RESEARCH ARTICLE

Effect of restricted feeding on nocturnality and daily leptin rhythms in OVLT in aged male Wistar rats

V. D. K. Reddy · Anita Jagota

Abstract Circadian system has direct relevance to the problems of modern lifestyle, shift workers, jet lag etc. To understand non-photic regulation of biological clock, the effects of restricted feeding (RF) on locomotor activity and daily leptin immunoreactivity (ir) rhythms in three age groups [3, 12 and 24 months (m)] of male Wistar rats maintained in light:dark (LD) 12:12 h conditions were studied. Leptin-ir was examined in the suprachiasmatic nucleus (SCN), the medial preoptic area (MPOA) and organum vasculosum of the lamina terminalis (OVLT). Reversal of feeding time due to restricted food availability during daytime resulted in switching of the animals from nocturnality to diurnality with significant increase in day time activity and decrease in night time activity. The RF resulted in % diurnality of approximately 32, 29 and 73 from % nocturnality of 82, 92 and 89 in control rats of 3, 12 and 24 m age, respectively. The increase in such switching from nocturnality to diurnality with restricted feeding was found to be robust in 24 m rats. The OVLT region showed daily leptin-ir rhythms with leptin-ir maximum at ZT-0 in all the three age groups. However leptin-ir levels were minimum at ZT-12 in 3 and 12 m though at ZT-18 in 24 m. In addition the mean leptin-ir levels decreased with increase in food intake and body weight significantly in RF aged rats. Thus we report here differential effects of food entrained regulation in switching nocturnality to diurnality and daily leptin-ir rhythms in OVLT in aged rats.

Keywords Restricted feeding · Diurnality · Leptin · OVLT · Locomotor rhythms

Introduction:

The mammalian circadian timekeeping system (CTS) coordinates the physiological adaptation of an organism to the daily rhythms of the external world. Any alteration of this internal temporal order leads to pathophysiology induced by circadian dysfunction (reviewed in Salgado-Delgado et al. 2011). The light–dark cycle is the major cue that entrains the biological clock (Jagota 2006); in addition, a number of non-photic cues like food availability cycles (Damiola et al. 2000), socio-professional routines (Lévi et al. 2010) etc. have been shown to exhibit the ability to entrain the circadian clock of an organism. The principal circadian clock resides in cells grouped in two suprachiasmatic nuclei (SCN), just above the optic chiasm in the anterior hypothalamus (Welsh et al. 2010; Jagota 2012). Rhythmic expression of mammalian clock genes are found in SCN as well as

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peripheral tissues indicating existence of both central and peripheral oscillators (Partch et al. 2013). The nutritional status, adiposity, glucose homeostasis, sympathetic and parasympathetic balance of the body is some of the key inputs in the regulation of circadian clock (Laposky 2008; Bechtold and Loudon 2013). When nocturnal animals are fed under restricted daytime feeding regimen, it misaligns the sleep-wake and fasting-feeding cycles of the organism to the light–dark cycle showing a shift in the activity to day time with a diurnal food anticipatory activity (FAA) which has been conceptualized by many workers as circadian rhythm driven by food entrained circadian oscillator (FEO) separate from light entrained SCN (Caba et al. 2008; Boulos and Terman 1980).

The central pacemaker has been suggested to entrain the organism to the light–dark cycle whereas the peripheral clocks respond to the feeding fasting cycles leading to an internal desynchrony (Kalsbeek et al. 2011). Circadian desynchrony promotes metabolic disruption (Barclay et al. 2012; Yoon et al. 2012). Leptin, a product of the obese (*ob*) gene is secreted into blood stream primarily by white adipose tissue which acts on several hypothalamic sites including dorsal medial hypothalamic (DMH) and median preoptic area (MPOA) (Zhang et al. 2011; Zhao et al. 2013). It is also a key controller of food intake and energy homeostasis as it acts as a bridge between energy metabolism and circadian clock (Froy 2010). Leptin levels decrease during fasting (Boden et al. 1996) and rise after food intake (Dallongeville et al. 1998). Leptin receptors (Ob-Rs) exist in multiple forms (Ob-Ra–f) (reviewed in Martin et al. 2008) however leptin receptor Ob-Rb present in hypothalamus (Mercer et al. 1996), was reported to be mediating the satiety effects of leptin (Lee et al. 1996).

In addition to regulating the photic cycles, SCN regulates different hypothalamic regions involved in energy homeostasis, which includes medial preoptic area (MPOA) (Choi et al. 1998). MPOA regulates the adipose tissue activity and leptin production (Froy 2010). MPOA is located in the periventricular regions of the anterior hypothalamus covering the organum vasculosum of the lamina terminalis (OVLT). OVLT has been reported to be connected to MPOA (Polston and Simerly 2006). It has been shown earlier that leptin can phase advance the SCN circadian clock in a dose-dependent manner in isolated in vitro brain slices (Prosser and Bergeron 2003).

Aging is the progressive deterioration in the behavioral, biochemical and physiological functions of an organism (Jagota 2005; Rattan 2012). With aging the circadian clock properties and functions are altered leading to the desynchronization of rhythms (Doi et al. 2011; Yu and Weaver 2011). We reported earlier age induced alterations in daily rhythms of serotonin (5-HT) levels in brain as well as SCN starting at middle age (Jagota and Kalyani 2008, 2010). In addition we have reported age related loss of sensitivity to melatonin in restoration of serotonin (Jagota and Kalyani 2010), lipid peroxidation and antioxidant enzymes (Manikonda and Jagota 2012).

Thus, in order to understand age induced alteration in the food entrained regulation of biological clock, we studied the effect of timed restricted feeding (RF) on food intake, body weight, gross locomotor activity rhythms and daily leptin-ir rhythms in SCN and MPOA region of male Wistar rats in various age groups.

Materials and methods

Male Wistar rats of three age groups (1–3) [3, 12 and 24 months (m)] (with $n = 44$ in each age group) were maintained in light–dark conditions (LD 12:12), lights on: 06:30 AM i.e. Zeitgeber time (ZT-0) and lights off: 6:30 PM (ZT-12) at $20 \pm 2°$ C with relative humidity of 55 ± 6 %, for 2 weeks prior to experiment (Fig. 1). All rats were kept individually in polypropylene cages contained within well ventilated light proof environmental cabinets isolated in animal facility. During light phase animals were exposed to 300 lux, automatically controlled by a 24 h timer. Dim red light was used for handling the animals in the dark. Cage changing was done at random intervals (Jagota and Reddy 2007).

