Circadian rhythms and the gut microbiota: from the metabolic syndrome to cancer

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Abstract

The metabolic syndrome is prevalent in developed nations and accounts for the largest burden of non-communicable diseases worldwide. The metabolic syndrome has direct effects on health and increases the risk of developing cancer. Lifestyle factors that are known to promote the metabolic syndrome generally cause pro-inflammatory alterations in microbiota communities in the intestine. Indeed, alterations to the structure and function of intestinal microbiota are sufficient to promote the metabolic syndrome, inflammation and cancer. Among the lifestyle factors that are associated with the metabolic syndrome, disruption of the circadian system, known as circadian dysrhythmia, is increasingly common. Disruption of the circadian system can alter microbiome communities and can perturb host metabolism, energy homeostasis and inflammatory pathways, which leads to the metabolic syndrome. This Perspective discusses the role of intestinal microbiota and microbial metabolites in mediating the effects of disruption of circadian rhythms on human health.

Non-communicable diseases are the leading cause of death globally[1]. The most common non-communicable disease is the metabolic syndrome, which is a condition characterized by obesity, insulin resistance, hypertension and hyperlipidaemia[1]. The metabolic syndrome can lead to the development of diseases, including type 2 diabetes mellitus (T2DM), cardiovascular disease, non-alcoholic steatohepatitis and cancer[2,3]. Therefore, it is imperative that we improve our understanding of the pathological mechanisms so that we can develop effective strategies to combat the causes and consequences of the metabolic syndrome.

An increase in the prevalence of the metabolic syndrome was first observed in 'westernized' nations that follow a Western lifestyle. The Western lifestyle is characterized by adverse lifestyle habits, including consumption of diets high in fat and red meat, and low in fibre, a sedentary lifestyle and circadian dysrhythmia. All of these habits are postulated to dysregulate the metabolic axis and lead to chronic inflammation and the metabolic syndrome[4]. Compelling evidence indicates that the Western lifestyle promotes the metabolic syndrome[1]. For example, in the USA, rates of obesity rose from 13% to 38% in less than five decades[5]. Furthermore, in populations that have adopted the Western lifestyle, such as those of urban China, the Middle East, India and Brazil, there has been an emergence of the metabolic syndrome[6,7].

Of the lifestyle factors that can lead to the metabolic syndrome, circadian disruption has received an increasing amount of attention within the scientific community. Circadian rhythms allow organisms to synchronize behaviour and physiology with regular and predictable routines in sleep–wake cycles and times of food consumption[8]. One of the most obvious behavioural consequences of circadian rhythms is sleep–wake cycles in which sleep is regulated by the circadian-controlled production of melatonin. At the molecular level, circadian rhythms are driven by the molecular circadian clock, which is found in nearly every cell in the body. The molecular clock includes the core transcription factors BMAL1, CLOCK, CRY1 and CRY2, and PER1, PER2 and PER3, as well as other components that fine-tune the core clock including REV–ERB and SIRT[9]. The 'central' circadian clock located in the suprachiasmatic nucleus in the hypothalamus is regulated by light exposure and acts as a conductor to regulate subordinate circadian clocks in the rest of the body. However, 'peripheral' clocks can also be regulated by factors other than the central clock. For example, time of food consumption regulates circadian clocks in the intestine and liver[10,11]. Alterations in time of light exposure and food consumption can disrupt circadian rhythms resulting in cellular and organ dysfunction, including metabolic dysfunction[9]. Shift work, rotating shift work, bright street lights, the use of light-emitting devices at night, social jet lag and even late night eating can all promote circadian disruption[9,12,13]. Circadian dysrhythmia is associated with the metabolic syndrome and a high risk of developing cancer[14,15]. Understanding the interaction between circadian rhythms and host metabolic function could provide opportunities to lessen the prevalence and consequences of the metabolic syndrome.

One mechanism by which circadian dysrhythmia can influence the development of the metabolic syndrome is via the intestinal microbiome. A wide variety of microorganisms inhabit the gastrointestinal tract, including bacteria, fungi and viruses. The bacteria that inhabit the gastrointestinal tract are the most studied among the human microbiome constituents and are known to contribute to the regulation of health and disease[16]. For the purpose of this Perspective, we operationally define microbiome and microbiota as the bacterial communities in the gastrointestinal tract. In this Perspective, we examine the link between circadian disruption, the gastrointestinal tract microbiome, metabolism, the metabolic syndrome and cancer.

Circadian rhythms and metabolism

The link between the molecular circadian clock and metabolism is well established[17,18]. For example, studies in rodents have shown that whole animal genetic disruption of the molecular circadian clock (that is, perturbations in *Bmal1, Clock* or *Rev-erb*), which disrupts circadian rhythms both centrally and peripherally, is associated with features of the metabolic syndrome, including adipocyte hypertrophy, fatty liver, abnormal lipid profile, hypoinsulinaemia, hyperglycaemia and obesity[19–22]. Similarly, manipulations of light–dark cycles to induce central circadian disruption predisposes rodents to glucose intolerance, reduced insulin sensitivity and pancreatic β-cell dysfunction[23,24]. These findings in rodents have been largely recapitulated in humans. Humans with variations and polymorphisms in *CRY2* and *PER2* demonstrate metabolic dysfunction, namely increased blood levels of glucose[25,26].

