UNIVERSITY OF TROMSØ UiT

FACULTY OF BIOSCIENCES, FISHERIES AND ECONOMICS
DEPARTMENT OF ARCTIC AND MARINE BIOLOGY

Peripheral Heterothermia in Reindeer (*Rangifer tarandus tarandus*)

Elin A. M. Olsson

BIO-3910 Master Thesis in Biology

Mai 2011

Cover Picture: One of the female reindeer at the research facility at the Arctic Biology building of the Institute of Arctic and Marine Biology at the University of Tromsø.

Table of Contents

Abstract....5

1. Introduction....7

The Reindeer as a Model Organism for studies of mammalian cold adaptations....7

Heat balance....8

Insulation....8

Peripheral heterothermia, blood flow and counter current heat exchange....9

Hypothesis....12

2. Material and method....13

2.1 Animals....13

2.2 Anatomy....15

X-ray studies of the vascular arrangement in the front and hind leg....15

Cross sections of the front leg....16

Dissection of the front and hind leg....16

Plastic cast of the vascular arrangement of the front leg....16

Samples for Electron Microscopy....17

Tissue type and weight....17

Anatomical names....18

2.3 Experiments....19

Preparations of animals for experiments....19

Temperature measurements....20

Thermocouples....20

Implantation of thermocouples....20

Brachial blood flow....22

Measuring blood flow with Ultrasound....22

Calibration of the flow probes....22

Implantation of the flow probe....24

Experimental protocol 26
Statistics and data analysis 27
3. Results 29
3.1 Anatomy 29
3.2 Subcutaneous temperature 34
3.3 Blood flow and heart rate 36
4. Discussion 41
5. Conclusion 47
References 49

Abstract

Reindeer (Rangifer tarandus tarandus) inhabit the Arctic and have evolved adaptions to meet the climatic challenges in this region. Peripheral heterothermia, which is a heat conserving mechanism in homeotherms during cold exposure, is well documented in the reindeer and manifests itself in tissue temperatures in the extremities well below 10°C. It is, however, unknown whether peripheral heteothermia in reindeer requires efficient vascular counter-current heat exchange, or is simply a result of reduced blood flow to the extremities. Vascular corrosion casts, x-ray pictures, dissections and electron microscopy did not reveal any counter-current rete in the front legs, but the vascular anatomy, with arteria brachialis and vena brachialis running in intimate contact with each other for the full length of the leg, still seems to allow quite efficient counter-current heat exchange. Recordings of brachial blood flow and subcutaneous temperature (T_{sc}) in the front legs of winter insulated reindeer subjected to ambient temperatures (T_a) of 20°C, -10°C and -30° suggest that the initial drop in T_{sc} seen in reindeer when exposed to moderate cold (-10°C) is primarily the result of use of a circulatory pattern enabling counter-current heat exchange, while decreasing blood flow appears to be a secondary mechanism when T_a falls further towards -30°C.

1. Introduction

The subject of this thesis is to investigate the anatomical and physiological basis for countercurrent heat exchange and regulation of peripheral blood flow in the extremities of reindeer (*Rangifer tarandus tarandus*).

The Reindeer as a Model Organism for studies of mammalian cold adaptations

To live in the Arctic is a challenge for most organisms. The long, cold winters are hard, not only because of the cold but also because of the scarcity of food experienced by the inhabitants of this barren region. Reindeer, being an arctic mammal, have evolved special adaptations, or refined the ones inherited from their ancestors, in order to cope with the environmental conditions. Not only do the reindeer have to keep warm; they also have to be very energy efficient to be able to maintain a stable body temperature without expending excess energy. The reindeer have not only managed to survive under these conditions, but actually prospers in the Arctic and surrounding temperate regions. There are seven subspecies of reindeer (Røed 2007) out of which five are found in the arctic region. The distribution as of 2007 can be seen in fig 1. If we include the world population of semi-domesticated reindeer, the world population in 2005 was estimated to about 5 million animals (Mathiesen *et al.* 2005).

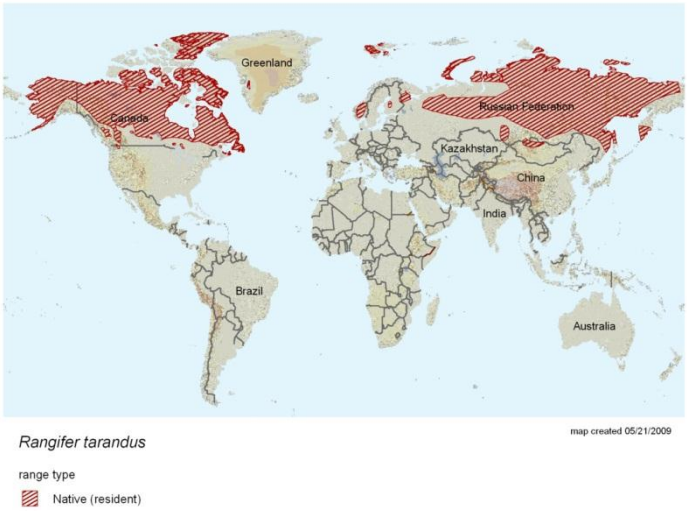

Fig 1: The world distribution of wild reindeer (*Rangifer tarandus*) (Henttonen and Tikhonov 2008).

In the winter when lack of food forces the reindeer to live of fat reserves of the body, it needs to reduce the energy expenditure to make sure that the catabolism does not go too far or too quickly. Energy needs of the reindeer can be decreased by reducing energy expenditure as much as possible, which is mainly done by changing behavior and by reducing the energy lost by the animal as heat. The behavioral change seen in many arctic animals in winter is referred to as "arctic resignation" and simply means that the animal reduces its

physical activity to a minimum (Blix 1989). The resting metabolic rate of both Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and Eurasian tundra reindeer (*Rangifer tarandus tarandus*) have also been shown to vary between summer and winter, but this may be explained in terms of the reduced feeding in the winter and not as a physiological adaptation to conserve energy during winter (Nilssen *et al.* 1984). Food intake is actually always lower in winter even when the animals have food *ad libitum* (Larsen *et al.* 1985). One aspect of the energy balance is heat balance since heat is a form of energy and it is the last form to be utilized and lost from the body.

Heat balance

The ability of a homeotherm to maintain heat balance can be mathematically expressed in the heat balance equation:

$$H_{tot} = H_c \pm H_r \pm H_e \pm H_s$$

Equation 1: H_{tot} is the rate of metabolic heat production. H_c is the rate of conductive and convective heat exchange. H_r is the rate of net radiation heat exchange. H_e is the rate of evaporative heat loss and H_s is the rate of heat stored in the body (Schmidt-Nielsen 1990).

Heat loss is an important form of energy loss, especially in winter, and it is a function of the temperature difference between the animal and the temperature of the environment. This heat is lost at the surface of the animal so reducing heat loss from the surface is of outmost importance. Heat loss at the surface is reduced by decreasing the temperature difference between the surface and the environment. The reindeer's adaptations to reducing heat loss from the surface will be discussed in the following sections.

Insulation

The deep body temperature of homeothermic animals is, by definition, maintained within narrow limits and can, for the sake of argument, be said to be constant. The difference between ambient and deep body temperature in arctic mammals is naturally particularly large during the winter. Therefore heat loss is potentially a very important mode of energy loss in winter. As has been previously described, heat can be lost or gained through four physical mechanisms: Conduction, convection, radiation and evaporation. The loss of heat

through the first three avenues can be reduced by a well-functioning insulation. This is in nature either by a thick fur or plumage, or in the case of the marine mammals, a thick blubber layer. The thickness of the fur of the reindeer varies during the year (Mootie 1955). The reindeer sheds its fur once a year in late spring and summer. The fur then continuously grows through the year until it is shed again next spring. It is therefore relatively short in the summer and thicker in the winter. The structure of the hairs in the fur is rather special in the reindeer. Each hair contains pockets of air (fig 2) which provides extra insulation since stagnant air is a very efficient insulator.

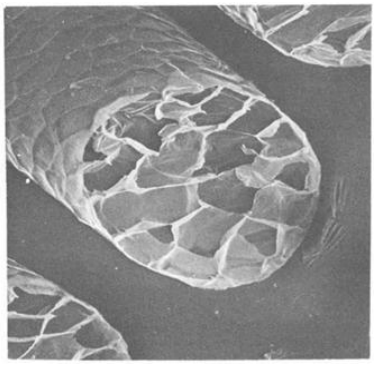

Fig 2: Scanning electron micrograph of a cut reindeer hair displaying the air filled structure (Timisjärvi *et al.* 1984).

