

## RESEARCH ARTICLE

# Regulation of brain temperature in winter-acclimatized reindeer under heat stress

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### SUMMARY

Reindeer (*Rangifer tarandus*) are protected against the Arctic winter cold by thick fur of prime insulating capacity and hence have few avenues of heat loss during work. We have investigated how these animals regulate brain temperature under heavy heat loads. Animals were instrumented for measurements of blood flow, tissue temperatures and respiratory frequency ( $f$ ) under full anaesthesia, whereas measurements were also made in fully conscious animals while in a climatic chamber or running on a treadmill. At rest, brain temperature ( $T_{\text{brain}}$ ) rose from  $38.5 \pm 0.1^\circ\text{C}$  at  $10^\circ\text{C}$  to  $39.5 \pm 0.2^\circ\text{C}$  at  $50^\circ\text{C}$ , while  $f$  increased from  $\sim 7$  to  $\sim 250$  breaths  $\text{min}^{-1}$ , with a change to open-mouth panting (OMP) at  $T_{\text{brain}} 39.0 \pm 0.1^\circ\text{C}$ , and carotid and sublingual arterial flows increased by 160% and 500%, respectively. OMP caused jugular venous and carotid arterial temperatures to drop, presumably owing to a much increased respiratory evaporative heat loss. Angular oculi vein (AOV) flow was negligible until  $T_{\text{brain}}$  reached  $38.9 \pm 0.1^\circ\text{C}$ , but it increased to  $0.81 \text{ ml min}^{-1} \text{ kg}^{-1}$  at  $T_{\text{brain}} 39.2 \pm 0.2^\circ\text{C}$ . Bilateral occlusion of both AOVs induced OMP and a rise in  $T_{\text{brain}}$  and  $f$  at  $T_{\text{brain}} > 38.8^\circ\text{C}$ . We propose that reindeer regulate body and, particularly, brain temperature under heavy heat loads by a combination of panting, at first through the nose, but later, when the heat load and the minute volume requirements increase due to exercise, primarily through the mouth and that they eventually resort to selective brain cooling.

Key words: *Rangifer*, brain temperature, thermoregulation, selective brain cooling, panting

### INTRODUCTION

When ungulates are under heat stress, it has been assumed that cold venous outflow from the nasal mucosa is drained either directly to the heart for general body cooling or by way of the angular oculi vein (AOV) to a cavernous venous sinus located just below the brain (Figs 1 and 2). Here, heat is exchanged with the arterial blood in a rete on its way to the brain for selective brain cooling (SBC) (Baker and Hayward, 1968). The reindeer (*Rangifer tarandus*) is an Arctic animal that has adapted to annual changes of  $80^\circ\text{C}$  in ambient temperature by growing a fur of excellent insulation value in the autumn to be shed in the following spring. That together with a reduction of surface temperature caused by vascular changes (Johnsen et al., 1985b) and an efficient nasal heat exchange mechanism (Blix and Johnsen, 1983) result in a  $30^\circ\text{C}$  reduction in lower critical temperature from summer to winter (Nilssen et al., 1984a). The animal, so equipped to withstand cold, consequently has few avenues of heat loss in winter and runs the risk of hyperthermia during exercise when metabolic heat production rises rapidly with running speed (Nilssen et al., 1984b) and softness of the surface over which the animal travels (Fancy and White, 1985). We have observed that moderately heat-stressed reindeer pant, first with the mouth closed, but, under severe heat stress, they resort to open-mouth panting (OMP) to dissipate heat from their big and richly vascularized tongue. In a previous study, we (Aas-Hansen et al., 2000) found, however, that the flow of air through the nose is reduced from 100 to only 2% of the respiratory minute volume when the animal resorts to OMP. That would seem to imply that SBC might be compromised when it is most needed.

In the present study, we have recorded a host of relevant parameters in winter-insulated reindeer standing in a climatic

chamber at an ambient temperature ( $T_a$ ) ranging from 0 to  $50^\circ\text{C}$  and while running on a treadmill at a  $T_a$  of 10 or  $25^\circ\text{C}$  in an attempt to understand how brain temperature is regulated in heat-stressed reindeer.

### MATERIALS AND METHODS

#### Animals

A total of nine adult female reindeer [*Rangifer tarandus tarandus* (Linnaeus)], with body masses ranging between 65 and 80 kg, were used in these experiments, which were approved by the National Animal Research Authority of Norway. Except during experiments, the animals were kept in outdoor pens under natural light and temperature conditions at the Department of Arctic Biology, University of Tromsø, Tromsø ( $70^\circ\text{N}$ ), located in the midst of the natural reindeer range of Norway. The animals were offered artificial reindeer feed (FK Reinför, Felleskjøpet, Trondheim, Norway), mineral lick-stones and water or snow *ad libitum*. After proper training of the animals, experiments were performed in the period between November and February when  $T_a$  in Tromsø averages  $-3.3^\circ\text{C}$ , with occasional drops to  $-17^\circ\text{C}$  and occasional rises to  $8^\circ\text{C}$ , whereas, in nearby inland localities, it can vary between 5 and  $-40^\circ\text{C}$ . At that time, fur insulation is at its prime, with an insulation value of approximately  $1.0 \text{ m}^2 \text{ }^\circ\text{C W}^{-1}$  (Scholander et al., 1950).

