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Circadian Rhythms, the Microbiome, and Food Intake Behaviour in Obesity

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Abstract:	Eating behaviour and circadian rhythms are closely related. The type, timing, and quantity of food consumed directly influence the gastrointestinal microbiota. Host circadian rhythms impact gut microbiota, regulating microbial oscillations and metabolic homeostasis. Gut microbiota influences host circadian rhythms and regulates food intake beyond homeostatic eating. This review discusses the impact of circadian disruptions in our obesogenic environment on gut-brain axis signalling. We also explore potential mechanisms underlying the effects of altered gut microbiota on food intake behaviour and circadian rhythmicity. Understanding the crosstalk between gut microbiota, circadian rhythms, and unhealthy eating behaviour is crucial to addressing the obesity epidemic, which remains one of the biggest societal challenges of our time.
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Editorial Board
Trends in Endocrinology & Metabolism

Dear Scientific editor,

We are submitting the manuscript "Circadian Rhythms, the Microbiome, and Food Intake Behaviour in Obesity", for consideration in Trends in Endocrinology & Metabolism.

Modern “fast-paced” lifestyles have shown to drive adaptations in human physiology and behaviour, which together have increased metabolic risk and stress levels in human society. These “unnatural” lifestyle factors have led to interference of the human internal clock and circadian rhythms, leading to disturbed sleep patterns and dysregulation of eating behaviour. Specifically, the increased intake of “unhealthy” fast foods and late-night snacking, further offsetting the circadian clock, has created a vicious circle impacting on human metabolic health.

This review aims to clarify the interaction between the circadian disruptions caused by our current lifestyles on our eating behaviour. We also discuss the potential mechanisms underlying the impact of the microbiota in the dysregulation of our appetite, with a specific focus on microbial regulation of eating when circadian rhythms are dysregulated. Understanding the complex crosstalk between gut microbiota, circadian rhythms and unhealthy eating are urgently needed to “halt” the obesity epidemic.

We believe that our findings are of fundamental interest to a broad reader community, particularly for those working in the field of the Microbiota, Nutritional Neuroscience and Chrononutrition.

We look forward to hearing from you in due course,

Sincerely yours,

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- The gut microbiota undergoes diurnal oscillations, regulated by feeding schedules, timing, and the host's internal clocks, thereby exerting a profound influence on metabolic health.
- The bidirectional interaction between microbiota and circadian rhythms impacts feeding behaviour and metabolic homeostasis.
- The generation of microbiota-derived metabolites, including Short-Chain Fatty Acids (SCFAs), also exhibit diurnal oscillations, and modulate the enteroendocrine signalling and central regulation of appetite.
- Disrupted circadian rhythms and poor dietary habits can trigger detrimental cycles that worsen metabolic risks, with the microbiota acting as a key communicator in this temporal dialogue.
- Directing interventions toward the gut microbiota holds promising potential for reinstating circadian rhythms and improving eating behaviours, thereby leading to overall enhancements in metabolic health.

Circadian Rhythms, the Microbiome, and Food Intake Behaviour in Obesity

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Abstract

Eating behaviour and circadian rhythms are closely related. The type, timing, and quantity of food consumed directly influence the gastrointestinal microbiota. Host circadian rhythms impact gut microbiota, regulating microbial oscillations and metabolic homeostasis. Gut microbiota influences host circadian rhythms and regulates food intake beyond homeostatic eating.

This review discusses the impact of circadian disruptions in our obesogenic environment on gut-brain axis signalling. We also explore potential mechanisms underlying the effects of altered gut microbiota on food intake behaviour and circadian rhythmicity. Understanding the crosstalk between gut microbiota, circadian rhythms, and unhealthy eating behaviour is crucial to addressing the obesity epidemic, which remains one of the biggest societal challenges of our time.

30 **Keywords**

31 Microbiota-gut-brain axis, circadian rhythms, eating behaviour, appetite, metabolic
32 homeostasis, lifestyle.

Biological Rhythms and Obesity: A Holistic Perspective

What are the factors (intrinsic and extrinsic) that determine when we eat, what we eat and how much we eat? The last few decades have seen increased societal pressures, environmental challenges and lifestyle changes in both developed and less developed countries, which have together accelerated changes in dietary patterns across the globe. Specifically, nutritional transitions towards westernized high-fat/high-sugar (HFHS), processed and ultra-processed foods, have led to a predominating consumption of more calorie-dense food with a lower nutritional value. Lifestyle-related changes in food intake behaviour, such as irregular meal times, reliance on fast food, overeating, and late-night snacking, continue to significantly impact metabolic health and contribute to the global prevalence of obesity. Data from the WHO shows that more than 1 billion people worldwide are obese, including 650 million adults, 340 million adolescents and 39 million children [1] and this number is still increasing. Estimations indicate that 8.4% of the health budget in developed countries will be spent to treat the consequences of being overweight and obese over the next thirty years [2]. This global increase in obesity prevalence is associated with other diseases and comorbidities, such as dyslipidaemia, hypertension and impaired glucose tolerance, which are metabolic risk factors for metabolic syndrome (MetS). The Organisation for Economic Co-operation and Development (OECD) estimates that obesity and associated disorders will reduce life expectancy by approximately three years in the OECD, EU28, and G20 nations between 2020 and 2050 [2].

Modern lifestyles, accompanied by changes in diet and temporal eating patterns, often coincide with alterations in natural light/dark cycles. A wide variety of artificial blue-light technologies at night-time and “social jetlag” produce discrepancies between work and free days, social and biological time, and lead to changes in the frequency and timing of meals. The resulting misalignment between external cues and the internal clock produces circadian disturbances, directly related to adverse metabolic health outcomes [3]. Although the mechanisms underlying these effects are not yet fully understood, disruption of circadian rhythms has been shown to lead to dyslipidaemia, adrenal gland hypertrophy, hepatic insulin resistance, and cardiometabolic abnormalities in healthy rats [4]. Additionally, studies in humans have demonstrated that circadian disturbances

64 can lead to metabolic disorders independent of diet. In fact, shift work has been shown
65 to be an independent risk factor for the development of obesity [5,6], and an association
66 between shift work and high cardiovascular risk has been reported [7–9]. Sleep cycle
67 disorders are also associated with the development of obesity and other chronic
68 diseases such as hypertension and type 2 diabetes (T2D) [10]. Therefore, obesity can
69 also be considered as a "chronobiological disease" [11,12]. Indeed, metabolic disorders
70 associated with MetS, along with other comorbidities such as depression, sleep
71 disturbances, cognitive dysfunction, and steatohepatitis have recently been grouped
72 and classified as "Circadian Syndrome"[13]. Regarding the inclusion of mental health-
73 related disorders in the definition of this new concept of “Circadian Syndrome”, it is
74 known that there is a close interconnection between circadian disruption and mental
75 health[14]. Additionally, the bidirectional relationship between mental health and
76 eating behaviour has also been demonstrated and cannot be ignored [15]. Further
77 research and literature reviews are warranted to further investigate and discuss the
78 association between circadian disruption and mental health, as well as on the
79 bidirectional relationship between mental health and eating behaviour.

80 One proposed mechanism for the poor metabolic health associated with the loss of daily
81 synchrony is an increased intake of unhealthy food and eating at irregular hours [16].
82 Circadian changes in temporal feeding patterns result in increased energy intake,
83 suggesting a fundamental role for circadian clock in regulating the metabolic
84 consequences of unhealthy eating behaviour[17]. Accordingly, numerous clinical studies
85 have observed that consuming hypercaloric meals at night and a greater frequency of
86 meals and snacks are related to a greater risk of overweight and obesity, leading to
87 subsequent adverse metabolic effects [12]. Therefore, biological rhythms play a key role
88 in the regulation of daily metabolism and energy homeostasis [17]. Vice versa, changes
89 in dietary patterns have also been shown to impact the function of the circadian clock
90 [18]. Noteworthy, recent evidence suggests a role for chrono-disruption in driving an
91 increase in hedonic eating, which may involve changes in central reward signalling [19].

92 Current lifestyle factors and the consumption of unhealthy foods have also been
93 associated with alterations in the gastrointestinal microbiota [20]. For example,
94 consumption of high amounts of sugar in the diet has been linked to the disruption of

gut microbiota composition [21]. Dysregulation of the microbiota is related to the development of metabolic disorders, including obesity and MetS [22]. This is associated with altered production of microbiota-derived metabolites, such as short-chain fatty acids (SCFAs), differential secretion of intestinal hormones, and increased intestinal permeability, leading to systemic inflammation and metabolic endotoxemia [23]. Disruption of the circadian system has also been linked to alterations in gut microbiota composition and diversity, subsequently impacting host metabolism and energy homeostasis [20]. It has been suggested that circadian rhythms and the gut microbiota have a converging role in modulating the metabolic response to diet in the host [24].

Here, we review the importance of the gut microbiota as a crucial mediator between host circadian rhythms and eating behaviour, specifically focusing on the homeostatic regulation, in an obesogenic context. A better understanding of the relationship between the microbiota, clock system, and food intake behaviour (e.g., diet composition, quantity, quality, eating patterns, meal timing, temporal energy intake, food choice) could provide potential new approaches and strategies to restore diurnal rhythmicity while promoting healthy eating and improving metabolic health.

Circadian rhythms

Living organisms present endogenous rhythms to adapt to the environment and to the challenges. These endogenous oscillations play an important role in their physiological and metabolic functions and allow them to be more energy efficient and survive in variable situations [25,26]. Daily oscillations are known as circadian rhythms and their synchronising centre is the clock located in the hypothalamic suprachiasmatic nucleus (SCN) [25,27] (Fig. 1). This master clock, pacemaker of the organism, along with other peripheral clocks, has the capacity to work autonomously. Nevertheless, the circadian system is reset by different external cues or *zeitgebers*, daylight being the most important one and dietary patterns constitute a second critical external cue, mainly for peripheral clocks. In this context, the central clock provides signals to the different peripheral oscillators via behavioural processes, including feeding, the autonomic nervous system and circulating humoral factors, such as melatonin and cortisol, in order to maintain rhythmicity and ensure the coordination of a physiology synchronized with

the time of day [27]. In turn, peripheral clocks are also modulated by behavioural cues such as physical activity or fasting/feeding cycles [28]. The peripheral clocks are present in almost all mammalian tissues where they maintain periodicity and regulate tissue-specific gene expression and functionality [27].

At the cellular level, circadian rhythms are set by the molecular clock, which has a common structure in all cells but with specific clock-controlled genes (CCGs) that allow the regulation of the characteristic functionality of each cell type [29] (Fig. 1). Consequently, the diurnal rhythmicity of tissue and cell functionality is a perfectly orchestrated and synchronised oscillation in a 24-hour period by the central clock and *zeitgebers* that modulate central and peripheral clocks [25]. The molecular mechanisms underlying the regulation of both central and peripheral clocks is the same (Fig. 1). The molecular clock controls cell functionality and metabolism directly because CCGs are either enzymes of metabolic pathways or transcription factors and nuclear receptors that control the expression of enzymes and transcription factors which ultimately regulate cell functionality and metabolism[30,31].

In a healthy state, diurnal oscillations orchestrated by master and peripheral clocks coordinate metabolic processes to preserve health. Indeed, all the periodic changes coordinated and synchronised by the clock system play a fundamental role in different pathways or functions of the body such as metabolism in liver and adipose tissue, gastrointestinal functions or blood pressure, among others [28,32]. In addition, the circadian clock influences the central regulation of food intake behaviour (as further described in Section 2.1) [33]. However, typical habits of our current lifestyle such as eating in the rest/sleep phase and lack of sleep, desynchronize the metabolic rhythms of the peripheral tissues with the central clock rhythms, which leads to a circadian disturbance and inefficiency in energy balance, key factors which drive obesity and other metabolic disorders [34]. The relationship between circadian rhythms, food intake behaviour (timing, quantity, quality) and metabolic health has gained great importance in the last years and has shaped the emerging field of chrononutrition [34] .