Feeding schedules, food and body weight measurement

The rats in three age groups (1–3) were separated further into two groups—(A) ad libitum fed (AL) with food and water provided ad libitum for 2 weeks ($n = 24$) (Group 1A, 2A and 3A) (B) restricted feeding (RF) (received food only during the light period, i.e., from 6:30 AM (ZT-0) to 6:30 PM (ZT-12)
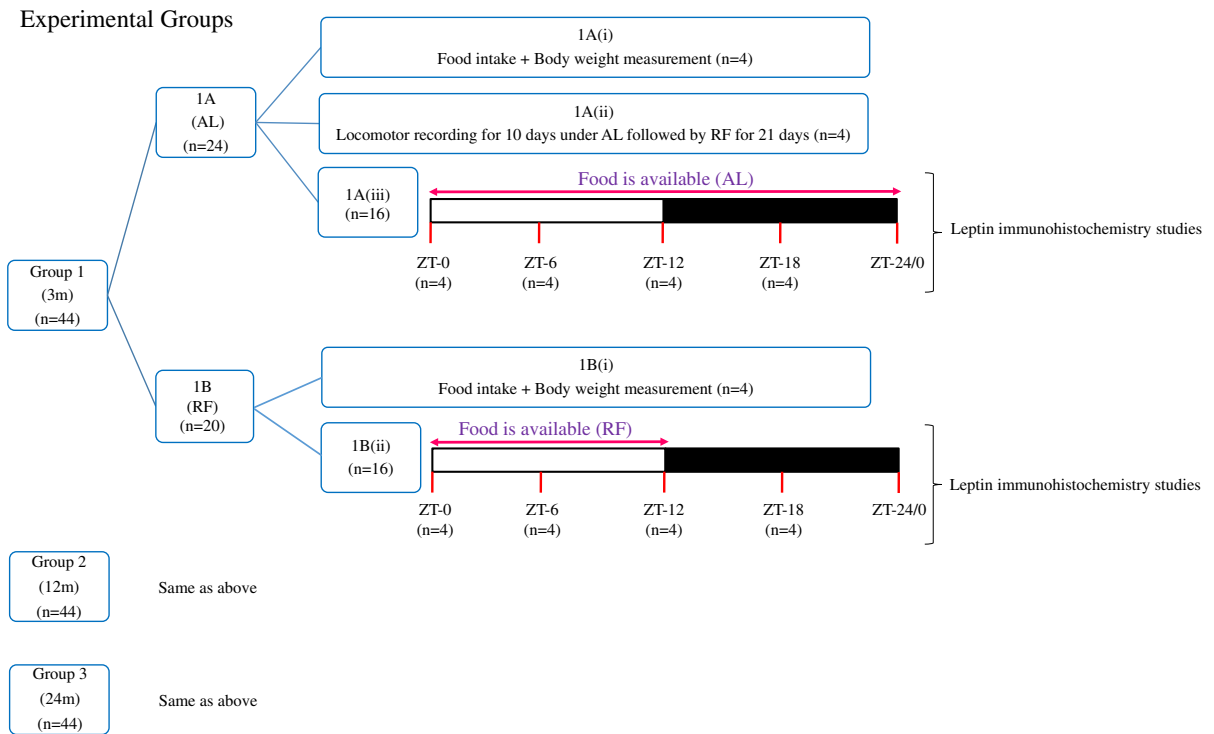
Experimental Groups

Fig. 1 Diagrammatic representation of the various age groups of animals used in the study. Three age groups (1–3) 3, 12 and 24 months (m) were further divided into two groups: A, ad libitum fed (AL) with food and water provided ad libitum for 2 weeks; B, restricted feeding (RF) (received food only during the light period zeitgeber times (ZT) 0 to ZT12

for 3 weeks) (Bodosi et al. 2004) ($n = 20$ each) (Group 1B, 2B and 3B). All animals were fed with standard rat diet. AL group with $n = 24$ was further divided into three groups of (i) $n = 4$, (ii) $n = 4$, (iii) $n = 16$. RF group was further divided into two groups (i) $n = 4$ and (ii) $n = 16$. Food intake was recorded every 6 h interval for both AL [1A (i), 2A (i), and 3A (i)] and RF [1B (i), 2B (i) and 3B (i)] groups by taking weight of the unconsumed food. In addition body weights were also recorded every third day till the end of the experiment for these groups. All experiments were performed as per Institutional Animal Ethics.

Gross locomotor activity

Animals of three age groups in AL group (1A (ii), 2A (ii) and 3A (iii) ($n = 4$ in each group) were housed individually in cages equipped with infra-red (I.R) motion detector sensors (IR 28 kit, INT, India). The gross locomotor activity was recorded using Chronobiology Kit (Stanford Software Systems, USA) (Schumann et al. 2005; Mammen and Jagota 2011) for

10 days. This was followed by RF and the gross locomotor activity was recorded for three weeks.

Data analysis

Actograms and mean activity profiles were prepared using the Chronobiology Kit and analyzed using the Kit Analyze software (Stanford Software systems, USA). The duration of the active period (α), circadian period (τ) and percentage diurnality were calculated (Schumann et al. 2005; Mammen and Jagota 2011). α started with activity onset and ended with activity offset. Results were represented as mean $\pm$ standard error (SE) (Table 1).

Leptin—immunohistochemistry

For studying daily leptin rhythms, animals 1A (iii), 2A (iii), and 3A (iii) and 1B (iii), 2B (iii), 3B (iii) with $n = 16$ in each group were sacrificed after anesthetizing and transcardial perfusing with heparinized

Table 1 Effect of restricted feeding on various circadian rhythm parameters

Age group (m)	Experimental group	Activity parameters			
		Circadian period (τ) (h)	Duration of active period (α) (h)	% Diurnality	% Nocturnality
3	AL	24.16 ± 0.16	12.66 ± 0.14	17.48 ± 0.24	82.49 ± 0.27
	RF	24.29 ± 0.08	15.37 ± 0.62*	31.8 ± 0.31*	68.53 ± 0.21*
12	AL	23.97 ± 0.22	12.52 ± 0.27	7.92 ± 0.26[x]	92.46 ± 0.18[x]
	RF	24.72 ± 0.06[x',*]	18.09 ± 0.45[x',*]	29.16 ± 0.13[x',*]	49.42 ± 0.22[x',*]
24	AL	23.95 ± 0.02	12.37 ± 0.16	11.44 ± 0.17[y, z]	88.88 ± 0.56[y, z]
	RF	23.55 ± 0.1[y',z',*]	13.03 ± 0.3[y',z',*]	72.75 ± 0.21[y',z',*]	18.42 ± 0.39[y',z',*]

Each value is mean ± SE (n = 4). P ≤ 0.05 [where * represents comparison between ad libitum (AL) and restricted feeding (RF) in the same age group; where x, y and z refers to comparison between 3 and 12; 3 and 24; 12 and 24 months (m) old AL rats, respectively; x', y' and z' refers to comparison between 3 and 12; 3 and 24; 12 and 24 m old RF rats, respectively]

