

Feature Review

Circadian Influences of Diet on the Microbiome and Immunity

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Host circadian rhythmicity and the timing of feeding are increasingly recognized to cross-regulate and entrain each other, and may play crucial roles in regulating multiple physiological functions including host immunity and metabolic health. Of relevance, these circadian diet-immune interactions may be modulated by the gut microbiota. We review current knowledge linking the circadian clock and dietary timing to host immune-microbiota interactions, exemplifying how this axis may impact on host immunity in health and in a variety of immune-mediated diseases. We also discuss current challenges in reaching mechanistic insights regarding the functions of the diurnally shifting diet-microbiome-host immune axis. We highlight the possible implications of circadian reprogramming by dietary timing patterns as a future intervention to modulate a variety of immune-related diseases.

Dietary Timing and Metabolism

The **circadian rhythm** (see [Glossary](#)), which characterizes multiple biological processes in living organisms, is a cycle that occurs with a periodicity of ~24 h. These rhythms are mostly governed by the circadian clock, an endogenous molecular machinery that is present in all cells and consists of a highly ordered and regulated network of genes and proteins, that can also be modulated by extrinsic factors including light, temperature, and diet [1–3]. The master clock, the **suprachiasmatic nucleus (SCN)** located in the mammalian central nervous system (CNS), aligns and controls the self-sustained and independent peripheral clocks in every tissue and organ [4]. It is well known that circadian rhythms coordinate a wide variety of important physiological processes including the sleep/wake cycle, body temperature, hormone release, and feeding/fasting patterns [5].

Many nutritional studies have focused on the effect of dietary content (e.g., high-fat diet) and amount (e.g., **calorie restriction, CR**) on host physiology, mainly metabolism [6]. In recent years the concept of dietary timing and its circadian influences is gaining increasing attention [7]. A broad range of diets based on alternating cycles of feeding/fasting are determined by the duration of fasting and the meal frequency. These include but are not limited to ultradian diet with multiple meals at regular intervals (e.g., food access every 4 h) [8], and **time-restricted feeding (TRF)** where daily meal consumption is restrained to a 4–12 h window without CR [9]. Moreover, the **infradian** diet is another eating pattern in which few or no calories are ingested for periods of time ranging from one to a few days, including **alternate-day fasting (ADF)**, periodic fasting (PF), and **fasting-mimicking diets (FMDs)** [9]. In addition to the fact that feeding behavior is partly controlled by the circadian clock, changes in feeding timeline can impact on the host circadian rhythm [10].

One recently highlighted factor that plays a pivotal role in regulating dietary timing–metabolic interactions is the gut **microbiome**, that is composed of a wide variety of microorganisms which extensively react to ingested dietary compounds, and in turn intimately interact with and signal to the host. Hence, it is imperative to investigate how feeding patterns signal to the gut

Highlights

Circadian clocks and daily feeding/fasting cycles are intimately connected. Circadian rhythms coordinate feeding patterns, and feeding/fasting cycles in turn have dramatic influences on circadian clocks.

The gut microbiome undergoes diurnal fluctuations in both composition and function, which are driven by feeding rhythms under the influence of host circadian clocks and diet. The gut microbiome also actively orchestrates host diurnal rhythms in the gut and remote tissues (such as the liver) by incorporating various signals from nutrients, hormones, and innate immune sensing.

Circadian clocks and host immunity are tightly interconnected. Both innate and adaptive immune responses, including immune cell oscillations and trafficking, are greatly influenced by various fasting/feeding patterns.

The role of timed food intake has been implicated in a wide variety of immune-related diseases, highlighting the promising translation potential of 'chrononutrition' in disease management.

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microbiome, how these interactions modulate downstream host physiology, and how the intestinal and other peripheral circadian clocks integrate with the microbiota in orchestrating diurnal function in response to fasting/feeding cycles.

Timed dietary regimens have been mostly studied in the context of metabolism, where they were suggested to prevent obesity and improve many other metabolic phenotypes ([11], reviewed in [12]). In addition, scheduled feeding timing was suggested to exert significant effects on human longevity, with potential to prolong lifespan [13]. The potential impact of diurnal dietary changes, and of timed feeding on host immunity, is only beginning to be unraveled. In this review we highlight recent findings regarding the interplay between feeding timing, circadian rhythmicity, gut microbiota, and the immune system. We also examine the putative implications of **chrononutrition** in a variety of immune-related diseases.

Interplay Between Dietary Timing and the Circadian Clock

The daily feeding/fasting cycle is under the influence of circadian clocks, including the light-entrainable master clock in the SCN and secondary brain clocks which are phase-controlled by the SCN [2, 14–16]. In addition, animals feature rhythmic behaviors to anticipate the time of food availability, which is driven by so far unclarified mechanisms [14, 17]. Mice deficient for the clock gene *Per2* [18] or *Reverba* (also known as *Nr1d1*; encoding Rev-Erbc) [19] exhibit impaired diurnal feeding rhythms, highlighting the importance of circadian clocks in orchestrating the timing of food consumption. Notably, mistimed food intake secondary to circadian irregularity has been linked to energy imbalance and the development of cardiometabolic disease in both rodents and humans [20–22]. In humans, consumption of food during the circadian evening and/or at night, independent of dietary amount or content, was found to be significantly associated with variations in body fat and body mass index [23].

Feeding times are essential, in turn, in synchronizing and entraining peripheral clocks. Temporally restricted feeding under light/dark or dark/dark cycles can change the phase of circadian gene expression in peripheral tissues, independently of the SCN and the light cycle [10, 24]. The liver is thought to be the first target of food-induced phase resetting. Both food availability and the time of feeding govern and orchestrate the diurnal liver transcriptome [25], acetylome [26], and the oscillations of hepatic triglyceride levels in mice [27]. Mechanistically, the activity of hepatic PARP-1, an ADP-ribosyltransferase, fluctuates in a daily manner in mice which can be regulated by feeding, and essentially connects the feeding pattern with the mammalian circadian system [28]. In addition to the liver, the murine gut contains functional clock genes (such as *Clock*, *Bmal1*, *Per1-3*, *Cry1-2*) which can be synchronized by timed feeding, and might be crucial in the circadian regulation of gastrointestinal functions, including cell proliferation, motility, migration, and absorption [29–32]. Mistimed feeding also impairs the peripheral clocks in skeletal muscles and brown adipose tissue to different extents in rats [33]. Similarly, TRF shifts the phase and changes the amplitude of the skin circadian clock, and greatly affects the skin transcriptome in mice [34]. Unlike secondary clocks, which harbor a **food-responsive oscillator**, the central SCN clock is almost unaffected by food timing under normal conditions [10]. Nevertheless, food-related cues may synchronize the SCN clock because SCN neurons are sensitive to metabolic cues (e.g., hormones) [35, 36]. For instance, some studies show that the central clock undergoes food-adaptation in the absence of photic inputs [37, 38] and under CR [39]. The SCN communicates with the peripheral clocks in a bidirectional manner through hormonal, neuronal, and metabolic signals [40]. For example, **ketone bodies** generated by the liver during CR can stimulate the SCN clock [41, 42]. In forebrain/SCN-specific *Bmal1* knockout mice, the lost circadian rhythms in peripheral tissues can be partially rescued by restricted feeding (only in the liver and kidney) [43]. As a consequence, desynchronization between the peripheral clocks and the

Glossary

Active phase: period of the day when an animal is usually active and eating (dark period of the light/dark cycle in mice).

Alternate day fasting (ADF): alternating 24 h fasting or extremely low caloric intake (25% of energy needs) with a 24 h *ad libitum* period of eating.

AMP-activated protein kinase (AMPK): a cellular metabolic sensor that is generally accepted to be the target of metformin modulation of hepatic gluconeogenesis.

Bone marrow (BM): primary lymphoid organ that supports the self-renewal and differentiation of hematopoietic stem cells (HSCs) into mature blood cells.

