Dietary Protein Modulates Circadian Changes in Core Body Temperature and Metabolic Rate in Rats

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Abstract: We assessed the contribution of dietary protein to circadian changes in core body temperature (Tb) and metabolic rate in freely moving rats. Daily changes in rat intraperitoneal temperature, locomotor activity (LMA), whole-body oxygen consumption (VO2), and carbon dioxide production (VCO2) were measured before and during 4 days of consuming a 20% protein diet (20% P), a protein-free diet (0% P), or a pair-fed 20% P diet (20% P-R). Changes in Tb did not significantly differ between the 20% P and 20% P-R groups throughout the study. The Tb in the 0% P group remained elevated during the dark (D) phase throughout the study, but VO2, VCO2, and LMA increased late in the study when compared with the 20% P-R group almost in accordance with elevated Tb. By contrast, during the light (L) phase in the 0% P group, Tb became elevated early in the study and thereafter declined with a tendency to accompany significantly lower VO2 and VCO2 when compared with the 20% P group, but not the 20% P-R group. The respiratory quotient (RQ) in the 0% P group declined throughout the D phase and during the early L phase. By contrast, RQ in the 20% P-R group consistently decreased from the late D phase to the end of the L phase. Our findings suggest that dietary protein contributes to the maintenance of daily oscillations in Tb with modulating metabolic rates during the D phase. However, the underlying mechanisms of Tb control during the L phase remain obscure.

Key words: thermogenesis, amino acid, low protein diet, behavior, food ingestion.

Core body temperature (Tb) in endotherms is maintained within a narrow range, and it serves as the optimal temperature for metabolic activities [1]. Therefore a disorder in Tb alters various organ functions that are based on a decrease in enzymatic activities, and it will alter physical properties. Such changes in Tb lead to various clinical abnormalities such as neurological, cardiopulmonary, and metabolic imbalances [2, 3]. Endotherms regulate their own Tb by balancing thermogenesis and heat dissipation, but illness can interfere with these thermoregulatory functions.

In rats in which Tb oscillates daily, food deprivation lowers Tb during the light phase (L: inactive state during the daytime), but not during the dark phase (D: active state during the nighttime) [4–7]. The daytime decrease in Tb is closely associated with reduced oxygen consumption compared with rats fed a normal diet [8]. However, irrespective of lowered metabolic heat production during the D phase, Tb at the control level is supposedly maintained via a suppression of the heat loss mechanism [5]. Thus diurnal changes in metabolic heat production, heat loss, and Tb are closely related to nutrient ingestion. Macronutrients affect these variables differently [9], but the degree to which the absence of individual macronutrients is responsible for a circadian Tb disorder is unknown.

Protein and amino acids are most efficient in elevating energy expenditure. Specifically, the proportion of energy expended relative to the energy content of a nutrient is 30%–40% for proteins or amino acids, 5%–10% for carbohydrates or glucose, and 0%–3% for fat [9, 10]. Nevertheless, energy expenditure either inferred or calculated using the energy balance method is increased in rats [11, 12] or pigs [13] fed a low protein diet. On the other hand, a low-protein diet does not change resting whole-body oxygen consumption (VO2) per unit of rat body weight, but it does cause enhanced thermogenesis in response to norepinephrine in rats [11]. Furthermore, the extent of the increase in VO2 after a liquid meal through a tube is enhanced in rats given a low protein diet [14]. The values in all of these experiments were obtained during the L phase. However, nocturnal rats feed during the D phase; further-
more, sympathetic activity is elevated during the D phase compared with the L phase. Therefore the thermogenic responses to the norepinephrine or to tube feeding might reflect physiological status during the D phase. Thus the absence of dietary protein appears to increase metabolic heat production during the D phase, but not the L phase. Furthermore, the regulation of metabolic heat production by dietary protein might extend to the modification of T_\text{b} fluctuation. To our knowledge, the influence of dietary protein on T_\text{b} and metabolic rate has not yet been investigated from a daily circadian viewpoint.