Interestingly, gastrointestinal microbiota is also influenced by diurnal rhythmicity through intrinsic circadian clocks of the host organism and by external cues (as further described in Section 4) [24]. In fact, it has been reported that approximately 35% of the

156 measured bacterial operational taxonomic units (OTUs) in humans have diurnal
157 oscillations [35]. In addition, many studies have pointed out that the gut microbiota and
158 their metabolites may modulate host diurnal oscillations, acting as a chrono-
159 communicator between the ingested food and the internal host clock system, ensuring
160 appropriate synchronization to the environment[36]. Nevertheless, the underlying
161 mechanisms of the bidirectional communication between the gut microbiota and the
162 brain and its role in the maintenance of diurnal rhythmicity are still poorly understood.

163 *Circadian rhythms and food intake*

164 Peripheral clocks mediate the metabolic response to food intake, and in turns, this
165 metabolic response influences the clock system [17]. As previously described, food and
166 meal timings are important *zeitgebers* capable of entraining many peripheral clocks to
167 fasting/feeding cycles [37]. In this context, recent studies have shown the importance of
168 temporal food intake on the clock system and metabolic response to food intake when
169 eating an “unhealthy” and higher palatable treat outside of the active phase in rodents
170 [38–40]. It was shown that late-night consumption of sweet snacks led to body weight
171 gain and a dysregulation of energy balance via the disruption of metabolic oscillations
172 and altered food intake in mice [38]. Male mice fed a milk-chocolate snack during the
173 rest phase instead of the active phase demonstrated decreased energy expenditures,
174 increased energy intake, higher levels of plasma lipids and serum glucose, altered
175 locomotor activity, differences in rhythmicity of core body temperature and altered
176 expression of genes involved in nutrient utilization and clock genes[38]. According to
177 this, a recent study by our group showed that hypothalamic clock genes and metabolic
178 effects following a low dose of sugar were strongly time-dependent, causing higher
179 circadian metabolic disruption when administered to rats at the beginning of their
180 resting period [40]. These studies may translate to the effects observed following late-
181 night snacking in humans, which is also associated with higher hedonics drive to eat in
182 the evening and adverse metabolic response [41]. Overall, these findings suggest that
183 eating at the wrong time, can induce a loss in diurnal rhythmicity impacting on metabolic
184 health. In contrast, a recent review highlighted that the metabolic response to eating is
185 enhanced when food intake episodes are aligned with the circadian clock[17], which
186 may result in improved synchronization of the endocrine response and endocrine-

mediated satiety signalling to the brain. In addition, the SCN neurons are also responsive to glucose and to the orexigenic hormone ghrelin, indicating that the master clock can be modulated by both nutrient and gut-related signals shown both in animal models and humans [42–44].

While eating behaviour and meal timings can influence the circadian system, there is also a significant impact in the opposite direction. In fact, circadian regulation of eating behaviour explains our consistent basic eating patterns, which persists in individuals isolated from external time cues[45]. According to this, homeostatic appetite oscillates in a diurnal manner, increasing in the biological evening and dropping in the biological morning independent of food intake and other behaviours [33,46]. For example, a study in humans investigated the diurnal rhythm of hunger rating food preferences and appetite via visual analogue scales. They showed that hunger peaks in the evening (around 8 PM), hypothesized to promote larger meals before the night-time fast, and a circadian trough (i.e. lowest point) in the morning (8 AM), thought to prevent fasting-induced hunger that could lead to arousal or early awakening [47]. Food intake is governed by a complex regulation of homeostatic processes and hedonic systems, both of which are closely interconnected but linked to circadian rhythms driven by regular intervals of food intake [48]. Homeostatic control of food intake guarantees that enough calories are ingested for survival [49]. It is well-known that the meal termination is induced by the physiological state of satiety or fullness when further eating[50]. Furthermore, hormonal and vagal signals from peripheral organs, such as adipose tissue, pancreas and gut, inform the nucleus of the solitary tract (NTS), hypothalamus and the ventral tegmental area (VTA) of the state of the body's energy balance and coordinate signals to promote food seeking behaviour[51,52] (Fig. 2). The VTA acts as an integration node for these peripheral, hypothalamic, and vagal signals to promote motivation and food intake, supporting the role of the reward system in the regulation of eating behaviours, driving the non-homeostatic regulation of food intake [53]. In the context of metabolic disorders, such as obesity, metabolic status is disrupted, altering the production and signalling of appetite gut hormones and finally, food intake and food reward regulation [54,55]. An altered eating behaviour involves the disruption of numerous mechanisms such as appetite, motivation, and energy demand [56]. In fact,

obesity has a considerable impact on the brain with changes in central regulation of food intake and even in the control of reward-driven impulsivity[54,56,57].

Metabolic status is transmitted in an oscillatory manner from peripheral tissues, via humoral and neurological signals, such as the anorexigenic leptin from adipocytes and orexigenic ghrelin from the stomach, which encode a corresponding discharge pattern in the appetite-stimulating neuropeptide Y (NPY) network in the hypothalamic arcuate nucleus (ARC) [58] (Fig. 2). Mice lacking NPY showed lower food intake at the start of the dark phase (activity phase for these animals), showing that diurnal rhythmicity of NPY in hypothalamus can be involved in the diurnal oscillations of food intake[59]. Hormones related to food intake regulation, such as leptin, ghrelin, cholecystokinin (CCK), and glucagon-like peptide 1 (GLP-1), the stress hormone cortisol, as well as the gut microbiota, all display diurnal oscillations (Fig. 3). For example, it has been shown that diurnal oscillatory secretion of leptin in rats is controlled by sympathetic input from the SCN to the adipocytes[60]. Circulating concentration of active, acylated, ghrelin is mostly regulated by nutritional status, and increases in fasting conditions and drops after food intake [61]. According to this, preclinical and human studies have shown that ghrelin exhibit a diurnal oscillation under fasting and postprandial conditions, reporting significant nocturnal rise for this hormone which is absent in conditions of obesity[62–66]. In addition, and in consonance to the diurnal oscillations displayed by ghrelin levels and hunger, postprandial satiety and appetite towards energy-dense food also oscillate in a circadian-manner [63].

The circadian-mediated effects on central regulation of appetite, may also be mediated via vagal afferents. However, the role of the circadian system in the regulation of vagal afferent function is not well known yet. Studies in mice show that circadian oscillations in gastric vagal afferent signalling are lost under high-fat diet (HFD) conditions and under conditions simulating shift work, which leads to a dysregulation in food intake [67,68]. Regulation of gastrointestinal processes by circadian clocks has been shown to be present throughout the digestive tract and its accessory organs[69]. This circadian regulation includes the microbiota and recently it has been reported that host intestinal clock drives the microbiota composition and functionality [70]. Therefore, we

hypothesize that the circadian clock plays an important role in the regulation of food intake and eating behaviour via the microbiota.

In relation to clock genes, it has been demonstrated that the diurnal oscillatory expression of the hypothalamic nuclear hormone receptors REV-ERB regulates rhythmic feeding patterns during the day[71]. Indeed, a loss of the feeding/fasting cycle, without changes in food intake or meal frequency, was observed after the deletion of *Rev-erbα* gene [72]. Overall, these findings suggest that the SCN clock and its rhythmic regulation have a major role in the daily timing of food intake via homeostatic energy intake regulation [72]. Noteworthy, recent evidence suggests a role for chronodisruption and an altered hedonic circadian appetite, involving changes in central reward signalling[19,48]. For instance, mutant mice without a functioning VTA clock, manipulated by injected bilaterally into the VTA of an adeno-associated virus, have unaltered rhythms for chow intake but are vulnerable to consumption of palatable snacks across the day [48]. These results suggest the involvement of a central clock in dopaminergic neurons of the VTA on the regulation of palatable food intake, which may explain increased consumption of high caloric foods when clocks are dysregulated [19,48].

The gut microbiota

Increasing evidence demonstrate a significant role of the gut microbiota in human physiology, health and well-being [73]. The microbiota comprises a set of microorganisms including bacteria, viruses, fungi, archaea and protozoa that colonize different parts of the body such as the skin, lungs, oral mucosa and saliva, reproductive and gastrointestinal (GI) tract [74]. However, the highest concentration of microbiota is found in the GI tract (~100 trillion of microorganisms make up the human microbiome, 95% of which are found in the GI tract), where it is responsible for the metabolism of dietary compounds impacting host health and disease via gut-brain axis signalling [22,56,73]. The gut contains numerous bacteria that depend on the host feeding to receive the necessary nutrients for proper growth and maintenance. Accordingly, the microbiota-gut-brain axis is a bidirectional communication between the gut microbiota and the brain via neuro-immune-endocrine pathways. Bacteria also contribute to host

physiology by digesting fibre, shaping the host immune system and producing essential metabolites [75]. Advancement in laboratory techniques and “omics” technology have led to a more precise understanding of the mechanisms and functionality of the microbiota [76]. This niche of microorganisms in humans is mainly composed of two bacterial groups, *Bacteroidota* and *Bacillota*, which represent about 99% of the known gut microbiota, whose alteration of their relative abundance has been associated with different metabolic disorders [56,77]. There is a large amount of evidence that show that the GI microbiota plays a crucial role not only at the digestive and metabolic level but also in neurodevelopment, neuropsychiatry, stress, brain reward signalling, neurocognition, ageing and reproductive health [78]. The gut microbiota is variable between individuals in both, a healthy and diseased scenario, and alterations in the composition by a disorder, functional changes or other factors such as diet, medication, genetics are infrequently consistent [79]. Therefore, it is important to note that studies have not been able to identify and attribute specific bacterial genus or species to a disease state or physiological system. Instead, studies may establish that the microbiota of an experimental group is different from a control group or correlate certain bacterial classifications with these disease states or physiological systems. Even when a certain bacterial genus or species is associated with a disease, this observation is often only correlative, lacking evidence of causality[80]. Nevertheless, a range of diseases and health conditions are associated with reduced microbial diversity, as a common and frequent microbial feature, which increases the likelihood of a bloom in opportunistic pathogens, including facultative anaerobes like *Enterobacteriaceae* [79].

Evidence also suggests that the gut microbiota is an important modulator of the communication pathways from belly to brain that regulate homeostatic feeding behaviours in the ARC[81]. Interestingly, diet and food intake are still the most important factors to crucially influencing the microbiota composition and dynamics [56]. In fact, it has been shown that the microbiome composition differs between lean and obese in rodents [82,83] and humans [84,85]. Noteworthy, western diets, rich in saturated fats and simple sugars, alter the gut ecosystem by reducing bacterial diversity and increasing the abundance of potential pathogens [86]. For instance, HFD and high-sugar diet (HSD), are associated with reduced microbial diversity, whereas low-fat, low-

sugar, high-fibre diets have been associated with higher gut microbial diversity [86]. In contrast, obesity-inducing HFD and HSDs are strongly associated with overgrowths of endotoxin-producing pathogens of the genus *Enterobacter* [87]. Endotoxins are complex lipopolysaccharides (LPS) present in the outer membrane of gram-negative bacteria and are well-known for the stimulation of inflammatory responses. Furthermore, high concentrations of LPS in bloodstream are associated with multiple metabolic and chronic disorders such as obesity, T2D, metabolic dysfunction-associated fatty liver disease (MAFLD), or even neurological disorders, which is denominated as metabolic endotoxemia [88]. It is important to highlight that the immune response induced by LPS is dependent on its lipidic structure and the microbial specie's origin [88,89]. In addition, many preclinical studies in rodents with HFD-induced obesity have demonstrated that dietary interventions can attenuate weight gain and adiposity and normalize the gut microbiota [90]. Moreover, translation studies in humans have shown significant correlations between body weight and gut microbiota composition, but supplementation with bacteria alone have only demonstrated limited success on body weight control [22]. In addition, recent studies in rodents are demonstrating a role for the gut microbiota in the orchestration of central mechanism of food intake behaviours [91–93], which will be further reviewed in the next section.

