Food Intake during the Normal Activity Phase Prevents Obesity and Circadian Desynchrony in a Rat Model of Night Work

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Shift work or night work is associated with hypertension, metabolic syndrome, cancer, and other diseases. The cause for these pathologies is proposed to be the dissociation between the temporal signals from the biological clock and the sleep/activity schedule of the night worker. We investigated the mechanisms promoting metabolic desynchrony in a model for night work in rats, based on daily 8-h activity schedules during the resting phase. We demonstrate that the major alterations leading to internal desynchrony induced by this working protocol, flattened glucose and locomotor rhythms and the development of abdominal obesity, were caused by food intake during the rest phase. Shifting food intake to the normal activity phase prevented body weight increase and reverted metabolic and rhythmic disturbances of the shift work animals to control ranges. These observations demonstrate that feeding habits may prevent or induce internal desynchrony and obesity. (Endocrinology 151: 1019–1029, 2010)

Shift work or night work forces people to be active in a phase of the light-dark (LD) cycle during which normally they would be sleeping. Several studies have indicated that the majority of night workers report difficulties tolerating this condition despite years of night work experience (1, 2). In the long term, this aberrant activity pattern leads to a propensity to develop obesity, metabolic syndrome, cardiovascular and gastric disorders, and increased mortality due to cancer (3, 4). At present, it is generally accepted that this anomalous activity schedule generates conflicting signals out of phase with temporal signals transmitted by the biological clock, the suprachiasmatic nucleus (SCN) (5).

The SCN is the main pacemaker in the circadian system, which is a complex feedback network that involves interactions between the SCN and peripheral oscillators (6). Due to its anatomical and functional relation with the retinal input, the SCN is mainly entrained to the LD cycle (7) and transmits rhythmic messages to the entire organism to couple physiology and peripheral oscillators to the daily cycle and for maintaining internal synchrony (8). Various cyclic factors in the environment can disturb the interaction of the SCN with the peripheral oscillators and promote that behavioral, hormonal, and metabolic oscillations shift out of the phase given by the SCN (5). This disturbed phase relation between the SCN and peripheral oscillations leads to internal desynchronization (1, 9).

It is well documented that shift and night work promotes changes in feeding patterns, resulting in increased food intake during the normal resting phase (1, 10–12). Shift and night workers tend to schedule their meals around their working hours, and it is common that they choose to ingest diets rich in carbohydrates (11, 13, 14). In recent years, evidence has indicated that feeding schedules exert a strong entraining influence on peripheral oscillators, on behavior, on visceral rhythms, and on metabolic rhythms, overriding rhythmic signals transmitted by the

Abbreviations: AL, Food ad libitum; C, control; FD, food restricted during the day; FN, food restricted food during night; LD, light-dark; MANOVA, multivariate ANOVA; NS, not significant; SCN, suprachiasmatic nucleus; TAG, triacylglycerol; W, night work; ZT Zeitgeber time.
Animals and housing

Male Wistar rats weighing 120–140 g (5–6 wk old) at the beginning of the experiment were housed in a soundproof monitoring room in individual transparent acrylic cages (40 × 50 × 20 cm) placed in isolated lockers housing eight animals each. Rats were maintained in a 12-h light, 12-h dark cycle with lights on defined as Zeitgeber time 0 (ZT0), constant temperature (22 ± 1°C), circulating air, and for all experimental conditions free access to water. All rats had free access to food (Rodent Laboratory Chow 5001, Purina, Minnetonka, MN) during baseline. During the experimental phase, food access was conditioned depending on the corresponding group, as stated below, whereas water was always available. Experiments were approved by the committee for ethical evaluation at the Faculty of Medicine, Universidad Nacional Autónoma de México, in strict accordance with the Mexican norms for animal handling, Norma Oficial Mexicana NOM-062-ZOO-1999, which conforms to international guidelines for animal handling. All efforts were made to minimize the number of animals and their suffering.

Experimental design

For this study, a total of 165 rats were used. Rats were randomly assigned to one of two groups: control (C) or night work (W). Control rats were housed in individual cages in the monitoring system and were left undisturbed during the baseline and 5 additional weeks, corresponding to the working manipulations. Working rats were monitored for 8–10 d for a baseline and were then submitted to the working protocol of 8 h daily from Monday to Friday for 5 wk (for details see below).

