Feature Article

Complex interactions of circadian rhythms, eating behaviors, and the gastrointestinal microbiota and their potential impact on health

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Human health is intricately intertwined with the composition and function of the trillions of microorganisms that make up the gastrointestinal (GI) microbiome. The GI microbiome is essentially a microbial organ that provides metabolic, immunologic, and protective functions for the host. Habitual diet, changes in macronutrient composition, and consumption of nondigestible dietary fibers have all been shown to impact the human GI microbiome. Intriguingly, the impact of diet on the microbiome may be related not only to what humans eat but also to the timing of food consumption. Emerging preclinical research suggests that gut microbes experience diurnal rhythms, and the health effects of eating patterns, including time-restricted feeding and meal frequency, may be related to the GI microbiome. Herein, the complex connections among circadian rhythms, eating behaviors, the GI microbiome, and health are reviewed, highlighting the need for additional translational research in this area.

INTRODUCTION

The impact of diet on the composition and function of the gastrointestinal (GI) microbial community and human health is an area of rapidly evolving research that is particularly important as the cost and prevalence of chronic diseases rise to staggering figures and standard, efficacious treatments such as dietary and physical activity modifications are infrequently prescribed and even less frequently followed.[1] The trillions of microbes that make up the GI microbiome form a microbial organ with a collective gene set 150 times larger than the human genome, providing metabolic, immunologic, and protective functions for the host.[2] In addition to contributing to immunologic development and metabolic function, the GI microbiome also influences nervous system development and function.[3] Furthermore, the composition and function of the GI microbiome have been shown to be linked to a growing list of metabolic diseases, including obesity, diabetes, and cardiovascular disease.[4,5]

Diet is an important mediator of the human GI microbiota; habitual intake,[6] rapid changes in dietary fat and fiber composition,[7] and consumption of dietary fibers[8–10] and other nondigestible food components have all been shown to impact both the composition and function of these resident microbes.[5,11] Intriguingly, the microbiome may be affected not only by what is eaten but also by when food is consumed. Host–symbiont bidirectional communication occurs via signaling along the gut–microbiota–brain axis by a variety of bacterial metabolites, which have been shown to

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impact centrally mediated feeding behaviors such as appetite.[12–14]

Increasingly, preclinical research has demonstrated that the bacteria in the GI tract vary over the course of a day, with relative abundances of bacterial taxa, proximity of bacteria to the colonic epithelium, and microbial metabolism all exhibiting diurnal rhythms.[15,16] Time of eating is considered a potential modulator of circadian rhythms with an effect on both bacterial abundance and function.[17] Furthermore, the gut microbiome appears to have a reciprocal relationship with the human body's circadian clock and eating patterns (Figure 1). Emerging research suggests that some of the observed health effects related to eating patterns, such as time-restricted feeding (TRF) and meal frequency, may also be related to the GI microbiome. Herein, preclinical and clinical research on circadian rhythms, eating behaviors, and the GI microbiome is reviewed.

CIRCADIAN RHYTHMS

Most of the life on Earth experiences a daily 24-hour light/dark cycle created by Earth's rotation in relation to the sun and, as a result, engages in a 24-hour cycle of feeding and fasting.[18] Circadian rhythms are cycles of gene expression, metabolism, and behaviors created by the internal clock that govern a multitude of metabolic functions such as hepatic lipid metabolism, cardiovascular function, obesity regulation, and glucose homeostasis.[19,20] Circadian rhythms are regulated in humans in 2 ways: (1) by light via the suprachiasmatic nucleus in the brain and (2) by clock proteins that are present in nearly every cell and provide a transcriptional rhythm based on a 24-hour day.[21] The suprachiasmatic nucleus also regulates the circadian release of digestive peptides, including vasoactive intestinal polypeptide and gastrin-releasing peptide.[21] Almost all human cells have circadian regulatory genes, including *Clock, Bmal1, RORα, Cry, Per,* and *Rev-erbα*.[21] In mice, 45% of transcripts have 24-hour oscillations.[21] The homeostatic sleep/wake cycles of the central nervous system, combined with pituitary gland activity, exert substantial influences on the endocrine system.[21]

