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Immunometabolism around the Clock

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Abstract

Almost every cell type has a molecular clock, which controls vast swathes of gene expression on a 24-hour cycle, providing circadian rhythmicity. An example of a circadian behaviour, common to most organisms, is the feeding/fasting cycle - shaping whole-body metabolism over evolutionary timescales. However, the exact mechanisms by which the clock controls cellular metabolism have only recently become clear. The molecular clock and related metabolic pathways are also key drivers of immunity. Thus, a natural convergence of circadian biology, metabolism, and immunology has emerged to form a new field which we term 'circadian immunometabolism'. Expanding our understanding of this field will provide new insights into chronic conditions such as obesity, cancer, diabetes, cardiovascular disease and arthritis.

Introduction

Life on our planet has evolved in a 24 hour cyclic environment due to Earth's daily rotation about its axis and its position relative to the sun. As such, most organisms have evolved molecular clocks that match their physiology to the solar cycle. Molecular clocks regulate timing of cellular activities depending on environmental cues such as light and food. Therefore, the molecular clock creates temporal harmony between tissues and the external environment. For many organisms, perhaps the most important factor in maintaining healthy tissues is a functioning immune system – thus the molecular clock and the immune system are inextricably linked. Five decades ago, Franz Halberg, demonstrated that lipopolysaccharide (LPS) (a component of bacteria) given at the end of the rest phase caused increased lethality in mice, compared to other times – suggesting a circadian-dependent immune function [1]. Since then, cells of both innate and adaptive immune systems have

been shown to exhibit circadian rhythms and humans also display this time-of-day variation to LPS [2]. Clocks in these cells impose temporal control over a range of responses, including inflammation [3, 4], cell trafficking [5, 6], bacteria [7], virus/parasite killing [8, 9], allergic responses [10] and natural killer cell mediated toxicity [11]. These topics have been reviewed extensively elsewhere [12, 13]. When *Bmal1*, the gene central to all circadian activity, is genetically knocked-out in mouse models, LPS-induced lethality is increased [14]. Circadian disruption occurs when molecular clock timing becomes mismatched with environmental cues, which occurs with night shift-work, When studied experimentally in humans and mice, shift-work caused disruption of both peripheral blood mononuclear cell rhythms (PBMCs), and inflammatory responses to LPS [15, 16]. Therefore, loss of circadian function in response to genetic manipulation or environmental factors negatively affects immunity and leads to dysfunctional responses to pathogens and insults.

In recent years, immunologists have dusted off their college biochemistry textbooks, as metabolism has emerged as a central controller of the immune system [17]. Pioneering work in immunometabolism has shown that metabolic and immune pathways can modulate the type, magnitude, and duration of immune responses. For innate immune cells, such as macrophages and dendritic cells, much of the immunometabolism work so far is in relation to the control of IL1β, an apical cytokine that is implicated in many diseases including arthritis, atherosclerosis, obesity, Alzheimer's and cancer [18-20]. The recent emergence of circadian immunometabolism means it is likely, although not yet proven, that molecular clocks in the immune system may help to integrate metabolic changes driven by rhythms to direct immune output. For example, restricted daytime feeding of mice limits their ability both to kill bacteria and produce serum cytokine responses [21]. Thus, it is crucial to understand how the molecular clock, metabolism and immune system coordinate homeostasis in immune cells - and whether circadian disruption impacts immunometabolism to drive pathology.

In this review we will address immunometabolism by first discussing how the three main macronutrients (glucose, lipids and proteins) are metabolised by innate immune cells. Then recent advances in clock modulation of metabolism will be reviewed along with the consequent effects on immunity. Many chronic diseases, which are at epidemic proportions, have inflammatory and metabolic components - and also involve the molecular clock. Thus these may represent prime candidates to target with circadian immunometabolism approaches.

The Inner Workings of the Molecular Clock

The word 'circadian' comes from the Latin words circa 'about' and dian 'day'. Circadian rhythms possess three core properties: (1) A period of approximately 24 hours, (2) an endogenous nature, whereby rhythms will continue to oscillate independent of external cues, and (3) potential for entrainment by external stimuli. Entraining stimuli are known as Zeitgebers, from the German word for time-giver, the most dominant of which are light and food. Light is sensed by melanopsin-expressing retinal ganglion cells, which signals to the suprachiasmatic nucleus (SCN), the master clock in the hypothalamus via the retinohypothalamic tract [22]. The SCN governs a number of important systemic factors including sleep/wake cycles, hormones, metabolism and, as recently shown, the immune system [13]. The SCN orchestrates peripheral tissue clocks via a number of factors (**Fig. 1**) [23]. The molecular clock consists of up to 14 proteins, working across a number of transcriptional and translational feedback loops (TTFL) (**Fig. 2**). While the TTFL is a common feature to all cells, downstream targets of clock proteins are remarkably cell-type and tissue specific [24]. Therefore, the impact of circadian immunometabolism will likely be systemic metabolic effects integrated into a cell type specific manner.

Circadian Disruption, Metabolism and Human Health

With night-time shift work, behaviours such as activity and eating occur during the 'biological night' and sleep must then occur in the 'biological day', a time when the SCN is receiving light inputs. This leads to disruption of peripheral clocks with respect to their master SCN clock (**Fig. 3**) [23]. Numerous studies have reported increased risk of breast cancer and other metabolic conditions such as heart disease and diabetes with shift-workers [25]. Circadian misalignment in humans lowers glucose tolerance by decreasing insulin sensitivity [26-28], which is a key early defect in type II diabetes (T2D) [29]. Social jetlag (SJL) is defined as the difference in sleep timing between free and work days, and is a mild but chronic form of desynchrony affecting many people in western societies [30]. SJL is driven by late bedtimes and early rising on week-days, and later bedtimes and rising on free-days. This is akin to travelling west at weekends and returning eastwards on Monday morning [31]. Most people suffer from SJL of up to 2 hours per week, and this brings increased risks of obesity, metabolic syndrome and T2D [31, 32]. Finally, other relatively recent social developments, such as exposure to light at night (LAN) through artificial light sources and altered feeding-times, cause circadian disruption and increased risk of chronic disease [33]. Circadian disruption is an inescapable feature of modern life. However its potential links with chronic disease, dysfunctional metabolism or immunity are worrying. Therefore, decoding how the molecular clock intersects with

immunometabolic pathways may provide crucial new insights into the mechanisms of chronic conditions that beset everyday living.