Most individuals, however, do not have abnormalities in the molecular circadian clock. Instead, they have common lifestyle factors that are known to cause circadian disruption centrally (such as shift work, jet lag and sleep deprivation) or peripherally (including late-night or irregular times of food consumption)[27,28]. These lifestyle factors are often associated with metabolic dysfunction and the metabolic syndrome[29–31]. It should be noted that not all clinical studies have found an association between circadian disruption and the metabolic syndrome. However, carefully controlled studies demonstrate that consuming calories closer to the onset of melatonin production (that is, near sleep onset) is associated with increased body adiposity, whereas consumption of higher calories (carbohydrate and protein) after ~1900 hours (~4 h before sleep onset) is associated with being less lean, which indicates that eating later in the day (which probably induces peripheral circadian dysrhythmia) is associated with obesity[32,33]. In addition, monitoring melatonin production (a gold standard to assess central circadian rhythms) demonstrates that central circadian disruption (low melatonin levels) is associated with insulin resistance, T2DM and obesity[34]. Taken together, compelling evidence links circadian dysrhythmia to metabolic dysfunction in both rodents and humans.

A close relationship exists between circadian rhythms and metabolism. Metabolic function is thought to be regulated by circadian rhythms. For example, many metabolic functions demonstrate diurnal rhythmicity (suggesting they are directly or indirectly controlled by circadian rhythms), including enzymes (expression and function) that are important for the regulation of cholesterol, other lipids, glucose and amino acids[35,36]. In addition, melatonin receptors (an output of central circadian rhythms) are involved in regulating gluconeogenesis, pancreatic β-cell signalling and insulin resistance[37,38]. Thus, low melatonin associated with central circadian disruption might be one mechanism by which circadian disruption promotes the metabolic syndrome. Indeed, studies have shown that humans with a polymorphism in a melatonin receptor tend to have increased fasting blood levels of glucose and HbA_{1c}, as well as an increased incidence of gestational diabetes and T2DM[39]. Conversely, evidence also indicates that metabolism can influence the function of the molecular circadian clock. For example, SIRT function (deacetylase activity) depends on the availability of cellular metabolites (such as NAD^+ and fatty acids) and it is in this way

that metabolism can influence the function of the molecular circadian clock[40]. Taken together, a high degree of crosstalk between the circadian clock and metabolism is present.

Metabolic syndrome and cancer

Similar to the metabolic syndrome, cancer rates are on the rise globally, including in populations that have adopted the Western lifestyle (for example, populations in China and the Middle East), suggesting that cancer in these countries could be a consequence of the metabolic syndrome and/or that there are overlapping mechanisms contributing to the development of these conditions[41–43]. The hypothesis that cancer is a consequence of the metabolic syndrome is supported by data demonstrating that the metabolic syndrome is associated with an increased risk of developing several types of cancer, including colorectal cancer[3,44]. Data that support the idea of there being overlapping mechanisms that contribute to the development of the metabolic syndrome and cancer demonstrate that circadian dysrhythmia promotes the development of the metabolic syndrome or cancer in rodent models and humans[45–50]. In either case, metabolic pathways are likely a converging mechanism.

Altered cellular metabolism is a key feature associated with cancer; therefore, it is possible that circadian dysrhythmia-induced metabolic dysfunction promotes carcinogenesis and the development of cancer. Numerous pathways that contribute to metabolic survival of cancer cells exhibit circadian oscillations[47,51], and it is hypothesized that circadian disruption might promote survival of cancerous cells by making them more proficient at utilizing available energy sources and performing lipogenesis[52]. Consistent with this hypothesis, circadian dysrhythmia disrupts hepatic circadian gene expression and alters metabolism, resulting in activation of β-catenin and hepatocellular carcinoma transformation[48]. It is possible that altered metabolism resulting from circadian disruption could activate oncoproteins and tumour growth[53], while at the same time those oncoproteins are able to further disrupt cellular circadian rhythms, which initiates a feedforward system by affecting cellular metabolism[54]. In addition to metabolism, a variety of other cancer-relevant cellular processes are under circadian control, but this is beyond the scope of this article. Taken together, these findings implicate metabolic signalling as a linchpin that connects circadian disruption to cancer.

Microbiota and circadian rhythms

Microbiota has circadian rhythmicity.

A diverse bacterial community (that is, the microbiota) thrives in the gastrointestinal tract, where there is a readily available supply of nutrients. The microbiota contributes to the mucosal integrity of the intestinal epithelial barrier, and food digestion and absorption, and also produces a number of hormones, making the microbiota an auxiliary 'metabolic organ'[55,56]. Since the early 1970s, it has been known that the function of the gastrointestinal tract has circadian rhythms[57], but newly emerging data demonstrate that the microbiome also exhibits diurnal variations[58,59]. In vitro data demonstrate that bacteria exhibit diurnal fluctuations in behaviour (for example, swarming activity of *Enterobacter aerogenes* in vitro)[60] and that the number of bacteria in the epithelial mucous layer varies during light and

dark periods (for example, *Mucispirillum schaedleri*)[61]. Evidence suggests that microbial rhythms are tightly interrelated to the host circadian rhythm.