#### Anesthesia and surgery

The following interventions were made under sterile surgical conditions using general isoflurane (Forene, Abbott Scandinavia AB, Solna, Sweden) anaesthesia, after premedication with Rompun Vet. ( $1 \text{ mg kg}^{-1}$ , i.m.; Bayer, Leverkusen, Germany) and Propofol

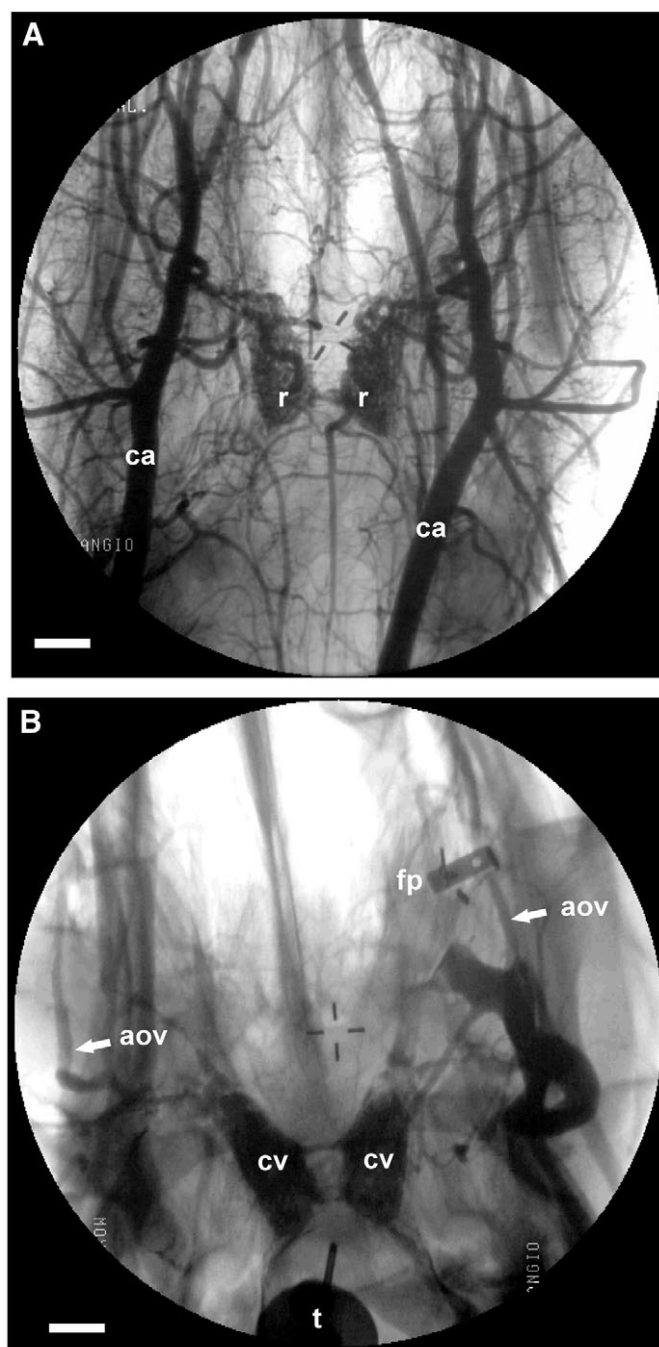


Fig. 1. Angiograms (dorsoventral view) of the head of a reindeer showing (A) carotid arteries (ca) and the carotid *retia* (r) and (B) the venous drainage, including the cavernous venous sinuses (cv) and the angular oculi veins (aov) involved in selective brain cooling (SBC). The ultrasonic blood flow probe (fp), as well as the base plate and the re-entrant guide tube for measurements of brain temperature (t) are also shown. Scale bars, 10 mm.

(2 mg kg<sup>-1</sup>, i.v.; Fresenius Kabi Norge A/S, Halden, Norway), followed by intubation: a blind-ended re-entrant stainless steel guide-tube (length 30 mm, diameter 1.5 mm) was implanted into the left cerebral hemisphere, 10 mm laterally to the midline, near the third ventricle. The upper end of the guide-tube had a stainless steel base-plate at a right-angle that was secured to the skull with a bone screw. The procedure was performed in five animals. The left common

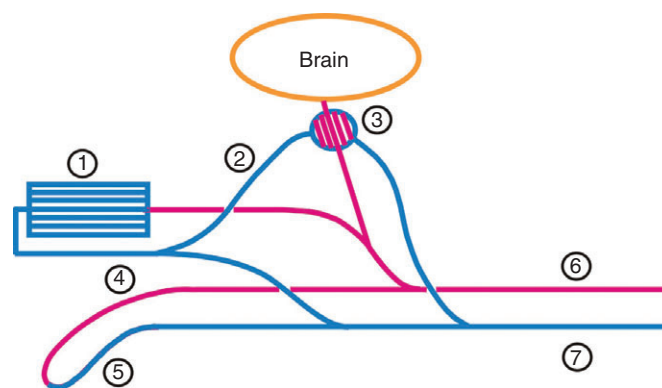


Fig. 2. Schematic drawing of the arterial (red) and venous (blue) vasculature of the head and neck involved in temperature regulation in the reindeer. Key: (1) venous rete in the nasal mucosa; (2) angular oculi vein; (3) carotid arterial rete in the cavernous venous sinus (see Fig. 1); (4) sublingual artery; (5) vessels of the tongue; (6) carotid artery; and (7) jugular vein.