Peripheral heterothermia, blood flow and counter current heat exchange

The total blood volume of the reindeer is 106-139 $ml \cdot kg^{-1}$, depending on season, age and gender, with adult males in autumn having the largest volume (Timisjärvi 1978). One of the principles behind peripheral heterothermia is that this blood volume can be distributed unevenly to different tissues, not only due to metabolic needs, but also in order to increase or reduce heat loss. The blood volume stays the same, so if the blood flow to a certain tissue increases; the flow to another tissue must decrease. Heat loss from the surface of the body of the reindeer is mainly through three of the four mechanisms mentioned in equation 1: convection, conduction and radiation. The last one, evaporative heat loss, is mostly found in the airways of the reindeer. Heat loss by the other three mechanisms is reduced by the already mentioned insulative fur, but the animal can also decrease blood flow to the skin in order to reduce the heat loss. The flow can only be

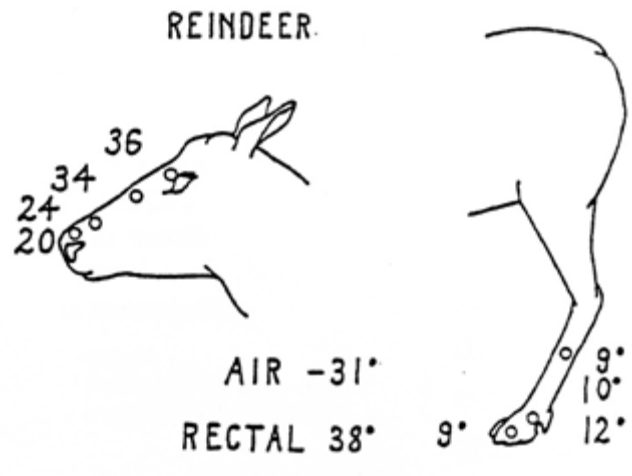

Fig 3: Topographical distribution of superficial temperatures in a reindeer (Irving and Krog 1955).

reduced a certain amount depending on the metabolic needs of the tissue. The reduced blood flow reduces the skin temperature, which reduces the surface temperature of the animal and therefore the temperature difference between the surface and the environment. Since it is this temperature difference that determines the rate of heat loss; the smaller the difference the smaller the heat loss. This is especially important in the extremities where the area to volume ratio is largest and the insulation poorest (Johnsen *et al.* 1985a), i.e. where the potential heat loss from the surface of the animal is greatest.

As the name "peripheral heterothermia" implies, the periphery, i.e. the extremities of the animal, has another temperature than the core of the animal (fig 3 and fig 4). As a result, the blood will be cooled in the extremities before flowing back to the core and the resulting cooling of the core would inevitably lead to hypothermia, unless prevented by a mechanism called vascular counter current heat exchange. In the reindeer, the counter current heat exchange was first suggested by Irving and Krog (1955), based on measured subcutaneous temperatures in dead reindeer after they had been shot (fig 3). The temperature measurements have been repeated by others and confirmed on live animals (fig 4) (Johnsen *et al.* 1985b; Folkow and Mercer 1986).

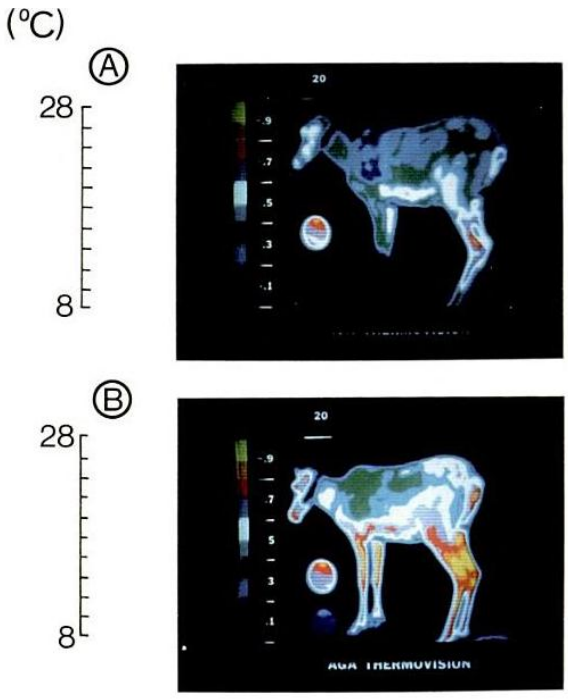

Fig 4: Typical radiative surface temperature of a reindeer, (A) before and (B) after a 45 min period of running (Johnsen *et al.* 1985).

There are two main ways to arrange vessels to achieve the counter-current heat exchange (fig 5). The simplest one is two blood vessels parallel to each other (fig 5A), with directionally opposite blood flows. This situation is by necessity found in the limbs of all vascularized animals, since blood flow must occur in both directions, i.e. to and from the extremities. The closer the counter-current blood vessels are to each other and the larger the area of heat transfer is, the more effective the heat exchange. The other more refined anatomical possibility is the counter current *rete*. A *rete* consists of several thin vessels in a bundle with some vessels carrying

blood in one direction, e.g. arterial blood, and the rest in opposite direction e.g. venous blood (fig 5B). This structure increases the area of heat exchange compared to the single vessel system and is thus a more effective way to attain heat exchange. The exchange in a *rete* can be completed in a much shorter distance than when there are only two parallel vessels. The complexity of counter current heat exchangers varies from just two parallel vessels to several hundreds of vessels in a *rete*. The *rete* solution is found in various aquatic/marine birds (Midtgård 1980) and mammals (Scholander and Schevill 1955; Pabst *et al*. 1995; Rommel and Caplan 2003), as well as some terrestrial mammals (Scholander and Krog 1956). It is however still not known whether reindeer legs have specialized counter current vascular arrangements or if the observed peripheral heterothermia in the cold is simply the result of vasoconstriction.

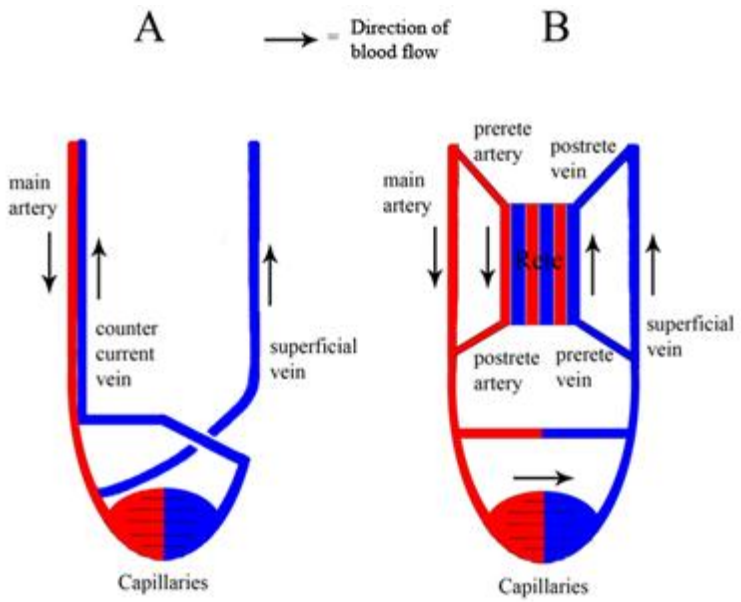

Fig 5: The two basic anatomical possibilities of vascular heat exchange, (A) simple two vessel arrangement and (B) a *rete* (Olsson 2008).

Hypothesis

The anatomy enabling counter current heat exchange in the extremities of reindeer has not been examined. The efficiency and to what extent the counter current heat exchange is used compared to other heat saving mechanisms, i.e. reduced blood flow, is unknown. Cold stressed reindeer may limit heat loss from their extremities by allowing tissue temperature to drop through a combination of

a) Efficient vascular counter current heat exchange
b) Reduced tissue perfusion

Thus the aims of this study were: a) Do anatomical studies of leg vasculature to investigate the basis for counter current heat exchange, b) Measure skin temperature along the leg of the reindeer under various thermal conditions as an indicator of peripheral heterothermia, and c) Measure blood flow in the brachial artery at the same ambient temperatures as the ones used for skin temperature measurements to in order to identify vasoconstrictor responses.

2. Material and method

2.1 Animals

In total twelve reindeer (*Rangifer tarandus tarandus*) were used in this study (table 1). All of the animals were born and maintained at the Arctic Biology research animal facility, University of Tromsø, Norway (70° N, 19° E). Between experiments the animals were kept outdoors, where both water and feed (Reinfôr, Felleskjøpet, Norway) was available *ad libitum*.

Seven of the reindeer were used for the anatomical studies, i.e. dissections, x-ray, cross sections of the leg, electron microscopy, tissue weight measurements and corrosion casts.

Two animals were used for measurements of brachial artery blood flow. Initially four animals were instrumented for this purpose, but due to low signal strength of the probe, experiments were only conducted on two of them.

Five animals were used in the leg skin temperature experiments, but due individual behavioral differences, only two of the animals gave reliable results.

The reason that the number of animals does not add up to twelve is that some of the animals used for brachial blood flow experiments were also used for the anatomical studies, see table 1.

Table 1: The reindeer used for this study. Age indicates the age of the animal when experiments were conducted or age at the time of death in the case of anatomical studies, if the age is "Adult" the reindeer was minimum 2 years old.

