A thymus tumour impairing hibernation

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Abstract

Background

Hibernation is a physiological and behavioural adaptation that permits survival during periods of reduced food availability and extreme environmental temperatures. This is achieved through cycles of metabolic depression and reduced body temperature (torpor) and rewarming (arousal). Rewarming from torpor is achieved through the activation of brown fat (BAT) associated with a rapid increase in ventilation frequency. Here, we studied the rate of rewarming in the European hamster by measuring both BAT temperature, core body temperature and ventilation frequency.

Results

Temperature was monitored in parallel in the BAT (IPTT tags) and peritoneal cavity (iButtons) during hibernation torpor-arousal cycling. We found that increases in brown fat temperature preceded core body temperature rises by about 47 min, and this was accompanied by a significant increase in ventilation frequency. The rate of rewarming was slowed by the presence of a spontaneous thymus tumour in one of our animals. Core body temperature re-warming was reduced by 6.2°C*h⁻¹ and BAT rewarming by 12°C*h⁻¹. Ventilation frequency was increased by 77% during re-warming in the thymus tumour animal compared to a healthy animal. Inspection of the position and size of the tumour indicated that the lungs and heart were obstructed.

Conclusions

We validated a minimally invasive method to monitor BAT temperature during hibernation. Using this method we showed compromised re-warming from hibernation in an animal with a thymus tumour, the likely cause of which is obstruction of the lungs and heart leading to inefficient ventilation and circulation.
Background

Hibernation is a physiological and behavioural adaptation that permits survival during periods of reduced food availability and extreme environmental temperatures. This is achieved through cycles of metabolic depression characterised by reduced body temperature (torpor) and rewarming (arousal). Entrance into torpor precisely is controlled by decreases in heart rate, ventilation frequency and oxygen consumption [1, 2]. Arousal occurs in a coordinated manner with increased ventilation frequency and oxygen consumption subsequently followed by heart rate, blood pressure and core body temperature rise [1]. Rewarming from torpor is achieved through the activation of subcutaneous brown fat reserves on top of the scapulae (classical BAT) and within the intra-scapular region (intra-scapular BAT) [3]. The primary function of brown fat is heat generation through non shivering thermogenesis [4], which has a high energy (oxygen) demand [5, 6]. During arousal from torpor temperature increases in brown fat correlate with increased oxygen consumption (respiration rate) in the brown bat (Eptesicus fuscus) and the golden-mantled ground squirrel (Callospermophilus lateralis), and precede rectal or muscle temperature increases [7–10].

Activation of brown fat is thought to originate in the thermo-sensing regions in the hypothalamus coupled to a sympathetic nervous pathway, which activates beta adrenergic receptors and in turn the brown fat mitochondria [11]. The thermogenic capacity of brown fat comes from the use of proton leak (uncoupling) in mitochondria as opposed to the coupled oxidative phosphorylation pathway which produces ATP [12]. The uncoupled pathway depends on the BAT-specific expression of the uncoupling protein, UCP1 transporter in the mitochondrial membrane. Initiation of this process requires good oxygen/energy supply to the BAT, and a marked increase in ventilation is an early event in the rewarming process [13]. The heat generated from BAT rewarms the anterior of the animal first, and then increases in heart rate and circulation are required to warm the rest of the animal. Subsequently, shivering thermogenesis is initiated to help the animal to reach normal body temperature (euthermy) quickly [5, 6, 14]. In Syrian hamsters (Mesocricetus auratus) the initiation of
shivering thermogenesis can only occur once the body temperature is above 16°C [5] and in the marmot (*Marmota marmota*) shivering is only observed when the subcutaneous BAT temperature reaches 16°C [4]. These data highlight the importance of non-shivering thermogenesis by BAT in the initial stages of the re-warming process.