Gut microbiota and eating behaviour

Importantly, not only physiological status, diet and feeding have been associated with influencing the gut microbiota composition, but the gut microbiota also exerts an impact on diet choices and eating behaviour [22,92–97]. At behavioural level, germ-free (GF) mice display increased food intake compared with conventional mice, which is correlated at molecular level with differences in the expression of orexigenic and anorexigenic neuropeptides in the hypothalamus of GF and conventional animals[98]. In addition, microbiota depletion using antibiotics in mice has been shown to suppress overconsumption of high-sucrose pellets [91,99].

The role of microbiota on gut hormones and endocrine signals that govern central regulation of homeostatic food intake are very well described. Mechanistic studies show that bacterial-derived metabolites, including the SCFAs, bile acids, amino acids or

hormones, and subcellular bacterial components, such as caseinolytic peptidase B (ClpB), LPS or muramidyldipeptide (MDP), activate the enteroendocrine cells (EECs) in the GI tract which secrete gut hormones involved in the regulation of satiety and appetite [81,100,101] (Fig. 2). For instance, one study showed that the chronic (9 weeks) administration of butyrate orally but not intravenous decreased food intake in mice under HFD compared to control, preventing diet-induced obesity, hypertriglyceridaemia, hyperinsulinaemia, and hepatic steatosis [102]. Oral butyrate induced the suppression of the activity of NPY neurons in the hypothalamus together with the reduction of neuronal activity within the NTS and dorsal vagal complex (DVC) in brainstem with no increase in circulating butyrate levels [102]. The mechanisms underlying the regulation of food intake and energy homeostasis by butyrate, equally to other derived metabolites, are still unclear. It has been suggested that butyrate can bind the G protein-coupled receptors free fatty acid receptor 2 (FFAR2 or GPR43) and 3 (FFAR3 or GPR41) as well as the olfactory receptor of family 51 subfamily E member 1 (OR51E1), which subsequently leads to secretion of gut hormones peripherally, which can signal to the brain via the systemic circulation or vagal afferent nerves [103]. In this regard, not only the microbiota-derived metabolites (postbiotics) can modulate the homeostatic food regulation, but also microbiota-targeting interventions, via pre- or probiotic administration, have been associated with regulating the expression of hypothalamic genes involved in the homeostatic regulation of appetite, directly impacting food intake in rodents [104,105] and humans [106,107]. For instance, a diet containing oligofructose (prebiotic) decreased daily food intake and epididymal fat mass together with an increase in the levels of GLP-1 and peptide YY (PYY) and a decrease of ghrelin levels in the serum of rats [105]. The administration of the probiotic *L. plantarum* CCFM023 reduced the food intake in rats, which was accompanied by decreased circulating levels of leptin and glucose and leptin [108]. The potential role of the microbiome in the regulation of food intake has been reviewed in detail elsewhere [109–112].

The microbiota is also able to produce amino acid sequences homologous to endogenous neuropeptides that regulate appetite and emotions a process called 'molecular mimicry'[113]. One of the clearest examples is with *Bacteroides* producing

molecules homologous to insulin, NPY, melanocyte stimulating hormone (α-MSH) showing their impact on appetite and emotion by mimicking these molecules [113]. These bacterial peptides have homologous sequences able to induce immunological cross-reactions with immunoglobulins (autoantibodies) present in the circulatory system[114]. Under physiological conditions, autoantibodies are found to act directly against hormones or peptides (e.g., ghrelin, leptin, insulin, PYY and NPY, among others) that regulate appetite. Autoantibodies have been detected in humans and rats [115], and their levels have been significantly reduced in GF animals, altering motivational behaviours [115]. Furthermore, it has been observed that *Rikenellaceae* and *Clostridiaceae* are able to produce a peptidase called ClpB in humans [116], can mimic the satiety enhancement of α-MSH[117]. This protease can produce autoantibodies that act against α-MSH reducing its anorexigenic effects, which can lead to a decrease in the satiety effects in the animal promoting food intake [117]. Interestingly, specific bacterial strains have been shown to impact on food intake through similar molecular mimicry[118]. For example, *Hafnia alvei*, a ClpB-producing commensal bacterium, was shown to decrease food intake in hyperphagic *ob/ob* mice [118].

Another approach to study the potential more causal impact of the microbiota on feeding behaviour is the faecal microbiota transplantation (FMT), which has been extensively investigated in the context of obesity, irritable bowel syndrome, anxiety and depression, among others [119]. A study using the FMT model proposed a role for microbiota in reward signalling, which is a driving factor of hedonic food intake [92,97,120]. In this recent study, the authors showed that the alteration in food pleasure induced by obesity was transferable via FMT from obese donor to lean mice[92]. The same authors showed that FMT from obese donors to lean recipients lead to excessive motivation for food reward using an operant conditioning paradigm[97]. At molecular level, recipient mice showed lower expression in dopaminergic and opioid markers in brain areas responsible for reward regulation, such as the nucleus accumbens (NAc) [92]. In another preclinical study, antibiotic administration induced overconsumption of palatable food which was normalized by the colonization with S24-7 and *Lactobacillus johnsonii* [91]. These studies suggest a link between the gut microbiota and food intake behaviours involving non-homeostatic systems and further studies are warranted.

Even though strong literature supports a role of the gut microbiota in the central regulation of food intake, the causal role and mechanisms by which this regulation occurs are still unclear. For this reason, further research is necessary in order to understand the underlying mechanisms of the gut microbiota mediated effects on eating behaviour and metabolic health and how well they translate to humans.

Gut microbiota and circadian rhythm

Table 1 shows the different studies that have analysed the diurnal oscillation of the microbiota in different animal models in recent years, highlighting the growing interest among the scientific community in this topic. This evidence suggests a crucial role of the microbiota and microbiota-derived metabolites in the maintenance of the host clock system and the diurnal rhythmicity for the gut microbiota. However, there is a critical gap in our understanding of how the circadian system and gut microbiota converge to regulate homeostatic and hedonic food intake behaviour under normal weight and in obesity fully understand the causality behind these initial correlative findings.

Convergence of circadian rhythm and microbiota on food intake behaviour

Similar to host tissues, the gut microbiota shows a diurnal oscillation in the relative abundance of its constituent microorganisms [35] (Fig. 3). This rhythmicity is orchestrated by the synchronization of external factors such as diet or eating behaviour and the host's own clocks (central and peripheral, such as the gut or the liver ones)[35]. Indeed, alteration of the molecular machinery of the clock system alters the rhythmicity of the microbiota and its activity [70,121,122]. Furthermore, the importance of the microbiota in maintaining the diurnal oscillations of the host’s peripheral tissues has been reported[123,124]. For instance, by converting fasting/feeding cycles into signals that can subsequently entrain the clock system, and even cause circadian disruption when these cycles are altered, or under an unhealthy diet [90,122,125]. In addition, this diurnal oscillation has also been observed in microbiota composition and functionality and in the content of microbiota-derived metabolites such as SCFAs [90,121,122,124,126–128]. This evidence suggests a crucial role of the microbiota and microbiota-derived metabolites in the maintenance of the host clock system and a complex bidirectional interrelationship between microbiota and feeding behaviour

driven by the mechanisms of energy homeostasis, enteroendocrine signalling and the central regulation of food intake. Our modern lifestyles, which are characterized by sleep disturbances and dysregulation of food intake behaviour, disrupt both our microbiota as well as the circadian clock [40,90,122,126,129]. Even though recent findings have suggested an individual correlation between unhealthy eating behaviours and an altered gut microbiota, as well as disrupted diurnal rhythmicity, there is no research yet showing the causal relationship behind these preliminary findings. However, we suggest here that characteristics of our current lifestyle, such as disrupted rhythmicity, altered microbiota, and changes in food intake, may act synergistically to create a vicious cycle promoting unhealthy eating behaviours and increasing metabolic risk (Fig. 4, Key Figure).

Taking a double hit: how microbiota changes and circadian disruption can negatively impact eating behaviour

We reviewed here the dual associations of food intake with the gut microbiota and the circadian clock, which we suggest that may act synergistically to maintain metabolic and physiological homeostasis. Indeed, there has been increasing interest in studying the interaction between the microbiota-gut-brain axis and biological rhythms. Emerging research is showing the contribution of the gut microbiota and circadian clock on food intake behaviour, i.e. what, when and how much food is consumed (Fig. 4, Key Figure). Unhealthy diet and altered eating patterns can negatively impact both the gut microbiota and the circadian clock. Nevertheless, no clear evidence exists that circadian mediated mechanisms can impact eating behaviour via microbiota-gut-brain axis signalling. What is emerging is that, converging pathways of microbiota-mediated mechanism and mechanism of circadian synchronicity exist, which may act independently or synergistically to impact on metabolic homeostasis and central regulation of eating behaviour with high relevance for metabolic health. Future studies should be aimed at investigating how the circadian system impacts on microbiota-gut-brain axis signalling in the context of metabolic homeostasis and homeostatic food intake behaviour.

Potential mechanism could involve interaction of microbiota with toll-like receptors (TLRs). The gut microbiota has a crucial role in maintaining the diurnal rhythmicity of TLRs, which have an important role in the communication of microbiota-associated molecular patterns (MAMPs), by which they distinguish between microbiota antigens and those from pathogens, with intestinal epithelial cells (IECs) [130]. Interestingly, Vijay-Kumar *et al.*, (2010) showed an association between hyperphagia an obese phenotype of TLR5 KO mice following faecal microbiota transplantation, suggesting a potential contribution of gut microbiota and malfunctioning immune system on metabolic disorders [131]. In addition, it has been observed that IEC clock genes are altered in the absence of gut microbiota, increasing ileal corticosterone synthesis leading to altered host metabolic homeostasis [130]. GF mice display alterations not only in IEC clock genes but also in circadian core expression in liver and white adipose tissue [123]. Furthermore, microbiota-derived metabolites that activate the aryl hydrocarbon receptor (AHR) and pregnane X receptor (PXR) are crucial for maintaining the rhythmicity and sexual dimorphism of growth hormone secretion, which maintains the diurnal rhythmicity of gene expression and metabolome in the liver in a sex-dependent manner [123]. This demonstrates an important role of the gut microbiota on both host energy metabolism as well as diurnal rhythmicity of different peripheral tissues such as the gut, liver or white adipose tissue and could be a key modulator of the host clock system sending signals from ingested food.

In addition, feeding rhythms of the host directly impact the gut microbiota by marking its diurnal oscillation, allowing it to adapt to the nutritional environment. This has been demonstrated in a study with Period 1/2 (*Per1/2)* gene KO mice or mice subjected to a jet-lag situation, which showed altered feeding cycles associated with abnormal fluctuations in the gut microbiota throughout the day [121]. This altered gut microbiota oscillation was partially restored with restricted feeding in *Per1/2* KO mice, demonstrating that eating behaviour is the main driver of diurnal microbiota oscillations[121]. Not only jet-lag exposure or lack of *Per1/2* expression, but also a HFD decreases gut microbiota oscillation leading to altered fluctuations of microbiota-derived metabolites and in turn affecting host metabolism[126,132]. Indeed, oscillations of the *Lachnospiraceae*-derived metabolite butyrate, disappear as a consequence of

491 altered fluctuations of this microbial family in GF mice HFD, affecting the expression of
492 liver clock genes and host metabolism [126]. Furthermore, it has been observed in
493 rodents that bacterial adherence to the intestinal epithelium and its proximity to the
494 mucosal surface are greater in the dark phase than in the light phase, showing a diurnal
495 oscillation of epithelial adherence dependent on feeding time [124]. These nutrient-
496 induced microbial oscillations throughout the day promote oscillations of luminal and
497 serum metabolites that are responsible for the diurnal rhythmicity of important
498 physiological processes in the liver [124]. The fact that the feeding cycle is maintained
499 in the absence of microbiota after antibiotic treatment suggests that this fasting/feeding
500 cycle is driven by the central clock, but findings obtained by different researchers by
501 means of FMT, managing to transfer obesity-associated hyperphagia in GF mice [131],
502 suggests a crucial role of the microbiota in modulating nutrient signalling with an impact
503 on feeding behaviour.

