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Intermittent fasting contributes to aligned circadian rhythms through interactions with the gut microbiome

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REVIEW ARTICLE

Abstract

The timing of food consumption is considered to be an important modulator of circadian rhythms, regulating a wide range of physiological processes which are vital to human health. The exact mechanisms underlying this relationship are not fully understood, but likely involve alterations in the structure and functioning of the gut microbiome. Therefore, this narrative review aims to clarify these mechanisms by focusing on intermittent fasting as a dietary strategy of food timing. A literature search identified 4 clinical and 18 preclinical studies that examined either (1) the impact of intermittent fasting on the gut microbiome, or (2) whether circadian rhythms of the host are subject to changes in the bacterial populations in the gut. Results reveal that intermittent fasting directly influences the gut microbiome by amplifying diurnal fluctuations in bacterial abundance and metabolic activity. This in turn leads to fluctuations in the levels of microbial components (lipopolysaccharide) and metabolites (short-chain fatty acids, bile acids, and tryptophan derivates) that act as signalling molecules to the peripheral and central clocks of the host. Binding of these substrates to pattern-recognition receptors on the surface of intestinal epithelial cells in an oscillating manner leads to fluctuations in the expression of circadian genes and their transcription factors involved in various metabolic processes. Intermittent fasting thus contributes to circadian rhythmicity in the host and could hold promising implications for the treatment and prevention of diseases associated with disordered circadian rhythms, such as obesity and metabolic syndrome. Future intervention studies are needed to find more evidence on this relationship in humans, as well as to clarify the optimal fasting regimen for balanced circadian rhythms.

Keywords: intermittent fasting, time-restricted feeding, alternate-day fasting, circadian rhythms, gut microbiome

1. Introduction

Most life forms on our planet have adapted to the daily 24-h light-dark cycle created by the rotation of the Earth in relation to the sun. As a result, organisms have developed internal timing mechanisms to synchronise important physiological activities, such as gene expression, metabolism, and behaviours, with daily environmental fluctuations (Kaczmarek *et al.*, 2017a; Teichman *et al.*, 2020). These cyclical patterns of approximately 24 h are regulated by the biological clock located in the body and are referred to as circadian rhythms (Voigt *et al.*, 2016). Circadian rhythms can be entrained by various environmental factors or 'zeitgebers' to keep the circadian clock aligned with the environment. Among these factors, light is the primary zeitgeber that strongly regulates the central clock, while other factors such as food timing, dietary composition, exercise, temperature, and infection influence the clocks in the peripheral organs (Teichman *et al.*, 2020). Disruption of circadian rhythms could lead to a variety of adverse health outcomes, ranging from sleep disorders to metabolic diseases, such as obesity (Patke *et al.*, 2017; Turek *et al.*, 2005). Therefore, it is essential to balance our circadian rhythms to optimise biological functions and thereby prevent disease.

As previously mentioned, the timing of food intake is considered to be an important modulator of the circadian

system and thereby may contribute to misaligned circadian rhythms (Page *et al.*, 2020; Voigt *et al.*, 2016). For instance, mice that consume a large amount of food outside their normal feeding window exhibit diminished oscillations in circadian genes and their transcription factors involved in metabolism. This desynchronisation between feeding-fasting patterns and circadian cycles could then contribute to weight gain and lead to the development of obesity and metabolic syndrome (Zarrinpar *et al.*, 2016). As circadian rhythms and metabolic processes are tightly connected to the gut microbiome, it is implied that these diseases may be established through interactions with the bacterial populations in the gut (Kaczmarek *et al.*, 2017a; Teichman *et al.*, 2020). According to Teichman *et al.* (2020), there seems to be a strong overlap between diseases related to the gut microbiome and circadian clock of the host. Thus, focusing on food timing in the prevention and treatment of these diseases could be of great importance.

One of the most widely studied and applied dietary strategies of food timing is intermittent fasting. Intermittent fasting is an umbrella term for a number of specific fasting regimens which are characterised by periods of food abstinence that are longer than the typical overnight fast. They can be divided into three categories: alternate-day fasting, whole-day fasting, and time-restricted feeding. Alternate-day fasting involves switching between *ad libitum* feeding days and fasting days, while whole-day fasting refers to fasting for one to two days per week and *ad libitum* eating on the other days (Tinsley and La Bounty, 2015). In some cases, fasting days permit food intake of up to approximately 25% of daily energy consumption (Brown *et al.*, 2013). The third category, time-restricted feeding, allows people to follow the same eating regimen each day, where a defined number of hours is assigned to the feeding window (4-8 h) and the remaining hours to the fasting window (Tinsley and La Bounty, 2015). Although alternate-day fasting and whole-day fasting are the most popular forms of intermittent fasting, time-restricted feeding has gained a lot of attention lately due to its easy incorporation into daily life (Harris *et al.*, 2018).

In the past years, an increasing amount of research has been conducted into the interplay between food timing, circadian rhythms, and the gut microbiome (Teichman *et al.*, 2020). It is suggested that the time of eating influences the diurnal cycles present in the gastrointestinal tract, which is reflected by alterations in the microbial structure. This represents a mechanism through which the gut microbiota can affect host metabolism and functioning (Patterson and Sears, 2017). Despite the fact that new findings have led to greater understandings, much is still unclear about the exact mechanisms that describe how these components come together to regulate the onset of, and risk of, certain diseases. Furthermore, few studies have investigated the role of intermittent fasting as a dietary strategy of food timing in this interaction. Therefore, this narrative review aims to clarify the working of these mechanisms and summarise the current knowledge on this complex relationship. The main objective here is to investigate how intermittent fasting influences circadian rhythms by intervening with the gut microbiome.

2. Methods

The focus of this review is on both human and animal studies that either assessed the effect of intermittent fasting regimens on the gut microbiota or whether circadian rhythms of the host are subject to changes in the bacterial populations in the gut. Two comprehensive PubMed searches were performed to identify articles that investigated this multidirectional relationship. First, studies regarding the interaction between intermittent fasting regimens and the gut microbiome were searched using the terms 'intermittent fasting', 'alternate-day fasting', 'time-restricted feeding', 'periodic fasting', 'whole-day fasting', 'food timing', and 'meal timing' in combination with the terms 'gut microbiome', 'gut microbiota', 'gastrointestinal microbiome', and 'gastrointestinal microbiota'. Articles with end points that measured changes in the structure or functioning of the gut microbiota were included. Second, the relationship between the gut microbiome and circadian rhythms was examined using the terms 'gut microbiome', 'gut microbiota', 'gastrointestinal microbiome', and 'gastrointestinal microbiota' in combination with the terms 'circadian rhythm(s)', 'circadian clock', 'circadian system', 'diurnal rhythm(s)', 'diurnal fluctuations', and 'diurnal oscillations'. To reduce the number of articles found, the keyword search was limited to the title and abstract of the article and studies published between 2015 and 2020. Inclusion criteria were articles with end points that included alterations in the circadian system or diurnal oscillations of metabolic processes. In addition, relevant studies from the reference list of research articles and reviews were included using the snowball method. Together these searches yielded a total of 129 studies of which 4 clinical and 18 preclinical studies were suitable for the current review.

