Vigilance states and body temperature during the circadian cycle in fed and fasted pigeons (Columba livia)

MICHAEL E. RASHOTTE,1 IURI F. PASTUKHOV,2 EUGENE L. POLIAKOV,2 AND ROSS P. HENDERSON1

1Program in Neuroscience, Department of Psychology, Florida State University, Tallahassee, Florida 32306-1270; and 2Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg 194223 Russia

Rashotte, Michael E., Iuri F. Pastukhov, Eugene L. Poliakov, and Ross P. Henderson. Vigilance states and body temperature during the circadian cycle in fed and fasted pigeons (Columba livia). Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1690–R1702, 1998.—Fasting induces nocturnal hypothermia in pigeons. Slow-wave sleep (SWS) and paradoxical sleep (PS) are associated with reduced heat production in pigeons. The possibility that fasting-induced nocturnal hypothermia is related to increased SWS and PS was examined by comparing body temperature (Tb) and vigilance states when pigeons were fed and fasted. The results showed that when Tb is decreasing near the beginning of the dark phase, the percentage of total recording time (%TRT) spent in SWS and PS was elevated in fasting due to increased frequency of episodes and increased duration of PS episodes. When Tb was low during the middle segment of the dark phase, SWS was elevated in fasting due to increased episode duration. However, fasting did not alter PS, which increased in %TRT across the segment due to increased episode frequency. When Tb was rising during the final hours of dark, SWS remained elevated in fasting and %TRT in SWS and PS was relatively high. SWS and PS may promote the fasting pigeon’s entry into, and maintenance of, nocturnal hypothermia.

In many avian species, conditions of food scarcity and/or cold can result in increased amplitude of the day-night rhythm in core body temperature (Tb), which approximates 1–2°C when conditions of food availability and ambient temperature (Ta) are benign (27). For example, the amplitude of the Tb rhythm in Columbiformes (pigeons and doves) progressively increases to 5–6°C during several days of fasting, primarily because Tb becomes progressively lower during the inactive (dark) phase of the day (11, 29, 40). Significant energy savings are achieved by nocturnal hypothermia (e.g., Ref. 32).

It has been proposed that fasting-induced nocturnal hypothermia in pigeons could be mediated, at least in part, by the separate influences of vigilance states and circadian phase on the threshold for endogenous heat production (11–14). With respect to vigilance states, Heller et al. (14) showed that when pigeons were in slow-wave sleep (SWS) or in paradoxical sleep (PS), their heat-production response to cooling of the spine (31, 35) was reduced. Specifically, in SWS a colder spinal temperature was required to initiate heat production than in the awake (W) state (and when the spinal cooling was continued beyond threshold, the rate of increase in heat production was lower in SWS). In the PS state, heat production was further dampened because pectoral shivering was greatly reduced [pectoral shivering is the main source of regulated heat production in pigeons (16)]. These findings and the characteristics of the dark-phase sleep architecture of pigeons suggest a possible role for vigilance states in the lowering of nocturnal Tb. Several studies with fed pigeons indicate that SWS predominates in the dark. Also, PS, which is reported not to occur at all in the light phase, occurs in hundreds of very short (~9 s) episodes, which, in total, occupy 8–10% of the dark phase (37–39). If the fed pigeon’s relatively modest reductions in nocturnal Tb were at least partly mediated by the influence of these vigilance states on thermoregulatory responsiveness, it may be that the fasting-pigeon’s deeper hypothermia is associated with increased SWS and PS in the dark phase. Data relevant to this possibility are presented in this paper.

With respect to the influence of circadian phase on nocturnal Tb, it has been demonstrated that a reduction in spinal thermosensitivity occurs during the dark phase of the circadian cycle and that this effect is independent of vigilance state. Heller et al. (14) showed that when a pigeon was in the W state, for example, spinal cooling resulted in greater heat production in the light phase than in the dark. A possible role for this factor in fasting-induced nocturnal hypothermia was proposed by Graf et al. (11), who found that as nocturnal hypothermia progressively deepened across the days of the fast, progressively colder spinal temperatures were required to initiate heat production in the dark phase. The light-phase threshold for response to cold remained relatively unaffected by the fast. Although the finding of Graf et al. (11) is typically viewed as indicating that, as fasting continues, there is a progressively greater influence of the circadian-phase factor that suppresses nocturnal heat production, their experiment did not characterize vigilance states during fasting. It remains possible, therefore, that the reduction of nocturnal Tb in their fasted pigeons might have been at least partially related to a fasting-induced increase in SWS and/or PS during the dark phase, as we have envisioned above.

The data are very limited with regard to the question of how vigilance states are affected by fasting in the pigeon or in other Columbiforme species. The only data...
available from pigeons is found in the reports of an experiment concerned with the effects of glucose infusions on \( T_b \) and vigilance states during fasting. In that case, Phillips and Berger (Ref. 25; also see Refs. 4, 6, 24) reported no changes in SWS or in PS during 4 days of fasting, even though nocturnal \( T_b \) decreased significantly. However, vigilance data during ad libitum feeding were not used in the comparisons (vigilance states were compared only across days 2–4 of the fast), and the severity of body weight loss (\(|-10\%|\) was not as great as in typical experiments on fasting-induced nocturnal hypothermia in pigeons. Phillips and Berger (25) showed that vigilance states in fasting were not differentially influenced by infusions of glucose or saline, although glucose infusion was associated with elevated \( T_b \).

In an experiment with ringed turtle doves (Streptopelia risoria), however, Walker et al. (40) reported that vigilance states are altered by fasting. In this case, vigilance states in ad libitum feeding were compared with those in fasting when the doves showed a strong nocturnal hypothermic response after a loss of \(|-20\%| \) body mass. The major finding was that, in the fasted state, there was about a 60% reduction in time spent in PS during the dark phase. The latter result was interpreted as indicating that fasting-induced nocturnal hypothermia is associated with reduced exposure to periods in which \( T_b \) is unregulated (i.e., PS periods), thereby favoring continuous thermoregulation during SWS (and W). The fasted doves showed no significant change in total sleep time/24 h or (judging from data presented in Table 1 of Walker et al.) in total time spent in SWS/24 h.