504 Bacterial growth dynamics are controlled by different factors such as nutrient
505 availability, immunity, gut motility and bacterial quorum sensing and appear to overlap
506 with host feeding/fasting cycles [112]. Interestingly, a model of appetite control based
507 on bacterial growth dynamics has been proposed, in which hunger-related signals are
508 associated with the bacterial depletion phase, while signals induced during the prandial
509 and post-prandial satiety phase are associated with exponential and stationary bacterial
510 growth [112]. Indeed, following food ingestion that induces bacterial growth,
511 *Escherichia coli* produces an α-MSH mimetic antigen called ClpB that activates host
512 satiety pathways [117,133]. In addition, the generation and release of bacterial ligands,
513 such as LPS or MDP, may be enhanced by nutrient-induced bacterial growth, which in
514 turn activates nutrient-sensing pathways in the gut. Although LPS and MDP suppress
515 food intake through immune-sensing pathways linked to satiety via regulation of GLP-1-
516 mediated signalling [134–136] and behaviour in a disease state, causality between
517 diurnal oscillations of LPS or MDP and feeding rhythms [137–139] has not been proven.
518 Therefore, there is a need to investigate oscillations in bacterial growth and the
519 production of metabolites derived from dietary signalling and bacterial derivatives
520 involved in modulating energy homeostasis and in turn feeding behaviour. In this regard,
521 a recent study in mice demonstrated that the gut microbiota could regulate host feeding

behaviour to stabilize its gut niche. The authors showed that hypothalamic neurons in mice could sense the wall components of the gut bacterial microbiota via neuronal nucleotide-binding oligomerization domain-containing protein 2 (*Nod2*) [140]. Female mice without *Nod2* (gene KO) in GABAergic neurons lose their ability to control food intake and body temperature, increasing their appetite and body weight gain. In addition, a reduction in nesting behaviour and range of daily body temperature was observed. Interestingly, this behaviour is associated with a response to circadian rhythms.

Since the gut microbiota as well as the circadian rhythms have been shown to potentially impact eating behaviour and share similar orchestrators that impact the bidirectional communication with the SCN, we suggest with the current review that the gut microbiota could be a key player in the circadian-mediated effects on central regulation of eating behaviour. In this line, future studies will need to consider and study the mechanism by which the circadian system and gut microbiota converge to regulate homeostatic and hedonic food intake behaviour under normal weight and in obesity.

Concluding Remarks

The modern obesogenic environment and lifestyle changes of the modern world are causing disruption of our biological circadian rhythms, our gut microbiota and food intake behaviours, creating a negative vicious cycle impacting on the metabolic health (Fig. 4, Key Figure). Both the circadian system and gut microbiota are bidirectional connected to the central nervous system via common orchestrators (i.e., parasympathetic vagal nerve of the autonomic nervous system, and through the neuro-immune-endocrine system). The gut microbiota is a biological factor that could directly or indirectly influence metabolic status and food intake, and, theoretically, its modulation could help to restore the disruption of biological rhythms. Restoration of eating behaviour through the gut-brain axis, taking into account the influence of the timing of food intake, would be a promising strategy due to the suggested key role of the gut microbiota in the control of metabolic homeostasis and food intake behaviour. We hypothesize that targeting the gut microbiota, due to its potential role as a chrono-communicator, in the context of metabolic disorders linked to unhealthy eating may

ultimately ameliorate the metabolic status via improving the disruption of the diurnal rhythmicity and the eating behaviours. However, future studies should investigate if and how microbiota oscillations relinked with the circadian regulation of metabolic health, and if both microbiota mediated effects and circadian effects converge on metabolic health via central regulation of homeostatic eating behaviour. A microbiota-targeted intervention (pre/probiotics) could be stand-alone or combined with previous strategies (i.e., melatonin or phenolic compounds) aimed to improve circadian rhythmicity, energy homeostasis, and eating behaviour (see Outstanding questions).

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Conflict of Interest

The authors declare no conflict of interest.

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931 ***Table 1. Clock system and microbiota functionality, a bidirectional relationship that impacts on food intake behaviour and host metabolism.***

Year	Experimental design	Animal strain/Human	Results	Diurnal oscillation	Rhythmic taxonomic levels	Ref
			Rhythmicity of microbiota			
2014	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 6 hours for 2 days, *ad libitum* food.	Wild-type mice.	Up to 15% of all bacterial operational taxonomic units (OTUs) exhibit diurnal oscillations.	Yes, around 60% of the microbial composition	*Clostridiales, Lactobacillales, Bacteroidales*, *Lactobacillus reuteri and Dehalobacterium spp*.	[121]
	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 6 hours for 2 days, *ad libitum* food.	*Per1/2*$^{-/-}$ and wild-type mice.	*Per1/2*$^{-/-}$ mice showed a near complete loss of rhythmic fluctuations in commensal bacterial abundance.	No, the rhythmic pattern was replaced by a random abundance fluctuation with a reduction in the number of diurnally oscillating bacterial taxonomic units	No oscillation.	
	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 6 hours for 2 days, access to food only during the light phase or only during the darkness phase for 2 weeks.	Wild-type mice.	Microbiota oscillations in the dark-phase-fed group were similar to *ad-libitum*-fed mice. In contrast, cyclic OTUs often displayed different phases between dark-phase-fed and light-phase-fed groups.	Yes, but most of the cycling OTUs appeared to show a phase shift of about 12 hours, suggesting a direct control of microbiota rhythms by feeding times.	*Bacteroides acidifaciens, Lactobacillus reuteri, Peptococcaceae, Candidatus Arthromitus*	
	Animals were kept for 4 weeks in a jetlagged situation: light was advanced 8 hours for 3 days and then returned to the normal light-dark cycle. Sampling was every 6 hours for 2 days.	Wild-type mice.	Jet-lagged mice showed an abrogation of bacterial rhythms with a reduced number of oscillating bacterial taxonomic units	No, environmentally induced abrogation of daily oscillatory patterns was associated with loss of diurnal rhythmicity in microbiota composition.	No oscillation.	
	Samples were collected from two volunteers at various times of the day on several consecutive days.	Human.	Up to 10% of all bacterial OTUs exhibit diurnal oscillations.	Yes.	*Parabacteroides, Lachnospira and Bulleida.*	
2015	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 2 days, *ad libitum* food.	Male and female C57BL/6 mice.	The fecal bacterial load oscillated diurnally during the light–dark cycle both in the group as a whole and when segregated by sex. The relative abundances of *Bacteroidetes* and *Firmicutes* varied during the light–dark cycle.	Yes.	*Clostridiales spp*. and *Turicibacter* (relative and absolute abundace), *Clostridiaceae spp.* (absolute abundance) and *Ruminococcaceae spp.*, *Clostridia spp.*, *Lachnospiraceae spp.*, *Oscillospira* and *Anaeroplasma* (relative abundance)	[141]

	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 2 days, ad libitum food.	Male and female homozygous *Bmal1* KO and wild-type mice.	Diurnal rhythmicity of the relative abundances of *Bacteroidetes* and *Firmicutes* in WT mice but none in *Bmal1* KO mice. Both relative and inferred absolute abundances of *Bacteroides* and *Lacotobacillaceae spp*. oscillate significantly in WT mice, and this oscillation was abolished in *Bmal1* KO mice.	No, oscillations are disrupted in *Bmal1* KO mice.	No oscillation.	
2016	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 6 hours for 2 days, *ad libitum* food.	C57BL/6 mice.	The numbers of bacteria colonizing the epithelial niche underwent marked diurnal changes, with epithelial layer adherence in the dark phase being up to 10-fold higher than in the light phase and the global bacterial composition featured marked diurnal oscillations. A total of 148 out of 633 detected operational taxonomic units (OTUs) featured rhythmic patterns of epithelial adherence.	Yes.	*Mucispirillum schaedleri, Lactobacillus reuteri* and *Bacteroides acidifaciens*	[124]
	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 6 hours for 2 days, *ad libitum* food. An animal group was treated with antibiotics.	C57BL/6 mice.	Antibiotic treatment nullified both the number of mucosa-associated bacteria and their oscillatory behaviour. In addition, the remaining antibiotic-persistent epithelioproximal microbiota lost their diurnal rhythms in composition.	No.	No oscillation.	
2018	Light-dark (LD) group and dark-dark (DD) group, *ad libitum* food. Samples were collected every 4 hours starting at ZT2.	Balb/c mice.	The oscillation was completely lost by DD, suggesting the diurnal fluctuation of microbial beta-diversity was dependent on a L/D cycle	No under DD conditions.	No oscillation.	[142]
2019	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 6 hours for 2 days, *ad libitum* food. Quantitative PCR analysis of bacteria in stools at the Phylum level.	$Arntl^{\Delta Camk2a}$ mice.	Surgical- and genetically-induced deregulation of brain rhythmicity led to disrupted circadian ILC3 oscillations, deregulated microbiome and altered lipid metabolism.	Yes.	$Arntl^{\Delta Camk2a}$ mice further exhibited altered epithelial reactivity genes and perturbed microbial communities, notably *Proteobacteria* and *Bacteroidetes*.	[143]

2021	A total of 1109 samples from 235 individuals were selected for microbiome analysis.	Meerkats (*Suricata suricatta*).	Diurnal oscillations in bacterial load and composition eclipsed seasonal and lifetime dynamics. Overall, the strength of diurnal oscillations identified here suggest that circadian rhythms in gut microbiomes are likely to play a major role in host biological function.	Yes	*Clostridium, Raoultibacter, Cellulomonas,* and *Bacillaceae*.	[144]
2021	Six cows of similar weight, parity, and milk performance were used. They were adapted to natural light for 2 weeks before sampling and fed twice daily at 07:00 a.m. and 14:00 p.m. Samples were collected at 02:00, 10:00, and 18:00 on the first day and 06:00, 14:00, and 22:00 on the second day of the experimental period.	Lactating cows.	The results not only showed that rumen fluid contains melatonin, but the diurnal variation of melatonin and the relative abundance of 9% of total rumen bacterial operational taxonomic units (OTUs) follow a circadian rhythm. Ruminal melatonin was closely associated with *Muribaculaceae*, *Succinivibrionaceae*, *Veillonellaceae*, and *Prevotellaceae* families in vivo.	Yes.	At the phylum level, *Proteobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, Melainabacteria, Gracilibacteria*, and *Tenericutes* followed a circadian pattern. At the family level, *Prevotellaceae, Succinivibrionaceae, Ruminococcaceae, Muribaculaceae*, and *Veillonellaceae* followed a clear circadian oscillation.	[145]
2022	Compared fecal microbiota rhythms of the same wild-type mice kept in a rhythmic 12-hour light/12-hour dark (LD) cycle and constant darkness (DD) for two weeks.	Wild-type mice.	Rhythms in microbiota are generated endogenously by the circadian system.	Yes, 77% zOTUs were rhythmic in LD condition and 63% in DD condition.	More than 95% of zOTUs show similar rhythmicity in DD than LD, e.g. the genera *Eubacterium* and members of the family *Lachnospiraceae.*	[70]
	Feces were collected from starved mice during the 2nd day in DD.	Wild-type mice.	Microbiota diversity became arrhythmic in the absence of food. However, rhythms persisted in the two dominant phyla, highly abundant families, including Muribaculaceae and Lachnospiraceae and the majority of zOTUs.	Yes.	Of note, circadian food intake behaviour enhanced the amount of rhythmic zOTUs by 16%.	
	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 24 hours.	Mice with IEC-specific deletion of the essential core clock gene *Bmal1* (*Bmal1*IEC$^{-/-}$)	The intestinal circadian clock controls microbiota composition and function.	Yes, diurnal oscilation disrupted by dysfunctional clock machinery.	63% zOTUs were rhythmic in control group and 26% in *Bmal1*IEC$^{-/-}$ group.	