Glycolysis

Cellular metabolism is a key regulator of the immune system. Increasing flux through metabolic pathways, such as glycolysis or lipid synthesis, boosts immune responses [34]. Glucose is the major energy source for innate immune cells and, depending on their activation state, they alter glucose metabolism to suit their needs [35]. Glucose is converted to ATP through a combination of two linked but distinct pathways – glycolysis and oxidative phosphorylation (**Fig. 4**). However, ATP production through glycolysis is quite inefficient – with each glucose molecule generating only 2 ATP molecules. Nonetheless, glycolysis also produces many other essential biosynthetic precursors for metabolic pathways. Increased glycolytic flux boosts the pentose phosphate pathway (PPP). The PPP diverts metabolites from glycolysis into NADPH reduction, or nucleotide and amino acid synthesis pathways (**Fig. 5**). Together, glycolysis and the PPP increase citrate levels and NADPH, which are then used by fatty acid synthase for the production of lipids [36] Other examples of glycolysis providing precursor metabolites is the production of 3-phosphoglycerate that is used in serine biosynthesis [37]. Thus, glycolysis is much more than a monotonic energy producing pathway and the intermediates it produces can impact immunity.

Immunometabolism of Macrophages and Dendritic Cells

Macrophages and dendritic cells are important components of the innate immune system, which can eliminate pathogens and resolve cellular and tissue damage. Pathogen recognition receptors (PRRs) on these cells recognise microbial or damage molecules and mobilize the innate immune response to remove them [38]. Having encountered such products, or proinflammatory cytokines such as INFγ, macrophages become classically activated to the "M1" state [39]. M1 macrophages are characterised by increased cytokine production, phagocytosis and bactericidal activity. This requires increased metabolic flux through glycolysis [40], as inhibition of glycolysis via 2-Deoxy-D-glucose drastically reduces these responses [41]. In the M1 state the tricarboxylic acid (TCA) cycle no longer completes the full loops necessary for metabolic flux (**Fig. 4**). Thus a 'break' occurs in the TCA cycle at two distinct points. These 'breaks' cause accumulation of important intermediate metabolites including succinate and citrate [41]. Succinate accumulation inhibits the prolyl hydroxylases, thus allowing stabilisation of the hypoxia transcription factor HIF-1α even in the presence of oxygen.

HIF1α then drives aerobic glycolysis through (1) increasing expression of glut transporters to increase glucose uptake [42] (2) increasing the expression of the glycolytic enzyme *pkm2* [43] and (3) increasing expression of pyruvate dehydrogenase kinase 1 (PDK1), enhancing phosphorylation of pyruvate dehydrogenase, and thus preventing pyruvate from entering the TCA cycle - thereby sustaining glycolysis [44] (Fig. 5).

Upon PRR engagement, dendritic cells (DCs) respond in a similar fashion to macrophages [45]. However, within minutes of encountering a pathogen, DCs require rapid lipid synthesis. This is achieved by increasing glycolytic flux to (1) generate citrate, a metabolite essential for fatty acid synthesis and (2) increase NADPH reduction, an essential cofactor for lipid synthesis, through the PPP [46]. This creates a futile loop of repeated lipid synthesis followed by their degradation to maintain biosynthetic precursors necessary for the response of DC's to the microbial product [46]. Therefore, macrophages and DCs display immense metabolic plasticity to suit the activation needs of the cell.

TCA cycle and Oxidative Phosphorylation in Immunity

In resting macrophages and DCs, pyruvate enters the TCA cycle and is metabolised to generate GTP, $FADH_2$ and NADH. These are used for oxidative phosphorylation in the electron transport chain (ETC) to generate ATP. The cycle begins when pyruvate moves into the mitochondria and is converted either to Acetyl-CoA by pyruvate dehydrogenase (Fig. 4). The TCA cycle works in tandem with oxidative phosphorylation to produce ATP from glucose much more efficiently than glycolysis alone. While the former can generate 36 molecules of ATP for every molecule of glucose, the latter can only produce two. As discussed above, the TCA cycle in M1 macrophages has two characteristic 'breaks'. The increased citrate fuels lipid synthesis, which are essential for the inflammatory response, by maintaining the cell membrane [41, 46, 47]. High citrate can also act as a negative feedback loop for the TCA cycle by inhibiting pyruvate dehydrogenase and succinate dehydrogenase to prevent unregulated synthesis of ATP [48].

Macrophages also contribute to resolution of inflammation and tissue repair. When carrying out this role they are known as alternately activated macrophages or 'M2 macrophages'. IL4 or IL13 polarise macrophages to the M2 state and are characterised by expression of arginase and the mannose receptor. Unlike M1s, M2s are highly dependent on mitochondrial respiration using both the TCA cycle and fatty acid oxidation to sustain oxidative phosphorylation. IL4 and IL13 can also drive the transcription of TCA and oxidative phosphorylation genes [49]. M2 function is dependent on the flux

through mitochondrial respiration as inhibition, by nitric oxide, drives macrophages to an M1 state [50]. Mitochondrial respiration in M2s is linked to increased STAT6 dependent expression of *Pgc-1β* [51]. Decreasing macrophage *Pgc-1β* decreases mitochondrial respiration with a concomitant rise in inflammation, with increased expression having the opposite effect [51]. Thus, it is clear that the change in glucose metabolism in macrophages and DCs under certain stimuli is an absolute requirement for the directed response of these cells.

Circadian Glucose metabolism

Whole body glucose tolerance was observed to be higher in rats during their active phase, lower during resting phase - and completely abrogated with SCN lesioning, demonstrating circadian control [52]. Similarly, whole body circadian glucose tolerance has also been demonstrated in humans and mice [27, 53, 54]. *Clock* $^{\Delta 19/\Delta 19}$, *Bmal1*$^{-/-}$, *Cry1*$^{-/-}$*/Cry2*$^{-/-}$ and *Rev-erbα*$^{-/-}$ transgenic mice display decreased glucose tolerance and increase in circulating glucose. In contrast *Per2*$^{-/-}$ mice display increased glucose tolerance with decreased circulating glucose [55]. Maintaining blood glucose levels within quite a narrow range is required for homeostasis. This is achieved by glycogenesis (glycogen formation) in the liver when glucose is high, and glycogenolysis (glycogen breakdown) when it is low [56, 57]. Glycogenesis is initiated by the clock controlled enzyme glycogen synthase. Furthermore, degradation of CRY2 by autophagy was shown to promote gluconeogenesis in the liver, implicating CRY2 as a suppressor of gluconeogenesis [58]. Taken together, these data suggest that the molecular clock drives glucose catabolism for energy during the active phase, and promotes glucose anabolism during resting - to prevent the futility of generating and degrading glucose simultaneously.

