Chapter 2

Circadian Body Temperature Rhythm and the Interaction with Energy State

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Additional information is available at the end of the chapter

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Abstract

We have revealed that circadian body temperature (T_b) rhythm is significantly influenced by fasting/fasting-related hormones. The effect of circadian mechanism and fasting/fasting-related hormones on thermoregulation was examined. Fasting decreases T_b during the light phase in rodents. For the regulation, the suprachiasmatic nucleus (SCN) and clock genes, such as *Cry* and *Clock*, are necessary. In addition, ghrelin and several hypothalamic nuclei, that is, the medial preoptic area, paraventricular nucleus (PVN), and arcuate nucleus (ARC), play a key role in the T_b rhythm. During the light phase, fasting and ghrelin affect the hypothalamic areas. The activity of the SCN increases and that of the ARC decreases. The SCN sends inhibitory signals to the PVN, which may result in a lower heat production in the interscapular brown adipose tissue (iBAT) and T_b. By contrast, during the dark phase, the activity of the SCN decreases and that of the ARC increases. The inhibitory signal from the SCN is less, and the PVN is activated. Heat production of the iBAT increases and T_b is maintained. There are functional and anatomical connections between the circadian and thermoregulation systems. The circadian system modulates thermoregulatory response to hypothermia and/or cold depending on time and feeding condition.

Keywords: fasting, ghrerin, leptin, brown adipose tissue, paraventricular nucleus, arcuate nucleus, suprachiasmatic nucleus

1. Introduction

The temperature of the cell(s), tissues, organs, and body is an important factor that determines the biological functions and survival of organisms, from prokaryotes to vertebrates, although the preferred temperature varies. Homeothermic animals can constantly regulate body temperature (T_b). Thermoregulation is the balance between heat loss and heat

production in the body. The temperature regulation is different among species, and the range of temperature regulation is fairly narrow compared to the larger temperature ranges in the living environment [1].

Homeothermic animals show a circadian T_b rhythm, although the goal of thermoregulation is to maintain a constant T_b. The T_b is higher in the active phase and lower in the inactive phase in both diurnal and nocturnal animals. The T_b rhythm is also observed under conditions wherein the influence of physical activity (i.e., heat production) is minimized. For example, circadian T_b rhythm is observed even in individuals forced to be on complete bed rest [2]. Results suggested that the circadian T_b rhythm can also be regulated.

Despite the usual small amplitude of the circadian T_b rhythm (e.g., less than 1°C in human beings [3]), the rhythm is still important for preserving energy during the inactive phase. The maintenance of higher T_b is energy-costly because more energy from the total daily intake is used for heat production [4]. Therefore, the circadian T_b rhythm may be important in saving energy in homeothermic animals when energy is not needed.

Environmental temperature significantly affects the thermoregulation system. We assessed the circadian T_b change in rats that were placed in an ambient temperature of 18, 25, or 32°C (unpublished data). Laboratory rats are usually housed at 25°C. However, such cold or heat stress did not alter the circadian T_b rhythm. Results suggested that the T_b rhythm is not simply a result of circadian change in heat loss or production in the body. Rather, it is a result of the coordinated thermoregulatory processes that maintain a specific T_b at a given time of the day. Moreover, the T_b rhythm is probably generated by the association between the circadian and thermoregulation system. However, the mechanism is not yet known.

As mentioned previously, the T_b rhythm remains unchanged even when environmental temperature is altered. However, our previous studies have revealed that the T_b rhythm is remarkably influenced by fasting/fasting-related hormones. It is hypothesized that a lack of energy affects thermoregulation and the T_b rhythm. However, several previous studies do not support this hypothesis. In this study, a review of the literature on the mechanism and physiological effects of the circadian T_b rhythm was carried out.

2. Association between fasting and circadian core body temperature rhythm

Fasting is a strong stimulus that changes the amplitude of the circadian T_b rhythm in both mammals and birds [5–8]. In a previous study by Tokizawa et al. [9], T_b was obtained with a thermometer placed in the abdominal cavity of mice, and it was continuously and noninvasively monitored with a telemetry. The most significant reduction was during the light (inactive) phase, whereas the reduction in the dark (active) phase was not remarkable (**Figure 1A**). In addition, no difference was observed in the spontaneous activity during both phases (**Figure 1B**). Results suggested that less heat production due to a decreased physical activity was not associated with the mechanism involved in the reduction of T_b during fasting. Moreover, a remarkable change in T_b was observed at a specific time, which may indicate an association with the circadian rhythm, although the phase shift of the rhythm was not observed.

