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The Relationship Between the Gut Microbiome and Sleep Examined Through Associated

Human Disease

by

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An undergraduate honors thesis submitted in partial fulfillment of the

requirements for the degree of

Bachelor of Science

in

University Honors

and

Biology

Thesis Adviser

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Portland State University

2022

Contents

Introduction

      The gut microbiome is a complex community of microbes living mainly within the large intestine (27) and is an essential part of human health and disease. The gut microbiome maintains a balance of species that is based on an individual and varies depending on certain genetic and environmental factors (i.e., geographic location, diet, etc.) (34). Bacteria, viruses, and parasites have been identified as integral members of the gut microbiome; however, we will focus on the bacterial species in this manuscript because they are the best-defined group and make up more than 98% of the present microbiota (27). Healthy microbiota homeostasis is defined generally as 90% of bacteria belonging to the two phyla Bacteroidetes and Firmicutes, the other 10% having high biodiversity of other species, and none of the species being highly competitive with another (34). An individual's gut microbiome can be dysregulated via antibiotic use, dramatic dietary changes, or disease (27). These dysregulations can cause certain bacterial species to become reduced and eliminated or increased and overgrown. It is possible for an individual's gut microbial homeostasis to be chronically dysregulated, and lack the diversity required to regain homeostasis (34). In both of these cases, the individual is considered in a state of gut dysbiosis, which is associated with many diseases and physiological disturbances (40). Much research is still being done on what species of bacteria are correlated with and cause pathologies, and which species are beneficial. Additionally, there has been significant research conducted in the last decade trying to understand the role of an individual's behavior such as eating habits, circadian rhythms, and sleeping patterns, that affect an individual's gut homeostasis.

Sleep is a vital part of human health that can be overlooked because of how basic and fundamental it is to everyday life. Regulation of sleep is studied more often than sleep itself and is most often studied through circadian rhythms. Mammals have a circadian rhythm programmed by the brain that allows their bodies to stay in a near twenty-four-hour sleep-wake cycle that coincides with the rhythm of the environment (41). Circadian rhythms affect practically every physiological system within the human body, including the gut microbiome (27). Species of bacteria vary throughout the day because of cyclical eating patterns that coincide with circadian rhythms and because of more direct effects of circadian rhythms (27). Like the microbiome, when circadian rhythm homeostasis is disturbed, acutely or chronically, it can lead to disease and pathologies (41). For example, in humans, circadian rhythms can be disturbed by artificial lighting, not enough sleep, and interrupted sleep, which can lead to stress and hypertension (32).

Importantly, circadian rhythms regulate neurotransmitters and hormones, levels of serotonin and norepinephrine are higher and increase wakefulness during the day, and melatonin peaks at night and promotes sleep in humans (49). Circadian rhythms create signals for a natural sleep-wake cycle, but they must be followed by an individual maintaining healthy sleep for them to remain regulated (49). Healthy sleep is defined as at least seven hours of uninterrupted sleep within a twenty-four-hour period, ideally at the same time each day (28). Disordered sleeping patterns stray from this definition; the further they are from it, the more issues they can cause. There are many sleep disorders, like insomnia and sleep apnea, that make maintaining healthy sleeping habits very difficult and cause disruptions to circadian rhythms (24, 31). Further, it is

challenging for most people to get the recommended amount of sleep (7 hours) with increasing

average stress levels and the increasing prevalence of shift work (40).

Recent research has shown that manipulating the sleep patterns of mice can affect gut

homeostasis, and the converse, gut dysbiosis can cause sleep disturbances (30, 31). Interestingly,

gut dysbiosis and disordered sleeping patterns are both associated with a similar set of diseases

and pathologies in humans, including obesity, diabetes, hypertension, and mental distress (7, 30,

50). These commonalities provide enough evidence to link these two seemingly unconnected yet

fundamental aspects of human health. This link has since been studied and found to play a

prominent role in human health beyond the initially shared disorders. Poor sleeping habits can

lead to disturbances of the gut microbiome in some obvious and some more discrete ways. For

example, sleeping less creates more hours in the day for an individual to eat, which could cause

obesity alone, but that combined with the disturbance of circadian rhythms that have some

control over the balance of the microbiome can cause more chronic issues (39). Over time if

poor sleeping habits continue, they can lead to more severe consequences for the bacteria and

cause diseases like irritable bowel syndrome (IBS) in the host (24). Gut dysbiosis can cause

sleep disorders as well. Sleep apnea, insomnia, and narcolepsy have all been shown to have a

component connected to specific imbalances in the microbiome (31, 24, 53). Together, these

findings have begun to define the crosstalk between the gut microbiome and sleep in humans,

and the many disorders that occur because of imbalances between them.

Metabolic Disorders, Gut Homeostasis, and Sleep Disorders

Metabolic disorders are often closely tied to the gut microbiome in various ways. Here the

methods through which the gut microbiome affects each metabolic disease discussed will be

established. Then the potential role that sleep disturbance has in the disorders will be examined.

The examples that were chosen to represent metabolic disorders are obesity, diabetes, and

irritable bowel syndrome.