Reindeer	Sex	Age	Studied	Weight
1	M	11 months	subcutaneous temperature (no reliable results), brachial flow (no results)	61 kg
2	M	10 months-1 year	brachial flow, subcutaneous temperature (no reliable results)	52 kg
3	F	1-1,5 years	subcutaneous temperature (no reliable results), brachial flow (no results)	76 kg
4	F	1-1,5 years	subcutaneous temperature	78,5 kg
5	F	1,5 years	dissection, flow probe calibration	89 kg
6	M	9 months	brachial flow, electron microscopy	55 kg
7	F	Adult	subcutaneous temperature	82,5 kg
8	F	Adult	corrosion cast	81 kg
9	F	Adult	x-ray, dissection, cross section	80 kg
10	M	Adult	x-ray, dissection, cross section	105 kg
11	unknown	Adult	Dissection	Unknown
12	M	1,5 years	tissue weight measurements	98 kg

2.2 Anatomy

Anatomical studies were carried out using five different approaches:

X-ray

Dissections

Cross sections

Plastic casts

Electron microscopy

X-ray studies of the vascular arrangement in the front and hind leg

The legs of two adult female reindeer (no. 8 and no.9), were used. The animals were sedated with an intramuscular injection of Domitor vet. (Medetomidine hydrochloride 1mg·ml^{-1}; Orion corporation, Espoo Finland), the dose was 1mg·10 kg^{-1} bodyweight. After sedation the animal was injected with 10 ml of Heparin (5000 IU·ml^{-1}, LEO Pharma AS, Oslo, Norway) in the jugular vein to keep blood from coagulating. The heparin was given time to circulate for 5 min and then the animals were stunned with a bolt pistol and killed by exsanguination. The front legs were separated from the body by a cut between the rib cage and the scapula, and the hind legs were separated from the body at the hip joint. The vascular system was washed with approximately 5 liters of 0,9% NaCl solution through the brachial artery in the front leg, and the femoral artery in the hind leg, to remove remaining blood. An x-ray picture was taken before the contrast medium, Mixobar colon (barium sulphate 1 g·ml^{-1}; Astra Tech AB, Mölndal, Sweden), was injected into the main artery in the legs i.e. *arteria brachialis* in the front leg and *arteria femoralis* in the hind leg. While the contrast medium was injected, the route of the contrast medium in the arteries was observed by taking a series of x-ray pictures. On the front leg, *vena brachialis* was also injected with Mixobar colon. An incision was made approximately half way between the elbow and the wrist, and *vena brachialis* was identified. The contrast medium was then injected in *vena brachialis* towards the elbow to visualize the path of the vein. The x-ray machine used was Series 9600 (OEC medical systems). The x-ray pictures were then assembled to one picture in Photoshop CS3 (fig 12).

Cross sections of the front leg

Cross sections of the front legs were made to investigate the position of the blood vessels in the leg in relation to each other, as well as to investigate areas of interest found in the x-ray pictures. The front leg of reindeer no. 10 was frozen after the animal was euthanized the same way as described in the previous section. This time however, the blood vessels were not washed with 0,9% NaCl solution, since the more blood that would be left in the vessels, the more visible they would be in a cross section. Cross sections were made of the frozen leg by cutting at intervals using a saw (see fig 10, 16 and 17).

Dissection of the front and hind leg

To further confirm the anatomy and arrangement of the blood vessels in the legs of the reindeer dissections were conducted on both front and hind leg. One front leg of three different animals (no. 5, no. 9 and no.10) and one hind leg from reindeer no. 11 were dissected to look at the anatomy of the vascular system in the legs. The vessels were carefully dissected and freed from surrounding tissue and photographs were continuously taken during the dissections.

Plastic cast of the vascular arrangement of the front leg

To get a better three dimensional understanding of the vascular arrangement both in the muscles and in the larger blood vessels, corrosion casts of the vascular system were made. The front legs used here came from the same female reindeer that was used for the x-ray analysis of the hind leg. The vessels were as already mentioned perfused with 5 liters of 0.9% NaCl solution to remove remaining blood. After that arteries on both front legs, and also the veins on one front leg, were injected with vinyl acetate (Mercox Embedding Resin Kits, SPI supplies, West Chester, Pennsylvania, USA) by hand, using a syringe. Arterial injections were made through the brachial artery and venous injections via smaller veins near the hoof in an attempt to fill the venous part of the vascular tree. The vinyl acetate used in the arteries was dyed red and the vinyl acetate in the veins was dyed blue. After curing of the vinyl acetate, the injected front legs were corroded in 10% NaOH until the tissues were gelatinous, but "landmarks" were still intact, to allow identification of different vascular

structures in relation to other tissue. Corroded tissue was carefully removed to expose the vinyl casts.

Samples for Electron Microscopy

To further investigate the vasculature of the muscles of the front leg, muscle samples were taken from a 9 months old semi-adult male reindeer (no 6, table 1) after the front leg was removed from the euthanized animal. The animal was first given an injection of 1 ml Zalopine (medetomidine 10 $mg \cdot ml^{-1}$, Orion Pharma, Orion Corporation, Espoo Finland) intramuscularly in the right thigh to sedate it, followed by 15 ml pentobarbital (100 $mg \cdot ml^{-1}$, Svaneapoteket, Tromsø, Norway) through a catheter in a vein in the right hind leg. The animal was then exsanguinated and the left front leg was parted from the body at the scapula. The vascular system of the leg was rinsed with 0,9% NaCl solution, followed by McDowell's solution (McDowell and Trump 1976) to prepare the tissue for electron microscopy. After fixation the fur and skin was removed from the area where the tissue was to be sampled. Slices approximately 1-2 cm thick were cut out with a scalpel and were immersion fixed in McDowell's solution for later electron microscopy. The samples were taken from a region in which x-ray pictures had revealed a *rete*-like vascular structure in the muscle tissue, posterior to *radius* and *ulna* (see fig 10) i.e. in the muscles *Pronator teres*, *Flexor carpi radialis*, *Flexor carpi unlaris* and *Flexor digitalis profundus* (hereafter referred to as posterior flexor muscles of the front leg). The electron microscope was a transmission electron microscope (CM 120, Philips, Eindhoven, Netherlands). Images were captured using a Moranda camera with accompanying software (Soft Imaging System GmbH, Münster, Germany). All electron microscopy and image capturing was done by Dr. E. B. Messelt, at the Institute of oral biology, Faculty of dentistry, at the University of Oslo.

Tissue type and weight

A front leg from reindeer no. 12 was separated from the body of the previously stunned and exsanguinated animal. The fur was cut off and the leg was weighed. The leg was then skinned and muscle tissue plus tendons was separated from the bones. All parts were weighed separately.

Anatomical names

All anatomical structures have been named by looking for homologues structures in anatomical descriptions of other artiodactyls (even-toed ungulates).

2.3 Experiments

Preparations of animals for experiments

Six 4 months old reindeer calves (no. 1-6, table 1), born at the facilities of Arctic Biology, University of Tromsø, Norway (70° N, 19° E) were held indoor in pens for a few weeks to make them comfortable with people and accustomed to standing in the box that was used to restrain animals during subcutaneous temperature and brachial blood flow measurements. They had *ad libitum* access to feed and close contact with people. After these weeks, the animals were kept outdoors. Here the work of taming them continued and the reindeer got used to being approached and caught. They were trained to walk on a leash into the climate chamber and to walk calmly in and out of the restraining box that would later be used to restrain the animals during the experiments. When the animals seemed to be used to the procedure and were able to do it without apparent stress, they were deemed ready to be used in experiments. In addition one previously trained adult female reindeer (no. 7, table 1) was used in the temperature experiments. All experiments were made on winter insulated animals.

Temperature measurements

Thermocouples

All temperatures were measured using copper-constantan thermocouples (type T). The basic principle behind thermocouples is that there will always be a specific electric potential between metals of different kind and this potential varies with temperature.

The thermocouples were made by taking one isolated copper wire and one isolated constantan wire (0,5mm x 0,8mm, Omega engineering Inc., Stamford, Connecticut, USA), remove the isolation on the outer 2-3 mm (or 1 cm for the rectal and ambient temperature thermocouple) with a scalpel, dip the ends in concentrated hydrochloric acid to remove any fat from the surface of the metals and then solder the two metals together. After this the tip was isolated by either dipping it in nail polish a few times or covering the tip with a plastic tube. The plastic tube of the rectal thermocouple was kept in place by epoxy glue, shaped as a round tip at the end to prevent damages to the animal. Two types of thermocouples were made for the subcutaneous temperature measurements. Type 1, where the thermosensitive tip of the thermocouple grade wire itself was inserted under the skin, and type 2 where the thermosensitive tip was placed at the tip of hypodermic needle with a diameter of 0,9 mm.

A high precision bath (model no. 6025, Hart Scientific, Peasant Grove, Utah, USA) which held a temperature of +40,0°C and a thermos with ice water, i.e. 0°C, was used to calibrate the thermocouples. The thermocouples were connected through a thermocouple amplifier with internal temperature reference (AD 595 CD: Analog devices, Norwood, Massachusetts, USA) to an analog-to-digital (A/D) converter and data acquisition system that stored the data every 20 seconds and on screen update intervals were 1 second. (Lab- Acq Pro and Insta-Trend Pro; Dianachart, Oak Ridge, New Jersey, USA).