3. Impact of intermittent fasting on the gut microbiome

Besides having a profound impact on health, intermittent fasting has been shown to affect the microbial populations in the gut (Kaczmarek *et al.*, 2017a; Patterson and Sears, 2017). Most research on this interaction has been conducted in the past five years. The majority of these studies assessed the mediating role of the gut microbiota in the treatment of certain disorders with different forms of intermittent fasting, including alternate-day fasting and time-restricted feeding. Evidence from animal studies provides a good

overview of the underlying mechanisms that describe how intermittent fasting exerts its effects on the gut microbiome. These findings are supported by recent pilot studies that demonstrate the existence of this relationship in humans. Detailed information of both preclinical and clinical studies is provided in Table 1.

Intermittent fasting increases gut microbiome β-diversity, but not α-diversity

Alterations in the microbial structure of the gut are generally reflected by α-diversity, which describes the species richness and evenness within a sample, and β-diversity, a measure of

Table 1. Studies on the impact of intermittent fasting on the gut microbiome.[1]

Reference	Study design	Population	Exposure	Outcome[2]
Preclinical studies				
Zarrinpar *et al.* (2014)	*in vivo*	male mice (12 weeks)	Ad lib | HFD | HFD + TRF (8 weeks)	HFD + TRF: α-diversity ↔, β-diversity ↑, bile acids ↑, improved rhythmicity in microbiota
Thaiss *et al.* (2016)	*in vivo*	SPF mice | *Per1/2-/-* mice (8-9 weeks)	Ad lib | TRF (light phase) | TRF (dark phase)	SPF mice: phase-reversed microbial attachment rhythms after TRF (light phase) compared to TRF (dark phase) *Per1/2-/-* mice: restored rhythmicity in amount and composition of microbiota and metabolites after TRF
Li *et al.* (2017)	*in vivo*	male mice (7-8 weeks)	Ad lib | ADF | HFD | HFD + ADF (30 days)	ADF: α-diversity ↑, β-diversity ↑, body weight ↓, SCFAs (acetate, lactate) ↑
Beli *et al.* (2018)	*in vivo*	male db/db mice | control mice (4 months)	Ad lib | ADF (7 months)	ADF: α-diversity ↔, β-diversity ↑, bile acids (TCDCA, TUDCA) ↑
Cignarella *et al.* (2018)	*in vivo*	female mice (7 weeks)	Ad lib | ADF (4 weeks)	ADF: α-diversity ↑, β-diversity ↑, body weight ↓, butyrate ↑, LPS-pathway ↓
Li *et al.* (2020)	*in vivo*	male mice (7 weeks)	Ad lib | TRF 12 h | TRF 16 h | TRF 20 h (30 days)	TRF: α-diversity ↔, β-diversity ↑, food intake ↓, body weight ↓
Liu *et al.* (2020a)	*in vivo*	male db/db mice | control mice (4 months)	Ad lib | ADF (28 days)	ADF: α-diversity ↑, β-diversity ↑, body weight ↓, serotonin ↑, tryptophan ↑, bile acids (CA, DCA, MCA, TUDCA) ↑, SCFAs (acetate, propionate, butyrate) ↑
Van der Merwe *et al.* (2020)	*in vivo*	male ob/ob mice (12 weeks)	HFID | HFD | HFD + TRF | HFD + ADF | HFD + CR (8 weeks)	TRF | ADF: α-diversity ↑, β-diversity ↑, food intake ↓, body weight ↓
Clinical studies				
Cignarella *et al.* (2018)	RCT	16 RRMS patients (±41 years)	Ad lib | ADF (15 days)	ADF: α-diversity ↑,β-diversity ↔, body weight ↓, leptin ↑
Gabel *et al.* (2020)	QET	14 obese adults (25-65 years)	TRF (12 weeks)	TRF: α-diversity ↔, β-diversity ↔, body weight ↓, food intake ↓
Ozkul *et al.* (2020)	QET	9 healthy adults (31-56 years)	TRF (29 days)	TRF: α-diversity ↑, β-diversity ↑, glucose ↓, total cholesterol ↓
Zeb *et al.* (2020)	RCT	80 healthy males (young aged)	Ad lib | TRF (25 days)	TRF: α-diversity ↑, β-diversity ↑, HDL ↑, LDL ↓, TAG ↓, *Sirt1* ↑, *Bmal1* ↑, *Clock* ↑

[1] Ad lib = ad libitum; ADF = alternate-day fasting; CA = cholic acid; CR = caloric restriction; db/db = diabetic; DCA = deoxycholic acid; HDL = high density lipoprotein; HFD = high-fat diet; HFID = high-fibre diet; LDL = low density lipoprotein; LPS = lipopolysaccharide; MCA = muricholic acid; ob/ob = obese; QET = quasi-experimental trial; RCT = randomised controlled trial; RRMS = relapsing-remitting multiple sclerosis; SCFAs = short-chain fatty acids; TAG = triglyceride; TCDCA = taurochenodeoxycholic acid; TUDCA = tauroursodeoxycholic acid; TRF = time-restricted feeding.

[2] Expression levels: no change (↔), increase (↑) and decrease (↓).

dissimilarity between samples (Beli *et al.*, 2018). Preclinical evidence has demonstrated that intermittent fasting regimens increase β-diversity, while research is inconclusive about the effect on α-diversity of the gut microbiota (Beli *et al.*, 2018; Cignarella *et al.*, 2018; Li *et al.*, 2017, 2020; Liu *et al.*, 2020a; Van der Merwe *et al.*, 2020; Zarrinpar *et al.*, 2014). Mice that were fed an alternate-day fasting diet for seven months showed an increase in β-diversity compared to control mice that had *ad libitum* access to food (Beli *et al.*, 2018). Similar results were found in a variety of other studies that investigated both time-restricted feeding and alternate-day fasting diets in healthy mice as well as diabetic and obese mice (Cignarella *et al.*, 2018; Li *et al.*, 2017, 2020; Liu *et al.*, 2020a; Van der Merwe *et al.*, 2020; Zarrinpar *et al.*, 2014). Interestingly, intermittent fasting led to greater improvements in the microbial diversity of genetically modified diabetic mice, which might have been due to the pre-existing disparities between the gut microbiome of these mice compared to wild type mice (Beli *et al.*, 2018).