Health implications of misaligned circadian rhythms

The natural condition of most living organisms on Earth is to spend one phase of a 24-hour day (either light or dark) in an active and feeding state and the other in a resting and fasting state.[18] Humans naturally spend the light phase in the active and feeding state and the dark phase in the resting and fasting state. However, with the development of artificial light, humans have deviated from the original pattern of eating only during the light portion of the day. Furthermore, individuals who work night shifts experience an almost complete reversal of food intake, with intake occurring primarily during the night and rest and fasting occurring during daylight hours. Dietary intake that is misaligned with the natural rhythms of the circadian clock has been shown to negatively impact human health. Specifically, disruptions to the normal sleep/wake cycle in relation to the night/day cycle of Earth, as seen in shift work, are associated with a 40%–60% increased risk for obesity and metabolic syndrome.[22,23]

A misaligned circadian rhythm is one in which the normal schedule of feeding and fasting is disrupted. Circadian rhythms can be misaligned through environmental conditions, such as shift work or jet lag, or through genetic manipulation of relevant genes as in the case of the creation of knockout models in preclinical research. The importance of cellular clock mechanisms to health has been demonstrated in *Bmal1* knockout mice and *Rev-erbα* and *Rev-erbβ* double-knockout mice, which display dysmetabolism in glucose and lipid homeostasis, respectively.[21] Restricting the food access of wild-type mice to only the light phase (when mice are normally not active or eating) resulted in a 23% increase in weight gain and an 8% higher body fat percentage in mice that had their environmental conditions manipulated compared with mice that had access to food during their normal active phase.[24] Thaiss et al.[25] reported that circadian-disrupted mice fed a high-fat diet had 17% greater body weight than nondisrupted mice on a similar diet. Antibiotic-treated mice were resistant to these detrimental metabolic changes, suggesting a connection between misaligned rhythms and the microbiome.[25]

Addition of dim light to an animal's habitual dark phase has also been shown to disrupt circadian rhythms and thus metabolism. Mice housed in dim light conditions have been shown to increase energy consumption during the light phase to 55% of total intake, compared with 36% in mice kept in standard light/dark phase conditions. Body mass and insulin resistance were also greater in mice exposed to both dim light and constant light situations when compared with mice exposed to standard light/dark conditions, despite the use of isocaloric diets and matched physical activity levels.[26] Chronic sleep fragmentation, a model of obstructive sleep apnea, also affects both the murine microbiome and health.[27] Poroyko et al.[27] demonstrated that mice exposed to chronic sleep fragmentation by tactile stimulation every 2 minutes during the sleep phase had increased food intake and decreased colonic barrier function and that transplantation of the Firmicutes-enriched microbiome of these animals into germ-free animals resulted in enhanced inflammatory response
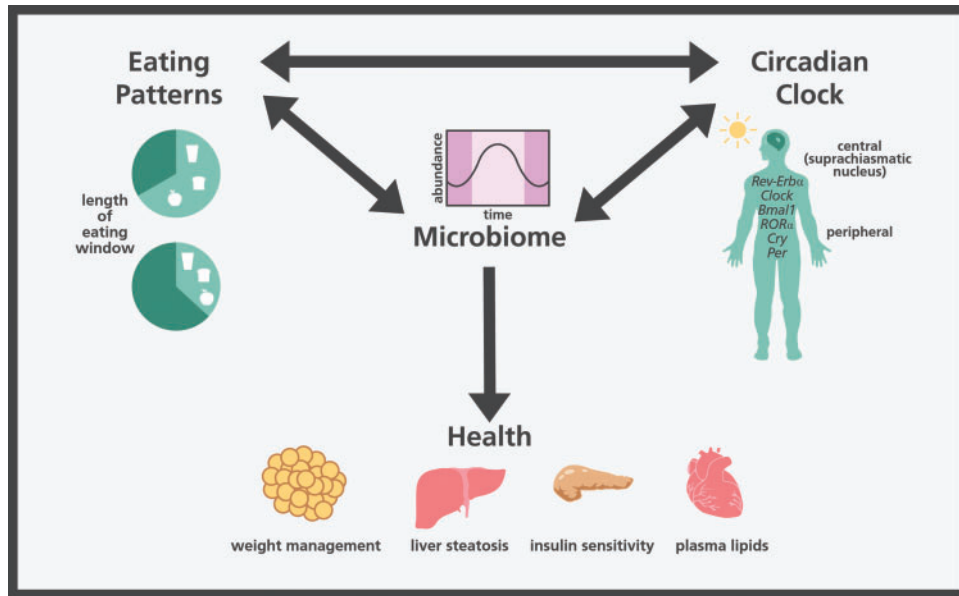
Figure 1 **Connections between the internal clock, eating patterns, the microbiome, and health.**