      The microbiome, sleep, and obesity. Obesity is defined as having a BMI over 30, this

condition of being extremely overweight often leads to major health issues. Obesity is often a

risk factor or starting point for many other serious illnesses such as cardiovascular disease,

cancer, and diabetes mellitus (39). The gut microbiome regulates obesity through regulating

absorption of nutrients from food sources. Because we depend on microbes to break down

nutrients that we would be unable to absorb otherwise (47), specific species within the

microbiome which are able to ferment specific large food molecules and produce short-chain

fatty acids, have control over the about of energy absorbed (47). Each family of bacteria is able

to get energy through different metabolic pathways and further produce products that affect the

body in various ways. For example, some bacterial species are beneficial and produce

metabolites like butyrate, propionate, and acetate which help the epithelial wall to establish and

maintain a steady amount of energy being absorbed (39). Microbial homeostasis in the gut

varies between people, this can affect an individual's healthy or homeostatic weight. Even

within one person, the amounts of various bacterial species has been shown to fluctuate in a

24-hour period, which usually coincides with a canonical circadian rhythm (39). This ties the

gut microbiome to circadian rhythms because in order to maintain a balanced level of energy absorption food intake must occur during the same time period each day (37).

There are multiple ways in which short sleep duration, fragmented sleep, and excess sleep duration have been shown to affect the gut microbiome and induce or encourage obesity (48). One of these studies analyzes energy balance and loss of sleep through an evolutionary perspective and suggests that if someone is not sleeping in line with the circadian rhythm created by the central nervous system, there must be some immediate stressor that the individual will need extra energy to be able to handle (48). This encourages excess uptake of short-chain fatty acids in the gut for quick energy but since most stressors today are not physical, that energy is not needed, and most of it will end up being transferred to fat stores throughout the body (33). Because the gut microbiome is considered an endocrine system, misalignment of the primary and secondary circadian rhythms can also cause issues regulating the hormones leptin (the hunger-suppressing hormone), ghrelin (the hunger hormone), and cortisol (a stress hormone) (47). Obese individuals tend to have high circulating levels of leptin which, because of its pro-inflammatory properties, causes chronic inflammation and is involved in a positive feedback loop of reducing sensitivity to leptin which will require more to achieve the same effect and increased production will continue to cause reduced sensitivity in the brain (39). Ghrelin levels are generally low in obese people leading to lower levels of hunger and less caloric intake. Studies have been done that show short sleep duration causes reduced levels of leptin and increased levels of ghrelin, causing an individual to feel hungry and eat more (4). Because this can directly contradict the signals that an individual's body, whether obese or healthy, is sending, it will cause excessive caloric intake, which will be put into fat deposits. Cortisol is also increased due to a lack of sleep, and though cortisol promotes leptin production so it should

theoretically reduce hunger, over time chronic stress and high cortisol levels cause an increase in fat mass, as has been well observed in mice (39).

Additionally, sleep fragmentation tends to cause a change in food choices. When sleep-deprived, it has been observed that both mice and humans prefer higher fat and higher sugar foods than when well-rested (47). It has also been observed in mice that those fed a high fat, high sugar diet while in a reversed light/dark cycle responded with changed intestinal microbiota while those fed a standard diet did not (47). The high fat, high sugar mice showed an increase in *Firmicute* species, which are better energy producers than their normal counterparts, the *Bacteroidetes,* and would therefore allow for more calorie absorption and weight gain. The change in the intestinal microbiome also showed a decrease in the biodiversity of other species, including those that produce buffering products that help the gut maintain stable energy levels when an event occurs, such as the consumption of very high sugar foods (39).

Sleep disruption exerts its effects on the microbiome in multiple different ways. It does so through direct changes in the composition of the microbiome, through misregulation of hunger and stress hormones, and through changes in food preferences. One direction of interesting future research that could be done on this topic is further examination of the hormones involved. Would treatment of obesity through artificial regulation of hunger and hunger-suppressing hormones be effective? What is the reason that cortisol has the opposite of its expected effect on hunger expression and fat mass?

The microbiome, sleep, and diabetes. While the link between the gut microbiome and diabetes is less causative than in obesity, patients with both type 1 and type 2 diabetes exhibit unique forms of microbiome dysbiosis that are not shared by healthy individuals (45). Type 1

diabetes (T1D) is an autoimmune disease where the insulin-producing cells of the pancreas are attacked and destroyed in early childhood (45). Patients have to be treated with an outside insulin source throughout their life. Interestingly early-life exposure to probiotics has been seen to reduce the risk of the destruction of those cells in children who are genetically at risk for developing T1D, suggesting a link between the microbiome and T1D (45). Further, children who are at risk for T1D and are actively showing signs of pancreatic autoimmunity have been observed to have lower levels of biodiversity in their gut microbiome and higher levels of potentially harmful species, including those that are not able to ferment large carbohydrates to produce short-chain fatty acids that are essential for maintaining the health of the gut epithelium (45).

In contrast, type 2 diabetes (T2D) is characterized by a high blood glucose level, insufficient insulin secretion, and insulin resistance (42). Patients tend to show microbiome dysbiosis through a low *Firmicutes/Bacteroidetes* ratio and reduced butyrate-producing bacteria that would typically help to keep the gut microbiome balanced and increase sensitivity to insulin (42). This dysbiosis causes a "leaky gut," which is when epithelial cells within the gut separate and become more permeable; as a result, there is intestinal inflammation and a disturbed immune response. This inflammation causes further insulin resistance and progression of the disease (43). Together this data suggests there is a link between microbiome homeostasis and both T1D and T2D pathologies.