Although the majority of the reviewed studies demonstrated an increase in α-diversity of the gut microbiome after a period of intermittent fasting (Cignarella *et al.*, 2018; Li *et al.*, 2017; Liu *et al.*, 2020a; Van der Merwe *et al.*, 2020), several studies were not able to find any effect (Beli *et al.*, 2018; Li *et al.*, 2020; Zarrinpar *et al.*, 2014). These different results might be due to differences in weight loss: studies that found an effect on α-diversity also reported a decrease in body weight after intermittent fasting, suggesting that weight loss is essential for detecting these alterations in the gut microbiome (Cignarella *et al.*, 2018; Li *et al.*, 2017; Liu *et al.*, 2020a; Van der Merwe *et al.*, 2020). However, α-diversity can vary widely throughout the day and when adjusting for all time points, no significant differences between intermittent fasting and *ad libitum* feeding groups could be observed (Zarrinpar *et al.*, 2014). This implies that α-diversity is rather a reflection of nutritional composition of the diet instead of the metabolism of the host (Zarrinpar *et al.*, 2018). Therefore, it is questionable whether α-diversity is a sufficient indicator for microbial functioning and metabolic health as previously assumed (Le Chatelier *et al.*, 2013). Changes in β-diversity are nevertheless significant, implying that fluctuations in the microbial composition are important for host metabolism (Zarrinpar *et al.*, 2014).

Given the preclinical findings on the effect of intermittent fasting on the diversity of the gut microbiome, it is presumable that intermittent fasting interventions in humans would generate similar results. Recently, a small randomised controlled trial was conducted that investigated the effect of an alternate-day fasting diet on the gut microbiome in sixteen multiple sclerosis patients. After fifteen days of intervention, α-diversity of the gut microbiome was increased in the fasting group, but no differences in microbial composition between the fasting and control group could be observed (Cignarella *et al.*, 2018). Although dietary assessment was not performed in this study, it should be considered that these findings may be influenced by differences in nutrient intake among the two groups. Since the fasting group was allowed to eat salad on fasting days, this may has contributed to a higher consumption of vegetables (Cignarella *et al.*, 2018). Similar changes in α-diversity of the gut microbiome were observed in two other clinical studies that even demonstrated significant alterations in the microbial community structure (Ozkul *et al.*, 2020; Zeb *et al.*, 2020).

Contradictory to the aforementioned studies, a quasi-experimental trial in fourteen obese adults that underwent time-restricted feeding for twelve weeks did not find any alterations in the gut microbiome. Again, subjects could adhere to their regular diet and were only bound to the feeding-fasting pattern. Although a reduction in body weight at the end of the intervention period was reported, this two percent weight loss was not clinically significant (Gabel *et al.*, 2020). Based on previous findings in obese adolescents, it is expected that a minimum weight reduction of 8% is necessary to observe improvements in gut microbiome diversity (Santacruz *et al.*, 2009). As described before, a similar pattern was found in genetically modified diabetic mice although these were not overweight (Beli *et al.*, 2018; Li *et al.*, 2020; Zarrinpar *et al.*, 2014). Therefore, it appears that an increased amount of body fat might diminish the positive effects of intermittent fasting on the gut microbiome, making the combination with weight loss essential when applied in overweight people.

Intermittent fasting causes a change in the microbial composition

Further analysis of the composition of the gut microbiome after intermittent fasting resulted in diverse outcomes between studies. Mice exposed to an alternate-day fasting diet demonstrated an enrichment in *Firmicutes*, and a reduction in *Bacteroidetes* and *Verrucomicrobia* at phylum level. Among these the genera *Lactobacillus* and butyrate producing *Odoribacter* were significantly enhanced (Beli *et al.*, 2018; Li *et al.*, 2017; Liu *et al.*, 2020a). This increased proportion of *Firmicutes* compared to *Bacteroidetes* has before been associated with an increased capacity for energy harvesting and subsequently a higher risk of obesity (Turnbaugh *et al.*, 2006). However, two out of the three reviewed studies that measured an elevated *Firmicutes*/*Bacteroidetes* ratio did not observe an increase in body weight and even reported weight loss at the end of the intervention, which casts doubt on previous findings (Li *et al.*, 2017; Liu *et al.*, 2020a). Moreover, the available evidence in humans suggests that this typical obesity associated microbiota is rather a result of dietary intake than being a cause of obesity (Bell, 2015). Another study in mice not necessarily found alterations at phylum level but specific

genera were altered after intermittent fasting. Bacteria from the *Actinobacteria* phylum, such as *Bifidobacterium*, were enriched in the alternate-day fasting group, while the relative abundance of *Ruminococcus* and *Christensenellaceae* (i.e. *Firmicutes* phylum) increased after time-restricted feeding (Van der Merwe *et al.*, 2020).

While the majority of animal studies indicate an enrichment of bacteria from the *Firmicutes* phylum after intermittent fasting, trials in humans were not able to demonstrate a similar pattern. However, time-restricted feeding interventions did cause alterations in bacterial abundance within the structure of the phylum (Ozkul *et al.*, 2020; Zeb *et al.*, 2020). Ozkul *et al.* (2020) followed nine adults who participated in Ramadan fasting for 29 days. Ramadan fasting is characterised by daily fasting intervals (12-18 h) from sunrise to sunset and thus can be considered as a representative model of time-restricted feeding. Comparison of stool samples before and after the intervention indicated an enrichment of *Akkermansia* (i.e. *Verrucomicrobia* phylum), *Bacteroides* (i.e. *Bacteroidetes* phylum), *Butyricicoccus*, *Feacalibacterium*, and *Roseburia* (i.e. *Firmicutes* phylum) at genera level after fasting. Interestingly, most of these bacteria play a major role in the production of short-chain fatty acids, such as butyrate. Although the relative abundance of *Bacteroidetes* was significantly increased after a period of time-restricted feeding, no reduction in the *Firmicutes*/*Bacteroidetes* ratio could be measured. However, it should be noted that the feeding and fasting pattern of Ramadan fasting is reversed as compared to natural human circadian rhythms, leading to some reservations about these results.

Intermittent fasting alters the levels of microbial components and metabolites

Considering that microbes in the gastrointestinal tract are responsible for the production of various metabolites, it is expected that these levels would be affected as well. Six preclinical studies measured a wide range of microbiota-derived metabolites and found significant changes after a period of intermittent fasting (Beli *et al.*, 2018; Cignarella *et al.*, 2018; Li *et al.*, 2017; Liu *et al.*, 2020a; Van der Merwe *et al.*, 2020; Zarrinpar *et al.*, 2014). Liu *et al.* (2020a) identified a total of 23 microbial metabolites whose levels were markedly modulated by the bacterial abundance in the gut after alternate-day fasting treatment. Among these were increased plasma levels of serotonin, tryptophan, various bile acids, and short-chain fatty acids (acetate, butyrate, and propionate) (Liu *et al.*, 2020a). Analysis of faecal contents from mice that underwent time-restricted feeding revealed a significant increase in primary bile acids and a trend towards increased levels of secondary bile acids compared to controls. Hence, intermittent fasting induced alterations in the gut microbiome result in an increased excretion of bile acids, and thereby increased concentrations of bile acids within the gut (Zarrinpar *et al.*, 2014). Another study reported a trend towards an increased abundance of several short-chain fatty acids – including acetate, butyrate, and formate – in the caecal content of mice consuming an intermittent fasting diet (Van der Merwe *et al.*, 2020). This elevation indicates either a reduced absorption of these short-chain fatty acids or increased fermentation of undigested carbohydrates. Since alternate-day fasting leads to the upregulation of the pyruvate fermentation pathway in the intestines associated with increased serum acetate and lactate levels, the latter seems most plausible (Li *et al.*, 2017).

