Physiology & Behavior 118 (2013) 129–137

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Brown adipose tissue thermogenesis precedes food intake in genetically obese Zucker (fa/fa) rats

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HIGHLIGHTS

- Zucker obese (fa/fa) rats have ultradian episodic increases in BAT temperature.
- The interval between episodic BAT temperature increases is prolonged in obese rats.
- Eating commences 15min after increases in BAT temperature in obese and lean rats.
- The action of leptin is not necessary for the occurrence of ultradian increases in BAT temperature.
- BAT thermogenesis is reduced in the Zucker obese rat.

ARTICLE INFO

Article history:
Received 28 October 2012
Received in revised form 22 March 2013
Accepted 7 May 2013

Keywords:
Body temperature
Brain temperature
Metabolic rate
Ultradian rhythms
Leptin receptors

ABSTRACT

In Sprague–Dawley rats, brown adipose tissue (BAT) thermogenesis occurs in an episodic ultradian manner (BAT on-periods) as part of the basic rest–activity cycle (BRAC). Eating occurs approximately 15 min after the onset of BAT on-periods. Zucker obese (fa/fa) rats eat larger less frequent meals than control rats. In chronically instrumented conscious unrestrained Zucker obese rats we examined ultradian fluctuations in BAT, body and brain temperatures, and the relation between BAT temperature and eating. The interval between BAT temperature peaks for the 12 hour dark phase was 121 ± 3 (mean ± SE) min for Zucker obese rats and 91 ± 3 min for control lean rats ($p < 0.01$). Corresponding values for the light phase were 148 ± 6 and 118 ± 4 min ($p < 0.01$). Mean BAT and body temperatures were lower in Zucker obese rats, in comparison with lean controls, during both BAT on-periods and BAT off-periods. Mean brain temperatures were lower during BAT off-periods. Amplitudes of the BRAC-related increases in all 3 temperatures were greater in the Zucker obese rats. Meal onset in Zucker obese rats commenced 15 ± 1 min after the onset of a BAT on-period, not significantly different for the delay observed in lean control rats (18 ± 1 min, $p > 0.05$). Thus periods between eating are increased in the Zucker obese rats, but the action of leptin, absent in these animals, is not crucial for the timing of eating in relation to increases in BAT and body temperature. Lack of the normal excitatory action of leptin on brain-regulated BAT sympathetic discharge could also contribute to lower BAT thermogenesis in Zucker obese rats.

1. Introduction

In Sprague–Dawley rats with ad libitum access to food, body temperature begins to increase approximately 15 min before the onset of each meal, and the increase is partially due to heat production in brown adipose tissue (BAT) [1–4]. This close temporal association between eating and increased brain temperature is part of a more general patterning of active/inactive behavioral states that Kleitman [5] called the basic rest–activity cycle (BRAC). Intervals between periods of BAT thermogenesis are approximately 1–2 h in the dark phase and approximately 2 1/2 h in the light phase of a 12 hour lights off/12 hour lights on day, so that the BRAC is an ultradian rhythm [2,6]. Intervals between active phases are highly variable, a feature of ultradian episodic events [7,8]. The BAT thermogenesis pattern continues substantially unchanged in Sprague–Dawley rats deprived of food for up to 24 h [4,9], so that eating itself does not generate the BRAC.

Zucker obese (fa/fa) rats [10] are homozygous for a missense mutation in the gene encoding the leptin receptor, so that although plasma leptin levels are elevated the actions of leptin are prevented because of the defective receptor [11–15]. Zucker obese rats eat less frequently than control rats, but average meal size is larger so that total daily food intake is increased [16–19]. Much is known concerning the actions of leptin on brain pathways regulating food intake [20–25], and leptin administration has effects on thermoregulatory systems [26,27]. The present paper determines whether the increased inter-meal intervals in obese Zucker (fa/fa) rats correspond to prolonged intervals between

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http://dx.doi.org/10.1016/j.physbeh.2013.05.021

episodic BRAC-related increases in behavioral activity and BAT thermogenesis, and whether these leptin-resistant obese animals also have an approximately 15 min delay between the onset of BAT thermogenesis and the commencement of eating.

2. Materials and methods

2.1. Ethics, animals and anesthesia

All experiments were conducted in accordance with the Animal Welfare Committee of Flinders University. Male Zucker obese (fa/fa) rats and heterozygous (Fa/fa) or wild type (Fa/Fa) Zucker lean rats, originally purchased from Harlan Laboratories (rms.na@harlan.com) were bred at the Flinders University Animal House. Genetic status was determined by PCR of ear tag tissues, performed in the laboratory of Dr Greg Barritt, Flinders University. Because of mortality associated with anesthesia and surgery for instrumentation, only obese animals weighing less than 700 g were used. A total of 23 Zucker obese rats (562 ± 21 g) and 19 Zucker lean rats (370 ± 9 g) were used in the study. We did not find statistically significant differences between heterozygous and wild type rats so results from these two subgroups were combined.

Animals were instrumented (see below) under general anesthesia (2% isoflurane in O_2, Veterinary Companies of Australia Pty. Ltd., NSW). Analgesia (Rimadyl, 5 mg/kg s.c., Pfizer Pty Ltd., West Ryde, NSW, Australia) and antibiotics (Baytril, 5 mg/kg s.c, Bayer Aust Ltd., Pymble, NSW, Australia, 0.1 ml s.c.) were administered, and the animal returned to the animal house for at least 1 week before experiments were carried out. Animals were individually housed in the presence of other animals with ad libitum food (standard rat chow pellets) and water. For experimentation animals were transferred to an insulated recording chamber, temperature 24–26 °C, 12 hour light and 12 hour dark. At the conclusion of the experiment animals were humanely killed by injection of Lethabarb (sodium pentobarbitone, 180 mg/kg i.p.).

2.2. Measurement of physiological variables and behavioral activity

BAT, body and brain temperatures were continuously measured with thermistors [2,4]. In each rat a temperature probe was positioned in interscapular BAT near the vein of Sulzer (BAT temperature), intracranially in a dorsal extradural position near the confluence of the sagittal and transverse sinuses (brain temperature), and in the mediastinum, just ventral to the trachea (body temperature). The hippocampal EEG was also recorded in some animals but there were technical artifacts in the EEG signal during eating, and the EEG results are not included in this paper.

