Review paper

Biological rhythms of the gut and microbiota

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Abstract

Numerous physiological processes occurring in the digestive system are subject to circadian rhythms, which are regulated by the endogenous biological clock. The motor activity of the small intestine, large intestine, and rectum operates in a 24-hour system, with significant differences between day and night periods. It is primarily correlated with the time of meals, hormone secretion rhythms, and other activities undertaken by the organism. In recent years, numerous scientific reports have emerged about the fundamental role of circadian rhythms in the proper functioning of the gut microbiota. In addition, the microbiota and its metabolites also influence the host's daily cycles, which affects the overall state of their organism. The aim of this review is to outline the mechanisms of action and interactions between biological rhythms, gut motility, and the functioning of the gut microbiota.

The overriding goal of the circadian rhythmic phenomenon is to optimize and maintain the homeostasis of the body. The endogenous biological clock drives many physiological processes in humans, including diurnal wake-sleep rhythms, hormone secretion, and metabolism [1]. Numerous activities in the digestive system show 24-hour repetition closely related to the systemic biological clock. The intestines, and the microbiota colonizing the intestines, in particular, are one of the organs most sensitive to circadian cycle fluctuations [2].

The small intestine motility is controlled by the migrating myoelectric complex (MMC), which consists of electric waves generated by the stomach and distributed along the intestinal smooth muscles inducing peristaltic contractions [3].

MMC undergoes strict hormonal regulation, with motilin and ghrelin, which are responsible for its generation. Gastrin, serotonin, and cholecystokinin are involved in the generation of slow electric waves that influence segmental motility of the smooth muscles of the duodenum, jejunum, and colon [3].

In the small intestine, the between-meal cycle, in which MMC participates, is divided into 4 key stages. The first MMC phase is a prolonged rest period (40–60% of the total time), the second phase shows the increased frequency of functional potentials and contractility of smooth muscles (20–30%), the third phase is the peak period of electric and mechanical activity of intestinal muscles, while the phase fourth involves activity decrease, transitioning smoothly to phase one [4]. The entire MMC cycle is consistent and operates on a 24-hour system, with individual periods of time showing significant differences between day and night. During the day phase II and III are longer and motor activity of the intestines is greater compared to the night. On the other hand, during sleep, the vast majority of MMC cycles involve a period of motor rest. The rhythmicity of MMC periods dependent on the diurnal cycle allows, e.g., facilitating defecation during waking hours and preventing it during sleep [5]. Other processes taking place in the small intestine, i.e. enzyme activity or self-renewal of intestinal epithelial cells, also show circadian rhythmicity, connected with periodic intake of food during 24 h [6].

Although MMC does not occur in the colon, its peristalsis also undergoes diurnal rhythms. Minimal motility occurs at night, with the peak occurring in the first hours after waking and after meals.

Literature data indicate that peristaltic movements of the large intestine are divided into 2 types of contractile activities: single or cyclic low-amplitude waves and high-amplitude waves related directly with formation and movement of relatively large faecal masses and defecation [7].

Rectum exhibits intermittent cyclic motor activity, which is referred to as periodic rectal motor activity (PRMA). In healthy individuals, PRMA is located primarily in the rectosigmoid region and follows clear circadian rhythms [8]. Studies suggest that PRMA most commonly occurs after motor events in the proximal colon, which may serve as an internal regulatory mechanism to prevent premature flow of colon contents [9]. Number, duration, and peak amplitude of anal peristaltic movements are significantly reduced at night, as opposed to the morning hours when its highest motor activity occurs [10].

Human microbiota is defined as all bacteria, parasites, viruses, fungi, and the least known so far: archaea, that inhabit the human body [11]. It is also often referred to as microbiome, which is not a mistake, but the term refers collectively to the microbial genome, the ecosystem, and the host environment. The estimated total number of bacteria in a model male (a person, aged 20–30 years, weighing 70 kg, 170 cm tall) is 3.8×10^{13}, and their biomass is estimated at about 0.2 kg [12]. The number of these microorganisms does not differ from the total number of cells in the human body, which is currently estimated at 3.0×10^{13}. Bacteria predominate other microorganisms by 2–3 orders of magnitude in the human microbiome, so the remaining ones do not significantly affect its total amount [13–15].

Human intestinal microflora is dominated by several basic types: *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia, Fusobacteria*, and *Tenericutes*, with the first 2 mentioned accounting for about 95% of the total microbiome [16].

Factors such as pH, oxygen gradient, and antimicrobial peptides have a significant impact on the presence of particular types of bacteria in the human body. Thus, development and multiplication are more favourable in the terminal portion of the alimentary tract, where the population of microorganisms exceeds all other organs by at least 2 orders of magnitude.

