The involvement of Cry1 and Cry2 genes in the regulation of the circadian body temperature rhythm in mice

Kei Nagashima,1,2 Kenta Matsue,2 Masahiro Konishi,2 Chisato Iidaka,3 Koyomi Miyazaki,3 Norio Ishida,3 and Kazuyuki Kanosue4

1Department of Integrative Physiology, School of Human Sciences, Waseda University, Tokorozawa, Saitama; 2Department of Physiology, School of Allied Health Sciences, Faculty of Medicine, Osaka University Suita, Osaka; 3Clock Cell Biology Research Group, National Institute of Advanced Industrial Science and Technology, Institute for Biological Resources and Functions, Tsukuba, Ibaraki; and 4Department of Physiology, School of Sports Sciences, Waseda University, Tokorozawa, Saitama, Japan

Submitted 15 June 2004; accepted in final form 20 August 2004

Nagashima, Kei, Kenta Matsue, Masahiro Konishi, Chisato Iidaka, Koyomi Miyazaki, Norio Ishida, and Kazuyuki Kanosue. The involvement of Cry1 and Cry2 genes in the regulation of the circadian body temperature rhythm in mice. Am J Physiol Regul Integr Comp Physiol 288: R329–R335, 2005. First published August 26, 2004; doi:10.1152/ajpregu.00395.2004.—The cryptochrome genes (Cry1 and Cry2) are involved in the molecular mechanism that controls the circadian clock, and mice lacking these genes (Cry1−/−/Cry2−/−) are behaviorally arrhythmic. It has been speculated that the circadian clock modulates the characteristics of thermoregulation, resulting in body temperature (Tb) rhythm. However, there is no direct evidence proving this speculation. We show here that Tb and heat production in Cry1−/−/Cry2−/− mice are arrhythmic under constant darkness. In contrast, both rhythms occur under a light-dark cycle and/or periodic food restriction linked with spontaneous activity and/or eating, although they are not robust as those in wild-type mice. The relationship between heat production and Tb in Cry1−/−/Cry2−/− mice is linear and identical under any conditions, indicating that their Tb rhythm is determined by heat production rhythm associated with activity and eating. However, Tb in wild-type mice is maintained at a relatively higher level in the active phase than the inactive phase regardless of the heat production level. These results indicate that the thermoregulatory responses are modulated according to the circadian phase, and the Cry genes are involved in this mechanism.

Circadian clock; thermoregulation; metabolism

Homeothermic animals regulate their body temperature (Tb) within a narrow range by controlling the balance of heat production and loss, in which various independent behavioral and autonomic thermoregulatory processes are involved (15, 19, 20). For example, rodents exposed to the cold curl up, huddle, erect their fur, and reduce tail blood flow to decrease heat loss. They also shiver and metabolize fat to increase heat production. In contrast, it is known that Tb follows a circadian rhythm. Under constant environmental conditions, Tb is higher in the active phase and lower in the inactive phase, which indicates the presence of a circadian clock (3, 12). Several investigators have speculated that the circadian clock modulates the characteristics of thermoregulation (3, 7, 12, 13, 25, 32), resulting in the Tb rhythm. However, there has not been any direct evidence proving this speculation published to date.

The suprachiasmatic nucleus (SCN) in the hypothalamus is the master clock of the circadian rhythm. Lesions of the SCN have been used to indicate that the circadian clock is associated with several physiological rhythms. Although these lesions also abolish the Tb rhythm in rodents (1, 11, 21, 24, 31, 32, 36), this finding may not indicate that the circadian clock regulates the Tb rhythm. It has been reported that behavioral or physiological responses such as locomotor activity, eating, and the secretion of several hormones accompany changes in heat production and Tb (23, 28, 29, 37, 38). Thus it could be interpreted that a lack of these rhythms in the presence of SCN lesions (1, 3, 16, 24, 36) abolishes the Tb rhythm with the heat production rhythm. We previously reported that the Tb rhythm was maintained in fasted rats; however, heat production and its circadian amplitude decreased (25). These results may indicate that, despite the strong influence of food intake on heat production rhythm, the circadian Tb rhythm is regulated independently of heat production.

Recent studies clarified the core molecular mechanisms of the circadian clock (8, 10, 18), which consists of autoregulatory transcription-translation loops with a periodicity of ~24 h. Moreover, the mechanism is situated in the central and peripheral tissues (4, 5, 9). The absence of this mechanism may abolish all internal rhythms, although it remains unclear yet how the molecular mechanism governs actual physiological and behavioral rhythms.

