Repeated light-dark shifts speed up body weight gain in male F344 rats

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Submitted 20 December 2004; accepted in final form 28 February 2005

Tsai, Ling-Ling, Yu-Che Tsai, Kai Hwang, Yu-Wen Huang, and Jeh-En Tzeng. Repeated light-dark shifts speed up body weight gain in male F344 rats. Am J Physiol Endocrinol Metab 289: E212–E217, 2005.—This study is aimed at verifying the causal relationship of chronic circadian desynchronization and changes in body weight control. Eight male albino F344 rats aged between 12–15 wk were subjected to twice weekly 12-h shifts of the daily light-dark (LD) cycle for 13 wk (3 mo). Continuous circadian phase shifts consisting of intermittent phase delay and advance and reduced circadian amplitudes were consistently displayed in all five experimental rats implanted intraperitoneally with heart rate, body temperature, and activity transponders. The experimental rat maintained a greater body weight during LD shifts and even after 10 days of recovery than that of the age-matched control rat, which was maintained on a regular LD cycle. Body weight gain was greater in the first 2 mo of LD shifts in the experimental rat than in the control rat. Relative to the baseline, food intake and activity percentages were increased and reduced, respectively, for the experimental rats. Features of these results, such as increased body weight gain and food intake, and reduced activity, suggest a causal relationship of chronic circadian desynchronization and changes in body weight control in male albino F344 rats.

shift work; circadian rhythm; reentrainment; energy regulation

ACUTE PERTURBATIONS of the circadian rhythm structure like the transmeridian flights and the shifts of working schedules are related to the so-called “jet lag” and “shift lag” syndrome, respectively, both of which are characterized by feelings of malaise, fatigue, sleepiness, insomnia, digestive troubles, irritability, impaired mental agility, and performance efficiency (3). These symptoms often last for a short term, and they disappear after several days of stay at the local time for the jet lag and during the day shifts for the shift lag. Long-term disruptions of circadian rhythms, like chronic shift work, are associated with several medical diseases. Cardiovascular diseases, gastrointestinal disorders, and negative pregnancy outcomes have been reported to be more common in shift workers than in day workers in several epidemiological studies (12, 24, 25). The mechanisms that are responsible for the association between work shifts and each disease are unclear. In addition to circadian rhythm and sleep factors, however, behavioral changes and social disturbances are also considered to be involved in disease mechanisms (11, 12) and coping ability of shift workers (17).

In most industrialized countries, cardiovascular diseases are the leading cause of death and disability. Bøggild and Knutsen (2) reviewed 17 studies and concluded that, compared with day workers, shift workers have a 40% increase in risk of cardiovascular disease. Conventional risk factors for cardiovascular disease are related mainly to metabolic syndrome, including obesity, elevated serum total and low-density lipoprotein (LDL) cholesterol, low high-density lipoprotein (HDL)-cholesterol, elevated triglycerides, and impaired glucose tolerance. Previous survey studies show that the current work shift status is related to elevated weight gain (5), body mass index (BMI; see Refs. 4, 7, 9, and 23), waist-to-hip ratio (WHR; see Ref. 23), and prevalence of overweight (20) and of obesity (4, 8). Furthermore, positive correlations of BMI (9, 21, 27), WHR (27), and prevalence of overweight (20) with duration of work shift have been reported. Elevated serum total cholesterol and prevalence of high serum triglycerides and of low HDL-cholesterol in shift workers have been reported in a population-based study of 27,485 people (8). Nonetheless, inconsistent results have also been reported in BMI (e.g., see Ref. 18), WHR (e.g., see Ref. 4), and serum cholesterol and triglycerides (reviewed in Ref. 2) for shift workers. Methodological problems related to duration and type of work shift, demographic variations between shift workers and reference groups, and even the timing of blood sampling, etc., could all lead to variant results (2).

Experimental work shift studies have been done in human subjects but only for a short period (e.g., see Ref. 10). The causal relationship of long-term circadian rhythm disturbances and changes in physiological systems has been studied in laboratory rodents undergoing a 12-h phase shift in the light-dark (LD) cycle on a weekly basis (13, 14, 19, 22). Body weight is lower in female Brown Norway rats (14) and female CD2F1 mice (19) subjected to weekly shifts in LD cycle. In contrast to most rat strains, e.g., Sprague-Dawley, bred in standard laboratory conditions, F344 rats are excellent in self-regulating their energy intake to maintain a lower body weight gain after maturity (15). The present study applied 12-h phase shifts in LD cycle two times per week to male F344 rats and found higher body weight gain in the experimental rats than in the controls rats. The relationship of food intake and activity level to repeated LD shifts was examined as well.