	Compared fecal microbiota rhythms of the mice kept in a rhythmic 12-hour light/12-hour dark (LD) cycle and constant darkness (DD) for two weeks. Also compared microbiota rhythms of the mice with *ad libitum* food and in fasted condition.	Wild-type mice and *Bmal1*IEC$^{-/-}$ mice.		Yes.	32% zOTUs were rhythmic in LD *Bmal1*IEC$^{-/-}$ group and 23% in DD *Bmal1*IEC$^{-/-}$ group. 24% zOTUs were rhythmic in *ad libitum* food *Bmal1*IEC$^{-/-}$ group and 17% in starvation *Bmal1*IEC$^{-/-}$ group.	
	Germ-free (GF) C57BL/6 mice were colonized with cecal content from *Bmal1*IEC$^{-/-}$ or control mice.	GF C57BL/6 mice.	50% of the rhythmic zOTUs in the controls lost rhythmicity within cecal content of *Bmal1*IEC$^{-/-}$ mice.	Yes, but diurnal oscilattion disrupted in *Bmal1*IEC$^{-/-}$ mice cecal content.	50% of the rhythmic zOTUs in the controls lost rhythmicity within cecal content of Bmal1IEC-/- mice.	
2023	150 Roman laying hens of 20 weeks of age were randomly divided into three treatment groups: a normal photoperiod (NP) with 16 h of light and 8 h of darkness (16 L: 8 D), an intermittent photoperiod 1 (IP1) with four 3 L:1 D cycles within 16 h followed by 8 h of darkness (16 [3 h -L/1 h -D]:8 D), and an intermittent photoperiod 2 (IP2) with sixteen cycles of 45 min -L/15 min -D within 16 h followed by 8 h of darkness (16 [45 min -L/15 min -D]:8 D), sampling every 6 hours for 24 hours.	Roman laying hens.	Intermittent photoperiod 1 (IP1, 16 [3 h -L/1 h -D]: 8 D) enhanced the circadian rhythms of cBmal1, cBmal2, cCry1, and cCry2 in the hypothalamus and increased the expression of cClock, cBmal1, and cCry2 in the liver and seven clock genes in the cecal wall. The intermittent photoperiod also significantly altered the composition and metabolic function of the cecal microbiota via the melatonin pathway.	Yes.	Diurnal oscillation of the microbiota under intermittent photoperiods were similar to those under the NP, and only a few microbes exhibited coscillations. *Eubacterium* under the NP treatment and *Ruminococcus* under the IP1 treatment exhibited significant diurnal rhythmicity, increasing during the day and decreasing at night, while the opposite rhythm was found for *Mucispirillum* under IP1 and IP2.	[146]
			Under metabolic disorder conditions			
2015	Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 6 hours for 2 days, *ad libitum* food, High-fat diet (HFD) and regular chow (RC) diet.	Germ-free and conventionally-raised specific-pathogen-free C57Bl/6J mice.	HFD reduced the number of OTUs exhibiting significant oscillation patterns in both fecal and cecal contents compared to RC.	Yes, in both fecal and cecal content.	*Lachnospiraceae* in RC, *Lactococcus* in HFD, *Oscillospira* in both conditions.	[126]
2019	Animals were fed *ad libitum* for 10 months. Samples were collected every 4 hours for 1 day. Mice were divided in diabetic and control groups.	Homozygous db/db mice and heterozygotes (db/m).	In diabetic mice many members of the gut microbiota lost their diurnal oscillations, especially members of the *Lachnosporaceae*, *Ruminococcaceae*, *Erysipelotrichaceae* families, while members of the *Prevotellaceae* family, and the classes *Actinobacteria* and *Proteobacteria* exhibited significant phase shifts.	Yes, but the oscillations were altered in diabetic group.	Bacteria of the genus *Bacteroides, Lactobacillus, Blautia, Akkermansia, Bifidobacterium, Allobaculum, Oscillospira,* and *Prevotella* (control mice). *Bacteroides*, *Blautia*, and *Prevotella* preserved their diurnal rhythmicity in diabetic mice.	[127]

2020	Diurnal rhythmicity of fecal microbiota profiles was studied in 1,943 subjects for whom time at sampling was available, T2D subjects (N = 277), nonT2D (N = 1,255), subjects with BMI < 30 (N = 1,393)	Human.	Disruption of microbial oscillation and the model for T2D classification was confirmed in 1,363 subjects. This arrhythmic risk signature was able to predict T2D in 699 KORA subjects 5 years after initial sampling, being most effective in combination with BMI.	Yes, arround 15% of all bacterial operational taxonomic units (OTUs) exhibit diurnal oscilations in noT2D group.	87 OTUs displayed oscillation in controls but lacked rhythmicity in T2D. They belonged to the genera *Akkermansia, Bacteroides, Bifidobacterium, Blautia, Clostridium, Coprococcus, Dorea, Prevotella, Roseburia*, and *Ruminococcus.*	[147]
2022	Mice were fed normal chow diet (NCD) or high-fat carbohydrate-free diet (HFD). After 2 months, a sub-group from NCD- or HFD-fed mice was treated for one additional month with antibiotics. C57BL/6 germ-free male mice were colonized by gavage with the mucosal microbiota from the ileum of mice fed for 3 months with a NCD or a HFD. Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 24 hours.	C57BL/6 WT male mice.	GLP-1 sensitivity is maximal during the dark feeding period, i.e., the postprandial state. Coincidently, the ileum expression of GLP-1 receptor and peripherin is increased and tightly correlated with a subset of clock gene. The abundance of ileum bacteria oscillated diurnally with a maximum during the dark period, along with expression patterns of a subset of clock genes. these resultas suggest that GLP-1 sensitivity relies on specific pattern of intestinal clock gene expression and specific gut bacteria.	Yes.	At phylum level, *Proteobacteria* and *Deferribacteres* showed large amplitude variation. At the family level, the abundances of *Lachnospiraceae*, *Lactobacillaceae*, and *Sphingomonadaceae* were most affected by the time of day.	[148]
2022	Mice were randomly assigned to one of two groups: a control group (Ctr, n = 40), which was fed a standard chow diet, and a high-fat diet group (HF, n = 40), which was fed a high-fat diet. After 5 weeks of intervention, the C57BL/6 female mice were mated with normal 9-week-old C57BL/6 male control mice. Pregnancy was timed based on vaginal plug formation.*ad libitum* food access. Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 24 hours.	C57BL/6 Pregnant female mice.	HF significantly disrupted the rhythmic pattern of hepatic and adipose circadian clock genes and downstream metabolic genes. HF altered the diurnal rhythm of the gut microbiota in a diverse manner, which was assessed across three categories: phase shift, loss rhythmicity, and gained rhythmicity.	Yes, but diurnal oscilation disrupted by HF.	At family level, *Clostridiaceae_1*, exhibited a diurnal rhythm on both feeding protocols, and its rhythmic pattern was dramatically phase-delayed in the HF group compared with that in the Ctr group. *Veillonellaceae* and *Prevotellaceae* lost rhythmicity in the HF treatment group, while *Erysipelotrichaceae* and *Ruminococcaceae* gained rhythmicity in the HF group	[129]

2023	Animals had *ad libitum* access to normal chow, and naïve and arthritic (Collagen induced arthritis (CIA)) mice were co-housed. Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 24 hours.	Male DBA/1 mice.	Mice with collagen induced arthritis exhibited extensive changes in rhythmic gene expression in the colon, and reduced barrier integrity. Re-modeling of the host gut circadian transcriptome was accompanied by significant alteration of the microbiota, including widespread loss of rhythmicity in symbiont species of *Lactobacillus*, and alteration in circulating microbial derived factors, such as tryptophan metabolites.Assessment of OTU rhythmicity revealed that a significant proportion of the microbial community exhibits 24 h rhythmicity within both naïve (26%) and arthritic animals (24%), but there is significant temporal re-organization in the setting of arthritis as only approximately 46% of rhythmic OTUs are shared between conditions.	Yes, but diurnal oscilattion disrupted in CIA group.	The most abundant phyla, the *Firmicutes* and *Bacteroidetes*, exhibited diurnal rhythmicity in naïve animals and were in antiphase to one another. Of note rhythmicity in the *Bacteroidetes* was dampened in the setting of CIA.	[149]
			Eating behaviour			
2017	Samples and data were collected from the control period (0 g supplemental fiber) of a previously completed trial of agave inulin consumption in healthy adults. Samples were collected only during the light phase.	Human.	Acetate, propionate, and butyrate concentrations decreased throughout the day. Thirty-five percent of bacterial OTUs were associated with time.	Yes, bacteria composition correlated to time.	*Roseburia, Veillonella, Haemophilus*, and an unspecified genus within the *S24-7* family decreased with clock time. *Adlercreutzia, Eggerthella, Anaerotruncus, Oscillospira, Ruminococcus, Holdemania, Desulfovibrio, Escherichia*, and an unspecified genus within the *Enterobacteriaceae* family increased with clock time.	[150]

2020	Animals were randomly grouped into 3 groups: (1) normal diet-*ad libitum* (NA group), (2) high-fat diet (HFD)-*ad libitum* (FA group), and (3) HFD-Time restricted Feeding (TRF) (FT group).	Male Kunming mice.	The numbers of *Bacteroidetes* and *Firmicutes* differed between mice that received a time-restricted high-fat diet and mice that received a high-fat diet *ad libitum* ($p < 0.05$). Mice fed a time-restricted high-fat diet showed distinct circadian rhythms of hepatic expression of SIRT1, SREBP, and PPARα compared with mice fed a normal diet *ad libitum*, as well as the circadian rhythm of the abundance of *Bacteroidetes* and *Firmicutes*.	Yes, diurnal oscilation disrupted by HFD and improved by Time Restricted Feeding.	The abundance of *Actinobacteria* was diurnally fluctuated in all three groups, and the abundance levels of *Bacteroidetes* and *Firmicutes* were diurnally fluctuated in FT mice. The relative abundance of family *Lachnospiraceae* showed oscillation in NA and FT mice, but not in FA mice. Abundance levels of family *Ruminococcacea* also showed oscillation in NA and FT mice while no rhythm in FA mice.	[90]
2021	Animals were fed either a lithogenic diet (LD) only during the sleep phase (time-restricted lithogenic diet feeding, TRF) or an LD ad libitum (non-time-restricted lithogenic diet feeding, nTRF) for 4 weeks. Light/dark cycle 12h, sampling every 4 hours for 24hours.	Male C57BL/6J mice.	TRF altered the circadian rhythms of and promoted gallstone formation in mice. This was associated with disruptions in hepatic cholesterol and bile acid metabolism and the gut microbiota, which exacerbated abnormalities in serum and biliary cholesterol concentrations.	Yes, diurnal oscillation in the nTRF group, but disrupted in the TRF group.	Compared with the nTRF group, the gut microbiota in the TRF group showed less fluctuation and no clear rhythm, especially with regard to *Bacteroidetes* and *Firmicutes*, which were the two most abundant phyla.	[125]
2021	Growing pigs were randomly assigned to daytime-restricted feeding (DRF) and nighttime-restricted feeding (NFR) groups. Light/dark cycle 12h, sampling every 4 hours for 24 hours.	Pigs.	NFR disrupted the diurnal oscillation of behaviour and clock genes and altered the serum ghrelin, dopamine, and serotonin levels. NRF also altered the diurnal oscilations and composition of the gut microbiota.	Yes.	At the pyhlum level, DFR group showed diurnal oscillation for *Firmicutes* and *Bacteroidetes*, while *Proteobacteria* and *Desulfobacterota* for NFR group.	[151]
2021	Rabbits with similar body weight were randomly assigned to daytime feeding (DF, n = 108) and night-restricted feeding (NRF, n = 108) groups and these rabbits were housed and fed in individual cages. Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 24 hours.	Female Ira rabbits.	NRF improved the diurnal rhythm and abundance of beneficial microorganisms, along with the production of beneficial metabolites, whereas reduced the abundance of potential pathogens (*Synergistes*, *Desulfovibrio*, and *Alistipes*). Furthermore, NRF strengthened the diurnal amplitude of body core temperature, and promoted the diurnal expression of intestinal clock genes (BMAL1, CLOCK, REV-ERBα, and PER1), and genes related to the regulation of the intestinal barrier (CLAUDIN-1), and intestinal epithelial cell self-proliferation and renewal (BMI1).	Yes.	At the phylum level determined that DF caused a diurnal rhythm in *Proteobacteria* as compared with the NRF group. At the genus level, *Intestinimonas* and *Flavonifractor* showed diurnal rhythm in the DF group. In contrast, NRF promoted diurnal rhythm of *Clostridium* (*Lachnospiraceae_NK4A136_group , Ruminococcaceae_UCG-013,* and *Roseburia*).	[152]