The specific mechanisms are still not well understood but are probably a combination of circadian clock-dependent and clock-independent mechanisms. For example, bacterial rhythms typically have a period of ~24 h, and can be regulated by melatonin and temperature (which are also regulators of circadian rhythms in mammalian cells)[60,62] and the microbiome diurnal pattern is lost in the absence of core molecular circadian clock genes (such as a *Clock* mutation) even when behavioural rhythms in the host are normal[59]. Altered microbiota rhythmicity has been found to be associated with other *Clock* network mutations, including both positive (such as *Bmal1* knockout) and negative (for example, *Per1* and *Per2* double mutant) loop components[61,63]. These data suggest that the fluctuations are dependent on an intact host circadian clock gene network. However, reinstating rhythms in the intestine of *Clock*-mutant mice by regulating the time of food consumption also reinstates robust microbial rhythms, which is consistent with food being a dominant cue for bacterial rhythms, independent of the *Clock* network[64,65]. Clearly additional studies are necessary to tease out the details of this relationship as fluctuations in bacterial communities and function have important biological consequences, including bacterial metabolite production, nutrient digestion and energy harvest, with both intestinal and extra-intestinal metabolic consequences for the host[66].

Based on these data, it is clear that signals from the host influence diurnal fluctuations in the gastrointestinal tract microbiota, but the microbiota can also regulate host circadian and metabolic homeostasis. The production of bacterial metabolites is rhythmic[61,67], which is critical because these metabolites can regulate host circadian rhythms and metabolism[68]. Indeed, ablation of the gastrointestinal tract microbiota disrupts the rhythmic expression of genes in the gastrointestinal tract[61]. This relationship remains rather mysterious but studies from 2019 suggest that the host diurnal rhythms might be driven by bacterial antigens or their metabolites[69,70]. For example, dietary choline is converted to trimethylamine by the intestinal microbiota and is converted to trimethylamine *N*-oxide (TMAO) in the liver[71,72], which might influence the expression of circadian clock genes (including *Clock* and *Bmal1*) in endothelial cells[73]. Thus, there is a dynamic interplay between the host circadian system and the microbiota. Disruptions in this relationship have important consequences for host metabolism and could be one mechanism by which the microbiota is involved in the metabolic syndrome and related pathologies (FIG. 1).

Development of the metabolic syndrome.

A variety of lifestyle habits that are risk factors for the metabolic syndrome alter the composition of the microbiota[16]. Indeed, a plethora of literature demonstrates that the metabolic syndrome is associated with intestinal microbiota dysbiosis. Obesity and the metabolic syndrome are associated with microbiota alterations, including an increase in the ratio of Firmicutes to Bacteroidetes and an increase in the relative abundance of Proteobacteria[74–77] as well as alterations in specific bacteria such as *Lactobacillus* and *Clostridium*[78–80]. Conversely, manipulations that improve features of the metabolic syndrome (for example, anti-obesity surgery, faecal microbiota transplant) are associated

with changes in bacterial communities such as reduced abundance of Proteobacteria, *Streptococcus*, *Clostridium* and Enterococcaceae, and increased *Akkermansia* and Bifidobacteriaceae[81–83]. Thus, the microbiota can both negatively and beneficially affect features of the metabolic syndrome. Studies demonstrate that changes in the gastrointestinal tract microbiota are sufficient to induce or mitigate features of the metabolic syndrome[16,58,59,68]. For example, obesity-prone (129S6) mice lose their phenotype when their microbiome is altered[84] and inoculation of microbiota from obese-prone rodents into a germ-free, non-obese recipient transfers the obese phenotype into the recipient[85]. Specific bacteria are associated with metabolism. For instance, *Akkermansia muciniphila* is negatively correlated with obesity, T2DM and hypertension[86–88] and administration of Akkermansia or purified components of Akkermansia have beneficial metabolic effects[89,90]. Indeed, dietary supplementation of *A. muciniphila* significantly improves insulin sensitivity, reduces insulinaemia and decreases total cholesterol[89,90]. Overall, it is clear that the gastrointestinal tract microbiota affects the host metabolism.