Calorie restriction (CR): daily reduction of caloric intake without malnutrition.

Chrononutrition: approach to coordinating the daily rhythms of the body with the timing of food intake.

Circadian rhythm: biological rhythm with ~24 h periodicity that persists in constant conditions.

Entrainment: phenomenon of synchronization of internal biological rhythms by external cues.

Experimental autoimmune encephalomyelitis (EAE): animal model for human CNS inflammation, including MS. The neurologic damage phenotype generates the activation of myelin-specific T cells that cross the brain–blood barrier and migrate into the CNS where they are activated by antigen-presenting cells. The inflammation leads to oligodendrocyte death, demyelination, and axonal damage.

Fasting-mimicking diet (FMD): type of modified fasting in which the dietary composition of macronutrients and micronutrients is specifically formulated to trigger responses such as reduced glucose and insulin-like growth factor 1 (IGF-1) concentrations, as well as increased ketone bodies, while maximizing caloric intake.

Fecal microbiome transfer (FMT): procedure in which the fecal microbiome from a healthy donor is transferred into the gastrointestinal tract of a recipient for treatment purposes.

Food-responsive oscillator: putative circadian pacemaker that can be entrained by a schedule of food restriction but not by a light/dark cycle.

Glucocorticoids (GCs): a class of steroid hormones that are secreted

central pacemaker by disrupted feeding-time leads to the development of metabolic disorders such as obesity, diabetes, and dyslipidemia [44].

Role of the Gut Microbiome in Dietary Timing–Circadian Interplay

The mammalian intestinal microbiota is composed of trillions of microorganisms of different kingdoms that are increasingly suggested to impact on host physiology in health and in disease [45]. In addition, the gut microbiota plays a modulating role in the crosstalk between dietary timing and host circadian rhythms. The murine gut microbiota presents diurnal compositional and functional fluctuations in a time-of-day-specific manner (Figure 1). Normal chow *ad libitum*-fed mice exhibit cyclical oscillations in cecal microbiome at the phylum level. *Firmicutes* species reach the highest abundance during nocturnal feeding and lowest abundance over daytime fasting; by contrast, *Bacteroidetes* and *Verrucomicrobia* show antiphasic trends [46]. The nature of the oscillating strains may differ between vivaria [47]. The functionality of the intestinal microbiota also features diurnal rhythms. For instance, functional pathways related to energy metabolism, DNA repair, and cell growth peak in dark phase, whereas detoxification, motility, and environmental sensing pathways peak in light phase [47]. In mice, the biogeographical aspects of colonic mucosa-associated commensal microbiome also undergo daily diurnal rhythmicity, namely *Mucispirillum schaedleri*, *Lactobacillus reuteri*, and *Bacteroides acidifaciens* [48]. In humans, the composition

mainly by the adrenal gland in a diurnal manner; can synchronize tissue rhythmicity and modulate the immune system.

Group 3 innate lymphoid cells

(ILC3s): characterized by the expression of transcription factor ROR γ t and the production of IL-17 and IL-22. ILC3s are involved in host defense against pathogenic microbes and in maintaining gut homeostasis.

Gut-associated lymphoid tissue

(GALT): composed of cells residing in the gut lamina propria, intraepithelial lymphocytes interspersed between epithelial cells, and immune cells located in organized lymphatic tissue (Peyer's patches and mesenteric lymph nodes).

Immune cell trafficking:

localization and redistribution of immune cells at distinct differentiation states into other anatomical sites throughout the body.

Inflammatory bowel diseases

(IBDs): chronic and relapsing inflammatory disorders in the gastrointestinal tract, mainly including ulcerative colitis (UC) and Crohn's disease (CD).

Infradian: rhythms with a period longer than 24 h (e.g., days, weeks, and months).

Innate lymphoid cells (ILCs): a subset of immune cells with constitutive presence and activity at the body's barrier surfaces of the body; they ensure the maintenance of tissue homeostasis and immune protection.

Intermittent fasting (IF): also known as intermittent energy restriction, an umbrella term for various meal-timing schedules that cycle between voluntary fasting (or reduced calorie intake) and nonfasting over a given period.

Intestinal epithelial cells (IECs): a monolayer of cells organized into villi and crypts in the intestine that have rapid self-renewing properties and play important roles in nutrient absorption, microbial interactions, and maintenance of tissue homeostasis.

Ketogenic diet: high-fat, adequate-protein, and low-carbohydrate diet.

Ketone bodies: a group of water-soluble molecules produced in the liver from fatty acids under circumstances of food deprivation, including fasting, starvation, and restricted feeding.

Microbial dysbiosis: dysregulated or impaired microbial composition associated with gain of normally outcompeted species or loss of normally dominating species, or alterations in the abundance of microbial species.

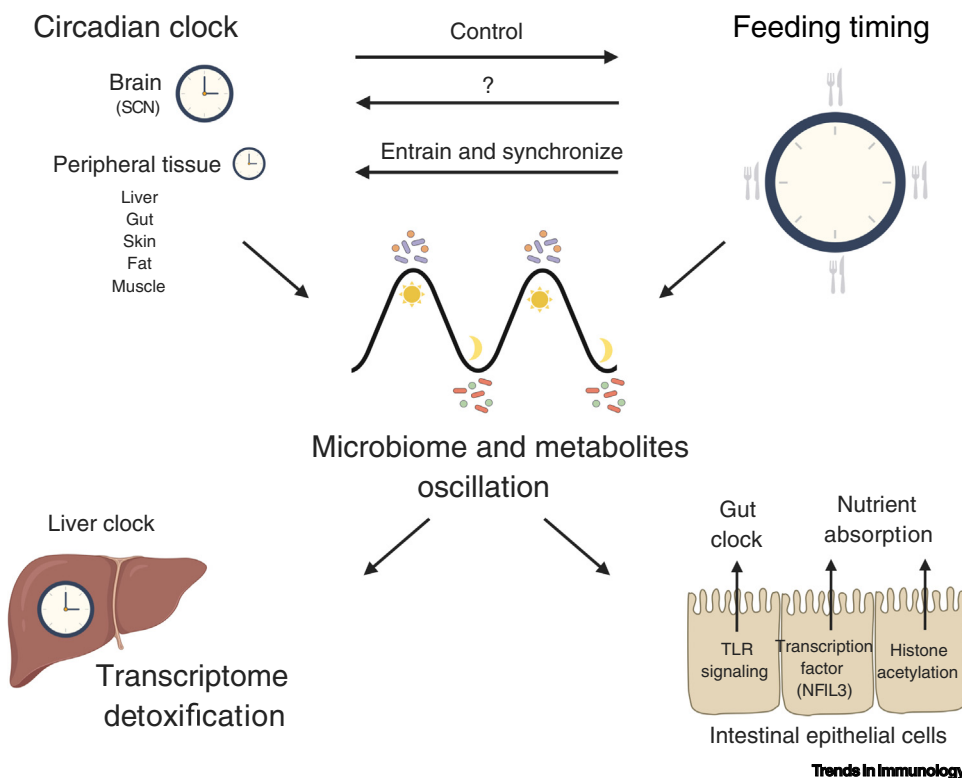


Figure 1. Interplay among Circadian Rhythms, Timing of Food Intake, and the Gut Microbiome in Mammals.

Circadian clocks, mainly the suprachiasmatic nucleus (SCN) in the brain, control the daily feeding/fasting cycles. The timing of feeding, in turn, entrains and synchronizes peripheral clocks, including those in the liver, intestine, skin, fat, and muscle. Under the influence of both circadian rhythm and feeding, the gut microbiome undergoes diurnal fluctuations in its composition, function, and metabolite production. The microbiome and its associated metabolites play essential roles in regulating circadian clocks in the liver and intestinal epithelial cells, as well as in modulating nutrient uptake and intestinal defense against pathogens. Abbreviations: NFL3, nuclear factor, interleukin 3 regulated; TLR, Toll-like receptor. Figure created with BioRender ([biorender.com](https://www.biorender.com)).

and function of the gastrointestinal microbiota also vary throughout the day, and this is associated with eating behaviors, including eating frequency and overnight-fast duration [47,49].