Here, we validated a minimally invasive method to monitor both BAT temperature (*T*$_{BAT}$) and core body temperature (*Tb*) in a well-established hibernation model, the European hamster (*Cricetus cricetus*) [15–17]. We demonstrate that increases in *T*$_{BAT}$ precede increases in *Tb* and that the ventilation frequency correlates with the rate of *T*$_{BAT}$ re-warming. Furthermore, in one animal bearing a thymus tumour, impaired ventilation led to a marked slowing of the re-warming process.
**Results**

*Re-warming in brown fat compared to the core body*

We placed Implantable Programmable Temperature Transponder (IPTT) tags subcutaneously to measure the temperature of classical BAT (\(T_{BAT}\)). In the same animals we surgically implanted iButtons into the intraperitoneal cavity to monitor core body temperature (Tb). To initiate the preparation for hibernation we transferred animals from long photoperiod (14L:10D, 14 hours of light per 24 hours) and 22°C (LP22), to short photoperiod (10L:14D, 10 hours of light per 24 hours) and 22°C (SP22). After 8 weeks, we reduced the room temperature to 10°C (SP10, Figure 1A). All animals showed a hibernation phenotype within 4 weeks of transfer to SP10, which was preceded by “test drops” in Tb of approximately 10 to 15°C below euthermy before initiating the multi-day torpor-arousal cycling characteristic of the hibernation season (Figure 1B). Once hibernating, the European hamster drops its Tb to near ambient room temperature for multiple days (approximately 25°C below euthermy) and arouses at intervals returning to euthermy (Figure 1B).

We confirmed the initiation of the hibernation season and torpor-arousal cycling for at least 2 weeks before we started monitoring \(T_{BAT}\) during the periodic arousals. These data were time matched with Tb from the iButtons. Plotting the data from 5 arousing individuals we observe that both \(T_{BAT}\) and Tb show similar curve trajectories until euthermy is reached (Figure 2A, non-linear asymmetric sigmoidal model, \(T_{BAT}: r^2 = 0.9778; \text{Tb: } r^2 = 0.9878\)). We found the maximum re-warming rate (RWR\text{MAX}) was also similar for BAT (RWR\text{MAX} = 20.9°C\text{h}^{-1}; 95% confidence interval (CI): 20.3°C to 21.5°C) and core body (RWR\text{MAX} = 21.0°C\text{h}^{-1}; CI: 15.4°C – 26.5°C), however Tb RWR\text{MAX} showed more individual variance. We show that brown fat re-warming precedes subsequent core body temperature re-warming by 47 minutes (CI: 44-49 minutes) (Figure 2A). The mean ventilation frequency (VF) for the 5 individuals was calculated for each quartile of re-warming showing a clear increase in VF during re-warming (Figure 2A). Using visual assessment, the onset of shivering in muscles adjacent to the BAT
during arousal was noted, an average BAT temperature of 15°C was recorded at the onset of shivering (Figure 2B).

We plotted data from one representative individual and calculated the $RWR_{\text{max}}$ in brown fat as 19.3°C*h⁻¹, a similar rate was observed in the core body temperature (18.1°C*h⁻¹, Figure 2C). We also monitored the ventilation frequency during arousal which increased from 13 breaths per minute (bpm) to a maximum of 86 bpm (Figure 2C). Ventilation frequency increases correlated with $T_{\text{BAT}}$ (pearson $r=0.899$, p-value= 0.002).

Brown fat re-warming is compromised by the presence of a spontaneous thymus tumour

We identified one individual in our study with a spontaneous thymus tumour (Figure 3A). When comparing the amount of time spent torpid or aroused the tumour bearing animal spent 63.5% aroused and 14.5% torpid, compared to an average of 25.9% aroused and 54.6% torpid for the healthy animals (Figure 3B). The $RWR_{\text{max}}$ of brown fat was reduced by 12°C*h⁻¹ relative to a healthy animal (Figure 3C). The $RWR_{\text{max}}$ of the core body was less compromised showing a reduction of 6.2°C*h⁻¹.

Ventilation frequency increases still correlated with $T_{\text{BAT}}$ (pearson $r=0.873$, p-value= 0.002) but the tumour animal showed a 77% higher maximum ventilation frequency compared to a healthy animal (Figure 2B compared to Figure 3C). Brown fat re-warming precedes core body temperature re-warming by 53.9 minutes in the tumour animal, this is outside the confidence intervals for the healthy animals, suggesting that the efficiency of BAT re-warming of the core is compromised.
Our study has validated a minimally invasive method to measure BAT temperature during hibernation. We show that there is a significant correlation between $T_{BAT}$ and $T_b$, with $T_{BAT}$ increases preceding that of $T_b$. Compared to intraperitoneal implantation of iButtons the IPTT tags offer a minimally invasive method to monitor temperature, furthermore there is a significant advantage to monitoring BAT temperature in hibernation studies instead of core body temperature because the earliest arousal events can be detected almost immediately.