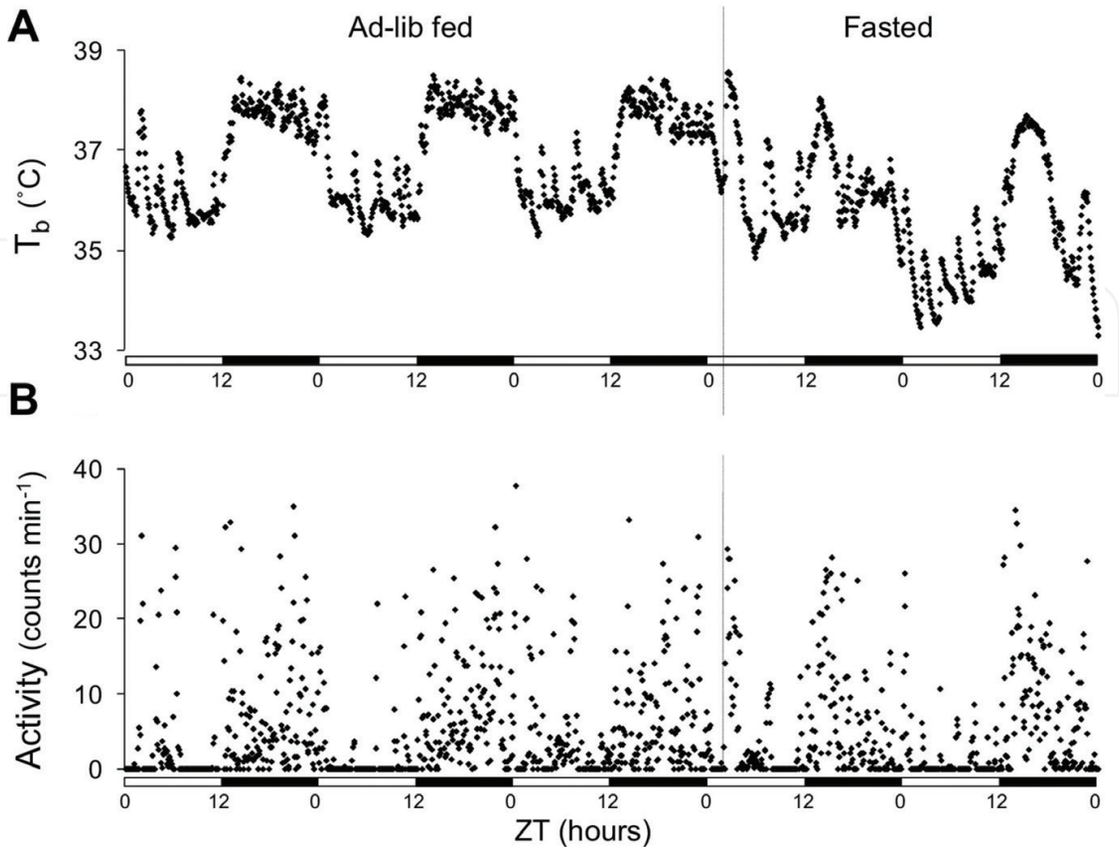

Figure 1. Circadian changes in T_b and spontaneous activity during *ad lib* feeding and fasting in settings with normal temperature (27°C) in mice. The x-axis indicates ZT. The lighting condition is shown at the bottom with the open bar denoting the light phase and the closed bar the dark phase. Data were plotted in 5-min bins. The vertical dashed line shows the time when the 48-h fasting period was started. The modified figure was obtained from the manuscript by Tokizawa et al. [9].

Compared to that during a normal T_b rhythm, the significant decrease in T_b during the light phase (i.e., from 36.0°C during feeding to 34.1°C during fasting) may lessen heat transfer from the body to the environment and preserve energy. In contrast, the maintenance of T_b during the dark phase may be essential in the maintenance of physical activity. On the basis of previous studies, the following questions might be raised: (1) Is the reduction in T_b during the light phase a regulated phenomenon by the thermoregulation system? (2) Is the circadian system involved in the phenomenon? and (3) Which factor that is associated with fasting causes the decrease in T_b, and how?

3. Physiological mechanism involved in the decrease in body temperature during fasting

T_b in homeothermic animals is controlled by heat production and heat loss mechanisms. However, whether such control is maintained and generates the circadian T_b rhythm during fasting is still not verified. Fasting is a strong stimulus that decreases metabolic heat production in rodents and pigeons [5, 6, 10–15]. In addition, fasting decreases thermal conductance

(i.e., opposite of thermal resistance) from the body core to the environment (heat loss [16–18]). If these physiological responses occurred in the same manner during the day of fasting, the reduction of T_b, specifically during the light phase, would not be observed. Therefore, the thermoregulatory responses must be different during the two phases.

Nagashima et al. [19] assessed the T_b, counts of locomotor activity, and oxygen consumption rate ($\dot{V}O_2$) in rats that were made to fast for 3 days. **Figure 2** illustrates the data before fasting and on the last day of fasting and recovery day. T_b and counts of activity were obtained as the

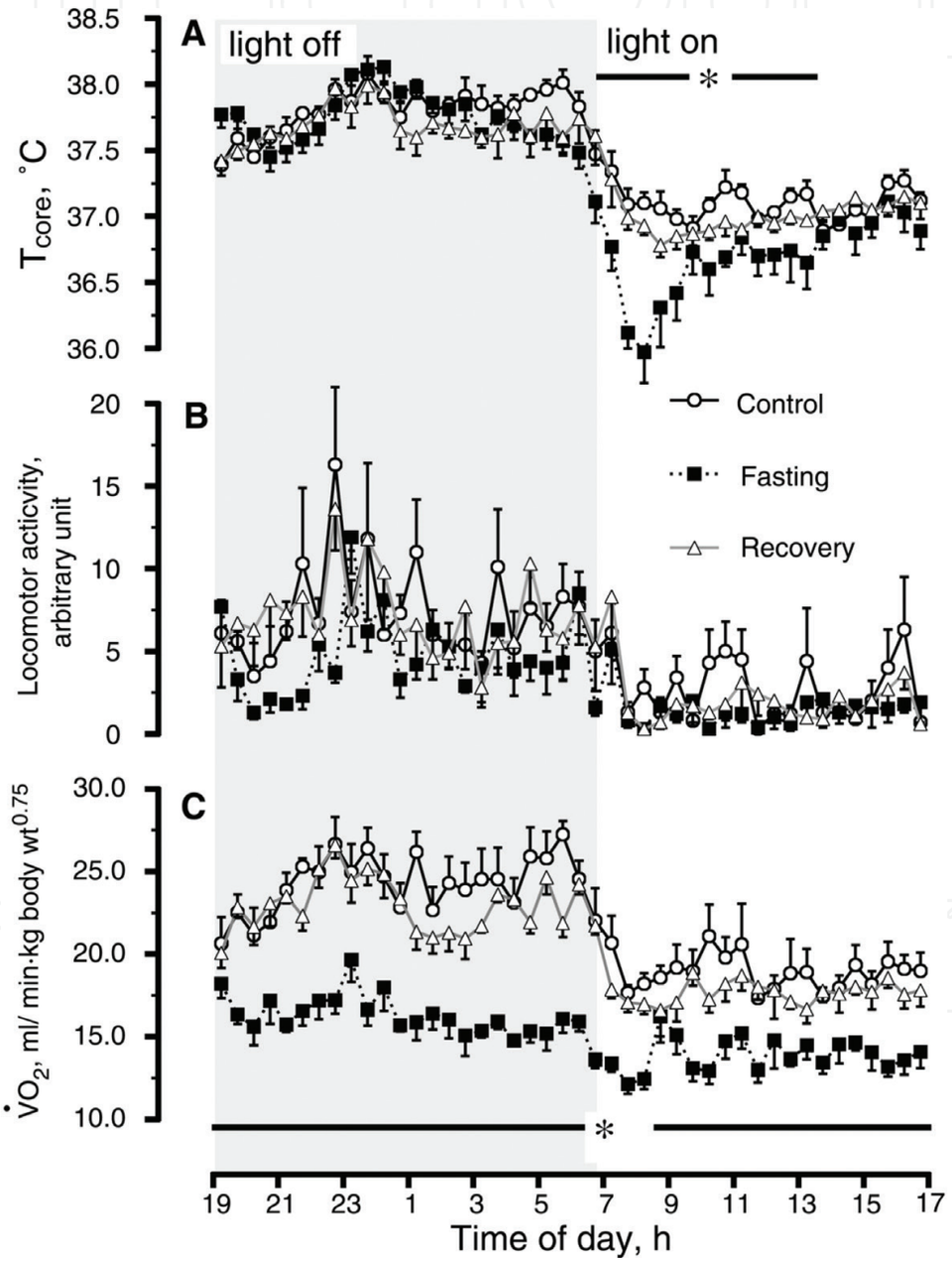

Figure 2. Body core temperature (*A*, T_{core}), counts of locomotor activity (*B*), and oxygen consumption rate (*C*, $\dot{V}o_2$) during feeding, day 3 of fasting, and day 4 of recovery. Stippled area, dark phase (1900–0700). Each point has an average of 30 min. The values are the means ± SE in six rats. *Significant difference from controlled feeding, $P < 0.05$. The figure was obtained from the manuscript by Nagashima et al. [19].