Implantation of thermocouples

The animals (no. 1, 2, 3, and 6, table 1) were sedated by an intramuscular injection in the thigh of 2,5-3 ml Rompun vet. (xylazin 20 $mg \cdot ml^{-1}$, Bayer Health Care AG, Animal health division, Leverkusen, Germany) and when the animal laid down the eyes and ears was covered with a blanket to keep the animal calm until the full effect of the anesthesia was reached. The three legs that were not going to have thermocouples inserted were immobilized. Three subcutaneous type 1 thermocouples were put into place by use of a catheter needle (see positions in fig 6 and 10). The needle was used to create a

subcutaneous canal for the thermocouple wire, which was inserted through the needle in the opposite direction of the insertion of the needle. The needle was then removed and the thermocouple pulled into place and secured by surgical tape and stitching to the skin. This was done at three positions along the leg (fig 12). The animal was then given an intramuscular injection of 4-8 ml Antisedan (antipamezole 5 $mg \cdot ml^{-1}$, Orion Corp, Turku, Finland) to wake up. The procedure took 25-45 minutes. When alert enough to walk, the animal was led into the climate chamber, where the thermocouple grade wires were connected to the recording instruments.

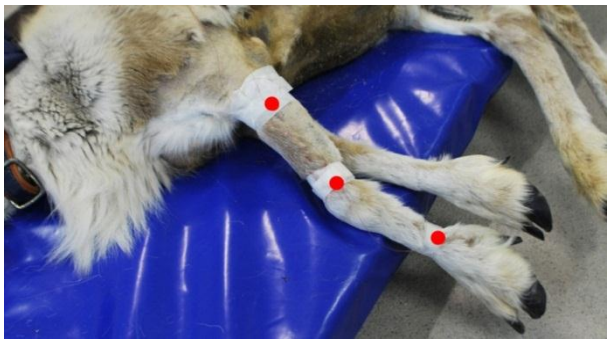

Fig 6: Positions of the thermocouples, indicated by red dots, on the reindeer leg.

Because sedation appeared to affect thermoregulation, even after antidote was given, a new procedure for placing thermocouples was developed. The animal was brought directly from its outdoor enclosure and held down with eyes covered to keep it calm, while the type 2 thermocouples that were mounted inside hypodermic needles were inserted subcutaneously and fixed with surgical tape (Positions of thermocouples, fig 6 and 10). After placing the animal in the restraining box inside the climatic chamber, all thermocouples were connected to the recording instruments.

Brachial blood flow

Measuring blood flow with Ultrasound

To measure blood flow in the brachial artery, we used a transit time flow probe (Butterfly flowmeter probe, 3mm, Medi-Stim ASA., Oslo Norway). The principle behind it is that a sound beam will travel faster downstream than upstream (Anonymous, 1997), see fig 7. In the flow probe body two piezoelectric crystals transmits ultrasound through the blood vessel towards the reflector on the other side. The sound beams are reflected on the reflector, return to the probe body and are picked up by a receiver. The blood flow is calculated from the difference in transit time of the ultrasonic pulses.

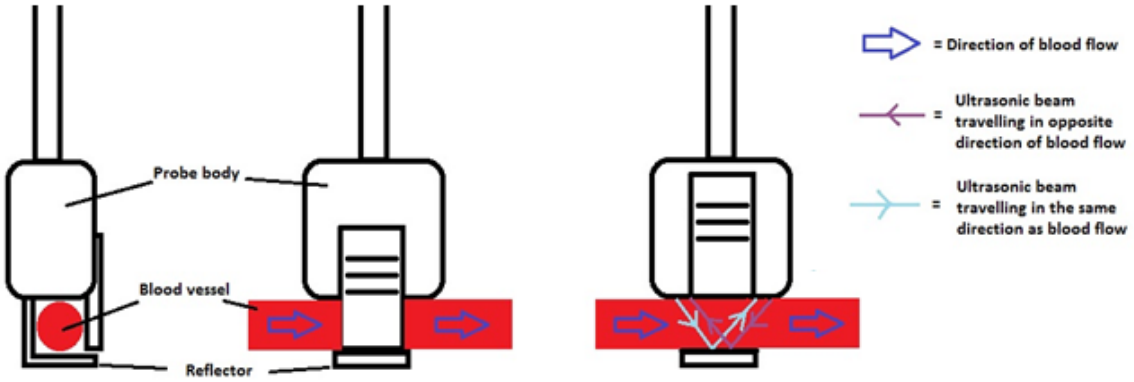

Fig 7: Schematic overview of the flow probe and the basic principle behind it.

Calibration of the flow probes

Prior to experiments, the butterfly flowmeter probe was calibrated using a silicone tube of suitable diameter and a peristaltic pump (21115 Multiperpex pump, LKB-produkter AB, Bromma, Sweden), as well as a measuring cylinder to measure the actual volume that passes the flow probe. Data were recorded using CardioMed flowmeter (type CM 4008, CardioMed AS, 0409 Oslo, Norway) that was further connected to PowerLab 4/25 data acquisition system and Chart 5 for Windows software (ADInstruments Ltd, Chalgrove, UK). Data analyses were also made using Chart 5. During the calibration, saline was used, and this could be adjusted for in the flowmeter, so that the calibration would also be valid for blood. The temperature of the liquid was also adjusted for. The volume in $ml \cdot min^{-1}$ pumped through during 30 s was compared to the analogue flowmeter output in mV to establish a

probe-specific relationship between flow (ml·min^{-1}) and voltage (mV). During the calibration, as well as during the experiments, the signal strength was good. To better mimic the experimental conditions additional calibrations were made using fresh blood vessels instead of silicone tubes, as collected from a killed animal directly after exsanguination. The tissue was kept moist by pouring 0,9% NaCl solution over it at regular intervals and the vessel, once removed from the leg, was kept in a petri dish with the same solution. A small part of the vessel, approximately 3-4 cm, was cut off from the rest and a silicone tube was inserted in the upper (proximal) end of the vessel and another in the lower (distal) end (fig 8). Two different pumps (FMI lab pump, Model QD, Fluid metering Inc., Oyster Bay, New York, USA) (Infusion/withdrawal pump, model 940, Harvard Apparatus, Millis, Massachusetts, USA) were used to maintain steady flow of four different speeds. The flow was also measured by weighing the saline that was collected from the collecting tube in the distal part of the artery to exclude possible errors from imprecisions of the pumps. After the data was collected, a plot of the data from the measurements was made and a linear trend line was added and forced through origo (since zero flow should mean zero mV) (fig 9). After that the mV was converted into its ml·min^{-1} equivalent, by use of the calculated conversion factor (slope of the trend line).

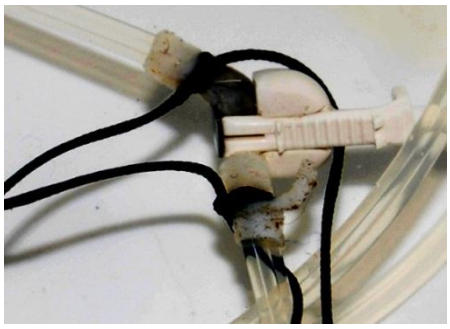

Fig 8: Photograph of the flow probe attached to the blood vessel segment during calibration.

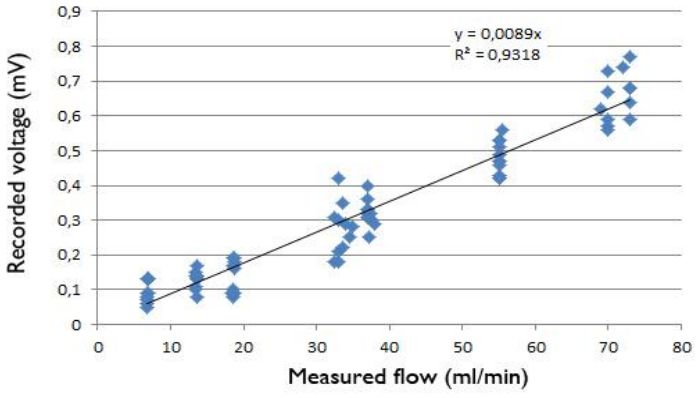

Fig 9: The calibration curve from the calibration when a section of *arteria brachialis* was used.

Implantation of the flow probe

Reindeer no. 1, 2, 3 and 6 were sedated with intramuscular injection of 1,8-2,8 ml Rompun vet. (xylazin 20 mg·ml^{-1}, Bayer Health Care AG, Animal health division, Leverkusen, Germany) before being carried to the operation table. 6-10 ml Propofol (10 mg·ml^{-1}, Alpharma AS, Oslo Norway), was injected intravenously through a catheter in a vein on the inside of the right thigh, whereafter the animal was intubated and maintained on gas anesthesia, Isofluran in air (Forene, Abbot Scandinavia, Solna, Sweden). The electrode pad for the diathermia knife (Valley lab Inc., Surgical Products Division, Boulder Colorado, USA) was placed on a shaved area of skin on the hind leg. The upper portion of the right front leg was shaved and cleaned with 5% Chlorhexidin and a subcutaneous injection of the local anesthetic Xylocain (10 mg·ml^{-1} Lidocain, AstraZeneca, Oslo, Norway) was injected. An incision was made where the pulse could be palpated through the skin and the brachial artery was carefully exposed. The butterfly flowmeter probe (3 mm, Medi-Stim ASA, Oslo, Norway) was placed around the vessel and secured under a muscle. A separate cut was made in the skin, for the exit of the cable (for exact position of flow probe, see fig 10). The wound was sutured with absorbable sutures in the muscles and silk sutures in the skin. The cable end and plug was attached to a harness that was put on the animal. The harness was of the type usually used on large sled dogs. 5 ml of the anticoagulant Fragmin (2500 IU·ml^{-1}, Pharmacia & Upjohn AS, Oslo Norway) was injected intramuscularly to reduce the risk of blood clotting, 1 ml of the analgesic Romefen (10mg·ml^{-1} Ketoprofen, Merial Norden A/S, Søborg, Denmark) was also injected intramuscularly and 5 ml of the antibiotic Streptocilln vet. (Boehringer Ingelheim Danmark A/S, København, Denmark) was injected intramuscularly to prevent infection. After the procedure the animal was given 5-8 ml intramuscular injection of Antisedan (antipamezole 5 mg·ml^{-1}, Orion Corp, Turku, Finland), and the tracheal tube was removed when the animal started to regain consciousness. The animal was allowed to lie down for a while, and when it was alert enough to walk, it was led back to its indoor pen where food (lichens and Reinfôr) and water was available *ad libitum* and the animal was left to recover for a minimum of 24 hours prior to the first flow measurements.