Feeding with an extremely low protein (2% casein) diet resulted in severely reduced food intake and lowered energy expenditure compared with rats given a diet with a normal protein content [15]. Reduced food intake decreases metabolic rates [5, 16–18]. All of these findings indicate that the effects of dietary protein itself must be distinguished from those of differences in caloric intake to understand metabolic changes in rats fed a diet containing low or no dietary protein.

Therefore we examined daily changes in the T_\text{b} of freely moving rats before and during 4 days of their consuming equivalent calories of a diet with or without protein. We also tested locomotor activity (LMA) and analyzed respiratory gases to determine associations between behavioral or metabolic states and T_\text{b}. We postulated that dietary protein contributes to modulate the daily metabolic rate and T_\text{b}, especially during the D phase.

MATERIALS AND METHODS

Animals and surgery. Seven-week-old male Sprague-Dawley rats, n = 30, 220–250 g (Charles River Japan, Inc., Yokohama, Japan), were housed under constant humidity (52%–56%) and temperature (22.8ºC–23.2ºC) on a 12:12 h light-dark cycle (lights on 07:00 h–19:00 h) throughout the study. The following surgical and experimental procedures were approved by the Committee on the Care and Use of Laboratory Animals of Otsuka Pharmaceutical Factory, Inc. After 3 days of feeding with a standard diet (AIN-93G diet, (Oriental Yeast Co., Tokyo, Japan), set at an airflow rate of 2 l/min and a measurement period of 15 s. The VO_2 and VCO_2 were corrected daily for differences in body surface area at the time of food replenishment and are expressed as ml/min/kg^{0.75} [20]. The respiratory quotients (RQ; respiratory exchange ratio) of the rats were calculated by dividing VCO_2 by VO_2. The values of T_\text{b}, LMA, VO_2, VCO_2, and RQ throughout the study were averaged every hour.

Measurement of intraperitoneal temperature, locomotor activity, and respiratory gases. The LMA signals indicating movement from one location to another and the T_\text{b} signals were passed by the telemetry transmitter to a receiver via a radio signal, and the information from the receiver was relayed to an automated data acquisition system by Dataquest A.R.T. (Data Sciences International) every 5 min. Whole-body oxygen consumption (VO_2) and carbon dioxide production (VCO_2) by the rats were measured, using an automated system, ARCO-1000 (Arco System, Chiba, Japan), set at an airflow rate of 2 l/min and a measurement period of 15 s. The VO_2 and VCO_2 were corrected daily for differences in body surface area at the time of food replenishment and are expressed as ml/min/kg^{0.75} [20]. The respiratory quotients (RQ; respiratory exchange ratio) of the rats were calculated by dividing VCO_2 by VO_2. The values of T_\text{b}, LMA, VO_2, VCO_2, and RQ throughout the study were averaged every hour.

Statistical analyses. Data were statistically analyzed using Statistical Analysis Software, version 9.13 (SAS Institute Japan, Inc., Japan). The values for each group are presented as means ± SEM. The differences among the
three groups were analyzed by an ANOVA or by two-way ANOVA for repeated measures to test the main effects of the factors of diet type and feeding times (or days for body weight and food intake) and their interactions, when appropriate, followed by Tukey’s post hoc test. Statistical significance was defined at $p < 0.05$.

**RESULTS**

The daily food intake during 4 days of feeding on the experimental diet was significantly higher in the 20% P group than in the 20% P-R and 0% P groups (respectively, $19.4 \pm 0.5$ g/rat/day, $n = 8$, vs. $16.3 \pm 0.7$ g/rat/day, $n = 11$, $p < 0.05$ and vs. $15.1 \pm 0.6$ g/rat/day, $n = 11$, $p < 0.05$). The daily food intake did not significantly differ between the 0% P and 20% P-R groups. The 20% P group gained more weight during the study than the 20% P-R and 0% P groups (respectively, $29.5 \pm 1.7$ g/4 days, $n = 8$, vs. $15.9 \pm 1.0$ g/4 days, $n = 11$, $p < 0.05$ and vs. $–4.9 \pm 1.1$ g/4 days, $n = 11$, $p < 0.05$). Moreover, the 0% P group lost more weight than the 20% P-R group ($p < 0.05$).