In both experimental models and humans, circadian disruption alters the gastrointestinal tract microbiota[91,92], which might increase the host's susceptibility to metabolic dysfunction[49,50,93]. For example, genetic modification of the circadian clock (such as in mice with modified *Clock*) increases susceptibility to metabolic dysfunction and is associated with a concurrent change in the gastrointestinal tract microbiota[91,94]. Experimental models have demonstrated that the metabolic syndrome induced by circadian disruption is (at least in part) mediated by the intestinal microbiome. Indeed, transfer of stool from circadian-disrupted rodents (with metabolic dysfunction) into non-disrupted rodents is sufficient to induce metabolic dysfunction in the recipients and microbial interventions can improve the metabolic syndrome[59,64]. Furthermore, in mice that consumed alcohol, colon carcinogenesis from eating at the 'wrong' time (that is, when the mice should have been resting) could be ameliorated by manipulating the microbiota composition[50]. Therefore, it is highly plausible that circadian disruption-induced effects on the intestinal microbiome can influence the development of the metabolic syndrome.

Mechanistic links

The microbiome can affect host circadian rhythms and metabolism through a variety of mechanisms, including contact-dependent and contact-independent mechanisms as well as changes in intestinal barrier integrity[16]. These mechanisms are discussed in this section and are partly summarized in FIG. 2.

Contact-dependent mechanisms.

Contact-dependent mechanisms require a direct interaction between the bacteria and the host cells. Epithelial cells that line the gastrointestinal tract and immune cells in the gastrointestinal mucosa express pattern recognition receptors (PRRs), which recognize conserved bacterial features. Through this direct contact mechanism, bacteria can influence the host circadian rhythms and metabolism. For example, segmented filamentous bacteria regulate rhythmic gene expression in the intestinal epithelium via PRRs[61,95] and animals with deficient PRR function (that is, deficient function of MyD88) have abnormal rhythms

in metabolic pathways in the small intestine[70]. Examples of PRRs include NOD-like receptors (NLRs) and Toll-like receptors (TLRs). NLRs are intracellular PRRs and activation of NLRs (for example, NOD1 and NOD2) modulates metabolic function with effects on obesity, fatty liver disease, adipocyte differentiation and adipogenicity[96,97]. TLRs are transmembrane receptors and require downstream components such as MyD88 (REF.[98]. A large body of evidence supports the idea that PRRs mediate the effects of the microbiota on the host clock and metabolism. Microbiota via TLRs signals to the epithelium clock in the small intestine, and subsequently regulates numerous genes associated with metabolic processes[99]. In this study, loss of microbiota signalling via TLRs disrupted the clock network, induced downstream corticosterone overproduction, and led to a pre-diabetic condition in mice[99].

Research on TLRs has provided compelling evidence that they affect metabolism. Activation of TLR4 initiates a series of events culminating in the production of pro-inflammatory factors that modulate insulin resistance, glucose uptake and fatty acid storage, as well as carcinogenesis[100,101]. Mice deficient in TLR2 have less adipose inflammation and less metabolic dysfunction than wild-type animals[102]. Furthermore, knocking out *TLR4* protects against the development of diet-induced obesity[103,104]. In addition, TLR5-deficient animals demonstrate less metabolic dysfunction than their wild-type counterparts[105]. Importantly, knocking out *TLR5* is associated with an altered microbiome, and transfer of stool from the metabolically improved TLR5-deficient mice into germ-free wild-type mice improves metabolic function[105]. Studies in humans support the observation that TLRs are important in metabolism wherein insulin resistance is associated with increased tissue expression of TLR2 and TLR4 (REF.[106]). Thus, compelling evidence indicates that bacterial signalling through PRRs can influence metabolism.

One consequence of stimulation of PRRs is inflammasome activation, and evidence implicates the NLR family pyrin domain containing 3 (NLRP3) inflammasome in mediating the effects of PRR on metabolism. Data demonstrate that NLRP3 activation contributes to insulin resistance via mechanisms including production of cytokines, such as IL-18 and IL-1β, that cause adipose inflammation and impair pancreatic β-cell function[107]. Compared with wild-type mice, *Nlrp3*-knockout mice demonstrated reduced adipose inflammation and enhanced insulin signalling[108]. While these data are compelling, the potential role of the NLRP3 inflammasome as a microbial clock sensor in coordinating the host circadian rhythmicity, inflammation and metabolism requires further investigation.

Contact-independent mechanisms: short-chain fatty acids.

The bacteria in the gastrointestinal tract are metabolically active and metabolites secreted from these bacteria can affect host circadian rhythms and metabolism via contact-independent mechanisms. Specifically, many investigations have focused on bacterial production of short-chain fatty acids (SCFAs), such as butyrate, acetate and propionate. SCFAs are metabolic byproducts of certain bacteria that are able to ferment dietary fibre, and they regulate a wide variety of processes that have metabolic benefits, including food and energy intake (via satiety), energy expenditure, hepatic metabolism, glucose uptake, gluconeogenesis and adiposity[109]. SCFAs act either through G protein-coupled receptors

(GPCRs) or by entering cells via transporters with subsequent epigenetic consequences via histone deacetylase activity[110].