Insulated wires from the temperature probes were passed subcutaneously and attached to a head socket screwed to the skull. Behavioral activity was continuously measured using an infrared light beam XY grid pattern (beams 5 cm apart) constructed by Biomedical Engineering, Flinders University. Interruption of an infrared beam triggered a TTL pulse output to MacLab (ADInstruments, Castle Hill, NSW, Australia) connected to an Apple Macintosh computer programmed with MacLab Chart software.

A flexible cable from the swivel device was attached to the animal's head socket. Continuous recording was then made for up to 72 h. Human interference was limited to correction of technical problems and replacement of food and water. When malfunctions occurred, results from periods of less than 72 h were used in our analyses. The temporal profile of the records from BAT, brain and body temperature probes was highly correlated, as we have previously reported for Sprague–Dawley rats [2,4]. If malfunction occurred in the BAT temperature recording we used the body or the brain signal to determine the peak time of BRAC episodes. We elected not to present group brain temperature results in the present paper.

2.3. Measurement of the timing of eating and the amount eaten at each meal

We developed instrumentation to record the timing of disturbance of the food container and amount of food eaten at each meal [4]. Because the present study was partially completed before this instrumentation was available the number of animals in the eating portion of the study is less than the total number of animals used for the temperature studies.

The process of food removal disturbed the food container, signaling the possible onset of eating. A meal was defined as a decrease of ≥0.2 g in the weight of the food container, with an intermeal interval of at least 35 min [4].

2.4. Processing of physiological signals

Signal analysis and graphic representation were performed using IgorPro (Wavemetrics, Lake Oswego OR, USA). A wavelet function was fitted to the records for temperature, using the discrete wavelet transform operation (DWT) in IgorPro. Records were then averaged into 1 min bins. To be defined as a peak, the BAT temperature wavelet function was required to have an amplitude of at least 0.5 °C and an interpeak interval of at least 35 min [2,4]. Duration of each BAT temperature increase (BAT on-period) was measured as the interval from onset of temperature increase to the time when the temperature had decreased by half the amplitude of the peak, or to the time of onset of the next BAT temperature increase, whichever was the sooner (BAT temperature end time). A BAT off-period was defined as the interval between the particular BAT end time and the onset of the next BAT temperature on-period.

2.5. Statistical analysis

Physiological variables were assessed with and without respect to ultradian fluctuations. For the latter we averaged the 12 hour dark and 12 hour light phases for each variable in each rat, and then group data (mean ± SE) was obtained by averaging the results across rats, for Zucker obese and lean control animals separately. To incorporate ultradian fluctuations into the analysis we first calculated mean values for each BAT on-period and each BAT off-period (defined as described in Materials and methods) for dark and light phases in Zucker obese and lean control rats. Group averages (mean ± SE) were then calculated by averaging across all ultradian episodes in each experimental sub-division, separately for BAT on-periods and BAT off-periods.

Individual BAT temperature records (12 hour dark periods) were autocorrelated in IgorPro by duplicating the record, sliding the duplicate to an earlier time in 1 min steps, and then calculating the Pearson product-moment correlation coefficient for the overlapping portions of the original and the duplicated records. Relations between variables were also assessed with linear regression, using Statview 5 (SAS Institute Inc., Cary, NC. USA). The statistical significance of mean differences between Zucker obese rats and control lean rats was assessed with analysis of variance (ANOVA). Post-hoc analysis was performed using Fisher's protected *t* test only if the ANOVA treatment effects were significant at $p \leq 0.05$.

3. Results

3.1. Patterning of sudden increases in temperature and activity in obese rats

Records from individual Zucker obese rats displayed sudden highly correlated increases in BAT, brain and body temperatures, occurring together with increases in behavioral activity (Fig. 1). For the 12 hour dark phase in obese rats the interval between peaks of the increases in BAT temperature was 121 ± 3 min, significantly longer than the