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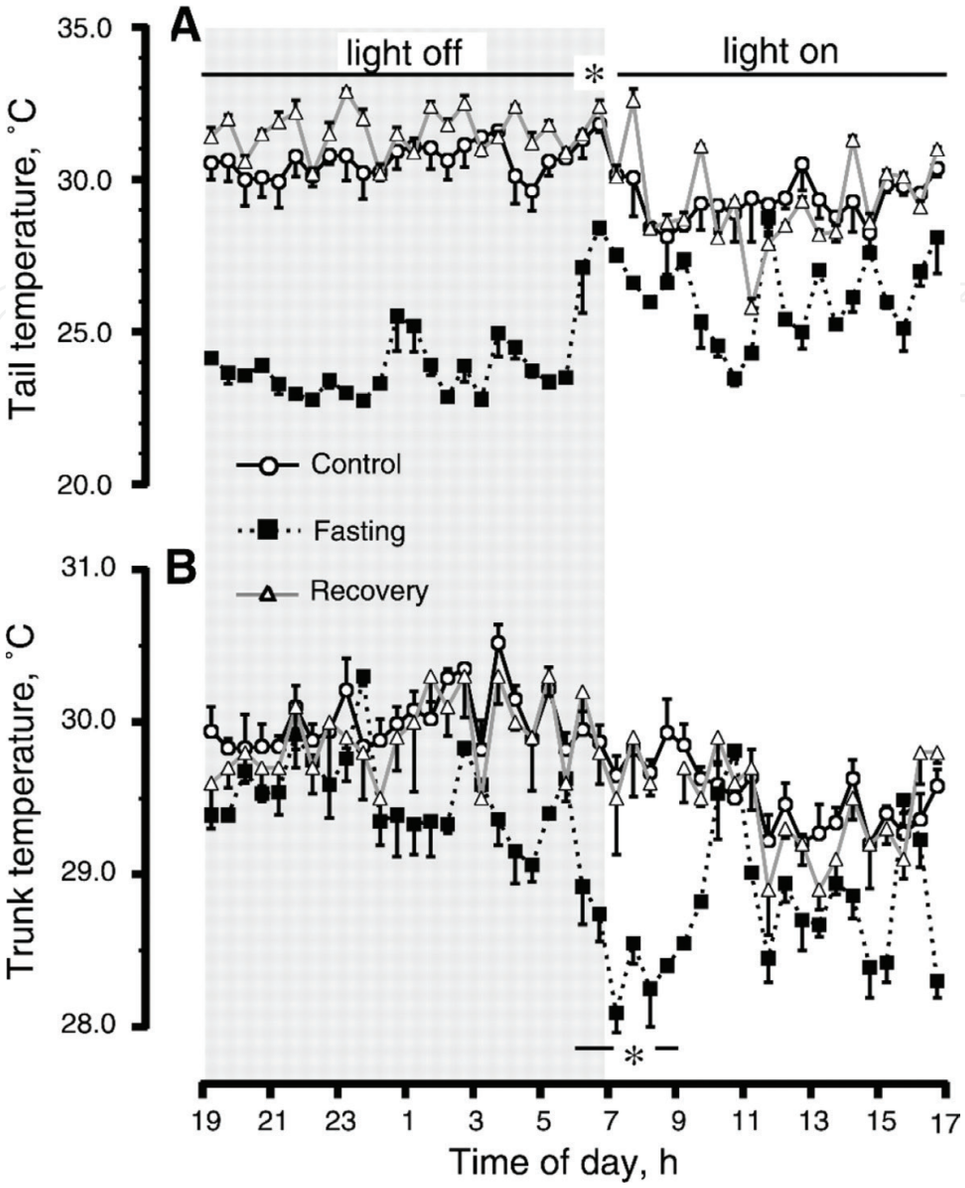

Figure 3. Surface temperatures of the tail (*A*) and trunk (*B*) determined by thermography in a controlled feeding condition, on day 3 of fasting, and day 4 of recovery. T_{tail} is the average of surface temperatures at one-third of the length of the tail from the root and the tip. T_{trunk} is the average of surface temperatures of the head and middle parts of the upper and lower back. Each point has an average of 30 min. The values are the means ± SE in five rats. *Significant differences from controlled feeding conditions, $P < 0.05$. The figure was obtained from the manuscript by Nagashima et al. [19].

study by Tokizawa et al. [9], and $\dot{V}O_2$ was assessed by indirect calorimetry. $\dot{V}O_2$ decreased during fasting in both dark and light phases. Although the rhythms of T_b and $\dot{V}O_2$ were significantly associated during feeding, the amplitude of the $\dot{V}O_2$ rhythm decreased and that of T_b increased during the fasting. The result suggested that T_b was maintained by the suppression of heat loss during fasting and the dark. The estimated thermal conductance during the dark phase of the fasting period decreased from that in the fed condition (1.10 and 1.65 $ml \cdot min^{-1} \bullet kg\ body\ wt^{-0.75} \cdot °C^{-1}$, respectively), which supported the hypothesis. On the contrary, such a change was not observed during the light phase.

Animals use several mechanisms to change the efficiency of heat loss. Among the mechanisms, the tail is a crucial site for the regulation of heat loss in rats and mice, with its physiological and anatomic characteristics, that is, a high density of arteriovenous anastomosis [6], the absence of fur, and a remarkable surface-to-volume ratio. Young and Dawson [20] reported that rats could dissipate 25% of basal heat production by changing the blood flow in the tail.

To assess the contribution of the tail in thermoregulation, Nagashima et al. [19] estimated the tail surface temperature by thermography (**Figure 3**). Tail temperature during the fasting day was lower than that during the fed control day. However, body trunk temperature during the fasting was lower than that during the fed control only around the light-onset period. Interestingly, different from the fed control, tail temperature in the fasting condition increased after light onset and remained at a higher level during the light phase. The results strongly suggested an attenuation of tail blood flow in the dark phase during the fasting condition and were blunted at the beginning of the light phase. Thus, the tail may help in determining the process of heat loss from the body in the fasting condition, which was the factor regulating the T_b rhythm.

4. Involvement of suprachiasmatic nucleus in T_b rhythm during fasting

The suprachiasmatic nucleus (SCN) in the hypothalamus is thought to be the master clock of the circadian rhythm. In addition, electrical or chemical lesions of the SCN destroy the T_b rhythm in rodents [21–26]. However, these findings may not indicate that the circadian clock regulates the T_b rhythm. Behavioral and/or physiological responses, such as locomotor activity, eating, and the secretion of several hormones, are also inhibited due to the SCN lesion, which may have direct influences on heat production and T_b [17, 27–29]. Simply put, SCN lesion inhibits such behavioral and/or physiological responses [3, 21, 24, 30], and the T_b rhythm is subsequently destroyed.