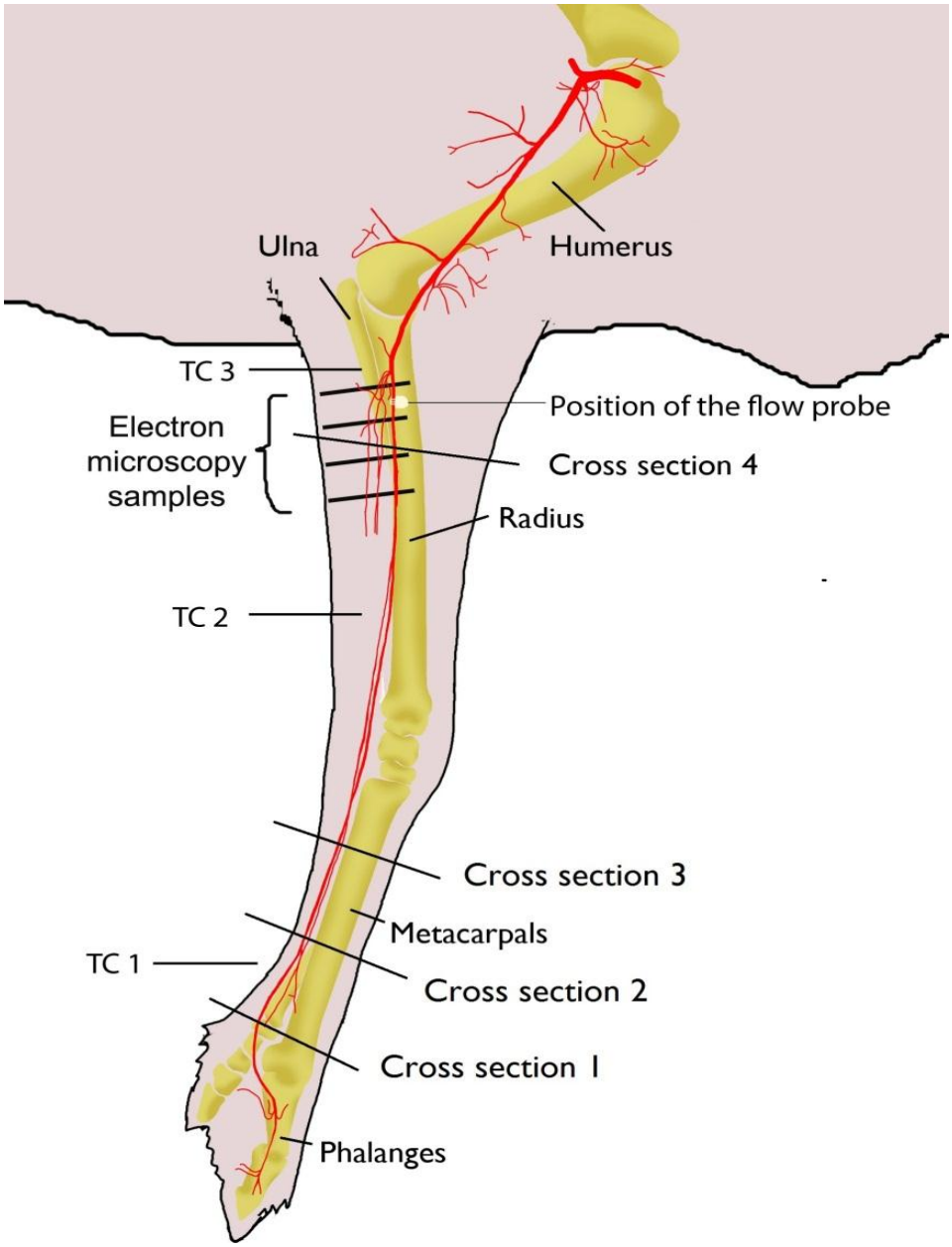

Fig 10: A summary of the procedures and their location on the front leg of the reindeer. The leg is redrawn from the x-ray pictures taken of the front leg of reindeer no.9, see fig 12. TC = thermocouple.

Experimental protocol

All animal experiments were conducted with the reindeer placed inside a restraining box (125x55x130 cm) which in turn was placed inside the climate chamber. The animal was led into the climate chamber from its indoor pen (flow experiments) or outdoor enclosure (temperature experiments) where it was kept between the experiments. The climate chamber was 25 m3 and the temperature in the camber was controlled within ±1°C in time and ±2°C in space. The humidity in the chamber could not be controlled. The time it took for the chamber to reach new ambient temperatures can be seen in fig 11. The flow probe was connected to the Power lab recording system tshrough the CardioMed flowmeter and the thermocouples were connected to their recording system. Food was available for the animal *ad libitum*, and water and lichens were offered to the animal several times between measurements. The blood flow and subcutaneous temperatures were measured in separate experiments at three different ambient temperatures (T_a), 20 °C, -10 °C and -30 °C. Flow was measured at steady state as well as during the changes between these temperatures, and temperatures were measured continuously during the temperature experiments. The time to reach steady state during flow measurements was based on previous experiments done at the department (Folkow and Mercer 1986). When the ambient temperature was reached, the animal was given 40-120 minutes to acclimate before a 30 min measurement was made. The time of acclimation depending on previous temperature experienced by the animal and the new ambient temperature. Subcutaneous temperatures and rectal temperatures were continuously measured during the full length of the temperature experiments.

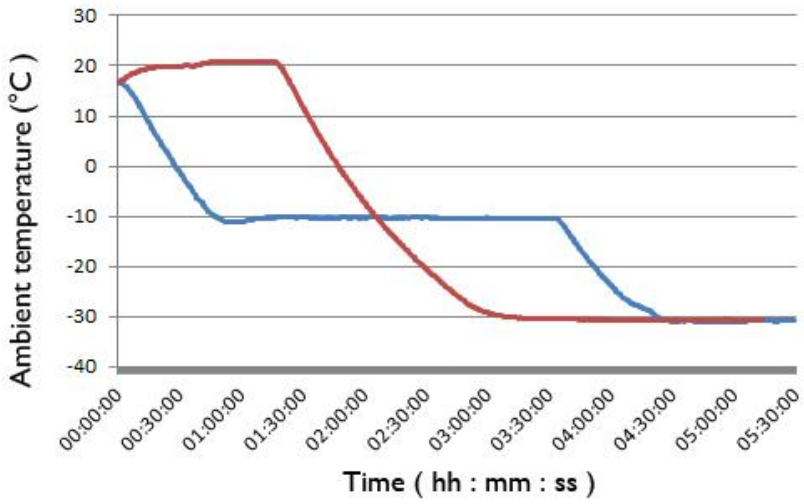

Fig 11: The response time of the climate chamber when changing ambient (chamber) temperature.

Statistics and data analysis

Mean heart rate was calculated from the brachial artery flow record by counting the number of beats seen in the flow traces during 6-7 30 second intervals. The mean heart rate of these 6-7 30 second intervals was then seen as the mean heart rate at the particular ambient temperature.

Mean blood flow was calculated by taking the mean of measured flow every 0,1 second for the 30 min measurement at the three ambient temperatures.

Figures and tables were made in either Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington, USA) or in IBM SPSS Statistics 19 (SPSS Inc., Chicago, Illinois, USA) and all statistical analyses were made in SPSS. Correlations between heart rate and ambient temperature, mean blood flow and ambient temperature, and range of blood flow and ambient temperature were made while controlling for which animal was used (individual effect) and which of the experiments the data were taken from (effect of different circumstances prior to and during the experiment). Correlations between subcutaneous temperature and ambient temperature were made while controlling for individual effect, but not for which experiment it came from since only one ambient temperature was tested per experiment.

F-tests for equal variance was used to see if equal or unequal variance should be used for the t-tests for equality of means for both flow and heart rate in pairwise comparisons.

3. Results

3.1 Anatomy

For descriptive purposes the anatomy of the vasculature of the reindeer legs can be divided into two parts: 1) one circulating the distal part of the leg mainly consisting of the hoof, bone, skin and tendons, and 2) one supplying the proximal part of the leg where the muscles of the limb are found (fig 12 and 13).