Control (C; n = 24) as well as the working rats (W; n = 24) were then subdivided into groups according to three feeding conditions, food *ad libitum* (AL), food restricted during the day (FD), and food restricted during the night (FN), resulting in the following groups: C-AL (n = 8), C-FD (n = 8), C-FN (n = 8), W-AL (n = 8), W-FD (n = 8), and W-FN (n = 8).

Work procedure

To induce activity, rats were placed in slow rotating wheels that are used for sleep deprivation (33 cm diameter × 33 cm long) with four concentric subdivisions, which allows housing individually four rats. Drums rotate with a speed of one revolution/3 min and force rats to stay awake. Due to the speed of the wheels, rats do not need to walk all the time; they can sit, groom, and even lie down. In addition, they can eat and drink freely [more details in Salgado-Delgado et al. (21) and supplemental video, published as supplemental data on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org]. A small bottle with water hanging from a concentric middle tube was available for all groups, whereas food, also hanging from the middle tube, was available for the W-AL and W-FD groups.

For a baseline, rats were monitored in their home cages for 8–10 d in LD conditions. Starting on a Monday, working rats were taken out of their home cages and were placed in the slow rotating drums. Rats worked from 0900–1700 h geographical time, which represented ZT2–ZT10. After 8 h in the drums, rats were returned to their home cages and remained undisturbed until the next day. On weekends, all rats remained undisturbed in their home cages in the registration room and with food *ad libitum*. This procedure was carried out for 5 wk.

Feeding schedules

Rats assigned to the *ad libitum* (AL) group had always free access to food in their home cages as well as in the rotating drums. Rats assigned to the group with restricted food during the day (FD) had access to food from ZT0–ZT12; therefore, food was removed from the feeder at the time of lights off and replaced in the feeder after lights on, and for the working group (W-FD), food was also available inside the rotating wheels. Rats assigned to the group with food restricted to the night (FN) had access to food from ZT12–ZT0; therefore, food was removed from the feeders at the moment of lights on and replaced after lights off.
For the working rats (W-FN), no food was available in the rotating drums.

Monitoring of behavioral rhythms

General activity in the home cage was continuously monitored with movement sensors placed under individual cages. The system for monitoring and collection of data was developed in our group with the contributions from Nico Bos in Amsterdam, The Netherlands, and the Mexican biomedical company Omnilava SA de CV. Behavioral events were collected with a digitized system and automatically stored every minute in a PC for further analysis. Analysis was performed with the program for PC SPAD9 (Sistema de Procesamiento y Adquisición de Datos, version 1.1.1) designed for this system (Ing Adrián Hernández, Instituto de Fisiología Celular UNAM, Mexico City, Mexico).

For the baseline and 4 working weeks, double-plotted actograms were obtained for each animal (n = 8 per group) by collecting the sum of activity for 15-min intervals. For 7 d of the baseline and for the first and fourth weekends (2 d), mean activity waves were constructed and the percentage of daily and nocturnal activity was calculated.

Food intake and body weight

Rats (n = 6–7 per group) were weighed before starting the baseline and at the end of the fourth week of the working protocol, and body weight gain was calculated for this interval. Ingested food was monitored twice every weekday during the baseline and during working weeks by weighing separately the nocturnal and the diurnal consumption.

Metabolic and hormonal rhythms

At the end of the fifth working week, blood samples were obtained over the course of 2 d to cover a 24-h cycle by 30-h intervals (ZT0, ZT3, ZT6, ZT9, ZT12, ZT15, ZT18, and ZT21). Blood samples (240 μl) were collected in Eppendorf tubes (1.8 ml) containing a clot-activator gel and were centrifuged at 2500 rpm during 10 min, and plasma was stored in 80-μl aliquots at −80°C until assay. Aliquots were processed with colorimetric methods for determination of glucose and triacylglycerols (TAG) and corticosterone was determined with ELISA.

Glucose was estimated from a 10-μl sample using a commercial colorimetric kit (no. 70478; Hycel de México, Mexico City, Mexico), which is based on the reaction between glucose and fenol-4-aminoazofenazo as chromogen, and TAG were evaluated with a commercial diagnostic kit (no. B01-4512-01, Sera-Pak Plus; Bayer, Sees, France) based on the reaction with 4-aminoantipiridin. Both were measured at 500 nm with a spectrophotometer (Novaspec II Visible; Amersham Pharmacia Biotech, Cambridge, UK). Corticosterone was determined in 25 μl serum with a kit for ELISA (rat corticosterone EIA kit; Diagnostic Systems Laboratories, Inc., Webster, TX).

