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Ontogeny of cardiac rate regulation and brown fat thermogenesis in golden hamsters (*Mesocricetus auratus*)

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Abstract It was shown previously in infant rats (*Rattus norvegicus*) that the ability to produce heat in the cold using brown adipose tissue (BAT) is closely related to the ability to maintain cardiac rate. When the limits of BAT thermogenesis were exceeded, interscapular temperature (which reflects the temperature of the interscapular BAT depot) and cardiac rate fell together. As an extension of this earlier study, the relation between BAT thermogenesis and cardiac rate was examined here in the golden hamster (*Mesocricetus auratus*), a species whose young do not exhibit BAT thermogenesis until the end of the 2nd week postpartum. It was found that 3 to 12-day-old hamsters were unable to increase shivering or nonshivering thermogenesis in the cold and exhibited decreases in cardiac rate that proceeded in lock-step with decreases in interscapular temperature. In contrast, as the thermogenic capability of hamsters increased after 12 days of age, cardiac rate was maintained within narrow limits across a wide range of air temperatures. These results support the hypothesis that heat produced by BAT helps to warm the heart and thus aids in the maintenance of cardiac rate during cold exposure.

Key words Brown adipose tissue · Golden hamster · Cardiac rate · Development Thermoregulation

Abbreviations *BAT* brown adipose tissue · T_a air temperature · T_{is} interscapular temperature · T_{back} back temperature · *IBI* interbeat interval

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Introduction

Soon after the discovery of the thermogenic function of brown adipose tissue (BAT), it was noted that the vascular anatomy of BAT is ideal for the delivery of warmed blood to the heart (Smith and Roberts 1964; Smith and Horwitz 1969). The influence of heart temperature on heart function had been known for years (Fairfield 1948; Adolph 1951; Lyman and Blinks 1959) and so it was reasonable to hypothesize that BAT thermogenesis aids in the maintenance of cardiac function during cold challenge (Smith and Roberts 1964). In a recent test of Smith and Roberts' hypothesis, it was demonstrated that the ability of newborn and week-old rats (*Rattus norvegicus*) to maintain cardiac rate during cooling is closely related to their ability to increase BAT thermogenesis (Blumberg et al. 1997). Specifically, it was found that as pups were challenged with successive decreases in air temperature (T_a), cardiac rate was maintained as BAT thermogenesis increased. In contrast, when T_a was decreased, such that BAT thermogenesis was maximized and physiological temperatures fell, cardiac rate decreased in lock-step.

One way to assess the strength of the association between BAT thermogenesis and cardiac function is to monitor their development in a single species. Rat pups are not useful for such a study because they exhibit robust thermogenic responses soon after birth (Taylor 1960; Blumberg and Stolba 1996). Golden hamsters (*Mesocricetus auratus*), however, do not begin exhibiting shivering or nonshivering thermogenesis until they are approximately 12 days of age (Hissa 1968; Nedergaard et al. 1986). Therefore, in the present study, the thermoregulatory and cardiac responses of 3 to 15-day-old hamsters were monitored. It was hypothesized that the maintenance of cardiac rate in the cold would be associated with the maturity of thermogenic responses.

Materials and methods

Subjects

A total of 15 male and female golden hamsters from five litters were used. All subjects were born to females in the animal colony at the University of Iowa and were 3–15 days of age at the time of testing. If a mother gave birth to more than eight pups, her litter was culled to eight pups within 3 days of birth (day of birth = day 0). Such culling was necessary for two litters; two of the remaining three litters contained exactly eight pups, and the fifth litter contained six pups. Litters and mothers were housed in standard laboratory cages (48 cm × 20 cm × 26 cm) in which food and water were available ad libitum. All animals were maintained on a 12:12 h light/dark schedule with lights on at 6:00 a.m.

Test environment

All experiments were conducted by placing an animal inside a double-walled glass chamber (height = 17 cm; i.d. = 12.5 cm). T_a within the chamber was controlled by pumping temperature-controlled water through its walls. Access holes and connectors in the side of the chamber and the lid allowed for the passage of air as well as the passage of thermocouple wires and EKG leads. A round platform constructed of polyethylene mesh was fitted inside.