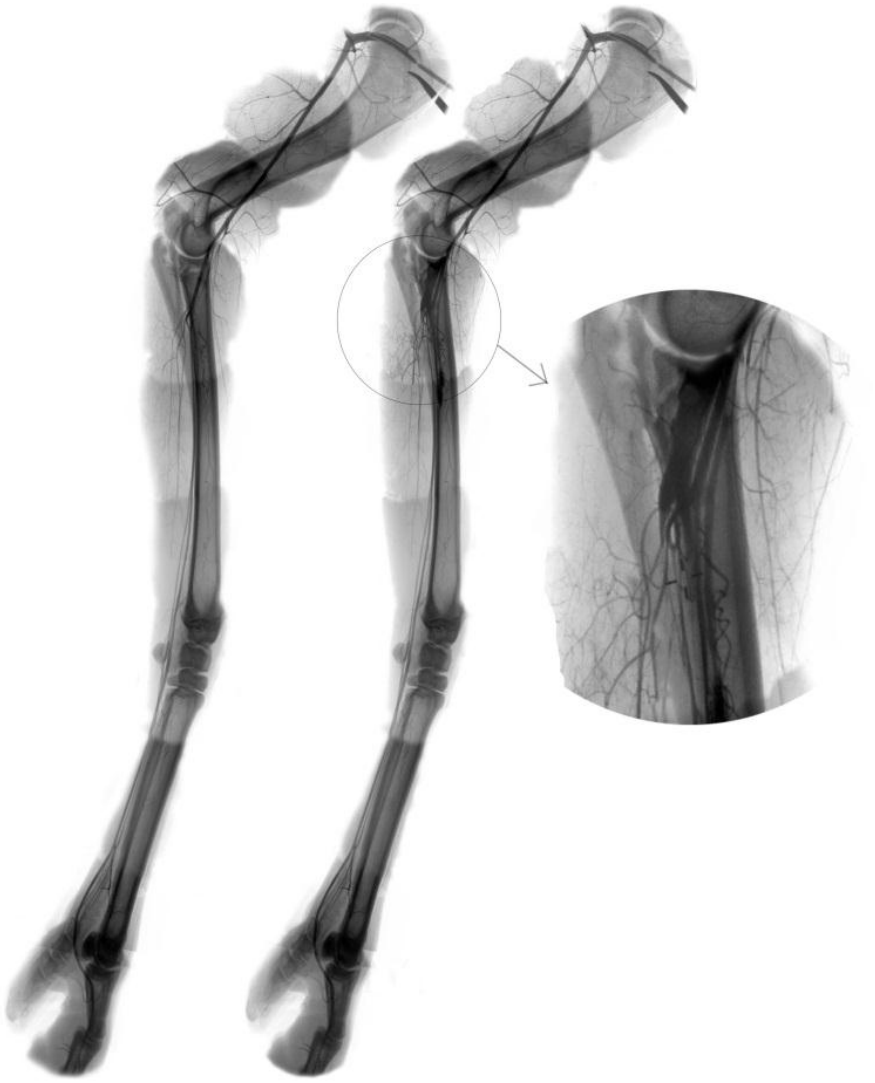

Fig 12: X-ray picture of the front leg with contrast solution in the blood vessels. Arterial filling to the left, arterial plus venous filling in the middle and to the right a close up of the venous and arterial filling just below the elbow.

Both start in the *arteria brachialis,* which branches into the two parts just below the elbow (fig 12). In the circulation of the distal part of the leg *arteria brachialis* continues a bit further and is then divided into *arteria mediana* and *arteria radialis*. Both *arteria brachialis* and *arteria mediana* is in close contact with respectively *vena brachialis* and *vena mediana* along their full length, and thus represent the counter current heat exchange system in the front leg. The homologous structures in the hind leg are *arteria femoralis* and *arteria tibialis* on the arterial side, and *vena femoralis* and *vena tibialis* on the venous side (fig 14). The superficial veins in the front leg were also studied and consist of mainly *vena cephalica* and *vena cephalica accessoria* (fig 15).

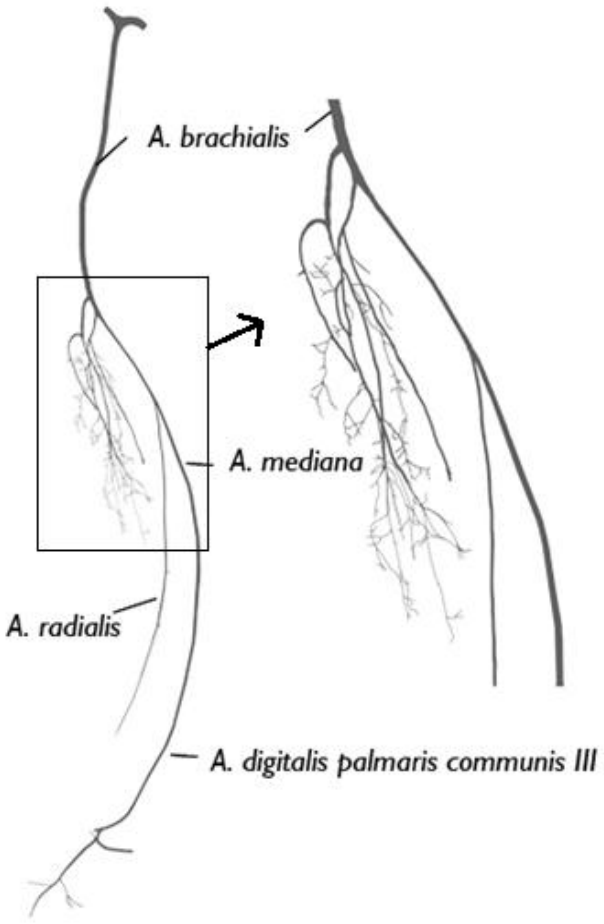

Fig 14: Vascular corrosion cast of the arteries of the front leg of the reindeer. Redrawn from photograph of the cast. To the right a close up of the vasculature in the posterior flexor muscles of the front leg.

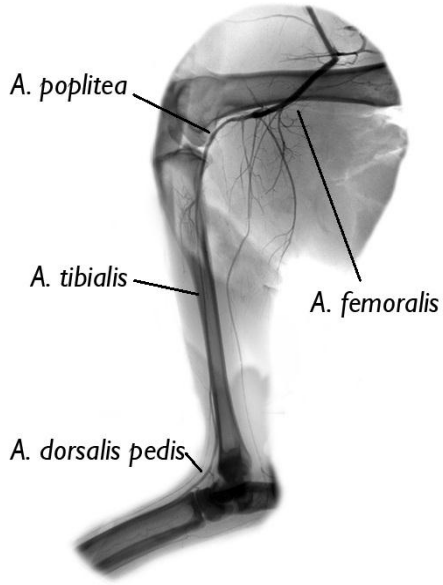

Fig 13: X-ray picture of the hind leg of a reindeer, arterial arrangement visualized with contrast solution.

The close proximity of the counter current vessels in the front leg can be seen in the cross section (cross section 4 in fig 10) picture (fig 16). The surface of the counter current artery (*arteria brachialis* and *arteria mediana* combined) was estimated from measured lengths and diameter of the vessels during dissection, as was the surface area of the counter current vein (*vena brachialis* and *vena mediana* combined) and was found to be approximately 44 cm^2 for the artery and 75 cm^2 for the vein. Since these vessels were found to be parallel, with

continuous contact along their full length, approximately half of the surface area of the artery was in contact with the vein. The close proximity of the counter current vessels were seen also in cross section no. 1-3 (fig 17), however, the vein was collapsed to such an extent that it is not possible to see it in the photographs. The vessel types were distinguished by looking at the thickness of the wall and the degree of collapse, with arteries retaining their rounded shape and having thicker vessel walls than veins.

After branching from *arteria brachialis* the other part of the vasculature of the legs, the one circulating the muscles in the proximal part of the leg, consists of a highly branched system of arteries and veins in the musculature. The vein to artery ratio was found to be approximately 5:1 (fig 18 and 19).

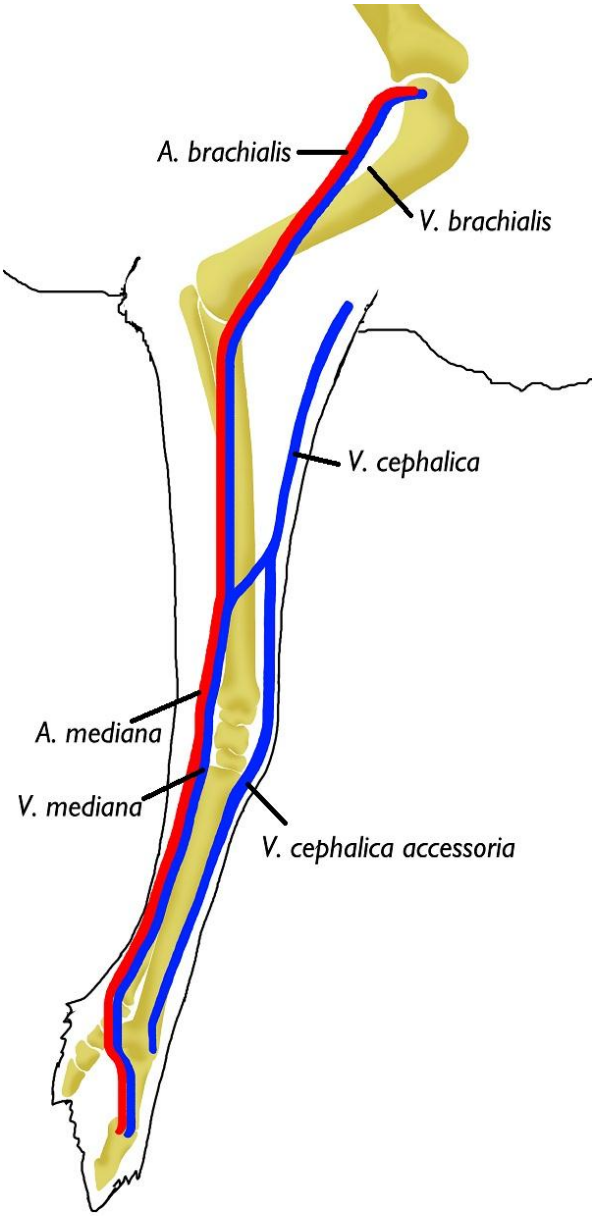

Fig 15: Venous vasculature in relation to the counter current arteries. *Vena brachialis* and *vena mediana* are counter current veins while *vena cephalica* and *vena cephalica accessoria* are superficial veins.