In the small intestine, bacteria from *Lactobacillaceae* and *Enterobacteriaceae* families are predominant, while in the colon, *Bacteroidaceae*, *Prevotellaceae*, *Rikenellaceae*, *Lachnospiraceae*, and *Ruminococcaceae* are observed in low numbers [17]. The intestinal microbiota plays many important functions in the human body, which include the following: influence on the communication of the brain-intestine axis, which is manifested in the host's cognitive abilities, playing a basic role in digestion and metabolism, protecting against pathogenic microorganisms by colonising gastrointestinal mucosa and producing various antimicrobial substances on it, which enhances the immune system, controlling proliferation and selection of epithelial cells, and forming/creating a biofilm, which is responsible for mucosa integrity as well as modifying insulin resistance and its secretion [18].

There are many more functions of the microbiome, and it is obvious that microflora has a crucial impact on the proper human homeostasis maintenance.

There are certain factors that have an important influence on the composition and functioning of the intestinal microbiome, including diet, age and genome of the host, type of labour/delivery, use of antibiotic therapy, and circadian rhythms, which is the main topic of this article [19].

For the purpose of this review, the use of the term microflora and its synonyms will be tantamount to the domain of bacteria because the role of the remaining microbiome microorganisms, i.e. parasites, fungi, viruses, and archaea, is so far less known in terms of biological rhythms. It has been known since the 1970s that circadian rhythms play a basic role in the functioning of the digestive system [20]. However, in recent years there have been an increasing number of scientific publications that prove the influence of day and night cycle also on the formation of microbiome oscillation in the human body [21]. It is worth noting that despite the important role of the host's circadian clock in maintaining diurnal rhythmicity of the intestinal microflora, it is not its direct determinant [19]. On the other hand, circadian fluctuations of the microbiome, and more precisely bi-products of microbial metabolism, i.e. short-chain fatty acids (SCFA) and lactate affect the modulation of peripheral clock cycle in the host *in vivo* [22]. Although the mechanisms of interactions of circadian rhythms of the microflora on the host organism and vice versa are still not fully understood, there is an inseparable bond between them.

It is absolutely certain that the coming years will bring new discoveries concerning the influence of microflora on functioning of the human body, and their practical application. However, we can already give some insight into the microscopic world. In light of recent studies on biological rhythms, some researchers have introduced the term "microbial oscillators" into the medical lexicon. It concerns primarily bacteria inhabiting the intestines, which show significant diurnal fluctuations in quantitative composition in the host organism and play the most important role in regulating circadian clock mechanisms of the host in the

entire microbiome. Oscillators in the darkness of the intestines do not directly perceive photic stimuli, and the food entering the gastrointestinal tract is for them the main environmental cue, which they convert into a signal [23]. Thus, they affect, directly or indirectly, the modulation of the circadian rhythm network and the host's metabolism with the help of putative mediators and their metabolites, which allows the host to obtain information on the time of a meal consumption and its quantitative and qualitative composition [24].

Certain taxonomic orders, namely *Clostridiales*, *Lactobacillales*, and *Bacteroidales,* which rhythmically oscillate in a 24-hour cycle, account for 60% of the microbiome composition, resulting in time-of-day-dependent changes in the configuration of the intestinal/gut microbiota. This, in turn, results in significant diurnal fluctuations in abundance in more than 15% of all bacteria that constitute operational taxonomic units [25].

Thaiss *et al.* found that in mice, with a light-dark rhythm conserved, intestinal microflora was concerned with energetic metabolism, DNA repair, and cell growth during the nocturnal period, while during the daytime – with detoxification, movement, and receiving signals from the environment. In another study, in rodents with properly functioning circadian clock and fed a normal diet, a reduction in microbial gene copies in the day was observed with their increase in the night, with the peak at the end of a 12-hour period of darkness [26].

These reports correlate with microscopic observations and quantitative PCR results, which showed more efficient colonisation of the intestinal epithelial layer by bacteria during the dark phase than during the light phase in mice [19]. It is worth mentioning at this point that different types of bacteria were more or less dominant depending on the time of the day and night. The average number of *Bacteroidetes* was higher at 11:00 p.m. (66%) and 11:00 a.m. (60%) and lower at other times while in case of *Firmicutes* it was higher at 3:00 (45%) and 7:00 a.m. (45%) and the lowest at 11:00 p.m. (29%) [27]. Metabolic products of microorganisms, such as SCFA – butyrate or acetate, also showed diurnal fluctuations [22].

Mechanisms that are responsible for the regulation of microbiota biological rhythms cannot be stated unequivocally, but the interaction of an apparatus dependent on and independent of the circadian clock of the host can be assumed with high probability. The biological rhythms of microorganisms mostly constitute 24-hour cycles and may depend on melatonin secretion and temperature fluctuations, i.e. the same factors responsible for the modulation of circadian rhythms in mammals [24]. Another study revealed that loss of basic genes of the biological clock (e.g. *Clock* gene mutation), while maintaining host behavioural rhythms, contributed to disorganisation of the diurnal pattern of the microbiome [25].