Although the experiment with doves provides evidence that fasting affects vigilance states in Columbiform species, it does not support the possibility we have raised above that fasting-induced nocturnal hypothermia might be mediated by the dampening effects of increased SWS and PS on the heat-production response. The dove experiment is not inconsistent with the possibility that a separate dampening effect related to circadian phase could be a factor, however.

Vigilance states during ad libitum feeding and fasting have been studied in only two other avian species, geese and emperor penguins, both of which have much greater body mass than the Columbiformes studied. Unfortunately from the present perspective, those studies did not include concurrent measurement of \( T_b \). In both species, however, fasting was associated with large changes in sleep architecture. Specifically, the geese (8) and the emperor penguins (7) both showed very large increases in SWS. There was little effect on PS in the geese, but there was a decrease in PS in penguins. The increase in SWS in the geese occurred largely at the expense of time in the drowsy state, whereas in the penguins it reduced time in W. The experiment with geese also identified the stage of the fast as being a critical variable: the large increase in SWS occurred when the geese were metabolizing lipids but did not persist late in the fast when they metabolized protein.

The present experiment utilized pigeons to investigate the relationship between \( T_b \) and vigilance states throughout the circadian cycle. As in the experiments by Walker et al. (40), we compared vigilance states in ad libitum feeding with those occurring after 20% of body weight was lost through fasting. That level of fasting promotes strong nocturnal hypothermia in pigeons (28, 29). The main goal was to inquire whether nocturnal hypothermia induced under such fasting conditions in pigeons is accompanied by the kinds of change in sleep architecture that might promote lowered \( T_b \).

**METHOD**

**Animals.** Six adult pigeons (Columba livia) of undetermined sex were purchased from a supplier of local animals in Tallahassee, FL. The pigeons were adapted to living in an indoor free-flying avairy with \( T_a = 19–22^\circ \text{C} \) and a 12:12-h light-dark cycle (LD 12:12). Food (Purina Nutriblend Gold; caloric value of 18.4 kJ/g, as determined by bomb calorimetry) and water were available continuously. The body weights of the birds averaged 348 g at the start of the experiment (range: 321–386 g).

**Experimental chamber.** During the experiment, each animal lived continuously in a chamber (48.2 by 35.5 by 35.6 cm) located inside a temperature-controlled enclosure where \( T_a \) was maintained at 21°C (±0.10°C) and LD 12:12 was in effect. During the light phase, an overhead incandescent lamp was lighted (illuminance = 40 lx of 2,250–K light measured at the approximate height of the pigeon’s head). The chamber was completely darkened in the daily dark phase. Food and water dispensers provided continuous access to food and water during ad libitum feeding, and only water during fasting.

**Electrophysiological recordings and \( T_b \).** Electrophysiological recordings to characterize vigilance states were made from bipolar electrodes placed in the skull to measure electroencephalogram (EEG) from the left and right hemispheres (in a midposterior location recommended by Van Twyver and Allison (38) for good recording of slow-wave activity) and electrooculogram (EOG) from the right eye. The electrodes were miniature gold-plated electronic connector pins bent into an L-shape that were inserted directly into small holes made in the pigeon’s skull at the electrode-placement sites. The pins were secured with dental cement. Each pin was manually crimped to a multistranded Teflon-coated wire (Cooner) in advance of the skull placement. In some cases we recorded muscle tone from electromyogram (EMG) electrodes placed in the neck muscles, and we recorded heart rate [electrocardiogram (ECG)] from subcutaneous electrodes inserted dorsal laterally from the spine. All wires from the recording electrodes were brought subcutaneously to an incision at the interscapular region of the back, and then to a connector on a lightweight (30 g) backpack secured by a comfortable harness running under each wing. The backpack, which the pigeon wore continuously, included a printed circuit board on which miniature amplifiers and circuitry were located to begin the processing of EEG, EOG, EMG, and ECG signals. It was connected to a swivel in the ceiling by a tether that did not restrict the animal’s movements or sleeping posture. \( T_b \) was measured by means of a thermosensitive radio transmitter implanted in the pigeon’s abdominal cavity under halothane anesthesia. The transmitters (model FT; Barrows, Palo Alto, CA) had a pulse rate of \( \sim 500 \text{ Hz at 37}^\circ \text{C} \), which was processed by software to provide mean \( T_b \) measurements with high resolution (0.01°C) averaged over every 30 s of the 24-h cycle.
Electronic signal processing. The details of signal processing at the pigeon's backpack and beyond are as follows. The differential amplifiers in the backpack provided a gain of 100 and single-ended analog outputs that went to a remote unit for further amplification and filtering. These outputs were routed to their destination by means of a cable tether connected to the backpack that led to a slip-ring assembly mounted in the ceiling of the chamber. The EEG filter passband was 0.16–30 Hz, and the EOG passband was 0.08–60 Hz. The EMG passband was 60–1,000 Hz, and its output was sent to a root mean square detector. A microcomputer sampled each signal 120 times per second with a 12-bit analog-to-digital converter. Full scale range was ±1 mV referred to the electrodes. The EEG and EOG samples were averaged to yield 60 data points/s, the EMG samples were averaged to 10 points/s, and all resulting averages were stored as signed binary integers with 0.25 mV resolution.

Vigilance states criteria. Scoring of vigilance states was done by visual inspection of records presented on a high-resolution computer screen. The records included raw EEG (from both hemispheres), EOG, and (in some cases) ECG and neck-muscle EMG data that could be displayed simultaneously with quantified measures of amplitude and frequency characteristics of the waveforms. All data were presented in 1-s epochs on the screen, and the full screen display used in scoring was typically 20–60 s of the records. When desirable, the screen display was zoomed (2–900 s) to improve the scoring procedure. A computer program automatically filed the time of onset and the duration of each scored state in a spreadsheet and superimposed a color-coded record of the scored states on the display. One person (Polakov) carried out the scoring used in this report. Three vigilance states (W, SWS, and PS) were scored using conventional criteria. Briefly, SWS was scored as high-voltage slow waves of EEG lasting at least 5 s without eye movements but with blinks greater than 5 s apart; PS was scored as low-voltage fast waves lasting at least 3 s that occurred during SWS and coincided with rapid eye movements; W was scored as mixed amplitude and frequency coinciding with blinks and other eye movements. Other electrophysiological patterns were scored as a transitional state (TS), which primarily included patterns intermediate to W, SWS, and PS and typically surrounded a change from one state to another. Our TS category is largely equivalent to the “drowsy” state scored in some papers (37). For some portions of the electrophysiological records, it was possible to display concurrently videotaped recordings of the pigeon's behavior in both the light and dark phases (infrared video camera). Analyses of these records indicated that all episodes scored as W were characterized by alert behavior (perched with eyes open and head erect, preening, walking, etc.), whereas episodes scored as PS were marked by eyes closed and head movements (including head dropping, head shaking). Behavior during SWS episodes was typically characterized by stillness during perching, with eyes closed, head resting on breast, etc.