Core temperature and intraabdominal fat

To monitor the core temperature and before starting the working and feeding protocols, a different series of rats (n = 5–6 per group) underwent surgery to insert intraduodenal temper-
strated in the actograms and in the mean activity wave forms obtained for weekends 1 and 4. This effect was specifically observed in the W-AL and W-FD groups (Fig. 2, top and middle), both groups consuming food during the day, as reported previously for the W-AL (21). During the fourth weekend, the nocturnal activity for the W-AL and W-FD had decreased and represented only 53% of the total 24 h activity, whereas the day activity had increased to a proportion of 47% (Fig. 2), which resulted in a loss of rhythm. In contrast, animals exposed to the same working schedule and with food restricted to the night maintained a nocturnal activity pattern with similar proportions as the baseline and to the control groups (Fig. 2, bottom).

**Food intake, body weight, and intraabdominal fat**

During the 4 experimental weeks, all groups consumed a similar amount of food, independently of the phase of food access (Fig. 3A and Table 2). Interestingly, rats fed exclusively (C-F and; W-FD) or voluntarily (W-AL) during the day gained more body weight than the controls fed ad libitum (C-AL) and than the C-FN and the W-FN rats, eating during the night (Fig. 3B and Table 3). At the end of the study, the difference of body weight as compared with the C-AL group was +17 ± 2.8% for the C-FD, +4 ± 4.2% for the C-FN, +12 ± 3.6% for the W-AL, +16 ± 0.9% for the W-FD, and +7.6 ± 0.8% for the W-FN. The two-way ANOVA indicated a significant difference in body weight due to the feeding schedule \([F(2,40) = 3.46; \; P < 0.04]\) but not due to the work schedule \([F(1,40) = 0.15; \; \text{NS}]\) or to the interaction of both factors \([F(2,40) = 1.26; \; \text{NS}]\). When normalizing ingested food for body weight, all groups ingested a similar proportion ranging between 7.6 and 6.6 g food/g weight (Table 3).

Likewise, the accumulated retroperitoneal and peritoneal fat (Fig. 3, C and D) were increased in groups that were fed during their normal resting phase (C-FD, W-AL, and W-FD) as compared with the C-AL, C-FN, and W-FN (Fig. 3, C and D). Also, the proportion of fat pads to their body weight was higher for groups that were eating during the day (Table 3). The two-way ANOVA for the retroperitoneal fat indicated a significant effect due to the feeding schedule \([F(2,40) = 18.90; \; P < 0.0001]\), no effect due to the work \([F(1,40) = 0.40; \; \text{NS}]\) and a significant effect due to the interaction of both factors \([F(2,40) = 11.21; \; P < 0.001]\). For the peritoneal fat, the two-way ANOVA indicated a significant effect due to the feeding schedule \([F(2,40) = 29.40; \; P < 0.0001]\), due to the work \([F(1,40) = 13.41; \; P < 0.001]\) and a significant effect due to the interaction of both factors \([F(2,40) = 14.61; \; P < 0.001]\).

**Metabolic and hormonal daily rhythms**

The control C-AL group exhibited a daily rhythm of glucose with a peak at the transition from light to dark phase. Similarly, the group C-FD showed this peak at the transition of light to dark phase (in ZT12), indicating that the diet restricted to the day does not modify the rhythm of glucose (Fig. 4A). Moreover, the C-FN rats with food restricted to the night exhibited a nocturnal peak with enhanced amplitude as compared with the other two control groups (Fig. 4A). Work combined with feeding during the normal resting phase abolished the daily rhythm of glucose in W-AL and W-FD rats (Fig. 4B), whereas food restricted to the night in the W-FN rats prevented this alteration and produced a high-amplitude rhythm with high values during the night, similar to the C-FN (Fig. 4B). The MANOVA indicated a significant difference between control and working groups \([F(2,296) = 52.49; \; P < 0.0001]\), a significant difference due to feeding schedules \([F(2,296) = 11.01; \; P < 0.0001]\), and a significant difference in time \([F(7,296) = 28.17; \; P < 0.0001]\).