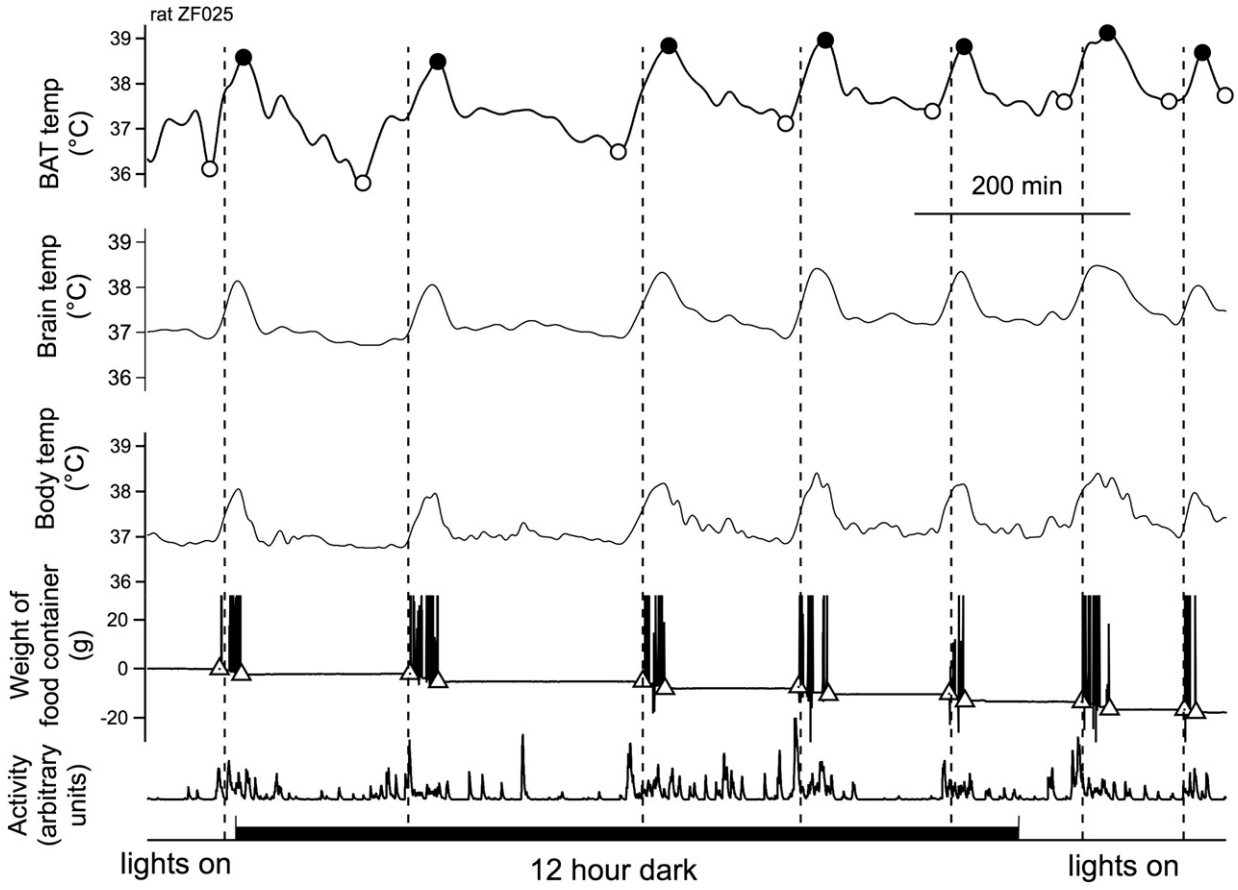

Fig. 1. Records (1 min bins) of parameters in an individual rat during both light and dark periods. The temperature traces are wavelets fitted to the original 1 Hz traces by the DWT function in IgorPro (see Methods). On the BAT record, open and filled black circles indicate onset and peak times of the increases. Transient changes in the weight of the food container indicate times when the rat is disturbing the container to remove food. Progressive decrease in the weight of the container indicates the amount of food eaten. Dotted and open triangle symbols indicate beginning and end of meal respectively. Ambient temperature 25 °C.

corresponding interval (91 ± 3 min) in lean controls (see Table 1). The corresponding interpeak interval for the light phase in obese rats was 148 ± 6 min, again significantly longer than the corresponding interval (118 ± 4 min) in lean controls (Table 1). The proportion of the total recording time occupied by BAT on-periods was 47 ± 2% (n = 24) for Zucker obese rats, not significantly different ($p > 0.05$) from 49 ± 1% (n = 19), the corresponding value for lean control rats.

Frequency distributions of BAT interpeak intervals for obese and lean animals (Fig. 2) show no obvious dominant interpeak intervals. We autocorrelated all BAT temperature records (1 per min bins), with the duplicate record advanced in time in 1 min steps over a 720 min period. The autocorrelation coefficients from each rat were then averaged across rats. For obese rats and for lean controls the autocorrelograms showed no evidence for a specific periodicity in the occurrence of peaks of BAT temperature (Fig. 3). For both groups the correlation coefficients decreased towards zero as the duplicate record was progressively advanced, with the value becoming maximally negative as the duplicate record was advanced by 720 min (12 h), presumably reflecting the 24 hour dark/light variation in temperature. We also used linear regression to determine whether there was any relation between the duration of a particular BAT on-period and the duration of the subsequent BAT off-period. No significant regressions were observed for either obese or lean animals, in either the dark or the light periods (data not shown).

Table 1
Group results (mean ± SE) for BAT temperature (temp) for Zucker obese rats (n = 24) and lean control rats (n = 19) during 12 hour dark and 12 hour light periods. Numbers in brackets indicate total number of BRAC episodes.

Physiological parameter	Genetic status	Dark	Light
BAT temp interpeak interval (min)	Obese	121 ± 3 (335) ¶¶	148 ± 6 (189) ¶¶
	Lean	91 ± 3 (372)	118 ± 4 (240)
Duration of BAT temp on-periods (min)	Obese	61 ± 2 (342) ¶¶	62 ± 2 (199) ¶¶
	Lean	47 ± 1 (384)	54 ± 2 (244)
Duration of BAT temp off-periods (min)	Obese	64 ± 3 (330) ¶¶	84 ± 5 (189) ¶¶
	Lean	44 ± 2 (372)	64 ± 3 (240) ¶¶

ns, not significantly different from the corresponding lean control value, $p > 0.05$.
¶¶ Significantly different from the corresponding lean control value, $p < 0.01$.

3.2. Group data for BAT, brain and body temperatures

When results were analyzed without regard to the ultradian fluctuations in temperature, there were no significant differences between Zucker obese rats and lean controls for average BAT, brain or body temperatures (data not shown). However when results were analyzed separately for BAT on-periods and BAT off-periods the variability of the measures was reduced, so that there were significant differences between obese and control rats. Average BAT and body temperatures were lower in obese rats in comparison with lean control rats, for both BAT on-periods and BAT off-periods, in both the dark and light phases (Fig. 4A, B). Average brain temperature was lower in the obese rats during BAT off-periods, for both dark and light phases (Fig. 4B).

BAT, brain and body temperatures at the onset of BAT on-periods were lower in Zucker obese rats in comparison with lean control rats for the dark period, and BAT and brain temperatures at the onset of BAT on-periods were also lower in obese rats during the light period (Table 2). The amplitude of all three temperature increases during the