Clock Controlled Glucose Metabolism and Immunity

Therefore, glucose metabolism directs innate immune function while in metabolically active tissues, such as the liver, glucose metabolism is clock controlled. This highlights the importance of considering the consequence of these three areas together (circadian biology, immunity and metabolism), and studies are just beginning to emerge. For example, PKM2, the enzyme which converts phosphoenolpyruvate to pyruvate during glycolysis, is transcriptionally inhibited by BMAL1. High levels of PKM2 were found in mice where *Bmal1* is conditionally deleted from myeloid cells. This caused lower survival in a caecal ligation puncture sepsis model, while glycolytic flux and lactate

production were increased [59]. When PKM2 was also knocked out (*Bmal1*$^{lysM-/-}$*Pkm2*$^{lysM-/-}$) mice showed increased survival, suggesting BMAL1 regulation of PKM2 plays a key role during sepsis [59]. Transcriptomic studies of peritoneal macrophages sampled every 4 hours showed cycling of genes involved in glycolysis and glucose metabolism, such as, *Pdk4*, *Ldha*, *Slc2a6* and *Slc2a9* [60]. Interestingly, many of these glycolytic genes are regulated by HIF-1α. Other clock controlled transcription factors are also important regulators of glycolysis and immunity [3]. For example, the activity of NRF2,an anti-oxidant transcription factor, was significantly reduced in *Bmal1*$^{lysM-/-}$ macrophages, which then increased ROS. Increased ROS stabilises HIF-1α, leading to increased and sustained IL1β expression in *Bmal1*$^{lysM-/-}$ macrophages [3]. Due to its importance in M1 macrophages [41], dendritic cells [61], and its suppression by BMAL1, HIF-1α is a likely candidate to connect the clock with immunometabolism [62, 63]. These studies provide an initial glimpse of the crucial connection points between the molecular clock and immunometabolism in innate cells. This potential circadian-based control over immunometabolism is particularly exciting, given the commercial interest in manipulating metabolism in immune cells for therapeutic purposes.

Lipids

Lipid metabolism in Immunity

Lipid metabolism is also critical for an efficient inflammatory response. Fatty acid and cholesterol synthesis are major pathways in lipid metabolism. The enzyme fatty acid synthase (FAS) plays a key role in synthesising palmitate, via catalysis of Malonyl-CoA, Acetyl-CoA and NADPH (Fig. 4) [47]. In a mouse model of sepsis, inhibition of FAS with C75 provided protection by reducing NLRP3 expression and bioactive IL1β [64]. As described above, DCs require the induction of a “futile” loop of lipid synthesis to respond to pathogens [46]. This places FAS as a key regulator of immune responses in innate immunity. Increased levels of membrane cholesterol can also enhance the proinflammatory response of macrophages by increasing receptor signalling at the membrane [65, 66]. Interestingly, FAS also promotes cholesterol synthesis, which facilitates PRR signalling [47]. Inhibition of FAS causes a reduction in cholesterol and IL1β production in response to LPS [47, 67]. Cyclodextran, a molecule capable of dissolving cholesterol crystals, can induce regression of established atherosclerosis, through increased LXR activity. LXRs drive expression of ABC transporters that lower cellular cholesterol [68]. Therefore, lipid metabolism is both a key regulator of immune responses and as discussed below a pathway that is strongly influenced by the molecular clock.

Circadian Lipid Metabolism

The molecular clock has profound effects on lipid metabolism and storage. For example, something as simple as the time of day at which mice feed can significantly affect their weight [69]. Altered lipid metabolism was first reported in *Clock*$^{\Delta 19/\Delta 19}$ mutant mice, which spontaneously develop obesity, hypertriglyceridemia and hypercholesterolemia [70]. A germline deletion of *Bmal1* also causes spontaneous development of hyperlipidaemia and increased fat content [71]. Synthesis or degradation of lipids occurs at different times of the day. BMAL1:CLOCK drives transcription of ATP citrate lyase, the first committed step of fatty acid synthesis during the active phase [72]. During the resting phase, the clock promotes lipid oxidation, through clock-controlled increase in expression of *Cpt1a* and *Cpt2a* to promote lipid degradation [72, 73]. The nuclear receptors and clock proteins REV-ERBα and REV-ERBβ, also play a potent role in lipid metabolism (**Fig. 2**). For example, both of these heme regulated nuclear receptors control lipid metabolism in the liver [74]. REV-ERBα is a potent transcriptional repressor both by the recruitment of the NcoR-HDAC3 complex and via modulation of enhancer RNAs. *Rev-erbα*$^{-/-}$ mice display 2.5-fold increase in adiposity and mild hyperglycaemia. These mice display constitutively upregulated levels of lipoprotein lipase (*lpl*), a gene important in lipid utilization and storage in muscle and adipose tissue [75]. Mice with deletion of both nuclear receptors have profound disruption of core clock genes and lipid homeostatic gene networks [76].

Circadian Lipid Immunometabolism

Rev-erbα is also a likely intermediary link between circadian lipid metabolism and immunity. Whether oscillations of lipid metabolites occur in innate immune cells in a circadian manner, and whether this affects innate immune responses is not yet clear. *Rev-Erbα* suppresses the inflammatory response in macrophages through direct promoter binding and suppressing genes, such as *Ccl2* [4, 77], similar to its regulator *Bmal1*. Inhibition of distal proinflammatory cytokine enhancers is another mechanism by which Rev-Erba suppresses inflammatory gene expression [78]. Protecting REV-ERBα from inflammation-induced degradation affords protection in models of lung inflammation [79]. Therefore, it will be intriguing to investigate whether Rev-Erb's effects on inflammation has an underlying link with alterations in lipid metabolism.

PROTEINS

Amino Acid Metabolism in Immunity

Cells require an adequate supply of amino acids for routine protein synthesis and cell growth. While some amino acids (non-essential) can be synthesized through metabolic pathways, others (essential) are required from external sources [80]. The mTOR complexes play a critical role in both sensing amino acid availability. In innate immune cells mTOR can be activated extracellularly by microbial products or cytokines or intracellularly by nutrient levels within the cell [81]. The mTOR pathway can either promote or inhibit inflammatory responses depending on the cause of inflammation [82, 83]. Independent of mTOR, metabolism of individual amino acids also plays a role in regulating inflammation. For example the specific way in which arginine is metabolized by either M1 or M2 macrophages can be used to distinguish one state from the other [39]. In M1 macrophages, arginine is metabolised to nitric oxide through inducible nitric oxide synthase (iNOS) and is essential for the bacterial killing [84]. In M2 macrophages, arginine is metabolised by arginase into ornithine, which is a metabolite for proline and polyamine synthesis and essential for the tissue repair function of M2 macrophages [85]. Amino acid metabolism also regulates other functions in innate immune cells, such as the use of glutamine as a carbon source to sustain the TCA cycle in M2 macrophages following IL4 stimulation [86].