Intermittent fasting regimens also affect the expression of various components on the surface of bacteria in the gastrointestinal tract. Cignarella *et al.* (2018) found that alternate-day fasting decreased the activity of the lipopolysaccharide biosynthesis pathway, which is responsible for the production of lipopolysaccharide on the outer membrane of gram-negative bacteria. It thus would appear as if intermittent fasting not only affects the microbial community structure of the gut, but also the activity of several metabolic pathways involved in both the production of microbial metabolites and components. However, for humans the findings are still inconclusive. Although several clinical studies reported an increase in short-chain fatty acid producing bacteria in the gut, no significant increase in serum butyrate levels could be measured (Cignarella *et al.*, 2018; Ozkul *et al.*, 2020).

Intermittent fasting restores the diurnal rhythmicity of the gut

Despite the fact that the bacteria in the gastrointestinal tract are not exposed to the light-dark cycle, they demonstrate great fluctuations in compositional and functional structures over the course of the day (Kaczmarek *et al.*, 2017b; Liang *et al.*, 2015; Thaiss *et al.*, 2014, 2016; Voigt *et al.*, 2014; Zarrinpar *et al.*, 2014). Analysis of faecal samples of 28 healthy males showed that 35% of bacterial operational taxonomic units undergo daily fluctuations, of which *Roseburia* and *Ruminoccocus* (i.e. *Firmicutes* phylum) were the most abundant genera associated with time. Furthermore, the concentrations of several bacterial metabolites – including acetate, propionate, and butyrate – decreased over the course of the day (Kaczmarek *et al.*, 2017b). Another study in mice reported that bacteria from the *Firmicutes* phylum were most abundant during the feeding phase, while *Bacteroidetes* and *Verrucomicrobia* peaked during the fasting phase (Zarrinpar *et al.*, 2014). These cyclical fluctuations in the gut microbiome may also explain large variation in results between studies investigating these microbes, emphasising the importance of collecting samples at consistent time points during the day.

Dysregulation of host circadian rhythms can inhibit intestinal oscillations and thereby affect physiological functions. For instance, deletion of circadian clock genes (Liang *et al.*, 2015; Thaiss *et al.*, 2014, 2016; Voigt *et al.*, 2014), restriction of food availability (Thaiss *et al.*, 2014, 2016; Voigt *et al.*, 2014; Zarrinpar *et al.*, 2014), and reversal of the light-dark phase (Thaiss *et al.*, 2014, 2016; Voigt *et al.*, 2014) in mice have all been shown to abolish the cyclical nature of the microbiome. Liu *et al.* (2020b) investigated whether this also applies to the human gut microbiome by inducing a shift in the sleep-wake cycle of 22 young adults. Although this acute circadian rhythm misalignment exerted limited influence on the gut microbiota, alterations in microbial functionality and the relationships within the gut microbiome could be observed. These data suggest that host circadian rhythms are indispensable for the maintenance of oscillations in the gut microbiota and synchronises these with changes in the light-dark cycle and eating behaviours.

Intermittent fasting has been shown to partially restore diminished cyclical oscillations in bacterial families that are essential for host metabolism (Thaiss *et al.*, 2016; Zarrinpar *et al.*, 2014). Mice lacking a functional circadian rhythm through deletion of the *Per1* and *Per2* genes that were given *ad libitum* access to food, demonstrated an irregular feeding pattern and a marked loss of microbial cycling. When these mice were placed on a time-restricted feeding diet, in either the light or dark phase, the cyclical oscillations of the microbiota were restored. In addition, the time of peaks and troughs was reversed in mice that were only fed during the dark phase compared to the light phase (Thaiss *et al.*, 2016). A similar effect was found in mice that were fed a high-fat diet. In these animals, diet-induced obesity altered their feeding and fasting rhythms and abolished much of their bacterial cycling. Although time-restricted feeding was not able to completely restore these fluctuations, it did induce the cycling of species that are thought to be obesogenic, such as *Lactobacillus* (i.e. *Firmicutes* phylum), and lower the relative abundance of another obesogenic microbe *Lactococcus* (i.e. *Firmicutes* phylum), especially during the fasting phase. Other presumed protective species belonging to the *Ruminococcacea* family, including the genus *Oscillibacter* (i.e. *Firmicutes* phylum), were increased in the fasting group (Zarrinpar *et al.*, 2014). These results further confirm the impact of feeding times on the microbial composition of the gut over the course of the day.

In humans, studies that investigated the effect of intermittent fasting diets with a full circadian characterisation of the gut microbiome are lacking. However, a recent randomised controlled trial aimed to measure the association between circadian rhythms and the bacterial populations in the gut induced by time-restricted feeding. A total of 80 healthy males were enrolled in the study of which 56 underwent daily fasting intervals of 16 h for 25 days while the remaining participants had no time restriction. Investigation of the microbiome revealed an increased α-diversity at different taxonomic levels in the fasting group, which was positively associated with activation of the *Sirt1* gene and increased levels of high density lipoprotein-cholesterol in the blood (Zeb *et al.*, 2020). Sirtuin 1 (SIRT1) modulates the circadian rhythm by controlling the acetylation of *Bmal1*, which is an important core clock gene (Nakahata *et al.*, 2008). Time-restricted feeding also had a striking effect on the composition of the gut microbiome. The fasting group demonstrated an enrichment in *Prevotellaceae* and *Bacteroidia* (i.e. *Bacteroidetes* phylum), while in the control group *Escherichia*, *Shigella* (i.e. *Proteobacteria* phylum), and *Peptostreptococcus* (i.e. *Firmicutes* phylum) were most abundant at genus level. Some of the enriched bacteria in the fasting group were inversely correlated with low density lipoprotein-cholesterol and triglyceride levels, exhibiting an anti-obesity response. Furthermore, these microbes had a positive association with *Sirt1* and the circadian genes *Bmal1* and *Clock*, meaning these were more expressed (Zeb *et al.*, 2020). These data imply that intermittent fasting may prevent metabolic risk through alterations in the gut microbiome that affect the circadian system of the host.

In conclusion, research in both humans and animals demonstrate that adhering to an intermittent fasting diet could contribute to the cyclical nature of the microbiome reflected by improved fluctuations in bacterial abundance and functioning. This newly emerged composition of the microbiome can then result in an increased production or conversion of metabolites – including tryptophan and its derivates, short-chain fatty acids, and bile acids – along with a decreased expression of lipopolysaccharide on the outer membrane of gram-negative bacteria in the gut. Considering that these microbial metabolites and components are important for a wide range of physiological processes in the host, they may also interfere with the circadian system. In the remaining of this review, it will be explored whether such an interaction between the gut microbiota and host circadian rhythms exists.