Procedure. Before the electrodes and T_b transmitter were installed under halothane anesthesia, the pigeons were well-adapted to the experimental chambers, including the LD 12:12 schedule, ad libitum feeding, and wearing a dummy backpack. After the surgery, they were allowed at least 10 days to readjust to the ad libitum feeding schedule. Then, electrophysiological data were recorded for at least one 24-h period to characterize the T_b and vigilance states in ad libitum feeding. Fasting began on the next day when food was withdrawn. Fasting continued until the pigeons had lost ~20% of their ad libitum feeding weight (5–12 days), whereupon electrophysiological data were recorded for another 24-h period to characterize T_b and vigilance states in fasting pigeons.

Statistical comparisons. Comparisons between conditions were made with Student's t-test (paired or unpaired observations, as the case required), by repeated-measures ANOVA, and by Pearson correlations, as described in the text. The minimal probability accepted for statistical significance was P ≤ 0.05.

RESULTS

Body weight was reduced by the targeted amount of 20% (Table 1) in 8 days on average (range: 5–12 days). Table 1 presents the average daily values of T_b and of each measure calculated for the vigilance states on the final day of feeding and fasting. Statistical comparisons indicated that fasting was associated with significantly lower daily T_b and with significant changes only in the PS vigilance state: the percentage of total recording time (%TRT) spent in PS was reduced because the number of PS episodes per 24 h was reduced. The average duration of PS episodes in feeding and fasting remained unchanged at ~6.6 s. In the entire 24-h cycle, fed pigeons slept (SWS + PS) 62.3% of TRT, were awake 23.3% of the time, and had a PS-to-SWS ratio (%TRT values) of 0.16. There was little change in the daily %TRT spent asleep and awake when the pigeons were fasted (SWS + PS = 64.6%; awake = 19.6%), but the PS-to-SWS ratio dropped to 0.12. In both feeding conditions, each of the two sleep states occurred with high frequency (~950 episodes/day in the fed condition; ~800 episodes/day in the fasted condition), and the duration of SWS episodes was about nine times greater than PS episodes (SWS = 58 s; PS = 6.6 s).

<table>
<thead>
<tr>
<th>Table 1. Mean body weight, T_b, and vigilance data (24-h cycle)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feeding Condition</strong></td>
</tr>
<tr>
<td>Ad libitum</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Fasting</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Table entries are means ± SE. All measures were calculated for n = 5 because a technical problem resulted in loss of data from the light phase for one bird. That bird's body weight was 347 g in ad libitum feeding and 265 g in fasting. Body weights for ad libitum feeding and fasting are from final day in each condition; birds were weighed at daily maintenance period, 2 h before lights off. Body temperature (T_b) and vigilance data were calculated over 24 h. W, wake; TS, transitional state; SWS, slow wave sleep; PS, paradoxical sleep. Statistically significant differences between fasting and ad libitum (paired t-tests): *P < 0.05; #P < 0.001.
A breakdown of the whole day data into separate light and dark phases of the daily cycles is shown in Fig. 1 and Table 2. This breakdown revealed that several important within-day effects were related to the feeding/fasting manipulation.

First, the T_b data indicated that fasting resulted in nocturnal hypothermia (Δ = −3.1°C), as expected, but also that T_b was lower during the light phase in fasting (Δ = −1.2°C). In both phases, the fasting-related reduction in T_b was statistically significant (Table 2).

Second, a far more detailed picture of the effects of fasting on vigilance states emerged. In particular, the original finding that fasting is associated with reduced PS (Table 1) turned out to reflect changes that were primarily localized in the light phase (Table 2, Fig. 1): statistical comparisons of the 12-h values for each PS measure in the dark phase revealed no significant changes between feeding and fasting; in the light phase, however, both the %TRT and the number of episodes of PS were statistically lower in fasting (Table 2). The failure to find a fasting-related reduction in PS during the dark phase was not related to a floor effect in ad libitum feeding that prevented a reduction from being detected. On the contrary, all three measures of PS in the dark-phase during ad libitum feeding were significantly higher than in the light (paired t-tests).

Table 2. Mean T_b and vigilance data in the light and dark phases of the daily cycle

<table>
<thead>
<tr>
<th>Phase</th>
<th>T_b, °C</th>
<th>W</th>
<th>TS</th>
<th>SWS</th>
<th>PS</th>
<th>%Total Recording Time</th>
<th>Number of Episodes</th>
<th>Episode Duration, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>41.6</td>
<td>±0.1</td>
<td>33.2</td>
<td>17.1</td>
<td>45.6</td>
<td>4.1</td>
<td>70.0</td>
<td>219.4</td>
</tr>
<tr>
<td>Fasting</td>
<td>40.4†</td>
<td>±0.2</td>
<td>36.5</td>
<td>22.1*</td>
<td>39.5</td>
<td>1.9*</td>
<td>107.0*</td>
<td>230.8</td>
</tr>
<tr>
<td>Dark (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>40.4</td>
<td>±0.1</td>
<td>13.8</td>
<td>11.5</td>
<td>62.2</td>
<td>12.5</td>
<td>70.8</td>
<td>317.2</td>
</tr>
<tr>
<td>Fasting</td>
<td>37.3‡</td>
<td>±0.2</td>
<td>3.1†</td>
<td>9.2</td>
<td>75.6‡</td>
<td>12.1</td>
<td>32.7*</td>
<td>262.3</td>
</tr>
</tbody>
</table>

Table entries are means ± SE for T_b and vigilance states in the 12-h light (n = 5) and dark (n = 6) phases on the last day of ad libitum feeding and fasting; see Table 1 for details. A technical problem, which resulted in light-phase data being unavailable for 1 bird, reduced n to 5 for light-phase means. Statistically significant differences between fasting and ad libitum data (paired t-tests): * P < 0.05; † P < 0.01; ‡ P < 0.001.
using 12-h values for %TRT, number of episodes, and episode duration: all df values = 4, all t values > 3.56, all P values < 0.024).