The highly ordered diurnal microbiota fluctuations are directly driven by feeding rhythm, orchestrated by either a host functional circadian clock or diet. Mice with deficiency in host molecular clock components (e.g., *Per1-2*), those exposed to an experimental 'jet lag' model (by an 8 h timeshift every 3 days for 4 weeks), or mice fed a high-fat diet, all exhibit greatly attenuated daily feeding/fasting rhythmicity relative to controls, and this consequently dampens the cycling oscillations in microbial composition and function [46,47]. TRF can partially but significantly rescue these cycling oscillations in mice; this is interesting because it highlights the flexible nature of microbiota rhythmicity, which can be impaired or restored by altered feeding behaviors [47]. Noteworthy, mice treated in an every-other-day fasting mode feature an improvement in **white adipose browning** and high-fat-diet-induced obesity by reshaping the composition of the gut microbiota [50] and its fermentation capacity [50].

Recent studies are beginning to reveal that the gut microbiome is not merely a passive player involved in the dietary timing–circadian interplay, but actively orchestrates host diurnal rhythms through various mechanisms (Figure 1). Diurnal fluctuations in intestinal microbiota biogeography and metabolite production in mice drive and program circadian epigenetic and transcriptional landscape of host tissues at both local and distant sites such as the liver [48]. Compared with conventionally raised mice, germ-free mice exhibit significantly impaired central and hepatic circadian clock gene expression profiles regardless of dietary challenges; this highlights the importance of gut microbiota in mediating diet-induced alterations of circadian clock function [51]. Under low- or high-fat diet, specific microbial metabolites, particularly short-chain fatty acids, directly regulate circadian clock gene expression within hepatocytes [51]. In murine **intestinal epithelial cells (IECs)**, the microbiota-associated molecular patterns and their interactions with **Toll-like receptors (TLRs)** are crucial for homeostatic maintenance of the gut circadian clock [52]. Microbiota ablation through antibiotic treatment or using germ-free mice results in overproduction of **glucocorticoids (GCs)** in IECs, and markedly affects the expression of circadian clock-related nuclear receptors, for example, decreasing the activator *Rora* and increasing the repressor *Nr1d1* (encoding Rev-Erb α) [52]. In fact, germ-free mice also exhibit elevated concentrations of adrenal GCs upon signaling via the hypothalamus–pituitary–adrenal axis relative to controls [53]. In addition, GCs are known as potent regulators of peripheral clocks [54]. It remains unknown, but it may be intriguing to unravel, the extent to which GCs are needed for these microbiome-induced changes in the circadian clock and, furthermore, which types of GCs are needed.

Of note, a recent study reported that the gut microbiota induces diurnal oscillations of the circadian transcription factor NFIL3 (nuclear factor interleukin 3 regulated) in IECs through Rev-Erb α . The microbiome-dependent diurnal expression of NFIL3 requires the dendritic cell–**group 3 innate lymphoid cell (ILC3)** signaling relay, as well as the transcription factor STAT3 (signal transducer and activator of transcription 3), because mice deficient in *Id2* (lacking all ILC subsets) or *Stat3* exhibit decreased expression of NFIL3 in IECs [55]. Indeed, ILCs comprise various innate immune subsets that play key roles in barrier defense by sensing extrinsic (e.g., pathogen) and intrinsic signals (host-derived signals such as neuropeptides). NFIL3 was thus shown to be mechanistically linked to the microbiota–IEC circadian clock crosstalk, subsequently regulating lipid uptake and storage, because mice with specific *Nfil3* deletion in IECs are less susceptible to high-fat diet-induced obesity compared with their wild-type littermates [55]. In addition, the gut microbiota controls rhythmic histone acetylation in IECs through HDAC3, whereas germ-free mice lack the oscillations of histone acetylation, and have lower expression of HDAC3

Microbiome: the spectrum of bacteria, viruses, fungi, and parasites that live inside or on the body.

Multiple sclerosis (MS): inflammatory demyelinating disease of the CNS characterized by varying degrees of axon and neuron injury that are presumably triggered by autoimmune mechanisms.

Natural cytotoxicity receptor positive (NCR): a group of activating natural killer (NK) cell receptors expressed in mammals that can recognize a wide array of bacteria-, virus-, parasite-, and tumor-derived ligands and modulate the cytotoxic functions of tissue NK cells.

Peroxisome proliferator-activated receptor α (PPAR α): ligand-activated transcription factor that regulates the expression of genes involved in fatty acid β -oxidation. PPAR α is a major regulator of energy homeostasis.

Peyer's patches (PPs): secondary lymphoid organs in the small intestine. PPs are constantly exposed to an enormous diversity of microbiome- and food-derived antigens (including pathogens).

Ramadan fasting: fasting from sunrise to sunset during the holy month of Ramadan, in which the most common dietary practice is to consume one large meal after sunset and one lighter meal before dawn. The feast and fast periods of Ramadan are ~12 h in length.

Rest phase: period of the day when an animal is usually sleeping and fasting (light period of the light/dark cycle in mice).

Secondary lymphoid organs (SLOs): organs containing B and T cells that initiate and organize adaptive immunity; these include spleen, lymph nodes, and GALT.

Sphingosine-1-phosphate (S1P): a bioactive lipid involved in lymphocyte egress through interactions between S1P and its receptor S1P1 that is expressed by naive T and B cells. The extracellular gradient of S1P concentrations mediates lymphocyte trafficking from lymphoid organs across lymphatic endothelial barriers.

Suprachiasmatic nucleus (SCN): small group of nerve cells lying in the ventral hypothalamus that possess properties of a circadian pacemaker.

T helper type 17 (TH17) cells: subpopulation of proinflammatory CD4⁺ T cells that are essential in host defense against extracellular pathogens.

transcripts and protein compared with conventionalized mice. The HDAC3 in IEC in turn regulates rhythmic expression of the lipid transporter gene *Cd36*, and modulates rhythmic nutrient uptake and lipid absorption in mice, whereas mice deficient in HDAC3 show lower lipid uptake in the intestine and are more resistant to obesity induced by high-fat diet [56]. The precise microbiota inputs that impact on these circadian immune aspects certainly merit further investigation.

Effects of Diet–Circadian Interactions on Host Immunity and Inflammation

The Circadian Clock and the Immune System Are Tightly Cross-Regulated

The immune system evolves to protect the organism from diseases. Most immune cells demonstrate circadian rhythms and harbor a molecular clock machinery [57–60]. Oscillation in the immune system may contribute to immune homeostasis by enabling temporal separation of (i) antagonistic pathways (e.g., tolerance and immunity), (ii) synergistic pathways that can cause damage (e.g., immune overactivation), and (iii) energetically inefficient pathways (i.e., constant immune activation is redundant and energetically expensive) [61–63]. The role of basal rhythmicity in the immune system is reflected in human diseases by time-dependent variations in the susceptibility, severity, and duration of immune responses [62]. Immune circadian rhythms are generated by both the SCN and the intrinsic clock present within immune cells. Globally, circadian information is transmitted from SCN to tissues and their residing immune cells by a variety of neural and endocrine signals [64]. The rhythmic secretion of such hormones (e.g., GCs) and neurotransmitters (e.g., noradrenaline) modulates the activation and functions of both innate and adaptive immunity in different cell types [65]. At the cellular level, a variety of immune cell subsets are regulated by circadian transcription factors. For example, **T helper type 17 (TH17) cells** and **natural cytotoxicity receptor positive (NCR⁺) ILC3s** are regulated by the core clock transcription factor *Rev-Erba* in mice [66,67]. Genes that are diurnally involved in immune function and trafficking regulation include those encoding chemokine receptors (e.g., CXCR4), adhesion molecules (e.g., ICAM-1) [68–70], pattern recognition receptors (e.g., TLRs, NODs, and NLRs [52,71–74]), cytokines [e.g., interleukin (IL)-6 [59,75–77]] and antimicrobial peptides (e.g., lipocalin-2 and cryptidins [78–80]). These collectively orchestrate the ability of innate and adaptive immune cells to protect from harmful substances by migration, recognition, and via spatially and temporally regulated activity. The potential benefits of circadian variations in the immune system are discussed in [Box 1](#).