Liu et al. [23] assessed the circadian T_b rhythm within 4 days of fasting in rats, of which SCN was electrically lesioned. The rats had arrhythmia of T_b and spontaneous activity, and no difference was observed during the light and dark phases and the non-fasting and fasting periods. Although the experiment did not answer the question of whether the circadian clock directly regulates the T_b rhythm, the result suggested that SCN may be important for the changes in thermoregulation and T_b due to fasting.

5. Importance of molecular circadian mechanism on T_b rhythm during fasting

Several genes in the central and peripheral tissues have expression rhythms with a periodicity of ~24 h. Among these genes, those with autoregulatory transcription-translation loops with a periodicity of ~24 h may be responsible for the core molecular mechanisms of the circadian clock (i.e., clock genes). These genes are observed in both the central and the peripheral tissues [31–33].

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Nagashima et al. [34] assessed the circadian T_b rhythm in mice that lack the cryptochrome 1 and 2 genes (*Cry*1 and *Cry*2), two of the core clock genes [35–38]. The mice loose the transcription-translation rhythms of the *Cry*1 and *Cry*2 and other clock genes and periodicity in a wheel-running behavior [38], as well as the electrophysiological activity of the SCN cells under constant dark conditions [39]. However, the behavior is suppressed under the light condition, and the daily rhythm is observed under both light and dark conditions. Therefore, despite the lack of the internal rhythm, lighting rhythm could induce T_b rhythm.

Table 1 summarizes the cosinor rhythm analysis [34] of T_b, spontaneous activity, and $\dot{V}O_2$ in normal wild-type mice that lack *Cry*1/*Cry*2 (*Cry*1$^{-/-}$/*Cry*2$^{-/-}$). The mice were placed under three different conditions: (1) constant darkness (DD) with ad lib feeding, (2) 12–12-h light-dark (LD; lights on at 1900) cycle with ad lib feeding, and (3) LD with food restriction. The food restriction protocol aimed to enhance the eating rhythm. Ordinary chow was given at 1900, which is the end of the light period, at 70–80% of the normal daily intake. Both *Cry*1$^{-/-}$/*Cry*2$^{-/-}$ and wild-type mice finished eating 10–12 h after the appearance of chow in the food-restriction regimen.

In the wild-type mice, no significant difference was observed between the mean, amplitude, and peak phase of the T_b rhythm under the LD condition with ad lib feeding and DD condition. Differences were found in the T_b and $\dot{V}O_2$ rhythms between ad lib feeding and food-restriction days (i.e., greater amplitude of the rhythms during the food-restriction day). The activity rhythm did not change throughout the three conditions. In *Cry*1$^{-/-}$/*Cry*2$^{-/-}$ mice, T_b, $\dot{V}O_2$, and spontaneous activity were arrhythmic under the DD condition. However, T_b, $\dot{V}O_2$, and spontaneous activity became rhythmic in the same peak phases during the LD conditions. As for the T_b and $\dot{V}O_2$ rhythms in the LD condition, the daily means were lower, and the amplitudes of the rhythms were higher in the food-restriction condition than those under the ad lib feeding condition. The study showed that the circadian T_b rhythm is observed even in mice that lack the internal circadian mechanism, when an external lighting and feeding stimuli that alter heat production are observed. The result showed that the heat production rhythm may be a key component for the T_b rhythm.

	T_{core}, °C			$\dot{V}O_2$, ml · min^{-1} · kg body wt$^{-0.75}$			Activity, Au		
	Fed	**Fast**	**Rec**	**Fed**	**Fast**	**Rec**	**Fed**	**Fast**	**Rec**
Mesor	37.5 ± 0.1	37.3 ± 0.1*	37.4 ± 0.1	21.63 ± 0.84	15.02 ± 0.55*	20.11 ± 0.78‡	5.0 ± 1.0	3.2 ± 0.3	5.0 ± 0.9
Amplitude	0.5 ± 0.1	0.7 ± 0.1*	0.4 ± 0.1*,‡	3.74 ± 0.44	1.57 ± 0.27*	3.06 ± 0.38*,‡	3.4 ± 0.6	2.6 ± 0.3	3.5 ± 0.5‡
Acrophase	18.2 ± 0.3	16.4 ± 0.3*	17.5 ± 0.3*,‡	18.2 ± 0.2	16.4 ± 0.3*	17.6 ± 0.4*,‡	17.7 ± 0.4	17.9 ± 0.4	17.3 ± 0.6
r	0.76–0.91†	0.80–0.85†	0.56–0.91†	0.51–0.85†	0.36–0.61†	0.45–0.74†	0.38–0.67†	0.39–0.63†	0.43–0.67†

Values are means ± SE. $\dot{V}O2$, oxygen consumption rate; Au, arbitrary unit; T_{core}, core temperature; Fed, *day* 4 of fed control period; Fast, *day* 3 of fasting period; Rec, *day* 4 of recovery from fasting. Acrophase is shown in zeitgeber time (ZT, ZT 0 = 0700).

*Significantly different from control, $P < 0.05$.

†Significant regression coefficient (r) for fitted cosine curve for daily change of variable, $P < 0.05$.

‡Significantly different from fasting period, $P < 0.05$.

Table 1. Analysis of cosinor rhythmometry for daily changes in core temperature, Vo_2, and locomotor activity.