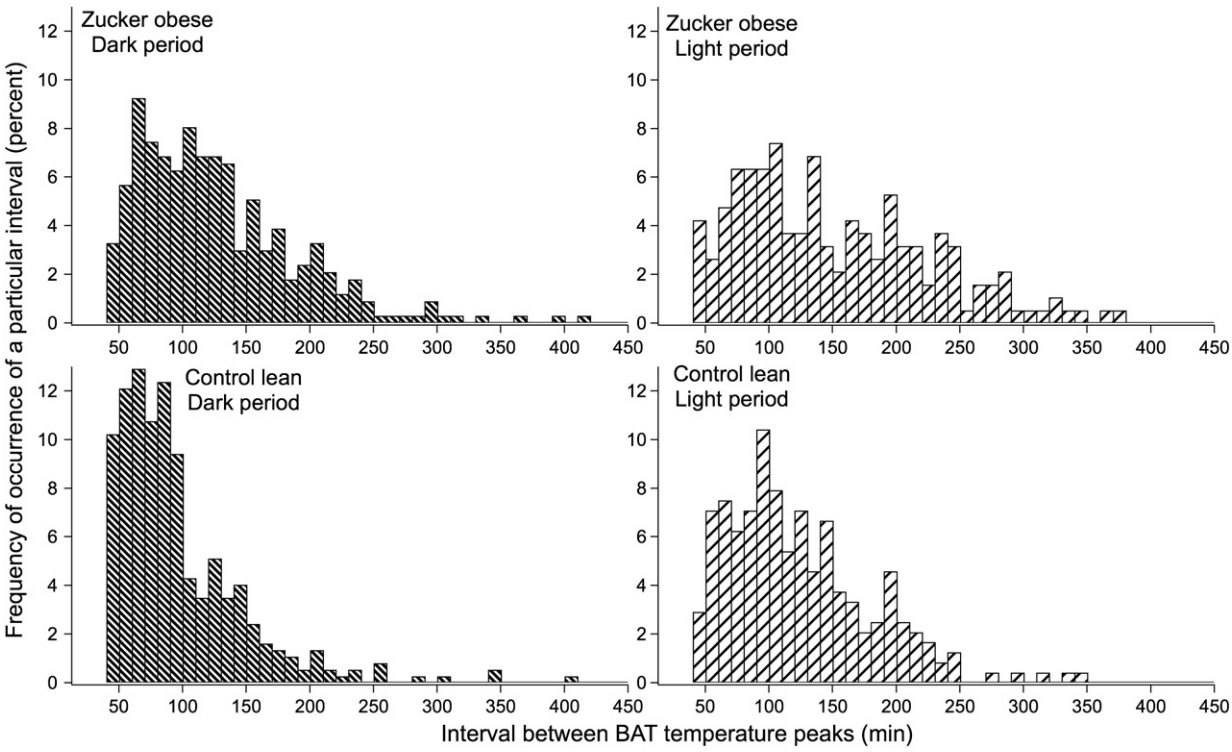

Fig. 2. Frequency distributions of the interval between peaks of episodic BAT thermogenesis in Zucker obese rats and lean controls for the dark and light periods.

BAT on-periods was greater for the obese rats for both dark and light periods, so that peak BAT, brain and body temperatures during BAT on-periods in obese rats were not significantly different from corresponding values in lean rats (Table 2).

3.3. Group data for activity

When results were analyzed without regard to the episodic increases in temperature, total daily activity was similar in obese versus lean control animals, for both the dark and light periods (data not shown). Average activity during BAT on-periods was similar in Zucker obese

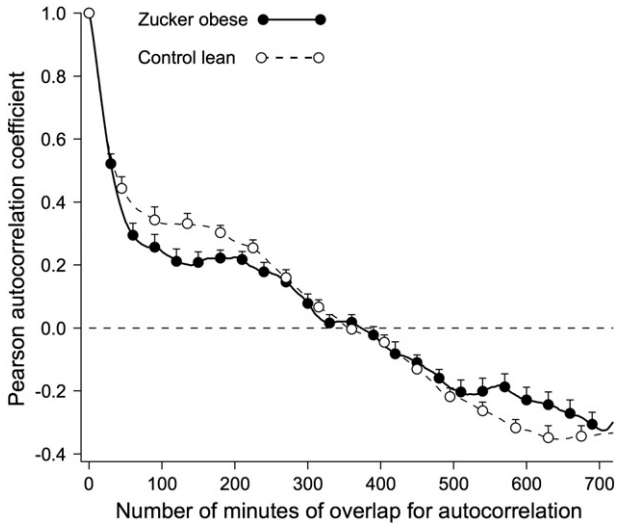

Fig. 3. Autocorrelation analysis of 12 hour dark period BAT temperature signals (1 min bins) for Zucker obese (thick line, filled circles) and lean control rats (thinner line, open circles). Values are mean ± SE for each indicated time point (n = 24 obese rats and 19 lean rats).

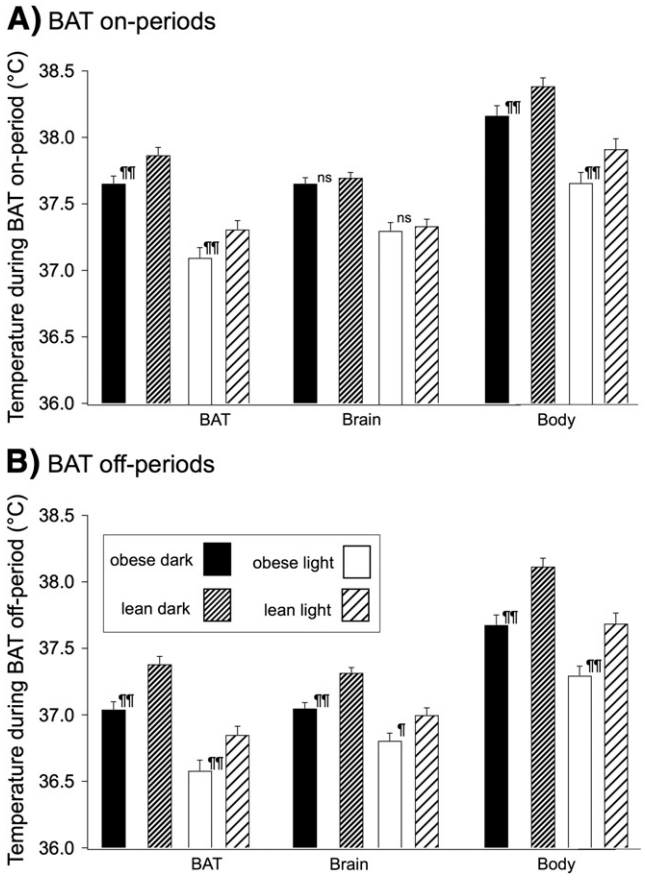

Fig. 4. Group data (mean ± SE) for mean BAT, brain and body temperatures during BAT on-periods (A) and BAT off-periods (B) for Zucker obese and control rats in both dark and light periods, as explained in the inset in B. ¶¶Significantly different from corresponding lean control group, $p < 0.01$.