4. Impact of the gut microbiome on circadian rhythms

In addition to environmental factors, such as diet and eating behaviours, the structure of the gut microbiome is subject to circadian fluctuations in the host (Liang and Fitzgerald, 2017). The question remains, however, whether the gut microbiota can also transmit signals to the biological clock, implying a reciprocal relationship between the intestinal bacteria and host circadian system (Teichman *et al.*, 2020). In recent years, an increasing amount of research has explored this field and demonstrated a clear association between the gut microbiome and diurnal oscillations in physiological processes of the host. Due to experimental difficulties in humans on this interaction, mainly animal studies have been conducted to date. Table 2 presents an overview of these studies with more detailed information.

Table 2. Studies on the impact of the gut microbiome on circadian rhythms.[1]

Reference	Study design	Population	Exposure	Outcome[2]
Mukherji *et al.* (2013)	*in vivo*	SPF mice | AIMD mice | GF mice[3] (8-12 weeks)	–	AIMD mice | GF mice: *RevErbα* ↑, *RORα* ↓, *Bmal1* ↓, *Cry1* ↓, *Per1* ↑, *Per2* ↑, PPARα ↑
Leone *et al.* (2015)	*in vivo*	SPF mice | CONV mice | GF mice[3] (8-10 weeks)	IP-injection of saline | saline + butyrate (10 times)	GF mice: *Bmal1* ↓, *Clock* ↓, *Per2* ↓, *Cry1* ↓ (liver and brain) After treatment: *Per2* ↓, *Bmal1* ↑ (liver)
	in vitro	hepatic organoids	administration of butyrate | acetate	*Per2* ↓, *Bmal1* ↑
Govindarajan *et al.* (2016)	*in vivo*	SPF mice[3] (8 weeks)	oral gavage of corn oil | corn oil + DCA | corn oil + CDCA (3 times)	*Per1* ↑, *Per2* ↑, *Per3* ↑, *Cry2* ↑, *Bmal1* ↓, *Clock* ↓, DBP ↑, NFIL3 ↓ (intestines and liver)
	in vitro	epithelial colorectal cells	administration of DCA | CDCA | TDCA | TCDCA	DCA | CDCA: increased rhythmicity in circadian genes
Montagner *et al.* (2016)	*in vivo*	SPF mice | GF mice[3] (10-12 weeks)	–	GF mice: altered rhythmicity of *Bmal1*, *Per1*, *Per2*, *Cry1*, *RevErbα*, *RevErbβ*, DBP, DEC2, TEF (liver)
Murakami *et al.* (2016)	*in vivo*	SPF mice | AIMD mice[3] (6 weeks)	–	AIMD mice: altered rhythmicity of PPARγ driven transcriptions (liver)
Thaiss *et al.* (2016)	*in vivo*	SPF mice | AIMD mice[3] (8-9 weeks)	–	SPF mice: rhythmicity in localisation and metabolite secretion of microbiome AIMD mice: altered rhythmicity of transcriptions and chromatin modifications (intestines and liver)
Wang *et al.* (2017)	*in vivo*	CONV mice | GF mice[3]	IP-injection of lipopolysaccharide (3 times) | oral gavage of LPS (6 times)	GF mice: *RevErbα* ↑, *Nfil3* ↓ After treatment: *RevErbα* ↓, *Nfil3* ↑
Heipertz *et al.* (2018)	*in vitro*	macrophages	administration of lipoteichoic acid (12 times)	Rhythmicity in IL-6 production and *Per2* expression
Tahara *et al.* (2018)	*in vivo*	SPF mice | AIMD mice[3]	oral gavage of water | SCFAs + lactate (12 times)	AIMD mice: *Per2* ↓ After treatment: altered rhythmicity of *Per1*, *Per2*, *Bmal1*, *Cry1*, *RevErbα* (kidney and submandibular gland)
Kuang *et al.* (2019)	*in vivo*	CONV mice | GF mice[3]	–	GF mice: HDAC3 ↓ Rhythmal recruitment of HDAC3 to target genes regulated by RevErbα
Weger *et al.* (2019)	*in vivo*	CONV mice | GF mice[3]	–	GF mice: altered rhythmicity of *Per1*, *Per3*, *Cry1*, *Cry2*, *Clock*, *Bmal1* (liver, intestines, and white adipose tissue)

[1] AIMD = antibiotic-induced microbiota-depleted; CDCA = chenodeoxycholic acid; CONV = conventionalised; DBP = albumin D site-binding protein; DCA = deoxycholic acid; GF = germ-free; HDAC3 = histone deacetylase 3; HFD = high-fat diet; IL = interleukin; IP = intraperitoneal; LPS = lipopolysaccharide; MAMPs = microbiota-associated molecular patterns; SCFAs = short-chain fatty acids; SCN = suprachiasmatic nucleus; PPARγ = peroxisome proliferator activated receptor γ; SPF = specific-pathogen-free; TCDCA = taurochenodeoxycholic acid; TDCA = taurodeoxycholic acid; TEF = thyrotroph embryonic factor.
[2] Expression levels: increase (↑) and decrease (↓).
[3] Mice had *ad libitum* access to food and water.

The gut microbiome impacts host circadian rhythms

An extensive amount of research demonstrates that the gut microbiome can influence both intestinal and peripheral clocks (Kuang *et al.*, 2019; Leone *et al.*, 2015; Montagner *et al.*, 2016; Mukherji *et al.*, 2013; Murakami *et al.*, 2016; Thaiss *et al.*, 2016; Wang *et al.*, 2017; Weger *et al.*, 2019). Montagner *et al.* (2016) investigated mRNA expressions of circadian genes and their output effectors in the liver of specific-pathogen-free and germ-free mice. They reported that daily fluctuations of *Bmal1*, *Per1*, *Per2*, *Cry1*, *RevErbα*, and *RevErbβ* were altered in the absence of the microbiome, as well as expressions of clock output regulators (albumin D site-binding protein (DBP), DEC2, and thyrotroph embryonic factor (TEF)). This had a profound influence on liver metabolism, leading to an increased vulnerability to oxidative stress and an activation of the hepatic immune response (Montagner *et al.*, 2016). Similar results were found by another research group who observed a suppression in both the levels and oscillatory behaviours of core clock genes (*Bmal1*, *Per2*, *Clock*, and *Cry1*) in the liver of germ-free mice. Dysregulation of circadian function in the liver caused by diet and host factors eventually lead to metabolic disturbances that include diet-induced obesity (Leone *et al.*, 2015). These changes in functional output are probably due to the ablation of microbial rhythms which causes a loss of oscillations in the epigenome and transcriptome in intestinal epithelial cells. This is turn affects the metabolic pathways in both the intestines and the liver (Thaiss *et al.*, 2016; Weger *et al.*, 2019). Rhythmic bacterial attachment or proximity to the mucus layer and production of microbial metabolites seem to play an important role in shaping these transcriptional oscillations (Thaiss *et al.*, 2016).