Studies have demonstrated that SCFAs regulate satiety via GPCR41 and GPCR43 (REF.[111]). Specifically, SCFAs decrease appetite by stimulating secretion of 'satiety' peptides such as peptide YY (PYY) and glucagon-like peptide 1 (GLP1) from intestinal cells with endocrine functions (enteroendocrine cells) via the GPCRs[109,112]. SCFAs also increase adipocyte expression and plasma levels of leptin, another satiety hormone, in part via GPCR41 and GPCR43 (REFS[113,114]). SCFAs are increasingly recognized as important players in the microbiota–gut–brain axis and circadian rhythms and act by multiple mechanisms, including modulating the host metabolism[115].

SCFAs also influence energy expenditure. Specifically, SCFAs are associated with increased oxygen consumption and fat oxidation[116], as well as reduced lipid synthesis and lipid accumulation in liver and adipose tissue[117]. These effects seem to be mediated through GPCR41 and GPCR43 as reduced energy expenditure has been observed in GPCR41 and GPCR43 knockout mice[117,118]. Finally, SCFA-stimulated production of GLP1 also stimulates fatty acid oxidation, reduces the amount of lipid in the liver and increases insulin secretion from pancreatic β-cells (hence the use of GLP1 agonists for the treatment of T2DM)[119].

Indirect effects of SCFAs on metabolism can be mediated via effects on the intestinal barrier. The intestinal barrier separates the pro-inflammatory contents inside the gastrointestinal tract from the mucosa and systemic circulation where they can initiate profound inflammation. The barrier is maintained by contacts between adjacent epithelial cells. Several factors can affect the intestinal barrier, including the intestinal microbiota directly and bacterial metabolites, such as SCFAs[120]. Intestinal epithelial cells preferentially use SCFA butyrate as an energy source, and abundant SCFAs fortify intestinal barrier integrity. Reduced SCFA production is commonly associated with barrier dysfunction, systemic inflammation and altered metabolism[111]. Specifically, barrier dysfunction is linked to obesity, hyperglycaemia, insulin resistance, T2DM and the metabolic syndrome[121].

Despite compelling evidence in rodents supporting the beneficial role of SCFAs in metabolic function, data from humans are inconsistent. For example, while increased dietary fibre consumption (and subsequent increases in SCFAs) improves metabolic function in humans, including by reducing blood pressure, improving lipid profile, reducing weight and increasing satiety[122,123], it has also been reported that individuals with obesity have increased circulating levels of SCFAs, which is inconsistent with a beneficial effect of SCFAs on metabolism[16,98]. However, this paradoxical increase in SCFAs in individuals with obesity could be secondary to bacterial overgrowth, increased microbial energy harvest, or a compensatory homeostatic mechanism as a sensor for excessive dietary energy[16,98]. This is just one example, but, broadly speaking, inconsistency in the findings of studies in humans regarding the effects of fibre consumption on metabolism could be explained by variations in fibre type, dose, and host genetic and microbial composition, among other factors[109]. Taking these findings together, SCFA signalling is a credible mechanism connecting the microbiota to metabolic dysfunction and the metabolic syndrome.

Other contact-independent mechanisms.

Consumption of fat initiates the release of bile acids into the small intestine; however, key enzymes in bile acid biosynthesis are regulated by circadian rhythms[12,124] and circadian disruption is associated with an abnormal bile acid profile[12]. Bile acids are recognized as important regulators of host metabolic function. For example, several primary bile acids improve metabolism by promoting GLP1 secretion, which induces satiety, stimulates fatty acid oxidation, reduces levels of lipid in the liver, and increases insulin secretion from pancreatic β-cells, via a mechanism that is TGR5-dependent or FXR-dependent[125–128]. Studies have shown that bile acids are involved in blunting the deleterious effects of high-fat diets in mice undergoing time-restricted feeding[58]. Other mechanisms might also be important. For example, sulfur in red meat is metabolized by sulfur-reducing bacteria to produce hydrogen sulfide, which can promote insulin resistance via inhibition of insulin secretion and sensitivity[129,130]. Hydrogen sulfide could have diurnal oscillations in the mouse intestine while in vitro could blunt the rhythmic expression of core clock genes[68]. Red meat also contains choline, which, upon microbial metabolism, is converted to TMAO in the liver, which, as described previously, might influence the expression of circadian clock genes (including *Clock* and *Bmal1*) in endothelial cells[71,72]. These are just a few of the contact-independent mechanisms by which microbiota signalling can regulate the host circadian rhythms and control metabolism.

Limitations and future directions

We are just starting to learn about the complex interactions among circadian rhythms, the gastrointestinal tract microbiota and host metabolism, and how these interactions affect human health. As these studies are still in their infancy, there are several limitations in extrapolating from the data and in the conclusions that can be drawn.

Circadian limitations.