Temperature measurements

Physiological temperature and T_a were measured using chromel-constantan thermocouples as described elsewhere (Blumberg et al. 1997). Mean T_a within the metabolic chamber was determined using two thermocouples located beneath the platform. Physiological temperatures were measured using thermocouples attached to the skin surface using collodion as an adhesive. One thermocouple was attached in the interscapular region above the brown fat pad, thus providing a measure of interscapular temperature (T_{is}). A second thermocouple was attached in the lumbar region distant from the BAT pad, thus providing a measure of back temperature (T_{back}). For those older subjects in which fur had developed, the thermocouples were positioned under the fur and secured with collodion.

Oxygen consumption measurements

Oxygen consumption was measured as described elsewhere (Blumberg et al. 1997). Briefly, compressed air was split into two lines. One line was circulated through the metabolic chamber at 300 ml/min and was drawn into the first channel of an electrochemical oxygen analyzer. The second line traveled directly from the air cylinder to the second channel of the oxygen analyzer. Oxygen concentration in each airstream was measured simultaneously and the percent difference in concentration was computed to 0.001%. This percent difference was fed into the data acquisition computer and transformed into a measure of oxygen consumption in milli-litres of O_2 per kilogram per minute.

Data acquisition

For both experiments, thermal and metabolic measures were acquired at least twice each minute using a customized data acquisition system for the Macintosh computer (LabView, National Instruments, Austin, Tex.). A second system was used to acquire EKG data at the rate of 1000/s.

Procedure

On the day of testing, the subject was removed from its cage and weighed. While the subject was in an incubator maintained at 35–36 °C, three EKG leads were attached either subcutaneously or transcutaneously depending on the age of the animal and the amount of fur present. For placement of transcutaneous leads (n = 10; age range: 11–15 days), the animal was first lightly anesthetized for approximately 1 min. For placement of subcutaneous leads (n = 5; age range: 3–11 days), the animal was not anesthetized and small openings in the skin were made using a 30 gauge needle. In all cases, collodion was used to secure the electrodes and to improve the electrical connection. The EKG leads were connected to a differential amplifier (A-M Systems, Everett, Wash.). The signal was filtered and amplified before being acquired by the computer.

Before testing, all subjects were postabsorptive, as evidenced by the presence of milk visible through their abdominal skin. After the thermocouples were attached, the subject was placed inside the metabolic chamber maintained between 32.8 °C and 36.8 °C; the lower T_as were used for the older subjects. Many of the older animals (≥10 days of age) with relatively mature locomotor abilities were first placed inside a polyethylene mesh cage (10 cm × 7 cm × 4 cm) to prevent them from tangling the electrode wires and thermocouples. Pipe cleaners were fitted through the holes of the mesh to decrease the area within which the animal could move.

Whether secured inside the mesh cage or allowed to roam freely within the chamber, each subject was given at least a 45 min acclimation period. After this period, cardiac rate data were acquired for 1 min. Then, T_a was decreased in succession in 2–5 °C increments, with the older animals experiencing the greater increments. There was no attempt to establish a fixed protocol for this experiment because of the large differences in the animals' age, size, insulation, and physiological abilities. Subjects were given at least 45 min to stabilize at each T_a, at which time cardiac rate data were acquired. Data were acquired at no more than five different values of T_a.

After the test, the subject was removed from the chamber, its EKG leads and thermocouples were removed, and it was returned to its home cage. After removal of the animal from the metabolic chamber, the oxygen consumption system was allowed to rezero to verify minimal drift in the system over the course of the test. If drift occurred such that VO_2 readings were affected by more than 10%, then a correction procedure was used to adjust the oxygen consumption values at each phase of the experiment. For this procedure, it was assumed that the system's drift was linear and constant throughout the experiment. Such correction of VO_2 values was necessary for 3 of the 15 tests.

Data analysis

Thermal and metabolic measures were imported into StatView 4.5 for the Macintosh and cardiac rate data were imported into DataDesk 5.0. A scatterplot of the cardiac rate data was produced for each session, the interbeat intervals (IBI) were determined, and mean IBI was calculated for each pup at each phase of the experiment. These means were calculated from a median number of 74 IBIs (range: 17–90).Mean thermal and metabolic data (i.e., T_a, T_{is}, T_{back}, and VO_2) at each T_a were calculated from two readings, taken 30 s apart, that were acquired simultaneously with the EKG measurements.