Circadian Feeding Cycles Influence Immune Function

Eating during the **rest phase** is associated with an alteration of both innate and adaptive immune responses ([Figure 2](#)). For instance, day-fed mice exhibit a reduction in bacterial killing and serum proinflammatory cytokine (e.g., tumor necrosis factor α , TNF- α) production in response to bacterial endotoxin [81,82], have impaired worm expulsion when infected with *Trichomonas muris* [83],

Time-restricted feeding (TRF): form of fasting that limits eating to a specified number of hours (usually between 6 h and 12 h) each day, with or without caloric intake reduction.

Toll-like receptors (TLRs): a group of pathogen recognition receptors that are involved in innate immunity; they recognize structurally conserved molecules derived from bacterial, viral, and fungal pathogens.

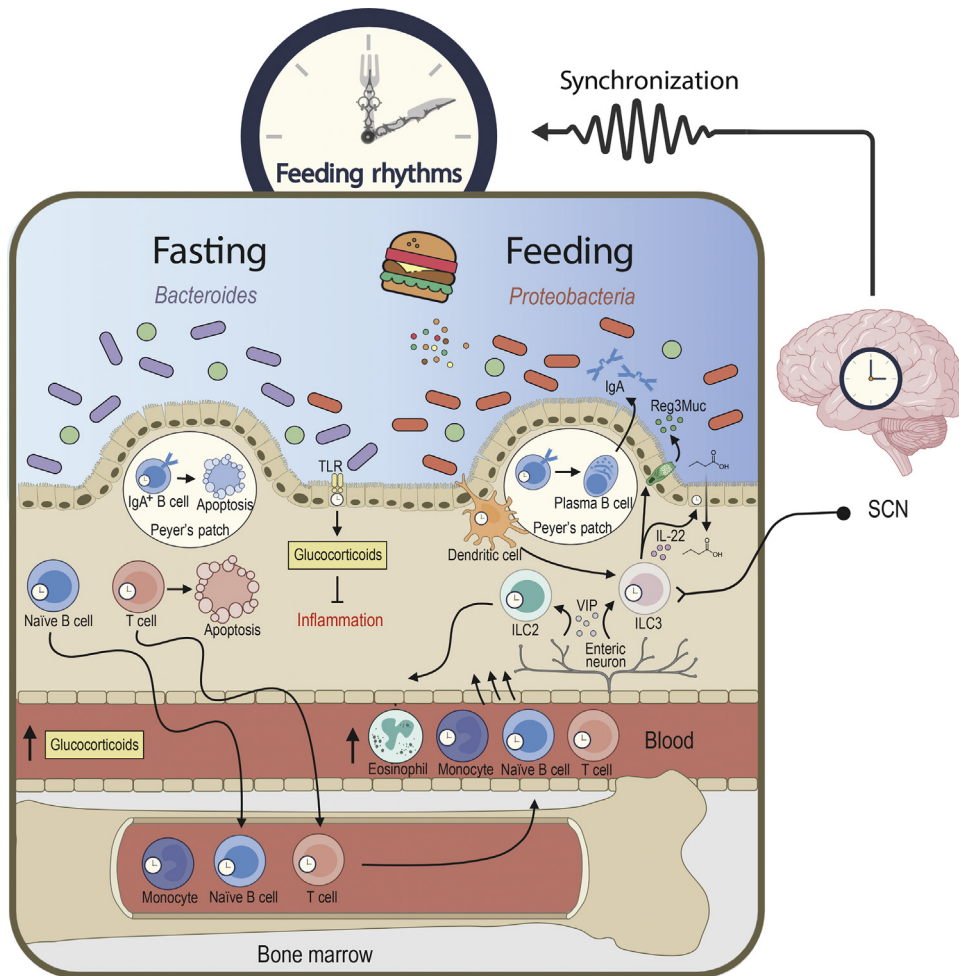
Type 1 diabetes (T1D): a chronic autoimmune disease of pancreatic β cells that is characterized by insulin deficiency leading to hyperglycemia. The pathogenesis may involve T cell-mediated destruction of β cells.

Vasoactive intestinal peptide (VIP): a vasoactive peptide hormone in the intestine.

White adipose browning: conversion of white fat tissue to beige fat to establish a balance between energy intake and energy expenditure; holds promise for potential obesity treatment.

Box 1. The Evolutionary Value of Circadian Rhythms in the Immune System

Circadian clocks are controlled by internal molecular genetic clocks that perform two major tasks: maintaining (self-sustained) rhythms under constant homeostatic conditions, and modifying rhythms in response to changing environments [187]. Likewise, the immune system harbors an intrinsic clock in many of its cells and branches, and is suggested to be regulated both homeostatically, by diurnal signals, and through the modulation of its circadian activity by responding to various environmental and microbial signals and stressors. The potential evolutionary advantage of immune system circadian activity is best exemplified in a feeding-dependent context, in which gut immune responsiveness and barrier function differ between times of nutrient exposure/absorption and times of fasting (such as during sleep) [99]. As such, lipid absorption and antimicrobial responses constitute two antagonistic pathways occurring in an antiphase manner in mice that are orchestrated, for example, by IL-22 secretion from ILC3 cells [99]. In turn, vasoactive intestinal peptide (VIP)–ILC3 circuits in the gut might be dynamically regulated by feeding and circadian rhythms. Such neuroimmune circuits might provide synchronization, and a functional time-dependent trade-off between efficient nutrient absorption and innate immune defenses, as part of an effort to maintain intestinal homeostasis.



Trends in Immunology

Figure 2. Nutritional Regulation of Mammalian Immune Cell Redistribution and Activity in the Gut. The central clock (suprachiasmatic nucleus, SCN) is involved in the synchronization of feeding/fasting cycles. During fasting, immune cells are reduced in peripheral tissues (such as the gut) by the induction of apoptosis. Conversely, they are elevated in the bone marrow through enhanced trafficking. Upon feeding, immune cells accumulate in peripheral tissues and their effector activities are locally enhanced via tissue-specific neuronal, hormonal, and metabolic signals. Figure created with BioRender ([biorender.com](https://www.biorender.com)). Abbreviations: IL-22, interleukin 22; ILC2, group 2 innate lymphoid cell; TLR, Toll-like receptor; VIP, vasoactive intestinal peptide.

and feature an inverted IgE/mast cell-mediated response to allergens, relative to night-fed mice [84]. In humans, **intermittent fasting (IF)** practiced during Ramadan (**Ramadan fasting**) results in a significant decrease in circulating immune cells (leukocytes, granulocytes, monocytes, and lymphocytes) as well as production of proinflammatory cytokines (IL-1 β , IL-6, and TNF- α); however, the numbers of circulating monocytes are significantly increased following cessation of Ramadan fasting [85]. Another intervention study in healthy Individuals showed that circadian misalignment by mistimed feeding and sleeping led to upregulated interleukin signaling (e.g., IL-3 and IL-5) and downregulated antigen presentation and interferon (IFN) pathways relative to controls [86]. By contrast, restricting feeding-time to the **active phase** of the diurnal cycle has resulted in improved gastrointestinal, cardiometabolic, and immune functions in human and animals (mice, rats, and fruit flies) [11,87–90]. Considering that mistimed food intake leads to loss of synchrony

between central and peripheral clocks [43], it is possible that coupling of central and peripheral rhythms is essential for an optimized immune response. In addition, dietary timing might influence host immunity via induction of autophagy, a fundamental process that transfers intracellular macromolecules to autophagolysosomes for degradation. Various forms of dietary restriction (e.g., CR, IF) can stimulate autophagy by improving insulin signaling or activating the **AMP-activated protein kinase (AMPK)** signaling pathway [91]. Because autophagy plays crucial roles in regulating innate and adaptive immune responses, as well as the production of inflammatory mediators [92], it may constitute an intriguing mechanism linking timed feeding with immunity outcomes.