The breakdown of the whole day data also revealed that vigilance states other than PS were affected by fasting. In the dark phase, fasting was associated with a significant increase in SWS and decrease in W (%TRT measure, Table 2). The increase in SWS resulted from increased duration of the episodes; the decrease in W resulted from a decrease in both the number and the duration of episodes (Table 2). Fasting had no significant effect on TS in the dark phase. In the light phase, the %TRT spent in TS increased (Table 2), but the latter effect was less straightforward than the others because it cannot be traced to significant changes in the component measures (frequency or duration of episodes), probably because the sample size is too small to detect subtle effects. Likewise, in the light phase fasting was associated with a significant increase in number of W episodes (Table 2), but this did not translate into a related change in %TRT. Individual differences in the pigeons’ light-phase response to fasting may be factors in these latter two outcomes (see below).

The nature of the relationship between sleep states and changes in T_b during the hours of the day when nocturnal hypothermia occurred was also clarified by the breakdown of the whole day data. Figure 2 presents a close-up view of the hourly values for T_b and for SWS and PS (%TRT) in the feeding and fasting conditions during the 12-h dark phase, bracketed by a 2-h segment of the light phase immediately before and after the dark. Segments of these plots were analyzed using two-factor repeated-measures ANOVAs. One repeated-measure factor was feeding condition (2 levels: ad lib and fasting). The other repeated-measure factor was hours. In one set of analyses (Early), only the initial part of the curves was analyzed to examine the part of the record when T_b was falling most rapidly; in these analyses, we utilized data from the last 2 h of the light phase (hours 23 and 24) and the first 2 h of the dark phase. A second set of analyses (Middle) utilized data from the part of the night when T_b was relatively stable (hours 3–9). The third set of analyses (Late) was concerned with the final part of the night when T_b was rising before lights on (hours 10–12). Analyses were performed with data from all five pigeons that had complete data for both the light and dark phases. In each analysis, feeding condition had df = 1, hours had df = 3 (Early), 6 (Middle), or 2 (Late), and the feeding condition × hours interaction had df = 3 (Early), 6 (Middle), or 2 (Late). The outcome of these ANOVAs is summarized in Table 3, which also shows the outcome of comparable ANOVAs conducted on the number and the duration of SWS and PS episodes (not plotted) in the Early, Middle, and Late segments of the curves.

Consider, first, the T_b and the %TRT measures for SWS and PS. Table 3 indicates that in the Early segment of the curves fasting was associated with significantly lower T_b, higher SWS, and higher PS (feeding condition factor) and, as can be seen in Fig. 2, that T_b decreased while SWS and PS increased (hours factor). Furthermore, the feeding condition × hours interaction was also significant in each case, which supports the impression conveyed by Fig. 2 that, compared with ad libitum feeding, fasting was associated with a greater rate of decrease in T_b and a greater rate of increase in SWS and PS. However, the significant feeding condition × hours interaction in the PS analysis likely also reflects the crossover of the PS curves in ad libitum and fasting by hour 2 of the dark phase (Fig. 2). These analyses, and the data plotted in Figs. 1 and 2, highlight the fact that during the pigeon’s entry into fasting-induced nocturnal hypothermia the %TRT spent

![Fig. 2. Mean (±SE) hourly values of T_b, %TRT SWS, and %TRT PS in final 2 h of light phase (hours 23–24), 12-h dark phase, and first 2 h of light phase (hours 13–14). Each panel shows a comparison of data obtained in ad libitum (Ad Lib) feeding and in fasting. Values for T_b are selected from T_b curves plotted in Fig. 1: each point is the mean of T_b values at completion of hour plotted (e.g., mean for hour 23 is based on T_b value for each pigeon recorded in 30-s intervals that completed hour 23 of the daily cycle). Values for sleep states are mean percentages of each hour spent in SWS and in PS. These values were plotted in Fig. 1, but in a stacked bar-graph format in that case. Error bars associated with several data points are smaller than the symbol and do not appear in the graph. Note that y-axis scale in B extends from 0 to 100%, whereas scale for PS is truncated to 0–25%. Also, vertical lines denoting beginning and end of dark phase have been slightly offset to the right of the actual times of light change to improve readability of the graph. Lights off occurred at completion of hour 24; lights on occurred at the completion of hour 12.](http://ajpregu.physiology.org/)
in SWS and PS is elevated, compared with these vigilance states during the relatively modest lowering of nocturnal Tb characteristic of the fed state.

The ANOVAs carried out on the number and duration of SWS and PS episodes during the Early segment provide information about the contribution of these measures to the changes in %TRT spent in SWS and PS that are plotted in Fig. 2. The outcome of these analyses, summarized in Table 3, indicated that fasting was associated with an overall greater number of SWS and PS episodes (feeding condition factor) and that the number of episodes in both sleep states increased substantially over these Early hours (hours factor) but not at different rates in ad libitum and fasting (feeding condition × hours interaction). According to this analysis, the increase in %TRT spent in SWS and PS during entry into nocturnal hypothermia is related to an overall increase in the number of episodes of each vigilance state. However, the analyses of the episode duration measure indicated that changes in duration also contributed to the changes in %TRT spent in PS, but not in SWS. That is, in the PS-duration analysis (Table 3), episode duration increased significantly across hours 23–2 (hours factor) and at a differential rate depending on the feeding condition (feeding condition × hours factor). The nature of the interaction is that the PS episode duration began the segment at a lower value in ad libitum (hour 23 = 4.52 s) than in fasting (hour 23 = 5.94 s), but ended the segment in the opposite relationship (hour 2 ad libitum = 7.32 s vs. hour 2 fasting = 6.18 s). These changes across hours parallel the changes in the plot of %TRT PS during the same hours (Fig. 2). The main effect of feeding condition was significant in the PS-duration analysis (Table 3). In the analysis of SWS episode duration, no effects reached statistical significance.