Table 2
Group results (mean ± SE) for BAT, brain and body temperatures and for behavioral activity (arbitrary units) for Zucker obese rats (n = 22) and lean control rats (n = 18) during 12 hour dark and 12 hour light periods. Numbers in brackets indicate total number of BRAC episodes.

Physiological parameter	Genetic status	Dark	Light
BAT temp at onset of on-periods (°C)	Obese	36.8 ± 0.1 (267)¶¶	36.3 ± 0.1 (152)¶¶
	Lean	37.2 ± 0.1 (364)	36.6 ± 0.1 (237)
Amplitude of BAT temp on-period increase (°K)	Obese	1.3 ± 0.03 (267)¶¶	1.4 ± 0.04 (152)¶¶
	Lean	1.0 ± 0.02 (364)	1.2 ± 0.03 (237)
BAT peak temp during on-periods (°C)	Obese	38.1 ± 0.1 (267) ns	37.6 ± 0.1 (152) ns
	Lean	38.3 ± 0.1 (364)	37.8 ± 0.1 (237)
Brain temp at onset of on-periods (°C)	Obese	36.9 ± 0.05 (169)¶¶	36.6 ± 0.06 (103)¶¶
	Lean	37.2 ± 0.04 (304)	36.8 ± 0.06 (187)
Amplitude of brain temp on-period increase (°K)	Obese	1.3 ± 0.04 (170)¶¶	1.3 ± 0.05 (103)¶¶
	Lean	0.9 ± 0.02 (304)	1.0 ± 0.03 (187)
Body temp at onset of on-periods (°C)	Obese	37.6 ± 0.1 (148)¶¶	36.1 ± 0.1 (86) ns
	Lean	38.1 ± 0.1 (224)	37.3 ± 0.3 (149)
Amplitude of body temp on-period increase (°K)	Obese	1.1 ± 0.03 (148)¶¶	1.1 ± 0.05 (86)¶¶
	Lean	0.7 ± 0.03 (224)	0.8 ± 0.04 (149)
Activity during BAT on-period	Obese	17.8 ± 0.6 (307) ns	16.3 ± 1.0 (178)¶¶
	Lean	16.7 ± 0.6 (353)	12.2 ± 0.6 (228)
Activity during BAT off-period	Obese	5.3 ± 0.4 (294)¶	5.7 ± 1.1 (170) ns
	Lean	6.9 ± 0.6 (342)	4.5 ± 0.6 (225)

ns, not significantly different from corresponding lean control value, $p > 0.05$.
¶¶ Significantly different from the corresponding lean control value, $p < 0.01$.
¶ Significantly different from the corresponding lean control value, $p < 0.05$.

rats and lean control rats during the dark period (Table 2) but activity was greater in the obese animals during the light period (Table 2). During BAT off-periods, average activity was reduced in Zucker obese rats in comparison with lean controls for the dark period, but the groups were not significantly different in the light period (Table 2).

3.4. Food consumption and its temporal relation with BAT temperature

With food available ad libitum, eating occurred episodically in obese rats and in lean controls. Zucker obese rats ate 11.3 ± 0.5 meals every 24 h, significantly less than 14.7 ± 0.5 meals per 24 h, the corresponding value in lean control rat ($p < 0.01$). Over a 24 hour period, obese rats consumed 28 ± 1 g of food, significantly greater than 22 ± 2 g, the corresponding value in lean control rats ($p < 0.05$). Duration of individual meals was 28 ± 1 min in the obese rats and 23 ± 1 min in lean control rats ($p < 0.01$), with no difference between dark and light periods.

There was a close association between the time of eating and the episodic increases in temperature and behavioral activity. As is evident in Fig. 1, when eating occurred it commenced some minutes after the beginning of a BAT on-period. Quantitative analysis established that eating commenced 15 ± 1 min after the onset of a BAT on-period in Zucker obese rats, compared with 18 ± 1 min in lean control rats ($p > 0.05$), with similar timing in dark and light periods. Fig. 5 summarizes these results and demonstrates that the stereotyped pattern of the changes in temperature and activity, and the relation with eating is similar in Zucker obese and lean control rats.

3.5. Preprandial and postprandial relations between amount eaten and time between meals

For the obese rats in the dark period there was no preprandial relation between the time since the end of the previous meal and the amount eaten during a given meal (linear regression $p > 0.05$). For the light period the corresponding linear regression was significant ($p < 0.05$) but the proportion of the variance due to the regression was small ($r^2 = 0.08$). Similarly, for the dark period in lean control rats there was no preprandial relation between the time since the end of the previous meal and the amount eaten during a given meal (linear regression $p > 0.05$). For the light period there was a significant relation ($p < 0.01$), with the regression accounting for a more substantial proportion of the variance ($r^2 = 0.54$).

For the dark period in obese rats there was a significant relation between the amount eaten at a given meal and the postprandial time till the next meal (linear regression $p < 0.01$), but the proportion of the variance due to the regression was small ($r^2 = 0.06$). There was no significant postprandial relation for the corresponding light period linear regression ($p > 0.05$). For the lean control rats there was no significant relation between amount eaten and postprandial delay for either the dark or the light period ($p > 0.05$ in both cases).

4. Discussion

4.1. Ultradian BRAC patterning of BAT thermogenesis occurs in Zucker obese (fa/fa) rats, but the interpeak interval is increased

Episodic increases in activity, associated with increases in BAT, brain and body temperatures, in both the dark and light phases of the daily cycle occur with ultradian BRAC patterning in Zucker obese rats in the same way with Zucker lean control rats and Sprague–Dawley rats [2,4]. The interval between BAT temperature peaks of active BRAC phases is notably longer in the obese rats, approximately 120 min in the dark period in comparison with approximately 90 min for the corresponding period in control animals, and in Sprague–Dawley rats. The large variability of the interpeak interval for periods of BAT thermogenesis, previously noted in Sprague–Dawley rats [2,4] is also present in the Zucker obese animals. Previous studies of the temperature and activity of Zucker obese (fa/fa) rats include figures suggesting the occurrence of ultradian fluctuations in these variables [28,29], but the focus of the authors is on circadian rhythms. Our current study documents prominent ultradian variations in BAT, brain and body temperatures, behavioral activity and food intake in Zucker obese rats. When no account is taken of these events the variability of "baseline" measures is substantially increased, reducing the power of statistical tests of possible temperature, metabolic rate, and activity differences between the Zucker obese animals and the controls.