Cryptochromes are photopigments for light entrainment of the circadian rhythm in plants and Drosophila and play a key role in the molecular feedback loop (14, 22, 35, 39) in mammals. The mice lacking the cryptochromes 1 and 2 genes (Cry1 and Cry2) lose periodicity in wheel-running behavior (39) as well as electrophysiological activity in the SCN cells under constant darkness (DD) (6). However, interestingly, lights have a masking effect on the behavior: the wheel-running activity is suppressed in the lights, and a light-dark (LD) cycle results in the display of the daily rhythmicity.

In the present study, we hypothesized that the core mechanism involved in the Tb rhythm would be regulated by the molecular mechanisms of the circadian clock. Moreover, the Tb rhythm would be controlled independently of heat production. To test this hypothesis, we compared a daily change in Tb in the knockout mice of Cry1 and Cry2 (Cry1−−/−−/Cry2−−/−−).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: K. Nagashima, Dept. of Integrative Physiology, School of Human Sciences, Waseda Univ., Mikajima 2–579-15, Tokorozawa, Saitama 359–1192, Japan (E-mail: k-nagashima@waseda.jp).
with wild-type mice in three different conditions: 1) the DD with ad libitum feeding (i.e., in a constant condition) and the LD with 2) ad libitum feeding and 3) restricted feeding during the dark phase (i.e., in the presence of factors modulating heat production). We surmised that, in the knockout mice, the $T_b$ rhythms would appear in the presence of factors modulating heat production. Moreover, the relationship between $T_b$ and heat production would be similar in all the conditions in the knockout mice but not in wild-type mice.

**METHODS**

**Animals.** Wild-type mice ($n = 5$, C57BL/6) and Cry1$^{-/-}$/Cry2$^{-/-}$ mice ($n = 5$, C57BL/6 background) were used for the experiments. The mice in both groups were male, 24–44 wk old, and weighed between 26 and 35 g. Normally, mice were individually housed in a plastic cage (16 × 26 × 13 cm) with water and food available ad libitum. The ambient temperature was 26°C, and the lighting cycle was 12 h light (300 lx at eye level, lights on at 0700) and 12 h complete darkness. All procedures in this study were approved by the Institutional Animal Care and Use Committee, Faculty of Medicine, Osaka University.

**Surgery.** A radio transmitter device used to measure $T_b$ and spontaneous activity ($17 \times 10 \times 8$ mm; Physiotel, model TA10TA-F40, DataScience, St. Paul, MN) was implanted in the abdominal cavity by sterile technique under general anesthesia induced by intraperitoneal injection of pentobarbital sodium (5.0 mg/100 g body wt). Lignocaine jelly (Astrazeneca, Tokyo) was applied to the wound, and penicillin G (1,000 U, Meiji Pharmaceutical, Tokyo, Japan) was intramuscularly injected to minimize post-surgical pain and infection. $T_b$ and spontaneous activity were monitored throughout the experiment. Spontaneous activity was estimated by the change in intensity of the telemetry signal. The signals passed through a receiver board (model CTR86, DataScience) underneath the cage and were stored in a personal computer every 5 min. The mice were allowed to recover for at least 4 wk before the experiments. Wild-type mice usually took 2 wk before showing clear circadian $T_b$ and activity rhythms.

**Indirect calorimetry.** To measure metabolic heat production, a mouse was transferred at 1300 to a Plexiglas metabolic box ($17 \times 17 \times 17$ cm) attached to an airflow system with a constant flow rate of 500 ml/min. The ambient temperature in the metabolic box was monitored and kept at 26.0 ± 0.2°C. The difference in oxygen tension between the inlet and outlet was continuously monitored by an electrochemical oxygen analyzer (model LCI-700, Toray, Tokyo), and the data were stored in a personal computer at 10-s intervals. Oxygen consumption rate ($V_O_2$, indirect calorimetry) was calculated as the product of the difference in oxygen tension and flow rate divided by the 0.75 power of body weight (Brody-Kleiber formula) and corrected to conditions of standard temperature (0°C), pressure (760 mmHg), and dry. In addition, we familiarized the mice with the system by placing them in the box several times during the recovery period after surgery. Body weight was measured before and after each trial. Normal daily food intake was not different between wild-type and Cry1$^{-/-}$/Cry2$^{-/-}$ mice (Student’s t-test, $P < 0.05$).