and insulin resistance in the recipient animals. Recent work has also examined sleep restriction in both mice and a small group of humans.[28] The authors found that, after mice and humans were restriced to 4 hours of sleep per night for 5 nights, there were minor changes in the murine microbiota but no changes in the human microbiota (n = 11). They concluded that, although weight and behavioral alertness were impacted in humans, the microbiota was resistant to sleep restriction–induced change. However, because of the small sample size of human participants and the acute nature of the study, the study may have not been adequately powered to detect changes in the highly individualistic human microbiota.[29]

Results from preclinical circadian rhythm misalignment studies are further supported by clinical studies that reveal associations between specific single nucleotide polymorphisms in the *Clock* gene and risk for metabolic syndrome.[30] There are 3 known polymorphisms of this gene, which are relatively equally distributed throughout the population, and haplotype is significantly associated ($P = 0.02$) with presence of metabolic syndrome.[30] The functional role of these single nucleotide polymorphisms has not yet been elucidated.[21] Furthermore, it has been demonstrated in shift workers, both observationally and in a laboratory setting, that working at times other than daylight hours negatively impacts health outcomes, including a 66% elevated risk of obesity and a 57% elevated risk of metabolic syndrome.[23] Shift workers also have been shown to have 12%–16% reduced energy expenditure,[31] and they are more likely to experience dyslipidemia, including elevated blood triglycerides and reduced high-density

lipoprotein cholesterol.[22] In 1 experiment, human participants experiencing circadian misalignment through a 12-hour reversal from their habitual schedule had 17% lower leptin concentrations compared with their values under circadian alignment. Furthermore, insulin concentrations, glucose concentrations, and mean arterial pressure were 22%, 6%, and 3% higher, respectively, under misalignment than under alignment.[32] A summary of clinical findings of misaligned circadian rhythms can be found in Table 1.[21,23,25,28,31,32]

Impact of misaligned circadian rhythms and diet on the microbiota

There is increasing evidence that the microbiota, diet, circadian rhythms, and internal clock mechanisms are connected (Figure 2). Interactions among these factors can be explored through the provision of continuous intravenous nutritional support, which eliminates effects of feeding time or even intestinal presence of food on the GI microbiome. Interestingly, mice given continuous parenteral nutrition have been shown to have substantial changes in microbial community structure, but the microbiota did not completely lose diurnal variation.[33] Specifically, although beta diversity indices, a measure of dissimilarity or distance between samples, revealed clustering by treatment group when enteral and parenteral nutrition were compared, samples also clustered by time of day within each treatment group. Additionally, relative abundances of different phyla varied between treatments, with Verrucomicrobia dramatically blooming at the expense of Firmicutes in the parenteral group. For Bacteroidetes, a cyclical

abundance pattern was observed over the course of the day, increasing during the light phase and decreasing during the dark phase; this occurred independent of the study treatment provided.

Research has also been conducted to investigate interactions among environmental circadian rhythm disruption, diet, and the GI microbiome in murine models.[34] Continuous circadian rhythm disruption was induced by reversing the 12-hour light/dark cycle every week for 12 weeks in mice receiving chow or a high-fat/high-sugar diet. Mice in the misalignment group experienced intestinal hyperpermeability and disrupted circadian gene expression in intestinal cells. Cycle shifting

Relationship	Reference
SNPs of *Clock* gene ↑ or ↓ risk of metabolic syndrome	Zarrinpar et al. (2016)[21]
Shift work ↑ risk of obesity and metabolic syndrome	Wang et al. (2014)[23]
Shift work ↓ daily energy expenditure	McHill et al. (2014)[31]
Shift work ↓ leptin ↑ insulin ↑ glucose ↑ mean arterial pressure	Scheer et al. (2009)[32]
Sleep restriction ↑ BMI ↓ alertness No change in microbiota	Zhang et al. (2017)[28]
Jet lag ↑ relative abundance of Firmicutes ↑ weight gain and blood glucose in mice receiving transplants from jetlagged humans	Thaiss et al. (2014)[25]