Figure 2 indicates that by hour 2 of the dark phase, when Tb had decreased to the vicinity of its nocturnal plateau, SWS had peaked at different levels in each feeding condition and was about to begin a generally decreasing trend across the subsequent hours. In contrast, PS had reached about the same level in the two feeding conditions by the end of the dark phase. In the analysis of %TRT spent in SWS, the numerically highest value of PS in the entire dark phase, SWS had peaked at different levels in each feeding condition and was about to begin a generally decreasing trend across the subsequent hours. In contrast, PS had reached about the same level in the two feeding conditions by the end of the dark phase.
these analyses, indicating that the changes shown in Fig. 2 across the Middle hours are statistically supported: overall, there was a decrease in $T_b$ in SWS and an increase in PS. The feeding condition $\times$ hours interaction did not reach an acceptable level of significance in any of these analyses, indicating that the trend of the changes observed in $T_b$, SWS, and PS across hours 3–9 of the dark phase was not differential in the ad libitum and fasting conditions. The correlation between SWS and PS (ignoring feeding condition) was negative and statistically significant ($r = -0.36, P = 0.003$), reflecting the fact that PS increased while SWS decreased during hours 3–9 of the dark phase, as is evident in the data presentation of Fig. 2.

Additional ANOVAs were conducted on the number of episodes and the duration of episodes of both sleep states in an attempt to clarify the basis for the changes in %TRT found in SWS and PS during hours 3–9. These analyses, summarized in Table 3, revealed that the increase in PS was solely attributable to an increase in the number of PS episodes across the hours (hours factor; hour 3 = 44.3 episodes/h, hour 9 = 69.5 episodes/h) and that this increase was not different in the ad libitum and fasting conditions (feeding condition $\times$ hours factor). In the case of SWS, the analyses summarized in Table 3 provide no indication of the basis for the significant decrease found in %TRT spent in SWS across the Middle hours of the dark phase. In these analyses, there was only a significant feeding condition factor in the SWS episode-duration analysis, which simply indicates that there was an overall longer duration of SWS in fasting, which is reflected in the means for SWS reported in Table 3 for this measure in ad libitum and fasting conditions.

Beginning at about hour 10 of the dark phase, $T_b$ began its prelight increase in both the fed and fasted conditions. Table 3 summarizes the analyses of measures during the last 3 h of the dark phase (hours 10–12). During this Late segment of the dark phase, fasting continued to be associated with significantly lower $T_b$ and higher %TRT spent in SWS (feeding condition factor). $T_b$ increased in both the ad libitum and fasting conditions (hours factor) but did so at a higher rate in fasting (feeding condition $\times$ hours factor), as is shown in Fig. 2. In the analysis of %TRT spent in SWS, there were no statistically significant effects relating to change across these hours (hours factor; feeding conditions $\times$ hours factor). The analysis of %TRT spent in PS during the Late segment yielded no statistically significant effects for any factor. During the recovery from nocturnal hypothermia, the majority of the increase in $T_b$ occurred between hours 10 and 12 of the dark phase, when %TRT spent in SWS and PS remained relatively high (Fig. 2). The onset of light at hour 12, which signaled the beginning of the daily active phase, resulted in a small further increase in $T_b$ and a relatively large and sudden decrease in the %TRT spent in SWS and PS (Fig. 2).

In the additional analyses of number and duration of the sleep-state episodes in hours 10–12 of the dark phase, none of the factors in the analyses of PS measures reached statistical significance (Table 3), which is consistent with the lack of effects found in the analysis of %TRT for PS reported above. The additional analyses also indicated that a greater number of SWS episodes in fasting (feeding condition factor) accounted for the greater %TRT spent in SWS during fasting in the Late segment, as reported above. There were no significant effects in the analysis of the duration of SWS episodes in the Late segment (Table 3). We also found a significant overall decrease in the number of SWS episodes in ad libitum and fasting during hours 10–12 (hours factor; overall mean for hour 10 = 55.9 episodes and for hour 12 = 44.0 episodes), but this effect was not sufficient to produce a statistically significant hours-related change in the %TRT measure of SWS (above).

Finally, an examination of the data of individual pigeons indicated that there were notable differences in response to fasting during the light phase of the daily cycle. In fact, the pigeons could be separated into two clear subgroups on the basis of the pattern of changes in $T_b$ that occurred during the light phase. Figures 3 and 4 present the data for these subgroups in the format of Fig. 1 to provide a more complete picture of how $T_b$ and vigilance states can be affected by fasting. In one subgroup (n = 2, Fig. 3), the light phase in fasting began with $T_b$ at a relatively high value, which was followed by only a small increase across the phase; this pattern of change in $T_b$ was accompanied by relatively high W and low SWS. In the other subgroup (n = 3, Fig. 4), the light phase began with $T_b$ at a relatively low value, which was followed by a gradual increase of ~1.5°C across the phase; this pattern was accompanied by relatively low W and high SWS. The very different patterns of change in the light-phase vigilance responses of these two subgroups in fasting is nicely captured by the percentage change in W and SWS relative to the light phase values in ad libitum feeding. With the use of values for the entire 12-h light phase in the calculations, pigeons with a relatively high $T_b$ throughout the light phase in fasting (Fig. 3) showed $\Delta W = +50\%$ and $\Delta SWS = -15\%$, indicating that fasting increased alertness in the light phase for these pigeons, perhaps suggesting that they persisted in food-searching activity. In contrast, the pigeons for which $T_b$ gradually increased from a low to a higher level (Fig. 4) showed $\Delta W = -45\%$ and $\Delta SWS = +24\%$, indicating that less wakefulness and more sleep occurred in the light phase during fasting than in ad libitum feeding.

**DISCUSSION**

A principal finding is that when pigeons have lost 20% body mass through fasting, their vigilance states are altered during the circadian cycle compared with the states characteristic of ad libitum feeding. These effects are particularly interesting with respect to the changes in $T_b$ associated with fasting-induced nocturnal hypothermia. Near the beginning of the dark phase, when $T_b$ is decreasing most rapidly toward its nocturnal plateau, the percentage of time spent by fasted
pigeons in SWS and PS was elevated in comparison to ad libitum feeding. These changes were attributable to increased frequency of the SWS and PS episodes and to a small increase in the duration of PS episodes. During the Middle segment of the dark phase, when \( T_b \) was near its nocturnal plateau, the fasted pigeons continued to show elevated SWS, which was attributable to increased duration of the episodes. Fasting did not affect PS in the Middle segment. Rather, in both ad libitum and fasting conditions there was an increase in the percentage time spent in PS across the segment, which was attributable to increased frequency of episodes. During the final segment of the dark phase, when \( T_b \) was increasing in both the fasting and ad libitum conditions, SWS remained elevated in the fasting condition and, overall, the percentage of time spent in SWS and PS was relatively high.