The daily rhythm in TAG followed mainly the feeding schedule. Animals predominantly eating in the night (C-AL, C-FN, and W-FN) showed peak values in the night at ZT15-18 (Fig. 5, C and D), whereas animals eating predominantly during the light phase (C-FD, W-AL, and W-FD) exhibited their TAG acrophase during the light phase with highest values at ZT6 (Fig. 5, C and D). The MANOVA indicated a significant difference between con-

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**TABLE 1. Daily peak values (ZT) among groups and phase relationship (ψ) between experimental groups and controls**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Activity</th>
<th>Glucose</th>
<th>TAG</th>
<th>Corticosterone</th>
<th>Temperature</th>
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<tr>
<td></td>
<td>ZT</td>
<td>ψ</td>
<td>ZT</td>
<td>ψ</td>
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<tr>
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<td>12</td>
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<td>12</td>
<td>12</td>
<td>12</td>
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<td>6</td>
<td>–6</td>
<td>12, 0</td>
</tr>
<tr>
<td>C-FN</td>
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<td>15</td>
<td>+3</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>W-AL</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>6</td>
</tr>
<tr>
<td>W-FD</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>6</td>
<td>12, 12</td>
</tr>
<tr>
<td>W-FN</td>
<td>12</td>
<td>15</td>
<td>+3</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Shifted daily peaks in one or more variables indicate internal desynchrony due to the feeding schedule and working schedule. They also point out desynchrony with the external cycle. NR, No rhythmicity.
and working groups \([F_{(2,266)} = 9.08; P < 0.005]\), a significant difference due to feeding schedules \([F_{(2,266)} = 29.31; P < 0.0001]\), and a significant difference due to time \([F_{(7,266)} = 6.09; P < 0.001]\).

Control (C-AL, C-FD, and C-FN) and forced activity groups (W-AL, W-FD, and W-FN) exhibited the expected daily corticosterone peak at light/night transition (Fig. 5, E and F). An additional increase of corticosterone was observed in groups feeding during the day (C-FD, W-AL, and W-FD) possibly associated with the anticipation of food consumption. Due to this additional rise, in both groups, mean levels of corticosterone were higher (C-FD, 215.63 mg/dl; W-FD, 222.66 mg/dl) compared with the ad libitum (C-AL, 127.59 mg/dl; W-AL, 130.34 mg/dl) and the night feeding groups (C-FN, 187.53 mg/dl; W-FN, 163.23 mg/dl). The MANOVA indicated a significant difference between control and working groups \([F_{(5,88)} = 35.97; P < 0.0001]\) a significant difference among feeding schedules \([F_{(7,88)} = 47.40; P < 0.0001]\), and a significant difference due to time \([F_{(35,88)} = 5.41; P < 0.0001]\).
Core temperature

All groups exhibited a clear daily rhythm of core temperature, which was influenced by the daily activity and by the feeding schedule. The daily rhythm in C-AL rats showed low values during the light phase, a rise approximately 2 h before the dark phase, and peak values in the first half of the night (Fig. 5, top left). The difference of mean day-night values was significantly different ($P < 0.017$). Control rats with food restricted to the day (C-FD) showed a shift of the temperature rhythm toward the light phase, whereas the C-FN rats with food restricted to the night showed a clear nocturnal pattern (Fig. 5, left middle and bottom). For the C-FD, the statistical analysis indicated no significant difference between day-night values ($P = 0.491$) and a significant difference for the C-FN ($P < 0.001$).

Working rats feeding during the day (W-AL and W-FD) presented a clear rhythm of temperature, with a phase advance of 6–9 h, respectively, and thus with a peak during the light phase (Fig. 5, second column), whereas W-FN rats that worked during the day and ate at night showed a nocturnal temperature rhythm similar to C-AL rats (Fig. 5). Mean values for the day and night confirmed a robust rhythm for the three groups, with a significant difference between day and night temperature, whereby the W-AL and W-DF groups exhibited highest values during the day (W-AL, $P < 0.016$; W-FD, $P < 0.0168$; and W-FN, $P < 0.0081$).