The increases in $T_{BAT}$ and $T_b$ are both correlated with increased ventilation frequency. Hyperventilation has been previously observed in the thirteen-lined ground squirrel but BAT temperature was not studied [13]. Our $T_b$ $\text{RWR}_\text{max}$ are similar to previous observations in rodents and European hamsters [18] but no data on BAT temperature is available.

Re-warming efficiency of the BAT in the tumour bearing animal was only 38% of that in the healthy animal, whereas the re-warming efficiency of the core body was 67% of that in the healthy animal. We also observed a greatly increased ventilation frequency compared to a healthy animal. We suggest that the size and position of the thymus tumour obstructed the lungs, reducing ventilation volume, thereby compromising the animal’s ability to deliver the necessary oxygen to support the activity of BAT. This probably resulted in increased ventilation frequency in an attempt to meet tissue demands. It has been observed that the early arousal events show a reliance on increased ventilation frequency and that increases in heart rate occur later as peripheral circulation increases [13]. The generally lower re-warming efficiency in both BAT and core body might be interpreted as evidence for compromised cardiac function leading to reduced circulation efficiency. In support of a decreased rate of re-warming of the BAT and core body due to decreased circulation we see re-warming rates in the thymus tumour animal comparable to that of the much larger Marmot (5000g compared to 500g) [18].

The discrepancy between BAT re-warming efficiency and core body re-warming is intriguing, and possibly relates to complicated relationship and order of events in arousal which start with
increased ventilation to increase oxygen availability, followed by vasodilation, and increased heart rate, to re-perfuse the circulation and re-warm the whole animal. Low oxygen availability increases vasodilation [19] therefore in the tumour bearing animal lower oxygen availability may have increased vasodilation leading to a compensated re-warming efficiency of the core body. Vasodilation would also benefit the BAT by supplying more oxygen in the blood. In support of this the tumour bearing animal in the initial stages of BAT re-warming from 10 to 15°C appears to show a gentler slope compared to 20 to 30°C (Figure 3C), indicating a lower efficiency in the early arousal stage which would not benefit from vasodilation, this distinction is not seen in the healthy animals. Another explanation for the discrepancy between BAT and core body re-warming efficiency may be the additional contribution of shivering thermogenesis to core body temperature increases.

The thymus is a lymphoid organ involved in T-cell maturation, it is located in front of the heart and found in all vertebrates (reviewed in: [20]). In general, thymus tumours are rare but are reported in domestic, laboratory and wild contexts (including wild European hamsters)[21]. Characterisation of European hamster thymus tumours has noted they closely match human thymic epithelial tumours [22]. Human thymus tumour cases often present with tumours that obstruct the heart and lungs, similar to our observations. Also in humans, an association of thymus tumour with hypothermia/defective re-warming has been reported twice [23, 24]. Impressively in one of the cases the patient experienced a body temperature between 32.8°C to 35°C (Ambient room temperature: 22°C) [23]. However, the cause of hypothermia in these cases is unknown.

Conclusions

In conclusion we have used temperature logging in BAT and intraperitoneal cavity to study progression of arousal in hibernating hamsters. We show compromised re-warming from hibernation in an animal with a thymus tumour, the likely cause of which is obstruction of the lungs and heart leading to inefficient ventilation and circulation.
Methods

Animals and ethics statement

European hamsters were bred from stock animals at the Chronobiotron, an animal facility dedicated to the study of biological rhythms. Animals were housed in an environment controlled room in separate cages according to legislations provided in European Commission directive 2010/63/EU. They were provided with ad libitum access to food (Safe®) and water throughout the study period. The animals were kept under a long photoperiod (14L:10D) and at 22°C (LP22) until the start of the experiment. To initiate the preparation for hibernation (pre-hibernal period) the animals were transferred to a short photoperiod (10L:14D) at 22°C (SP22). After 8 weeks, the temperature was lowered to 10°C (SP10), all animals exhibited torpor-arousal cycling, and were kept in this condition to the end of the experiment (10 weeks, Figure 1A). The experimental procedure was validated by a local ethical committee and further validated by the Ministry of Higher Education, Research and Innovation (APAFIS#21424-2019070219421923 v3).