The relationships we report here between \( T_b \) and vigilance states in the fed and fasted pigeon during the dark phase are correlational. Consequently, we cannot distinguish whether the fasting-related changes we observe in SWS and PS contribute in a causal way to the changes in \( T_b \) that are characteristic of nocturnal hypothermia in pigeons. It is possible that causality goes the other way around, or that these vigilance measures covary with \( T_b \) as a result of another unmeasured variable. However, because SWS and PS are associated with reduced heat production in pigeons, our results are consistent with the idea proposed originally by Heller et al. (14) and Graf et al. (11) that SWS might...
act cooperatively with the separate circadian suppression of heat production to lower nocturnal $T_b$ in this species, and we suggest that this be extended to include PS. That is, the increased SWS and PS around the time of the light-dark transition in fasting pigeons could work in concert with the circadian factor to promote the pigeon’s entry into nocturnal hypothermia. The elevated SWS in fasted pigeons during the Middle and Late segments of the dark phase could contribute to the observed hypothermic $T_b$ in those segments. Even the increase in PS across the Middle segment of the dark phase, which was characteristic of both ad libitum and fasting, might play a role in maintaining nocturnal $T_b$ at a reduced level through a suppressive action on shivering, at least in moderate and low ambient temperatures where shivering thermogenesis would be important.

However, if SWS and PS contribute to the reduction in $T_b$ during the dark phase, as we are proposing, it might be expected that those vigilance states would decrease in favor of W or TS states during the last 3 h of the dark, when $T_b$ is rising. This is especially the case in fasting, when $T_b$, although still hypothermic, rises several degrees. Although our data hint at a late-night decrease in SWS and PS (Figs. 1 and 2), the statistical analyses of these effects did not provide corroborative support. In any case, we propose that SWS and PS may play an important thermoregulatory role late in the dark phase after the rise in $T_b$ has been initiated by a light-entrained circadian oscillator (e.g., Ref. 30). That is, SWS and PS may moderate the rate at which $T_b$ rises before lights on by reducing shivering and altering heat-retention mechanisms (such as vasoconstriction in the unfeathered legs) that contribute to the late-night increase in $T_b$ (10). By this account, the occurrence of SWS and PS in the latter part of the night would ensure that $T_b$ does not reach a high level before lights on (see Ref. 3 for a related proposal concerning a possible role for SWS in arousal from hibernation in mammals).

Ad libitum feeding. We begin a detailed discussion of our findings by considering some features of the ad libitum feeding data. The circadian cycle of vigilance states in fed pigeons and doves has been described in several other papers, and there are some notable differences in result among these reports. We wish to draw attention to the status of our findings with respect to those previously reported. Considering the entire 24-h period, we found that pigeons feeding ad libitum spent 62.3% TRT in sleep (SWS + PS; Table 1). This is a larger percentage than was reported in the two classic studies of sleep in pigeons (i.e., Van Twyver and Allison (38): 44.3%; Walker and Berger (39): 45.2%), but it is essentially the same as reported in a more recent paper (i.e., Tobler and Borbély (37): 60%). Only two other Columbiforme species have been studied to date, the much smaller-sized doves Streptopelia risoria (40) and Zenaida asiatica (1); during ad libitum feeding Streptopelia spent ~71.1% of the time in sleep (SWS + PS), whereas Zenaida spent ~38% of the time in sleep.

An examination of the methods used in all these papers suggests that the differential values obtained for 24-h sleep time when Columbiforme species are feeding ad libitum cannot be accounted for by differential ambient conditions that might affect sleep time. A review of these papers indicates that conditions such as LD cycle (all were LD 12:12), $T_a$ (all were 21–24°C), illumination level used to define the light phase, time of adaptation to the testing chamber, and so on are not systematically related to the different total sleep time (TST) reported in these papers. We agree with others (e.g., Ref. 25) that the epoch length used in scoring the vigilance stages may be an important factor in the different outcomes. It is noteworthy, therefore, that the epoch durations were relatively long in the two classic studies where 24-h TST in pigeons approximated 45% [i.e., Van Twyver and Allison (38): 60-s epochs for SWS and for PS; Walker and Berger (39): 25-s epochs for SWS and 2.5-s epochs for PS], but were relatively short in the two more recent studies where the pigeon’s 24-h TST approximated 60% [i.e., Tobler and Borbély (37): 4-s epochs for SWS and for PS; the present experiment: 1-s epochs for SWS and for PS]. Furthermore, the number of episodes of SWS and PS in 24 h when the pigeons were feeding ad libitum was far larger in the present experiment (~1,000 episodes for each of these states; Table 1) than was reported in the classic studies (e.g., 400–500 episodes; Ref. 39).

The different outcomes in the experiments discussed above may also be related to whether the scoring system included a TS (or drowsy state). When this intermediate state is not differentiated from W, SWS, and PS, its occurrence must be included in one of the other states and this, perhaps combined with a long epoch time, could result in inflation of sleep time. Tobler and Borbély (37) explicitly raised this possibility in discussing why the percentage time pigeons spent sleeping per 24 h (60%) in their study was so much higher than reported in the two classic studies (38, 39). However, only one of those studies did not score drowsy state (39); the other one scored and then excluded it from sleep-time calculations (38). Of course, in the case where drowsy state was not scored, it is impossible to know in which of the W, SWS, and PS states it was assigned and in what proportions. Furthermore, if we redefine sleep time in the present experiment to include TS + SWS + PS (instead of just SWS + PS), our pigeons would be reported to sleep 76.7% of the 24-h period, far more than reported when Tobler and Borbély (37) included drowsy state as part of sleep.