### TABLE 2. Mean ± SEM food intake (grams) per week for control and forced activity groups

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
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<td>Night</td>
<td>Day</td>
<td>Night</td>
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<td>Night</td>
<td>Day</td>
<td>Night</td>
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<td>Night</td>
<td>Day</td>
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<table>
<thead>
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<th>Night</th>
<th>Week 2</th>
<th>Day</th>
<th>Night</th>
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<th>Night</th>
<th>Week 4</th>
<th>Day</th>
<th>Night</th>
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<tbody>
<tr>
<td>C-AL</td>
<td>18.3</td>
<td>3.83</td>
<td>18.43</td>
<td>4.6</td>
<td>19.56</td>
<td>5.82</td>
<td>16.2</td>
<td>21.05</td>
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<tr>
<td>C-FD</td>
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<td>21.8</td>
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<td>24.2</td>
<td>0</td>
<td>24.2</td>
<td>16.02</td>
<td>20.3</td>
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<td>C-FN</td>
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<td>11.64</td>
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<td>23.5</td>
<td>0</td>
<td>22.8</td>
<td>24.9</td>
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<tr>
<td>W-AL</td>
<td>18</td>
<td>11.92</td>
<td>11.04</td>
<td>16.02</td>
<td>9.28</td>
<td>20.28</td>
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<tr>
<td>W-FN</td>
<td>18</td>
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</table>

FIG. 3. Mean daily food intake ± SEM (A), body weight gain (B), and retroperitoneal (C) and peritoneal (D) fat of control (n = 6–7 per subgroup) and working groups (n = 6–7 per subgroup) with food ad libitum (light gray bars), food during the day (white bars), and food during the night (dark gray bars); striped bars, working groups. All the groups showed a similar daily pattern of food consumption (A); however, groups that ate during the normal resting phase (C-FD, W-AL, and W-FD) showed major body weight gain (B) and higher accumulation of fat (C and D). Different letters indicate significant differences among groups ($P < 0.001$).
Phase relationship with the external cycle

When comparing daily peaks among groups, evidently, the feeding schedule and the working protocol produced a shift of temperature and TAG and a loss of glucose rhythms (Table 1), modifying the phase relationship among variables and ZT12, indicating internal desynchrony.

Discussion

Our present data indicate the importance of the moment of food intake for the maintaining of circadian internal synchrony or the induction of desynchrony, body weight gain and adipose tissue accumulation in a rodent model of night work. As described in a previous paper, the night worker’s activity during the normal resting phase influenced and moved the food intake toward the working hours, abolished the rhythm in general activity and glucose, whereas it significantly shifted the TAG and temperature rhythm. This was not observed in rats exposed to the activity schedule during the night (21). In the present study, restricting food to the normal resting phase, independently of the working conditions (C-FD, W-AL, and W-FD), also shifted the TAG daily rhythms and the temperature rhythm toward the day, increased body weight, and promoted accumulation of abdominal fat deposits. In contrast, restricting food to the normal activity phase (W-FN), thus to the night, prevented disturbances due to the workers’ activity protocol; it enforced the nocturnal rhythm of activity and of glucose and TAG and prevented the increased body and fat weight gain.

In contrast to metabolic rhythms, the nocturnal corticosterone peak was not modified by the activity protocol or scheduled feeding. Data reported here indicate that in the control (C-AL, C-FD, and C-FN) and worker activity

<table>
<thead>
<tr>
<th>Body weight, basal</th>
<th>Body weight, wk 4</th>
<th>Body weight gain</th>
<th>Proportional food ingestion (g)/body weight (g)</th>
<th>% Retroperitoneal fat</th>
<th>% Peritoneal fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-AL 180.3 ± 3</td>
<td>353.6 ± 1.7</td>
<td>173.3 ± 3.2</td>
<td>7.60</td>
<td>1.01</td>
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<td>C-FD 193.5 ± 1.5</td>
<td>396.5 ± 5.7</td>
<td>203 ± 5.8</td>
<td>6.60</td>
<td>1.52</td>
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<tr>
<td>C-FN 182.7 ± 2</td>
<td>362 ± 6.57</td>
<td>179.3 ± 4.7</td>
<td>6.85</td>
<td>1.10</td>
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<tr>
<td>W-AL 182.8 ± 2.09</td>
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<td>194.7 ± 5.2</td>
<td>6.94</td>
<td>1.38</td>
<td>1.45</td>
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<td>W-FD 172.5 ± 1.1</td>
<td>374.3 ± 2.3</td>
<td>201.8 ± 1.8</td>
<td>6.65</td>
<td>1.42</td>
<td>1.48</td>
</tr>
<tr>
<td>W-FN 178.5 ± 2.8</td>
<td>365.7 ± 1.34</td>
<td>187.2 ± 1.6</td>
<td>6.78</td>
<td>0.65</td>
<td>0.64</td>
</tr>
</tbody>
</table>

The proportion of food ingestion and retroperitoneal and peritoneal fat are expressed as a proportion of body weight after 4 wk of work. Data that were significantly different in statistical analysis are highlighted in bold.