0.9 % saline at variable time points (ZT-0, 6, 12 and 18). The brains were removed rapidly and post-fixed immediately in 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2–4 h. Brains were then transferred into a gradient of sucrose solutions (20 and 30 % sucrose prepared with 0.1 M phosphate buffer (pH 7.4)) subsequently and stored at 4° C till it sinks to the bottom in both the solutions. 20 μm sections from these brains were taken using cryostat (Leica CM 1850). The sections were transferred into phosphate buffer saline (PBS) and left for 1 h at room temperature to remove sucrose. These sections were then incubated in 3 % H_2O_2 for 30 min followed by blocking using normal goat serum. The sections were incubated in primary antibody (dilution 1:350) (Rabbit polyclonal IgG primary antibody [Ob (Y-20) Sc-843, Santa Cruz Biotechnology, Inc., Santa Cruz, CA] at 37° C for 3 h and then with corresponding secondary antibody (dilution 1:450) (Goat anti-rabbit IgG-HRP: HP03, Bangalore Genei, India) at 37° C for 4 h. Finally the sections were incubated in substrate solution containing 0.1 % DAB (Diamino Benzedene) and 3 % H_2O_2 in PBS for about 10 min at 37° C till color develops. The reaction was stopped using 2 M HCl for 30 s and washed immediately with PBS. Sections were dehydrated using absolute alcohol and cleared by methyl benzoate and mounted using D.P.X. All sections were then arranged in a rostro-caudal axis, photographed using DP-12 digital camera attached to Olympus microscope (BX-41). Leptin-ir levels were compared using densitometric analysis with Image Pro AMS software (Media Cybernetics, USA) (Mammen and Jagota 2011).

Statistical analysis

Statistical Analysis Data was analyzed using Jandel Scientific Sigma stat software by Student's t test and one way ANOVA followed by Post hoc Duncan's test for multiple comparisons.

Results

The food intake increased with age as observed in various AL age groups. Food intake was highest in 24 m old rats (37.76 ± 0.06 g per day) but significantly less in 3 m old rats (28.86 ± 0.25 g per day). Food intake showed two peaks at ZT-6 and 18 respectively in 24 m AL rats. In RF animals, the food intake was more between ZT-0 and ZT-6, i.e. immediately after the food was provided. In both 3 and 12 m RF rats the food intake significantly decreased compared to respective AL group; however in 24 m RF rats food intake significantly increased to 38.56 ± 0.01 g when compared to AL group (Fig. 2a, b). Body weights of 3 and 12 m old RF rats significantly decreased under RF when compared to their respective AL group by approximately 12 and 5.5 % respectively. Whereas, the body weights of 24 m old RF rats significantly increased approximately by 3 % compared to their AL group (Fig. 2c).

Studies of gross locomotor activity rhythms

The locomotory activity profile of all the 3, 12 and 24 m old AL animals was restricted largely to the night
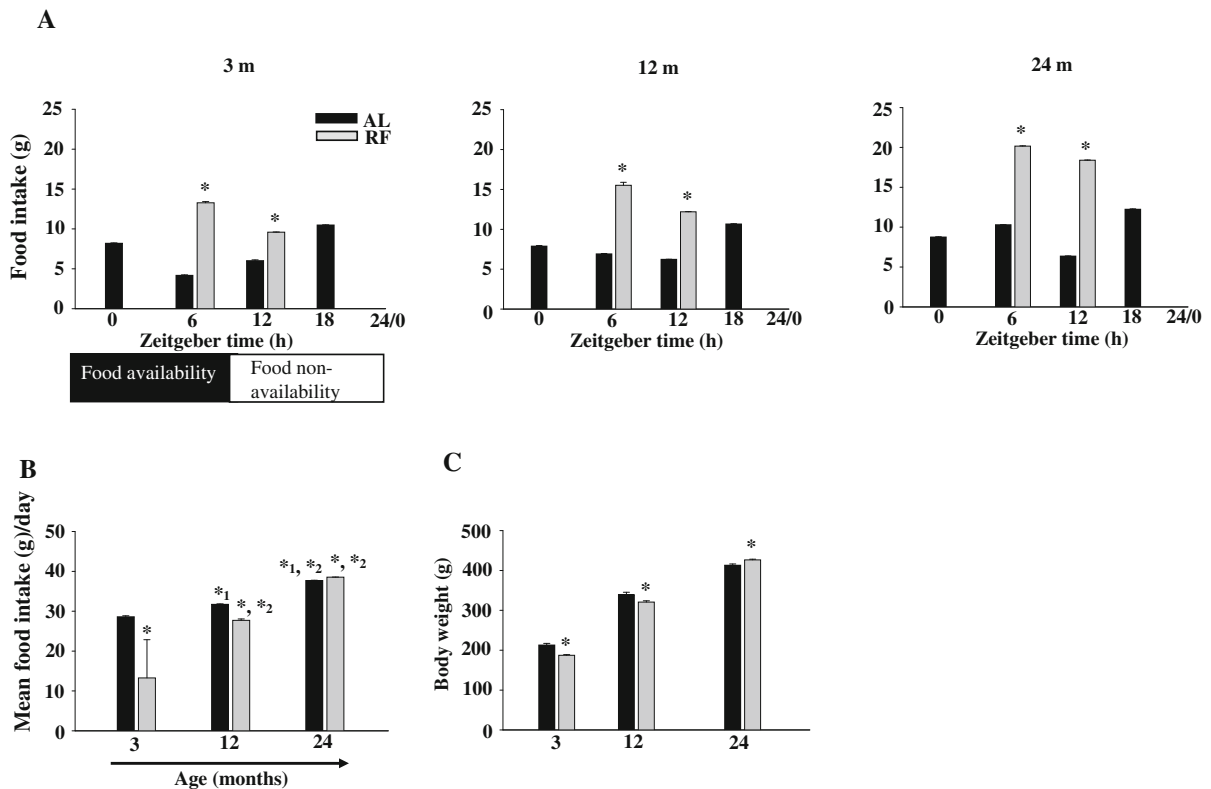
Fig. 2 Food intake profile in ad libitum (AL) fed and food restricted (RF) male Wistar rats in variable age groups: 3, 12 and 24 months (m) ($n = 4$ in each age group) at various zeitgeber times (ZT) 0, 6, 12 and 18. **a** Food intake; **b** mean food intake over 24 h; **c** body weights (measured every third day for 21 days). *, *$_1$, and *$_2$ indicate significant difference ($p \leq 0.05$) in comparison to AL in the same age group, in 3 and 12 m in the same experimental group respectively