While bacteria in the gastrointestinal tract contribute to the working of peripheral clocks, it is implied that the central clock located in the brain could also be subject to microbial alterations in the gut. Removal of the gut microbiome of specific pathogen free mice caused significant differences in the expression of circadian genes in the brain, reflected by reduced oscillations of the clock transcripts *Bmal1*, *Per2*, *Clock*, and *Cry1* (Leone *et al.*, 2015). Together, these findings indicate that the gut microbiome is essential for a proper functioning of both the peripheral and central clocks, although the effects on the central clock need to be investigated in more detail.

Altered circadian rhythms due to microbial components and metabolites

Emerging evidence suggests that the connection between the gut microbiome and the biological clock is mediated by pattern-recognition receptors that directly bind bacterial components and metabolites (Heipertz *et al.*, 2018; Mukherji *et al.*, 2013; Wang *et al.*, 2017). Mukherji *et al.* (2013) identified Toll-like receptors on the surface of intestinal epithelial cells that recognise microbiota-associated molecular patterns. Binding of substrates to these receptors generates a whole cascade of reactions that eventually activate the nuclear receptor RevErbα and cause suppression of the circadian clock (Mukherji *et al.*, 2013). Another study outlined a complete signalling pathway that describes how microbes in the gut can impact host circadian rhythms and metabolism. The researchers demonstrated that body composition is controlled by the gut microbiota through activation of the transcription factor NFIL3 (nuclear factor, interleukin 3 regulated). Transcription of *Nfil3* oscillates diurnally in intestinal epithelial cells and is triggered by structures present on the outer membranes of gram-negative bacteria – including flagellin and lipopolysaccharide – via the epithelial cell circadian clock (Wang *et al.*, 2017). Similar effects on Toll-like receptors were observed for lipoteichoic acid, which is the gram-negative counterpart of lipopolysaccharide. *In vitro* administration of lipoteichoic acid at different time points to macrophages caused a rhythmic production of proinflammatory cytokines that closely mimicked the response to caecal bacteria and was inversely proportional to the expression of *Per2* (Heipertz *et al.*, 2018). Besides the above-described bacterial components, it is likely that more microbiota-associated molecular patterns that have not been investigated yet can interfere with the circadian rhythmicity of the host.

Metabolites that are produced by the microbes in the gut have also been described as potential circadian modulators. These bacterial metabolites include secondary bile acids, short-chain fatty acids, hydrogen sulphide, certain vitamins, and tryptophan derivates (Parkar *et al.*, 2019). Some of these have been studied extensively and may be the fundamental link through which intermittent fasting regimens could impact host circadian rhythms. As described before, intermittent fasting has been associated with changes in the levels of tryptophan and its derivates, short-chain fatty acids, and bile acids. Therefore, these metabolites and their effects on the biological clock of the host will be further discussed in more detail.

Short-chain fatty acids

Fermentation of undigested carbohydrates by microbes in the gastrointestinal tract leads to the formation of short-chain fatty acids, of which acetate, butyrate, and propionate are most abundant. Most of the bacteria that are responsible for the production of short-chain fatty acids belong to the *Firmicutes* phylum. Besides that these metabolites function as an energy source for specific host cells, they are also essential for the transmission of signals from microbes in the gut to the host (Frazier and Chang, 2020). Analysis of caecal and faecal contents from mice showed that several short-chain fatty acids – including butyrate, propionate, and to a lesser extent albeit – exhibit diurnal fluctuations,

leading to rhythmicity in these signals (Leone *et al.*, 2015). As germ-free and antibiotic-treated mice generate metabolites that do not show any rhythmicity, the presence of a gut microbiome is essential for these fluctuations and thus for a proper functioning of the circadian system (Thaiss *et al.*, 2016).

Leone *et al.* (2015) were the first to assess the effect of short-chain fatty acids on the expression of circadian genes. Direct administration of butyrate and acetate to an *in vitro* hepatic model led to a significant increase in the amplitude and shifted the phase of the expressions of *Bmal1* and *Per2*. Since this effect was more pronounced for butyrate, it is probable that different short-chain fatty acids affect the host to a different extent. Further assessment in germ-free mice showed similar results. An intraperitoneal injection of butyrate caused an increase in *Bmal1* and reduction in *Per2* in the liver of these mice, while no effect was observed in the expression in the brain (Leone *et al.*, 2015). This suggests that the signalling of microbial metabolites to the central clock located in the brain may require additional signals. Another study investigated the effect of oral administration of a mixture of short-chain fatty acids and lactate on circadian rhythms in several tissues – including the kidneys, liver, and submandibular glands – of antibiotic-treated mice. They found altered phase shifts in circadian genes in these tissues when administered at specific time points. However, this was only observed for concentrations of the mixture that were higher than the *in vivo* situation, so whether this applies to the living situation is unknown. Further administration of single short-chain fatty acids led to less significant phase shifts, indicating that a combination of these metabolites is needed for sufficient circadian signalling (Tahara *et al.*, 2018).

One of the mechanisms that explains how short-chain fatty acids could affect circadian rhythms of the host is by suppressing histone deacetylases. These proteins are responsible for the removal of acetyl groups from histones, regulating the expression of certain genes (Nakahata *et al.*, 2008). Treatment of hepatic cells with varying concentrations of butyrate has been found to inhibit the expression of histone deacetylase SIRT1 (Pant *et al.*, 2017). Downregulation of the *Sirt1* gene leads to disturbances in the acetylation of the core clock gene *Bmal1* and subsequently to alterations in the circadian cycle (Nakahata *et al.*, 2008). Remarkably, derivates of metabolites, such as butyryl-CoA, actually act as histone deacetylase activators, implying that different effects on these proteins must be considered (Vogelauer *et al.*, 2012). Recently, Kuang *et al.* (2019) revealed a complete signalling pathway that illustrates how the gut microbiota controls diurnal metabolic rhythms by altering histone modifications. Analysis of intestinal epithelial cells showed that histone deacetylase 3 oscillates diurnally in conventionalised mice, while this is abolished in the absence of the microbiome. Further investigation demonstrated that this rhythmicity is regulated by the circadian factor RevErbα that rhythmically recruits histone deacetylase 3 to target genes, leading to diurnal fluctuations in metabolic gene expression.

Bile acids

Bile acids are synthesised from cholesterol in the liver and secreted in the gastrointestinal tract to facilitate digestion and absorption of dietary fats and lipid soluble vitamins (Frazier and Chang, 2020). Most of these bile acids are resorbed back from the distal ileum by the liver, generating a circulation of bile acids between the intestines and the liver. However, some bile acids escape this circulation and undergo microbial metabolism in the gut. This includes deconjugation and dehydroxylation by bacteria producing the bile salt hydrolase enzyme that are mostly present in the *Firmicutes* phylum, such as *Lactobacillus* (Parkar *et al.*, 2019). These newly formed secondary bile acids can then act as signalling molecules in the host by activating the nuclear receptor FXR and G-protein-coupled receptor TGR-5 (Frazier and Chang, 2020; Zhang *et al.*, 2011). Bile acid signalling occurs in a highly fluctuating manner and tightly regulates host glucose and lipid metabolism. Since serum and livers from mice that had 24-h access to food demonstrated rhythmicity in both primary and secondary bile acids, with peak levels of secondary bile acids during the fasting phase, daily energy homeostasis may be influenced by the diurnal fluctuations in bile acid secretion (Zhang *et al.*, 2011).