**Experimental protocols.** $T_b$ and spontaneous activity of wild-type and Cry1$^{-/-}$/Cry2$^{-/-}$ mice in a cage were continuously measured by telemetry ($n = 5$ in each group). To examine the effect of lighting on $T_b$ and spontaneous activity, mice were exposed to DD for 3 days after 12:12-h light-dark (LD, lights on at 0700). Before DD exposure, the two groups were exposed to LD for at least 10 days.

We placed the same mice separately in a semiclosed Plexiglas chamber for 3 days at least 1 wk after the first experiment and continuously measured their oxygen consumption rate ($V_O_2$, i.e., metabolic heat production) by indirect calorimetry together with $T_b$ and spontaneous activity. This was repeated three times under different conditions with at least a 1-wk interval: 1) DD with ad libitum feeding, 2) LD with ad libitum feeding, and 3) LD with food restriction. The food restriction protocol was aimed at enhancing the eating rhythm. Ordinary chow was given at 1900, the end of light period, at 70–80% of the normal daily intake. We verified that both Cry1$^{-/-}$/Cry2$^{-/-}$ and wild-type mice finished eating 10–12 h after the appearance of chow in the food-restriction regimen, which induced a fed-fasting rhythm.

**Statistics.** The circadian period of each parameter was estimated with the chi-square periodogram (34). The mean, amplitude, and peak phase of the circadian rhythm were estimated by cosinor rhythmometry (26). Regression analysis was conducted by the least-squares method. Differences among means were analyzed by the Student’s t-test or ANOVA with repeated measurement. Post hoc testing to verify significance for a specific period was conducted using the Newman-Keuls procedure. The null hypothesis was rejected at $P < 0.05$.

**RESULTS**

$T_b$ rhythm during a light-dark cycle and under constant darkness. Both $T_b$ and activity rhythms in wild-type mice were robust (Fig. 1, A and B), and their circadian period ($\tau$) was both 24.0 ± 0.1 under the LD condition and 23.9 ± 0.1 under the DD condition (the chi-square periodogram, $P < 0.0013$ in each condition; means ± SE, $n = 5$).

Compared with wild-type mice, daily rhythms of both $T_b$ and activity in Cry1$^{-/-}$/Cry2$^{-/-}$ mice were weak and accompanied by ultradian-like rhythms even under the LD condition (Fig. 1, C and D), although the dominant $\tau$ was 23.9 ± 0.2 and 24.0 ± 0.2 for $T_b$ and activity, respectively (the chi-square periodogram, $P < 0.0013$ in both parameters; $n = 5$). Under the DD condition, no significant ($P > 0.05$) daily rhythm occurred for $T_b$. However, $T_b$ was associated with spontaneous activity in Cry1$^{-/-}$/Cry2$^{-/-}$ mice under the DD or LD condition (regression analysis, $0.33 < R^2 < 0.55$ and $0.35 < R^2 < 0.56$, respectively, $P < 0.001$).

Heat production and $T_b$ in light-dark and/or fed-fasting cycles. Wild-type mice showed robust circadian rhythms for $V_O_2$ and $T_b$ during each trial (Fig. 2, A and B; the chi-square periodogram, $P < 0.0073$, $\tau = 23.9 ± 0.2$ under the DD condition and 24.0 ± 0.1 in the 2 trials under the LD condition, $n = 5$). The mean, amplitude, and peak phase of $T_b$ rhythm under the LD condition with ad libitum feeding remained similar as those under the DD condition (Table 1; ANOVA, $P > 0.05$). Significant differences were found in the $T_b$ and $V_O_2$ rhythms between ad libitum feeding and food-restriction days (ANOVA, $F = 7.122$ and 6.450, $P < 0.05$). The activity rhythm was unchanged throughout the three trials.

In Cry1$^{-/-}$/Cry2$^{-/-}$ mice, $V_O_2$ was arrhythmic (the chi-square periodogram, $P > 0.05$) under the DD condition, seen by $T_b$ and spontaneous activity (Fig. 2, C and D, and Table 1). $V_O_2$ and $T_b$ were rhythmic (the chi-square periodogram, $P < 0.043$; dominant $\tau = 24.0 ± 0.3$ in $V_O_2$, $n = 5$) with the similar peaks phases [cosinor rhythmometry, zeitgeber time (ZT) 15.4 ± 0.3 and 15.5 ± 0.2, respectively] on the ad libitum feeding day under the LD condition, although they are not visually clear. The food restriction altered $V_O_2$ and $T_b$ rhythms. The daily means were lower (ANOVA, $F = 5.402$ and 5.590, $P < 0.05$), and the amplitudes of the rhythms were greater (ANOVA, $F = 5.992$ and 6.613, $P < 0.05$) than those under the ad libitum feeding condition (Table 1). The circadian amplitudes of $T_b$ and $V_O_2$ rhythms in the two trials under the LD condition were smaller (ANOVA, $F = 6.218$ and 6.320,
than those in wild-type mice. Food restriction did not affect the activity rhythm (P > 0.05).

