Heterothermy is associated with reduced fitness in wild rabbits

Shane K. Maloney, Maija K. Marsh, Steven R. McLeod, Andrea Fuller

Supplementary Files S1: Material and methods

*Temperature loggers*

The miniature temperature data loggers (MLog-T1; Sigma Delta Technologies, Perth, Australia) had a storage capacity of 2 Mb, a measurement range from 0 to 60°C, a resolution of 0.06°C, and each was powered by a 3V CR1632 Lithium coin cell battery. The loggers were launched, covered with self-amalgamating butyl rubber tape, and coated in multiple layers of inert wax (EXP987, Sasol, South Africa). The resulting waterproofed loggers weighed approximately 10 g. Each logger was calibrated against a certified mercury-in-glass thermometer (Wika Australia, Rydalmere, Australia, a National Association of Testing Authorities certified testing facility) in an insulated water bath, to an accuracy of 0.05°C at 2°C intervals between 34 and 42°C, both before implantation into an animal and again at retrieval. For every logger, a linear calibration equation (y = mx + b) provided very good fit to the data obtained from the calibration before implantation (r2 > 0.98 in each case), and so the data from each animal were adjusted according to its specific linear calibration. Many of the loggers had stopped recording at retrieval, but several that were still logging after the year of field data recording revealed a small calibration drift of 0.12°C on average. A correction of 0.12°C drift per year was thus routinely applied to all the time series datasets.

*Trapping, field surgery and monitoring*

The trapping of rabbits (*Oryctolagus cuniculus*, Linnaeus, 1758) for the implantation of temperature loggers was conducted over several nights in early December. The rabbits were captured in wire cages baited with diced carrot. Two-hundred traps were placed at least 5 m apart near several active warrens at the study site. Pre-baiting was supplied prior to trap opening. The traps were set at dusk and checked the next morning shortly after dawn. All of the captured rabbits were sexed, weighed, and inspected for the presence of fleas, abnormalities, and clinical signs of myxomatosis. All of the animals utilized for experimentation were free of fleas and myxomatosis. A pair of metal ear tags containing a unique identification code was attached, one to each ear, of all newly captured rabbits.

Surgery was conducted with the rabbits under general anaesthesia [1]. For induction, a subcutaneous injection of 0.25 mg/kg medetomidine (Domitor; Pfizer Animal Health, West Ryde, Australia) was given into the scruff, and an intramuscular injection of 5 mg/kg alfaxalone (Alfaxan-CD RTU; Jurox, Rutherford, Australia) was given into lumbar muscle. Each rabbit was placed in dorsal recumbency, the abdominal skin was clipped of fur, and the skin was sterilised. Anaesthesia was maintained using 1.5 to 3% isoflurane (Attane; Bayer Australia Ltd. Pymble, Australia) in oxygen administered via a facemask. Toe pinch and palpebral reflexes were assessed regularly and the dose of isoflurane was adjusted to maintain a surgical depth of anesthesia. Heart rate and hemoglobin saturation were monitored continuously via a pulse oximeter applied to the ear, and ventilation rate was measured by counting chest movements.

Access to the peritoneal cavity was obtained via midline incision of approximately 2 cm. After insertion of the temperature logger, the peritoneum and muscle layers were sutured with 5/0 vicryl (Ethicon; Johnson & Johnson, North Ryde, Australia) and the skin was closed using 4/0 vicryl. The medetomidine was reversed with a subcutaneous or intramuscular injection of 5 mg/kg atipamezole (Antisedan; Novartis Pty Ltd, Macquarie Park, Australia). Long-acting penicillin (0.25 ml) (Benacillin; Troy Laboratories, Glendenning, Australia) and the long-acting anti-inflammatory carprofen (2.5 mg/kg) (Rimadyl; Pfizer Animal Health, West Ryde, Australia) were injected subcutaneously. The rabbits were then fitted with radio collars (Sirtrack E2C) and placed in cages in a quiet location for recovery. They regained consciousness and maintained an upright stance after an average of 28 ± 16 minutes [1]. The rabbits were then released at the site of their capture and all hopped immediately to a burrow. Retrieval of other loggers occurred 12 months after surgery, when the animals were opportunistically trapped and killed with an overdose of sodium pentobarbitone (60 mg/kg; Lethabarb, Virbac, Peakhurst, NSW).