time with 82.49 ± 0.27, 92.46 ± 0.18 and 88.88 ± 0.56 % nocturnality, respectively (Fig. 3; Table 1). The α of all the three age groups of AL animals was approximately 12 h. Interestingly all the age groups of animals have shown onset of bouts of activity mostly after the food was provided in RF condition. In RF 3 m old animals, the activity increased during the early feeding periods ZT 0-6, but declined after ZT-6 time point (mid-day) and then the onset of activity started $\sim$3–4 h before the normal onset which starts at ZT-12. From ZT-12 onwards the activity in RF animals was similar with decreased robustness compared to AL animals. In RF 12 m old rats the activity was observed throughout the day and night. In RF 24 m animals the activity profile was distributed more during the day (ZT-0 to 12) than in night (ZT-18 to 0). Under RF, the % nocturnality decreased to 68.53 ± 0.21, 49.42 ± 0.22, and 18.42 ± 0.39 in 3, 12 and 24 m old rats respectively

and % diurnality increased significantly ($p \leq 0.05$) from 17.48 ± 0.24, 7.92 ± 0.26 and 11.44 ± 0.17 to 31.8 ± 0.31, 29.16 ± 0.13 and 72.75 ± 0.21 in 3, 12 and 24 m old rats, respectively. In RF 12 m old rats, α increased significantly ($p \leq 0.05$) (18.09 ± 0.45 h) when compared to RF 3 m (15.37 ± 0.62 h) but in RF 24 m old rats, α decreased significantly compared to 3 and 12 m old rats ($p \leq 0.05$) (13.03 ± 0.3 h). In 24 m old rats the activity was shifted more towards the light phase and the night time activity decreased. The τ was approximately 24 h in 3, 12 and 24 m AL rats, i.e. 24.16 ± 0.16, 23.97 ± 0.22 and 23.95 ± 0.02 h, respectively. But in RF rats the τ significantly ($p \leq 0.05$) increased from 23.97 ± 0.22 to 24.72 ± 0.06 h in 12 m old rats whereas in 24 m old rats τ significantly ($p \leq 0.05$) decreased from 23.95 ± 0.02 to 23.55 ± 0.1 h however no significant change was observed in the τ between AL and RF in 3 m old rats.

Fig. 3 A Double plotted actograms of gross locomotor activity in ad libitum *(*AL) and food restricted (RF) condition: in 3 (**Ai**), 12 (**Aii**) and 24 (**Aiii**) m male Wistar rats. **B** Activity profiles of animals in 3 (**Bi**), 12 (**Bii**) and 24 (**Biii**) m (*n* = 4) under AL and RF conditions. Dashed lines on activity profile indicates lights on and off for animals in both groups. Additionally black and white *bar* at the bottom of mean profiles indicates food availability and non availability period for RF rats
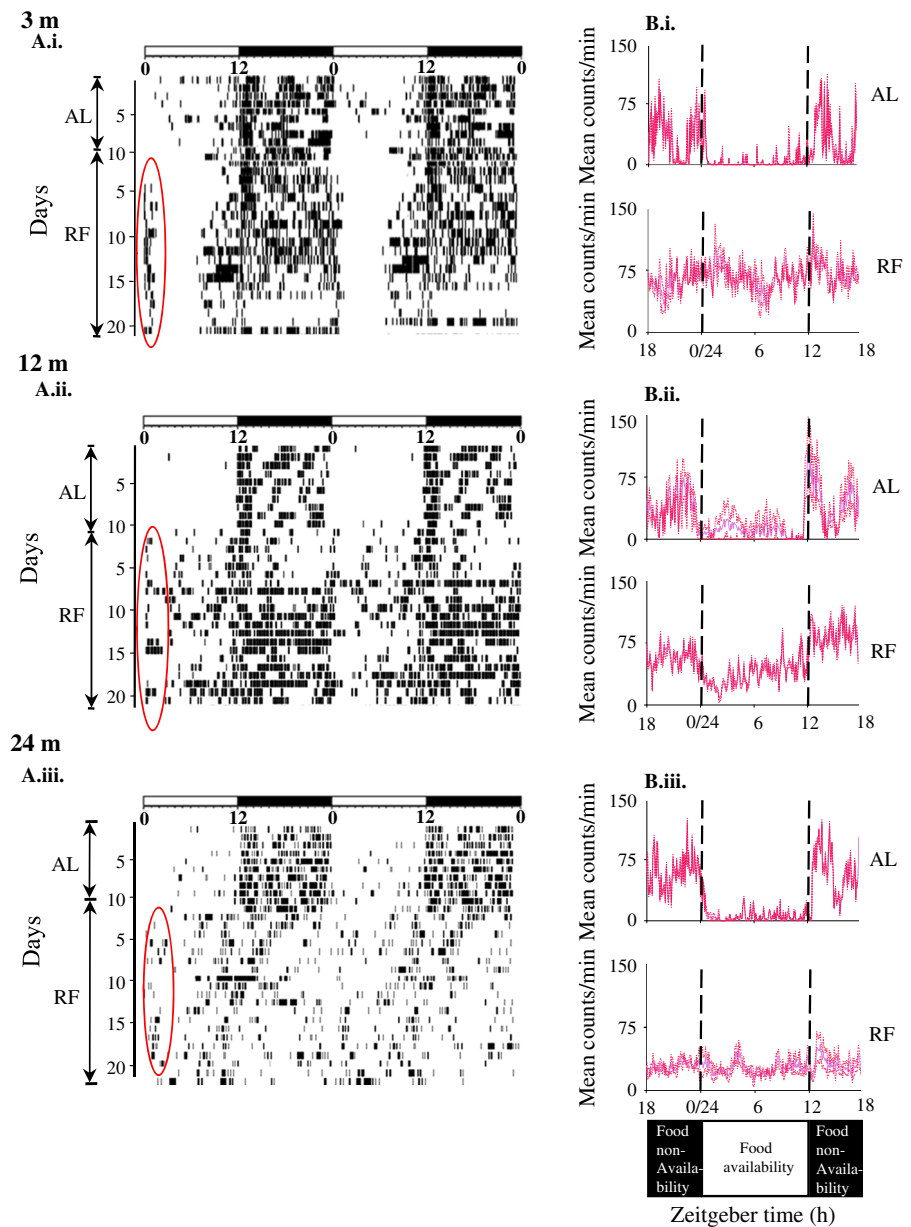
Studies of daily leptin rhythms

During the preliminary screening, all the coronal sections of SCN were screened for the leptin immunoreactivity (-ir), but leptin-ir could not be detected in this region (Fig. 4A), however when scanned serially from SCN towards optic chiasm, leptin-ir was detected in OVLT region located in the anteroventral region of the third ventricle (AV3V) in all the three age groups, i.e. AL rats (Fig. 4Bi, Ci, Di).