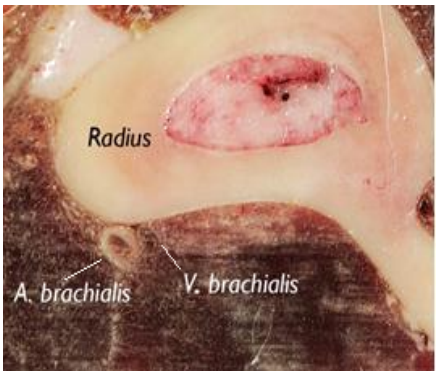

Fig 16: Close up of a part of cross section 4 (see fig 10), illustrating the close proximity of the two counter current vessels in the front leg of a reindeer.

The tissue composition of the front leg was also studied by dissection, cross section (fig 17) and by measuring tissue weights (table 2). And it is apparent from fig 17 that distal to the wrist the leg consists mostly of bone, skin, cartilage and tendons. Almost all of the muscle is found proximally to the wrist.

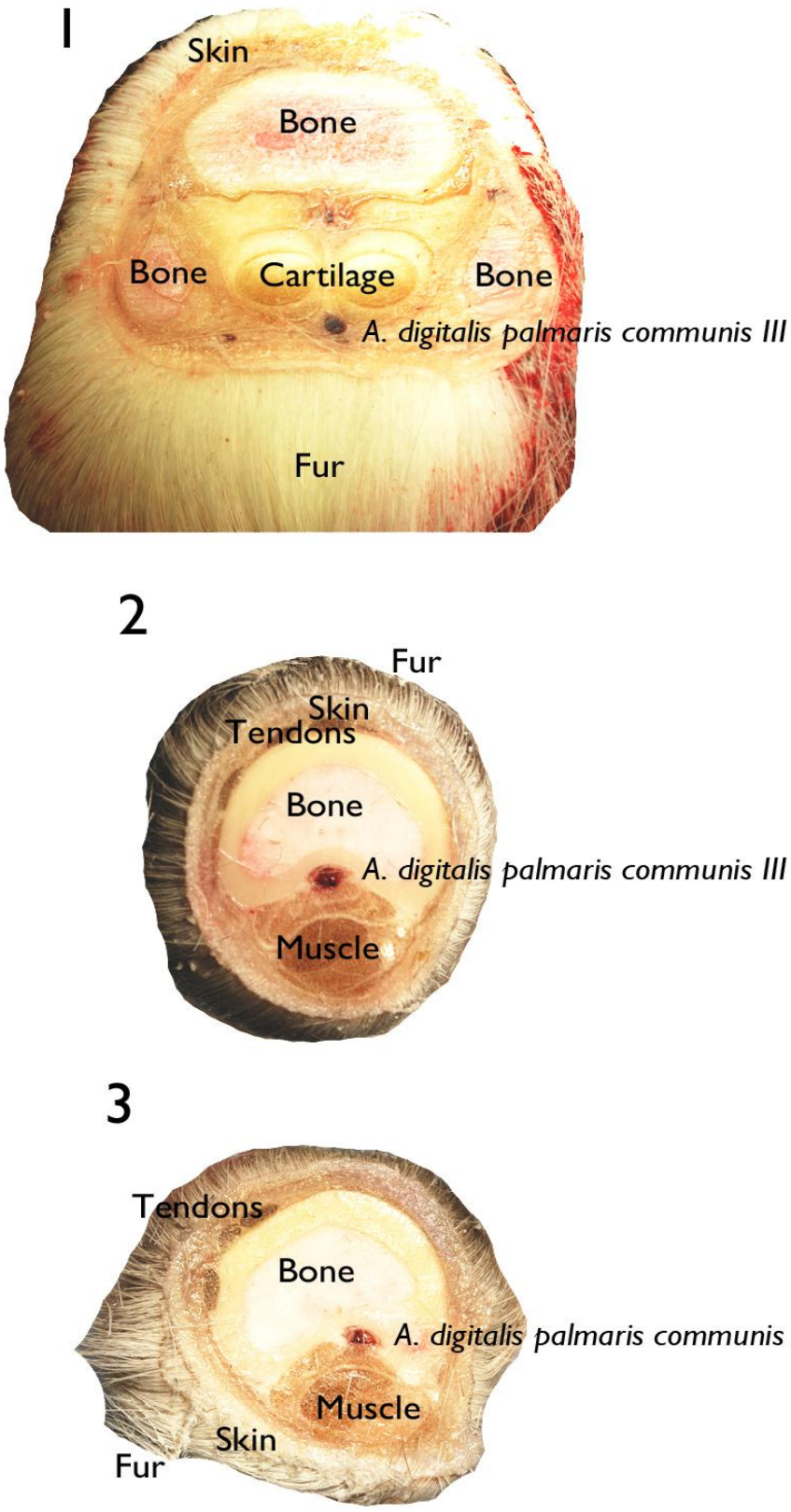

Fig 17: The tissue in the distal part of the reindeer front leg consists mainly of bone, tendons, skin and cartilage. See fig 10 for positions of the cross sections.

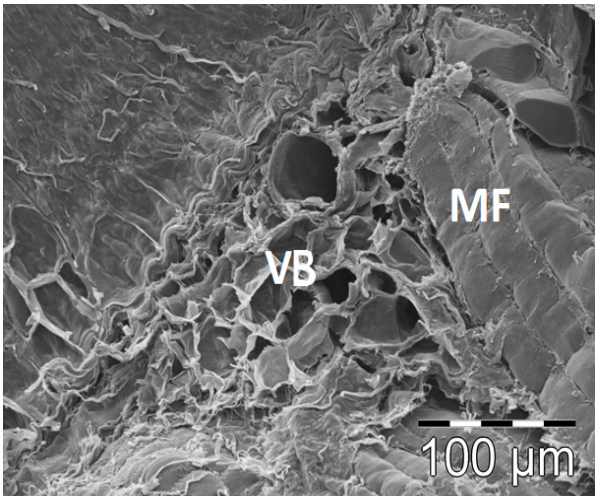

Fig 19: Electron microscopy picture of the vascular bundle (VB) found in the posterior flexor muscles of the front leg, MF = muscle fibers.

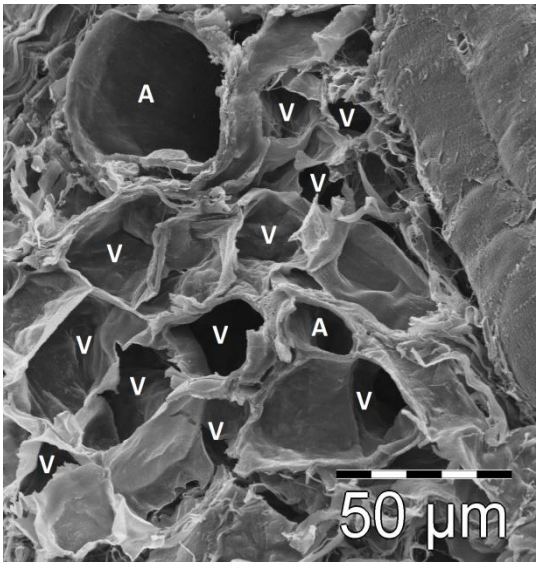

Fig 18: Close up of the vascular bundle in fig 18. Arteries are labeled "A" and veins "V".

Table 2: **Tissue weights in the front leg of the reindeer.**

Tissue type	Weight (g)	Weight (%)
Bone + hoof	905	48
Skin	207	11
Muscles and tendons	758	41
Only muscle	548	29
Total	1870	100

3.2 Subcutaneous temperature

Experiments done when the sedative Rompun had been used to sedate the animal during the insertion of the thermocouples gave results that could not be used since an unstable rectal temperature was observed. The rectal temperature was below normal at an ambient temperature (T_a) of -10°C and -30°C and above normal at T_a 20°C. They were unable to thermoregulate properly in spite of administration of antidote. When the animals were not sedated prior to experiments they had a steady body core temperature, 38,4 ± 0,3°C, that did not vary with ambient temperature.