FIG. 4. Daily values for glucose, TAG, and corticosterone (mean ± SEM) for control groups (A, C, and E) and working groups (B, D, and F) (n = 5–9 per group) after 4 wk with food ad libitum (gray circles), with food during the day (FD; white squares), or with food during the night (FN; dark gray triangles). White and black horizontal bars, LD cycle; striped bar, time in the activity wheel. Asterisk (ad libitum), cross (FD), and α (FN) indicate statistical difference between the highest and the lowest values of the same group (P < 0.01).
groups (W-AL, W-FD, and W-FN), the corticosterone rhythm remained fixed to temporal cues transmitted by the SCN, confirming that the daily rhythm of corticosterone is driven by the SCN (27). In working rats with food ad libitum or restricted to the day (W-AL and W-FD), we observed an additional peak of corticosterone at ZT3, suggesting an anticipatory response as reported for rodents anticipating restricted food access (28, 29). Also, both groups exposed to day feeding exhibited high mean corticosterone levels compared with the ad libitum and night feeding groups. Animal models of obesity invariably have increased levels of corticosterone, whereas adrenalectomy leads to the reversal of obesity (30). Because high levels of corticosterone increase lipogenesis and stimulate abdominal and sc fat (31, 32), in the present experiment, this could be a factor promoting body weight increase and abdominal fat in rats scheduled to feed during the day.

In rodents, timed activity during the normal resting phase induced by restricted or scheduled access to a running wheel is a nonphotic Zeitgeber that results in shifted activity patterns (33, 34). The present study confirms that activity during the normal resting phase disturbs behavioral temporal patterns as early as wk 2 of work (21). The main effects are a decrease of nocturnal activity and a shift of food ingestion to the day, leading to low-amplitude or suppressed hormonal rhythms. Despite disturbed behavioral and peripheral rhythms, in our model of night work, the SCN remains rhythmic and locked to the LD cycle (21).

The differential effect of working on food intake, activity, metabolic rhythms, and corticosterone indicate a loss of phase relation among SCN and food-driven rhythms, which all together results in internal desynchrony (Table 1).

The use of the slow rotating wheels is a common strategy to study sleep deprivation; rats are placed in slow rotating wheels for intervals of 20 h or more to decrease the sleep hours, whereas placing the rats in such slow moving wheels for less than 10 h is considered an induced activity protocol because rats can profit from at least 14 h for sleep recovery (35, 36). Due to the slow speed of the wheels, rodents are able to sit, groom, eat, drink, and even lie down. In this and in a previous study, we have provided evidence that corticosterone levels are similar between the working group and the nonworking controls, allowing us to discard a chronic stress condition due to the daily working schedule. In fact, after their working period, rats are able to catch up their lost rest in their activity period similar to human shift workers (25, 37). For the short working periods of 8 h, as employed here, authors have not observed that rats were exhausted from this protocol (see supplemental material). Additional research should be conducted to address sleep specifically.

FIG. 5. Daily temperature curves (mean ± SEM) for the control groups and working groups (n = 5–6) with food ad libitum (gray circles, top row), with food during the day (white squares, middle row) and food during the night (gray dark triangles, bottom row) after 4 wk. White and black horizontal bars, LD cycle; striped bar, food access. Bar charts, Mean values for the day and night temperature. Control groups are represented by filled bars and working groups by striped bars. The asterisk indicates statistical difference between day and night temperature (P < 0.01).
Restricting food intake to a few hours daily has proven to be a strong entraining signal for peripheral oscillators for behavior and metabolism (16, 38, 39). In such conditions, feeding signals override the temporal cues transmitted by the SCN and represent a powerful uncoupling force for peripheral functions because the rhythm of the SCN remains preferentially entrained to the LD cycle (16, 40). Our present data demonstrate the power of food intake to drive metabolic temporal patterns, especially in the TAG rhythm, and to uncouple them from the nocturnal activity and the nocturnal corticosterone peak.