Figure 1 shows the changes in $T_b$ (A) and LMA (B) during 4 days of feeding on the experimental diet. Each point (20:00 h–17:00 h) represents $T_b$ and LMA averaged for 1 h. Values are means ± SEM. Points labeled with * (20% P-R or 0% P vs. 20% P) or † (0% P vs. 20% P-R) indicate significant differences ($p < 0.05$).
Feeding on the 0% P diet resulted in a slight but significant increase in Tb during the early D phase on day 1 compared with both the 20% P groups ($p < 0.05$). Thereafter, Tb during the D phase in the 0% P group obviously increased on day 2 and continued to increase until day 4 ($p < 0.05$).

Fig. 2. Oxygen consumption (VO$_2$ in A), carbon dioxide production (VCO$_2$ in B), and respiratory quotient (RQ in C) in rats fed a 20% P diet (△, 20% P group; $n = 8$), pair-fed rats with the 20% P diet (○, 20% P-R group; $n = 11$), and rats with a 0% P diet (●, 0% P group; $n = 11$) throughout the experimental period. A solid bar indicates dark phase. Each point (20:00 h–17:00 h) represents VO$_2$, VCO$_2$, and RQ averaged for 1 h. Values are means ± SEM. Points labeled with * (20% P-R or 0% P vs. 20% P) or † (0% P vs. 20% P-R) indicate significant differences ($p < 0.05$).
group during the L phase significantly increased compared with each of the 20% P groups on days 1 and 2 \((p < 0.05)\). It then obviously decreased during the early L phase on day 4 compared with both 20% P groups \((p < 0.05)\). Changes in \(T_b\) did not significantly differ between the 20% P-R and 20% P groups throughout the study. The LMA of the 0% P group during the D phase gradually increased and significantly differed from each of the 20% P groups on days 3 and 4 \((p < 0.05)\). However, changes in LMA during the L phase in the 0% P group did not significantly differ from those in both 20% P groups.

Figure 2 shows the changes in \(V_O_2\) (A), \(V_CO_2\) (B), and RQ (C) during the experimental period. The \(V_O_2\) and \(V_CO_2\) during the D phase of the 0% P group gradually increased and significantly differed from the 20% P-R group on days 3 and 4 \((p < 0.05)\). The \(V_O_2\) and \(V_CO_2\) of the 20% P-R group tended to decrease during the late D phase, and both significantly differed from the 20% P group on day 4 \((p < 0.05)\). By contrast, \(V_O_2\) and \(V_CO_2\) of the 0% P group tended to gradually decrease during the L phase, and differences in \(V_O_2\) and \(V_CO_2\) became significant on day 4 \((p < 0.05)\) compared with the 20% P group, but not with the 20% P-R group. The RQ during the D phase was decreased in the 0% P group on day 2 compared with the 20% P group \((p < 0.05)\). Thereafter, the decreases gradually expanded throughout the D phase on days 3 and 4. The decrease in RQ during the late D phase in the 20% P-R group was slight, but it was significant throughout the study as compared with the 20% P group \((p < 0.05)\). By contrast, RQ during the early L phase was lower in the 20% P-R group from day 1 and in the 0% P group from day 2 than in the 20% P group \((p < 0.05)\). The RQ during the late L phase in the 20% P-R group continued to decrease throughout the study, but during the late L phase in the 0% P group it increased and did not significantly differ from that of the 20% P group.