In T1D patients, sleep is very important, but it can also cause a lot of fear of possible complications with the disease (54). Nocturnal hypoglycemia is when blood sugar drops to too low levels during the night and, most concerningly, when the person is asleep (54). It is challenging to self-monitor glucose levels through the night, and they tend to fluctuate randomly

(54). Unfortunately, severe episodes of nocturnal hypoglycemia can be fatal (54). Without a way to continually monitor blood glucose levels throughout the night and be alerted when there is a problem, patients sleep poorly out of fear or only sleep for short periods of time, so they are able to monitor levels through the night (54). Despite this poor subjective sleep quality, objectively during episodes of nocturnal hypoglycemia, adults and children are observed to have fewer arousals out of sleep and increased sleep efficiency (54).

Sleep restriction, sleep fragmentation, and circadian misalignment all lead to reduced insulin sensitivity, as seen in studies done with both mice and humans (10, 33, 35). This both increases the risk of developing T2D and worsens the disease in those who already have it (35). A possible mechanism of this is through cortisol levels which oscillate in time with circadian rhythms but are higher after sleep deprivation leading to altered gut microbiota as it does in obesity (39).

Further, obstructive sleep apnea (OSA) is often found to co-occur with T2D and causes significant sleep fragmentation and poor sleep quality. Addressing OSA through treatment with a CPAP has been shown to slow specific diabetes symptoms like retinopathy and even reverse others like macular edema (35).   Targeting the gut microbiome through probiotics also has the potential to reduce disease severity in both T1D and T2D through the reestablishment of beneficial commensal species that increase insulin sensitivity and glucose tolerance (23).

From the available research, it seems that sleep issues are not considered a major factor in diabetes, however, some connection has been seen. Future research is needed to establish a possible causal connection. An intriguing experiment that could be done to establish a connection between the development of T1D and sleep issues would be to cause sleep

fragmentation in mice with a genetic risk factor for development and observe actual rates and ages of development.

The microbiome, sleep, and irritable bowel syndrome. There are three main types of irritable bowel syndrome (IBS); they are characterized by constipation in IBS-C, diarrhea in IBS-D, and both occurring at different times in IBS-M (38). Studies done on these types have shown varying results of gut microbiome composition, making it difficult to point to any one species of bacteria and be able to define what role it plays in the disease, if any, but one thing that is consistent is that levels of biodiversity in IBS patients is lower than in healthy individuals (11). There is one type of bacteria that stands out in the microbiome of IBS patients, methane-producing bacteria (11). These bacterial species are found in higher-than-average amounts in patients with IBS-C and lower than average amounts in IBS-D correlating to increased constipation with increased methane-producing bacteria and increased diarrheal disease with decreased methane-producing bacteria (11). Another thing that suggests IBS is being controlled by the gut microbiome is the large number of patients that also exhibit forms of psychological distress like depression and anxiety (16), which will be shown to be tied to the gut microbiome in a later section.

Symptoms of IBS often onset after some disruption of the gut microbiome (38). One that has been studied is gastroenteritis or an enteric viral infection (38). These infections will often lead to the flushing out of some of the bacteria present in a previously healthy gut microbiome and leave the person with lower biodiversity and open to opportunistic infections (38). Taking antibiotics would have a similar effect and may cause more harm than good (38).

Studies have been done showing that the rates of IBS in nurses who work rotating shifts are significantly higher than in those working fixed day shifts (16). It has been suggested that sleep deprivation and sleep debt could cause enough of a disruption in the gut microbiome that dysbiosis leading to IBS could occur (36). The most likely way for this to happen would be through stress caused to the body by poor sleep, which would cause the immune system to be suppressed, thus having less control over the intestinal microbiome homeostasis, and allowing it to run mostly unchecked for long periods of time (36). After this time, when the immune system is able to recover, it would attack the intestines with increased intensity in an attempt to regain control, and this could lead to many of the IBS symptoms (36).

Sleep loss as a potential starting point for IBS has only been theorized and not proven yet. More research would have to be done for it to be considered an actual cause. Symptoms of IBS, however, have been shown to be worsened by misaligned circadian rhythms and poor sleep quality, so there is evidence for an important medical connection (36). Future research would be difficult because in order to show that sleep deprivation was the cause it would have to be observed as the dysbiosis developed. Sleep restriction studies could be done on mice but they are likely to suffer from other negative effects before a case occurred where the correct dysbiosis happened to cause IBS.

Sleep Disorders are Affected by Gut Dysbiosis

This section examines the potential role of the gut microbiome in sleep disorders through

analysis of bacterial diversity and abundance in individuals with said sleep disorders.

Speculation is made on the mechanism through which dysbiosis is causing sleep issues or if the

gut is disturbed as a result of the sleep disorders instead. The sleep disorders investigated here

are insomnia, narcolepsy, and obstructive sleep apnea.