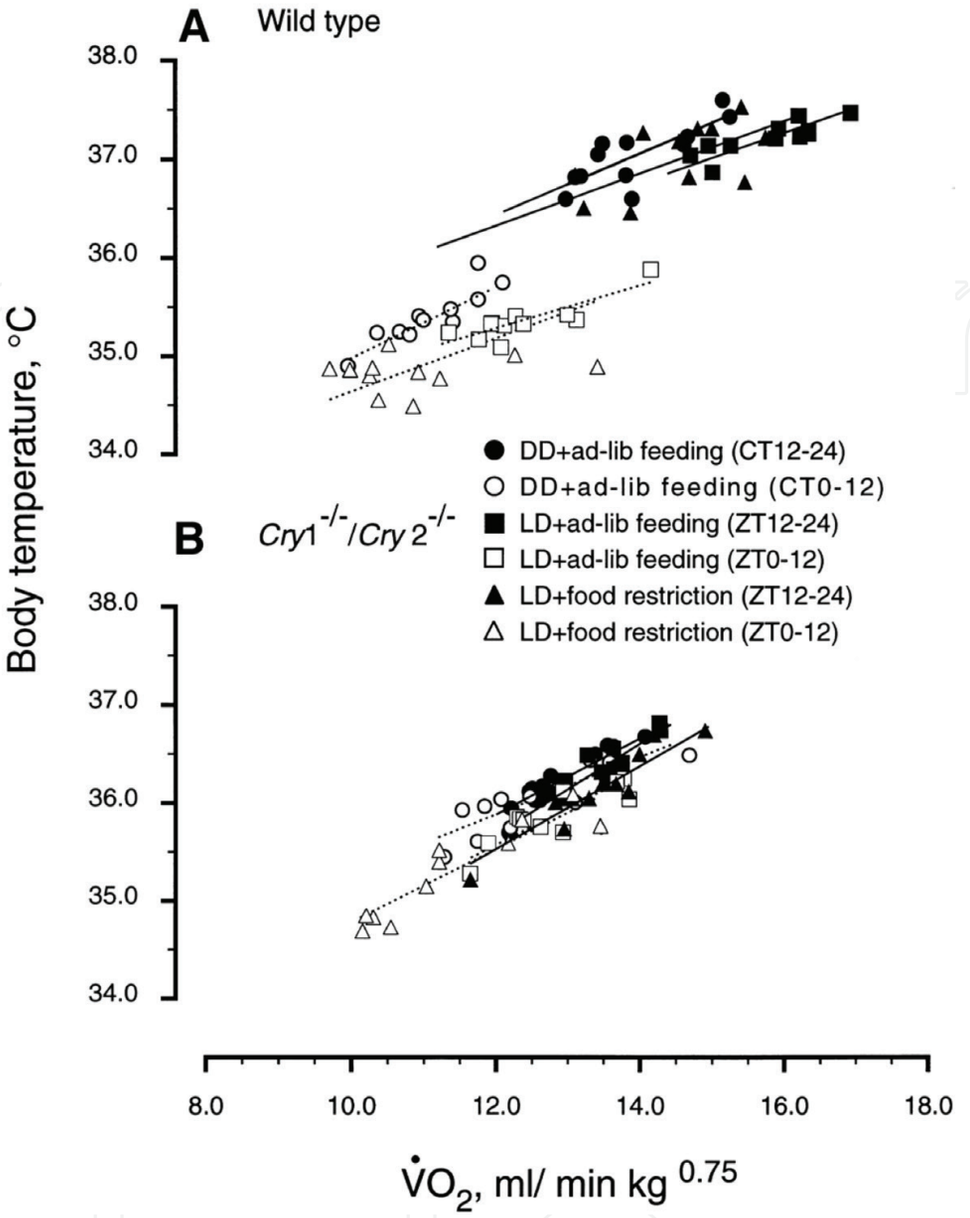

Figure 4. Relationship between metabolic heat production ($\dot{V}O_2$) and T_b in wild-type mice (*A*) and *Cry*1$^{-/-}$/*Cry*2$^{-/-}$ (*B*) based on three trials. The values are the means (±SE) in five mice for 30 min, and data corresponding to each hour are used for this illustration. Regression lines were applied for the averaged values in each group. The figure was obtained from the manuscript by Nagashima et al. [34].

Figure 4 shows the results of the regression analysis of the $\dot{V}O_2$ and T_b of each group. If heat production was the sole determinant of circadian T_b rhythm, these regressions must be identical regardless of trial, circadian phase, and group. In addition, model equations fitted to all the data in the three trials within the same phase and the group was established. Model equations were also created for all possible associations among the three trials. The analysis showed that the data could be classified into four groups: those at circadian time (CT; a standard of time based on the free-running period of a rhythm). The onset of activity of diurnal organisms defines circadian time 0, CT 0. The onset of activity of nocturnal organisms defines circadian time 12, CT 12, or zeitgeber time (ZT; under standard light–dark cycles, the time of lights on defines zeitgeber time 0 and the time of lights off defines zeitgeber time 12), 12–24

and CT or ZT 0–12 in *Cry*1$^{-/-}$/*Cry*2$^{-/-}$ mice and wild-type mice, respectively. Moreover, the model indicated that the two regressions in the wild-type mice were different (i.e., for the active and inactive phases), and those in the *Cry*1$^{-/-}$/*Cry*2$^{-/-}$ mice were similar. The results suggested that the circadian phase can be a significant factor in determining the T_b rhythm in wild-type mice but not in the *Cry*1$^{-/-}$/*Cry*2$^{-/-}$ mice. Thus, T_b was kept higher in the active phase than in the inactive phase regardless of the $\dot{V}O$ level in wild-type mice. Moreover, the regression slopes of $\dot{V}O$ and T_b in both phases were smaller than those of the *Cry*1$^{-/-}$/*Cry*2$^{-/-}$ mice (wild-type: 0.24 and 0.25 in the active and inactive phases, respectively; *Cry*1$^{-/-}$/*Cry*2$^{-/-}$: 0.44). Results showed that the wild-type mice can maintain their T_b within a narrower range than the *Cry*1$^{-/-}$/*Cry*2$^{-/-}$ mice over the same variation of $\dot{V}O$ in each phase. Thus, the circadian T_b rhythm in wild-type animals is not a simple byproduct of the heat production rhythm but a phenomenon regulated by the circadian system.

The mammalian sirtuins (SIRT) modulate the circadian epigenome and provide specificity in transcriptional control [40–43]. It was reported that the circadian clock regulates mitochondrial oxygen consumption rate in the oxidoreductase factor nicotinamide adenine dinucleotide (NAD^+)/SIRT3-dependent manner. In addition, NAD^+-dependent enzymes are important in fasting and oxidative metabolism. Peek et al. [40] evaluated NAD^+ biosynthesis, lipid and glucose oxidation, and acetylation of mitochondrial proteins in normal and circadian (Bmal1)-mutant mice. They reported that lipid oxidation and mitochondrial protein acetylation exhibited circadian oscillations that corresponded with the clock-driven NAD^+ cycle in the liver; however, rhythmic NAD^+ and oxidative cycles were self-sustained in fasted mice. These results suggest a strong interaction between circadian and metabolic rhythms and its destruction during fasting but do not explain for the change in circadian T_b rhythm during fasting.

6. Role of the circadian system and associated brain areas in controlling the circadian T_b rhythm during fasting

Tokizawa et al. [9] tested the hypothesis that thermoregulation is modulated by the circadian system (including the SCN and clock genes), depending on the time of day and feeding condition. Moreover, the physiological and neural responses of the mice during exposure to cold (20°C) under ad lib feeding and during 48-h fasting conditions and the dark and light phases were compared. The differences in the responses between wild-type and *Clock*-mutant mice were also examined. *Clock* is also a gene that organizes the core loop of molecular circadian oscillation. The mutation of *Clock* causes the disappearance of oscillation [44]. However, mutant mice show a T_b rhythm under light-dark conditions because of the masking effect of light.