2022	Mice were housed individually and randomly assigned to one of two experimental conditions: a normal light/dark cycle (LD) or dim light at night (dLAN). After 2 weeks in experimental light conditions, fecal pellets were collected every 4 hours for 24 hours. A temporal restricted feeding cycle with a high-fat diet to eliminate daytime feeding.	Male littermate age-matched controls (Opn4Cre/+), male melanopsin knockout mice (Opn4Cre/Cre), and male ipRGC-eliminated mice (Opn4DTA/DTA). Mice were maintained on a C57BL6/J background.	Light-dark cycle information transmitted by the intrinsically photosensitive retinal ganglion cells (ipRGCs) is essential for daily oscillations of gut microbes under temporal restricted high-fat diet conditions. Furthermore, aberrant light exposure such as dim light at night (dLAN) can alter the composition, relative abundance, and daily oscillations of gut microbiota.	Yes.	Not specified.	[153]
2022	Mice were split into three groups: mice fed a normal chow diet with *ad libitum* food access (n = 18, NA condition), a HFD with ad libitum food access (n = 18, FA condition), or a HFD with time-restricted food access (n = 18, FT condition). Time-restricted food access refers to restricting food access to a period of 8 hours during the dark (ZT 13–21). Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 24 hours.	Wild-type male C57BL/6 mice.	The dynamic rhythms of ileal microbiome composition and transcriptome are dampened in Diet-induced obesity. TRF partially restores diurnal rhythms of the ileal microbiome and transcriptome, increases GLP-1 release, and alters the ileal bile acid pool and farnesoid X receptor (FXR) signalling, which could explain how TRF exerts its metabolic benefits.	Yes, diurnal oscillation in the NA and FT group, but disrupted in the FA group.	Lactobacillus, Lactococcus, Staphylococcus and Streptococcus displayed diurnal oscillation in the FT group.	[122]
	CDKO mice were kept in similar light tight boxes with *ad libitum* food access (n = 18, CDKO-NA). Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 24 hours.	Whole body clock mutant Cry1;Cry2 double KO were backcrossed to C57/B6 background.		No.	No oscillation.	

2022	Food was withheld for the fasting group beginning 24 hours prior to the respective sacrifice time. *ad libitum* food access. Light/dark cycle of 12 hours of light and 12 hours of darkness, sampling every 4 hours for 24 hours.	Male C57BL/6J mice.	Core clock rhythmicity remains robust in the fasted state, with tissue-specific changes to rhythm parameters, particularly in the BAT. The abundances of bacterial phyla in the fecal microbiota are rhythmic. Phyla-specific alterations were observed upon fasting, however, bacterial abundances retained significant 24 h rhythmicity even in the absence of rhythmic nutrient availability.	Yes.	*Bacteroidetes, Firmicutes*, and *Actinobacteria* were all rhythmic in both conditions, *Verrucomicrobia* and *Proteobacteria* were rhythmic only in fed condition. Only *Ternericutes* became arrhythmic in the fasted condition. At the family levels, *S24-7, Alcaligenaceae, Bacteroidaceae* and one unknown family were all rhythmic in both conditions, *Verrucomicrobiaceae* were rhythmic only in fed conditions, *Coriobacteriaceae*, *Anaeroplasmataceae* and one unknown family were rhythmic only in fast conditions	[154]
2023	Flies were randomly assigned to timed feeding group or *ad libitum* feeding group. The w^{118} iso^{31}, per^{01} fly lines.	Drosophila.	Timed feeding does not increase resistance of flies to stress and germ-free flies lacking a microbiome reset more rapidly with shifts in the light:dark cycle, suggesting that the microbiome stabilizes the cycle in the host gut to avoid rapid fluctuations, functioning as a shock absorber, with changing environmental conditions.	Only in clockless per^{01} flies the diurnal oscillation is displayed and under timed feeding conditions. No diurnal oscillation in *ad libitum* conditions.	*Firnicutes, Lactobacillaceae*, *Proteobacteria* ,*Acetobacter* , *Proteobacteria,* and *Gluconacetobacter*	[128]
			Supplements			
2019	An initial colonization in young adult male C57BL/6J mice gut using freshly fecal sample from 6 healthy volunteers (25–30 years old). Animals were randomly assigned to 3 groups (27 mice per group): a regular light/dark cycle group (CONT), constant dark group (CD), and constant dark with Oolong Tea Polyphenols (OTP) group (CD-OTP), sampling every 6 hours for 2 days, *ad libitum* food.	C57BL/6J mice.	The circadian rhythms observed in the phylum-level in the CONT group were not significant in the CD group. Interestingly, we found that OTP can significantly improve the suppression of phylum-level cyclical changes caused by continuous darkness.	Yes, but diurnal oscillations disrupted by constant darkness.	*Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria*	[155]

2020	Animals were randomly grouped into the control (Cont), HFD, and HFD plus melatonin (MelHF) groups. Mice in the MelHF group received the HFD and melatonin-containing water for 2 weeks. Samples were collected every 4 h within 24 hours.	Female ICR mice, a melatonin-deficient strain.	Administration of exogenous melatonin improved the composition and diurnal rhythmicity of the gut microbiota in HFD-fed mice.	Yes, diurnal oscilation changed by constant darkness and by melatonin treatment.	*Firmicutes, Bacteroidetes, Parasutterella, Oscillibacter, Rikenella, Lachnoclostridium, Anaerotruncus, Lactobacillus, Alloprevotella, Parabacteroides, Ruminiclostridium, Alistipes* (cont). *Bacteroidetes, Oscillibacter, Rikenella, Lachnoclostridium, Lactobacillus, Lachnospiraceae, Intestinimonas, Ruminiclostridium* (HFD). *Firmicutes, Ruminococcacea, Anaerotruncus, Lactobacillus, Helicobacter, Lachnospiraceae, Ruminiclostridium, Roseburia* (MelHF).	[156]
2020	Mice were randomly grouped into 3 groups (n = 42): Control treatment, mice fed a low-fat diet and gavaged with saline; HFD treatment, mice fed an HFD and gavaged with saline; miRNA treatment, mice fed an HFD and gavaged with miRNA-10a-5p (100 pmol/mice per day). Samples were collected every 4 hours within 24 hours.	C57BL/6J mice.	The diurnal oscillations of three genera of microbes, Oscillospira, Ruminococcus and Lachnospiraceae, disrupted by HFD feeding, maintained by administration of miRNA-10a-5p, suggesting that miRNA-10a-5p may improve HFD-induced glucose intolerance and insulin resistance through the modulation of the diurnal rhythm of Lachnospiraceae and its metabolite butyrate.	Yes, diurnal oscilation disrupted by HFD and improved by MiRNA.	Diurnal oscillation restored for *Oscillospira, Ruminococcus, Lachnospiraceae.*	[157]
2020	Colonization in mice gut using microflora present in a freshly voided fecal sample from 6 healthy volunteers. Mice were randomly divided into 3 groups (15 mice per group), including the control group (CONT group), the constant darkness group (CD group), and the constant darkness with flavonoid group (CPF group).	Germ-free male C57BL/6J mice.	CPF significantly ameliorated the imbalance of intestinal microbiota by decreasing the F/B value, as well as alleviated the disrupted diurnal oscillation and phase shift of the specific intestinal microbes and liver clock genes expression induced by circadian rhythm disorder.	Yes.	*Bacteroidetes, Firmicutes and Proteobacteria* exhibited different amplitude or pattern in diurnal oscillations in different groups. The relative abundance of *Ruminococcaceae* showed a robust diurnal oscillation in the CONT group, but there were no obvious fluctuations in the CD group and the CPF group. The diurnal oscillations of *Lactobacillaceae* and *Lachnospiraceae* which belong to *Firmicutes* in the three groups were similar to those at the phylum level.	[158]

2021	The 20 mice from CON group were housed under light/dark cycle at 12 h (light-on at 7:00 a.m. and light-off at 7:00 p.m., everyday). The rest were housed under jetlag condition by changing light/dark cycle twice a week and dietary supplement ofpterostilbene (PSB) and resveratrol (RES) (0.25%) are given to jetlagged mice. Sampling every 6 hours for 24 hours.	C57BL/6 male mice.	Chronic jetlag led to gut microbial dysbiosis with increased abundance of diseases-related microorganisms at different time points. Supplementation of RES and PSB could not reverse the impact of chronic jetlag but still, partially inhibited some microbial growths and facilitated existence of health-beneficial microorganism.	Yes.	At the phylum level, CON group showed clear oscillation for *Firmicutes*. JLG, RES, and PSB showed obvious oscillation patterns in *Bacteriodetes*, *Firmicutes*, and *Proteobacteria*.	[159]

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- What are the exact mechanisms underlying the impact of changes in the diurnal oscillation of the gut microbiota on metabolic disorders, and can restoring these rhythms alleviate metabolic risks?
- In what ways does the gut microbiota participate in the communication pathways linking the circadian system with the central regulation of eating behavior, given that they both involve the hypothalamus as a primary regulator/orchestrator?
- How do lifestyle factors, such as sleep disturbances and changes in eating patterns, intersect with disruptions in circadian rhythms and the gut microbiota, amplifying metabolic risks?
- Do interventions aimed at the gut microbiota, such as pre-/probiotics, have the potential to effectively restore disrupted circadian rhythms and enhance metabolic health? Moreover, can these microbiota-focused interventions mitigate disturbances in eating behavior and thus further improve metabolic health?
- Can targeting the gut microbiota be a viable strategy for restoring disrupted circadian rhythms and enhancing metabolic health in clinical settings?"
- What are the enduring impacts of interventions directed at the gut microbiota on circadian rhythms, eating behavior, and metabolic health, and how do these interventions translate to broader human populations?