       The microbiome and insomnia. Insomnia is a prevalent sleep disorder characterized by

difficulty initiating or maintaining sleep or having poor sleep quality (26). Insomnia patients

show higher levels of metabolic disease compared to healthy patients (14), potentially due to the

mechanisms caused by sleep deprivation discussed above. While sleep deprivation can be a

causal factor in gut dysbiosis, it has also been thought to work the opposite way. Several studies

have been done that show that chronic insomnia patients, those who have experienced symptoms

for longer than three months, have shifted bacterial abundances and lowered biodiversity in their

gut microbiome when compared to healthy patients (26). The bacteria *Bacteroides* and

Clostridials were specific to general insomnia patients, *Lachnospira* and *Bacteroides* were

specific to acute insomnia (patients with symptoms lasting between 1 week and three months),

and *Faecalibacterium* and *Blautia* were specific to chronic insomnia patients (26). Amounts of

these signature bacteria were found to correlate with patients' sleep quality as well as plasma

levels of IL-1β (26), which was used as a marker for levels of inflammation. High levels of

inflammation are also characteristic of insomnia due to the increased amount of those certain

bacteria, which are known to cause excess production of inflammatory cytokines (24). Having

excess inflammatory cytokines causes a large amount of inflammation and is seen by the body as

a stress signal (24). This signal travels to the brain through the immunoregulatory pathway and can potentially be perceived as a danger and cause a change in primary circadian rhythm control by the suprachiasmatic nucleus to keep the body awake and prepared for the potential threat (24).

The excess of pro-inflammatory gut bacteria like *Faecalibacterium* and *Clostridials* present in people with insomnia can cause metabolic disorders in ways different from those discussed previously (14). Dysbiosis causes increased gut permeability which allows for greater absorption of nutrients and sugars than should be possible, and it also allows for some normally harmless bacteria to cross the epithelium and enter metabolically active tissues (26). These tissues, like the liver and the pancreas, are essential in the production of metabolic enzymes. When exposed to high levels of inflammation and potentially bacterial infection, they cannot function correctly (24). Chronic inflammation of these tissues inhibits homeostatic metabolic signaling and mediates processes like insulin-regulated glucose uptake or proper hunger hormone secretion. Over time this can lead to issues like T2D and obesity (14), which were examined before through a different method of development. Reduction in inflammation and a return to homeostasis in the gut microbiome can be observed with probiotic supplementation, meaning that they could be used to treat both insomnia and its other related diseases at once (14).

In insomnia, it is difficult to determine if the characteristic shift in the microbiome is the cause of the sleep disorder or if it is a result of sleep loss. In order to determine this long-term studies would need to be done by looking at the change in the microbiome over time. Patients with acute insomnia could be observed to determine if their insomnia becomes chronic and how the microbiome changes if it does. If acute insomnia resolves itself it would still be interesting the see how the microbiome changes when sleep is improved. Even through this observation, it would be difficult to determine what is causing what. Experiments using fecal microbiota

transplants could be done by taking fecal matter from a mouse with insomnia and giving it to a germ-free mouse and observing the recipient for sleep issues could help determine the direction of the causal relationship if one exists.

The microbiome and narcolepsy. Type 1 narcolepsy is characterized by excessive daytime sleepiness, cataplexy, sleep paralysis, and disturbed sleep (53). These symptoms result from the irreversible destruction of orexin neurons in the brain (22). The cause of this destruction is not well understood, it has long been hypothesized that it occurs due to autoimmunity, but not all sources agree (19). Determining who is susceptible to developing type 1 narcolepsy has also proven difficult. There has been a gene linked to the increased the risk of type 1 narcolepsy development by about 200 times (53), but the disease rarely runs in families, and there is a low concordance rate between monozygotic twins meaning that there must be a significant environmental component to development as well (19). The onset of the disease usually occurs in adolescence and is difficult to observe since it cannot be predicted who will be affected, and symptoms only become severe after the damage is done (19). It has been shown that infection with influenza A or *Streptococcus pyrogens* can lead to pathology in the human immune system that could potentially lead to the autoimmunity seen in type 1 narcolepsy, but this has not been proven as there seem to be more factors present (53).

Since the gut microbiome plays a large role in the development of the immune system in childhood, it has been proposed that it also plays a role in the development of the potential autoimmune destruction of orexin neurons (22). Adults with type 1 narcolepsy have been shown to have characteristic gut dysbiosis compared to healthy controls (22). Hence, it is possible that

this dysbiosis was present in the development and led to the disease. It is also possible that this dysbiosis results from the circadian disruption caused by the disease.

Type 1 narcolepsy is a very complicated disease and development relies heavily on unknown environmental factors. Since the gut microbiome is a potential environmental factor it is possible that dysbiosis plays a role in the development of the disease. Much more research needs to be done to support the claim that gut dysbiosis leads to type 1 narcolepsy, but as stated earlier, it is challenging to do so because it is a relatively rare and unpredictable disease. Even so, more studies could be done by tracking narcolepsy patients and their gut microbiome over time as well as possible screening for development periodically in those who are genetically susceptible to attempt to catch the development in action.

The microbiome and obstructive sleep apnea. Obstructive sleep apnea (OSA) is a sleep disorder characterized by repetitive airway constriction or collapse during sleep causing intermittent hypoxia, microarousals, and sleep fragmentation (17). OSA is a relatively common condition, and its connection with hypertension which will be discussed later has been very well studied in mice and moderately studied in humans (3), enough to suggest that conclusions made from mice studies are generally able to be applied to humans. Most studies on OSA and the gut microbiome show how sleep fragmentation and intermittent hypoxia alter the gut microbiome (31). This is the case because it is much easier to observe the gut microbiome of an individual who has OSA already than to try to induce OSA onto a previously healthy individual through microbiota transplant (3).