METHODS

Subjects. Specific pathogen-free, male inbred F344 rats (F344/N) aged 6–8 wk were purchased from the National Laboratory of Animal Breeding and Research Center (Taipei, Taiwan, R.O.C.). Rats were randomly assigned to experimental (LS) and control (LC) groups. Eight age-matched LS-LC rat pairs were used, and each pair was studied at the same time. Five of the rat pairs were implanted aseptically under isoflurane anesthesia with heart rate, temperature, and activity transponders (HR E-Mitter; Mini-Mitter, Bend, OR) in the abdomen at least 2 wk after their arrival and 2 wk before baseline data collection. The transponder was energized by an energizer/ receiver device (ER4000; Mini-Mitter), and it transmitted heart rate,

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body temperature, and activity signals that in turn, were detected by the same device via telemetry. Heart rate, body temperature, and activity signals were sampled at 2 Hz and converted to heart rate (beats/min), temperature (°C), and activity counts, respectively, using custom software written in LabView 6.1 (National Instruments, Austin, TX). Converted heart rate and temperature data were averaged, and activity counts were summed up every 30 s; thus, a total of 2,880 data sets were collected daily.

When they were 10–13 wk old, rats were housed individually in plastic cages (45 × 25 × 50 cm³) with Lignocel soft wood bedding on the bottom. The plastic cages were placed in two adjoining sound-attenuated recording rooms, one for the LS rat and the other for the LC rat, with a counterbalanced arrangement. Both rooms were illuminated by fluorescent lamps, and the location of each cage was adjusted to maintain the illuminance level at each cage bottom to be ~300 lux when the fluorescent lamps were turned on. The lamps were connected to a power switch, under the control of a digital timer. Each room was also equipped with a dim red lamp (2–4 lux) so that routine measurement and cleaning could be performed when the fluorescent lamps were turned off. The two rooms were ventilated by a single air-conditioning system to maintain a similar environmental temperature and relative humidity at 22–24°C and 55–75%, respectively. Food pellets (3.25 kcal/g) and water were available ad libitum. Routine husbandry work was performed every 2–3 days to maintain the well-being of rats. All the animal facilities and care followed the guidelines provided by the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996), and all procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee.

Experimental design. Baseline data collection started 1 wk after the rats were transferred to plastic cages. The experimental period for the LS rat included a 1-wk baseline period with lights on at 0900–2100, 13 wk of 12-h phase shifts in the LD cycle two times per week, and 10 days of recovery period with lights on at 0900–2100. LD shifts always involved a 24-h period of lights off. The LC rats were maintained on the same lighting schedule with lights on at 0900–2100 throughout the experimental period except that lights-on periods were extended to 24 h every Monday during the LD shift period to maintain an equal amount of exposure to light for both the LC rats and the LS rats.

Measurements for food intake and water intake were performed at around 2100 on Monday, Wednesday, and Friday for the LC rat throughout the experimental period. For the LS rat, measurements were made at the same time as the LC rat during baseline and recovery periods on Monday and Friday but at around 0900 on Wednesday during the LD shift period. That is, routine husbandry work was performed during light-to-dark transitions or at the time corresponding to that on the previous day as the condition on Monday during LD shifts (Fig. 1). Wasted food pellets were searched and weighed, and the weight was added back to the weight of the food feeders. There was no evidence of food waste dependent on lighting periods on Monday and Friday but at around 0900 on Wednesday during the LD shift period. That is, routine husbandry work was always involved a 24-h period of lights off (Fig. 1). The LC rats were extended to 24 h every Monday during the LD shift period to maintain an equal amount of exposure to light for both the LC rats and the LS rats.

Cannulation and blood sampling. Each rat was implanted aseptically under isoflurane anesthesia through the jugular vein with a polyurethane tubing (0.040 in. OD × 0.025 in. ID, MRE 040; Braintree Scientific) after 10 days of recovery. Later (2 days), beginning at 0900, 400 µL of blood were withdrawn every 3 h for 24 h. The blood sample was kept on ice for 30–60 min. Serum samples were then taken by centrifugation (7,000 rpm, 10 min) and were frozen at −70°C until analysis. Triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol in the serum were analyzed by an autoanalyzer (Hitachi 7170).

Organ weight and examination. After blood sampling (4 days), rats were killed under isoflurane. Heart, liver, lung, spleen, kidney, adrenal gland, and testis were removed and weighed soon after the rat stopped breathing for a few minutes. Routine microscopic examinations were performed on samples of the above-mentioned organs, aorta, stomach, and duodenum. Tissues were fixed in 10% formalin solution and, after being processed by an automatic dehydration machine (Shandon Pathcenter), were embedded in paraffin. Sections 3 µm thick were stained with hematoxylin and eosin and examined by our pathologist (J.-E. Tzeng).