Another example can be the deletion of transcription activator gene *Bmal1* or transcription inhibitor genes *Per1* and *Per2,* which are the core of the molecular clock, where in both cases disorders of intestinal microflora oscillation occurred in mice [19, 27]. Nevertheless, it was possible to restore intestinal circadian rhythm in the mice mentioned above with mutation by correlating the time of the day with availability of a meal or its absence, which could indicate the dominant role of the food as a signal for 24-hour microbiota, regardless the core of the molecular clock of the host [28]. Such time-restricted feeding (TRF) is an aspect in the food signal control strong enough to prevent or even reverse metabolic imbalance in mice as well as in humans [28]. A context connected with food intake by the host is therefore important for the functioning of the circadian rhythms of the intestinal microbiome. Thaiss *et al.* showed that feeding time is the most important factor responsible for diurnal oscillations correlated with microflora distribution in the host's alimentary tract. They also proved that wild-type mice fed only in the dark or only in the light period were characterised by the phase reversal of cycles associated with microbial adhesion to the mucosa [19]. The above-mentioned determinant is connected with time-restricted feeding (TRF), which involves restriction of food intake in particular time intervals, e.g. 8–10 h/24 h for a meal followed by absolute fasting. One study explored the topic and revealed that TRF stimulated mRNA expression of *Sirt1*, *Bmal1*, and *Clock* genes and increased population of beneficial microorganisms (e.g. *Prevotellaceae*, *Prevotella*, *Bacteroidia*) [29]. The results of this study revealed positive metabolic changes such as a decrease in total cholesterol, triglycerides, liver enzymes levels, and fatty acid synthesis accompanied by increased serum concentration of HDL. This evidence will be of great use in future intervention strategies related to prevention or treatment of metabolic diseases.

However, putting the topic of host food consumption in a different perspective, one study showed that in response to a high-fat (HF) diet, mice experienced a diurnal change in microflora configuration that caused reprogramming of the liver circadian clock, thereby contributing to the development of obesity, impaired glucose tolerance, increased insulin resistance, and impaired lipid metabolism [26]. In another study, the above result was replicated in microbiota-deprived mice that received transfer of intestinal flora from mice fed a fat-rich diet, which confirmed the influence of microorganisms on regulation of hepatic lipid metabolism in response to

HF, mainly by activating the host circadian clock related to PPARγ signalling pathway [24]. Production of intestinal microbiome metabolites also exhibits circadian rhythmicity, which in turn affects the 24-hour clock of the host [19, 26]. In a widely cited study, it was shown that certain metabolites produced by microorganisms, e.g. ornithine or proline, did not show diurnal oscillations of their concentrations in the serum of mice that were either devoid of microbiota or treated with antibiotics [19]. As in the previous paragraph, it was also shown that a high-fat diet (HF) adversely affected diurnal oscillation of metabolite metabolism produced by microflora. It suppressed butyrate production, which was caused by bacteria from the *Lachnospiraceae* family, compared to mice fed a low-fat diet [26]. Leone *et al.* observed that the exposure to butyrate and, to a lesser extent, to acetate of microflora-free mice *in vivo* and hepatocytes obtained from rodents free of specific microorganisms *in vitro*, resulted in significant changes in diurnal oscillations of major circadian clock genes, while enhancing their expression amplitude (*Per2* and *Arntl*). Moreover, the authors stated that administration of sodium hydrosulphide (NaHS), which increased H_2S production by microbiota, attenuated 24-hour fluctuations of *Per2* gene [26]. Other scientific studies have shown that bioactive SCFA, polyphenolic metabolites, vitamins, and bioamines of intestinal microflora are responsible for the modulation of host circadian clocks. Thus, a conclusion was drawn in favour of the supplementation of prebiotic fibre, which would hypothetically alleviate jet lag-induced disorders and resynchronize circadian rhythms [22].

Abnormalities in the microbiome as well as any disturbances in the host's circadian rhythms are associated with numerous diseases, such as obesity, diabetes, ulcerative colitis, cancer, or Alzheimer's disease [25]. In case of Alzheimer's disease, it is suggested that long-term sleep deprivation as well as disturbances of the human circadian cycle mediate chronic intestinal microbiota dysbiosis by altering dietary habits, lifestyle, and host metabolism, which contributes to its onset and progress [30]. The topic of interdependence between microflora and disease is too broad and beyond the scope of this article. Thus, it is worth reading about the newest findings concerning the issue.

Although in recent years there have been an increasing number of reports on the circadian rhythm-dependent mutual influence of microbiome and host's organism, we do not know much about the mechanisms responsible for regulating particular processes.

There is a need for further observation and analysis of the functioning of the human microflora to better identify the factors affecting them and incorporate appropriate therapeutic strategies into clinical practice.

Conflict of interest

The authors declare no conflict of interest.

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