Relationship between metabolic heat production and Tb. We assessed the relationship between V\(\dot{O}_2\) and Tb in each mouse (Table 2). Each value was averaged for 30 min. Regression analysis was carried separately on the data for active [circadian time (CT) or ZT 12–24] and inactive (CT or ZT 0–12) phases in wild-type mice and the corresponding periods in Cry\(^1\)/Cry\(^2\) mice. All analyses showed significance. We used regression analysis for the averaged V\(\dot{O}_2\) and Tb of each group to make further comparisons (Fig. 3), because all regressions within the same trial, period, and group showed a similar tendency: the slopes and intercepts were within the 95% confidence limits for the averaged V\(\dot{O}_2\) and Tb. We hypothesized that if heat production was the sole determinant of circadian Tb rhythm, these regressions would be identical regardless of trial, circadian phase, and group. To test this, we first built a model equation fitted to all the data in the three trials within the same phase and group (see APPENDIX). Model equations were created for all possible relationships among the three trials: model A) all three regressions were similar; model B) all were different; and model C) two were similar. We calculated the Akaike information criterion (AIC) (Ref. 2) and selected the model with the lowest AIC as the most suitable one. In wild-type mice, the AIC in model A was the lowest in both phases (AIC = −117.87 and −90.50 in CT or ZT 12–24 and CT or ZT 0–12, respectively). Model A had the lowest AIC in both phases in the knockout mice (−271.01 and −204.82 in CT or ZT 12–24, and CT or ZT 0–12, respectively, in contrast to −242.62 and −182.11 in model B). Thus the data could be roughly separated into four groups: those at CT or ZT 12–24 and CT or ZT 0–12 in Cry\(^1\)/Cry\(^2\) and wild-type mice, respectively. Then, we applied a similar analysis to the four data groups simultaneously to evaluate the influence of the differences between mice groups and circadian phases on Tb. Here, the model indicated that the two regressions in wild-type mice were different, and those in the knockout mice were similar, showing the lowest AIC (−664.04 in contrast to −531.71 in the model where all regressions were similar (model A)). These results are summarized in Fig. 4.
Fig. 2. Daily changes in oxygen consumption rate (V\textsubscript{O\textsubscript{2}}, A and C) and T\textsubscript{b} (B and D) on the last day of ad libitum feeding under the DD condition, ad libitum feeding under the LD condition, and food restriction under the LD condition. Each data point is a mean (±SE) of 5 mice for 30 min. For better assessment, the data corresponding to each hour are presented. Zeitgeber time (ZT) 0 and circadian time (CT) 0 denote the time the lights were turned on under the LD condition and the corresponding time under the DD condition. The V\textsubscript{O\textsubscript{2}} and T\textsubscript{b} changes in wild-type mice were rhythmic for the 3 trials (r = 23.9 ± 0.2 and 24.0 ± 0.1 under the DD and LD conditions, respectively). In Cry\textsuperscript{1/-}/Cry\textsuperscript{2/-} mice, the changes were arrhythmic under the DD condition. However, they became rhythmic (r = 24.0 ± 0.3) under the LD condition. On the food restriction day, the means for the T\textsubscript{b} and V\textsubscript{O\textsubscript{2}} rhythms in both groups were smaller and the amplitudes were greater than those on the ad libitum feeding day.

**DISCUSSION**

This study showed that Cry\textsuperscript{1/-}/Cry\textsuperscript{2/-} mice lack internal circadian T\textsubscript{b} rhythm (i.e., a loss of the daily change in a constant environment) similarly to the SCN-lesioned animals. There were clear T\textsubscript{b} and heat production rhythms in any condition in wild-type mice. In addition, the T\textsubscript{b} rhythm in the knockout mice appeared, linked with the heat production rhythm, in the two trials under the LD condition.