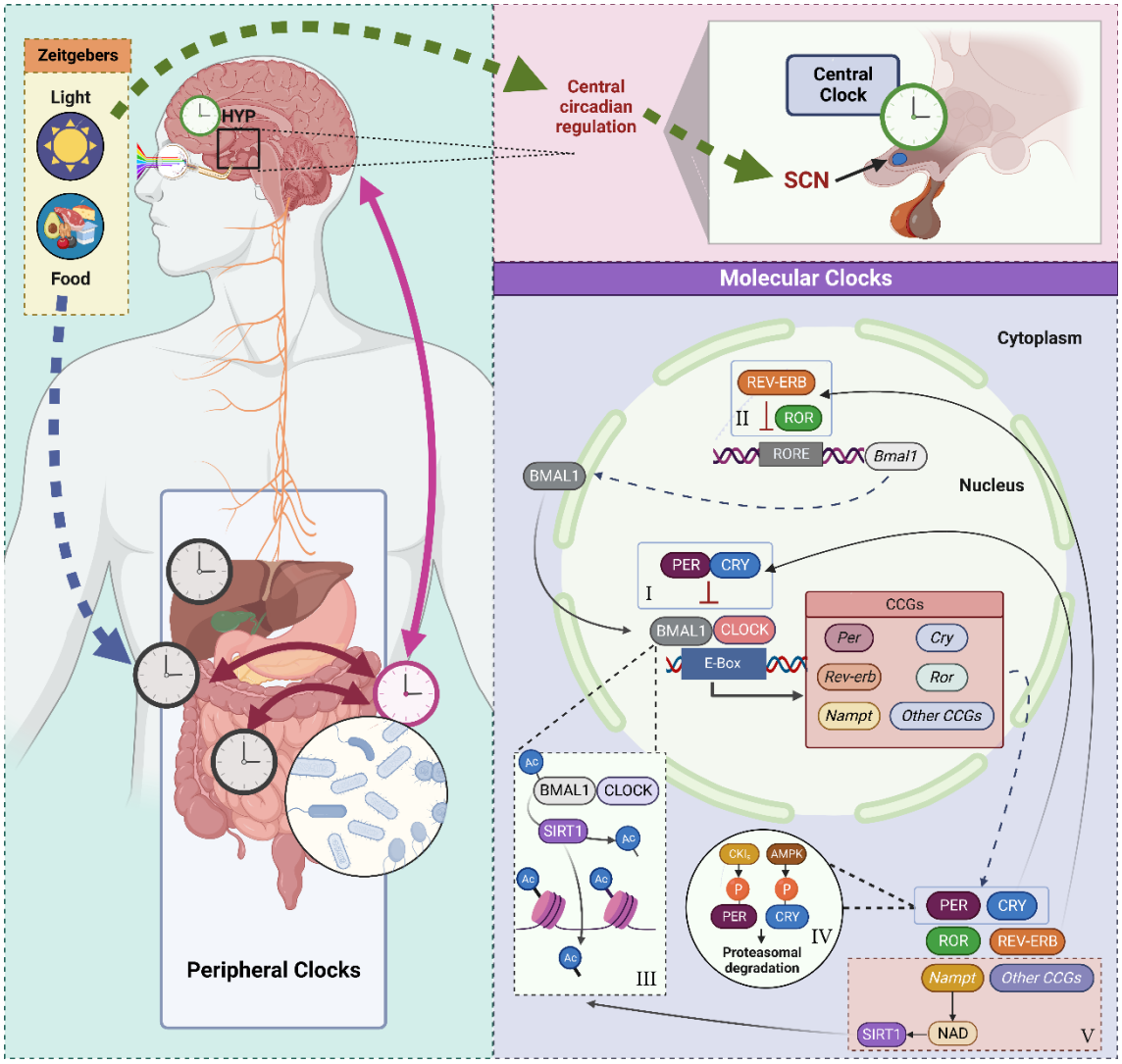

Figure 1 | Anatomical and molecular regulation of circadian rhythms, both centrally and peripherally. The master central clock of biological rhythms lies in the suprachiasmatic nucleus (SCN) of the hypothalamus (white box). The central clock (clock with green circle) can work autonomously, but it is tightly linked and coordinated with peripheral clocks (clocks with black circles) located in peripheral tissues, such as the liver, intestine, or gut, involving gut-brain axis communication (pink arrow). Light is the most important *zeitgeber* or external cue of the circadian system, which resets the master clock (green discontinuous arrow). Food constitutes a second critical external cue, mainly for the peripheral clocks (blue discontinuous arrow). Host biological rhythms and food also affect the gut microbiota (pink clock), which is, in turn, a potential modifier of the central and peripheral clocks. The molecular mechanisms underlying the regulation of both the central and peripheral clocks are the same (signalling cascade on the bottom right). The core of the clock are the transcription factors circadian locomotor output cycles kaput (CLOCK) and the brain and muscle Arnt-like protein-1 (BMAL1), upon binding to E-box (5'-CACGTG -3') and E-box-like promoter sequences, and their repressors period (PER) and chryptocrhome (CRY). The heterodimer CLOCK/BMAL1 activates the transcription of PER and CRY, which in turn repress the transcriptional activity of CLOCK/BMAL, establishing a negative feedback loop (Box I). Other feedback loops reset the transcriptional activity of the clock. One loop includes the nuclear receptors retinoic acid orphan receptor (ROR) and orphan nuclear hormone receptor reverse-erb alpha (REV-ERB), such that the expression of these two nuclear receptors is controlled by CLOCK/BMAL1 and, in turn, ROR and REV-ERB control the expression of BMAL1, activating or inhibiting it, respectively, upon binding ROR response element (RORE, 5'-AGGTCA-3') sequences (Box II). Moreover, metabolism controls the functionality of the molecular clock through the activity of sirtuin-1 (SIRT1) and adenosine monophosphate activated protein kinase (AMPK), which act as energy sensors for the cell. SIRT1 is a nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase that can deacetylate histones as well as BMAL1, which represses the transcription of CLOCK/BMAL1 (Box III). AMPK and casein kinase1ε (CK1ε), which is activated by AMPK, phosphorylate CRY and PER, respectively, inducing their degradation in the proteasome (Box IV). Because CLOCK/BMAL activates the transcription of clock-controlled genes (CCGs), the state of the molecular clock determines the daily rates of expression of these CCGs, and thus the metabolic and functional rates of the cells. Moreover, the molecular clock controls metabolism via indirect mechanisms, such as the expression of the rate-limiting enzyme of the NAD+ salvage pathway, nicotinamide phosphoribosyltransferase (NAMPT), which fluctuates throughout the day. Therefore, the circadian oscillation in the concentration of NAD+ marks the SIRT1

activity and the activation or inhibition (by acetylation/deacetylation) of transcription factors and nuclear receptors keys in metabolism regulation (Box V).

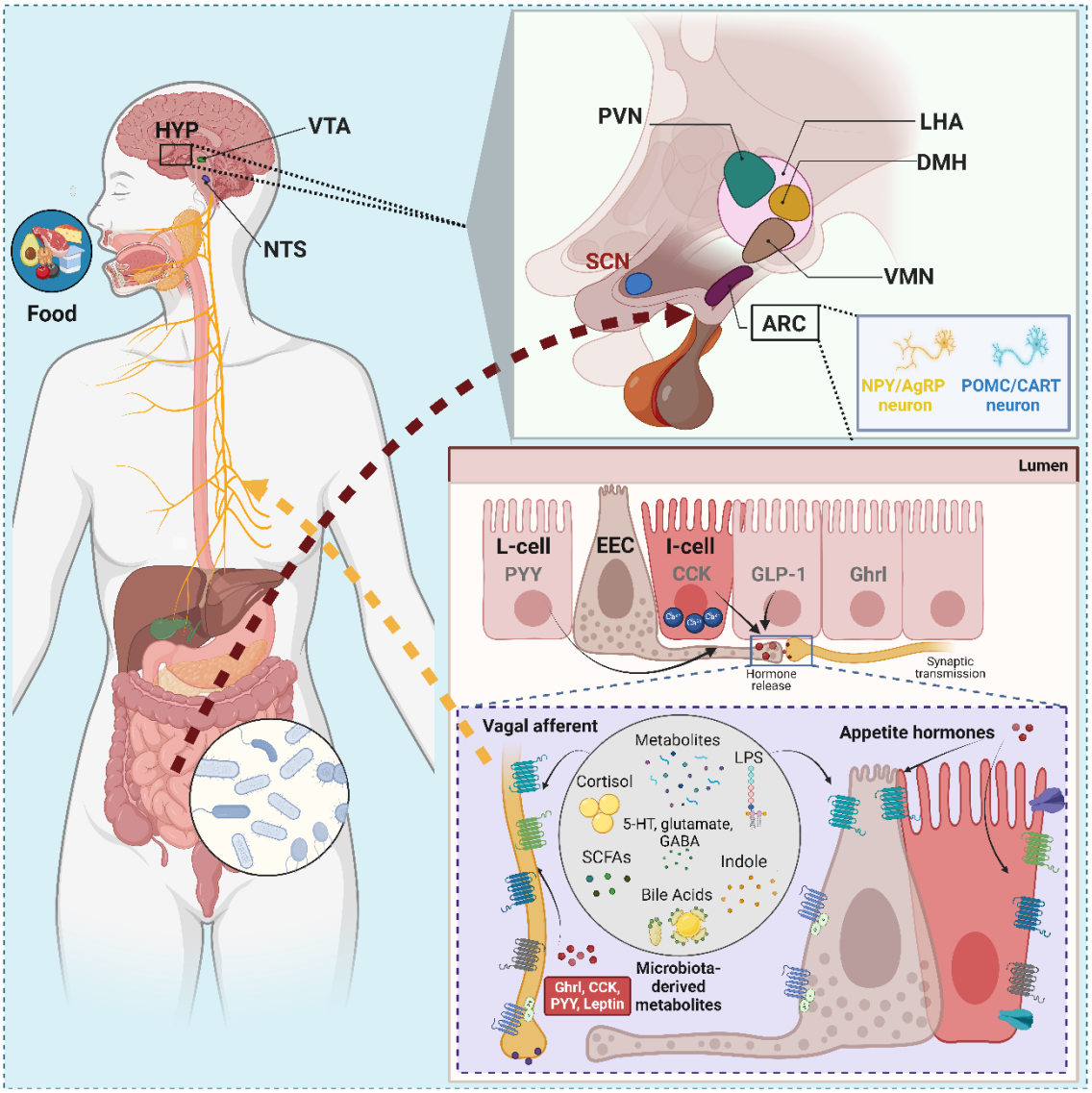

Figure 2 | Homeostatic regulation of eating behaviour. Nutrient receptors are located on enteroendocrine cells (EEC), which are the primary chemosensory cells in the gut. The gut microbiota metabolize nutrients and produce microbiota-derived metabolites, including short-chain fatty acids (SCFAs), bile acids, neurotransmitters and monoamines (serotonin (5-HT), glutamate, gamma-aminobutyric acid GABA) (grey circle). Microbiota-derived metabolites can activate receptors such as G protein-coupled receptor (GPCRs), ion channels or fatty acid receptors (FFARs) , which leads to the paracrine secretion of gut appetite hormones (including peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) by enteroendocrine L cells, cholecystokinin (CCK) by I cells in response to fatty acids and amino acids, and ghrelin (Ghrl) by X/A-like oxyntic gland cells or P/D1 in humans and EECs). Microbiota have also been shown to produce some of these gut hormones directly or gut hormone mimetics. Gut hormones display not only peripheral metabolic effects but can also modulation the central regulation of appetite, via the activation of vagal nerve afferents (in yellow). This is the main parasympathetic pathway for transmitting nutrient signals to the nucleus tractus solitarius (NTS) (blue circle, top left). Nutrient-related information is transmitted from the NTS to the hypothalamic arcuate nucleus (ARC), which is a critical node in the homeostatic regulation of food intake (white box). These signals can also inform other brain regions, such as the ventral tegmental area (VTA) (green circle, top left) which is a key region involved in the non-homeostatic regulation of feeding, mainly the food reward aspect, through dopaminergic signalling as major mediator. Enteroendocrine hormones released in the gut can also reach the hypothalamus via humoral pathways (i.e., via the circulatory system) (red discontinuous arrow). The ARC region consists of two distinct neuronal populations, the anorexigenic proopiomelanocortin anorexigenic (POMC)/cocaine- and amphetamine-regulated transcript (CART) and the orexigenic neuropeptide Y (NPY)/agouti-related peptide (AgRP) expressing neurons (blue box). Both POMC/CART and NPY/AgRP neuron populations project to other hypothalamic regions including the paraventricular nucleus (PVN), lateral hypothalamic area (LHA), ventral medial nucleus of the hypothalamus (VMN) and dorsomedial hypothalamic nucleus (DMH). The PVN, VMN and DMH are considered anorexigenic since they are stimulated by POMC/CART and inhibited by NPY/AgRP neurons. In contrast, the LHA

nucleus is related to stimulation of food intake and contains two subpopulations of neurons, one expressing orexin and a second expressing melanin concentrating hormone (MCH). Conversely, these are stimulated by NPY/AgRp and inhibited by POMC/CART neurons. The NTS also contains POMC neurons and, through monoamine neurotransmission, transmits sensory information to ascending brain areas, including the PVN and the dorsal vagal complex (DVC), which send efferent outputs involved in the vagal response. These processes aim to control feeding behaviour and whole-body energy homeostasis via efferent outputs.