Many challenges and limitations to studying circadian rhythms currently exist: accurate assessment of circadian rhythms in humans is labour intensive and requires controlled environments that can only be achieved in a laboratory, participant-to-participant variability is high and evaluating circadian rhythms in the human gastrointestinal tract is challenging. While the assessment of central circadian rhythms in humans is fairly straightforward (such as via melatonin and body temperature), studies have to be conducted in tightly controlled environments that limit exposure to light and regulate time of food consumption and meal size (factors known to alter rhythms). Additionally, there is a high degree of variability among humans due to genetics, age, sex and diet, all of which influence circadian rhythms and make robust signals in data difficult to discern. However, the biggest challenge for the purpose of this Perspective is lack of feasibility in evaluating circadian rhythms in the gastrointestinal tract. Frequent tissue sampling, while an option in rodents, is not possible in humans. In this context, the use of intestinal organoids to assess circadian rhythms ex vivo could be promising. Organoids harbour phenotypic and genotypic characteristics of the intestinal tissue from which they are extracted and they exhibit robust circadian rhythms ex vivo, which means that organoids could be utilized for indirect measurement of the circadian rhythmicity in the gastrointestinal tract[131]. In summary, while studies in rodents are fairly

straightforward, studies in humans are challenging and the use of new technologies, such as intestinal organoids, might aid in conducting careful studies of circadian rhythms in the gastrointestinal tract.

Microbiota limitations.

The studies discussed in this Perspective had several limitations. Firstly, the majority of the studies discussed (including those that investigated microbiota rhythmicity) used 16S ribosomal RNA gene sequencing. While this technique is adequate to broadly assess bacterial populations, studies using this technique cannot address other components of the microbiome, such as viruses and fungi. It is possible that these other components also exhibit a diurnal oscillation, are affected by circadian disruption and are critical regulators of metabolism. Future studies should utilize shotgun metagenomic sequencing, which will provide additional resolution (to the species level), allow characterization of non-bacterial components of the microbiome and also provide critical information about function[132]. RNA sequencing might also be a useful technique to fully elucidate how gene expression is altered diurnally, by circadian disruption and in the metabolic syndrome.

Secondly, most of the data available on gastrointestinal tract microbiota (and bacterial rhythms) come from analysis of stool; however, there are several niches inside the gastrointestinal tract and stool is only one. For example, mucosal-associated bacteria represent a niche that can be critically important for host interactions. In addition, microbial populations and functions vary among the different regions of the gastrointestinal tract (for example, small intestine versus colon), and certainly this regionality could have important implications for metabolism, circadian dynamics and bacteria–host interactions[133].

Finally, with the exception of a few characterizations[99,134], studies have yet to carefully evaluate how manipulation of the gastrointestinal tract microbiota influences the host epithelium, including circadian rhythms. Additional studies are necessary to precisely define the mechanisms that contribute to the complex interaction between the microbiome and the host. In conclusion, much is yet to be learned and the coming years are likely to be highly informative.

Experimental models.

Most of the mechanistic evaluations of circadian rhythms and microbiota have been performed in mouse models. Although there are numerous advantages to using rodents (low cost, easy environmental control and/or manipulation, and availability of genetic models), there are limitations in the translatability of murine data to humans. Besides the cross-species microscopic and macroscopic differences in the intestine, the microbial composition is different between mice and humans, making direct translation of data from mice to humans a challenge[135]. In addition, the influence of specific bacteria or bacterial populations on host pathology is often assessed in gnotobiotic models, such as germ-free mice, but such assessments are fraught with problems, which include the abnormal immune function of these models. To move the field closer to the bedside, the development of human-based ex vivo systems wherein bacteria (that is, enterotype)–host (that is, organotype) interactions could be studied has been long awaited. In fact, organ-on-a-chip is a new technological

advance that will open doors in this area of research. Several gut-on-a-chip platforms, such as HuMiX and OrganoPlate, are currently being developed, and the availability of such platforms will allow integrated analysis of host–microbiota interactions ex vivo[136,137]. Inclusion of host non-epithelial components in such systems, although technically challenging, could allow information to be obtained on the role of the cues from the microenvironment. In conclusion, extrapolating rodent model data to humans is challenging; however, continued development of ex vivo approaches in the future are expected to lead to an improved understanding of the host–microbiota interaction.

Conclusions

Epidemiological data suggest that the 'Western lifestyle' might contribute to the prevalence of non-communicable diseases. Chief among these lifestyle factors are lack of physical activity, poor diet, sleep disturbances and circadian rhythm disruption. It is interesting that all of these lifestyle factors are known to alter microbiome communities in the gastrointestinal tract, but of these factors, circadian rhythms have only started being appreciated for their important effects on health and disease in the past decade. Circadian rhythms are critical for optimal function of a host, and disruption of rhythms contributes to susceptibility and development of pathology and disease. Circadian rhythms are influenced by environmental factors, such as light exposure and time of food consumption; therefore, light exposure at night (from light-emitting devices and light pollution), irregular sleep–wake schedules (through jet lag, social jet lag and shift work) disrupt central circadian rhythms, and eating food late at night or at irregular times disrupts circadian rhythms in the gastrointestinal tract. An increasing number of studies have demonstrated that circadian disruption negatively influences microbiota communities in the gastrointestinal tract, which may be associated with metabolic dysfunction and might contribute to the development of the metabolic syndrome and cancer.