There is very little research to support that gut dysbiosis may cause the development of OSA. However, one study done on rats showed that the gut microbiome can alter the body's

response to higher-than-normal carbon dioxide levels in the blood (5). This was determined by inducing gut dysbiosis through the use of antibiotics and FMT in the rats and observing a decreased ventilatory response to increased carbon dioxide levels (5). This is significant to OSA because it suggests that in periods of intermittent hypoxia or lack of breathing that occur, the gut microbiome could be playing a role in allowing them to continue longer than they otherwise would and potentially allow for more damage to be caused. Another study was done using FMT that used fecal matter that had either been exposed to hypoxic air or regular room air that was then placed in young mice (3). The mice that received the FMT from hypoxic air showed increased sleepiness compared to the mice who received the room air transplant (3). This could be a partial explanation for the excessive daytime sleepiness experienced by patients with OSA (31).

These two rodent studies, combined with studies in humans that show that taking probiotics and prebiotics improve the symptoms of OSA, suggest that the gut microbiome and gut dysbiosis play a role in the development and level of severity of OSA (5). Probiotics could be a possible method of actually resolving OSA rather than just treating the symptoms and comorbid diseases through things like CPAP treatment. Future studies should be done by imitating OSA in mice. This could be done by creating chambers that are able to have alternating oxygenated and hypoxic air while a healthy mouse is sleeping and periodically sampling the gut microbiome for changes in species present and abundance of those species.

Other Associated Disorders

Through the examination of diseases related to the gut microbiome and diseases related to sleep, it has been noted that some are shared. Here diseases that are not directly in either system are discussed based on their connection to the gut microbiome and sleep separately and on the crosstalk that occurs between them. Many issues could be looked at here but the focus is on hypertension, Alzheimer's disease, and psychiatric diseases, more specifically depression and anxiety.

      Hypertension. High blood pressure or hypertension is very common and is used as an indicator of cardiovascular disease, the leading cause of death in the US and many other countries (6). Hypertension is also very closely related to OSA, which is present in 35% of patients with primary hypertension and 65-80% in those with drug-resistant hypertension (9). As discussed above, it is generally accepted that OSA causes alterations in the gut microbiome. In the case of hypertension, the most interesting change is a decrease in SCFA producers. SCFAs are made through microbial fermentation of indigestible dietary fiber, some examples are acetic acid and butyric acid (12). In the gut, these SCFAs help to stabilize the epithelial barrier between the host and the environment and regulate host cytokines (9). Lowered amounts of SCFAs were correlated with epithelial damage and hypertension through enhanced vascular tone of the kidney (12). Along with the decrease in SCFA producers, it has been seen that there is an increase in lactate production, and it is known that high plasma lactate levels are tied to high blood pressure (9).

Some rat models found that induced OSA alone, even for periods of up to 8 weeks, was not enough to cause hypertension (9). One way hypertension was able to be induced during

induced OSA was when rats were fed a high-fat diet (12). This diet mimicked conditions that are often found to occur in patients with both OSA and hypertension, like obesity, diabetes, and aging (18). Neither OSA nor a high-fat diet could induce hypertension alone, but together they led to the idea that if even one of these factors can be treated in humans, it could significantly reduce their chances of developing hypertension.

Another mechanism through which OSA and other sleep disorders could cause adverse effects on the cardiovascular system that would be seen as hypertension is through the buildup of oxidative stress (20). While awake, much oxygen is used by the brain, which causes the production of oxidants. Some oxidants are potentially harmful and are called reactive oxygen species (ROS). It is normal to accumulate ROS throughout the day, and once they reach a certain threshold, they may even act as a sleep promoter (20). While sleeping, there is a higher level of antioxidants at work in the body, and they break down the ROS so that they return to a low baseline after sleep. In sleep disorders such as insomnia or OSA, ROS levels stay heightened due to lack of sleep or poor quality sleep (20). High ROS levels over time lead to damage throughout the body, including lipids, proteins, and DNA at a cellular level, and also impairment of the cardiovascular system (51). In clinical studies on humans, antioxidants have not been shown to improve hypertension, but biomarkers that indicate oxidative stress have been observed as directly correlating with blood pressure (51). This method of disease development is also tied to the gut microbiome through diet, considering that rats previously fed with a prediabetic diet were shown to return to healthy blood pressure after being switched to a normal diet (51). This suggests that even if regulated and healthy sleep is not possible, reduction of the disease can be achieved through improvements in diet and possible prebiotic usage.

Both poor diet and sleep quality together can increase the risk of hypertension through a reduction of SCFA production and an increase in ROS. Treatment of either one of these issues can reduce the risk significantly showing that even some small lifestyle changes can be very beneficial. Understanding the tie between drug-resistant hypertension and OSA is a compelling direction for future research. Since they are so closely related it would be interesting to see how treatment of OSA through a CPAP machine would affect blood pressure in these patients.