The leptin-ir in OVLT region was found to be highest in 3 m AL animals when compared to 12 and 24 m AL animals (Fig. 4Biii, Ciii, Diii). The leptin-ir showed daily rhythm pattern both in 3 and 12 m though there was decrease in robustness in later. Interestingly leptin-ir was maximum at ZT-0 in all the groups studied but was minimum at ZT-12 in 3 and 12 m though at ZT-18 in 24 m. In 24 m old rats the leptin-ir further decreased in comparison to 3 and 12 m with a significant change in the pattern of the

rhythm with two peaks at ZT-0 and ZT-12 though there was no significant difference in leptin-ir levels between 12 and 24 m old rats.

The variable RF age groups showed differential leptin-ir daily rhythm profile in OVLT region. RF in 3 m old animals resulted in decrease in mean leptin-ir though rhythmicity persisted but the maximum levels appeared delayed by 6 h at ZT-6 as compared to ZT-0 in AL. In 12 m, however there was significant decrease in leptin-ir maximum though minimum and mean (24 h) levels did not show significant change in OVLT with RF. In 24 m RF, the rhythmicity in leptin-ir persisted but there was significant decrease in mean levels with the maximum levels at ZT-0 and minimum at ZT-12 (Fig. 4E).

Discussion

The amount of food intake increased from 3 to 24 m in AL rats. The amount of food intake and body weights significantly decreased in 3 and 12 m RF rats when compared to AL groups. In 24 m AL rats, two peaks of food intake appeared at ZT-6 and 18 which could be comparable to anticipation of two daily meals and are related with two oscillator structure (Jagota et al. 2000) described by Mistlberger et al. 2012. In 24 m RF rats the amount of food intake and the body weights were significantly high compared to 24 m AL group. Interestingly some researchers have reported increased food intake and body weights of 9 weeks old mice fed under high fat diet during the light phase for 6 weeks demonstrating role of food on such parameters (Arble et al. 2009).

Locomotor activity in the present study is synchronized to feeding time in RF rats. We demonstrate here that altered meal timing switch the animals towards diurnality with a phase shift in the locomotor activity towards the day differentially with age. FAA was observed 3–4 h before the normal onset of activity in 3 m AL animals. In all the three RF age groups of animals the % diurnality increased and the % nocturnality decreased. The FAA in nocturnal rodents has been related to availability of food restricted to few hours in daytime (Mistlberger et al. 2012). This is in correlation with the earlier studies showing food deprivation and caloric restriction results in the nocturnal animals becoming partially diurnal (Challet 2010). Such synchronization of daily rhythms of

locomotor activity to the feeding time was also shown previously in Glithead seabream fish (*Sparus aurata*) (Montoya et al. 2010) and Wistar rats (Carneiro and Araujo 2011). In 24 m old RF animals the gross locomotor activity decreased significantly and became arrhythmic. This is similar to the finding of some researchers for Mongolian gerbils when fed during their inactive phase under LD14:10 conditions (Karakas et al. 2006). In addition, in mice, switching from nocturnal to diurnal with availability of food only during the day time has been reported (Chabot et al. 2012). We demonstrate here that such food entrained switching from % nocturnality to % diurnality is quite robust with 6.5 folds increase in 24 m RF rats.

The timing of nursing and FAA in rabbit pups has been reported to differentially affect *Per1* expression in SCN and DMH (Caba et al. 2008). Some researchers have related nocturnal to diurnal switch change in input pathway to SCN (Doyle et al. 2008). In the present study also there appears to be change from photic to non-photic entrainment with FEO. Interestingly leptin has been reported to stimulate DMH neurons (Lee et al. 2013).

The leptin-ir levels in SCN, MPOA and OVLT were studied but, to our surprise we could not find any leptin-ir in the SCN and MPOA region. However, we scanned all the coronal sections serially for any leptin-ir up to the end of the optic chiasm and interestingly we localized leptin-ir in OVLT region.

The mean 24 h leptin-ir levels in OVLT region decreased with age in AL groups as highest levels were observed in 3 m old rats. The peak leptin-ir levels in OVLT region of all the three AL age groups were observed at ZT-0 representing the beginning of the resting phase of these nocturnal animals, also a post feeding phase for these rats. Plasma leptin in diurnal humans has also been reported to exhibit a strong diurnal rhythm with a peak occurring at near midnight during their resting phase. In addition the plasma leptin levels have been reported to be entrained to meal timings and a shift of 6.5 h of meal timings was shown to result in shift of plasma leptin rhythm by about 5.7 h indicating a rapid phase alteration in the leptin rhythms with shift in the meal timings (Schoeller et al. 1997).