A dynamic crosstalk exists between microbiota communities in the gastrointestinal tract and the host, and disruption of this complex interaction is associated with inflammation and metabolic dysfunction. Therefore, it is possible that circadian-directed interventions might beneficially alter gastrointestinal tract microbiome communities to promote host resilience and prevent or delay the onset of chronic metabolic and inflammatory disorders. As proof of concept, light therapy (a circadian-based strategy (known as chronotherapy) that can increase the robustness of central circadian rhythms) improves features of the metabolic syndrome, such as insulin sensitivity[138,139]. While clinical trials (such as NCT03777722) to evaluate the utility of light therapy as a treatment for the metabolic syndrome are ongoing, it will be critical to interrogate microbiota composition before and after light treatment, which could provide mechanistic insights about the role of the microbiota. Another circadian treatment approach to increasing the robustness of circadian rhythms in the intestine and/or liver is optimization of time of food consumption. Therefore, limiting food intake to only daylight hours (10–12 hours food availability, 12–14 hours of fasting) has the potential to optimize circadian homeostasis and microbiota community structure and/or function by improving healthy metabolic function in the host. We propose that these circadian-directed interventions have the promise of preventing the metabolic syndrome and its pro-inflammatory consequences, such as cancer, in high-risk populations. The addition of

microbiota-directed interventions such as prebiotic or probiotic supplements (and/or consumption of primarily plant-based diets) could be considered to further optimize the microbiota community and circadian rhythms, especially in high-risk populations such as shift workers.

This Perspective summarizes compelling evidence to support the concept that circadian disruption negatively effects metabolism through a mechanism involving (at least in part) the gastrointestinal tract microbiome. Therefore, the use of circadian and/or microbiota-directed interventions might be able to ameliorate metabolic dysfunction associated with the Western lifestyle. Well-designed randomized clinical trials are needed to establish the therapeutic effectiveness of such approaches to decrease the burden of the metabolic syndrome and its associated pathologies.