Circadian amino acid metabolism

At the cellular level, rhythmic protein synthesis is reliant on BMAL1 and its control of mTOR activity. Through its effector kinase S6K1, mTOR promotes protein translation and ribosomal biogenesis. Phosphorylated BMAL1 by S6K1 then associates with mTOR, stimulating protein synthesis further [87]. A phosphoproteomic screen showed that the phosphorylation of mTOR occurred with circadian rhythmicity, where levels were highest during the active phase and lowest during the inactive phase [88]. Conversely, Per2 suppressed mTOR and reduced translation [89]. In the liver, expression of factors involved in ribosomal biogenesis are also controlled by the clock [90] This relationship between circadian proteins, mTOR and protein synthesis is an example of how oncoming active phase can be anticipated and how nutrient sensing and protein synthesis can be timed appropriately.

Circadian amino acid metabolism and immunity

It remains unclear whether circadian rhythmicity in amino acid synthesis plays a role in innate immune system. We hypothesise that circadian variation in mTOR activity has immuno-regulatory properties due to its central importance in the innate immune system. Whether metabolism of key amino acids, such as arginine, glutamine, and tryptophan are rhythmic, and whether their associated immune function have a time of day dependency remains an open and exciting area of study.

MITOCHONDRIA

Mitochondrial Dynamics, Metabolism and Circadian Rhythms

A key feature of mitochondria is their ability to alter their morphology or size depending on a cell's circumstance and/or environment. Collectively, these processes are known as mitochondrial dynamics and have profound effects on cell metabolism [91] – and also display circadian rhythmicity **(Fig. 6**). In fibroblasts, post-translational modification of DRP1 (a pro-fission protein), is under clock control and this can affect mitochondrial dynamics and bioenergetics [92]. Furthermore, loss of clock control (via *Bmal1 deletion*) leads to loss of the circadian pattern of mitochondrial fission/fusion in hepatocytes. This leads to enlarged dysfunctional mitochondria that respond inefficiently to metabolic input with increased ROS levels. This scenario also leads to insulin resistance and increased levels of fat in the liver [93]. Importantly, mitochondria have signalling functions in innate immune cells, they can promote both inflammasome activation and MAVS oligomerisation in response to bacterial and viral infections, respectively [94, 95] and production of mitochondrial ROS is a critical inflammatory signal [95]. Therefore, mitochondrial biology is a key regulator of immunity [96], however, whether the molecular clock modulates immunity through its control of mitochondria has yet to be determined.

Chronotherapy and defining Internal Circadian Time

Chronotherapy is where careful timing of medical interventions (within the daily cycle) is used to maximise their efficacy and minimize side-effects. However, circadian timing is highly individualised. The current gold standard for measuring an individual's circadian time is dim-light melatonin onset (DLMO). DLMO sampling must be done under controlled dim-light conditions every 30-60 minutes over 5-6 hours. This is clearly problematic for routine clinical practice. Interestingly, recent advances have shown that individual circadian time can be determined from monocytes harvested from peripheral blood by measuring gene expression of 12 specific targets using nanostring technology

[97]. This test called 'BodyTime' is the first reliable assay that can determine circadian time from a single sample. It is becoming clear that while methods of disease treatment are obviously important, the timing of when they are delivered is also crucial – thus technologies such as BodyTime will be an invaluable tool. It has also become apparent that many existing drug targets are rhythmic [98]. Therefore, retrospective re-timing of currently accepted dosage regimes may improve efficacy and reduce toxicity, especially in drugs that have short half-life [98]. Development of other diagnostic tests like BodyTime, which are feasible for use in the clinic, will enable chronotherapy to become a significant arm of the personalized medicine revolution and allow for the translation of fundamental circadian immunometabolism findings into useful clinical practice.

Concluding Remarks

There is now a rapid and complementary growth occurring in the fields of circadian biology and immunometabolism. The ever-increasing evidence of circadian rhythmicity in the immune system suggests that time-of-day and clock status is central to immunity. Simultaneously, our understanding of how metabolism is a guiding force for immune function also continues to grow. These two fields are intimately linked as various studies have shown that metabolism is extensively regulated by the molecular clock. Now is an opportune time to begin viewing these previously disparate fields of biology as three points of the same triangle, where circadian biology, immunology and metabolism each represent a cornerstone (**Fig. 7**). Until now, much research focussed on two of these three fields. Future progress will depend on considering the contribution of all three. The birth of a new field is an exciting time, however it also means that many basic questions remain unanswered (see outstanding questions). Most chronic diseases (cancer, obesity, arthritis, and atherosclerosis) have been linked to circadian disruption, metabolic disorder or inflammation independently. In order to fully understand the complexity of these diseases, we must interrogate the contribution of all three to each other and to disease. This comes at a time when compounds that manipulate metabolism are beginning to emerge as a new class of anti-inflammatories. Combining this with our increased understanding of the molecular clock in regulating metabolism will facilitate development of novel targets and chronotherapeutic approaches. These very questions and challenges make circadian immunometabolism an incredibly exciting field of research with genuine potential for novel personalised treatments for chronic disease.

Highlights

- Circadian rhythms are controlled by cell autonomous transcription translation feedback loops (termed the molecular clock) that regulates gene transcription on a 24 hr timescale. There are profound changes in metabolism and immune function over a 24 hr period.

- Alterations in metabolism have emerged as essential regulators of immune responses, a field called immunometabolism. As the molecular clock is a key regulator of metabolism and immunity, understanding how the molecular clock impacts on immunometabolism represents an exciting next step.

- Circadian disruption is prevalent across society and is linked with a range of chronic conditions. Therefore, understanding the integration of circadian biology, immunology and metabolism into a new field which, we term Circadian Immunometabolism, presents a unique opportunity to better understand the pathology of chronic diseases and how behavioural interventions or therapeutic options for the treatment of these conditions.

Outstanding Questions

- Is there a hierarchical biological order to circadian immunometabolism (see Figure 7)? In order to maintain circadian and metabolic homeostasis, is one process upstream of the others, or are they equivalent? Does this relationship differ depending on immune cell subtype?
- Is the initial trigger in chronic low grade inflammatory disorders circadian disruption driven metabolic changes of immune cells? Which chronic inflammatory diseases are most sensitive to circadian disruption? Can management of circadian rhythms improve low grade chronic inflammatory disorders?
- Under disease driven metabolic disturbance such as diabetes and obesity, what happens to the molecular clock in immune cells?
- The mitochondria are “the powerhouse of the cell” but their shape and size are strongly regulated by the molecular clock in non-immune cells. Is clock control of mitochondrial dynamics a major regulator of circadian immunometabolism?
- Can pharmacological agents targeting clock components in immune cells represent a novel approach for the treatment of chronic disease?