Interestingly, the leptin-ir levels in the OVLT region in 3 and 12 m AL rats increased from ZT-12 to ZT-24 after the onset of feeding peak which starts from ZT-12 onwards (i.e. onset of night) and leptin
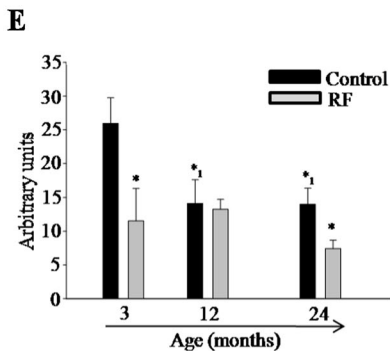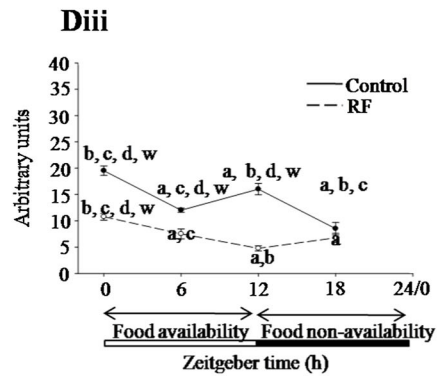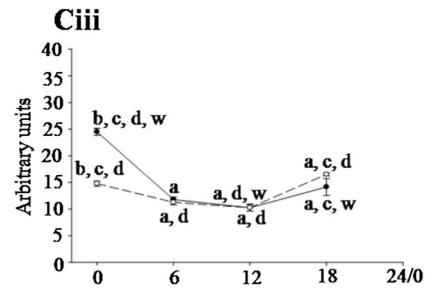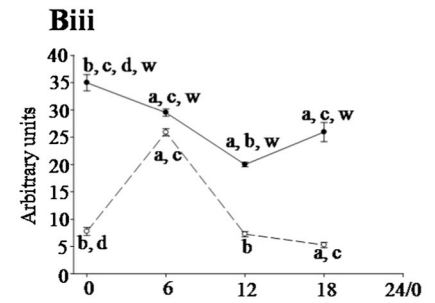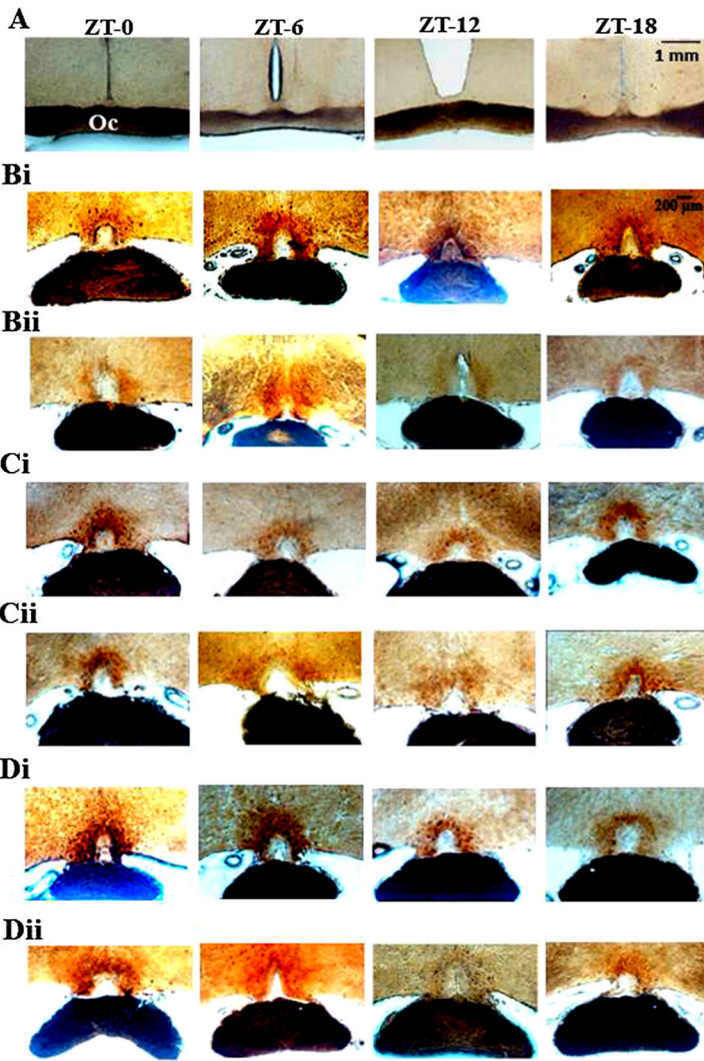
◄ **Fig. 4** Leptin-ir in the coronal brain sections (25 μm) of 3 m old male Wistar rats killed under various zeitgeber times (ZT) 0, 6, 12 and 18: **A** no leptin-ir was observed in the SCN region (scale bar = 1 mm); **B, C, D** Leptin-ir in the organovasculosum of the lamina terminalis (OVLT) of 3, 12 and 24 m old ad libitum (AL) fed rats (**Bi, Ci, Di**); food restricted (RF) rats (**Bii, Cii, Dii**) (scale bar = 100 μm) and their densitometric analysis using Image Pro AMS software ($n = 4$ at each time point) (**Biii, Ciii, Diii**); **E** mean 24 h leptin-ir $p_a \leq 0.05$; $p_b \leq 0.05$; $p_c \leq 0.05$; $p_d \leq 0.05$ (a, b, c and d refers to comparison with ZT-0, 6, 12 and 18); $p_w \leq 0.05$ (w refers to comparison at same time point in AL and RF in same age group). *, *$_1$, *$_2$ indicate significant difference ($p \leq 0.05$) in comparison to AL in the same age group; 3 and 12 m in the same experimental group

peak is 12 h after the onset of the feeding or at the offset of feeding (i.e. onset of day), i.e. off set of active period (ZT-0/24). In RF 3 m animals, the leptin-ir levels showed peak 6 h after onset of feeding, i.e. at ZT-6. In 12 and 24 m RF rats the onset of feeding and leptin levels were parallel. Thus, our results demonstrate that 3 m rats show more entrainment towards non-photic cue restricted feeding. In control rats upon aging the mean leptin-ir levels significantly decreased from 3 to 12 m but no significant difference was observed between 12 and 24 m old rats.

In 24 m old AL animals the locomotor activity increased during the natural inactive phase of the animals. This increased day time activity and increased body weight is correlating with the sleep loss human subjects having relatively low serum leptin levels and increased ghrelin and body mass index (BMI) (Taheri et al. 2004). Interestingly RF has also been related with shifts of corticosterone, temperature, activity and brain amine periodicity (Krieger 1974) in addition to decreased plasma insulin (Díaz-Muñoz et al. 2000).

The decreased mean 24 h leptin-ir levels in OVLT in 3 and 24 m old RF rats is in correlation with the decreased serum and hypothalamic leptin levels after 12 h of food deprivation in rats (Vujovic et al. 2011). This decrement in mean 24 h leptin-ir levels is also in correlation with the decreased plasma leptin levels in food deprived sheep under short and long days (Marie 2001). The robust increase in diurnality in aged rats might in addition to decreased leptin levels would have resulted in the increased circadian desynchrony responsible for increased food intake and body weight gain in 24 m old rats.

In the mammalian circadian clock function serotonin is implicated in the photic and non-photic

regulation of circadian rhythms (Jiang et al. 2000). Serotonin has been reported to regulate ghrelin levels which influence leptin levels (Takeda et al. 2013). In addition, as serotonin innervation and varicosities into OVLT regulating circulating factors have been reported earlier by some researchers (Bosler and Descarries 1988). We have previously reported progressive decline with age in mean 5-HT and reduced amplitude of daily rhythmicity with disintegration at middle age (Jagota and Kalyani 2008, 2010). Such a decrease in the serotonin levels with age thus could be related to decrease in leptin levels and decrease in amplitude of leptin pulse in 12 and 24 m in OVLT region. Reduced leptin levels have been related by some researchers to obesity due to insatiable appetite leading to increased food intake (Korkmaz et al. 2009). Decrease in leptin as well as circadian misalignment could lead to reduced sleep efficacy (Scheer et al. 2008). Some researchers have reported that monoamines could play an important role in addition to neuropeptide Y (NPY) in mediating many of leptin's central and neuroendocrine effects (Clark et al. 2006). These alterations in the male rat can be related to changes in middle age female rats such as serotonin uptake, serotonin transporter (SERT) binding sites, maintenance of normal cyclic release of LH, alpha-1 adrenergic receptors, decreased sensitivity of GnRH neurons to VIP input (i.e. decreased *c-fos* expression) and changes in glucose utilization (Cohen and Wise 1988; Krajnak et al. 2003). These changes have been related to mood, memory and sleep (Krajnak et al. 2003). Smith et al. 2005 have related onset of menopause with the alterations in the biological rhythms and CNS function due to reduced exposure to estradiol.