These observations notwithstanding, whether feeding regimes constitute major entraining cues of oscillation in immune cells remains unclear to date. A suggested example of an intestinal feeding rhythm-immune interaction that may also involve microbiome signals includes ILCs, eosinophils, and their effector functions. Blood eosinophil numbers are modulated by feeding [93] as well as by neuroimmune circuits, and by ILCs in mice [94,95]. Among murine ILC subtypes, group 2 ILCs (ILC2s) and ILC3s are also sensitive to temporal regulation of food-derived stimuli [96], and are situated in proximity to enteric neurons that secrete **vasoactive intestinal peptide (VIP)** in response to food stimuli [97,98] (Figure 2). VIP, in turn, can modulate infection-induced ILC cytokine secretion by signaling through a VIP receptor (VIPR2), which is particularly abundant on the surface of ILC2 and ILC3 cells [96,99,100]. Moreover, VIPR2 is essential for rhythms in SCN neurons and is involved in entrainment with feeding regimes in mice [101–103]. As such, a feeding time-responsive network includes cross-regulation by ILCs, eosinophils, and enteric neurons through their various effector molecules. Following food intake, intestinal ILC2s triggered by local neuron-secreted VIP can stimulate VIPR2 intracellular signaling to elevate the responsiveness of ILC2s. Stimulated ILC2s secrete, in turn, higher amounts of IL-5, which enhances the expansion and chemotaxis of circulating eosinophils, relative to fasted mice [100]. A similar regulatory mechanism has been recently reported for ILC3s, where VIP can upregulate murine ILC3 production of IL-22 [96]; however, another group observed downregulation of IL-22 concentrations following VIPR2 activation in the murine gut [99]. ILC3s have been suggested to be reactive to rhythms derived from the microbiota in the murine intestine [55], in addition to being reactive to their intrinsic clock machinery, as well as to food and light in mice [77,96,104]. Moreover, disruption of circadian ILC3 oscillations in *Bmal1*-deficient mice has resulted in **microbial dysbiosis** and more severe intestinal inflammation and damage in a dextran sodium sulfate (DSS)-induced colitis model [77,104]. Noteworthy, the microbiota is also robustly influenced by food-timing [29,46,47,87,105,106]. Nevertheless, the exact mechanism(s) by which the microbiome impacts on these neuronal-immune networks remains largely unexplored and constitutes an exciting avenue of future research.

Infradian Feeding Cycles Modulate Immune Trafficking

Feeding/fasting cycles that extend to more than 24 h (e.g., IF, ADF, FMD) and CR reset both the central and peripheral clocks [9,107]. Recent studies reveal drastic redistributions of immune cells during cycles of fasting or CR in mice and humans [108–110] (Figure 2). Immune cells are the most dynamic cells in the body, and regulating their redistribution among various compartments is fundamental for optimal immune surveillance [111,112]. Indeed, **immune cell trafficking** is mediated by several protein interactions, some of which are controlled by circadian rhythmicity [62].

In the innate immune system, CR and fasting reduce monocyte and macrophage circulating numbers and activity [108,113–116]. Likewise, fasting reduces the number of circulating monocytes by blocking monocyte egress into the blood from the **bone marrow (BM)** [108]. This

inhibition vastly depends on hepatic energy-sensing factors (AMPK and **peroxisome proliferator-activated receptor α , PPAR α**) and their ability to reduce CCL2 production in the BM [108]. At steady-state, monocytes exhibit circadian oscillations in their circulating numbers in both human and mice [57,68,117]. The chemokine CCL2 is expressed rhythmically in inflammatory monocytes and is regulated by the circadian core clock. Indeed, *Bmal1* deficiency in myeloid cells leads to disturbed diurnal trafficking of monocytes in mice [57]. Although monocyte trafficking may be regulated by nutrient intake [108], monocyte diurnal variation cannot be solely entrained by feeding timing because *ad libitum* and day-fed mice display similar monocyte oscillations [57].

Similarly, CR and fasting induce changes in immune cell distribution of the adaptive immune system. Both T and B cells fluctuate diurnally [60,68,118]. During fasting, T and B cell numbers are reduced in blood and increased in the BM in mice, potentially through arrested development of naïve T and B cells [119,120]. During multiple cycles of fasting, immune cells, including lymphocytes, undergo apoptosis and regeneration upon refeeding in rodents [121–123]. CR triggers the adrenal gland to diurnally release GCs into the blood and potentially results in rhythmic T cell migration and apoptosis. Of note, blood GC concentrations increase during the rest (fasting) phase, with a peak at the beginning of the active phase in mice [124]. Moreover, GCs sensed by circulating CD4⁺ and CD8⁺ memory T cells can induce cell death and CXCR4 expression, a chemotactic receptor for CXCL12 in mice. In addition, CR signaling may induce the expansion of red blood cells in the BM, which secrete **sphingosine-1-phosphate (S1P)**. Together, S1P and CXCL12 can promote memory CD4⁺ and CD8⁺ T cell migration to BM in mice [109]. In addition, under homeostasis, GCs induce the expression of *Cxcr4* in circulating CD4⁺ and CD8⁺ T cells in mice, inducing T cell recruitment to **secondary lymphoid organs (SLOs)** instead of to BM. Conversely, reduced GCs concentrations can lead to a rise of T cell numbers in the circulation [70]. Furthermore, in mice, the rhythmic expression of *Cxcl12* is regulated by the central clock [125], and that of *S1pr1* (S1P receptor) by the transcription factor BMAL1 [69].

Considering other tissues, the effect of fasting on mucosal immunity has been largely investigated in the gut [126]. Indeed, the gastrointestinal tract is strongly influenced by nutrient availability because temporal fasting and CR result in intestinal atrophy [127,128], depletion of **gut-associated lymphoid tissue (GALT)** lymphocytes [128], and decreased titers of IgA in mice [129,130]. Moreover, fasting/refeeding cycles in mice promote the proliferation and regeneration of IECs during refeeding [127,131]. One elegant example of fasting-induced gut immune responses relates to B cell migration in and out of **Peyer's patches (PPs)** in the small intestine. Specifically, naïve B cells in PPs exhibit diurnal migration towards the BM in mice during fasting and then out of BM upon access to food [110]. Furthermore, inhibition of glycolysis (upon administration of 2-deoxy-D-glucose) led to reduced CXCL13 production in stromal cells *in vitro* and in PPs *in vivo*, indicating that intracellular nutritional signals can regulate CXCL13-producing stromal cells that help naïve B cells to navigate between PPs and BM. Furthermore, proliferating B cells and IgA⁺ B cells undergo apoptosis during fasting, resulting in attenuated antigen-specific IgA production in the gut, which may consequently aggravate antigen-induced diarrhea in mice [110]. Indeed, CXCL13 elicits its effects by interacting with chemokine receptor CXCL5, which is expressed on B cells. In mice, CXCL5 is controlled by transcriptional regulation of *Bmal1*, a core clock component that is crucial for dynamic migration of B cell subsets in PPs [68].

Clinical Implications of Diet–Circadian Interactions for Immune-Related Diseases

The importance of circadian rhythms in modulating disease pathogenesis and development has led to the emergence of a promising intervention strategy regarded as 'chronomedicine', which

involves circadian modulation of potential pharmacological treatments and other disease management features. This intervention is suggested to play important roles in immune- and microbiome-associated diseases. For example, fasting and vegetarian diets have long-term beneficial effects in patients with rheumatoid arthritis (RA), a systemic autoimmune disorder mainly involving the joints. These dietary impacts have been reviewed elsewhere [132]. Recent findings on the role of timed food intake in other immune-related diseases open the window for future translational studies to focus on the role of 'chrononutrition' in immunity and inflammation, including CNS diseases, **inflammatory bowel disease (IBD)**, infections, immune-mediated metabolic diseases, cancers, and aging (Table 1).