During ad lib feeding at a low temperature setting (20°C), the T_b of the wild-type mice was similar to that of the wild-type mice during both the dark and light phases at normal temperature settings (27°C) (**Figure 5A** and **B**). However, $\dot{V}O_2$ was higher during the light phase at settings with a temperature of 27°C (ZT2.5–3.5; **Figure 5E**). During fasting at low temperature settings, the reduction in T_b increased during both the light and dark phases (ZT1–4 and 14.5–16, respectively; **Figure 5A** and **B**). However, T_b increased during the light phase. $\dot{V}O_2$ increased in settings with a temperature of 27°C at ZT13.5–14.5 during the dark phase (**Figure 5F**) but remained unchanged during the light phase (**Figure 5E**).

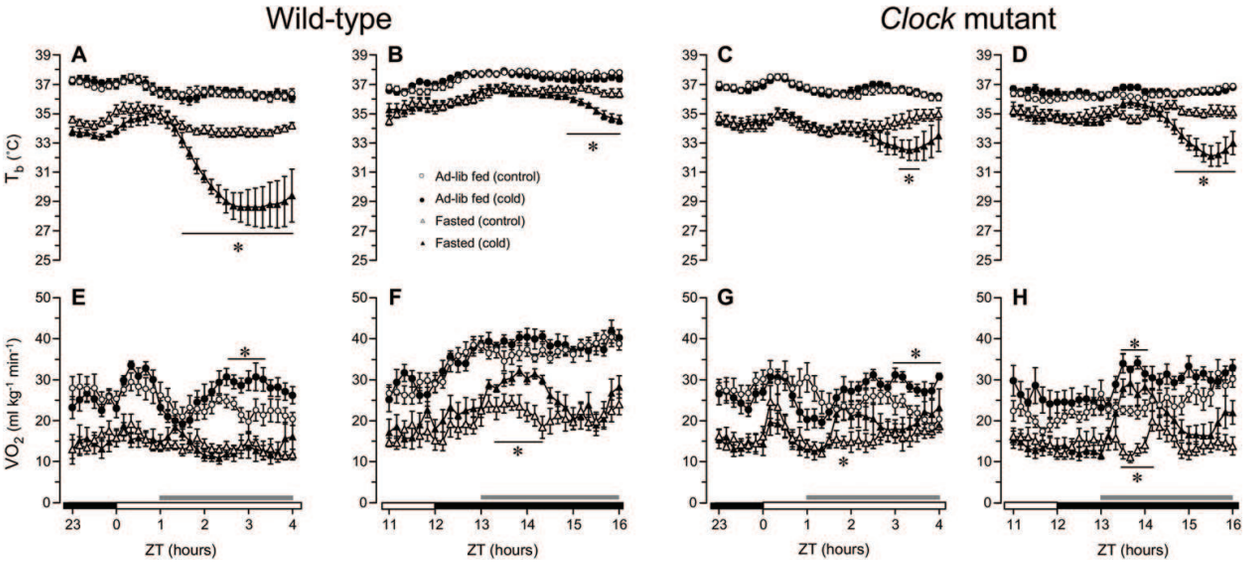

Figure 5. T_b and $\dot{V}O_2$ at low-temperature settings (27 or 20°C) with *ad lib* feeding or fasting in the wild-type and *Clock*-mutant mice (*A–H*). The values given are the means ± SEM ($n = 8$). $^{*}P < 0.05$, 20°C (low-temperature setting) versus 27°C (control setting) in each feeding condition. $^{\#}P < 0.05$, versus the dark phase in wild-type mice and both phases in *Clock*-mutant mice; $^{\S}P < 0.05$, versus dark phase in wild-type mice and light phase in *Clock*-mutant mice. The modified figure was obtained from the manuscript by Tokizawa et al. [9].

During ad lib feeding at low temperature settings, T_b in *Clock*-mutant mice was also maintained at the 27°C level (**Figure 5C** and **D**). $\dot{V}O_2$ increased above the 27°C level during both the dark and light phases (ZT3–4 and 13.5–14; **Figure 5G** and **H**), and no difference was observed between the two phases. During fasting at low temperature conditions, T_b decreased below the 27°C level at ZT3–3.5 during the light phase and ZT14.5–16 during the dark phase (**Figure 5C** and **D**), and no significant difference was observed between the phases. $\dot{V}O_2$ was higher than the 27°C level at ZT2 and 13.5–14 (**Figure 5G** and **H**). This increase in $\dot{V}O_2$ was higher during the dark phase than during the light phase.

The study indicated that the physiological response of mice to low temperature conditions is different during ad lib feeding and fasting in wild-type animals and during the dark and light phases. However, such differences were not observed or significantly decreased in *Clock*-mutant mice. The thermoregulatory mechanism of heat production is attenuated or inhibited during fasting and light phases. For such response, the circadian system is important. To support this result, the expression of uncoupling protein 1 (UCP1) mRNA [45–47] in the interscapular brown adipose tissue (iBAT, one of the effector organs for thermoregulatory heat production) was suppressed during the light phase and fasting in wild-type animals. However, cold exposure increased the expression during both dark and light phases in *Clock*-mutant mice.

7. Histological evidence on functional and anatomical connection between the circadian and thermoregulation systems

On the basis of the physiological findings, the association between the circadian and thermoregulation systems may be significant during fasting. The center of thermoregulation is thought to be in the hypothalamus [48, 49]. However, whether functional and anatomical

neural connections between the SCN exist (the center of the circadian system) and the hypothalamic subregion is involved in the thermoregulation remains unclear.

Liu et al. [23] assessed the cFos (i.e., early gene expression protein) expression in the SCN of rats. cFos can be a marker of neuronal activation in the brain [50]. The number of cFos immunoreactive (cFos-IR) cells changes daily, which is higher during the light phase and smaller during the dark phase. A 4-day fasting did not change the phase difference in the number of cFos-IR cells. However, the number increased during the dark phase and decreased during the light phase. Whether such change in the SCN causes altered T_b rhythm during fasting remains unknown. However, results showed that the SCN receives some information that is associated with fasting and alters T_b rhythm and/or the thermoregulatory responses.

Tokizawa et al. [9] evaluated cFos expressions in the SCN and other hypothalamic areas in wild-type and *Clock*-mutant mice. The mice were exposed to settings with a temperature of 20°C and/or 48-h fasting. Neural associations were also observed between the SCN and the hypothalamic areas involved in thermoregulation. Data are summarized in **Figure 6**. Fasting increased the number of cFos-IR cells in the SCN in both wild-type and *Clock*-mutant mice. The number was smaller in *Clock*-mutant mice than in wild-type mice. cFos-IR cells also increased during fasting in other hypothalamic areas, such as the medial preoptic nucleus (MPO), dorsomedial hypothalamus (DMH), paraventricular nucleus (PVN), and dorsal subparaventricular zone (dSPZ), in wild-type mice. Differences were observed in the number of cFos-IR cells during the dark and light phases (**Figure 6A**, **B**, **D**, **E**). Small increases were also observed in the DMH, ARC, and PVN in *Clock*-mutant mice. However, no phase differences were observed. Neural outputs from the SCN, including the SPZ, reached these hypothalamic areas [51, 52]. Thus, the activation of the SCN during fasting may be linked with the activation of the hypothalamic areas.