Table 1 shows the weight of rat tissues after 4 days of feeding on the experimental diets. The liver weighed significantly more in the 20% P group than in the 0% P group \((p < 0.05)\). The gastrocnemius muscle also weighed more in both of the 20% P groups than in the 0% P group \((p < 0.05)\). However, the weight of the soleus muscle, the perirenal and retroperitoneal WAT, and the epididymal WAT did not significantly differ among the three groups. Moreover, none of the tissue weights per unit of body weight significantly differed among the three groups (data not shown).

**DISCUSSION**

The major finding of this study is that dietary protein is involved in the maintenance of daily oscillations in rat \(T_b\) through modulating metabolic rates, especially during the D phase.

The \(T_b\) during the D phase in the 0% P group was significantly elevated on days 3 and 4 when compared with the 20% P-R group in a manner that was almost synchronous with those of LMA, \(V_O_2\), and \(V_CO_2\). These results suggest that behavioral changes as a result of feeding on the 0% P diet might have contributed to the increased heat production especially late in the study and thus concomitantly elevate \(T_b\) during the D phase. Since rodents increase LMA during food restriction to forage for food and/or to achieve and maintain a stable \(T_b\) [6, 21, 22], the reduced food intake observed in the 0% P group might have been responsible for the increases in LMA. However, the values for LMA during the D phase did not significantly differ between the 20% P and the 20% P-R groups, suggesting that LMA during the D phase is not modified by the degree of caloric restriction in the 20% P-R and 0% P groups. Furthermore, \(V_O_2\) and \(V_CO_2\) during the late D phase in the 20% P-R group tended to be rather lower than in the 20% P group. This observation is similar to previous findings showing that metabolic heat production during the D phase is reduced in rats that are fasting and/or under food restriction [5, 17, 18]. The increase in LMA, metabolic rates, and \(T_b\) during the D phase in the 0% P group would have thus resulted from the absence of dietary protein and not from the reduced food intake. Furthermore, the results shown here suggest that the absence of dietary protein leads to a loss of energy as heat by increasing LMA, and also that dietary protein contributes to conserve energy by reducing metabolic rates during the D phase. As a potential explanation for the physiological importance of the increased LMA during the D phase, it might have acted as a strategy under conditions of the 0% P diet to promote anabolism and to overcome a negative protein balance, because the activity in general has a gross
positive impact on protein balance [23, 24]. Another interpretation is that the central nervous system requires the amino acids found in protein (such as tryptophan, phenylalanine, tyrosine, histidine, glutamine, and arginine) as substrates for the synthesis of various neurotransmitters and neuromodulators to ensure adequate function. Therefore the brain identifies inadequate protein intake and consequently enhances protein-seeking behavior [25].

The T_b during the L phase was decreased by 4 days of feeding on the 0% P diet. On day 4 of the L phase, VO_2 and VCO_2 were lower in the 0% P group than in the 20% P group, which decreased almost in accordance with the decline in T_b. Therefore the inability of the rats in the 0% P group to maintain metabolic rates seemed to be the mechanism that caused the significant reduction of T_b at that time. Furthermore, our results showing that the changes in VO_2 and VCO_2 of the 20% P-R group did not significantly differ from those in either the 0% P group or the 20% P group suggest that the effects of both calorie restriction and the absence of dietary protein were the major causes of prominent reduction in VO_2 and VCO_2 in the 0% group. However, during the L phase T_b was higher in the 20% P-R group than in the 0% P group, although neither VO_2 nor VCO_2 significantly differed between them, suggesting that dietary protein might have also modulated other primary mechanisms and determined T_b rhythms during this period. We found no concurrent changes in LMA and T_b or metabolic rates during the L phase in any group. Thus behavioral thermoregulation was probably not involved in the T_b modification by dietary protein during the L phase. This implies that different thermoregulatory systems function during the D and L phases. When ambient temperature is low, T_b at resting levels falls until the threshold for thermogenesis is reached. Thereafter heat production is activated, and resting T_b consequently stays close to and above the threshold [6, 26]. The decreases in T_b of the 0% P group might thus represent lowered T_b thresholds for thermogenesis because of the absence of dietary protein. A decreased T_b is usually closely associated with decreased metabolism (Q_{10} effect). The lesser reduction in metabolic rates irrespective of the extent of the fall in T_b in the 0% P group might be explained as follows. T_b fell until it reached the lowered threshold for heat production, which subsequently activated the thermogenic response to counteract the excessive fall in T_b and consequently masked the Q_{10} effect in the 0% P group. Not only does T_b similarly decline in rats fed a low (6% casein) protein diet, but the increase in VO_2 is greater in such rats placed in a mildly cold environment than in controls given a normal protein (25% casein) diet [27]. Further, amino acid infusion increases the threshold T_b for heat production against cooling in humans [28]. Taken together, feeding on a normal protein diet might have maintained the thresholds T_b for the activation of thermogenesis resulting in T_b maintenance during the L phase.