CNS Disease

Multiple sclerosis (MS) is a T cell-mediated demyelinating and neurodegenerating disorder involving the CNS (brain and spinal cord). An association between circadian rhythmicity disruption and MS was suggested by a link between shift work at a young age and increased risk for MS [133], and by the negative correlation between the clock-regulated hormone melatonin secretion and the seasonality of MS relapses [134]. Furthermore, in the **experimental autoimmune encephalomyelitis (EAE)** mouse model of MS, deletion of the clock gene *Bmal1* in myeloid cells (*Bmal1^{Myeloid-/-}*) or midday immunizations led to the expansion and infiltration of inflammatory monocytes and increased pathogenic IL-17 and IFN-producing T cells into the CNS, thus exacerbating T cell-mediated disease symptoms of EAE [135]. Different dietary patterns, including a **ketogenic diet** [136], acute severe CR [137], chronic CR [138], and IF [139] have conferred

Table 1. The Influence of Dietary Timing Patterns on the Host Immune System and the Gut Microbiome in Different Mouse Disease Models^a

Mouse disease models	Dietary timing	Impact of dietary timing on the immune system	Impact of dietary timing on gut microbiota	Impact of dietary timing on disease outcomes	Refs
Multiple sclerosis: EAE	ADF or FMD	T _{reg} ↑ T _{H1} , T _{H17} , and APC ↓ IFN-γ, IL-17, and TNF-α ↓ Demyelination ↓	<i>Bacteria richness</i> ↑ <i>Lactobacillaceae</i> ↑ <i>Bacteroidaceae</i> ↑ <i>Prevotellaceae</i> ↑ Ketone metabolism ↑ Glutathione metabolism ↑ FMT from fasting-treated mice ameliorates the course of EAE	Clinical severity ↓ Pathology improved	[140,121]
Parkinson: MTPT-induced PD	FMD	Number of glial cells ↓ TNF-α and IL-1β ↓	<i>Firmicutes</i> ↑ <i>Tenericutes</i> ↑ <i>Opisthokonta</i> ↑ <i>Proteobacteria</i> ↓ Butyric acid and valeric acid ↑ FMT from FMD-treated mice confers neuroprotection for PD	Motor function ↑ Dopaminergic neurons ↑	[143]
IBD: chronic dextran sulfate sodium colitis	FMD	Serum lymphocytes ↓ Serum granulocytes and neutrophils ↑	<i>Lactobacillaceae</i> ↑ <i>Bifidobacteriaceae</i> ↑ FMT from FMD-treated mice improves DSS induced clinical severity	Intestinal inflammation ↓ Intestinal regeneration ↑ Intestinal pathology improved	[131]
Colon cancer: alcohol-induced polyposis in genetic susceptible mice	Eating at rest phase ('wrong'-time eating)	Hyperpermeability protumorigenic mucosal inflammation CD3 ↓ T _{reg} /T _{H17} ratio ↓	Dysbiosis Alpha diversity ↓ Butyrate ↓ <i>Turcibacteraceae</i> ↑ <i>Turcibacter</i> ↑ <i>Lachnospiraceae</i> ↓	Colon carcinogenesis ↑	[168]

^aAbbreviations: ADF, alternate day fasting; DSS, dextran sodium sulfate; EAE, experimental autoimmune encephalomyelitis; FMD, fasting-mimicking diet; FMT, fecal microbiome transfer; IBD, inflammatory bowel disease; PD, Parkinson disease.

neuroprotection in rodent EAE models, mainly by reducing inflammation and autoimmune responses. Moreover, a recent study found that administering FMD with periodic 3 day cycles was effective in reducing disease severity in the EAE model, and this was associated with increased GC concentrations in the serum and reduced proinflammatory cytokines in the spinal cord, as well as autoimmunity suppression, by inducing apoptosis of autoreactive T lymphocytes and remyelination via oligodendrocyte regeneration relative to controls [121]. Likewise, preliminary data from a completed, randomized, controlled pilot clinical trial comparing the effects of prolonged fasting and ketogenic low glycemic load treatment on the health-related quality-of-life of relapsing-remitting MS patients (48 subjects) suggested that FMD might be a feasible, safe, and potentially effective treatment strategy for MS (NCT01538355) [121]. Moreover, **fecal microbiome transfer (FMT)** from intermittent fasting-treated mice to myelin oligodendrocyte glycoprotein (MOG35-55)-immunized recipient mice greatly reduced EAE severity compared with controls, suggesting that IF might improve the clinical course and pathology of EAE through regulation of the gut microbiota [140]. Indeed, IF increases the abundance of antioxidative microbial metabolic pathways (including glutathione metabolism), as well as gut bacteria richness, and results in higher abundance of the bacterial families *Lactobacillaceae*, *Bacteroidaceae*, and *Prevotellaceae* relative to regularly fed mice [140]. Lastly, MS patients undergoing intermittent energy restriction in an ongoing randomized pilot trial (16 participants) showed altered blood adipokines (primary outcome) as well as gut microbiota abundance, which partially recapitulated their observations in mice, and suggested an interesting translational potential (NCT02411838) [140].

In addition to MS, restricted feeding is also effective in mouse models of neurodegenerative disease. In HD-N171-82Q mutant mice (a model for Huntington's disease), ADF slows disease onset and improves the survival of these mice by normalizing blood glucose and brain-derived neurotrophic factor [141]. Another TRF mode (6 h feeding/18 h fasting) restores circadian locomotor activity rhythms and improves neurological symptoms in the Q175 mouse model of Huntington's disease relative to controls [142]. Moreover, in the MPTP-induced Parkinson's disease mouse model, FMD inhibited neuroinflammation by reducing brain glial cell numbers and the production of striatal TNF- α and IL-1 β , and shifted the gut microbiota to the more abundant *Firmicutes*, *Tenericutes*, and *Opisthokonta*, but less abundant *Proteobacteria* populations, relative to controls. In addition, this diet increased the presence of more favorable microbiome-associated metabolites such as butyric acid and valeric acid [143]. In a triple-transgenic Alzheimer's disease (AD) mouse model (3xTgAD), relative to controls, IF and CR regimens have exhibited improved cognitive functions, albeit through unclear mechanisms [144]. A recent study using the *App*^{NL-G-F} mouse model of AD showed that IF resulted in hippocampal synaptic adaptations in a manner that was dependent on the mitochondrial protein deacetylase sirtuin 3 (SIRT3) because SIRT3 knockdown in neurons of *App*^{NL-G-F} mice increased neuron excitability and decreased the number of synapses [145]. The effect of TRF on other neurologic diseases including stroke and epilepsy has been recently reviewed in detail elsewhere [146]. Although encouraging findings have been obtained, mechanistic insights are scarce, and it is imperative for future studies to investigate how dietary timing can alter immunity and neuroinflammation in these neurologic disorders.

Inflammatory Bowel Disease

IBDs, mainly encompassing ulcerative colitis (UC) and Crohn's disease (CD), are chronic and relapsing inflammatory disorders involving the gastrointestinal tract. Although the pathogenesis of IBD remains elusive, it is thought to stem from gut innate and adaptive immune dysfunction triggered by both genetic and environmental factors, in particular by the gut microbiome [147]. Environmental stressors that influence circadian rhythms, particularly sleep disturbances and shift-working, have been correlated with disease activity, disease course, and the risk of relapse, as extensively reviewed elsewhere [148,149].

