance that may interact with gut microbes

Leptin: a hormone produce by adipose cells that regulates appetite and energy homeostasis

Lipogenesis: a process by which fat is produced in the body

Lipopolysaccharides (LPS): also known as endotoxin; a molecule found as the outer part of gram-negative bacteria

Low-density lipoprotein cholesterol: a type of cholesterol that is associated with higher risk of cardiovascular disease

Lumen: the cavity of a blood vessel or an organ, such as those in the gastrointestinal tract

Lymphocyte: a type of white blood cell that includes T cells and B cells

Macronutrient: a nutrient that is required in large amounts by the body, such as carbohydrates, protein, and fat

Macrophage: a large phagocyte that engulfs foreign substances and helps eliminate them

Metabolic cross-feeding: a process by which one microbe is able to use the metabolites of another microbe

Metabolic syndrome: a cluster of risk factors (including obesity and high blood pressure) that increase risk of cardiovascular disease and type 2 diabetes

Metabolism: the chemical processes that sustain life

Metabolite: a substance produced or used during metabolism

Metabolomics: the study of metabolites present in or produced by an organism

Metaproteomics: the study of protein components such as those present in microbes and their metabolites

Micronutrient: any nutrient required by the body in small amounts, typically referring to vitamins and minerals

Microvilli: microscopic protrusions on the surface of villi that expand the surface area of the small intestine

Mitochondria: organelles in eukaryotic cells that produce energy

Monosaccharide: the simplest form of carbohydrate

Mucin: a type of protein that is part of a gel-like substance found in the mucosa

Mucosa: a lining along the gastrointestinal tract, composed of a layer of epithelial cells and a mucus layer

Myelin sheath: a covering that insulates the nerves

Neurochemical: a molecule that affects the nervous system

Neuron: a type of cell within the nervous system that is able to transmit nerve impulses

Neurotransmitter: a molecule that transmits signals between neurons

Neutrophil: a highly mobile while blood cell that is one of the first responders during an innate immune reaction

Nitric oxide: a compound that can dilate blood vessels and therefore protect against high blood pressure

Noradrenaline: a hormone and neurotransmitter that is released during stressful situations

Oligosaccharide: a carbohydrate typically containing between two and ten linked monosaccharides

Opportunistic: microorganisms that can become pathogenic under the right circumstances and generally cause acute infections

Paneth cells: a type of epithelial cell in the small intestine that can produce antimicrobial compounds

Parasympathetic nervous system: the part of the autonomic nervous system that regulates digestive function

Pathobiont: a pathogenic microorganism that is associated with chronic inflammatory conditions

Pathogen: a microorganism that causes disease

Peptide YY: a compound produced in the intestine after a meal to reduce appetite

Peristalsis: wavelike movements through the gastrointestinal tract that push contents through the lumen

Phagocyte: a type of cell that can engulf and remove harmful microorganisms

Phosphatidylcholine: a part of cell membranes that is also found in certain foods and can interact with gut microbes to create a toxic compound known as TMAO

Phytate: a compound found in many plants that can interfere with the absorption of dietary minerals

Phytochemical: any biologically active chemical compound found in plants

Pituitary gland: a gland in the endocrine system that regulates metabolism, blood pressure, and growth

Planktonic: organisms that float within the lumen of the GI tract rather than adhering to host cells

Polyphenols: a group of compounds found in plants that can benefit human health

Polysaccharide: a long-chain carbohydrate made up of many monosaccharide units linked together

Polysaccharide A: a substance produced by certain gut bacteria that reduces inflammation by inhibiting the activity of pro-inflammatory cytokines, while supporting the action of anti-inflammatory cytokines

Prebiotic: a non-digestible food that encourages the growth of beneficial microorganisms in the colon

Prokaryote: a single-celled organism, such as bacteria and archaea, that lacks a nucleus and other organelles that are present in eukaryotes

Proteolytic: a term referring to the metabolism of protein

Proximal: anatomical locations that are close to the center of the body

Quorum sensing: a system of communication used by bacteria to determine their gene expression based on their own population density

Reactive oxygen species: unstable molecules containing oxygen that can damage cells

Resistant starch: a type of starch that escapes digestion in the small intestine and is fermentable by colonic bacteria

Retrogradation: a reaction that causes gelatinized starches to recrystallize and become more resistant to digestion

Reverse cholesterol transport: a process by which cholesterol is transported back to the liver from other body tissues

Saccharolytic: a term referring to the metabolism of carbohydrates

Secondary bile acids: a substance produced by bacteria from bile in the gut

Serotonergic system: the part of the nervous system that uses the neurotransmitter serotonin

Serotonin: a neurotransmitter synthesized mainly in the GI tract that regulates appetite, mood, and GI function

Short-chain fatty acids (SCFAs): byproducts of bacterial fermentation that have a variety of health effects

Soluble fiber: a type of dietary fiber that absorbs water and is fermented by gut microbes

Starch: a polysaccharide that is found in plants as a stored energy source

Statins: a group of medications used to lower cholesterol and reduce cardiovascular disease

Symbiogenesis: an evolutionary theory stating that eukaryotic cells originated from the union of two prokaryotic organisms

Symbiosis: a close biological relationship between two different organisms, typically characterized by mutually beneficial interactions

Sympathetic nervous system: a part of the autonomic nervous system that is responsible for stress responses such as fight-or-flight, and generally slows digestive functions while increasing heart rate

T cells: a lymphocyte that plays a role in the body's specific defense mechanisms

Tight junctions: the area between cells that holds these cells together and forms a highly selective semi-permeable barrier

Toll-like receptors (TLRs): cell receptors that can recognize microbes and their metabolites

Triglycerides: the primary storage form of fat in the body

Trimethylamine-N-oxide (TMAO): a compound produced by bacteria that may increase the risk of cardiovascular disease

Tryptophan: an amino acid used in the synthesis of serotonin

Ulcerative colitis: an inflammatory bowel disease that causes abdominal pain, gas, diarrhea, and blood in the stool

Vagus nerve: a part of the autonomic nervous system that is one of the primary pathways of communication used by gut microbes within the gut-brain axis

Zonulin: a protein that influences the permeability of tight junctions within the GI tract

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