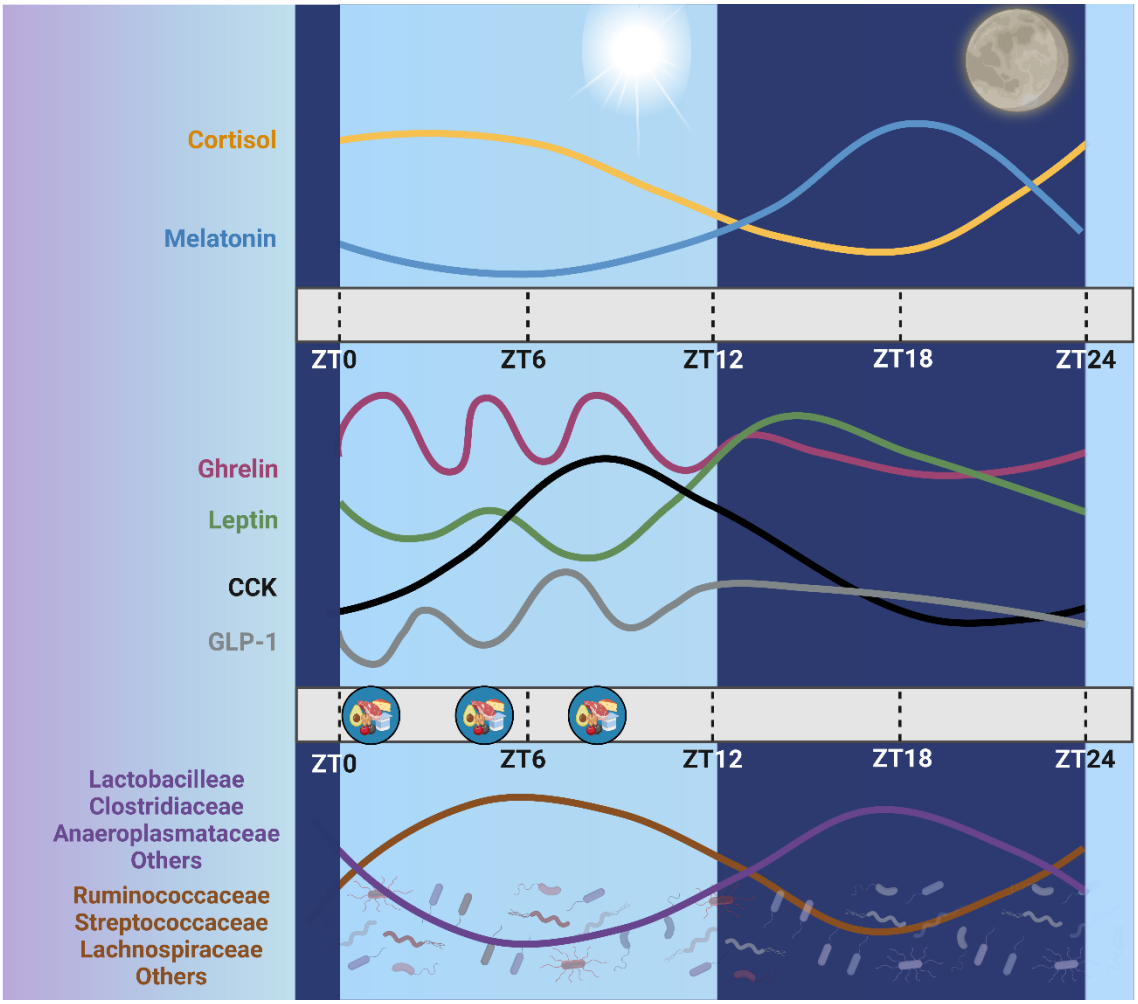

Figure 3 | Daily oscillations in healthy conditions of different hormones crucial for biological rhythms and food intake, as well as circadian variations of the gut microbiota. Melatonin (in blue) and cortisol (in yellow) are circulating humoral factors that fluctuate daily to coordinate and maintain the biological rhythms. The highest levels in blood of melatonin are in the middle of the night, declining towards dawn, and the maximum circulant cortisol levels are in the early morning. Serum cortisol levels decrease towards the afternoon. The serum levels of appetite hormones depend on fasting and feeding cycles and time. Plasmatic ghrelin levels (in pink) rise in pre-prandial period (in general around 7- 8 am, 11-1 pm, and 5-7 pm) and decrease in postprandial state (morning after breakfast time, 1-2 pm after lunch, and evening after dinner time). Moreover, smaller peaks have been shown around midnight [1]. On the contrary, circulating levels of leptin (in green) are higher during the night in humans, to permit fasting and nocturnal rest, than during the morning, which favours the food intake when the energetic demands are high. Gastric leptin levels present circadian rhythms in humans as well, also showing a peak/spike at night and a trough during the day. Specifically, the highest plasmatic levels of leptin in humans are between 10 pm and 3 am and the lowest between 8 am and 5-6 pm [2]. Plasmatic levels of cholecystokinin (CCK, in black) are low in the fasting states and increase after meals, remaining with high levels in postprandial conditions for several hours after the meal end. For instance, CCK plasmatic levels remain high up to 5-6 hours after lunch (around 1 pm) and after dinner (around 6-8 pm), with the most remarkable peak around 6 pm [3]. Glucagon-like peptide 1 (GLP-1, in grey) is an anorexigenic hormone and accordingly, its plasmatic levels peak at postprandial periods (9-10 am, 1-3 pm and 8-9 pm) with the highest levels after the main meal with less significant increases after dinner. The lowest concentrations are fasting times before breakfast, lunch, and dinner, owing to the lowest levels of nutrients in the stomach [4]. Gut microbiome also presents daily rhythmicity, including not only bacteria and other microorganisms but also their derived metabolites. For instance, certain families (such *as Lactobacilleae, Clostridiaceae, Anaeroplasmataceae*, among others) were shown to peak at active/light phase, while other families (including *Ruminococcaceae, Streptococcaceae, Lachnospiraceae,* among others) peak at the resting/dark phase [5]. It has been suggested that bacteria may anticipate and adapt to an environment with increased availability of nutrients, in order to use them for their growth. Approximate oscillations have been obtained from literature. The onset of light is defined as zeitgeber time 0 (ZT0).

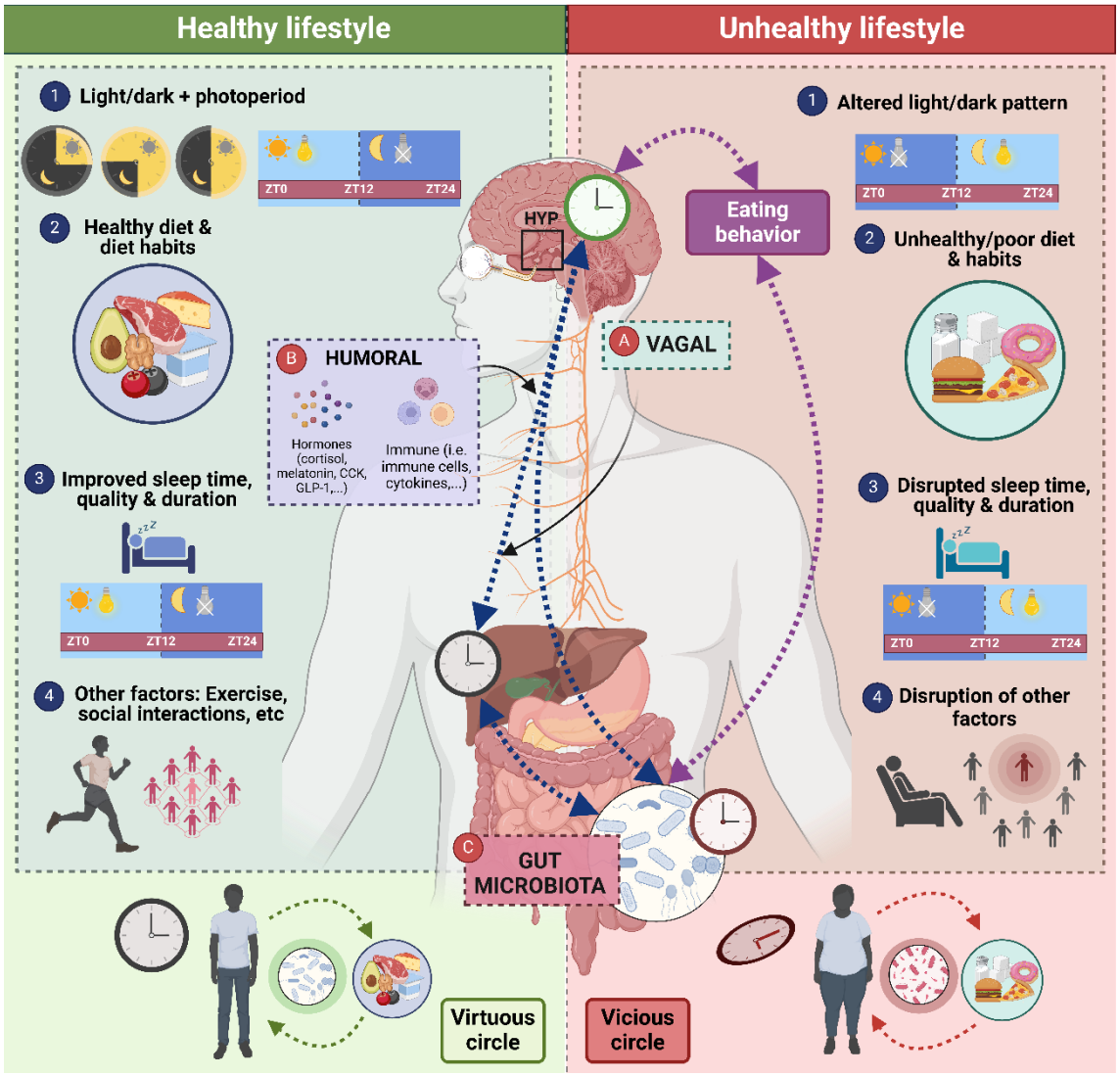

Figure 4, Key Figure | Links between the biological rhythms, eating behaviour and the gut microbiota and healthy or unhealthy lifestyle that impact their regulation. The central and peripheral clocks are bidirectionally connected through the vagal afferents of the parasympathetic nervous system (vagus) (A), and via circulating humoral factors (B) (including melatonin and cortisol), and the microbiota (C). (A) and (B) are also crucial mediators in the bidirectional communication between the gut microbiota (C) and the centra nervous system. Different external cues depending on our lifestyle (shown in numbers, 1-4) can reset the circadian system. Nevertheless, the physiological activities that central clock and peripheral clocks control trigger changes in feeding patterns and metabolism. The biological rhythms and the external cues also impact on the gut microbiota and their clock (pink clock), and the gut microbiota is in turn, a potential modifier of the central and peripheral clocks. The disruption of the circadian rhythms, enhanced for instance by obesogenic diets, sedentarism or accelerated lifestyle, factors impacting the gut microbiota and the regulation of eating behaviour as well, creating a vicious circle.

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