Microbial metabolism of bile acids in the intestines is essential for the signalling pathway between the gut microbiome and the peripheral clocks of the host, which is illustrated by the following research insights. Govindarajan *et al.* (2016) examined the expression of circadian genes *in vitro* and *in vivo* after treatment with primary and secondary bile acids. Direct administration of secondary bile acids (deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA)) to human epithelial colorectal cells induced significant oscillations in the expression of circadian genes, while their conjugated moieties (taurodeoxycholic acid (TDCA) and taurochenodeoxycholic acid (TCDCA)) were not able to generate the same effect. Furthermore, oral gavage of DCA and CDCA dissolved in corn oil to mice increased the expression of the circadian activator DBP, with concomitant activation of *Per1*, *Per2*, *Per3*, and *Cry2* expression. The translation of a competitive inhibitor NFIL3, on the other hand, was reduced in both the intestines and liver (Govindarajan *et al.*, 2016). Another study in antibiotic-treated mice demonstrated that the gut microbiota regulates the liver circadian clock by inducing the activation of the transcription factor peroxisome proliferator activated receptor gamma (PPARγ) (Murakami *et al.*, 2016). PPARs have previously been reported to increase the expression of the circadian gene *Bmal1* which

causes the upregulation of PPARα, illustrating a positive feedback loop. This mechanism is regulated by bile acids (cholic acid (CA) and CDCA) and short-chain fatty acids (butyrate and propionate) which have been identified as strong stimulators of PPARs and its target genes (Oh *et al.*, 2019).

Tryptophan derivates

Tryptophan is an essential amino acid that is utilised for protein synthesis and forms an important precursor for the neurotransmitter serotonin. Conversion of dietary tryptophan into serotonin largely occurs in the gastrointestinal tract in which the gut microbiota plays a profound role. Spore-forming bacteria, such as *Clostridia* (i.e. *Firmicutes* phylum), stimulate enterochromaffin cells to convert the end product tryptamine into serotonin (Gao *et al.*, 2019). Microbes also directly contribute to the serotonin biosynthesis pathway by expressing tryptophan synthase. This enzyme is responsible for the production of 5-hydroxytryptophan, which can be further metabolised into serotonin. Several bacteria including *Lactococcus*, *Lactobacillus*, *Streptococcus* (i.e. *Firmicutes* phylum), *Escherichia coli*, and *Klebsiella* (i.e. *Proteobacteria* phylum) have been identified to take part in this pathway (O'Mahony *et al.*, 2015). Microbes in the gut are also involved in the regulation of the brain serotoninergic pathways via the microbiota-gut-brain axis. Research in germ-free mice showed a significant increase in the concentration of serotonin in the hippocampus, in combination with elevated tryptophan plasma levels (Clarke *et al.*, 2012). This implies the existence of a humoral route through which the microbiota can affect serotonin concentrations in the brain. Previous studies demonstrated that the serotonin system is extensively interconnected with the central clock via neuronal networks. For instance, serotonin inhibits the response of the circadian system to light by acting both presynaptically on retinal afferent terminals and postsynaptically on the suprachiasmatic nucleus (Ciarleglio *et al.*, 2011). In addition, manipulation of brain serotonin levels in mice led to an alteration in circadian behaviour patterns expressed by an advance in the onset and a delay in the offset of daily activity (Whitney *et al.*, 2016).

Whereas research in animals indicates a clear connection between the gut microbiota and the serotonin system, it remains elusive whether this also applies to humans. Diminished activity of serotonergic pathways in the brain has long been thought to play an important role in the onset of depression (Cowen and Browning, 2015). In fact, serotonin seems to be directly involved in the regulation of mood and cognitive functions (O'Mahony *et al.*, 2015). Interestingly, patients with major depressive disorder exhibit significant differences in their faecal microbial composition compared to controls, characterised by a decreased α-diversity and alterations in the relative abundance of *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* (Jiang *et al.*, 2015). An increased prevalence or severity of major depressive disorder has been linked to disturbances in circadian rhythms or a phase delay in circadian rhythm onset, which can be treated with the administration of selective serotonin reuptake inhibitors (Teichman *et al.*, 2020). These notions indicate that alterations in the serotonin biosynthesis pathway in the intestines, caused by the absence of a functioning gut microbiota, could lead to disturbances in host circadian cycles. Intermittent fasting may restore these cyclical fluctuations, since various studies show improved scores on depression and cognitive function after a period of intermittent fasting (Fitzgerald *et al.*, 2018; Hoddy *et al.*, 2015).

5. Discussion

The aim of this review was to examine the influence of intermittent fasting on the gut microbiome and whether this affects host circadian rhythms. Analysis of both preclinical and clinical studies indicates that intermittent fasting regimens directly influence the microbial populations in the gastrointestinal tract by amplifying its cyclical fluctuations. This in turn contributes to the β-diversity of the gut microbiota and changes its composition, reflected by an increased abundance of bacteria that are responsible for the production of metabolites, such as short-chain fatty acids and tryptophan derivates, as well as the conversion of primary bile acids to secondary bile acids. In addition, restricting the time of feeding affects the expression of certain components (lipopolysaccharide) on the outer membrane of gram-negative bacteria that, together with these metabolites, can act as signalling molecules to the circadian system of the host. Diurnal fluctuations in bacterial abundance and metabolic activity, caused by the cycling between periods of feeding and fasting, could lead to temporal changes in the levels of bacterial components and metabolites. In the lumen, these components and metabolites bind pattern-recognition receptors on the surface of intestinal epithelial cells in an oscillating manner, leading to fluctuations in the transcription of circadian factors in various metabolic pathways. With this, intermittent fasting optimises host metabolism by acting as a time indicator for the circadian system to synchronise environmental fluctuations with internal processes.

Although there is mounting evidence to suggest that the gut microbiome influences the peripheral clocks, it is questionable whether this also applies to the central clock located within the suprachiasmatic nucleus. Based on recent findings, it would appear as if serotonin activity in the brain is affected by bacterial metabolism of tryptophan in the gut, leading to alterations in the circadian cycle via neuronal connections with the central clock. However, signalling of bacterial metabolites via the microbiota-gut-brain axis

may require additional factors, since no direct effect of short-chain fatty acids on clock expression in the brain could be observed. This implies that the central clock may be indirectly affected by the gut microbiota via interactions with the peripheral clocks. Another point of interest is whether different types of microbial components and metabolites exert different effects on host circadian rhythms (Den Besten *et al.*, 2013). This seems to hold for various short-chain fatty acids that all influence the circadian cycle to a different extent, generating a more robust effect when combined. The same does not apply to all types of bile acids, given that microbial conversion of primary to secondary bile acids is essential for generating circadian gene expression patterns. To summarise the conclusions described above, a schematic overview of the interactions between intermittent fasting, the gut microbiome, and host circadian rhythms is provided in Figure 1.