Rats fed during the day, with or without work, developed together with the suppression of metabolic rhythms a significant propensity to obesity characterized by accumulation of abdominal fat. The fact that the daily total consumption of food was similar in all groups indicates that the main factor promoting obesity was the disturbance in metabolic rhythmicity as is demonstrated by the flattened glucose curves and the shifted TAG rhythm in night working rats. Our data confirm previous reports in rodents and humans that indicate that eating during the normal resting phase leads to a loss of blood glucose rhythm and overweight (25, 41, 42).

Our data agree with previous studies reporting that in night-active persons the low amplitude and desynchronized timing of endocrine and metabolic rhythms may result from shifted eating patterns (43–46). As observed with our rodent model, several studies suggest that in human populations, shifting activity and the main food consumption toward the night results in propensity to obesity and increased accumulation of abdominal fat (42, 47–49). The increase of abdominal fat is considered a predisposing factor for metabolic syndrome, including diabetes and a higher incidence of cardiovascular disease (48–51). Also, and in agreement with Kreier et al. (52), the increase of abdominal fat can be seen as a symptom of unbalance in metabolism.

In view of the worldwide increase in obesity, the mechanisms that underlie the relationship between circadian rhythms, metabolism, and obesity need to be established. Energy-related signals provided by food also exert entraining effects on clock genes in peripheral organs (53). Recent studies report that clock genes have reciprocal interaction with genetic regulators of metabolism, especially with those mediating the formation of fatty tissue and carbohydrate metabolism (54). Thus, out-of-phase feeding patterns can lead to disturbed oscillatory expression of metabolic genes and lead to alterations in cellular energy balance. In this way, metabolic disease and obesity in the night worker may result from circadian disruption or desynchrony of metabolic genes in peripheral tissues (55–57).

Nocturnal activity patterns are usually accompanied by increased nocturnal food ingestion. This has especially been observed in night workers, who ingest up to 70% of their daily intake during their work hours (11–13). The reason nocturnal workers and rats exposed to activity during the sleep phase voluntarily shift the temporal pattern of food ingestion needs to be better studied. The orexin system in the hypothalamus may be the functional system in the brain that couples forced wakefulness with the drive to eat during working hours. The orexigenic system, localized mainly in the lateral hypothalamus, has anatomical connections with monoaminergic neurons (58) and promotes sustained wakefulness. Also, orexigenic neurons establish dense and reciprocal connections with other hypothalamic nuclei regulating feeding behavior. Therefore, it is suggested that under conditions of reduced food availability and low energy stores, orexigenic neurons may play the role of maintaining wakefulness and arousal allowing more time to search for food (59, 60). It is also possible that enhanced activation of orexin neurons during sustained wakefulness can induce food ingestion as a secondary effect; however, this effect is still not well understood (61). For the night worker, this last mechanism may explain the drive of night workers and our W-AL rats to eat voluntarily during the work hours.

In the present study, we provide evidence that internal desynchrony and propensity to obesity in the night working rats is prevented by restricting food to the night, which for rodents represents the normal active phase (group W-FN). This, together with a previous study reporting that nighttime-restricted feeding normalized clock gene expression in diabetic mice with initial dampened locomotor and shifted clock gene daily rhythms (62), allows us to suggest that the strong influence of feeding schedules may prevent internal desynchrony in individuals exposed to shift work by coupling food intake to the biologically correct active period. The relevance of the phase for food ingestion is furthermore indicated in a recent study providing evidence that nocturnal mice fed a high-fat diet only during the 12-h light phase gained significantly more weight than mice fed only during the 12-h dark phase (22). The present study supports the hypothesis that disturbed feeding patterns lead to overweight and metabolic unbalance.

In conclusion, the present study demonstrates that working during sleep hours induces food ingestion and internal desynchrony. Food intake during sleep hours in control and working rats also promoted internal desynchronization and promoted increased body weight and abdominal fat accumulation. Food intake restricted to the activity phase reverted the disturbance of daily rhythmicity and prevented overweight, demonstrating the relevant
contribution of feeding habits to prevent internal desynchrony in the night worker as well as to prevent obesity related to nocturnal feeding habits. The findings support the notion that uncoupling feeding-related processes from the light cycle is deleterious for health.

Acknowledgments

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