- What technical advances and models are required in order to study the combination of circadian rhythms, metabolism and immune cell function in models of human disease?
- How will the findings on circadian immunometabolism in mice (which is a nocturnal animal), translate to diurnal humans?
- Which drug classes and inflammatory disease conditions are likely to benefit the most from chronotherapy approaches?
- Does fuel dependency and metabolism of immune cells alter throughout the day in line with feeding and fasting rhythms and if so how does these temporal alterations in metabolism regulate immune function?

- How much can fuel dependency and intracellular metabolism explain time-of-day differences observed in immune function, and how does restricted feeding alter immune cell function?

Clinician's corner

- Understanding circadian biology is more pertinent than ever given our 24/7 society. Many of us spend our days and nights indoors under artificial light day and have erratic eating patterns. This is causing circadian disruption and may be a larger contributor to the increase in chronic inflammatory pathologies observed in the western world than is currently realised.

- Treatment of inflammatory disorders by neutralising proinflammatory cytokines with antibodies has been one of the most successful recent advances in human health. Manipulation of immune cell metabolism presents a unique opportunity to manage the immune system in the treatment of inflammatory disease. An understanding how the circadian system impacts on immunometabolism could provide more efficacious and personalised approaches in the treatment of chronic inflammatory disease.

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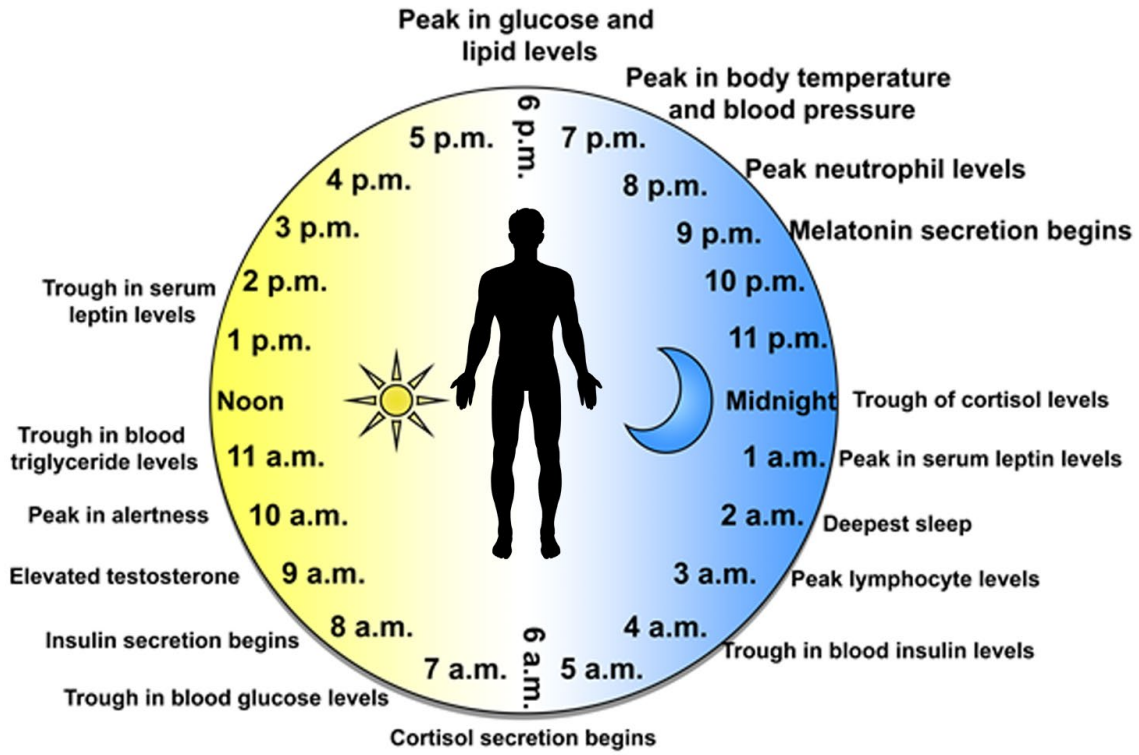

Figure 1. Diurnal Rhythms in Human Physiology and Metabolism. An overview of behaviours, physiology immune and metabolic events, which cycle throughout the day in humans.