Results

The body weights of the 15 hamsters used in the experiment are shown in Fig. 1. From 3–15 days of age, body weight increased from approximately 4 to 26 g. Figure 1 also indicates which subjects were from the same litter.

T_{is}, VO_2, and IBI as a function of T_a are shown in Fig. 2. The data for the 15 subjects are segregated into

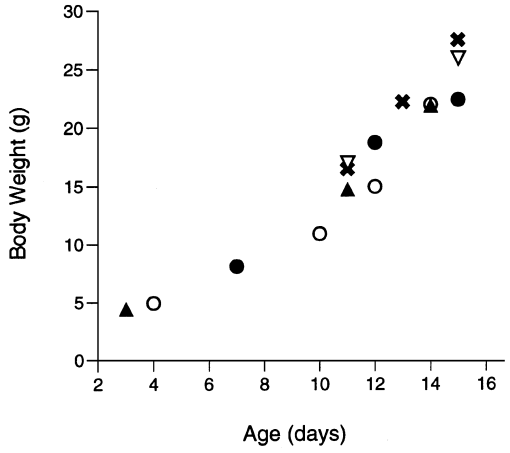
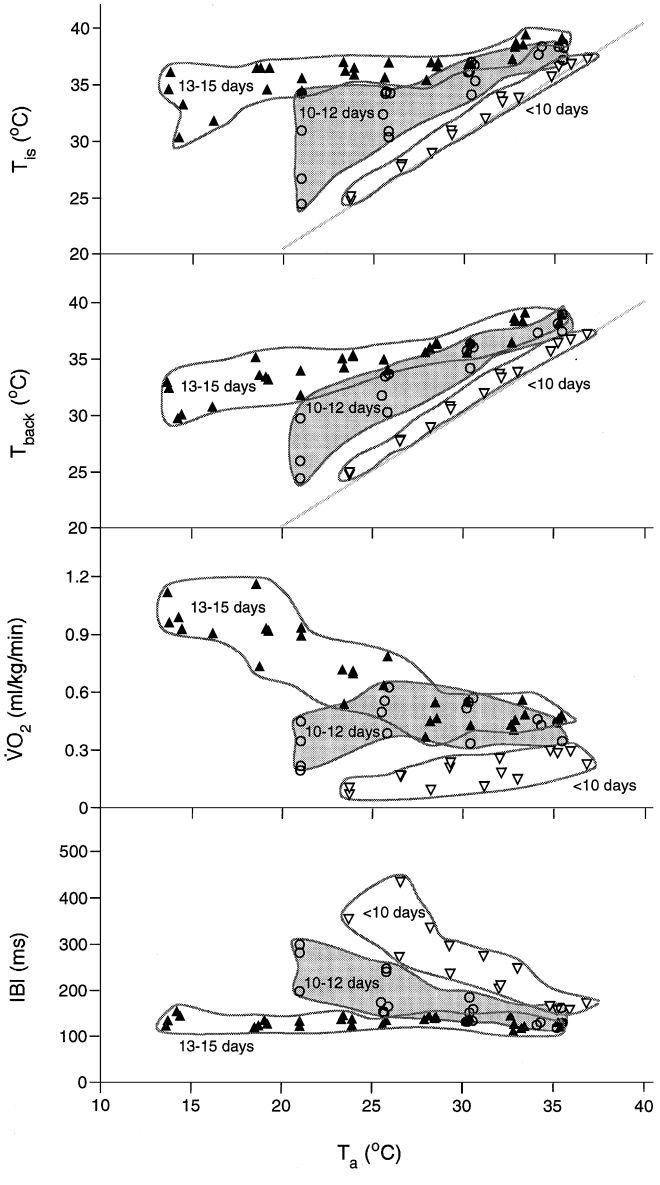


Fig. 1 Body weight (g) vs age (days) for the 15 hamsters in the experiment. Each of the five litters used is represented by a different symbol



three age groups that are distinguishable based on their physiological responses to cold exposure: < 10 days of age (n = 3), 10–12 days of age (n = 6), and 13–15 days of age (n = 6). First, it can be seen that the youngest animals used in the study (3, 4, and 7 days of age) did not exhibit observable thermoregulatory responses as T_a decreased. Specifically, both T_{is} and VO_2 declined steadily in the cold, as did T_{back}. Moreover, IBI increased with each successive decrease in T_a.