*Temperature logger data analysis*

Body temperature data were obtained from 23 rabbits. Body temperatures were obtained for between 257 and 410 days, with an average of 319 days. Many of the loggers began to fail (due to low battery power) in October, and so data were analysed until the end of September, up to when data were obtained from 21 rabbits. With Lithium batteries and a voltage regulator in the measurement circuit, there is no change in calibration as the battery drains, it just stops working, and so we are confident that the obtained data are reliable. At the time of surgery those 21 rabbits had a body mass of 1.60 ± 0.11 (SD) kg, not different from the average body mass of the 44 rabbits that were initially implanted.

Pregnancy was defined from the pattern of decrease in body temperature that occurs from about mid-gestation and returns to normal at birth [2-5]. While the patterns on the temperature records accorded with previous observations, we also tested the assessment of temperature records against physical observations of the reproductive status of the rabbits when they were trapped opportunistically throughout the year. At each trapping period, each individual rabbit was identified by its ear tag, and a judgment of pregnancy status was made by palpation of the abdomen, with an outcome recorded as “pregnant” or “not-pregnant”. A different member of the research team marked a plot of the temperature record of each individual rabbit to demarcate periods of gestation, with shading in the second half of gestation to indicate the period of lower body temperature (similar to the open boxes on Figure 1). The dates of trapping of each individual rabbit were then marked onto the temperature traces. If the date was inside the shaded area of one of the boxes the temperature record was scored as “pregnant”. If the date was outside of a shaded area, then the temperature record was scored as “not-pregnant”. There were 20 occasions where an individual was scored as pregnant from both trapping observations and temperature record, 35 occasions where an individual was scored as not-pregnant from both trapping observations and temperature record, five occasions where an individual was scored as pregnant from the trapping observations but not-pregnant from the temperature record, and one occasion where an individual was scored as not-pregnant from the trapping observations but pregnant on the temperature record. A Chi-square contingency analysis revealed that the likelihood of such a distribution being due to chance was very low (χ2 = 38.97, P < 0.00001) and we therefore conclude that the same pattern of body temperature decrease during gestation occurs in rabbits as has been reported in other mammals.

Based on the temperature records, none of the rabbits was pregnant in February, four fell pregnant in March, and fifteen more in April. The number of pregnancies was totaled for each individual between March and September. The heterothermy status of each individual was defined as the amplitude of the daily body temperature rhythm as determined by fitting a cosinor function [6] to the data for the 28 days of core body temperature in February (before any of the animals were pregnant). The mesor and 24h minimum of the body temperature rhythm were obtained from the same analysis, and the daily minimum was calculated as the mesor minus the amplitude.

The number of pregnancies observed in each individual rabbit was regressed on each of the parameters from the cosinor model of the core body temperature data in February (mesor, amplitude, and daily minimum). Results are presented as the F-value of the regression analysis, P-value, and r2.

The weather during the experiments was unremarkable, with maximum temperatures in February of 34°C and minimum temperatures in July of -5°C, with rainfall of 508 mm.

**References**

[1] Marsh, M.K., McLeod, S.R., Hansen, A. & Maloney, S.K. 2009 Induction of anaesthesia in wild rabbits using a new alfaxalone formulation. *Vet. Rec.* **164**, 122-123.

[2] Fewell, J.E. 1995 Body-temperature regulation in rats near term of pregnancy. *Can. J. Physiol. Pharmacol.* **73**, 364-368.

[3] Eliason, H.L. & Fewell, J.E. 1997 Thermoregulatory control during pregnancy and lactation in rats. *J. Appl. Physiol.* **83**, 837-844.

[4] Naccarato, E.F. & Hunter, W.S. 1983 Brain and deep abdominal temperatures during induced fever in pregnant rabbits. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **245**, R421-R425.

[5] Williams, C.T., Sheriff, M.J., Schmutz, J.A., Kohl, F., Tøien, Ø., Buck, C.L. & Barnes, B.M. 2011 Data logging of body temperatures provides precise information on phenology of reproductive events in a free-living arctic hibernator. *J. Comp. Physiol. B* **181**, 1101-1109.

[6] Nelson, W., Liang Tong, Y., Lee, J.K. & Halberg, F. 1979 Methods for cosinor-rhythmometry. *Chronobiologia* **6**, 305-323.