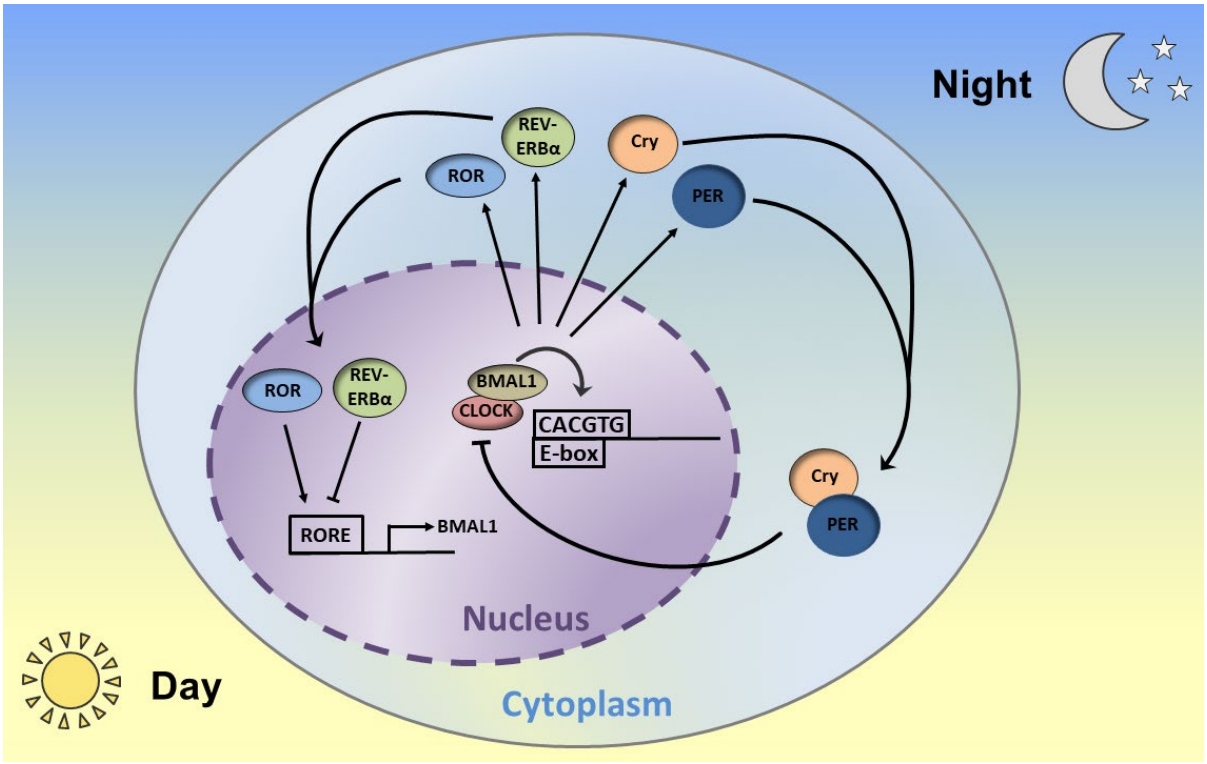

Figure 2. Transcription translation feedback loops of the molecular clock. Central to these loops is a heterodimeric partnership between two transcription factors; circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like 1 (BMAL1). BMAL1 is the master clock gene as its deletion completely ablates all rhythmic activity throughout the organism. The bHLH-PAS domains allow BMAL1:CLOCK heterodimers to bind canonical E-boxes containing the consensus sequence CACGTG, or non-canonical E-boxes (examples of which include sequences such as CACGTT). This binding allows the transcription of Period (*Per1, Per2, Per3*) and Cryptochrome (*Cry1, Cry2*), components of the negative arm of the molecular clock. In turn, CRYs and PERs form large complexes in the cytoplasm, which in time translocate back to the nucleus and suppress their own expression by disrupting the BMAL1:CLOCK heterodimer, and the cycle begins again. Further regulatory input into the clock occurs through the nuclear receptors REV-ERBα and REV-ERBb, and retinoic acid ophan receptor RORα, b and g [5, 6]. REV-ERBs and RORs are transcriptional repressors and activators, and drive cyclic expression of Bmal1 by binding to RORE elements in *Bmal1* gene promoter. ROREs are also present in other clock gene promoters such as *Clock* and *Cry1* to fine tune their expression. Transcription is not the sole mechanism to regulate these rhythms, as post-translational modifications including phosphorylation and acetylation have also shown to be important in maintaining circadian oscillations [7, 8].

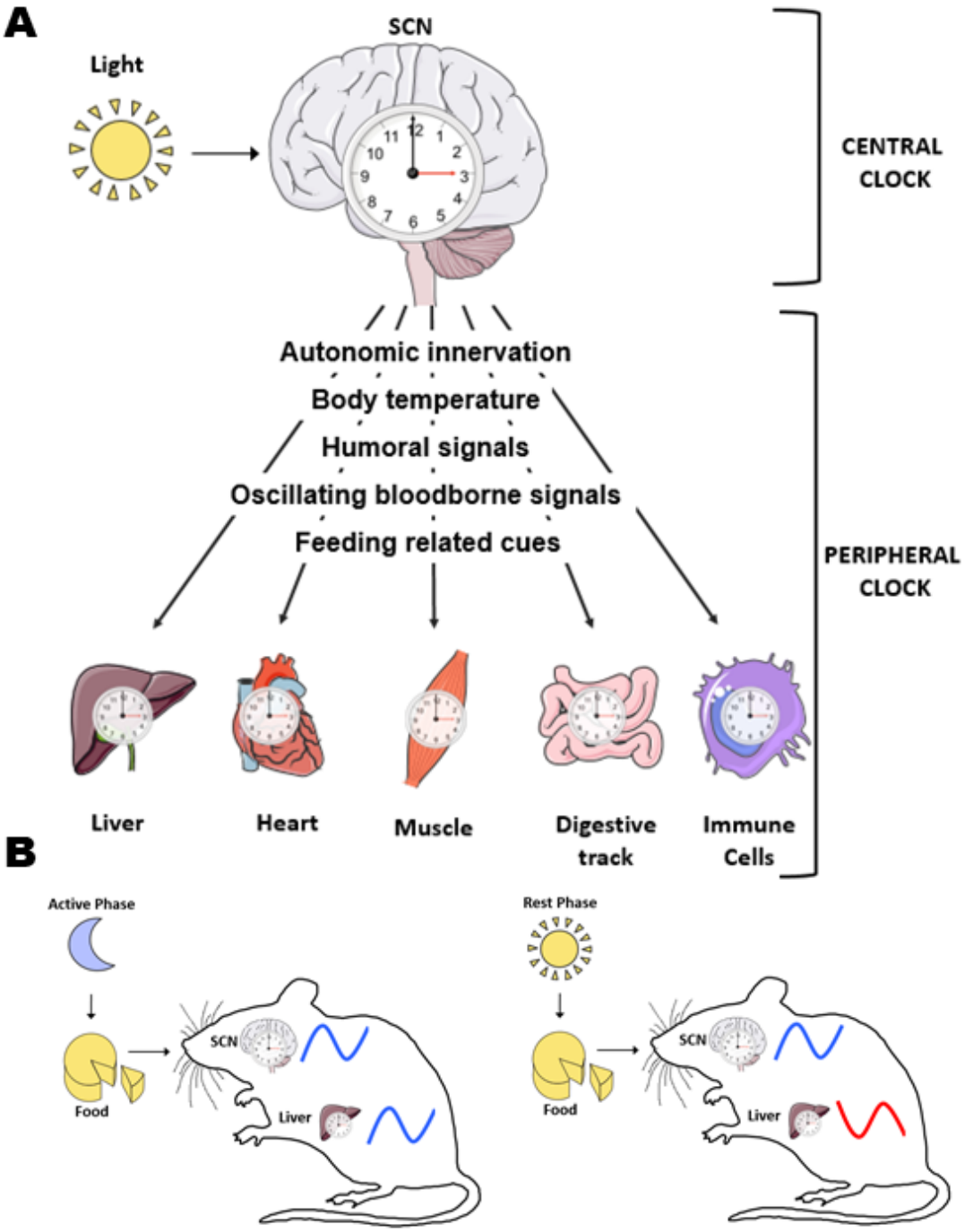

Figure 3. Communication between the central SCN clock and peripheral clocks. (A) Light provides entrainment signals for the central clock through the SCN. The SCN then coordinates signals to the peripheral clocks ensuring that the whole organism is synchronised with the external environment. Other entrainment signals can regulate the peripheral clocks, such as body temperature, hormones or feeding/fasting. (B) Mice which are restricted to feeding on a high fat diet during their inactive phase gain more weight mice restricted to a high fat diet during their active period [69]. Introduction of light during the dark leads to increased weight gain in mice. When mice are fed during their inactive phase, they do not consume more calories; however, they continue to increase their body mass [99]. Feeding is no longer aligned with the correct form of metabolism to process the food intake as it should. Time restricted feeding could be a non-pharmacological approach to reducing obesity without the need to reduce caloric intake [69].