carotid artery was surgically exposed by a 5 cm incision and a (16G/1.70×130 mm) BD Secalon-T catheter (Becton Dickinson Critical Care Systems, Singapore), equipped with a lock, inserted into the artery through a puncture made by the internal needle, which was then withdrawn and the catheter further advanced in the rostral direction until the tip was located 20 mm caudal to the angle of the lower jaw. The catheter was then secured in place with a purse-string suture in the wall of the vessel, around the catheter, using absorbable Biosyn (3-0) suture (Covidien, Mansfield, MA, USA). Likewise, another catheter was inserted into the exposed left jugular vein and advanced caudally until its tip was located 150 mm caudal to the angle of the lower jaw, without the use of a purse-string suture. Both catheters were then secured to the skin, and the wound was closed with silk (0) sutures. The catheters, which were filled with heparin (5000 IU ml<sup>-1</sup>; LEO Pharma A/S, Ballerup, Denmark) between experiments, were kept open and functional for a period of 2–3 weeks. The procedure was performed in three animals. Ultrasonic Butterfly flow-probes (Medi-Stim AS, Oslo, Norway) of appropriate size were chronically implanted around the left lingual artery in three animals, around the left carotid artery in three animals and around the right AOV for measurements of blood flow in one animal. Small Teflon arches that eventually protruded through the skin were placed over the AOVs and secured to the skull on both sides with bone screws. By using these arches, the AOVs could be occluded by inserting a small wedge in the gap between the arch and the skin over the vessel in three animals. Relevant anatomical features and the location of implanted devices were verified by X-ray (Series 9600; OED medical systems) analysis and ultimately by autopsy. Thus, all the animals were not subjected to all of these interventions, but each animal was subjected to a combination of some of them, as later specified.

#### Preparation for experiments and recordings

In preparation for an experiment, thin (0.2 mm) copper-constantan thermocouples were introduced into the brain and the blood vessels (carotid artery and jugular vein) through the guide tubes described above, and in addition a thermocouple probe was inserted 200 mm into the rectum for recording of rectal temperature ( $T_{\text{rec}}$ ). The thermocouples were connected through a thermocouple amplifier with internal temperature reference (AD 595 CD; Analog Devices,

Norwood, MA, USA) to A/D converters and data acquisition systems (Lab-Acq Pro and Insta-Trend Pro, Dianachart, Oak Ridge, NJ, USA; or Powerlab 4/25, AD Instruments, Chalgrove, UK). All thermocouples were calibrated (accuracy  $\pm 0.05^\circ\text{C}$ ) in a calibration bath (model 6025, Hart Scientific, Pleasant Grove, UT, USA) and a  $0^\circ\text{C}$  ice-point dry-well reference chamber (model 5115, Hart Scientific), and the recorded temperatures were linearized according to the method of Tøien (Tøien, 1992). Blood flow was measured by use of a Cardio Med CM 4008 Physiological Trace System (Medi-Stim AS, Oslo, Norway) and recorded on a PC using the Powerlab data acquisition system as described for the temperature records. The flow probes were calibrated *in situ* post mortem by use of a peristaltic pump and a measuring cylinder. Respiratory frequency ( $f$ ) was recorded by inserting a silicone tube (outer diameter 4 mm, inner diameter 2 mm) 25 mm into one of the nostrils of the animal. The air-filled tube was secured to the nose by use of tape and connected to a pressure transducer (Transpac IV, Abbot Ireland, Sligo, Ireland). The transducer, in turn, was connected via a Gould Universal amplifier to a Gould thermal array recorder (TA 4000, Gould Electronics, Cleveland, OH, USA). The recorded pressure differences made it possible also to determine when the animal employed open- or closed-mouth respiration.

#### Experimental protocol

During experiments, the animal was placed either inside a  $25\text{ m}^3$  climatic chamber (type 24/50 DU; Weiss Technik, Giessen, Germany), at ambient temperatures ( $T_a$ ) ranging from  $0$  to  $+50^\circ\text{C}$ , or on a treadmill (length 3.5 m, width 1.0 m) with a slope of  $0$  or  $10^\circ$  located in a separate room that was kept at a  $T_a$  of  $10$  or  $25^\circ\text{C}$ , where in both cases the temperature was controlled within  $\pm 1^\circ\text{C}$  in time and  $\pm 2^\circ\text{C}$  in space. The treadmill was driven by a variable-powered motor providing constant speed, independent of load at a very low [ $\sim 60\text{ dB (A)}$ ] noise level. Humidity could not be controlled during the experiments, and the measured relative humidity varied with ambient temperature between 35 and 60%. The animals were instrumented and kept in the climatic chamber for at least 1 h, or until the recorded variables had stabilized, before any recordings commenced. They were accustomed to run for at least 20 min on the treadmill at a speed of  $9\text{ km h}^{-1}$  over a period of several weeks (and seemed to enjoy it).

#### Statistics

Data on AOV flow were only obtained from one animal, but this animal also contributed data on  $f$ ,  $T_{\text{brain}}$  and carotid blood flow, that were cross-referenced with those obtained from the other animals in order to ascertain that its thermoregulatory responses were similar to those of the other reindeer. This was done by fitting regression lines (exponential for  $f$  and  $T_{\text{brain}}$ , linear for carotid blood flow) to the larger data sets plotted against  $T_a$  and checking whether the data for the AOV-flow animal were within the 95% confidence intervals. The analyses were made using Sigmaplot for Windows (version 11.0, Systat Software, Chicago, IL, USA). Results are otherwise presented as means  $\pm$  s.d. if  $N > 2$ , or  $N = 1$  and  $n > 2$ ; or means  $\pm$  ranges if  $N = 2$  ( $N$ =number of animals;  $n$ =number of experiments).