Abbreviations: BMI, body mass index; SNP, single-nucleotide polymorphism.

resulted in significant weight gain in mice consuming chow ($P = 0.04$), but there were no significant changes in GI microbial composition of these animals ($P = 0.81$). There were, however, significant differences in microbial community composition between high-fat shifted and high-fat nonshifted groups ($P = 0.04$). The high-fat shifted mice had decreased alpha diversity, a measure of the number of different microbial species (richness) within a sample and their relative abundances (evenness), and a higher ratio of Firmicutes/Bacteriodetes compared with the other high-fat diet fed mice who were not exposed to a light/dark cycle shift. This specific microbial ratio has been associated with obesity or ill health in both rodent models and humans.[34] These results suggest that a combination of a high-fat diet and circadian disruption may be what drives microbial dysbiosis in mice.

A "jet lag" model has also been used to explore the effects of shorter (eg, ≤12 h) circadian cycle disruptions. In 1 study, mice exposed to an 8-hour shift in the light/dark cycle every 3 days for 4 weeks experienced a loss of rhythmicity in oscillating GI bacterial taxa.[25] Microbiome community composition was impacted after 4 weeks of this jet lag model intervention, and dysbiosis was even more pronounced by 16 weeks. Fecal transplant of jet-lagged mouse microbiome into germ-free mice resulted in weight gain and glucose intolerance in the recipient animals.[25]

Interestingly, there is evidence of bacteria containing clock genes[35] and regulating the behavior of their host in a circadian manner.[36] For example, *Enterobacter aerogenes* is purported to contain an endogenous circadian clock that synchronizes with the human host through melatonin secreted into the GI tract.[37] In mice, reprogramming of the hepatic circadian clock that
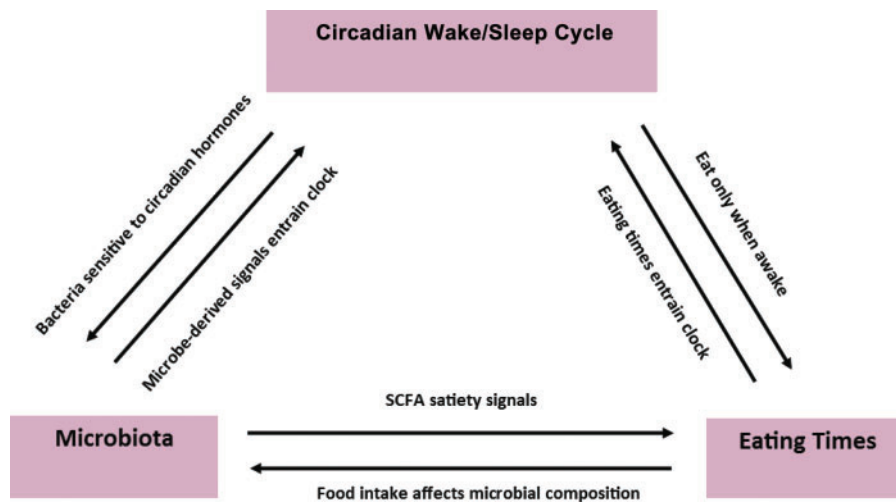

Figure 2 **Integration of circadian homeostasis with eating patterns and the microbiota.** *Abbreviation*: SCFA, short-chain fatty acids.

occurred following a high-fat diet intervention has been attributed to microbiota-driven induction and activation of the transcription factor PPARγ.[38] Both liver and intestinal circadian genes are affected by unconjugated bile acids, known products of microbial metabolism.[39] The absence of a microbiota, both in germ-free and antibiotic-induced murine models, has been shown to alter intestinal epithelial cell transcription of nuclear receptors and clock components such as *Rev-erbα, RORα, Bmal1, Cry1, Per1,* and *Per2*.[40] Additionally, these microbiota-deficient mice have decreased insulin concentrations and elevated blood glucose, triglyceride, and free fatty-acid concentrations as a result of intestinal corticosterone overproduction.[40] Circadian regulation within ileal and colonic epithelial cells has been found to be completely disrupted in animals without a microbiota.[40] The researchers theorized that microbe-associated molecular patterns are released from the microbiota in a continuous fashion, but Toll-like receptors translate this information into rhythmic signals of gene expression.[40] Conversely, Leone et al.[33] asserted that circadian shifts in bacterial composition result in corresponding shifts in concentration of bacterial metabolites, such as butyrate, which peaks during fasting, and hydrogen sulfide, which peaks during feeding. Fecal butyrate has been shown to cycle in mice on a standard, but not high-fat, diet, whereas hydrogen sulfide has been shown to exhibit cyclical behavior in the ceca of mice on a high-fat, but not normal, diet. It has been previously demonstrated in vitro that these metabolites can directly impact the cycling of hepatic clock genes *Per2* and *Bmal1*.[33] Taken together, the negative consequences observed following circadian disruption may be related to inflammatory processes caused by alterations in intestinal barrier function, increased abundances of proinflammatory bacteria, and etiologies of circadian disruption–related diseases.[17]