Acknowledgements

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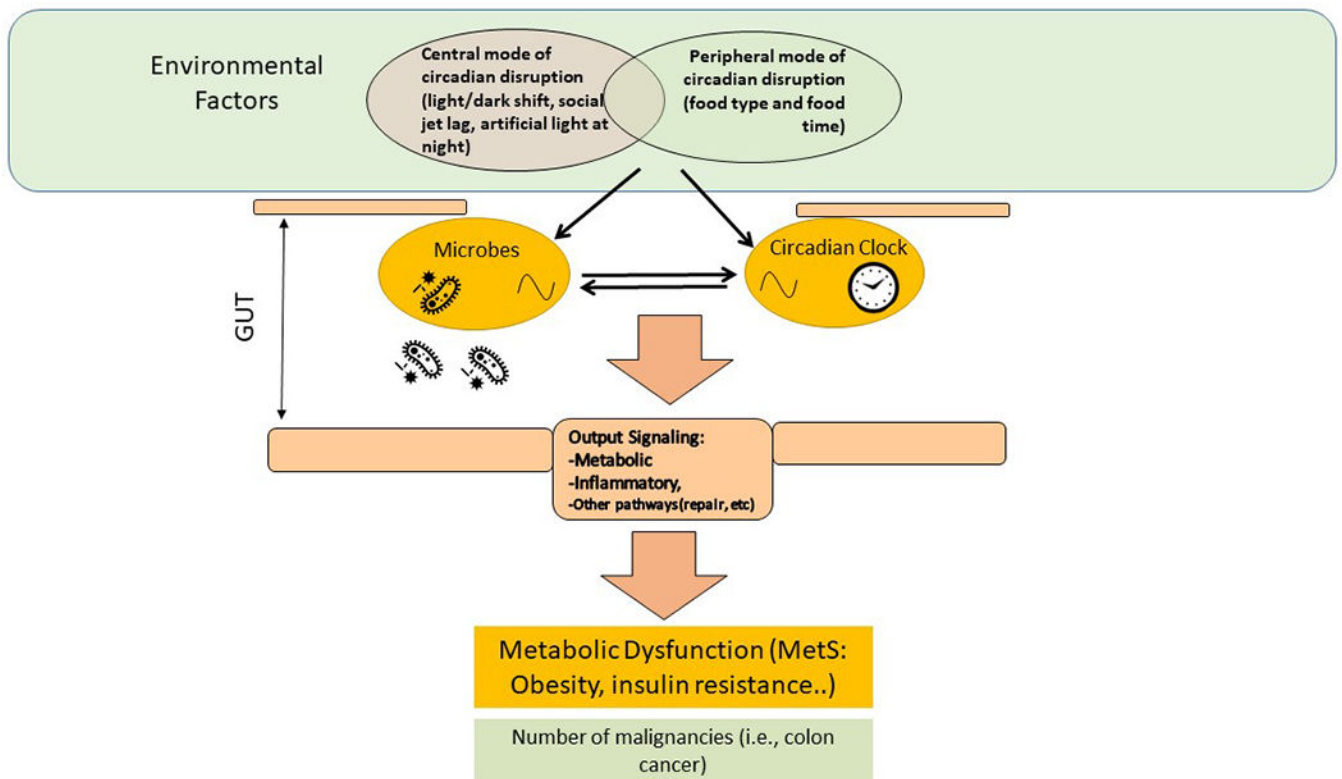
Fig. 1 |. The bidirectional bacteria–host relationship.
Host circadian rhythms influence intestinal microbiota structure and function, and microbial rhythms influence circadian rhythms in the digestive system; both systems being intact is key to maintaining host metabolic homeostasis. Modern lifestyle factors that lead to circadian rhythm disruption (for example, artificial light exposure at night, irregular sleep–wake cycles, eating late at night) interrupt this homeostasis by affecting the microbiota and the host circadian clock, shifting metabolism towards increased calorie extraction and decreased energy expenditure, providing an environment that promotes the metabolic syndrome (MetS), chronic inflammation and a number of malignancies.
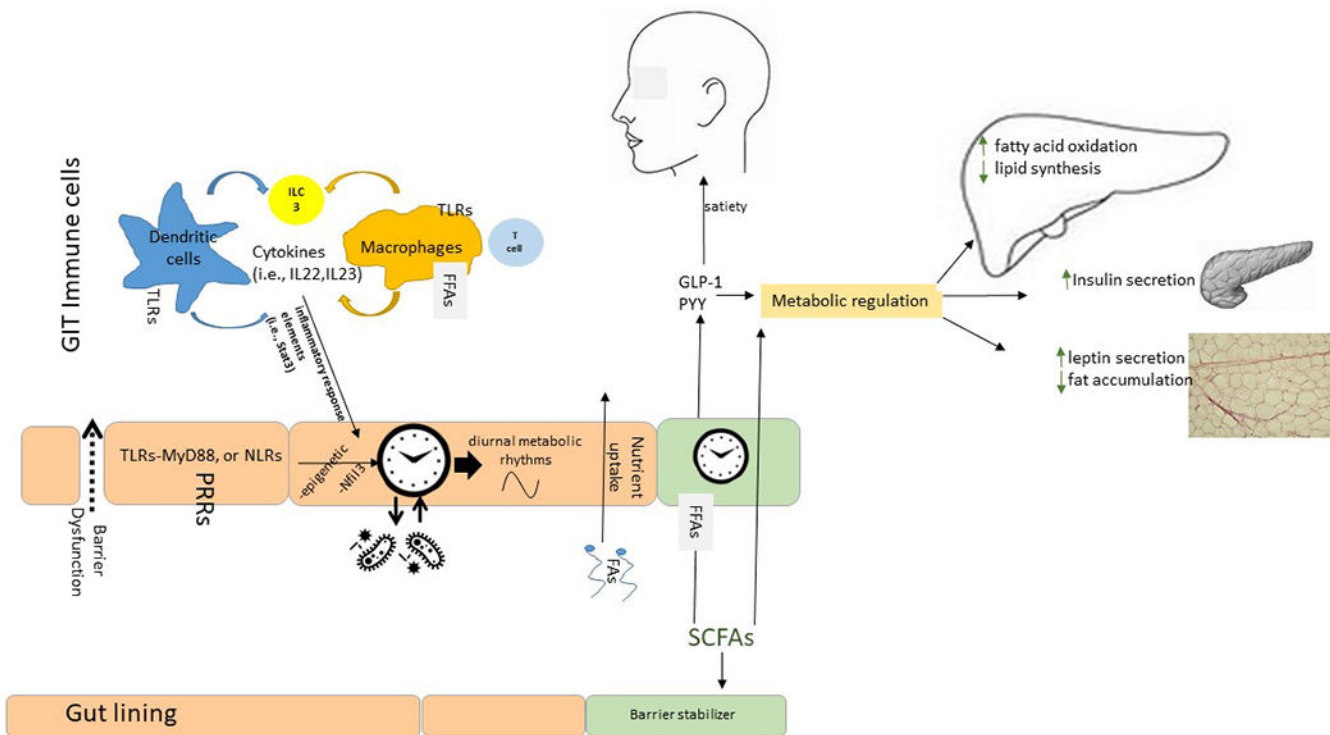
Fig. 2 |. Microbiota signalling to the host circadian and metabolic systems occurs via both contact-dependent mechanisms and contact-independent mechanisms.
Pattern recognition receptors (PRRs) on intestinal epithelial cells and immune cells represent a contact-dependent signalling pathway where the activation of PRRs eventually leads to alterations in gene expression via nuclear receptors (such as nuclear factor, IL-3-regulated (NFIL3)), epigenetic alterations or other mechanisms (contact-dependent mechanism). Bacterial products (for example, metabolites) are responsible for contact-independent mechanisms; of these, bacterial fermentation of fibre and the production of short-chain fatty acids (SCFAs) regulate satiety and the function of metabolic organs (liver, pancreas and adipose tissue). FFARs, free fatty acid receptors; GLP1, glucagon-like peptide 1; MyD88, myeloid differentiation primary response protein 88; NLRs, NOD-like receptors; PYY, peptide YY; TLRs,Toll-like receptors.