#### RESULTS

We found that  $T_{\text{brain}}$  in resting reindeer was stable at  $38.6 \pm 0.2^\circ\text{C}$  ( $N = 5$ ;  $n = 11$ ) at a thermoneutral  $T_a$  of  $10^\circ\text{C}$  (Nilssen et al., 1984a) up to a  $T_a$  of  $30^\circ\text{C}$ , where after it rose linearly with increasing heat loads to  $39.0 \pm 0.1^\circ\text{C}$  ( $N = 5$ ;  $n = 7$ ), which was the threshold for OMP, at a  $T_a$  of  $35$ – $40^\circ\text{C}$  (Fig. 3A). In that situation,  $f$  increased from  $7$  to  $\sim 250\text{ breaths min}^{-1}$ , in some cases reaching  $300\text{ breaths min}^{-1}$  (Fig. 3B).

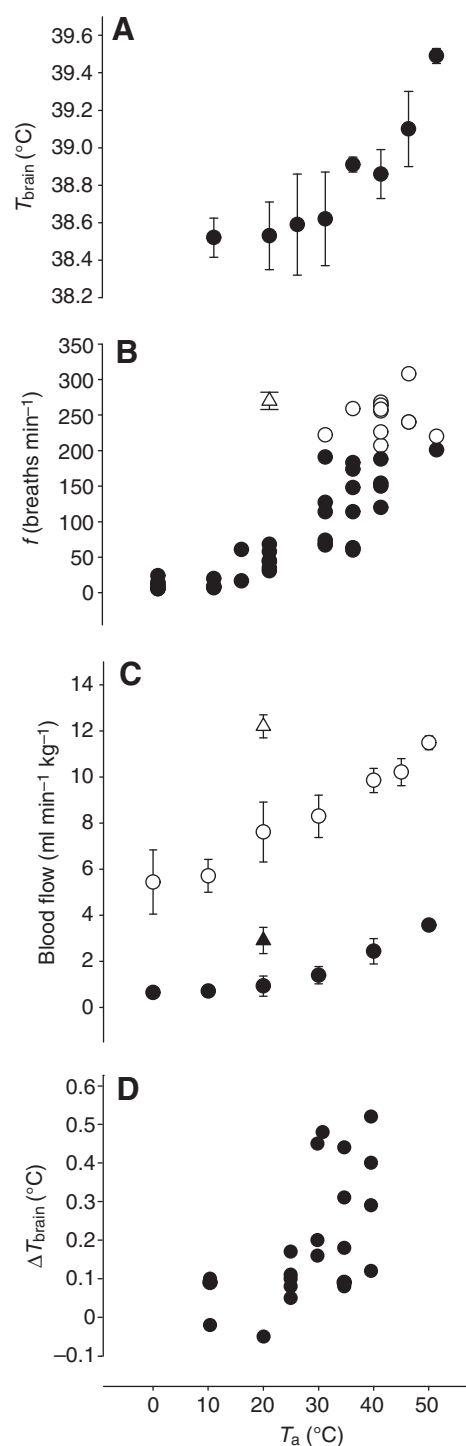


Fig. 3. Changes in (A) brain temperature in five animals; (B) respiratory (breathing) frequency (filled circles, closed mouth at rest; open circles, open mouth at rest; open triangles, open mouth during exercise) in three animals; (C) left carotid (open symbols) and lingual (filled symbols) arterial blood flow during rest (circles) and exercise on a treadmill (triangles) at  $9\text{ km h}^{-1}$  in three animals; and (D) differences in brain temperature after bilateral occlusion of the angular oculi veins in three animals, at rest at different ambient temperatures. Data are means  $\pm$  s.d.

Left carotid arterial blood flow also increased linearly from  $5.5 \pm 1.4\text{ ml min}^{-1}\text{ kg}^{-1}$  at  $T_a 0^\circ\text{C}$  to  $11.5 \pm 0.3\text{ ml min}^{-1}\text{ kg}^{-1}$  at  $T_a 50^\circ\text{C}$  ( $N = 3$ ), while a maximum value of  $12.2 \pm 0.5\text{ ml min}^{-1}\text{ kg}^{-1}$  was



reached within less than 2 min during trotting at  $9 \text{ km h}^{-1}$  ( $T_a=20^\circ\text{C}$ ) on the level treadmill ( $N=1$ ;  $n=6$ ) (Fig. 3C). The left sublingual arterial flow to the tongue increased from  $0.6 \pm 0.2 \text{ ml min}^{-1} \text{ kg}^{-1}$  at  $T_a 0^\circ\text{C}$  to  $0.9 \pm 0.4 \text{ ml min}^{-1} \text{ kg}^{-1}$  at  $T_a 20^\circ\text{C}$  ( $N=2$ ), where after it rose linearly with increasing  $T_a$  to a maximum of  $3.6 \text{ ml min}^{-1} \text{ kg}^{-1}$  ( $N=1$ ) at  $T_a 50^\circ\text{C}$ , which is comparable to the value ( $2.9 \pm 0.6 \text{ ml min}^{-1} \text{ kg}^{-1}$ ) that was reached within less than 2 min during trotting at  $9 \text{ km h}^{-1}$  ( $T_a=20^\circ\text{C}$ ) on the level treadmill ( $N=1$ ;  $n=6$ ) (Fig. 3C).