T_b, but not metabolic rates and LMA, became elevated in the 0% P group early in the study (days 1 and 2); this was especially prominent during the D phase on day 2 and immediately after lights off. Increased sympathetic nervous system (SNS) activity, which has been identified in rats given a low protein diet [11, 29, 30], might explain the T_b elevation. Although we did not assess the indices of SNS activity, changes that reflect the response to protein deprivation can be indirectly determined. Similar to the T_b elevation, the decreases in RQ during the D phase were also significant on days 2 in the 0% P group. This suggests that increased SNS activity in rats on the 0% P diet enhances lipolysis [31]; as a consequence, it shifts substrate utilization (increased lipids oxidation) regardless of the intake of a diet rich in carbohydrates. Increased SNS activity decreases blood flow in the rat tail [32, 33], a crucial site for heat loss regulation [34], implying that the absence of dietary protein also contributes to the suppression of the heat loss mechanism and consequently increases T_b. Acute changes in food characteristics, including not only nutrient composition, but also the smell and taste of the diet, would also contribute to the modulation of SNS activation [35]. Therefore the effects of protein deprivation on T_b might have been more readily obvious after food replacement. The SNS probably played a role in the T_b increases, especially early in the study and/or after lights off. However, its effects require definitive characterization.

The present study evaluated the contribution of dietary protein to circadian substrate utilization. Decreases in RQ throughout the D phase were prominent, especially in the 0% P group, which might reflect a shift in substrate utilization (increased lipid oxidation). The shift of substrate utilization from carbohydrate to lipid was probably caused by reduced glucose-stimulated insulin secretion. In fact, this secretion after an intravenous glucose load is severely blunted in protein-calorie-restricted rats, but it was only moderately decreased in rats on an isocaloric but standard diet when compared to the freely fed rats on the standard diet [36]. Excess carbohydrates ingested from the 0% P diet might then be stored in glycogen during this period in the 0% P group. By contrast, the distinctive RQ elevations were indicated in the 0% P group during the late L phase compared with the 20% P-R group, despite a similar caloric restriction between the two groups. This may be interpreted to mean that ingested carbohydrates are stored as lipids during this period and that substrate utilization is shifted from lipids to carbohydrates partly derived from glycogen stored during the D and early L phases. This interpretation is supported by the finding that liver triacylglycerol and glycogen concentrations increase in rats fed a low protein diet [37]. Similar to published results [17, 18], however, the finding that RQ decreased earlier during the D phase and largely dropped throughout the L phase in the 20% P-R group compared with the 20% P
group indicates that the rats consumed calories from food during the early D phase and relied more on fat stores during the L phase.

In conclusion, our findings demonstrated that dietary protein plays an important role in modifying circadian core body temperature during both the light (inactive) and dark (active) phases and that the maintenance of normal metabolic rates by dietary protein intake might partly account for such maintenance during the dark phase. Furthermore, our findings support the notion that a lack of dietary protein contributes to potential circadian Tb disruptions that can cause various clinical health problems.

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REFERENCES