Hamsters at 10–12 days of age showed some improvement in their ability to maintain T_{is}, T_{back}, and cardiac rate in the cold (Fig. 2). VO_2 did not increase substantially in these subjects, suggesting that they were not exhibiting either BAT or shivering thermogenesis. Therefore, the improved thermoregulation likely resulted from increased body size and insulation.

From 12–15 days of age, there was a pronounced change in the physiological responses of the animals to cold. The ability of these subjects to maintain T_{is} and T_{back} improved substantially, even at T_as as low as 14 °C. The substantial increases in VO_2 are suggestive of increased heat production. Finally, cardiac rate was maintained within narrow limits by these animals throughout the 20 °C range in T_a tested.

The increase in VO_2 exhibited by the 13 to 15-day-olds could have reflected increases in BAT thermogenesis, shivering thermogenesis, or both. To assess BAT thermogenesis at different T_as, T_{is}-T_{back} is plotted against T_a in Fig. 3 for each of the three age groups; for this figure, data were collected from each subject at 20-min intervals throughout the experiment. Because T_{is} reflects the temperature of the depot of BAT underlying the skin in the interscapular region and T_{back} is measured from the lumbar region some distance from BAT, the difference between T_{is} and T_{back} provides an estimate of BAT thermogenesis (Dawkins and Hull 1964; Blumberg and Stolba 1996). In Fig. 3, the horizontal lines represent points of equality between T_{is} and T_{back}, and points above the line are suggestive of BAT thermogenesis. It can be seen that all of the points for subjects in the two youngest age groups fall on or just above the horizontal line. In contrast, most of the points for the 13 to 15-day-olds at the lower T_as are substantially above the horizontal line; these data, in conjunction with the clear increases in VO_2 seen in Fig. 2, indicate the presence of BAT thermogenesis in the cold (Heim and Hull 1966).

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Fig. 2 Cloud diagrams for interscapular temperature (T_{is}; °C), back temperature ($T_{back;}$ °C), VO_2 (ml/kg/min), and interbeat interval (*IBI*; ms) as a function of air temperature (T_a; °C). These diagrams outline all of the data points for hamsters less than 10 days of age (*open triangles*), 10–12 days of age (*open circles*), and 13–15 days of age (*filled triangles*). Isometric lines are included in the top two panels. The 13 to 15-day-olds were better able than younger animals to maintain T_{is} in the cold. This ability was accompanied by increases in VO_2 and the maintenance of cardiac rate across a wide range of air temperatures