Right AOV flow was negligible until  $T_{\text{brain}}$  reached  $38.9 \pm 0.1^\circ\text{C}$  ( $N=1$ ;  $n=5$ ) at a  $T_a$  of  $40^\circ\text{C}$ , but it rose rapidly after that to  $0.81 \text{ ml min}^{-1} \text{ kg}^{-1}$  when  $T_{\text{brain}}$  had reached  $39.2 \pm 0.2^\circ\text{C}$  ( $N=1$ ;  $n=5$ ) (Fig. 4), the rapid increase coinciding with a change to OMP.

As these results were only obtained from one animal, we tested whether this animal provided typical thermoregulatory responses by cross-referencing data on  $f$ ,  $T_{\text{brain}}$  and carotid flow with those from the other animals. The analysis revealed that all data points for the AOV-flow animal fell within the 95% confidence intervals that were obtained after nonlinear regression analysis of data on  $f$  versus  $T_a$  ( $f=24.4e^{0.05T_a}$ ;  $R^2=0.69$ ,  $n=42$ ) and  $T_{\text{brain}}$  versus  $T_a$  ( $T_{\text{brain}}=38.3e^{0.0004T_a}$ ;  $R^2=0.45$ ,  $n=16$ ) from the other reindeer. Carotid blood flow was higher in the AOV-flow animal than in the others and did not fall within the 95% confidence intervals obtained after linear regression of carotid blood flow versus  $T_a$ , ( $\text{flow}=4.0+0.13T_a$ ;  $R^2=0.88$ ,  $n=24$ ), but it fell within the 95% prediction intervals throughout the  $T_a$  range and showed the same qualitative change (increase with increasing  $T_a$ ).

Moreover, bilateral occlusion of both AOVs had only negligible effect on  $T_{\text{brain}}$  until  $T_{\text{brain}}$  reached  $38.8 \pm 0.2^\circ\text{C}$  ( $N=3$ ;  $n=6$ ) at a  $T_a$  of  $30$ – $35^\circ\text{C}$ , but, from that stage on, occlusion induced OMP and a rise in  $T_{\text{brain}}$  and  $f$  (Fig. 3D and Fig. 5). We therefore feel confident that the AOV-flow animal provided data that are representative for reindeer.

At rest, left jugular venous blood temperature ( $T_{\text{jug}}$ ) dropped linearly from  $38.6^\circ\text{C}$  at  $T_a 0^\circ\text{C}$  to  $37.3^\circ\text{C}$  at  $T_a 30^\circ\text{C}$  and then rose linearly to  $37.5^\circ\text{C}$  at a  $T_a$  of  $50^\circ\text{C}$  ( $N=1$ ;  $n=3$ ), whereas left carotid blood temperature ( $T_{\text{car}}$ ) was fairly stable at  $38.5 \pm 0.1^\circ\text{C}$  ( $N=4$ ;  $n=7$ ) throughout the same range of ambient temperatures. During exercise at  $9 \text{ km h}^{-1}$ , by contrast, left  $T_{\text{jug}}$  dropped approximately  $2.5^\circ\text{C}$  within a few minutes (Fig. 6A) and was maintained at this level both at a  $T_a$  of 10 and  $25^\circ\text{C}$  when the treadmill was level, but it rose linearly at a  $T_a$  of  $25^\circ\text{C}$  when the slope was increased to 10 deg.  $T_{\text{car}}$  increased rapidly by approximately  $0.2^\circ\text{C}$  at the commencement of running

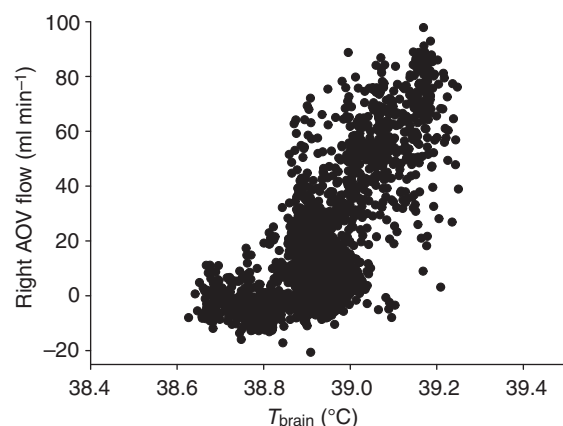


Fig. 4. Typical example of right angular oculi vein (AOV) blood flow recorded every 5 s at different brain temperatures at ambient temperatures increasing from 20 to  $50^\circ\text{C}$  in a single animal.