Calibration of IPTT tags

The accuracy of each IPTT tag (BMDS IPTT-300®) was verified from manufacturer to be ±1°C of actual temperature in the range between 21 and 30°C. During torpor-arousal the tags would measure between 10-38°C therefore to ensure accuracy between the iButtons and IPTT tags we created an individual calibration curve for each IPTT tag allowing for post hoc correction at a 0.1°C resolution. A similar method was used by Wacker et al [25]. Each tag was calibrated using a water bath containing two iButtons (thermochron DS1922L, Maxim integrated) set to 16-bit resolution (0.0625°C) and sampling rate of 2 seconds, the IPTT tag was scanned every 2-3 seconds. The water bath was then placed on a heating plate with a magnetic stirrer and heated, the temperature range recorded was 5°C to 39°C. The resulting data was plotted in Graphpad Prism v8.0 and a regression analysis was done to allow for calibration of the IPTT tag recordings.

iButton and IPTT tag surgery
Surgeries were performed on each animal to implant iButtons (thermochron DS1922L, Maxim integrated) and IPTT tags. Each animal was anesthetized by 3% isoflurane, surgery was performed under 3% isoflurane and 95% oxygen. The IPTT-tag was implanted subcutaneously into the classical brown adipose tissue (BAT) depot, using a standard IPTT-tag injector (supplied by the manufacturer). Post-mortem verification ensured the tag was in contact with the BAT. The iButton was implanted in the abdominal cavity with the use of laparotomy. Local anaesthetic agents (lidocaine and bupivacaine) was injected intraperitoneally (2.5 mg/kg) before the incision was made. Analgesic agent (Metacam®, injectable, 2mg/kg) was dorsally injected subcutaneously. In addition, analgesic agent was dissolved in drinking water and provided for 3 days post-surgery (Metacam® drinkable 1.5 mg/ml, 1 mg/kg). The animals were extensively monitored after surgery and allowed to recover for two weeks.

**Behaviour and temperature monitoring**

To keep track of individual torpor-arousal patterns, behavioural recordings were made twice per day; 1 to 2 hours after lights on and 9-10 hours after lights on. We defined torpid as a ventilation frequency (VF) < 10 per minute, curled up position in hibernacula, immobile, unresponsive and an IPTT reading <13.5°C. Signs of arousal were; increased IPTT tag temperature and increased ventilation frequency. We identified animals beginning to arouse and monitored IPTT tag temperatures every two minutes, ventilation frequency was counted in regular intervals for one minute. iButton core temperature recordings were collected post-mortem and time matched with the calibrated IPTT measurements. Graphpad Prism v8.0 was used for statistical analysis and data plotting.
References

1. Fishman AP, Lyman CP. Hibernation in Mammals. Circulation. 1961;24:434–45. doi:10.1161/01.CIR.24.2.434.

2. Snapp BD, Heller HC. Suppression of Metabolism during Hibernation in Ground Squirrels (Citellus lateralis). Physiol Zool. 1981;54:297–307. doi:10.1086/physzool.54.3.30159944.

3. Zhang F, Hao G, Shao M, Nham K, An Y, Wang Q, et al. An Adipose Tissue Atlas: An Image-Guided Identification of Human-like BAT and Beige Depots in Rodents. Cell Metab. 2018;27:252-262.e3. doi:10.1016/j.cmet.2017.12.004.

4. Smith RE, Hock RJ. Brown Fat: Thermogenic Effector of Arousal in Hibernators. Science (80- ). 1963;140:199–200. doi:10.1126/science.140.3563.199.

5. Cannon B, Nedergaard J. Brown Adipose Tissue: Function and Physiological Significance. Physiol Rev. 2004;84:277–359. doi:10.1152/physrev.00015.2003.