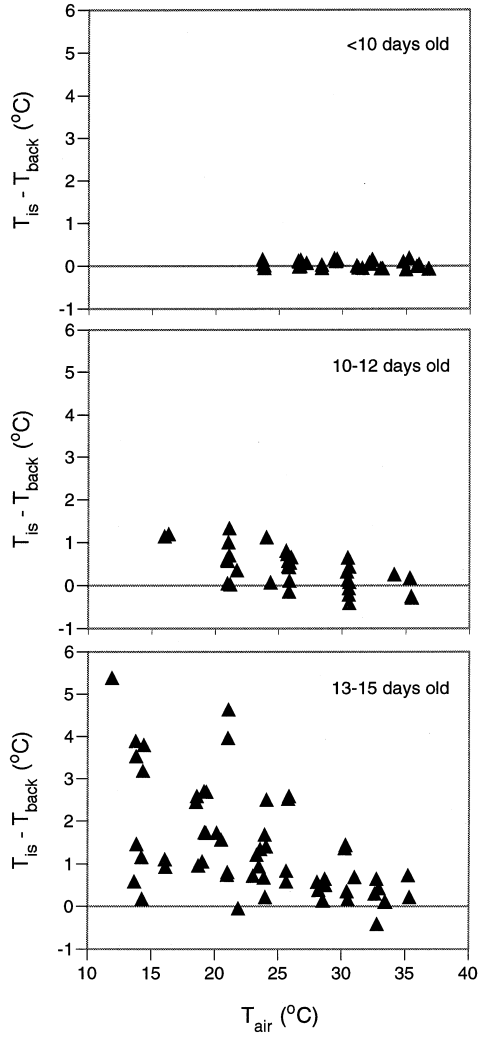

Fig. 3 T_{is}-T_{back} (°C) vs T_a (°C) for hamsters less than 10 days of age (three subjects), 10–12 days of age (four subjects), and 13–15 days of age (six subjects). Data points collected from each subject at 20-min intervals during the experiment are presented. For the 13 to 15-day-olds, points lying above the horizontal line at the lower T_as suggest activation of brown adipose tissue thermogenesis

That the 13 to 15-day-old hamsters were producing heat using BAT does not preclude the possible contribution of shivering. Although shivering thermogenesis was not explicitly measured in this experiment, its presence could be inferred from increased noise in the EKG records when overt body movements were not observed. This noise was occasionally apparent in the 10 to 12-day-olds, although the VO_2 data in Fig. 2 suggest that these subjects were not exhibiting appreciable levels of either shivering or nonshivering thermogenesis. In the 13 to 15-day-olds, the EKG records became increasingly noisy at the lowest T_as tested, eventually necessitating the termination of the experiment. In some cases, T_{is} decreased toward T_{back} as this noise became evident, perhaps owing to disinhibition of shivering during periods of decreased BAT thermogenesis (see Brück and Wünnenberg 1970).

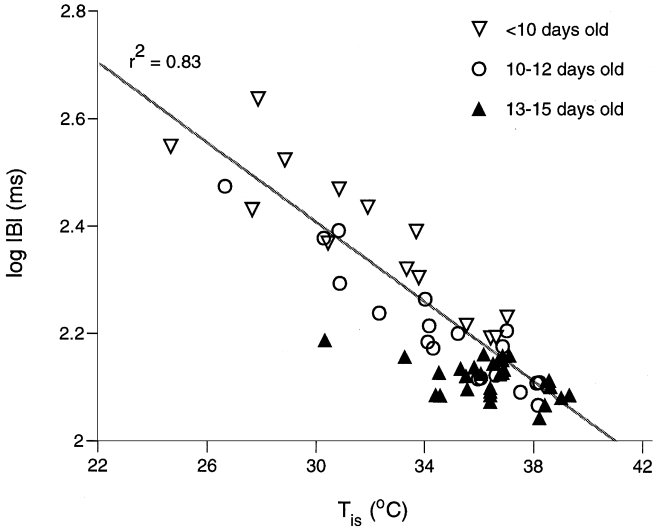

Fig. 4 Log IBI (ms) vs T_{is} (°C) for each of the 15 hamsters and at each phase of the experiment. The regression line is fitted only to the data for the 3 to 12-day-olds. The goodness of fit indicates a strong relation between cardiac rate and T_{is} for these younger subjects. The 13 to 15-day-olds were better able to maintain T_{is} in the cold, and cardiac rate was maintained as well

It was shown previously in infant rats that ganglionic blockade results in a near-perfect correlation between T_{is} and log IBI, indicating that when BAT thermogenesis is prevented and autonomic control of the heart is blocked, cardiac rate is temperature-dependent (Blumberg et al. 1997). Similarly, as shown in Fig. 4, linear regression of log IBI on T_{is} for the 3 to 12-day-old hamsters indicated a highly significant relationship ($r^2 = 0.832$, $F_{1,34} = 168.3$, $P < 0.0001$). Because the inclusion of multiple data points from each subject in this analysis violates statistical assumptions of independence, a linear regression was performed on the data for each of the 3 to 12-day-olds and a *t*-test was used to determine whether the mean slope deviated significantly from 0. The mean slope of −0.033 did differ significantly from 0 ($t_8 = 9.6$, $P < 0.0001$), thus adding confidence in the validity of the overall regression analysis. Finally, Fig. 4 shows that the data for the 13 to 15-day-olds exhibit a relatively narow restricted range and fall on or near the regression line for the younger subjects.