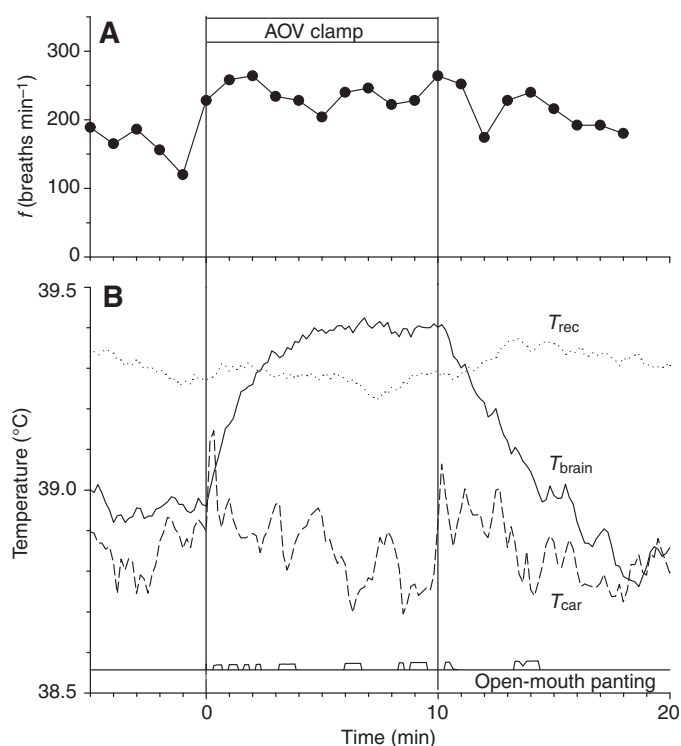


Fig. 5. Typical example of the changes in (A) respiratory frequency ( $f$ ) and (B) temperatures of brain ( $T_{\text{brain}}$ ; solid line), carotid arterial blood ( $T_{\text{car}}$ ; dashed line) and rectum ( $T_{\text{rec}}$ ; dotted line) with indications of concomitant occurrences of open mouth panting (bottom) in response to bilateral clamping of the angular oculi veins (AOV clamp) at an ambient temperature of  $35^\circ\text{C}$ .

and was thereafter maintained at this level, or even returned to the resting level during 20 min running at both  $T_a$  values, when the treadmill was level, but rose gradually, after the initial drop, at a  $T_a$  of  $25^\circ\text{C}$ , when the angle was at 10 deg. Thus,  $T_{\text{jug}}$  was  $35.7 \pm 0.6^\circ\text{C}$  and  $T_{\text{car}}$  was  $38.7 \pm 0.6^\circ\text{C}$  ( $N=2$ ;  $n=4$ ) during exercise at all temperatures and slopes, except at  $T_a 25^\circ\text{C}$  and 10 deg slope, when both temperatures increased throughout the run, with an arterio-venous temperature difference of  $2.8^\circ\text{C}$ . The biggest difference ever recorded between jugular and carotid temperatures was  $3.7^\circ\text{C}$  on a level treadmill at a  $T_a$  of  $10^\circ\text{C}$ .

Respiratory frequency increased slightly from  $260 \pm 20$  to  $260 \pm 6$ ,  $267 \pm 9$  and  $273 \pm 3 \text{ breaths min}^{-1}$  ( $N=2$ ) after 5, 10, 15 and 20 min of running, regardless of ambient temperature and slope of the treadmill. Moreover, all animals always showed periods of closed-mouth breathing, of 10–15 s duration, approximately once every minute, when  $f$  was reduced to a seemingly fixed level of approximately  $250 \text{ breaths min}^{-1}$ , which caused  $T_{\text{jug}}$  to rise  $0.5$ – $1.0^\circ\text{C}$ , whereas  $T_{\text{car}}$  rose  $0.2$ – $0.5^\circ\text{C}$  (Fig. 6B). The frequency of the closed-mouth breathing episodes was independent of  $T_a$  and slope of the treadmill and presumably occurred in order to moisten the tongue. The highest  $f$  recorded was  $299 \text{ breaths min}^{-1}$ .

We found, as others (e.g. Mitchell et al., 1987) have observed before, that the vasculature involved in the regulation of brain temperature could be influenced by psychological stimulation of the animal. In fact, on one occasion, lingual arterial and angular oculi venous blood flow dropped precipitously but transiently, from 2 to  $0 \text{ ml min}^{-1} \text{ kg}^{-1}$  and from 0.6 to  $0 \text{ ml min}^{-1} \text{ kg}^{-1}$ , with a concomitant drop in left carotid arterial blood flow from 10 to

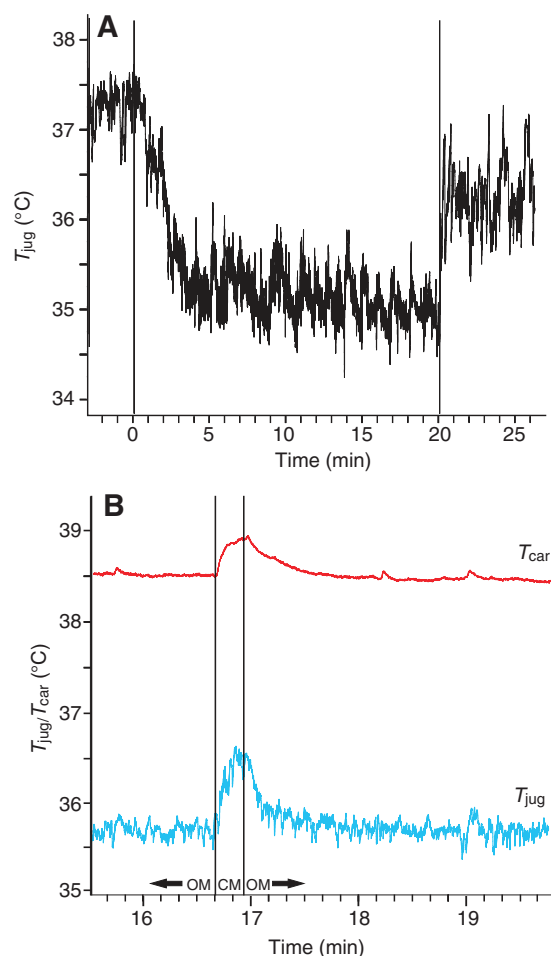


Fig. 6. Typical changes during exercise on a level treadmill at  $9 \text{ km h}^{-1}$  at a  $T_a$  of  $20^\circ\text{C}$  in (A) left jugular temperature before, during (between vertical lines) and after exercise, and (B) left jugular venous ( $T_{\text{jug}}$ ) and carotid arterial ( $T_{\text{car}}$ ) temperature in response to changes from open mouth (OM) to closed mouth (CM) panting.