Alzheimer's disease. The main disease-causing feature of Alzheimer's disease (AD) is the build-up of a harmful mutated protein called amyloid-beta (Aβ), which forms plaques in the brain. These plaques grow with time and cause symptoms like amnesia, aphasia, and disorientation (25). AD can develop due to genetic components, environmental conditions, or a combination of both. Generally, people who exhibit early-onset AD, before the age of 65, are more likely to have developed it due to genetic factors, and those who exhibit late-onset AD, after age 65, develop it through environmental factors (2). Patients with neurodegenerative diseases, including AD, have been shown to have disrupted gut homeostasis. This leads to the idea that gut dysbiosis could play a role in AD development and progression through disruptions of the blood-brain barrier (BBB). This barrier ensures that potentially harmful bacteria entering the bloodstream through the gut never make it into the brain (44). In AD, it does not function properly. The BBB is somewhat broken down, and damaging things are let through and can severely affect the brain (44). Bacterial strains such as *E. coli*, *Salmonella*, and *Citrobacter* can enter the brain tissue, since these species have the capability to produce Aβ and introduce it to the brain, they also can start plaque formation due to the chemical properties of Aβ (15). Aβ is chemically similar to a protein required for normal brain function and neuron firing and can

disrupt the production of that protein and cause it to form Aβ instead. Over time this causes loss of brain function and build-up of Aβ plaques. Usually, Aβ in the blood can be cleared, but in the brain, it cannot (15).

Initial disruption of the BBB can be caused by sleep loss and circadian disruption (44). Sleep loss is known to play a role in the reduction of gut microbiota biodiversity and inflammation, as discussed above. It also plays a role in the degradation of the mucosal membrane of the gut leading to a "leaky gut" where more microbes can enter the bloodstream (15). A similar mechanism to the breaking down of the gut mucosa seems to happen with the BBB. Studies have shown that certain gut dysbiosis profiles and sleep loss patterns have been observed to be causing an increase in Aβ and AD risk even decades before the onset of diagnosable symptoms (25). It is theoretically possible then, to be screening for AD and treat patients who have early warning signs to prevent the disease from ever developing. Since AD rates have been rising for no known reason, this ability would be very valuable (2). Studies in humans have shown that sleep is beneficial in the rate of Aβ clearance. Healthy individuals tend to have decreased amounts of Aβ in cerebrospinal fluid in the morning, but sleep deprivation, as well as sleep disruption, reversed the decrease and caused accumulation (25). It has also been seen that AD patients have low levels of melatonin and have a hard time regulating circadian rhythms meaning that not only is sleep deprivation a potential risk factor for AD, but it is also a symptom after development (2). This suggests there could be a positive feedback loop that could be interrupted to slow disease progression through either attempts at implementing a regulated sleep schedule or through the reestablishment of gut microbiome homeostasis.

Aβ plaques form when the BBB is degraded and allows microbes to enter the brain tissues and introduce this harmful mutated protein. Since this initially happens long before

symptoms are noticeable, once they have been noted it is likely too late to stop the feedback loop that progresses the disease. Future research should be done to determine ways to help maintain the BBB so that preventative treatment may be possible. This treatment would have to be tested on a large group over a long period of time and rates of AD would have to be compared. Because of the large sample size and large amount of time needed, this research would be difficult to do but the outcomes it could possibly give would be very beneficial and could help reduce the rising rates of AD.

      Psychiatric diseases. The gut-brain axis is the avenue through which the gut microbiome and the central nervous system interact (52). The gut microbiome affects the brain through cytokine production and the immune system, hormone production and the endocrine system, direct production of neuropeptides that will act on the brain, and other mechanisms (24). The central nervous system affects the gut microbiome through control of other endocrine organs and conscious decision-making (50). Psychiatric diseases, specifically depression and anxiety, are tied to the gut microbiome through this axis. Certain bacteria in the gut can produce essential neuropeptides like GABA, serotonin, and BDNF (8). These peptides are used by neurons in the brain as regulators for mood and cognitive function. In a state of gut dysbiosis, there would be reduced levels of these neuropeptides (8). The brain would lose balance and control over mood stabilization and other related functions, potentially leading to these psychiatric diseases (24). The cause of this gut dysbiosis could be many different things, but one theory is that sleep deprivation plays a role.

      Depression and sleep loss through insomnia often go together. 90% of patients with depression also have some type of sleeping problem (52), and 20% of patients with insomnia

also have depression symptoms (24). This connection is thought to be because of the regulation of neuropeptides like serotonin which is known to be connected to amounts of REM sleep as well as the development of depression (24). GABA is also known to have an abnormal expression in patients with depression and anxiety, and experiments in mice have shown that administration of specific probiotics increases the expression of GABA receptors in the brain, which lead to reduced depression and anxiety behaviors (24). This study also found that mice who did not have a vagus nerve, which is the nerve that connects the brain to the digestive system, did not have the same positive effects (1). This suggests that the vagus nerve is vital to communication between the gut microbiome and the central nervous system. Mice who were given antibiotics that affected the gut microbiome were also seen to have an increase in depressive behaviors suggesting that gut dysbiosis was the cause (24). Mice infected with certain strains of pathogenic bacteria showed increased levels of c-FOS expression, a gene used to measure brain activity, in areas of the brain implicated in anxiety (1). This suggests that stress put on the gut microbiome by harmful bacteria also puts stress on the brain, making the individual experience anxiety. Studies in humans attempting to create a gut profile of those with major depressive disorder have given the same results. Most studies showed some sort of species overgrowth, but they disagree on what species those are (13). Studies done on differences between the metabolic products of people with major depressive disorder and healthy people did agree that there is a reduction in SCFA production and an increase in isocaproic acid concentrations (52). These two things help support the idea that the specific species present in the gut are not as important as metabolic pathways remaining intact and able to function.