4.2. Zucker obese (fa/fa) rats have lower BAT, brain and body temperatures in comparison with lean controls

When we classified our records according to active or inactive phases of the BRAC (i.e. BAT on-periods versus BAT off-periods), it was clear that Zucker obese rats had lower average body and BAT temperatures during both BRAC phases, and lower brain temperatures during BAT off-periods. The Zucker obese rats had fewer BAT on-periods but the average duration of each on-period was larger, so that the overall percentage of time spent in BAT on-periods and BAT off-periods (approximately 50%) was similar in Zucker obese and lean control rats. Thus our results suggest that temperature and heat production are reduced in Zucker obese rats. A previous study, using 5 min recording points from intra-abdominal probes [28], reported that Zucker obese rats have lower mean daily temperatures in comparison with control lean rats (37.2 °C versus 37.5 °C). Demes et al. [30] found no significant differences in temperature between Zucker obese and control rats.

BAT on-period behavioral activity level was similar in both groups during dark period, and increased in obese rats during the light period. BAT off-period behavioral activity level was increased in lean rats during the dark period and similar in both groups during the light period. Overall activity level during 24 h was similar in both groups. Thus the lower temperatures in the Zucker obese rats are unlikely to be related to reduced heat production in skeletal muscle. Previous studies have reached differing conclusions concerning the amount of bodily activity in Zucker obese rats, with the variability due to different measuring procedures being noted [16,31–33]. Taken together

with these previous studies, our findings suggest that BAT thermogenesis is reduced in the Zucker obese rats.

Since BAT thermogenesis contributes to the increases in body and brain temperatures [3,34], it is likely that the overall metabolic rate of the obese rats is lower than that of control rats, perhaps contributing to their obesity. A previous study demonstrated that during the dark period Zucker obese rats have reduced oxygen consumption, normalized for body mass [35].

4.3. BAT thermogenesis in Zucker obese rats

Previous studies have provided evidence suggesting that BAT function is reduced in Zucker obese rats in a number of situations. Anesthetized Zucker obese rats were found to have absent or impaired BAT sympathetic nerve discharge responses to cooling [36,37]. Stress-induced increases in body temperature have been shown to be reduced in Zucker obese rats and the febrile response to lipopolysaccharide is altered in a complex manner [38,39]. These and other studies are discussed by Ivanov and Romanovsky [40] who emphasize that the response of BAT to different stimuli is best assessed in a cool environment. In their study [40] the increase in body temperature in response to intravenous administration of lipopolysaccharide and to a stress stimulus were found to be normal in Zucker rats at thermoneutral temperatures, but reduced in a cool environment. Ivanov and Romanovsky [40] agree with previous investigators [41–43] who concluded that BAT is morphologically and/or functionally defective in Zucker obese rats.

Our study found, in agreement with previous investigations, that in comparison with lean controls, average BAT temperature is reduced in Zucker obese rats, in both the resting and the active phases of the BRAC. However we also found the amplitude of the episodic increases in BAT temperature to be greater in the Zucker obese rats, and the peak BAT and body temperature values to be similar in obese and lean control rats. These observations suggest that at ambient temperatures of 24–26 °C the central and peripheral neural pathways regulating BAT, as well as the response of the BAT to sympathetic activation, are essentially intact in Zucker obese rats. In this respect our finding contrasts with those of previous investigators.

Previous studies have demonstrated robust ultradian fluctuations in CO_2 emission in Sprague–Dawley rats [8]. The amplitudes of the fluctuations in CO_2 emission vary, but their magnitude is substantial, approximately 30% of the baseline, even more marked when constant light exposure reduces or abolishes circadian metabolic rhythms. Given the major contribution of BAT metabolism to overall metabolic rate [34] it is likely that ultradian increases in BAT thermogenesis contribute to the fluctuations in CO_2 emission, and thus to metabolic rate. This may also be the case in the Zucker obese rats, so that our demonstration of