$4 \text{ ml min}^{-1} \text{ kg}^{-1}$  even during severe heat stress ( $T_a=40^\circ\text{C}$ ) in response to a person passing through the door into the climatic chamber. Such responses will, of course, be variable and depend on the perception of the animal of the disturbance, and we have therefore only presented this extreme example for the record (Fig. 7).

## DISCUSSION

Since the discovery by Baker and Hayward (Baker and Hayward, 1968) of SBC in sheep some 40 years ago, and the finding that the small subtropical Thomson's gazelle (*Gazella thomsonii*) is able to maintain a brain temperature that is  $3^\circ\text{C}$  lower than carotid (body) temperature during very fast running (Taylor and Lyman, 1972), it has been assumed that this is the way by which heat-stressed ungulates regulate brain temperature.

More recently, however, it has been argued persistently in a series of papers on other subtropical ungulates that SBC as described above (Baker and Hayward, 1968; Johnsen et al., 1987) acts instead to modify thermoregulatory responses to conserve water (Jessen et al., 1994; Maloney and Mitchell, 1997; Jessen, 1998; Jessen, 2001). Since then, the same authors have also suggested that the same heat exchange system can be employed for heating of the brain during cold stress by way of unknown

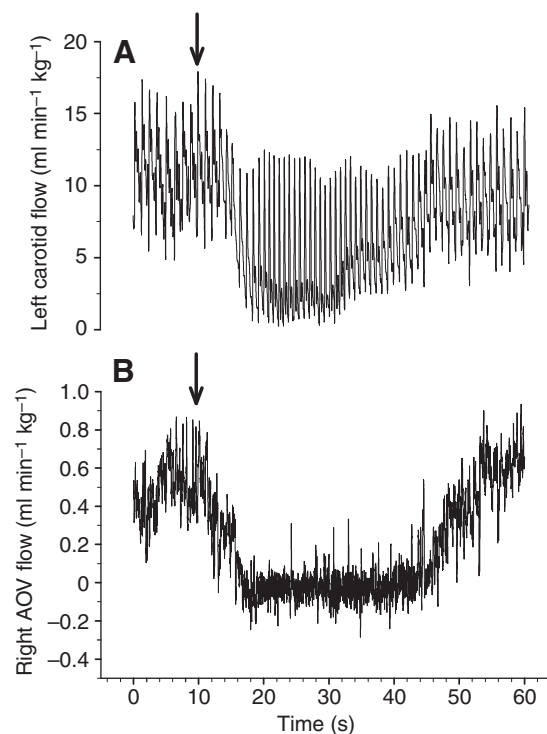


Fig. 7. Typical example of dramatic psychological influences on the thermoregulatory mechanisms in a heat-stressed, but trained and otherwise calm, reindeer at a  $T_a$  of  $40^\circ\text{C}$  in a climatic chamber: changes in (A) left carotid artery and (B) right angular oculi vein (AOV) blood flow in response to a person opening the door to the climatic chamber (arrows).

mechanisms (Lust et al., 2007). For further references, see papers from Mitchell and colleagues and Hébert and associates (Mitchell et al., 2002; Mitchell and Lust, 2008; Hébert et al., 2008). In reindeer, with a great thermal gradient between the body core and its Arctic environment, and easy access to water or snow, we find this rather unlikely. However, while the jury is still out, and as Arctic reindeer, African antelopes and the pronghorn (*Antilocapra americana*) separated phylogenetically some 25 million years ago (Hassanin and Douzery, 2003), we feel for the time being compelled to assume that what appears to be the same vascular system might be utilized to different ends in the different species in very different thermal environments.

Published data on normal thermoneutral brain temperatures of winter-acclimatized reindeer are few and far between, but Mercer and associates (Mercer et al., 1985) reported a value of  $38.1 \pm 0.2^\circ\text{C}$ , whereas in the present study we found  $38.5 \pm 0.1^\circ\text{C}$  (Fig. 3A). This difference is difficult to explain except for the fact that the animals used by Mercer and colleagues (Mercer et al., 1985) were exceptionally tame and that even minor apprehension in winter-insulated reindeer might influence body temperature in animals, even at rest.

Our data suggest that, in resting winter-acclimatized reindeer under moderate heat loads ( $T_a < 30^\circ\text{C}$ ), respiratory frequency is increased and carotid blood flow is increased to supply blood to the nasal mucosa (Fig. 3B,C) to provide cooled blood by way of the facial vein for general body cooling (Johnsen et al., 1985a; Johnsen et al., 1987). This will in most cases meet the needs without much loss of water (Blix and Johnsen, 1983). With increasing heat loads (artificially high  $T_a > 30^\circ\text{C}$ , or exercise),  $f$  is much increased (Fig. 3B) together with carotid blood flow to support sublingual arterial flow

(Fig. 3C) to provide blood to the tongue (and certainly the nasal mucosa) for additional dissipation of heat by OMP.