Depression and anxiety both occur when neuropeptide regulation is disturbed. In some cases this occurs because of issues with the gut-brain axis. The gut-brain axis is established

differently for every individual which could make treatment through the gut microbiome both easier and harder. It could be easier because there would not have to be a specific probiotic species found that fixes the connection, but harder because every person may need a different species in order for the treatment to be effective. Future research could be done using proteomics to determine possible missing enzymes required for certain metabolic pathways that produce neuropeptides. Once a missing link is determined, a probiotic supplement containing bacteria that produces the correct enzymes could be tested and the patient could be observed for improvements in mood and behavior.

Treatment Implications

Establishing connections between the gut microbiome and sleep for all of these diseases allows

for speculation on new treatments to occur. New uses for probiotics, prebiotics, and fecal

microbiota transplants can be proposed. To an extent, regulating sleep schedules can also be

proposed as a method of treatment for seemingly unrelated health issues.

      Probiotics and prebiotics. Probiotics are living commensal bacteria that are eaten and

administered to the gut through the digestive tract (14). Certain strains can be consumed to

compete with pathogenic bacteria and prevent overgrowth or to restore a metabolic pathway that

may have been interrupted due to dysbiosis (12). Prebiotics are certain sugars and fibers that can

be taken in through a normal diet or supplementation but cannot be digested by the host only by

the bacteria present in the gut (14). Both have few side effects and can be used to prevent

dysbiosis from occurring or to restore homeostasis when dysbiosis has occurred (12). Their use

in many areas of human disease has been an active area of study in recent years, and an overview

of some of the results in disorders also related to sleep follows.

Certain strains of probiotics given to mice fed a high-fat diet were able to prevent weight

gain and metabolic syndrome that would normally occur as a result of their diet (23). A certain

probiotic mixture called VSL#3 that is currently prescribed to human patients with ulcerative

colitis, irritable bowel syndrome, and ileal pouchitis has been shown to improve lipid profiles

and insulin sensitivity suggesting that it could be used as an antidiabetic therapy (23). Most

studies done using probiotics use naturally occurring bacteria strains, but some trials are being

done that are using genetically modified strains of *E. coli*. One study has been done with *E. coli*

that expresses large amounts of a naturally occurring chemical that reduces hunger and food

intake (23). In mice, this has successfully reduced obesity and diabetes even when they are being fed a high-fat diet (23). There are many possible options for probiotics that could prevent the development and progression of obesity and diabetes, but many of them have not yet been well enough studied in humans for their use to be widespread.

Sometimes administration of a certain bacterial species is not enough to repair a damaged metabolic pathway. Just because a probiotic is taken does not mean that the species will be able to survive in the gut and perform its proper function if it does not have the right nutrients to do so, this is why prebiotics are essential. One study done in a rat model of OSA noted that the specific SCFA that, when artificially introduced to the gut, reduced the development of OSA-induced hypertension was acetate (12). While attempting to find a probiotic that would increase acetate production and have the same effect as the infused acetate, they found that the acetate-producing bacteria *C. butyricum* effectively increased gut levels of acetate. However, this only occurred when administered along with the prebiotic starch Hylon (12). When used together the probiotic and prebiotic were able to reduce damage to the gut wall, blood pressure, and neuroinflammation levels (3). While this is a very specific study, the same principles could be studied more and potentially applied to humans to prevent the development of hypertension in patients with sleep apnea even if they have low adherence levels to other treatment methods discussed below.

Psychobiotics rely on the fact that some species of bacteria produce neuropeptides as metabolic products when present in the human gut. Species in the families *Lactobacillus* and *Bifidobacterium* are known to produce GABA, serotonin, and acetylcholine (8). Serotonin biosynthesis in the gut can also be regulated by microbes which affect the enterochromaffin cells in the gut epithelium (8). This knowledge was the basis for many mouse studies done with

various strains of probiotic bacteria from the previously mentioned groups, most of which showed positive effects on stress, anxiety, and depression behaviors as well as levels of cognitive dysfunction (8). With so many positive effects in mice, the research was quickly extended to human subjects. Human studies showed similar results to animal studies and reported positive effects on stress levels and memory (8). These clinical trials, however, were performed on healthy subjects. Hence, it is yet to be seen if probiotics would have a positive effect on patients who have anxiety or depressive disorders caused by methods like dysfunctional serotonin pathways (1). Psychobiotics can also be helpful in the treatment of Alzheimer's disease. Probiotic supplementation improved learning and memory in rats with induced AD as well as reduced the number of Aβ plaques, inflammation, and oxidative stress (8). Unfortunately, positive effects were only observed in rats with mild to moderate AD, in rats with severe AD, probiotic treatments were not shown to be beneficial (1). This suggests that there is a limit to this treatment method's effectiveness, and when being tested in humans, age and disease progression will play a prominent role in the determination of who is eligible for trials. If trials in humans do show similar results to those done in rats, it is possible that disease progression could be significantly slowed if caught in its early stages (1). More research is still needed, but it is possible that psychobiotics could be used as an alternative treatment for various mental health and neurodegenerative disorders.