One ambient condition that deserves special attention for its likely role in affecting the vigilance states reported in studies of avian sleep is the brightness of the light used to define the light phase. For example, we were easily able to find PS during the light phase, but other papers have reported that, during the light phase, PS is scarce or absent in pigeons (Refs. 25, 37, 39; but see Ref. 38) as well as in doves (1, 40). We note, however, that the brightness of light used to define the light phase in those other experiments (when reported) appears to have been in a range known to suppress
sleep in pigeons (5, 26). The light intensity in our experiment was relatively low. It is possible, therefore, that a lower light intensity might have yielded a quite different picture of light phase vigilance states in the earlier experiments. We have successfully used the present relatively low-intensity light with pigeons to define the daily light phase in experiments concerned with the relationship between daily cycles in T_b, energy metabolism, and feeding, as well as the relationship between light- and food-entrainable circadian oscillators (e.g., Refs. 2, 28, 30).

There was one other distinguishing feature of our procedure that seems not to have had an important effect. Because pigeons typically sleep while perching, with their head and neck in an erect position, we developed a method for recording EEG and EOG data from chronically implanted skull electrodes that did not require the traditional head connector attached to a tether (e.g., Refs. 37–39). Head movements are known to accompany PS in pigeons (e.g., Ref. 38), and our goal was to eliminate mechanical constraint on head or neck movements that might influence the duration of PS states. Our method, as described in Procedure, involved running wires from all skull electrodes (and from neck muscle EMG electrodes) subcutaneously to a lightweight backpack that the pigeon wore continuously. The backpack included miniature amplifiers for the EEG, EOG, and EMG signals. Because the duration of PS episodes in our experiment was similar to that reported when a head connector was used, our methodological innovation did not have a substantive effect on this measure of the pigeon’s vigilance states.

Fasting versus feeding comparisons. The earlier experiment by Phillips and Berger (25), which investigated how the pigeon’s T_b and vigilance states are affected by fasting and glucose infusion, appears at first glance to have yielded a different result than we have obtained. They reported that nocturnal T_b fell significantly during fasting, but that vigilance states did not change. However, Phillips and Berger measured T_b and vigilance states on only the 2nd through 4th days of fasting, at a time when there was relatively shallow nocturnal hypothermia and a relatively small loss of body weight (~10% loss from ad libitum feeding (estimated from information in the various reports of that experiment)). In contrast, we compared vigilance states when the pigeons were feeding ad libitum and when they were showing relatively deep hypothermia after a 20% loss of body weight. Because the time course of changes in vigilance states during fasting is unknown, it is possible that the quantitative properties of the vigilance states we observed on the final day of fasting are, in fact, present from the 2nd day of the fast onward. In that event, there would be no discrepancy between the findings of the two experiments. However, Phillips and Berger also reported that the vigilance states of their fasting pigeons were not differentially affected by saline or glucose infusions, even though the glucose infusion resulted in elevated T_b. To the extent that glucose infusion mimics the ad libitum feeding condition, this aspect of their finding would remain an important point of difference between the two experiments. It is likely, however, that in a feeding situation the bulk properties of ingested food have an important influence on the pigeon’s T_b (9, 33), and this factor may differentiate the glucose infusion from feeding. These various possibilities remain to be investigated.

The outcome of the only other experiment carried out on this question with a Columbiforme species is in agreement with our general finding that vigilance states are changed by fasting. However, the specific changes reported in that experiment are not wholly consistent with our present results, and this raises the possibility of species differences. Walker et al. (40) subjected ringed turtle doves to about the same level of fasting as imposed in our experiment (i.e., 20–22% loss of body mass relative to ad libitum feeding) and observed a strong nocturnal hypothermic response. Their main comparison was also the same as ours: the doves’ vigilance states were compared in ad libitum feeding and on the final day of fasting. Walker et al. (40) calculated the %TRT (24-h period) spent in each state and reported that fasted doves showed no change in TST, but that there was a statistically significant reduction in time spent in PS. We obtained the same result in pigeons when we analyzed %TRT (24 h) values for the vigilance states (Table 1). However, the locus of the reduction in PS during the circadian cycle was different in the two experiments: it occurred in the dark phase for doves (40) but in the light phase for pigeons (Table 2). It is important to note that the fasted doves could not have shown a decrease in PS during the light phase because PS did not occur in that phase when the birds were feeding. The complete absence of PS in the light phase might be related to the illumination used in the experiment, as discussed above. When the effect of fasting on SWS during the circadian cycle is considered, there seems to be better agreement between the two experiments: it appears that in both pigeons and doves, SWS was strongly elevated near the beginning of the dark phase and remained elevated throughout the dark phase. These effects were supported in pigeons by statistical analyses. Walker et al. (40) noted the occurrence of these SWS effects in some doves.

The finding that PS is reduced during the dark phase in fasted doves was interpreted by Walker et al. (40) as consistent with the idea that PS reduction could be a “kind of homeostatic adjustment” that would promote regulation of the low T_b during nocturnal hypothermia. The idea is that because thermoregulatory processes are turned off during PS, the suppression of PS during fasting should help keep nocturnal T_b well regulated at its hypothermic level. In pigeons, however, the total time spent in PS was similar whether the pigeons were fed or fasted, and the %TRT in PS increased across the hours of the dark phase as a result of an increase in the frequency of episodes. An increasing trend in PS across the dark period has previously been reported in pigeons (37) and other avian species (e.g., Ref. 36). It is interesting that during the Middle hours of the dark phase when T_b was near its nocturnal plateau in the fasted pigeons, the kinetics of SWS and
PS were complementary (Fig. 2 and associated analyses): %TRT spent in SWS decreased across these hours whereas %TRT spent in PS increased.

In contrast to the dark phase, where we found that PS was unaffected by fasting, PS was reduced during the light phase in fasted pigeons. This change in PS could possibly serve the kind of homeostatic regulatory function envisioned by Walker et al. (40) in their discussion of the fasted doves, which showed a reduction of PS in the dark. It is unclear, however, why that function should be confined to the light phase in the fasted pigeon.