6. Nedergaard J, Cannon B, Lindberg O. Microcalorimetry of isolated mammalian cells. Nature. 1977;267:518–20. doi:10.1038/267518a0.

7. Hayward JS, Ball EG. Quantitative aspects of brown adipose tissue thermogenesis during arousal from hibernation. Biol Bull. 1966;131:94–103. doi:10.2307/1539650.

8. Horwitz B, Smith R, Pengelley E. Estimated heat contribution of brown fat in arousing ground squirrels (Citellus lateralis). Am J Physiol Content. 1968;214:115–21. doi:10.1152/ajplegacy.1968.214.1.115.

9. Smalley RL, Dryer RL. Brown Fat: Thermogenic Effect during Arousal from Hibernation in the Bat. Science (80- ). 1963;140:1333–4. doi:10.1126/science.140.3573.1333.

10. Rauch JC. Sequential changes in regional distribution of blood in Eptesicus fuscus (big brown bat) during arousal from hibernation. Can J Zool. 1973;51:973–81. doi:10.1139/z73-141.
11. Morrison SF, Madden CJ, Tupone D. Central Neural Regulation of Brown Adipose Tissue Thermogenesis and Energy Expenditure. Cell Metab. 2014;19:741–56. doi:10.1016/j.cmet.2014.02.007.

12. Staples JF. Metabolic suppression in mammalian hibernation: the role of mitochondria. J Exp Biol. 2014;217:2032–6. doi:10.1242/jeb.092973.

13. Landau BR, Dawe AR. Respiration in the Hibernation of the 13-Lined Ground Squirrel. Am J Physiol Content. 1958;194:75–82. doi:10.1152/ajplegacy.1958.194.1.75.

14. Hayward JS, Lyman CP. Mammalian hibernation III. 3rd edition. New York: Elsevier; 1967.

15. Fenyk-Melody J. The European Hamster. In: The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents. Elsevier; 2012. p. 923–33. doi:10.1016/B978-0-12-380920-9.00036-5.

16. Canguilhem B, Vaultier J-P, Pevet P, Coumaros G, Masson-Pevet M, Bentz I. Photoperiodic regulation of body mass, food intake, hibernation, and reproduction in intact and castrated male European hamsters, Cricetus cricetus. J Comp Physiol A. 1988;163:549–57. doi:10.1007/BF00604908.

17. Gautier C, Bothorel B, Ciocca D, Valour D, Gaudeau A, Dupré C, et al. Gene expression profiling during hibernation in the European hamster. Sci Rep. 2018;8:13167. doi:10.1038/s41598-018-31506-2.

18. Geiser F, Baudinette R V. The relationship between body mass and rate of rewarming from hibernation and daily torpor in mammals. J Exp Biol. 1990;151:349–59. http://www.ncbi.nlm.nih.gov/pubmed/2380659.

19. Umbrello M, Dyson A, Feelisch M, Singer M. The Key Role of Nitric Oxide in Hypoxia: Hypoxic Vasodilation and Energy Supply–Demand Matching. Antioxid Redox Signal. 2013;19:1690–710. doi:10.1089/ars.2012.4979.

20. Miller JFAP. The golden anniversary of the thymus. Nat Rev Immunol. 2011;11:489–95.
21. Ghadially FN, Illman O. Naturally occurring thymomas in the European hamster. J Pathol Bacteriol. 1965;90:465–9. doi:10.1002/path.1700900214.

22. Brandes K, Fend F, Monecke S, Teifke JP, Breuer W, Hermanns W. Comparative Morphologic and Immunohistochemical Investigation of Spontaneously Occurring Thymomas in a Colony of European Hamsters. Vet Pathol. 2004;41:346–52. doi:10.1354/vp.41-4-346.

23. Johns RH, Reinhardt AK. Association between thymoma and persistent hypothermia: a case report. J Med Case Rep. 2009;3:73. doi:10.1186/1752-1947-3-73.

24. Ho WKW, Wilson JD. Hypothermia, hyperhidrosis, myokymia and increased urinary excretion of catecholamines associated with a thymoma. Med J Aust. 1993;158:787–8. doi:10.5694/j.1326-5377.1993.tb121967.x.