There is emerging evidence that the circadian clock impacts eating time among humans.[25] It has also been established that habitual diet and dietary alterations affect the GI microbial composition.[5] Given these relationships, it is purported that changes to the circadian clock and/or feeding time may impact the human gut microbiome. However, to date, there is only preliminary data on time shift–induced microbiota dysbiosis in humans. Jet lag induced by flying ahead 8 time zones resulted in changes in microbial abundances, including a higher relative abundance of Firmicutes, in 2 individuals.[25] Observed microbial changes in these jet-lagged individuals resolved within 2 weeks after landing.[25] Fecal transplant from jet-lagged human participants into germ-free mice resulted in weight gain that was 37% greater and peak blood glucose concentrations that were 35% higher during an oral glucose challenge than the

levels in mice that received fecal material from the same individuals taken before jet lag occurred.[25] Although the sample size was small in this study, the similarities to results from animal trials are promising.

TIME-RESTRICTED FEEDING

Time-restricted feeding is defined as the consumption of as much food as desired by a person or animal during a specific window of time, followed by denial of food access during a subsequent period.[41] A common theory for the observed benefits of TRF is that it mimics natural eating patterns based on circadian rhythms, the way humans ate before artificial lighting and high-energy foods became available 24 hours a day.[21,26] TRF among humans results in food being consumed during the light phase when the body is in the active state and not consumed during the dark phase when the body is ready to rest and repair. Thus the body receives the energy it needs when it is metabolically expecting and prepared for it. Evidence suggests that there are protective effects on weight, blood lipids, and glucose homeostasis associated with eating only within a specific window of the day (eg, TRF).[42,43]

Health implications of time-restricted feeding

Much of the research on TRF has been conducted in animal models, commonly through the use of diet-induced-obesity murine models involving a high-fat diet intervention. When mice are given ad libitum access to normal chow, intake occurs almost entirely during the dark phase, and the mice consume an adequate amount of energy for their needs and maintain normal body weight.[44,45] However, when mice are given ad libitum access to high-fat chow, the tendency to eat only during the dark phase disappears.[15,45,46] Contrary to their natural rhythm, the mice eat around the clock, which results in obesity and metabolic dysfunction. Introducing TRF in the context of a high-fat diet has been shown to reverse many detrimental metabolic consequences.[41,46] Mice provided 8-hour access to a high-fat diet, for example, were protected against obesity, hyperinsulinemia, hepatic steatosis, and inflammation, despite consuming an equivalent number of calories from identical diets as the animals in the 24-hour-access treatment arm.[46] Time-restricted feeding has also been linked to 26%–62% lower fat mass, 60% lower postprandial glucose concentrations, and a 93% reduction in insulin resistance in mice undergoing a 6-month TRF intervention compared with ad libitum–fed controls.[41]

In humans, TRF has been shown to result in modest weight reductions (1%–3%) when food intake is limited to a window of 10–12 hours; small to moderate

(5%–31%) improvements in plasma lipid measures (low-density lipoprotein [LDL], high-density lipoprotein, triglyceride, and total cholesterol) when food intake is limited to windows of 7–8 hours and 10–12 hours; and improved insulin sensitivity and fasting blood glucose concentrations across interventions with food intake limited to windows ranging from 4 hours to 10–12 hours.[42] Recently, a study in healthy, overweight adults demonstrated that decreasing the food intake window from approximately 14 hours per day to 10–11 hours for a 16-week period resulted in an average weight loss of 3.27 kg.[43] Clinical studies of TRF are summarized in Table 2.[25,42,43]