The model analysis for wild-type mice (Figs. 3A and 4) showed that two factors, i.e., the V\textsubscript{O\textsubscript{2}} and circadian phase, were linked as a dominant factor determining the T\textsubscript{b} rhythm in wild-type mice. The first reason is that T\textsubscript{b} was kept higher in the active phase than the inactive phase regardless of the V\textsubscript{O\textsubscript{2}} level. The second reason is that the regression slopes for V\textsubscript{O\textsubscript{2}} and T\textsubscript{b} in both phases were smaller than those for Cry\textsuperscript{1/-}/Cry\textsuperscript{2/-} mice (Student’s t-test, P < 0.01). In other words, wild-type mice could maintain their T\textsubscript{b} within a narrower range than the knockout mice over the same variation of V\textsubscript{O\textsubscript{2}} in each phase. Thus the T\textsubscript{b} rhythm in wild-type animals is not a simple by-product of the heat production rhythm but a regulated phenomenon by the factor of circadian phase.

In contrast to wild-type mice, the model analysis for the relationship between V\textsubscript{O\textsubscript{2}} and T\textsubscript{b} (Figs. 3B and 4) indicates that heat production predominantly determines T\textsubscript{b} in Cry\textsuperscript{1/-}/Cry\textsuperscript{2/-} mice. Because the relationship was similar in all the trials, the factors of lighting, feeding, and phase themselves should have no specific effect on T\textsubscript{b} (i.e., heat production

**Table 1. Cosinor rhythmometry for daily changes in T\textsubscript{b}, V\textsubscript{O\textsubscript{2}}, and spontaneous activity**

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>Cry\textsuperscript{1/-}/Cry\textsuperscript{2/-}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DD</td>
<td>LD</td>
</tr>
<tr>
<td></td>
<td>Ad lib feeding</td>
<td>Food restriction</td>
</tr>
<tr>
<td>T\textsubscript{b}, °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>36.3 ± 0.1</td>
<td>36.3 ± 0.1*</td>
</tr>
<tr>
<td>Amplitude</td>
<td>1.12 ± 0.1†</td>
<td>1.50 ± 0.2*†</td>
</tr>
<tr>
<td>Peak phase CT</td>
<td>17.5 ± 0.2</td>
<td>17.6 ± 0.2</td>
</tr>
<tr>
<td>V\textsubscript{O\textsubscript{2}}, ml/min·kg\textsuperscript{-0.75}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>12.73 ± 0.88</td>
<td>14.18 ± 0.98*</td>
</tr>
<tr>
<td>Amplitude</td>
<td>1.95 ± 0.12</td>
<td>2.00 ± 0.14†</td>
</tr>
<tr>
<td>Peak phase CT</td>
<td>16.6 ± 0.2</td>
<td>16.9 ± 0.2</td>
</tr>
<tr>
<td>Activity, units</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.21 ± 0.20</td>
<td>2.20 ± 0.19</td>
</tr>
<tr>
<td>Amplitude</td>
<td>1.27 ± 0.17</td>
<td>1.30 ± 0.12†</td>
</tr>
<tr>
<td>Peak phase CT</td>
<td>17.6 ± 0.2</td>
<td>17.9 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 mice. DD, constant darkness; LD, 12:12-h light-dark cycle; T\textsubscript{b}, body temperature; V\textsubscript{O\textsubscript{2}}, oxygen consumption; CT, circadian time; ZT, zeitgeber time. *Significantly different from value in the trial under the LD condition with ad libitum (Ad lib) feeding, P < 0.05; †significantly different from value of Cry\textsuperscript{1/-}/Cry\textsuperscript{2/-} mice, P < 0.05.
determines $T_b$). Moreover, there was also a linear relationship between spontaneous activity and $\dot{V}O_2$ in the knockout mice under the ad libitum feeding condition during DD or LD (the regression analysis, $R^2 = 0.51$ and 0.59, respectively, $P < 0.05$), which indicates that their $T_b$ is linked with spontaneous activity. Although the food restriction regimen had no additional effect on the activity rhythm, both the heat production and $T_b$ rhythms became robust (Fig. 2). The means for the energetic effect on the activity rhythm, both the heat production and $T_b$ rhythms were smaller during the food restriction than ad libitum feeding; however, the amplitudes were greater (Table 1). These results show a suppression of heat production during the food deprivation. It is well known that fasting is a strong stimulus decreasing heat production (25, 27). Moreover, we previously suggested that eating rhythm is a potent stimulus generating heat production rhythm in normal rats (25). Thus eating is another factor modulating $T_b$ in the knockout mice.