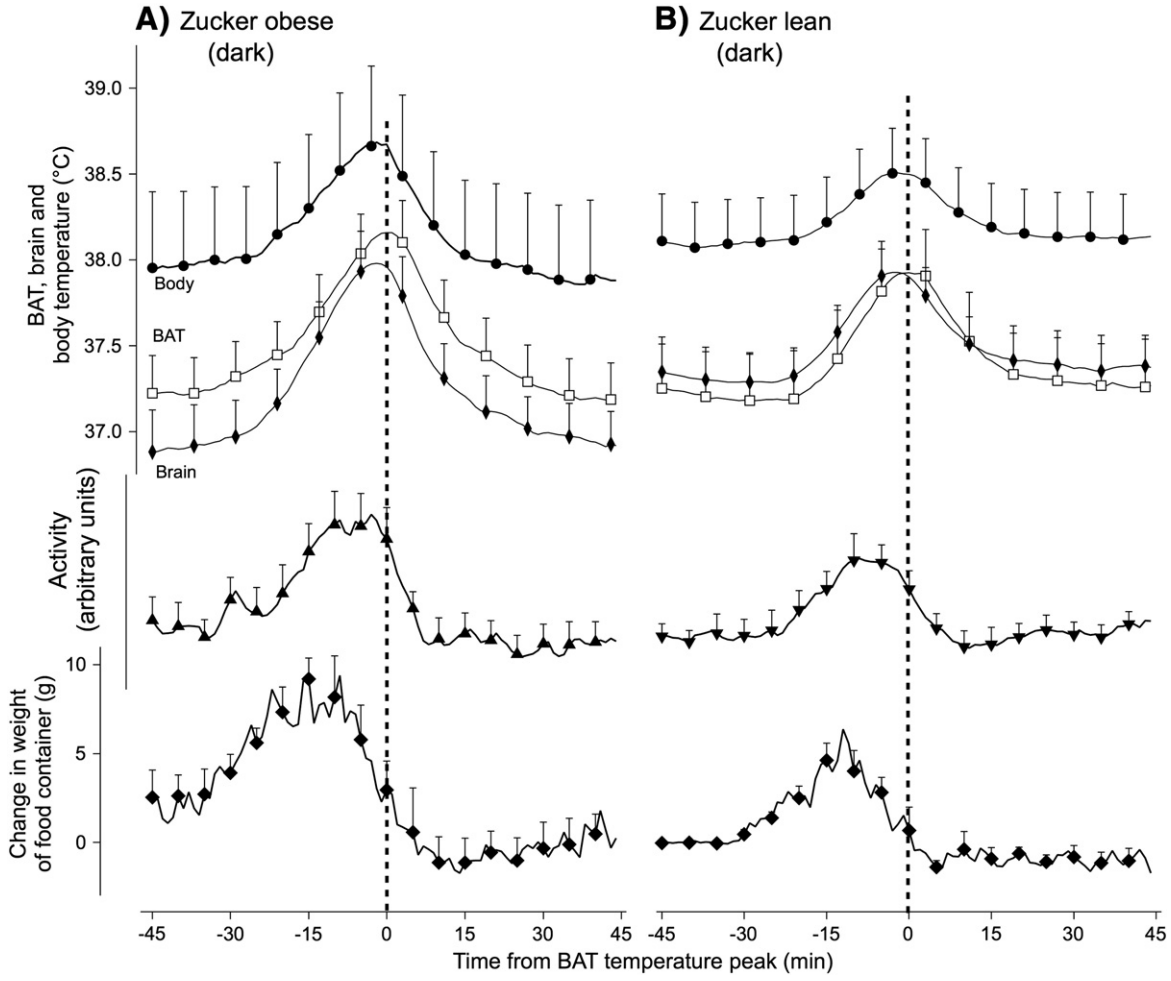

Fig. 5. Group data showing BAT (solid squares), brain (solid diamonds) and body (open circles) temperatures, activity, and change in weight of the food container in Zucker obese (A, C) and lean control rats (B, D). Each trace in the figure is mean ± SE (1 min bins) of 90 min epochs, beginning 45 min before and ending 45 min after the peak of each BAT temperature increase during the dark (A, B) and light (C, D) periods. Variables were first averaged in individual rats and then across rats (N = 16 obese and 19 lean rats for the temperature and activity traces, and N = 8 obese and 5 lean rats for the food traces).

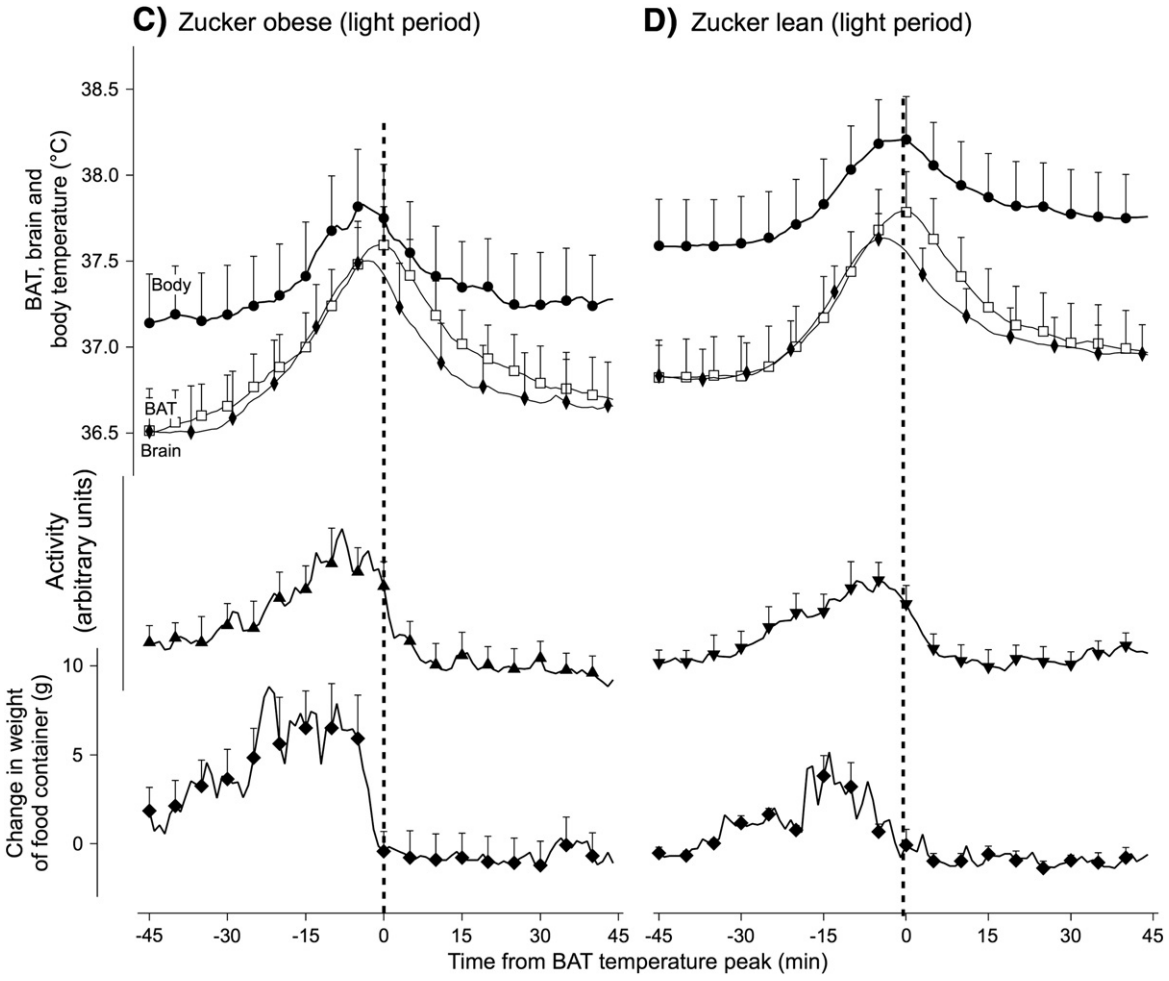

Fig. 5 (*continued*).

reduced BAT thermogenesis may be a marker of reduced overall metabolism in these animals, presumably contributing to their obesity.