Changes in dietary components, especially a high-fat, high-animal protein, and low-fiber westernized diet, have been implicated as potential environmental drivers influencing the onset and progression of IBD [150]. However, the role of dietary timing in IBD is only beginning to be revealed. Cell proliferation in murine colonic epithelial cells exhibits daily fluctuations and is tightly controlled by a circadian feeding rhythm, suggesting that the timing of food intake is important for the rhythmicity of colonic cell proliferation [106]. Of relevance, cycles of FMD in a chronic DSS-induced colitis model in mice partially reversed IBD-associated intestinal inflammation and tissue damage, enhanced intestinal renewal, and promoted the expansion of protective bacteria populations including *Lactobacillaceae* and *Bifidobacteriaceae* compared with controls [131]. Furthermore, associated with this study, the completed, randomized, Phase I clinical trial of IBD patients (100 participants) suggested that FMD could suppress systemic inflammation and leukocytosis (primary outcome, safety, and feasibility of the Prolon diet, NCT02158897ⁱⁱⁱ) [131]. Conversely, a recent retrospective study of 222 IBD patients (unregistered) showed no significant association between fasting and the disease course [151]. Prospective clinical research investigating the possible effects of feeding/fasting on IBD remains scarce to date, although one ongoing randomized clinical trial is testing the efficacy of FMD in UC patients [75 participants; the primary outcome measures inflammatory parameters such as the concentrations of blood C-reactive protein (CRP), erythrocyte sedimentation rate (SR), and/or amounts of fecal calprotectin] (NCT03615690^{iv}). More large-scale clinical trials are warranted to investigate the translational potential and putative mechanisms governing the effects of time-restricted feeding in IBD patients.

Bacterial and Viral Infections

Conflicting effects of diurnal nutritional regimens on host defense against infection have been noted by different studies, depending on the types and timing of infection, the duration of fasting, and the pathophysiological changes upon fasting. These are reviewed elsewhere [152]. In brief, one study suggested that dietary restriction in mice impacted anti-infectious immunity by inducing memory CD8⁺ T cell homing to the BM, which in turn mediated enhanced protection against *Yersinia pseudotuberculosis* (*Yptb*) infection. Specifically, upon receiving secondary challenge with systemic or oral *Yptb*, previously infected mice on dietary restriction exhibited lower bacterial burden in spleen than did mice fed *ad libitum* [109]. In another study, short-term fasting for 20 h did not influence monocyte mobilization against *Listeria monocytogenes* infection in mice, as demonstrated by comparable splenic bacterial load between fasted and feeding mice upon intravenous infection with *L. monocytogenes* [108]. However, another study showed that in an oral ovalbumin (OVA)-induced diarrhea model in mice, repeated fasting abolished plasma IgG and fecal IgA titers, thus exacerbating OVA-induced diarrhea symptoms [110]. In mice, the innate immune response to bacterial endotoxin is distinct, depending on the time of food intake [81]. Fasting has been shown to be protective in bacterial infection but harmful in viral infection because fasted mice exhibit less lethality upon *L. monocytogenes* infection and lipopolysaccharide-induced sepsis, but more mortality upon influenza virus infection or poly(I:C)-mediated viremia, compared with nutrition-supplemented mice. This might seem surprising, but these two types of infection have been associated with distinct patterns of glucose utilization versus ketogenesis, respectively [153]. Similarly, IF aggravates the immune and sickness responses to intraperitoneally injected poly(I:C) in mice, accompanied by elevated peripheral cytokine concentrations (e.g., TNF- α , IFN- α , IFN- γ) [154]. Of note, the effects of Ramadan fasting on patients with infectious diseases suggest that patient fasting-based decisions may be rationally made based on underlying disease status (summarized in a systemic review [155]). Given these discordant observations, and other inconsistent results observed in mouse models, robust and extensive testing is evidently warranted, and it remains to be seen how dietary timing might impact on human immunity when exposed to different infectious pathogens.

Metabolic Disease

In addition to fighting infections, the immune system has been proposed to contribute to modulating metabolism, metabolic health, and features of cardiometabolic disease [156]. Several studies have demonstrated a role of timed feeding in regulating cardiometabolic disease in humans, focusing on metabolic parameters such as glucose metabolism and body weight. These have been systematically reviewed elsewhere [157, 158].

Type 1 Diabetes

This chronic autoimmune disease, that features destruction of insulin-producing pancreatic β cells and resultant insulin insufficiency, commonly appears during childhood or adolescence. Chronic CR for 9 weeks significantly inhibits the production of plasma inflammatory cytokines including TNF- α and upregulates plasma IL-10 and haptoglobin in a streptozotocin-induced **type 1 diabetes (T1D)** rat model, relative to controls [159]. In mice, ADF can exert favorable effects on glucose metabolism by reducing blood glucose and insulin concentrations, and, as a result, ADF can mediate protection against neuronal injury by increasing neuron survival in mice injected with excitotoxin kainic acid in the dorsal hippocampus, an effect which was independent from caloric intake [160]. Moreover, in streptozotocin-induced T1D rats, IF increased glucose tolerance, plasma insulin, and β cell mass relative to controls [161]. Cycles of FMD were also suggested to restore glucose homeostasis and insulin production by generating insulin-producing pancreatic β cells in both the streptozotocin-induced T1D mouse model and the leptin receptor-deficient (*Lep^{db/db}*) mouse model of type 2 diabetes, as well as promoting islet insulin generation by reprogramming pancreatic cells in T1D patients [162].

Atherosclerosis and Heart Disease

The effect of dietary timing on cardiovascular diseases and associated risk factors, including hyperlipidemia, high blood pressure, and chronic elevation in inflammatory biomarkers in both mouse models and human trials, has been summarized elsewhere [163]. A recent single-arm, paired-sample, ongoing clinical study (35 participants) found that a 10 h TRF improved cardiometabolic health in patients with metabolic syndrome, including features of weight, blood pressure, and concentrations of atherogenic lipids (NCT03182985^v) [88]. In mice, the gut microbiome can regulate myocardial ketone body metabolism during fasting, and fasted germ-free mice exhibit reduced hepatic ketogenesis, decreased enrichment of ketone body metabolic pathways in the myocardium, and reduced myocardial mass, highlighting the significance of the gut microbiota in mediating some beneficial effects of restrictive feeding on cardiovascular health [164]. Furthermore, a rhythmic pattern of recruitment of myeloid cells to atherosclerotic lesions was reported to be regulated by the oscillatory release of myeloid cell-derived CCL2, in both high-fat-diet-fed mice and hypercholesterolemic *Ldlr^{-/-}* mice [165]. Based on this, timed pharmacological treatments that target the CCL2-CCR2 axis might potentially impede the development of atherosclerosis [165], although this remains to be rigorously tested. It is therefore reasonable, but remains to be investigated, whether chrononutrition could impact on the course of cardiovascular diseases by targeting multiple immune-related pathways. It should be noted that, unlike adults, childhood TRF in mice may lead to multiple adverse effects including irreversible metabolic disorders, suppressed immune function, and gut microbiome alterations [166]. Therefore, this suggests that the indication of TRF in treating metabolic diseases should be carefully considered and tested among different age groups.