This review is limited since it is largely based on findings in rodent models and to some extent in humans. Mice differ from humans in terms of the anatomy of the gastrointestinal tract as well as that they practice coprophagy. In addition, the included studies have used various methods for measuring changes in the structure and functional output of the gut microbiome, making it somewhat questionable to combine their results and generate conclusions. For instance, in rodent studies, the bacterial content of the cecum is frequently examined, whereas in human studies faecal samples are mostly used. Methodological differences might also explain the diverging results in α-diversity between studies. Furthermore, the relative abundance of bacterial species is often used to define the microbial composition, while it is highly likely that intermittent fasting affects overall bacterial levels regardless of changes in proportions. Besides these methodological limitations, performing circadian rhythm or food-timing interventions in humans can be difficult because of several experimental challenges. First of all, humans generally tend to decrease their energy intake when the feeding period is restricted, simply because of the reduced time to consume food. Additionally, differences in nutrient intake among intervention groups or changes over time, could affect the gut microbiome and interfere with the study outcomes. In order to control for energy intake and dietary composition, it is important to ensure that eating patterns are strictly monitored and equal among groups.

Much uncertainty remains about intermittent fasting regimens and the mechanisms regulating interactions between the gut microbiome and host circadian clock. Large-scale randomised controlled trials in free-living populations are necessary to confirm preclinical findings and establish a causal link between intermittent fasting, the gut microbiome, and circadian rhythms. These studies should include not only measures of the composition and functional capacity of the microbiome, but also the central and peripheral clocks and their output regulators. Furthermore, it is needed to clarify the optimal fasting

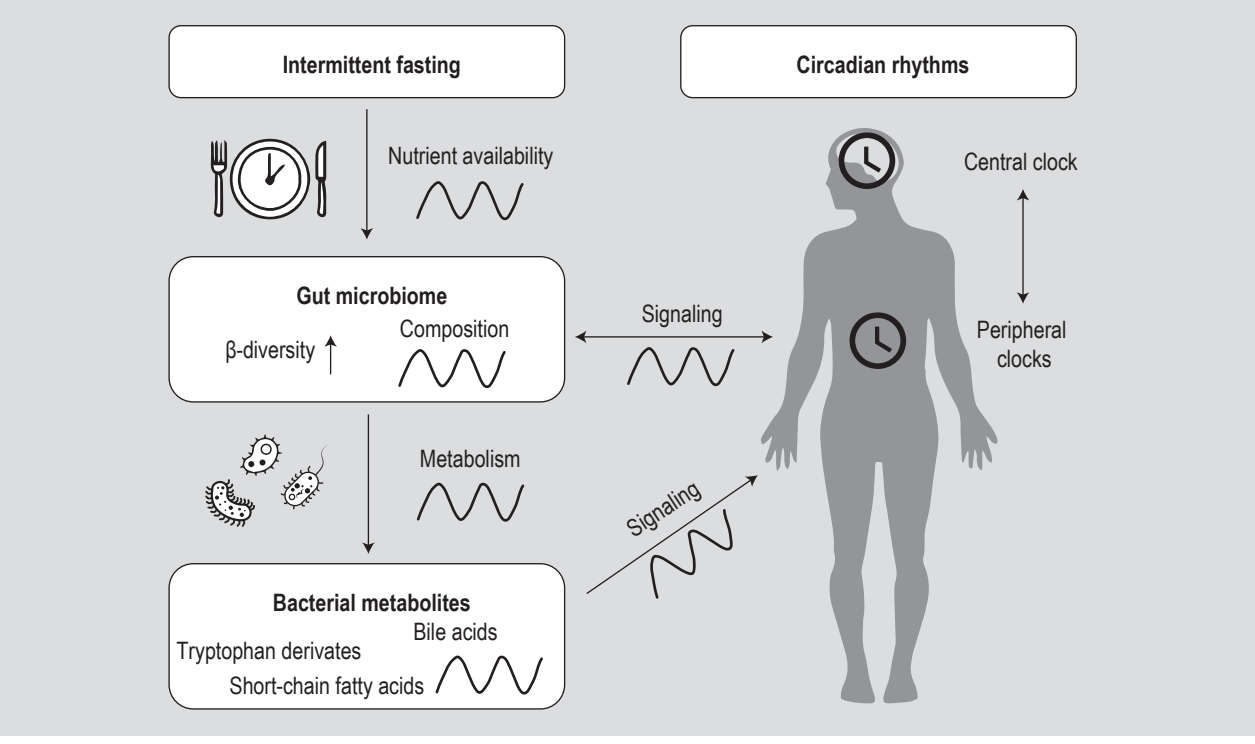

Figure 1. Proposed multidirectional relationship between intermittent fasting, the gut microbiome, and circadian rhythms based on the review.

regimen for balanced circadian rhythms, including the duration and timing of the fasting interval, the amount of fasting days per week, and the maximum allowed energy intake on fasting days. For example, comparisons could be made between daily fasting hours, ranging from 16 to 20 h, or between periodic fasting of one to two days. In addition, future studies should incorporate measures of energy expenditure to assess whether diet-induced thermogenesis varies between early (8:00 – 16:00) or late (12:00 – 20:00) feeding windows. These differences may relate to host circadian rhythms and subsequently impact weight management, emphasising the importance of coordinating food intake with metabolic processes. Finally, the feasibility of intermittent fasting regimens as treatment or prevention strategy should be considered. Adhering to such a diet may be difficult for individuals who lack certain personality traits or are not convinced of its beneficial effects. Nevertheless, it appears as if fasting regimens are more promising for long-term weight management than caloric restrictive diets. Unrestricted food consumption within a reduced feeding window may allow more flexibility and thereby increase adherence (Heilbronn *et al.*, 2005).

6. Conclusions

The circadian system is essential for the regulation of physiological and behavioural processes, and synchronises these with daily environmental fluctuations such as the light-dark cycle and eating behaviours. With this, it tries to optimise the functioning of cells, organs, and systems, contributing to human health. There is a growing body of evidence which indicates that the gut microbiome interacts with host circadian cycles in a bidirectional manner. Diurnal oscillations in the microbial composition of the gut are controlled by the central and peripheral clocks, while bacterial components and metabolites regulate the expression of circadian genes. Dysbiosis in the gut microbiome could lead to diminished intestinal cyclical fluctuations, impairing host circadian rhythms and contributing to misalignment in metabolic processes. The timing of food intake is seen as an important indicator that influences the regulation of several peripheral clocks, in particular the intestinal clock. The notion that the gut microbiome plays a profound role in this is strongly supported by animal studies, while human evidence is promising. Alternating between periods of feeding and fasting contributes to rhythmicity in bacterial abundance and functionality in the gut, leading to fluctuations in the levels of microbial components and metabolites. These in turn act as signalling molecules that communicate with the central and peripheral clocks to synchronise host metabolism. Intermittent fasting regimens may thus be a promising intervention strategy through which disordered circadian rhythms can be realigned, and obesity and its associated metabolic diseases can be treated or even prevented.

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