4.4. The temporal relation between increased temperature and onset of eating is preserved in Zucker obese rats

The meal pattern in obese rats was similar to the pattern described for the Zucker obese rats in Fig. 1 of Castonguay et al. [18]. Eating is integrated into the BRAC in a temporally precise manner in Zucker obese rats, as also occurs in Sprague-Dawley rats. If eating occurs, it commences about 15 min after the onset of an active phase, when temperatures are increasing at their maximum rates. As eating ceases, these physiological parameters abruptly begin decreasing towards baseline values. In agreement with previous studies we found that obese rats had larger, longer, less frequent meals. Nevertheless, the previously observed relation between temperature and eating [1,4] was also present in obese rats. Eating commenced approximately 15 min after the onset of a period of BAT thermogenesis. Since eating is integrated into the BRAC in a temporally precise manner, meal intervals are related to BRAC intervals.

No specific periodicity is apparent using frequency histogram and autocorrelation analysis, and there is no relation between the duration of a particular active episode and the delay until the onset of the next active episode. Similarly, there was a weak or absent relation between meal size and meal interval. Other researchers have provided evidence for the unpredictability of meal occurrence [44]. Our present results in Zucker obese rats are in accord with the idea that in our experimental conditions activity patterns, including food intake, are generated by stochastic processes similar to those described in a number of species by Lehmann [45].

We consider that the association between eating and thermogenesis principally reflects a coordinating central command process that integrates the timing of both variables. We hypothesize that BAT thermogenesis contributes to the rise in brain temperature during active BRAC periods to facilitate the complex synaptic processing that mediates active engagement of the individual with the external environment, including the search for food.

4.5. Role of leptin in determining temperature, meal size and meal interval

We have previously shown that the interval between active BRAC phases is not substantially changed when food is removed from the container for up to 24 h [4]. Thus we consider that rather than eating, of itself, "causing" the BRAC, the process of food ingestion is centrally programmed as part of the BRAC. Our present study demonstrates that Zucker obese rats demonstrate the same 15 min delay between the onset of an active phase of the BRAC and the onset of eating. The interval between active BRAC phases, and thus the time between meals, is longer in the obese animals. Time spent eating is longer in the obese rats, but the additional eating time per meal (approximately 5 min longer in obese animals) does not account for the longer duration of the BRAC phases (approximately 30 min in the dark period).

It is not clear how this longer interval between active BRAC episodes is related to the absence of functional leptin receptors. Exogenously administered leptin acts as a short term satiety factor, leading to termination or reduction of eating, with meal size being affected rather than

meal interval [25,46,47]. Thus in Zucker obese rats, meal duration could be increased because the normal inhibitory action of leptin is lacking, but the longer intermeal interval displayed by the obese animals is difficult to relate to the absence of leptin receptors. In the ad libitum food condition there are no studies showing that adipose tissue secretes leptin in a meal-related manner, so that leptin-mediated satiety actions in our rats would presumably reflect interactive effects, with amplification of satiety signals that are meal-related, such as cholecystokinin [48,49]. Potential brain neural pathways mediating the effects of leptin are complex, with receptors for leptin distributed widely in the forebrain, midbrain and hindbrain [21,22,24]. Projections of hypothalamic neurons expressing the leptin receptor have been identified by Gautron et al. [23] and BAT-controlling hypothalamic and brainstem neurons expressing leptin receptors have been identified by Zhang et al. [50]. Emphasis for a direct action of leptin on hindbrain neurons has extended the framework for our understanding of the possible brain mechanisms whereby the lack of the normal action of leptin contributes to obesity and decreased metabolic rate [21,51–53].

Presumably Zucker obese rats have non-functioning leptin receptors in all these brain areas. There are few studies of possible contributions of these different areas to the effect of leptin on eating and metabolism. The arcuate NPY neurons are overactive in Zucker obese rats so that this could contribute to obesity [54]. Increased meal duration in Zucker obese rats could also reflect the failure of the satiety action of gastric leptin secreted in a meal-related exocrine fashion into the gastric lumen [55,56]. Whether Zucker obese rats lack leptin receptors in the gastric system has not been investigated. Leptin receptors are also present in other peripheral sites including the perikarya of vagal afferent neurons in the nodose ganglion [21,51].

Since we observed a positive correlation between meal size and subsequent (post-prandial) meal interval in the Zucker obese rats, the larger meal size could contribute to the longer intermeal interval. However the relation was not a strong one, with only 6% of the intermeal interval variance being attributable to previous meal size. The longer meal interval in Zucker rats may reflect the altered timing of the BRAC rather than being primarily eating-related. In our previous study we showed that food deprivation, a procedure that presumably reduced plasma leptin levels [57], slightly decreased the interval between periods of active BAT thermogenesis [2], as also documented by Closa et al. [9]. Thus it is unlikely that non-functioning leptin receptors can explain the increased BRAC interval that occurs in Zucker obese rats. In this respect it would be most interesting to study the BRAC, and the relation between BAT on-periods and the onset of eating in rats with diet induced obesity.

5. Conclusion

Our paper is the first to report the relation between onset of eating and ultradian increases in BAT thermogenesis in Zucker obese (fa/fa) rats. The daily life of these obese animals is patterned according to the basic rest–activity cycle, as is the case in Zucker lean control rats and in Sprague–Dawley rats. Food intake commences approximately 15 min after the beginning of an active phase, as is also the case in lean control rats and in Sprague–Dawley rats. In obese rats, during active phases brown adipose tissue temperature increases to levels similar to those observed in control rats, but the resting level is reduced in comparison with control rats. Since active phases of the rest–activity cycle occur significantly less often in the obese rats, the number of meals per day is reduced, even though total food intake is increased. The absence of functional leptin receptors does not change the basic organization of the rest–activity cycle.

Acknowledgments

This work is supported by the NHMRC, the ARC, and the Flinders Medical Centre Foundation. We thank Pam Simpson and Robyn Flook for technical help. Professor Greg Barritt and Dr Zabin Zhou, Dept. of Medical Biochemistry, Flinders University kindly performed the genetic analysis.

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