Fecal microbiota transplant. A more extreme yet still relatively noninvasive treatment option compared to probiotics and prebiotics is a fecal microbiota transplant (FMT). FMTs are done by processing fecal matter from a healthy donor and administering it to a diseased recipient through methods such as a nasogastric tube, colonoscopy, or enema (14). Currently, the only

approved use of FMT is to treat antibiotic-resistant *Clostridium difficile* infections, which is an overgrowth of pathogenic bacteria that cause life-threatening damage to the lower intestine (3). FMTs have been suggested and researched to treat many other diseases such as obesity, diabetes, insomnia, sleep apnea, and hypertension. Almost any illness that has an established tie to the gut microbiome and dysbiosis could be treated using FMT.

Obesity has been suggested as an issue that FMT could be very beneficial in treating. It has been well observed in mice that obesity can be transferred to a lean mouse through an FMT from an obese mouse, and the same is true in the other direction, an obese mouse receiving an FMT from a lean mouse becomes more lean (23). In humans, this could be used as a less invasive alternative to bariatric surgeries like gastric bypass surgery, which is used to treat extreme obesity. It has been observed that patients who do undergo gastric bypass surgeries often experience a long-term shift in their gut microbiome that causes them to resemble lean subjects more closely (23). This shift causes a decrease in the *Firmicutes/Bacteroidetes* ratio which is generally seen as positive, improved insulin sensitivity, and improved glucose tolerance. Though the mechanism of this is not known, it suggests that part of the efficacy of these surgeries is the impact they have on the gut microbiome and that a similar result could be achieved without surgery through FMT. However, an FMT alone would not completely rid a person of obesity, it would have to be followed by an improved diet, increased exercise, and healthy sleep habits. Otherwise, the beneficial microbes and the biodiversity given to the recipient of an FMT could be lost, and their gut microbiome could revert to the same place they started. FMT is a promising treatment option that has relatively few adverse effects, is non-invasive, and is less expensive than repeated drug therapies that might be used instead (23). It can also potentially treat many related metabolic diseases caused by the microbiome at once.

Some barriers that cause it not to be a viable option for treatment and a difficult subject of study are a lack of standardized protocol, issues with donor selection and the definition of a healthy gut microbiome, and costs of donor screening (23). In order for this treatment to be used clinically outside of *Clostridium difficile* infections, much more work and research will have to be done on it.

Improved sleep. To improve sleep quality in patients with OSA a therapy called continuous positive airway pressure (CPAP) is often used. This therapy prevents the airway collapse that causes intermittent hypoxia and arousals in OSA by opening the airway with air pressure through the nose, mouth, or both (21). This treatment effectively turns off the disease and with it, improvements in daytime sleepiness and cognitive function are seen. Improvements in hypertension and cardiovascular disease are also noted when OSA patients are treated with CPAP (21). Statistically significant reductions in hypertension are seen in those treated with CPAP, the reductions also lead to substantial risk reduction for coronary artery diseases and stroke. Despite these benefits, adherence to this treatment is often low (21). Patients may experience discomfort from the device used and discontinue use without consulting their doctor. Because of this, much effort has gone into creating new devices that cause as little discomfort as possible to increase treatment adherence, but more is needed as the rates are still low.

Though most research has shown sleep is likely to be a disease-causing issue for many of the disorders discussed here, there is very little research done on how improved sleep can improve public health through effects on the gut microbiome. To effectively study this, longitudinal studies rather than studies done at one point in time would be needed. Most conclusions made about sleep's effect on the gut microbiome were made by comparing a group

of people who had a specific sleep disorder or other condition to a healthy group (5, 10, 14).

This cannot prove any type of causal relationship, only a correlation that can be speculated upon.

Future research would need to have a longer-term of study to draw more solid conclusions.

Conclusion

The major finding of this study is that sleep loss, sleep fragmentation, and disrupted circadian rhythms are able to affect the gut microbiome in a significant way but there is not enough evidence to say that the gut microbiome can cause sleep disturbances. Most of the evidence found in studying these specific diseases suggests a one-way relationship. It is still possible, however, that the gut microbiome does affect sleep, just not in these specific disease states. It was found that sleep loss causes significant stress to the body which leads to gut microbiome alterations that can then turn into harmful dysbiosis of not given time to properly heal and become balanced again. This dysbiosis can lead to many metabolic disorders even beyond the ones discussed and play a role in many other disorders, even ones that may seem to have no connection with the gut.

This research is important in establishing the consequences of circadian rhythm misalignment caused by shift work, delayed sleep, early morning starts, and general chronic sleep loss (29). In 2017, 35% of Americans reported their sleep quality as good, fair, or poor (46), and these issues are only going to become more common in modern society as time goes on. Chronic sleep deficiency is dangerous, especially in shift workers who have to continually disrupt circadian rhythms by changing their sleep-wake cycles. They are much more likely to suffer from metabolic diseases and even some cancers (29). Understanding why this is dangerous and the mechanisms behind it is important so that treatments can be developed and recommendations that protect people from potential harm can be made.

Much future research on this topic is still needed. Since the gut microbiome is tied to so many different body systems, there will always be more research to do. Some specific examples of possible future projects are present throughout this paper, but there is still one large question

that needs to be answered. Most studies done on the gut microbiome are observations of disease states, because of this a good understanding of what a healthy gut microbiome is has been hard to achieve. While knowledge of these disease states is important it is also important to have a baseline to compare it to. Since the gut microbiome is so complex and varies so much from person to person there may never be a defined ideal microbiome but there should be some kind of defined standard developed.

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