Discussion

The present experiment indicates that young hamsters acquire the ability to maintain cardiac rate in the cold as metabolic heat production begins to develop. The increase in heat production in the 13 to 15-day-olds is due in part to BAT thermogenesis, as has been shown previously (Hissa 1968; Nedergaard et al. 1986). In addition, it is likely that these older hamsters were shivering at the lowest T_as, a capability that has also been demonstrated in hamsters beginning at approximately 12 days of age (Hissa 1968). Either form of heat pro-

duction, in conjunction with autonomic control of the heart (Adolph 1971), could help protect cardiac rate by maintaining heart temperature, although the location of BAT and its large vascular connection with the heart suggest that BAT is ideally suited for the selective maintenance of heart temperature (Smith and Horwitz 1969). Nonetheless, additional experiments are necessary to assess the relative efficacy of BAT and shivering thermogenesis in young hamsters.

Hamsters and rats exhibit very different developmental profiles. For example, although the 16-day gestation of hamsters is 6 days shorter than rats, hamsters develop fur at an earlier age than rats and begin eating solid food earlier as well (Schoenfeld and Leonard 1985). The two species also differ with regard to the ontogeny of their autonomic and behavioral thermoregulatory mechanisms. Rat pups exhibit thermogenic responses shortly after birth (Taylor 1960) but do not exhibit rapid behavioral responses to cold when placed on a thermal gradient (Kleitman and Satinoff 1982). In contrast, newborn hamsters are incapable of increasing heat production in the cold but are capable of rapidly selecting appropriate temperatures in a thermal gradient (Leonard 1974, 1982; Schoenfeld and Leonard 1985). It is interesting that despite these species differences in the development of thermoregulatory responses, both hamsters and rats exhibit a strong association between BAT thermogenesis and cardiac rate regulation.

It is now apparent that neither young hamsters nor infant rats increase cardiac rate as BAT thermogenesis and oxygen consumption increase in the cold, thus raising the question of how sufficient oxygen is delivered to BAT to sustain high levels of thermogenesis. Without increasing cardiac rate, these animals could support increased utilization of oxygen by increasing stroke volume (and thereby increasing cardiac output) and/or by redistributing blood flow to BAT. There is evidence in adult animals that cardiac output increases during BAT thermogenesis (Foster and Frydman 1979). There is also evidence in young rabbits that increased BAT thermogenesis can be supported by a redistribution of blood flow to BAT without an increase in cardiac output (Harris et al. 1985); redistribution of blood flow to BAT would be especially important when there are limitations in the ability to increase stroke volume, as is the case in some infants (e.g., Teitel et al. 1985). With regard to young hamsters during cold exposure, this question remains open for further study.

In conclusion, converging evidence supports Smith and Roberts' (1964) hypothesis that BAT thermogenesis contributes significantly to the regulation of cardiac rate. First, cardiac rate is dependent on heart temperature in vitro (Adolph 1951) and exhibits strong temperature-dependence in vivo in hamsters younger than 13 days of age (Fig. 4) and in ganglionically blocked infant rats (Blumberg et al. 1997). Second, BAT thermogenesis is associated with the maintenance of cardiac rate in the cold in 2-week-old hamsters (Figs. 2, 3) and infant rats (Blumberg et al. 1997). Finally, as mentioned above, blood flows directly from the interscapular BAT depot to the heart (Smith and Roberts 1964; Smith and Horwitz 1969). In order, however, to demonstrate a causal relationship between BAT thermogenesis and cardiac rate, it must be shown that selective manipulation of BAT thermogenesis can modulate cardiac rate. Such manipulation is now possible with the availability of adrenergic agonists that bind with the β_3-adrenoceptor, a receptor subtype that is prevalent in adipose tissue and that mediates the thermogenic response of BAT (Zhao et al. 1994). Indeed, recent experiments on infant rats using a selective β_3-agonist lend support to the hypothesis that heat provided by BAT directly modulates cardiac rate (G Sokoloff, RF Kirby and MS Blumberg unpublished manuscript).

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