Data analysis. Daily body temperature and heart rate data were averaged for each hour accordingly, and circadian variations of these hourly mean data were analyzed using a 24-h cosine fitting model (1). Amplitudes of daily temperature and heart rate rhythm were averaged over weeks accordingly, to represent the mean amplitudes in baseline, successive weeks during LD shifts, and recovery. Circadian phase (acrophase) was defined as the time of the highest point of the daily fitted cosine curve.

Food intake and water intake data were summed up over three successive measurements and then divided by 7 to represent the daily mean values over a certain week. Daily mean food intake and water intake were then averaged across the weeks accordingly to represent mean values in the baseline, successive months during LD shifts, and recovery. Mean food and water intake during LD shifts and recovery was subtracted from baseline values and then divided by baseline values according to represent food and water intake changes from baseline. Body weight data were subtracted by the baseline value or the value in the previous month to indicate successive body weight gain.

Two-factor mixed-design ANOVA with group (LS vs. LC) by time (baseline, 3 mo during LD shifts, and recovery) was performed on body weight, food intake, water intake, mean heart rate, mean temperature, and total activity data. Post hoc comparisons were performed by Tukey’s test. A two-tailed independent t-test was performed to...
examine the difference between LS and LC rats in amplitude of circadian rhythms, body weight gain, and percent changes in food intake, water intake, mean heart rate, mean temperature, and total activity. A paired $t$-test or one-sample $t$-test was used to examine the differences from baseline across the experimental period. All statistical analyses were performed using SYSTAT 7.0 for Windows. An $\alpha$-level of 0.05 was used for all statistical tests.

**RESULTS**

Changes of circadian rhythms during the LD shift period. There were few qualitative differences between LS rats in daily rhythms of heart rate, body temperature, and activity throughout the experimental period. Thus the raster plot of temperature data is graphically represented in Fig. 2. The temperature...
Body weight, food intake, and water intake. The LS rat had a greater body weight than the LC rat during LD shifts and still weighed more after 10 days of recovery in a regular LD cycle (Fig. 3). Thus repeated 12-h LD shifts two times a week induced continual circadian desynchronization in F344 rats. After the return from repeated LD shifts, the LS rat reentrained to the regular LD cycle within 1 wk of the recovery period, with circadian phase back to baseline level and relatively faster than amplitudes. In contrast to the LS rat, the LC rat maintained a stable circadian phase and amplitude throughout the experimental period (Figs. 2A and 3).

Conversely, both the LS and LC rats showed reduced total body weight and food intake in each experimental period (M, month) for LS-LC rat pairs. Data are means ± SE. For body temperature rhythm, significant differences from baseline did not reach the level of statistical significance (*P < 0.05, 1-sample t-test). Thus mean percentage change in food intake was significantly higher in the LS rat in the 1st and 2nd mo of LD shifts and in recovery, whereas the LC rat ate less in the 1st mo of LD shifts. Despite increased body weight and weight gain, the LS rat did not eat more than the LC rat throughout the experimental period (Fig. 5). However, relative to its own baseline level, the LS rat ate more in the 1st and 2nd mo of LD shifts and in recovery, whereas the LC rat ate less in the 1st mo of LD shifts. Thus mean percentage change in food intake was significantly higher in the LS rat in the 1st and 2nd mo of LD shifts and in recovery than in the LC rat. Similarly, the LS rat tended to drink more during LD shifts and in recovery relative to its own baseline level, but the value of the mean percentage change from baseline did not reach the level of statistical significance (Fig. 6).

Heart rate, body temperature, and activity. Both mean heart rate and mean body temperature in the LS and LC rats were maintained at baseline levels during LD shifts and in recovery. Conversely, both the LS and LC rats showed reduced total
activity counts progressively over the experimental period (Fig. 7). The LS rat had a lower value of the mean percentage change of activity from baseline in the 2nd mo of LD shifts than that of the LC rat.

**Organ weight and pathology.** The mean weights of heart, lung, liver, spleen, kidney, adrenal gland, and testis, as well as the ratios of organ weights to body weights, were not different for the LS and LC rats. No prominent pathological conditions and significant lipid infiltrations were found in any organ in either rat group.

Because of technical problems, only a few blood samples were collected. Thus the data of blood lipids were not reported.