The following summary picture of how fasting affects \( T_b \) and vigilance states during the circadian cycle in pigeons and doves is suggested by our experiment and that of Walker et al. (40). First, when \( T_b \) is decreasing during entry into nocturnal hypothermia, %TRT spent in SWS is elevated in pigeons and in at least some of the doves studied; in pigeons, there is also a short-term elevation in PS during this part of the circadian cycle. Second, during the hours when \( T_b \) is near its hypothermic nocturnal plateau, SWS is elevated in pigeons and PS is reduced in doves. The pigeon's elevated SWS occurs because the duration of SWS episodes increases. Some data presented in Walker et al. (e.g., see their Fig. 1) indicated that SWS is also elevated during the entire dark phase in individual fasting doves, but it is not clear if that result is characteristic of the doves as a group. In pigeons, there is a complementary relationship between the changes that occur in SWS and PS across the dark-phase hours: %TRT in SWS decreases whereas %TRT in PS increases. Third, in the final hours of the dark phase, when \( T_b \) is recovering from its hypothermic plateau in anticipation of lights on, there is no clear pattern of change in vigilance states in the fasted pigeons. Comparable data from the doves are not summarized in Walker et al. (40), although their Fig. 1 provides some indication that increased %TRT in W may be associated with increased \( T_b \) late in the dark phase. Fourth, in the light phase, PS is reduced in fasted pigeons whereas there is no PS in doves, whether they are fed or fasted. The light-phase data from pigeons also provide additional indications that SWS is related to low \( T_b \); specifically, SWS is higher for the subset of fasting pigeons in which light-phase \( T_b \) is lower (Fig. 4 vs. Fig. 3). There is no apparent relationship between \( T_b \) and the accompanying PS in the light phase for pigeons.

Hypothermic/hypometabolic states in mammals, including torpor, hibernation, and estivation, are closely related to changes in vigilance (6, 12, 15, 17, 40). In torpor and hibernation, for example, there is a sharp decrease in the total time spent in W and PS, and a considerable increase in the time spent in SWS (12, 15, 21). An interesting possibility has been raised using data from ground squirrels that the lower boundary of euthermia (i.e., the starting point of entry into torpor) might be identified by a distinctive change in PS (18, 22, 23). The initial part of the entry into torpor by Citellus major and Citellus parryi is accompanied by an increase in the total time spent in PS, and a maximum in PS is reached when brain temperature (\( T_{br} \)) has decreased to 36–35.5°C. When \( T_{br} \) falls below 35°C, however, there is a distinct change: PS episodes become less frequent and shorter in duration, and they eventually disappear completely when \( T_{br} \) reaches 27–25°C. Pastukhov et al. (22, 23) have proposed that the point at which the PS maximum occurs during entry into torpor may mark the lower boundary of the euthermic state. By this standard, euthermia gives way to the beginnings of shallow torpor in ground squirrels when \( T_{br} = 36–35°C \).

The question arises as to whether the proposed boundary marker for euthermia in ground squirrels is useful in identifying a lower boundary for the euthermic state in pigeons. Viewed from the ground squirrel perspective, the fasted pigeon's daily nocturnal hypothermia (as indexed by \( T_b \) rather than by \( T_{br} \)) does not appear to represent a departure from euthermia. That is, in pigeons the daily maximum in PS occurred late in the dark phase at about the time \( T_b \) was preparing to increase in anticipation of lights on (Fig. 2), rather than during the period of falling \( T_b \) or \( T_{br} \) as found in the squirrels. On these grounds, it might be argued that a lower boundary of the euthermic range of \( T_b \) was not encountered in the fasted pigeons studied in this experiment, suggesting that the pigeon's euthermic range is at least 42–36°C. It would be interesting to examine the relationship between \( T_b \) (and \( T_{br} \)) and vigilance states in pigeons that show far deeper nocturnal hypothermia than found in the present experiment. However, we do not know of cases in which fasting-induced nocturnal hypothermia has resulted in \( T_b \) below ~35°C in pigeons. Until further evidence is obtained on these questions, the above considerations suggest that nocturnal hypothermia in pigeons may be best viewed as a shallow hypometabolic state that falls within (or very close to) the euthermic range. It does not appear to be comparable to the shallow torpor state shown by spontaneously fasting mammals as part of their entry into torpor (22, 23). Changes in vigilance states in fasted pigeons, on the contrary, seem more similar to changes observed in hibernating mammals during the dark phase in summer (i.e., during the euthermic season) and in various other euthermic mammalian species when well fed (23).

**Perspectives**

Our data highlight the desirability of further experiments in which the proposed role of changes in vigilance states in fasting-induced nocturnal hypothermia is investigated more fully. The proposed interplay between vigilance states and the separate circadian reduction in thermosensitivity during the dark phase deserves special attention, as does the time course of changes in vigilance states during the development of fasting-induced nocturnal hypothermia. Such information would deepen understanding of the hypothesized relationship between changes in sleep stages (SWS and PS) and the suppression of heat production that we envision as promoting daily nocturnal hypothermia in pigeons and doves. We have also raised the question of
identifying a boundary marker between euthermia and hypothermic states in pigeons, and this problem deserves further attention in birds and in mammals more generally. From this point of view, it would be desirable to analyze the changes in central (especially, in brain) and peripheral temperatures during nocturnal hypothermia in relation to SWS and PS episodes, and also in relation to short periods immediately preceding PS episodes. We have earlier shown that the direction and value of $T_e$ changes during and preceding PS could be an additional important marker of the transitional periods from euthermia to torpor and hibernation in hibernating mammals (19, 20).

Our present experiment joins several others in encouraging research into the ways various nutritional states affect the circadian organization of vigilance states, $T_b$, $T_r$, and energy metabolism. We noted questions about the separate influences of food and of glucose on $T_b$ and vigilance states, and consideration of hormonal influences related to fasting would provide a more complete picture (34). Research into these questions would likely enhance the understanding of how animals adapt to food scarcity. Also, there is a need for more information about the effects of ambient conditions such as light intensity and ambient temperature as factors influencing the circadian distribution of vigilance states. Systematic variation of such conditions can be useful in experimentally dissecting the relationship between vigilance states and hypothermic/hypometabolic states.

We thank Paul Hendrick of the Technical Support Staff in the Program in Neuroscience at Florida State University for expert help in constructing recording electrodes and electronic signal processing. We thank Paul Hendrick of the Technical Support Staff in the Program in Neuroscience at Florida State University for expert help in constructing recording electrodes and electronic signal processing. This work was supported by a grant for international scientific collaboration between the laboratories of M. E. Rashotte and I. F. Pastukhov from the National Science Foundation (NSF IBN-9421921).

Address reprint requests to M. E. Rashotte.

Received 18 May 1998; accepted in final form 28 July 1998.

REFERENCES