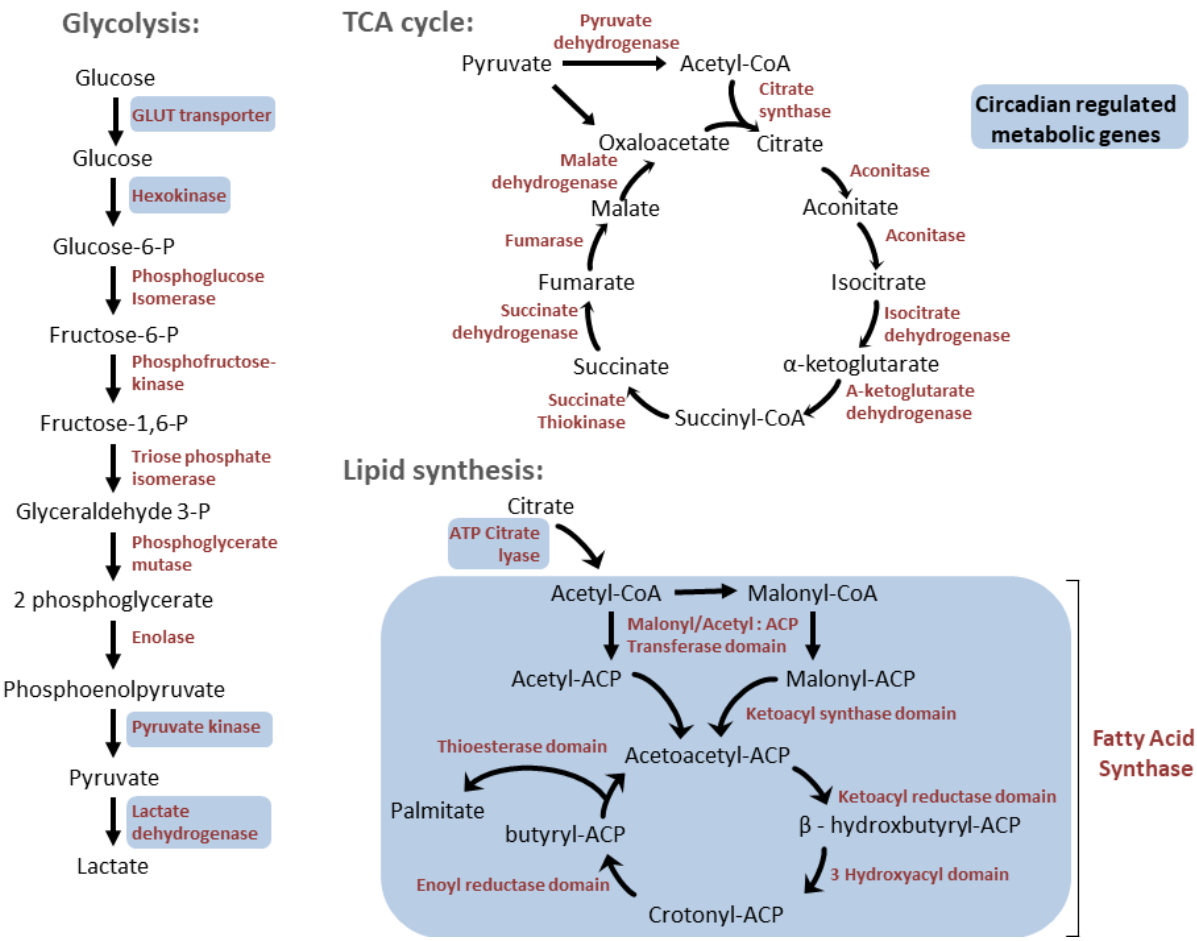

Figure 4. Circadian rhythms of carbon metabolism pathways of glycolysis, TCA cycle and lipid synthesis. Intermediate reactions of glycolysis showing the breakdown of glucose to either pyruvate or lactate. The intermediate reactions of the TCA cycle showing the reactions that sustain the cycle. The intermediate reactions occurring in the several enzymatic domains of the enzyme, fatty acid synthase (FAS), which result in palmitate synthesis.