Interestingly OVLT is also a circumventricular organ (CVO) where blood brain barrier (BBB) is weak (Johnson and Loewy 1990). In addition OVLT has been reported to contain angiotensin II (AGII) and is rich in Gonadotropin-Releasing Hormone (GnRH) cells and fibers and plays important role in regulation of body fluid and cardiovascular function. Leptin has been reported to indirectly regulate GnRH neuronal function (Quennell et al. 2009). The decline in leptin-ir levels in the RF groups is parallel to decrease in the night time activity and increase in the day time activity with aging in the present study. Thus our work will be a step towards understanding the role of RF acting as a non-photic cue in entraining the daily leptin levels and

rhythms in the OVLT region as well as the gross locomotor activity rhythms in the aging rats.

We have reported earlier age induced change in circadian alignment in lipid peroxidation and antioxidant enzymes (Manikonda and Jagota 2012) which could be the result of reduced leptin (energy metabolism regulatory hormone) levels and attenuated rhythms with aging as leptin has been reported to play important role in inflammation (Lee et al. 2013). Age induced decrease in leptin levels and daily rhythms in OVLT could be responsible for age induced increase in inflammation.

References

Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW (2009) Circadian timing of food intake contributes to weight gain. Obesity 17:2100–2102

Barclay JL, Husse J, Bode B, Naujokat N, Meyer-Kovac J, Schmid SM, Lehnert H, Oster H (2012) Circadian desynchrony promotes metabolic disruption in a mouse model of shift work. PLoS ONE 7:e37150

Bechtold D, Loudon A (2013) Hypothalamic clocks and rhythms in feeding behaviour. Trends Neurosci 36:74–82

Boden G, Chen X, Mozzoli M, Ryan I (1996) Effect of fasting on serum leptin in normal human subjects. J Clin Endocrinol Metab 81:3419–3423

Bodosi B, Gardi J, Hajdu I, Szentirmai E, Obal F Jr, Krueger JM (2004) Rhythms of ghrelin, leptin, and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation. Am J Physiol Regul Integr Comp Physiol 287:R1071–R1079

Bosler O, Descarries L (1988) Monoamine innervation of the organum vasculosum laminae terminalis (OVLT): a high resolution radioautographic study in the rat. J Comp Neurol 272:545–561

Boulos Z, Terman M (1980) Food availability and daily biological rhythms. Neurosci Biobehav Rev 4:119–131

Caba M, Tovar A, Silver R, Mogado E, Meza E, Zavaleta Y, Juárez C (2008) Nature's food anticipatory experiment: entrainment of locomotor behavior, suprachiasmatic and dorsomedial hypothalamic nuclei by suckling in rabbit pups. Eur J Neurosci 27:432–443

Carneiro BTS, Araujo JF (2011) Influence of scheduled restricted feeding on reentrainment of motor activity rhythm after a 6-h light-dark advance in rats. Psychol Neurosci 4:317–322

Chabot CC, Connolly DM, Waring BB (2012) The effects of lighting conditions and food restriction paradigms on locomotor activity of common spiny mice, *Acomys cahirinus*. J Circadian Rhythm 10:1–7

Challet E (2010) Interactions between light, mealtime and calorie restriction to control daily timing in mammals. J Comp Physiol B 180:631–644

Choi SJ, Wong LS, Yamat C, Dallman MF (1998) Hypothalamic ventromedial nuclei amplify circadian rhythms: do they contain a food-entrained endogenous oscillator? J Neurosci 18:3843–3852

Clark KA, MohanKumar SM, Kasturi BS, MohanKumar PS (2006) Effects of central and systemic administration of leptin on neurotransmitter concentrations in specific areas of the hypothalamus. Am J Physiol Regul Integr Comp Physiol 290:R306–R312

Cohen IR, Wise PM (1988) Age related changes in the diurnal rhythms of serotonin turnover in microdissected brain area of estradiol-treated ovariectomized rats. Endocrinology 88:2626–2633

Dallongeville J, Hecquet B, Lebel P, Edme JL, Le Fur C, Fruchart JC, Auwerx J, Romon M (1998) Short term response of circulating leptin to feeding and fasting in man: influence of circadian cycle. Int J Obes Relat Metab Disord 22:728–733

Damiola F, Minh NL, Preitner N, Kornmann B, Fleury-Olela F, Schibler U (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev 14:2950–2961

Díaz-Muñoz M, Vázquez-Martínez O, Aguilar-Roblero R, Escobar C (2000) Anticipatory changes in liver metabolism and entrainment of insulin, glucagon, and corticosterone in food-restricted rats. Am J Physiol Regul Integr Comp Physiol 279:R2048–R2056

Doi M, Ishida A, Miyake A, Sato M, Komatsu R, Yamazaki F, Kimura I, Tsuchiya S, Kori H, Seo K, Yamaguchi Y, Matsuo M, Fustin JM, Tanaka R, Santo Y, Yamada H, Takahashi Y, Araki M, Nakao K, Aizawa S, Kobayashi M, Obrietan K, Tsujimoto G, Okamura H (2011) Circadian regulation of intracellular G-protein signalling mediates intercellular synchrony and rhythmicity in the suprachiasmatic nucleus. Nat Commun 2:327–336

Doyle SE, Yoshikawa T, Hillson H, Menaker M (2008) Retinal pathways influence temporal niche. Proc Natl Acad Sci USA 105:13133–13138

Froy O (2010) Metabolism and circadian rhythms—implications for body weight. Open Neuroendocrinol J 3:28–37

Jagota A (2005) Aging and sleep disorders. Indian J Gerontol 19:415–424

Jagota A (2006) Suprachiasmatic nucleus: center for circadian timing system in mammals. Proc Indian Natl Sci Acad B 71:285–300

Jagota A (2012) Age induced alterations in biological clock: therapeutic effects of melatonin. In: Thakur MK, Rattan SI (eds) Brain aging and therapeutic interventions. Springer Netherlands, London, pp 111–129

Jagota A, Kalyani D (2008) Daily serotonin rhythms in rat brain during postnatal development and aging. Biogerontology 9:229–234

Jagota A, Kalyani D (2010) Effect of melatonin on age induced changes in daily serotonin rhythms in suprachiasmatic nucleus of male wistar rat. Biogerontology 11:299–308

Jagota A, Reddy MY (2007) The effect of curcumin on ethanol induced changes in suprachiasmatic nucleus (SCN) and pineal. Cell Mol Neurobiol 27:997–1006

Jagota A, de la Iglesia HO, Schwartz WJ (2000) Morning and evening circadian oscillations in the suprachiasmatic nucleus in vitro. Nat Neurosci 3:372–376

Jiang ZG, Teshima K, Yang Y, Yoshioka T, Allen CN (2000) Pre- and postsynaptic actions of serotonin on rat suprachiasmatic nucleus neurons. Brain Res 866:247–256