Homeothermic animals control $T_b$ by balancing heat loss and heat production. For example, an activation of heat loss responses results in a negative heat balance, decreasing $T_b$ until the heat balance is equilibrated again. Because this model shows that the influence of heat production on the circadian $T_b$ rhythm is small, the difference in heat loss between the active and inactive phases may determine the $T_b$ rhythm. Several studies may support this conclusion: the circadian $T_b$ rhythm persists in completely bed-rested (13) humans, fasting humans and rats (7, 25), and hibernating squirrels (32), in which the amplitude of heat production rhythm disappears or is attenuated. Circadian changes in nonthermoregulatory factors that affect heat loss, such as the basal tone of the sympathetic nerve, may be involved in the $T_b$ rhythm in part. However, the difference in the regression slope for heat production and $T_b$ between wild-type and the knockout mice (Fig. 4) indicates modulation of the thermoregulatory responses of heat loss in the presence of the circadian clock.

Refinetti (30) reported that there was a heat balance rhythm with a 180-degree phase difference from the $T_b$ rhythm; however, the amplitude was small. The results clearly show that the rhythm of heat balance is not a factor determining the $T_b$

---

### Table 2. Slopes and intercepts of the regression lines for $\dot{V}O_2$ and $T_b$

<table>
<thead>
<tr>
<th>Slope</th>
<th>Intercept</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD</td>
<td>0.24 ± 0.04</td>
<td>33.8 ± 0.46</td>
</tr>
<tr>
<td>LD + ad libitum feeding</td>
<td>0.22 ± 0.04</td>
<td>33.9 ± 0.59</td>
</tr>
<tr>
<td>LD + food restriction</td>
<td>0.24 ± 0.02</td>
<td>33.5 ± 0.42</td>
</tr>
<tr>
<td>Cry1−/−/Cry2−/−</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD</td>
<td>0.40 ± 0.04</td>
<td>31.1 ± 0.38</td>
</tr>
<tr>
<td>LD + ad libitum feeding</td>
<td>0.40 ± 0.05</td>
<td>30.9 ± 0.50</td>
</tr>
<tr>
<td>LD + food restriction</td>
<td>0.40 ± 0.04</td>
<td>30.9 ± 0.37</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 mice. Significant regression: *$P < 0.0001$, †$P < 0.002$. 

---

**Fig. 3. Relationship between metabolic heat production ($\dot{V}O_2$) and $T_b$ for the 3 trials in wild-type mice (A) and Cry1−/−/Cry2−/− (B). Values are means (±SE) of 5 mice for 30 min, and the data corresponding to each hour are used for this illustration. Regression lines were applied for the averaged values in each group. The regression analysis for each mouse is also summarized in Table 2.**

**Fig. 4. Summary of the model analyses of the relationship between metabolic heat production ($\dot{V}O_2$) and $T_b$. Data are separated into 3 groups: active phase data on wild-type mice, inactive phase data on wild-type mice, and all the data on Cry1−/−/Cry2−/− mice. Each regression line is drawn within the range of $\dot{V}O_2$ and $T_b$.**
rhythm and also support our data that the heat production rhythm was not a prime mechanism involved in the Tb rhythm. Moreover, we speculate that the heat balance is mostly equilibrated throughout the day (i.e., the influence of heat production on Tb is reduced by heat loss responses), except for the dawn and sunset when Tb increases and decreases. This is also confirmed by the present data that the regression slope for heat production and Tb in each phase was smaller in wild-type than Cry1^{-/-}/Cry2^{-/-} mice.

Mice lacking the Clock gene, another key regulator of the circadian molecular loop, also lose activity and Tb rhythm under the DD condition (17, 33, 40). This result would indicate how the molecular mechanisms of the circadian clock modulate the Tb rhythm and also support our data that the heat production rhythm was not a prime mechanism involved in the Tb rhythm. However, it remains unclear how the molecular mechanisms of the circadian clock modulate neural and/or humoral signals, which regulate the thermoregulatory responses.

In conclusion, the thermoregulatory responses change according to the circadian phase, which is an important mechanism involved in circadian Tb rhythm. The activity of the circadian clock should be under the DD condition (17, 33, 40). This result would indicate how the molecular mechanisms of the circadian clock modulate neural and/or humoral signals, which regulate the thermoregulatory responses.

**ACKNOWLEDGMENTS**

We thank Drs. Y. Ohno and T. Nakamura for valuable suggestions in the model analysis.

**GRANTS**

This study was partially supported by the Ministry of Education, Science, and Culture of Japan, Grants-in-Aid for Scientific Research, No. 14570058.

**REFERENCES**