**DISCUSSION**

This study demonstrates that repeated reversal of the external LD cycle two times per week resulted in continual phase shifts of circadian rhythms with reduced amplitudes and increased body weight gain in male F344 rats. To the best of our knowledge, this is the first experimental evidence vindicating the causal relationship of chronic circadian desynchronization and changes in body weight control in normal young rats. This finding corresponds to the results of epidemiological studies showing elevated weight gain (5) and prevalence of overweight (20) and of obesity (4, 8) in human shift workers.

A previous study applying weekly 12-h phase shifts in the LD cycle to female CD2F1 mice showed that the temperature rhythm of mice responded to each LD shift by a gradual phase delay and reentrained to the LD schedule by the 4th or 5th day (19). The wheel-running activity rhythm in cardiomyopathic male Syrian hamsters reenters a 12-h phase shift in the LD cycle by the 1st or 2nd day, with a phase delay or by the 4th or 5th day with a phase advance (cf., Fig. 2 in Ref. 22). In this study, a 12-h phase shift in the LD cycle was repeatedly applied to male F344 rats at twice-weekly intervals, and this treatment resulted in a mix of intermittent phase delay and advance; it was also intermingled with a masking effect of light and dark onset activity, heart rate, and body temperature. However, the masking effect probably added little help to speed up reentrainment, since the amplitude of circadian rhythms was reduced even during the later period of LD shifts when the masking effect was most prominent, and it still took several days for the temperature rhythm to reentrain to the LD cycle in recovery (see Fig. 2, C and D). Thus twice-weekly 12-h shifts of the daily lighting schedule over months consistently resulted in continuous disruptions in circadian rhythmicity.

In contrast to previous findings of reduced body weight in female CD2F1 mice (19) and female Brown Norway rats (13, 14) subjected to weekly LD shifts, this study shows that the mean body weight and body weight gain in male F344 rats subjected to twice-weekly LD shifts were higher than in the LC rat. Compared with the LC rat, the LS rat maintained its mean body weight at a higher level across the 3 mo of LD shifts and even after 10 days of recovery in a regular LD cycle. However, the body weight gain was higher in the LS rat only for the first 2 mo of LD shifts. Increased weight gain was concomitant with increased food intake percentage relative to the baseline level in the LS rat, particularly in the first 2 mo of LD shifts and in recovery. Thus the tendency for the LS rat to gain more weight could result from an increase in energy intake. However, a decrease in energy output could also contribute to the increased weight gain in the LS rat. Relative to the baseline, the activity level in both the LS and LC rats was progressively reduced during LD shifts and in recovery. The decline of spontaneous activities could be age-related (16, 26). Nonetheless, the mean percentage change of activity in the LS rat was significantly lower than in the LC rat in the 2nd mo of LD shifts. An increase of energy intake concomitant with a decrease of energy output leads to the possibility of an elevated body weight set point that inevitably speeds up body weight gain. However, the treatment of repeated LD shifts at twice weekly intervals seemed not to increase body weight gain unlimitedly, since the mean weight gain in the LS rat was progressively reduced across the LD shift period and, in the last month, reached to the level similar to that of the LC rat. It was not known whether the gradual adaptation to LD shifts and/or the quantitative change of phase shift pattern during the later period of LD shifts, i.e., enhanced phase advance and masking effect, were causally related to the slow down of body weight gain in the LS rat.

A survey study in hospital staff members showed that workers who worked during the late (evening and night) shift...
reported a higher weight gain than the day-shift workers (5). There was a trend for the late-shift workers to report eating more and exercising less since beginning the late shift. Furthermore, it has been reported that prevalence of overweight is related to duration of work shift (20). The LS rat maintaining a greater body weight continuously during LD shifts and even in recovery appeared to display an effect similar to that of prolonged work shift on human subjects. In comparison with the results of previous studies in mice (19) and rats (13, 14), the present study seemed to provide a more appropriate animal model for human work shift, at least on the aspect of body weight control. Whether sex difference (female mice and rats in previous studies vs. male rats in the present study), strain difference (Brown Norway vs. albino F344 rats), and/or frequency difference in LD phase shifts are involved in the specific finding of this study will await future studies to clarify.

In summary, this study demonstrates that circadian desynchronization caused by repeated 12-h phase shifts of the LD cycle at twice-weekly intervals resulted in elevated body weight gain concomitant with increased food intake and reduced activity level in male F344 rats. These results suggest a causal relationship of chronic circadian desynchronization and changes in body weight control in male albino F344 rats.

ACKNOWLEDGMENTS

We are grateful to Wen-Yin Huang, Bessy Hung, Su-Fen Lai, Li-Chun Lin, Hau-Min Liu, Pei-Yu Ting, and Hui-Chun Wu for technical assistance.

GRANTS

This study was supported by the National Science Council, Republic of China, Grants NSC90-2413-H-194-019 and NSC91-2413-H-194-014.

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