Krönert and Pleschka (Krönert and Pleschka, 1976) found a temperature difference between the lingual artery and vein of 1°C in dogs breathing with the mouth closed at thermoneutrality, whereas the arterio-venous temperature difference rose to 1.5°C during heat stress when the dogs were panting with their mouth open. By then, sublingual arterial blood flow had risen five times, and respiratory heat loss over the tongue had increased almost 10 times. In our reindeer, the temperature difference between the carotid artery and the jugular vein during OMP was in most cases at least 2.5°C (Fig. 6B). This difference results from the cooling effect of both the nasal mucosa and the tongue, combined with the (fairly constant) warming from the venous drainage of the brain. Pleschka and colleagues (Pleschka et al., 1979) have also found in dogs that blood flow to the nasal mucosa increases steadily with increasing heat load, whereas the flow in the lingual arteries rises rather abruptly when OMP occurs. The sublingual arterial blood flow in reindeer is similar to that of the dog, when adjusted for differences in body mass, and respiratory frequency is also of the same order during severe heat stress. Thus, as Krönert and Pleschka (Krönert and Pleschka, 1976) found that dogs can increase respiratory heat loss over the tongue about 10 times with only a 1.5°C arterio-venous difference, the capability for increasing total respiratory heat loss in reindeer should be at least as good. In fact, using mean values for carotid arterial flow, the difference between carotid arterial and jugular venous blood temperature, assuming a specific heat of whole blood of  $3.6 \text{ J ml}^{-1} \text{ }^{\circ}\text{C}^{-1}$  (Nybo et al., 2002), and a body mass of 80 kg, a maximal (total) respiratory heat loss of at least 360 W is indicated in our reindeer during exercise at  $9 \text{ km h}^{-1}$ . This is about 45% of the heat production of 800 W at that speed and is of the same order of magnitude as previously recorded rates of respiratory evaporative heat loss (Folkow and Mercer, 1986).

Finally, when the heat load in reindeer becomes even more severe, we have shown that AOV flow starts to increase at a  $T_{\text{brain}}$  of  $38.9 \pm 0.1^{\circ}\text{C}$  (Fig. 4) to achieve SBC and that occlusion of both AOVs results in increased  $T_{\text{brain}}$  (Fig. 3D and Fig. 5) at a  $T_{\text{brain}}$  of  $38.8 \pm 0.2^{\circ}\text{C}$ . Kuhnén and Mercer (Kuhnén and Mercer, 1993), using an intra-ruminal heater, found the  $T_{\text{brain}}$  threshold for SBC in resting reindeer to be  $38.7 \pm 0.2^{\circ}\text{C}$ . This seems to imply that the threshold for SBC at rest is a  $T_{\text{brain}}$  close to  $39.0^{\circ}\text{C}$ , whereas the threshold during exercise, according to Kuhnén and Mercer (Kuhnén and Mercer, 1993), seems to be slightly elevated (to  $39.5 \pm 0.3^{\circ}\text{C}$ ).

With regard to SBC, Aas-Hansen and associates (Aas-Hansen et al., 2000) have shown that only a small fraction of the respiratory minute volume passes the nasal mucosa during OMP. It seems therefore that SBC might be compromised when it is most needed. However, in wild reindeer, overheating in winter is likely to happen only during running, and, even though the nasal fraction is much reduced during OMP, the total minute volume is much increased during exercise. In fact, the nasal fraction during running at  $8 \text{ km h}^{-1}$  is almost the same as the total minute volume at rest at  $T_a = 20^{\circ}\text{C}$  (Ø. Aas-Hansen, L.P.F. and A.S.B., unpublished). One should also keep in mind that reindeer in winter, unlike African antelopes, have a thermal gradient between body core and the environment that is huge and that might occasionally even amount to  $90^{\circ}\text{C}$ . This provides a great additional potential for dry heat loss, and, as absolute humidity is very low at low  $T_a$ , the potential for evaporative heat loss is also greater. Accordingly, Johnsen and associates (Johnsen et al., 1985b) found that the amount of heat stored during running

at  $9 \text{ km h}^{-1}$  was less than 5% of total metabolic heat production (Nilssen et al., 1984b) at a  $T_a$  of  $-30^{\circ}\text{C}$  and was less than 10% at a  $T_a$  of  $2^{\circ}\text{C}$  in winter. This suggests that the reindeer has a rather remarkable ability to regulate body temperature, even without the use of SBC, in spite of its formidable winter fur insulation. But that does not imply that SBC is unimportant if the animal has to run, for instance, in deep snow, or in emergencies, when they for short distances can achieve a top speed of approximately  $60 \text{ km h}^{-1}$ , which we were unable to simulate experimentally.

## LIST OF ABBREVIATIONS

AOV	angular oculi vein
$f$	respiratory frequency (breathing frequency)
OMP	open-mouth panting
SBC	selective brain cooling
$T_a$	ambient temperature
$T_{\text{brain}}$	brain temperature
$T_{\text{car}}$	blood temperature in carotid artery
$T_{\text{jug}}$	blood temperature in jugular vein
$T_{\text{rec}}$	rectal temperature

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