Impact of time-restricted feeding on the microbiota

In addition to impacting health, restricting the time of food access has also been shown to affect the GI microbial community structure in mice. In healthy mice fed a standard chow diet ad libitum, 17% of bacterial operational taxonomic units (OTUs) showed cyclical behavior, with 20%–83% of bacterial sequences at a single point in time belonging to cyclical OTUs.[15] Cyclical behavior among bacteria may be a result of microbial adaptation to the availability of food in the intestine at different points in the day.[25] Firmicutes were found to peak during the normal murine feeding phase (eg, dark), and Bacteroidetes peaked during the normal fasting phase (eg, light).[15] Interestingly, a reduced Bacteroidetes/Firmicutes ratio is associated with increased body weight and obesity in rodent models and humans. Fecal transplants in gnotobiotic mice suggest that these perturbations increase energy harvest and weight gain.[4] Another major phylum that displayed cyclical behavior is Verrucomicrobia, which followed the same pattern as Bacteroidetes of peaking in relative abundance during the fasting phase. Interestingly, this phylum contains the species *Akkermansia muciniphila*, which has been associated with positive health outcomes, such as improved glucose homeostasis and decreased inflammation.[47–49]

Preclinical research has demonstrated that feeding pattern alterations also disrupt the cyclical nature of OTUs. Mice fed a high-fat diet ad libitum alter their daytime fast/nighttime feed behaviors.[15,45,46] These mice also lose much of their normal OTU cycling.[15] Although TRF of a high-fat diet may be beneficial metabolically, it did not completely restore OTU cycling.[15] This is of note because others have reported that microbiome alterations can persist for longer than dietary interventions and even longer than the metabolic consequences of dysbiosis.[50] TRF did, however, decrease the relative abundances of several presumed obesogenic microbes, such as *Lactobacillus* and *Lactococcus* species,

and increase the abundances of presumed obesity-protective bacteria, such as *Oscillibacter* and other *Ruminococcaceae* species.[15]

Mice lacking a circadian clock through knockout of the *Per1* and *Per2* genes that were given ad libitum food access ate irregularly and had lower alpha diversity.[25] These circadian clock–absent mice also showed a distinct microbial community and lacked microbial cycling. When placed on a TRF regime, in either the light or dark phase, the cycling of the microbiota was restored. Several bacteria, including *Bacteroides* and *Lactobacillus reuteri*, showed cycling in these *Per1/Per2*–deficient animals under TRF, but the time of peaks and troughs was reversed in animals under TRF in the dark phase compared with TRF in the light phase. This further confirms the impact of feeding times on GI microbial composition within murine models.

To the best of the authors' knowledge, there are no published studies on the effect of TRF on the human GI microbiota. Given the preclinical findings related to relationships between the microbiota and TRF, it is probable that TRF interventions in humans would impact the microbiota; however, additional TRF research is needed to make a determination.

Health implications of eating frequency

Some observational evidence links differences in eating frequency with varying health effects in humans (Table 3).[51–56] Results from a study of nearly 2000 adults revealed an inverse relationship between adiposity and number of eating occasions after controlling for energy intake per kilogram of ideal body weight.[51]

Table 2 **Summary of human studies of time-restricted feeding**

Relationship	Reference
4-h feeding window ↑ insulin sensitivity	Rothschild et al. (2014)[42]
7–8-h feeding window ↑ insulin sensitivity ↑ HDL cholesterol ↓ LDL cholesterol ↓ triglycerides ↓ total cholesterol	Rothschild et al. (2014)[42]
10–12-h feeding window ↓ weight ↑ insulin sensitivity ↑ HDL cholesterol ↓ LDL cholesterol ↓ triglycerides ↓ total cholesterol	Rothschild et al. (2014)[42]
10–11-h window vs 14-h window ↓ weight	Gill et al. (2015)[43]
Human microbiome displays cyclical behavior, likely as a result of feeding times	Thaiss et al. (2014)[25]

Table 3 Summary of human studies of eating frequency

Relationship	Reference
↑ eating frequency ↓ adiposity	Metzner et al. (1977)[51]
>6 meals/d vs 4 meals/d ↓ CVD mortality	Chen et al. (2016)[52]
No breakfast ↑ type 2 diabetes mellitus risk	Mekary et al. (2012)[53]
1–2 meals/d vs 3 meals/d ↑ type 2 diabetes mellitus risk	Mekary et al. (2012)[53]

17 meals/d vs 3 meals/d
    ↓ total cholesterol
    ↓ LDL cholesterol
    ↓ apolipoprotein B
    ↓ insulin
	Jenkins et al. (1989)[54]

1 meal/d vs 3 meals/d ↑ weight loss	Kant (2014)[56]

Vast majority of weight
    maintenance research
    no relationship
	Raynor et al. (2015)[55]

Abbreviations: CVD, cardiovascular disease; LDL, low-density lipoprotein.