Cancer

Evidence in mice suggests that, in a model of pancreatic adenocarcinoma, targeting circadian clocks by meal timing inhibits cancer growth through tumor transcriptomic reprogramming, as exemplified by the increased circadian expression of cellular stress genes (*Hspa8*, *Cirbp*,

Ccna2) [167]; this also highlights the potential role of nutritional timing in cancer treatments. By contrast, mistimed food intake, coupled to alcohol consumption, has been suggested to promote colon carcinogenesis in the *TS4Cre × APC^{lox46B}* mouse model of colorectal cancer. This phenotype was driven by decreasing the amounts of short-chain fatty acid-generating bacteria, in addition to butyrate, as well as inducing proinflammatory and protumorigenic immune profiles in the colon (e.g., increased TH17 and decreased regulatory T cell numbers relative to controls) [168]. These findings potentially link feeding-timing, microbiome, immunity, and cancer development. In humans, ongoing trials are testing the effect of dietary timing on the pathogenesis and growth of different tumor types (summarized in [169]). Short-term fasting greatly enhances the response to cancer chemotherapy in various tumor types, as reviewed recently [170]. Furthermore, periodic fasting causes death in chemotherapy-resistant lung adenocarcinoma (A549) cells *in vitro*, and athymic mice xenografted with A549 cancer cells exhibit longer survival and less tumor growth when subjected to periodic fasting cycles, compared with controls [171]. Moreover, multiple cycles of prolonged fasting in mice can reverse the immunosuppression caused by chemotherapeutic drugs [122]. In addition, fasting has been proposed to lead to substantial changes in metabolic profiles and growth factor concentrations, thus reducing the ability of cancer cells to survive, while protecting normal cells from the side effects caused by anticancer treatments [172]. The combination of FMD and chemotherapy can enhance CD8⁺ T cell-mediated killing of cancer cells in a mouse breast cancer model (4T1) and a melanoma model (B16) [173]. The gut microbiota might also modulate the host response to different cancer immunotherapies, such as checkpoint blockade anti-PD-L1 drugs [174, 175] and CTLA4 blockers in both mice and humans [176]. However, whether timed feeding plays a similar role in cancer immunotherapy responsiveness – potentially through modulation of the gut microbiome and its interactions with host immunity – remains to be investigated. Similarly, the tumor types that can potentially benefit from timed feeding remains a pressing unresolved question.

Aging

A growing body of evidence suggests that lifespan can be extended by either CR or ADF [177]. In male mice, a prolonged daily fasting protocol is associated with improved health and longevity as well as delayed onset of liver pathologies, and this was independent of dietary composition and caloric content [178]. A recent randomized controlled trial of healthy and non-obese humans suggested that long-term ADF might be safe to practice, and can improve aging-associated markers including cardiovascular and metabolic parameters (60 participants; primary outcome, insulin sensitivity) (NCT02673515^{vi}) [179]. It is hypothesized that CR and IF exert these beneficial effects, at least in part, by resetting the circadian rhythm because IF restores the disruptive rhythms of clock gene expression (*mPer2*, *mClock*) induced by disruptive light conditions in mice [180, 181]. In addition, dietary restriction increases lifespan in the nematode *Caenorhabditis elegans* by regulating the conserved p38–ATF-7 innate immunity pathway [182]. In this study, longevity was associated with reduced insulin/insulin-like growth factor-1 (IGF-1) signaling which also involved reducing food consumption, and in turn downregulated p38–ATF-7 signaling. Thus, this report mechanistically linked nutrient signals with appetite, innate immunity responses, and aging [182]. Whether the potential impacts of timed dietary regimens on human longevity and age-related disorders is mediated via effects inflicted on the host immune function and microbiome certainly merits further studies and may represent a fascinating area for future investigation.

Concluding Remarks

Our understanding of circadian reprogramming by time-specific nutritional intake has been rapidly expanding in recent years. This is also the case for advances on dietary timing–circadian crosstalk. Indeed, these processes actively participate in the regulation of microbiome and

Outstanding Questions

Can dietary timing regulate innate immune responses including immune cell recruitment, activation of inflammatory cascades, and antimicrobial responses?

Can dietary timing influence adaptive immune functions such as antigen presentation, T cell effector functions, antibody production, and immunological memory development?

Can intestinal barrier function be controlled by the timing of food intake?

Does the gut microbiome play a causal role in mediating the effect of dietary timing on host immunity?

What is the mechanism that integrates dietary timing, gut microbiota, and host circadian rhythms to regulate diurnal host immune functions?

Can the efficacy of dietary timing intervention in rodent disease models be effectively translated to human immunological disorders? How can we best overcome this experimental barrier?

Do the gut microbiome responses and host immune responses to the timing of feeding differ between individuals? Can such personalized responses be predicted or modeled?

immunity. However, many unknowns and challenges remain, particularly in disentangling the complex impact of the aforementioned processes on physiology and pathology. First, current studies have tried to clarify the impact of the fasting/feeding cycle on immune events including trafficking and chemotaxis, although the effect of dietary timing on many other innate and adaptive immune responses, as well as on intestinal barrier function and antimicrobial responses, remains to be determined (see Outstanding Questions). Second, the molecular mechanisms underlying the influence of feeding schedules on host circadian and immune responses need to be mechanistically investigated. For example, much of the role of circadian clock-associated molecules and transcriptional factors (including but not limited to Bmal1, Clock, Rev-Erb proteins) in mediating diet–immunity interplay remains elusive. Third, the role of gut microbiota in mediating the effect of timed feeding on host immunity has yet to be determined. Increasing numbers of studies are noting gut microbiome alterations upon time-restricted feeding in both mice and humans. It is imperative to clarify whether the microbiome mediates the effect of feeding patterns on host physiology by using FMT approaches in germ-free mice, and other recently introduced modalities enabling the study of causality (for example, host responses to microbiome-associated metabolites or colonization with engineered microbial strains). Fourth, the host–microbiome interface in the gut is a complex multilayer system that comprises the mucus layer, IECs, and enteric neuronal and immune system components. How signals related to these different compartments, cell types, and their intrinsic circadian clocks are integrated to regulate an immune response to feeding-regulated nutritional signals remains an attractive challenge for future research. Last, although many studies claim a beneficial role of TRF in metabolic diseases as well as on immune-mediated disorders, most of these have either been performed in rodent models or offer small-scale preliminary human findings. Laboratory mice remain the main model in studying circadian influences on immunity and the microbiome, but are limited by their translational potential to human diseases. The repeatedly failed translations from mouse to human could possibly stem from divergences in microbiome configurations, dietary components, and even opposite immune and other circadian rhythms that are present in mice as compared with humans [168,183]. Large-scale randomized controlled clinical trials are warranted to investigate the potential of timed-feeding schedules as a putative treatment strategy in the context of human diseases.

Despite these challenges, the emerging concept of circadian medicine based on the restoration or modulation of physiological features related to circadian rhythms may hold promise as a potential future intervention in a wide range of human diseases by means of chronopharmacology and chrononutrition (e.g., TRF) [184]. In addition to studies on metabolic diseases, the presumed beneficial role of meal-timing in modulating immune-mediated diseases is only beginning to be unraveled. This is also the case for our understanding of the intricate interactions between immune cells and the gut microbiome. Because the relationship between dietary timing, circadian rhythmicity, gut microbiome, and the immune system constitutes an intricate and poorly understood signaling network, future studies integrating multi-omic approaches, including metagenomics, transcriptomics, proteomics, epigenomics and metabolomics, will be essential to disentangle such complex interactions. The advent of 'personalized nutrition' [185,186] hypothetically suggests that, akin to glucose homeostasis, diet and the microbiome may modulate immune responses in a highly individualized manner. Future studies may attempt to generate predictors of personalized responses of host immune parameters to timed feeding, in large-scale populations, using machine-learning and other artificial intelligence algorithms based on gut microbiome and clinical metadata. These may enable noninvasive tailoring of the timing of dietary interventions to the individual in different clinical contexts. Notably, the translational potential of such dietary interventions in human immune-mediated disorders requires high-quality clinical trials, as well as careful consideration of potential biases such as concomitant exposure

to anti-inflammatory or immunosuppressant medications. Although challenging, implementing these approaches may pave the way towards a better understanding of the fascinating pleiotropic effects of dietary timing on the gut microbiome and immune function.

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Declaration of Interest

E.E. is a paid scientific consultant for DayTwo and BiomX.

Resources

ⁱ<https://clinicaltrials.gov/ct2/show/NCT01538355>

ⁱⁱ<https://clinicaltrials.gov/ct2/show/NCT02411838>

ⁱⁱⁱ<https://clinicaltrials.gov/ct2/show/NCT02158897>

^{iv}<https://clinicaltrials.gov/ct2/show/NCT03615690>

^v<https://clinicaltrials.gov/ct2/show/NCT03182985>

^{vi}<https://clinicaltrials.gov/ct2/show/NCT02673515>

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