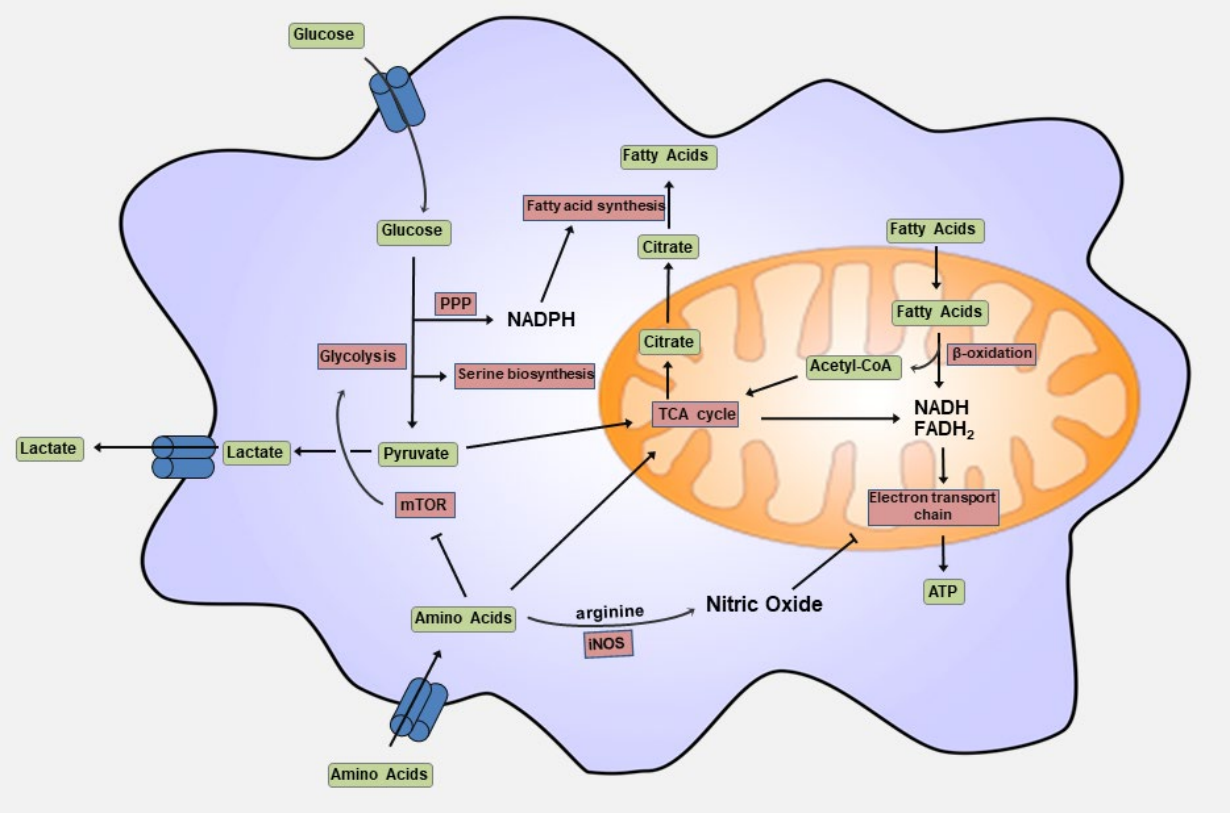

Figure 5. The interconnectivity of the major cellular metabolism pathways. Representation of the major metabolic pathways of macromolecules. Imported glucose is converted to pyruvate by glycolysis. Pyruvate is then converted to lactate in the absence of oxygen; however, if oxygen is present it gets transported to the mitochondria where it is converted to acetyl-CoA. This then enters the TCA cycle to produce the cofactors, NADH and $FADH_2$, which are used by the electron transport chain to create a proton gradient for ATP synthesis. Beta-oxidation and amino acid metabolism also contribute acetyl-CoA to the TCA cycle to produce ATP. The TCA cycle contributes citrate for the synthesis of lipids. Glycolysis provides metabolites for the pentose phosphate pathway that generates NADPH, a cofactor necessary for lipid synthesis. The amino acid arginine can be converted to nitric oxide by iNOS that can inhibit oxidative phosphorylation.

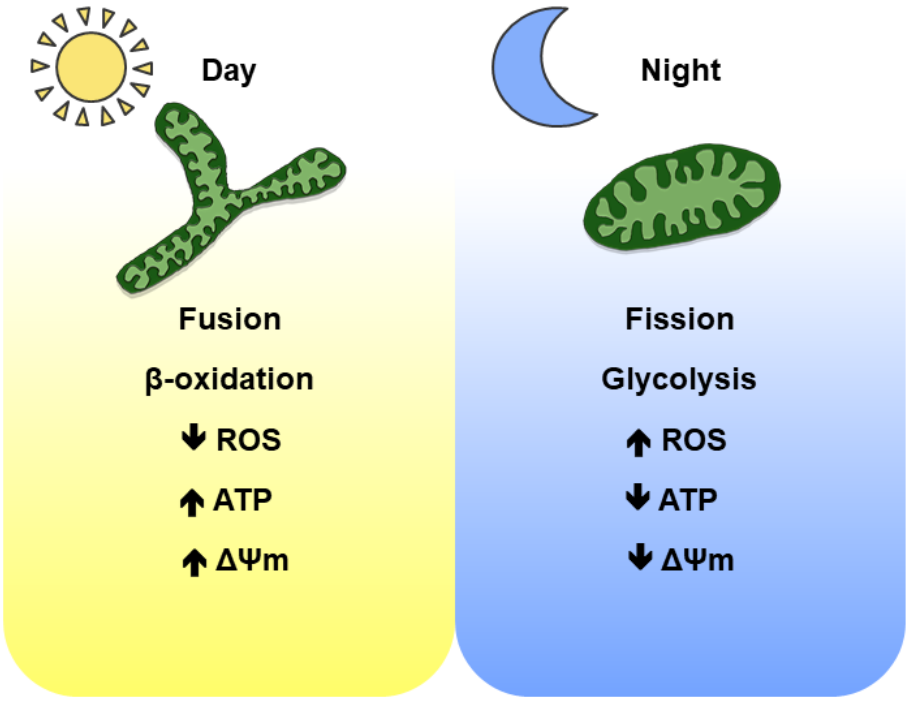

Figure 6. Circadian rhythms of mitochondrial dynamics. The shape and size of mitochondrial vary over the course of the day. During the day, mitochondria have a fused morphology. This morphology leads to increased oxidative phosphorylation, β-oxidation, membrane potential and ATP production. During the night, mitochondria are in a fragmented morphology. This morphology leads to decreased mitochondrial oxidative phosphorylation, membrane potential and ATP production. This morphology is also associated with increased glycolysis and ROS.

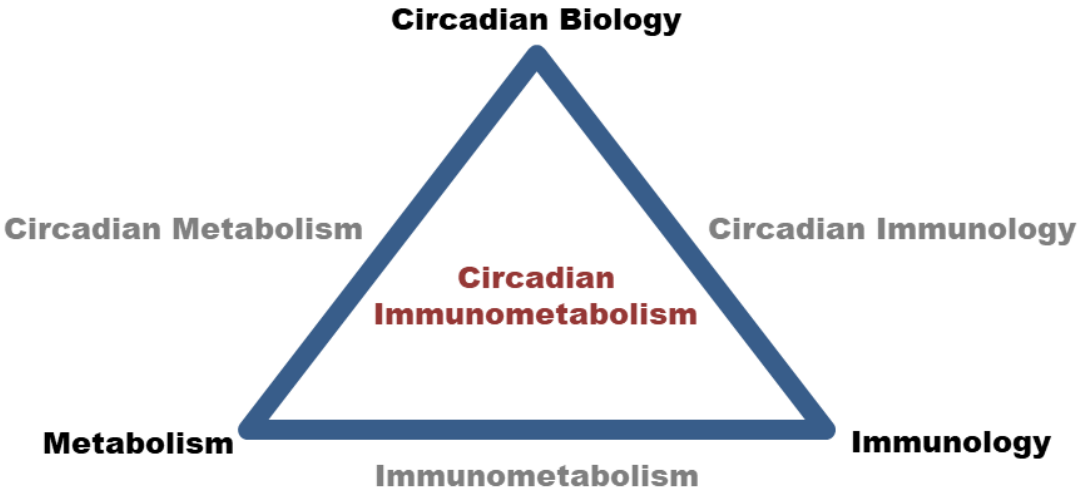

Figure 7. Graphical representation of the convergence of circadian biology, metabolism and immunology.