Furthermore, analyses of the 1988–1992 National Health and Nutrition Examination Survey III cohort followed through 2006 revealed a lower hazard ratio (0.68) for cardiovascular disease–related mortality for those eating >6 meals per day compared with eating 4 times per day.[52] This association was stronger for those consuming >2500 calories per day. Another large epidemiologic study (n = 29 206) reported that men who skipped breakfast had a 21% higher risk of diabetes and men who ate 1–2 meals per day had a 25% higher risk of diabetes compared with those who consumed 3 meals per day.[53]

Intervention trials focused on the impact of eating occasions on metabolic health are lacking. One small crossover study (n = 7) investigated serum markers of metabolic health following 2 isocaloric interventions: (1) a snacking pattern consisting of 17 small meals eaten 1 hour apart and (2) a 3-meal pattern.[54] The researchers reported that participants in the snacking pattern group had reductions of 8%–15% in total cholesterol, LDL, apolipoprotein B, and serum insulin concentrations compared with participants in the 3-meal pattern group. A potential confounding aspect of this study was that the eating time window differed between the 2 treatments—participants in the 3-meal pattern group, ate during an 11-hour period, whereas those in the hourly snacking pattern ate during a 17-hour period. As discussed earlier, restricting food intake to a smaller time interval is associated with improvements in metabolic health. Intriguingly, those in the snacking pattern group had improved metabolic health despite the longer eating duration.

Raynor et al.[55] conducted a systematic review of human and animal studies on eating frequency and weight status and concluded that the relationship between eating frequency and weight is unclear. More than 60% of the studies reviewed found no effect of eating frequency on consumption/intake, body weight, or body mass index. Inconsistent findings related to eating frequency and body weight may be due to reporting bias. It has been suggested that underreporting of food consumption and eating occasions in adults who are overweight or obese may result in an erroneous connection between fewer meals and higher weight.[56,57] A review by Kant of 4 prospective cohort studies and 12 controlled trials of eating frequency and body weight found mixed results among the cohort studies with 1 reporting a benefit, 2 reporting a detriment, and 1 showing no effect; the majority of the randomized controlled trials found no relationship between eating frequency and weight loss. Only 1 study reported a significant difference (loss of 0.6 kg; $P = 0.01$) in participants consuming 1 meal per day over 8 weeks compared with participants consuming 3 meals per day over 8 weeks (gain of 0.8 kg).[56]

The connection between the number of eating occasions and health is not fully understood. Although beneficial clinical results from greater eating frequency have been reported in the areas of adiposity, insulin, blood lipids, and risk of diabetes and cardiovascular-related death, effects on weight status are less clear. Plausible mechanisms underlying the inverse relationships between more frequent eating occasions and lower glucose, insulin, total cholesterol, and LDL cholesterol concentrations include a lower glycemic load as food is spread throughout the day, slower stomach emptying from smaller meals leading to a lower need for insulin, decreased insulin leading to decreased stimulation of enzymes for cholesterol synthesis, and increased LDL receptors as a result of the lower circulating cholesterol.[58]

Impact of eating frequency on the microbiota

Although there is considerable research on eating frequency and health, the impact of eating frequency on the GI microbiome has only recently been explored. In horses, the cecal microbiota is impacted by feeding frequency, with higher frequency being associated with increased relative abundance of the genus *YRC22* and decreased relative abundances of *Prevotella, Lactobacillus, Streptococcus, Coprococcus,* and *Phascolarctobacterium*.[59] Additional research is necessary to determine whether changes in eating patterns affect taxa associated with glucose response, lipid metabolism, and adiposity in humans.