25. Wacker CB, Daniella Rojas A, Geiser F. The use of small subcutaneous transponders for quantifying thermal biology and torpor in small mammals. J Therm Biol. 2012;37:250–4. doi:10.1016/j.jtherbio.2011.11.007.
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Author contributions

FM - Experimental design, analysed the data, collected samples, prepared figures, and revised manuscript. VJM – Experimental design, analysed the data, collected samples, prepared manuscript. BB – performed surgeries, technical expertise, revised the manuscript. DGH - conceived and designed the experiments, supervised, and revised the manuscript. VS - conceived and designed the experiments, supervised, provided funding and revised the manuscript. SHW – conceived and designed the experiments, supervised, provided funding and prepared the manuscript.
Figure 1: European hamster displays hibernation after exposure to short photoperiod and low ambient temperature. A.) Study design to induce the hibernation phenotype. Animals were transferred from long photoperiod (14 hours of light per day, 14:10) and 22°C to short photoperiod (10 hours of light per day, 10:14) for 8 weeks. The ambient temperature (Ta) was lowered to 10°C, a temperature of 9.6 ± 1°C was observed. B.) Core body temperature (Tb) measurement from a European hamster displaying torpor-arousal cycling. The grey dotted line indicates the average ambient temperature (Ta) over the experiment.

Figure 2: Brown adipose tissue re-warming precedes core body temperature increases. A) Core body temperature (Tb – iButton, diamond symbol) and BAT temperature recordings (T_BAT - IPTT tag, circle symbol) from 5 individuals during an arousal from torpor (each individual represented by a different colour). The mean ventilation frequency (VF) at each quartile of arousal, as defined by a non-linear curve fit for all individuals, is indicated by black arrows. Mean maximum re-warming rate (RWR_max) for core body temperature (Tb) = 21.0°C (confidence intervals: 15.4 – 26.5) & brown adipose tissue (T_BAT) = 20.9°C (CI: 20.3 – 21.5). B) BAT temperature recording corresponding to onset of BAT adjacent skeletal muscle shivering. Observations and recordings were done in 8 separate arousal events in 5 different animals. C) A representative individual showing core body temperature (Tb), BAT temperature (T_BAT) and ventilation frequency (VF) changes during arousal from torpor. Grey dotted line shows room temperature (Ta). Inset graph shows the linear portion of the arousal that was used to calculate maximum re-warming rate (RWR_max) in °C per hour.

Figure 3: Rewarming from torpor is compromised in an animal with a thymus tumour. A.) Post-mortem image of exposed thoracic cavity of a European hamster with a thymus tumour (indicated by the white arrow). For reference, white * is placed on lungs and ^ on heart. B.) Heat-map of core body temperature recorded by iButtons over the course of the experiment. The percentage time spent in above 35.5°C (Euthermic – aroused) and below 12°C (Torpid) is indicated, the remainder is time spent
between 35 to 12 °C (entering or arousing from torpor). C.) Core body temperature (Tb), BAT temperature (TBAT) and ventilation frequency (VF) changes during arousal from torpor in an animal with a thymus tumour. Grey dotted line shows room temperature (Ta). Inset graph shows the linear portion of the arousal that was used to calculate maximum re-warming rate (RWRmax) in °C per hour.
A. LP 14:10
Ta 22 °C

SP 10:14
Ta 22 °C

SP 10:14
Ta 10 °C

Pre-hibernal period
8 weeks

Hibernation period

B.

\[ T_b(°C) \]

0 5 10 15 20 25 30 35 40

0 1 2 3 4 5 6 7 8

Weeks at 10 °C

Fig. 1
A. \[ T_{\text{BAT}} \text{(IPPT)} \quad \bullet \]
\[ T_b \text{(iButton)} \quad \diamond \]
\[ V_F \quad \downarrow \]

\[ RWR_{\text{MAX}} T_{\text{BAT}} = 20.9 \text{ C}^\circ \text{h}^{-1} \]
\[ RWR_{\text{MAX}} T_b = 21.0 \text{ C}^\circ \text{h}^{-1} \]

B. 

C.

Fig. 2