The preoptic area in the hypothalamus is thought to be important in thermoregulation because it has several thermosensitive neurons in the core body and skin temperatures [53]. In addition, stimulatory and inhibitory signals are sent from the area to other brain areas, which regulate the effector organs of thermoregulation, such as vasodilation, shivering, and non-shivering thermogenesis [48, 49]. The sympathetic outflow may originate from the PVN [54–56]. The DMH receives thermal input from the skin and is associated with the control of BAT thermogenesis [57]. Whether all cFos-IR cells are associated with circadian change of T_b and/or thermoregulation is not verified. However, the changes in cFos expression between the phases may, in part, be responsible for the decrease in T_b that was observed during fasting and the light phase.

Fasting increased the cFos expression in the ARC in mice [58, 59]. The ARC is involved in the regulation of food intake and energy expenditure, which responds to peripheral nutritional signals, such as the levels of leptin and insulin [60]. However, no study on the direct association between ARC and thermoregulation was conducted. The ARC had phase differences in cFos expression in wild-type mice (**Figure 5C**), which increased during fasting. The ARC has neural input from the SCN [61–63]. Therefore, the fasting signals received by the ARC increased during the dark phase and were attenuated during the light phase in wild-type mice, and this may be related to the signals from the SCN.

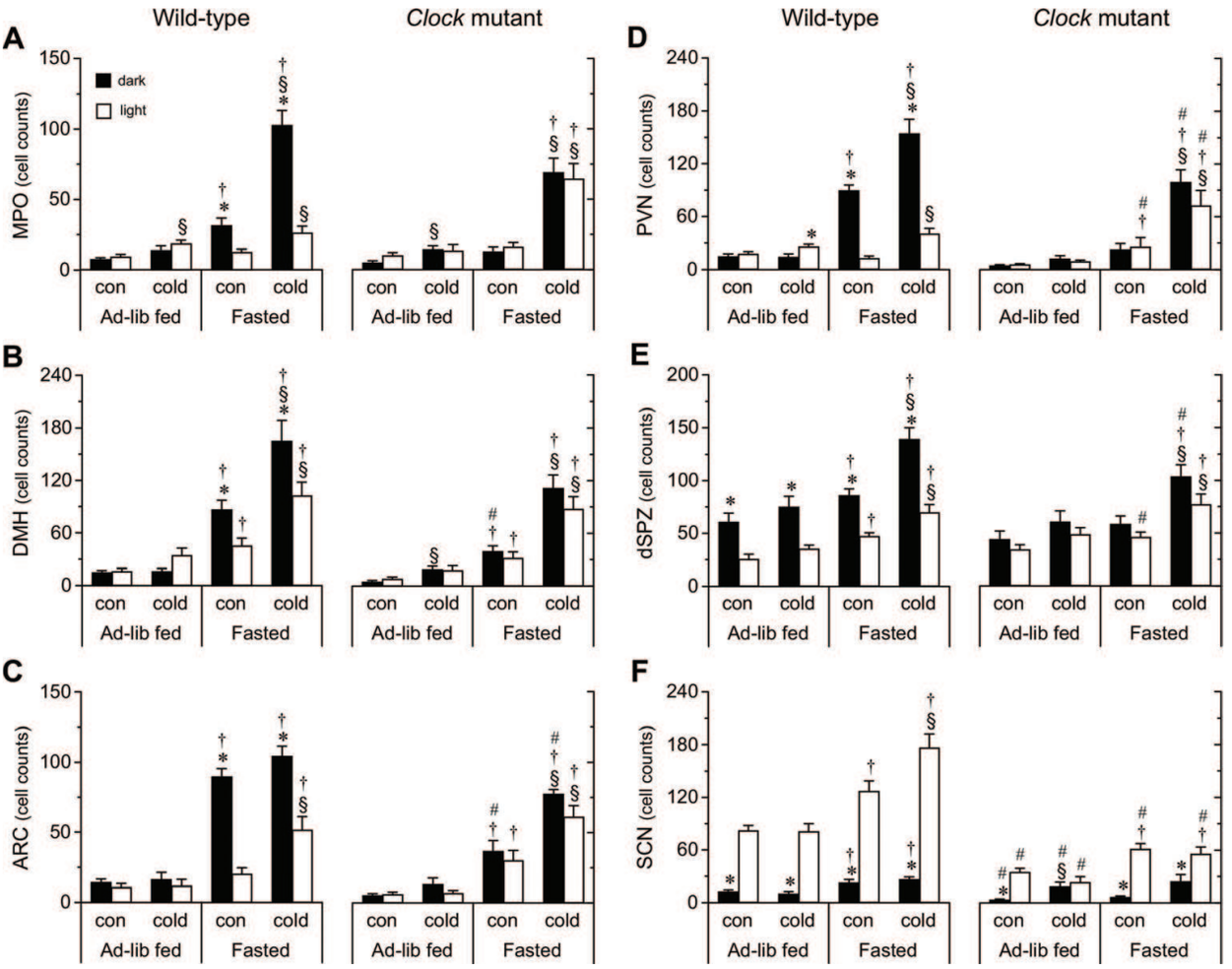

Figure 6. Counts of cFos-IR cells in the MPO (*A*), dorsomedial hypothalamus (*B*, DMH), ARC (*C*), PVN (*D*), dorsal subparaventricular zone (*E*, dSPZ), and SCN (*F*) under various conditions. Values are the means ± SEM ($n = 8$). $^{*}P < 0.05$, dark versus light phase; $^{\S}P < 0.05$, 20°C (low-temperature setting) versus 27°C (control setting); $^{\dagger}P < 0.05$, fasting versus *ad lib* feeding; $^{\#}P < 0.05$, wild-type versus *Clock*-mutant mice. The figure was obtained from the manuscript by Tokizawa et al. [9].

The increase in cFos expression in *Clock*-mutant mice that was attributed to low temperature during fasting was also observed in all the hypothalamic areas besides the SCN. However, no differences were observed between the two phases. In wild-type mice, the number of cFos expression in the SCN increased during the light phase. However, the increases in the MPO and PVN in wild-type mice were lower than those in Clock-mutant mice. Therefore, exposure to cold while fasting increases neural activity in the SCN during the light phase. In addition, normal molecular circadian mechanisms may be necessary for the response. On the basis of the results of cFos expression in the hypothalamic areas, the SCN may send inhibitory signals to the MPO and PVN, which may result in attenuated thermoregulatory responses. Moreover, since the inhibitory signals are stronger in some conditions, such as in fasting, the attenuation of thermoregulation becomes stronger.