Johnson AK, Loewy AD (1990) Circumventricular organs and their role in visceral functions. In: Loewy AD, Spyer KM (eds) Central regulation of autonomic function. Oxford University Press, New York, pp 247–267

Kalsbeek A, Scheer FA, Perreau-Lenz S, La Fleur SE, Yi CX, Fliers E, Buijs RM (2011) Circadian disruption and SCN control of energy metabolism. FEBS Lett 585:1412–1426

Karakas A, Serin E, Gunduz B (2006) Food restriction affects locomotor activity in Mongolian gerbils (Meriones unguiculatus). Turkish J Biol 30:23–28

Korkmaz A, Topal T, Tan DX, Reiter RJ (2009) Role of melatonin in metabolic regulation. Rev Endocr Metab Disord 10:261–270

Krajnak K, Rosewell KL, Duncan MJ, Wise PM (2003) Aging, estradiol and time of day differentially affect serotonin transporter binding in the central nervous system of the female rats. Brain Res 990:87–94

Krieger DT (1974) Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. Endocrinology 95:1195–1201

Laposky AD, Bass J, Kohsaka A, Turek FW (2008) Sleep and circadian rhythms: key components in the regulation of energy metabolism. FEBS Lett 582:142–151

Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM (1996) Abnormal splicing of the leptin receptor in diabetic mice. Nature 379:632–635

Lee SJ, Verma S, Simonds SE, Kirigiti MA, Kievit P, Lindsley SR, Loche A, Smith MS, Cowley MA, Grove KL (2013) Leptin stimulates neuropeptide y and cocaine amphetamine-regulated transcript coexpressing neuronal activity in the dorsomedial hypothalamus in diet-induced obese mice. J Neurosci 33:15306–15317

Lévi F, Okyar A, Dulong S, Innominato PF, Clairambault J (2010) Circadian timing in cancer treatments. Annu Rev Pharmacol Toxicol 50:377–421

Mammen AP, Jagota A (2011) Immunocytochemical evidence for different patterns in daily rhythms of VIP and AVP peptides in the suprachiasmatic nucleus of diurnal Funambulus palmarum. Brain Res 1373:39–47

Manikonda PK, Jagota A (2012) Melatonin administration differentially affects age-induced alterations in daily rhythms of lipid peroxidation and antioxidant enzymes in male rat liver. Biogerontology 13:511–524

Marie M, Findlay PA, Thomas L, Adam CL (2001) Daily patterns of plasma leptin in sheep: effects of photoperiod and food intake. J Endocrinol 170:277–286

Martin SS, Qasim A, Reilly MP (2008) Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. J Am Coll Cardiol 52:1201–1210

Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Trayhurn P (1996) Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. FEBS Lett 387:113–116

Mistlberger RE, Kent BA, Chan S, Patton DF, Weinberg A, Parfyonov M (2012) Circadian clocks for all meal-times: anticipation of 2 daily meals in rats. PLoS ONE 7: e31772

Montoya A, López-Olmeda JF, Garayzar AB, Sánchez-Vázquez FJ (2010) Synchronization of daily rhythms of locomotor activity and plasma glucose, cortisol and thyroid hormones to feeding in Gilthead seabream (Sparus aurata) under a light–dark cycle. Physiol Behav 101:101–107

Partch CL, Green CB, Takahashi JS (2013) Molecular architecture of the mammalian circadian clock. Trends Cell Biol 24:90–99

Polston EK, Simerly RB (2006) Ontogeny of the projections from the anteroventral periventricular nucleus of the hypothalamus in the female rat. J Comp Neurol 495: 122–132

Prosser RA, Bergeron HE (2003) Leptin phase-advances the rat suprachiasmatic circadian clock in vitro. Neurosci Lett 336:139–142

Quennell JH, Mulligan AC, Tups A, Liu X, Phipps SJ, Kemp CJ, Herbison AE, Grattan DR, Anderson GM (2009) Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. Endocrinology 150:2805–2812

Rattan SI (2012) Biogerontology: from here to where? The Lord Cohen Medal Lecture-2011. Biogerontology 13:83–91

Salgado-Delgado R, Tapia Osorio A, Saderi N, Escobar C (2011) Disruption of circadian rhythms: a crucial factor in the etiology of depression. Depress Res Treat 2011:839743

Scheer FA, Shea TJ, Hilton MF, Shea SA (2008) An endogenous circadian rhythm in sleep inertia results in greatest cognitive impairment upon awakening during the biological night. J Biol Rhythms 23:353–361

Schoeller DA, Cella LK, Sinha MK, Caro JF (1997) Entrainment of the diurnal rhythm of plasma leptin to meal timing. J Clin Invest 100:1882–1887

Schumann DM, Cooper HM, Hofmeyr MD, Bennett NC (2005) Circadian rhythm of locomotor activity in the four-striped field mouse, Rhabdomys pumilio: a diurnal African rodent. Physiol Behav 85:231–239

Smith RG, Betancourt L, Sun Y (2005) Molecular endocrinology and physiology of the ageing central nervous system. Endocrine Rev 26:203–250

Taheri S, Lin L, Austin D, Young T, Mignot E (2004) Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. PLoS Med 1:e62

Takeda H, Nakagawa K, Okubo N, Nishimura M, Muto S, Ohnishi S, Sakamoto N, Hosono H, Asaka M (2013) Pathophysiologic basis of anorexia: focus on the interaction between ghrelin dynamics and the serotonergic system. Biol Pharm Bull 36:1401–1405

Vujovic P, Lakic I, Laketa D, Jasnic N, Djurasevic SF, Cvijic G, Djordjevic J (2011) Time-dependent effects of starvation on serum, pituitary and hypothalamic leptin levels in rats. Physiol Res 60(Suppl 1):S165–S170

Welsh DK, Takahashi JS, Kay SA (2010) Suprachiasmatic nucleus: cell autonomy and network properties. Annu Rev Physiol 72:551–577

Yoon JA, Han DH, Noh JY, Kim MH, Son GH, Kim K, Kim CJ, Pak YK, Cho S (2012) Meal time shift disturbs circadian rhythmicity along with metabolic and behavioral alterations in mice. PLoS ONE 7:e44053

Yu EA, Weaver DR (2011) Disrupting the circadian clock: gene-specific effects on aging, cancer, and other phenotypes. Aging 3:479–493

Zhang G, Bai H, Zhang H, Dean C, Wu Q, Li J, Guariglia S, Meng Q, Cai D (2011) Neuropeptide exocytosis involving synaptotagmin-4 and oxytocin in hypothalamic programming of body weight and energy balance. Neuron 69:523–535

Zhao K, Ao Y, Harper RM, Go VL, Yang H (2013) Food-intake dysregulation in type 2 diabetic Goto-Kakizaki rats: hypothesized role of dysfunctional brainstem thyrotropin-releasing hormone and impaired vagal output. Neuroscience 247:43–54