Independent of eating frequency, certain bacterial taxa are associated with improved glucose, lipids, and

adiposity. For example, *A. muciniphila*, a mucin-degrading intestinal bacterium, has been associated with improved glucose homeostasis[47] and inversely correlated with inflammation in animal models.[48] In humans, an increased abundance of *Akkermansia* was found to inversely relate to fasting glucose, waist-to-hip ratio, and subcutaneous adipocyte diameter.[49]

LIMITATIONS

This review is limited by the novelty of the field it explores. Recent advances in microbiome investigation technologies have only lately made this research possible. Additionally, performing circadian rhythm or food-timing interventions on humans can be challenging. Self-reported behaviors are subject to bias and error, and in-house experiments are costly and difficult to execute. Furthermore, human studies involving eating pattern alterations (eg, TRF) must be carefully monitored to assess and control energy intake because humans generally decrease energy intake when given a reduced eating window, whereas animals will not.

Limitations of rodent models in microbiota research include the anatomy of the GI tract as well as coprophagy. Rodents are cecal fermenters, meaning most of their bacterial fermentation takes place in the cecum. In humans, most bacterial fermentation occurs in the large intestine. Additionally, the distribution of goblet (mucin-producing) cells is consistent throughout the human colon but decreases along the length of the mouse colon, which could affect the distribution of mucin-degrading bacteria.[60] Furthermore, rodents practice coprophagy, consuming their fecal material as well as fecal material of other animals. Lastly, in rodent studies, in addition to fecal samples, the bacterial content of the cecum is frequently examined, whereas in human studies fecal samples are used to characterize the microbiota.

The use of undefined animal diets limits comparisons among rodent studies as well as translation to clinical populations. Chow varies in composition and sources of nutrients due to price and availability of ingredients, whereas refined diets, such as the American Institute of Nutrition diet, have defined nutrient composition. The high-fat diets used in animal research are infrequently representative of the proportion and composition of fat in human diets. In high-fat chow, up to 60% of total energy comes from fat, with 24% of total energy coming from saturated fat. In contrast, NHANES data from 2009 to 2012 show that men and women aged >20 years get an average of 33% of their total energy from fat and 10.6% of their total energy from saturated fat.[61]

Despite these limitations, the murine model is still a powerful tool in microbiota research. Mice have a different core microbiome than humans, but many of the species comprising the microbiome are similar and present in relatively similar abundances. Interventions also tend to show similar shifts in the microbiota of both mice and humans for many conditions, although it may take longer for diet to affect shifts in humans than in mice.[60] Colonization of germ-free mice with fecal transplants from humans overcomes some of these challenges, but the cross-talk between host and symbiont is not identical. Overall, the mouse is a valuable model that allows isolation of variables that would be impossible in humans. Helpful conclusions from these studies can be drawn, although the drawbacks to the translation of results should be kept in mind.

CONCLUSION

Research on disrupted circadian rhythms suggests a reciprocal relationship between the microbiome and the internal clock. Animal evidence supporting the detrimental health effects of disrupting the normal circadian rhythm is robust.[21,24,25] Human evidence is preliminary but promising. In both models, the microbiome has been suggested as a potential mediator between circadian misalignment and negative health consequences. Further work is needed with interventions rather than observational studies to establish a causal link between misaligned rhythms and the microbiome, investigating not only microbial composition but also the functional capacity of the microbiome and/or metabolomics.

Results from TRF studies indicate that restricting the time of food access may be protective against weight gain, insulin resistance, and dyslipidemia.[41,46] These results also indicate there is a connection between the microbiome and metabolic health. Results demonstrate an absence of diet-induced obesity in germ-free mice and a lack of microbial cycling in mice without an internal clock. To date, evidence from human studies has not been robust because of issues such as small sample sizes and methodologic limitations inherent to human research, including the inability to conduct germ-free experimentation.

There is extensive research on the connection between eating frequency and health but almost no published research on the connection between eating frequency and the microbiome. Additional adequately powered, well-controlled, randomized trials investigating the impact of eating frequencies on the GI microbiome as a primary outcome are necessary to translate preclinical research findings to human populations.

In general, additional well-designed randomized controlled trials of eating behaviors, including trials that focus on circadian rhythm alignment, TRF, and eating frequency, with their effect on the microbiome as a primary outcome, will vastly increase knowledge and

strengthen the evidence for using "when to eat" as a novel intervention to prevent or treat disease through GI microbiota manipulation.

Acknowledgments

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