On the basis of the speculated neural connection between the SCN and MPO and/or PVN, Tokizawa et al. [9] conducted an experiment, which directly evaluated the associations. A cholera toxin b-subunit was injected (CTb; monosynaptic retrograde neural tracer) to the MPO or PVN during the light phase, and the presence in the SCN was assessed 3 days later. Cold exposure

and fasting were also conducted in the same manner as the previous cFos study. The injection of CTb in the PVN resulted in a widely spread labeling in both the dorsomedial and the ventrolateral parts of the SCN of both wild-type and *Clock*-mutant mice. In both mice, 5–10% of CTb-labeled neurons in the SCN were also cFos-positive at 27°C during the light phase and ad lib feeding. In the cold exposure during fasting, the ratio of the double-labeled neurons of CTb and cFos increased to 25–30% only in the wild-type mice. Moreover, the double-labeled neurons are GABAergic. When CTb was injected to the MPO, the dorsomedial part of the SCN was labeled. However, the ratio of the double-labeled neurons remained at the same level (15–20%) during ad lib feeding and during fasting and cold exposure. These results suggested that the SCN may send inhibitory signals to the PVN and MPO during the light phase. Fasting and/or cold exposure increased the inhibitory signals only in the PVN. Moreover, in the process, a normal molecular circadian mechanism would be necessary. The sympathetic outflow may originate from the PVN [54–56]. Therefore, such inhibitory signals may attenuate metabolic heat production and/or skin vasoconstriction (i.e., cold defense mechanisms), thus decreasing T_b during fasting.

8. Signals attenuating thermoregulatory responses during fasting

In Section 6 of the cFos study, the ARC also seems to play a key role in the circadian change of thermoregulation during fasting, which decreases plasma leptin and increases plasma ghrelin [64]. A reduction of leptin results in hypothermia [65]. Gluck et al. [66] showed that ghrelin induces hypothermia. Decreases in both hormones are signals activating neuropeptide Y (NPY) neurons in the ARC [67], which strongly reduces heat production. The receptors for leptin and ghrelin are found in NPY neurons [68, 69]. These experimental results suggest that leptins and/or ghrelins are the factors that modulate thermoregulatory heat production and decrease T_b during fasting. Moreover, the neurons in the SCN have leptin and ghrelin receptors [68, 70, 71]. In addition, the neural activity of the SCN is modulated in the presence of leptin and ghrelin [72, 73].

Tokizawa et al. [74] reported that the thermoregulatory response to cold was attenuated in *ob*/*ob* mice (genetically deficient of leptin). However, the response was not different between the light and dark phases. On the contrary, ghrelin injection to normal mice inhibited thermoregulatory heat production during cold exposure and reduced T_b. Such a response was observed only during the light phase. Therefore, ghrelin plays a key role in the phase-specific (light phase) modulation of thermoregulation and T_b during fasting.

The administration of ghrelin suppresses sympathetic nerve activity and iBAT temperature in rats [75, 76]. In the study by Tokizawa et al., ghrelin levels after the injection and 48-h fasting did not differ between the two phases. Therefore, a central mechanism that modulates the sensitivity to plasma ghrelin levels must exist, which may affect thermoregulatory responses to cold exposure. Ghrelin induced phase-specific changes in cFos expression in the hypothalamic areas: increased cFos-IR cells in the SCN during the light phase, the ARC during the dark phase, and the PVN during the cold exposure in the dark phase. Ghrelin injection activates NPY neurons in the ARC in both phases, and cFos-IR cell counts are higher in the dark than in the light phase. In the SCN, the ghrelin effect was limited to the light phase, and 25% of the cFos-IR cells were NPY neurons. NPY acts as nonphotic stimuli in the SCN [77]. The activation of the hypothalamic nuclei, that is, the SCN, ARC, and PVN, seems to be involved in the changes in the thermoregulatory metabolic heat production. **Figure 7** shows the summary of the findings.

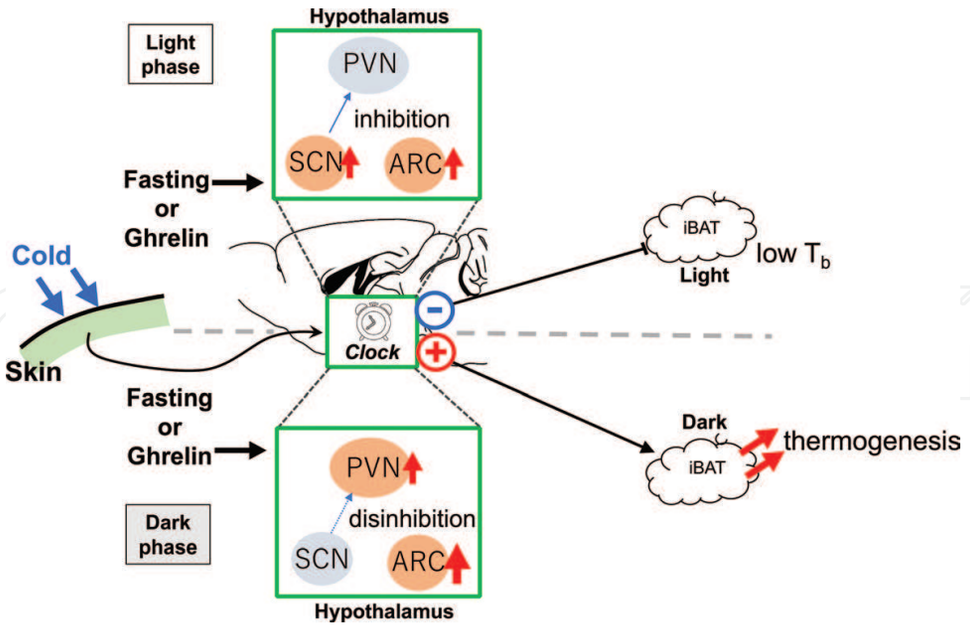

Figure 7. Summary of the histological findings together with physiological studies. During the light phase, fasting and an increase in plasma ghrelin level affect the hypothalamic areas. The activity of the suprachiasmatic nucleus (SCN) increases and that of the arcuate nucleus (ARC) relatively decreases. The SCN sends inhibitory signals to the paraventricular nucleus (PVN), which may result in a lower metabolic heat production of the interscapular brown adipose tissue (iBAT) and a lower body temperature. On the contrary, during the dark phase, the activity of the SCN decreases and that of the ARC relatively increases. The inhibitory signal from the SCN is less, and the PVN is activated. Metabolic heat production of the iBAT increases and body temperature is maintained.

9. Conclusion

This is a review article showing our previous series of studies involved in fasting-induced change in T_b rhythm. Interestingly, the change in T_b rhythm is a regulated phenomenon by molecular mechanisms such as *Cry and Clock* and neural mechanisms such as the SCN, MPO, and ARC in the hypothalamus. These studies are important in considering physiological importance and mechanism of the circadian body temperature and metabolic rhythms.

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Conflict of interest

There is no conflict of interest in this review.

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