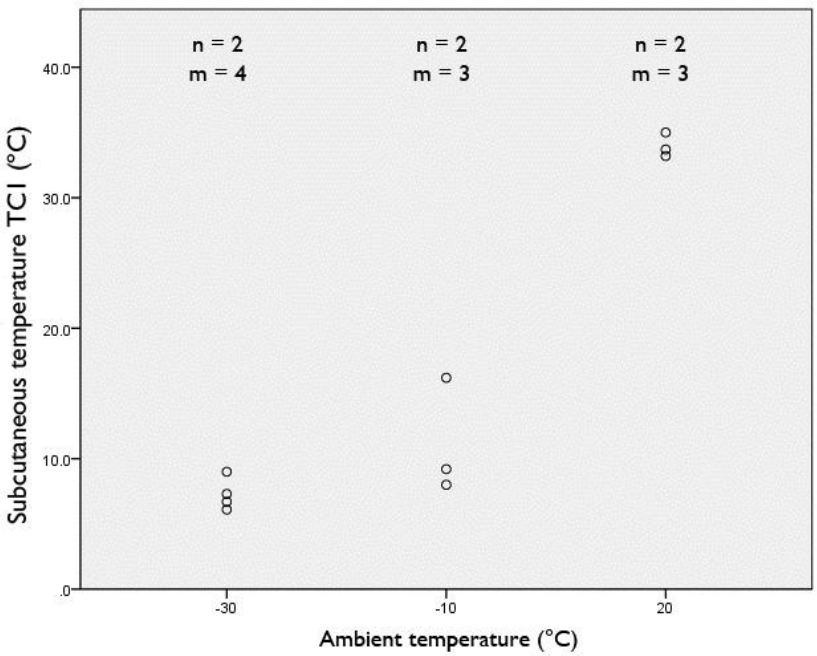

Fig 21: Lowest subcutaneous temperatures measured at steady state. "n" = number of animals, "m"= number of experiments. All data from TC1 (fig 10).

The data used for statistical analyses and graphs were retrieved from TC 1 (fig 10) and not the other two thermocouples. This was due to that there were many experiments where the reindeer managed to either destroy or pull out one or several thermocouples, either

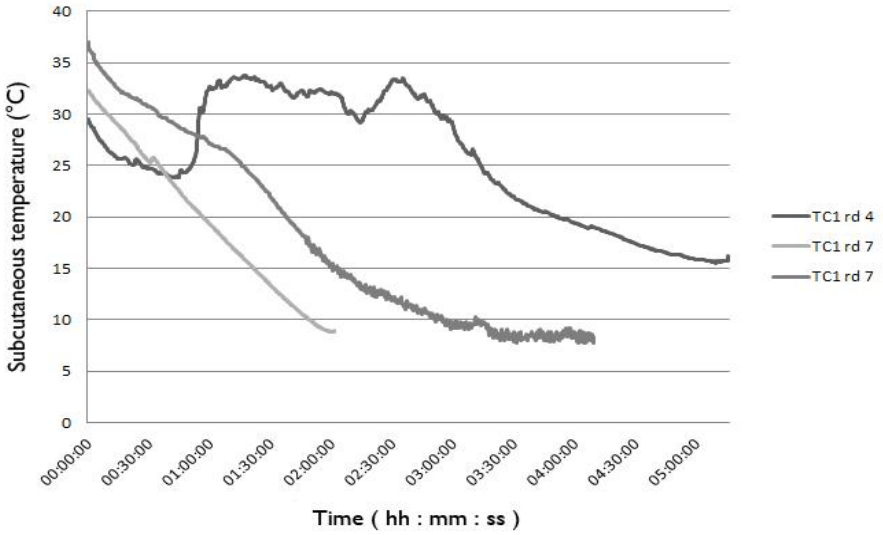

Fig 20: Results from three experiments done at T_a -10°C, illustrating the rate of subcutaneous temperature decrease. TC = thermocouple, rd = reindeer. T_a at the start of the experiments was 0°C, -10°C was reached after 20 minutes.

just from getting to a standing position after being held down or while the experiment was conducted and the animal shifted position in the restraining box. We got enough successful measurements of the subcutaneous temperature at the position of TC 1 to be able to do statistics and to draw consistent conclusions. There were no statistical difference between subcutaneous temperature at T_a -10°C as at -30°C (fig 20, 21 and 22). What we did see at T_a -30°C that was not observed at -10°C was rhythmic heating of the skin (fig 22). It was also clear that even if the subcutaneous end temperature was the same at T_a -10°C and -30°C, the time it took to reach this temperature differed. The average time for the animal to decrease the subcutaneous temperature by 10°C at T_a -10°C was approximately 50 min. (fig 21) At -30°C the same temperature change took on average approximately 25 min (fig 22). At T_a 20°C a steady high subcutaneous temperature was observed. The correlation coefficient between subcutaneous temperature and ambient temperature was 0,950, and it was significant, p-value <0,000.

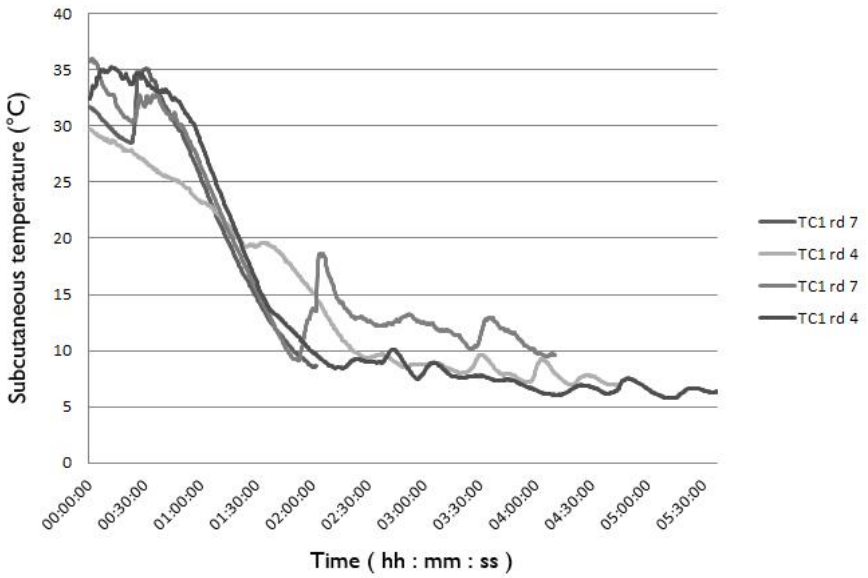

Fig 22: Results from four experiments done at T_a -30°C illustrating the rate of subcutaneous temperature decrease and subsequent rhythmical temperature changes. All temperatures measured by TC 1 (fig 10). TC = thermocouple, rd = reindeer.

3.3 Blood flow and heart rate

Reindeer no. 6 was euthanized while it still had the flow probe in place around the blood vessel. A dissection was made to confirm the location of the flow probe and the patency of the vessel. Pictures were taken to document the results and the position shown in fig 10 was confirmed. Since the flow probe was positioned distal to the branching off to the muscle circulation from *arteria brachialis*, measured brachial blood flow does not include blood flow to the muscle.

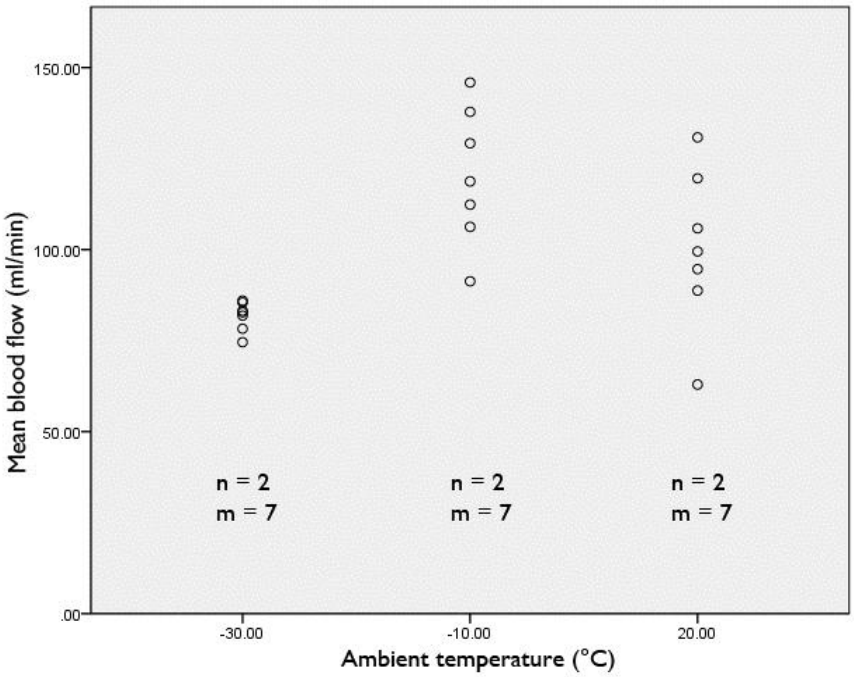

Fig 23: The mean blood flows of the measurements are indicated with circles. "n" = number of animals, "m" = number of experiments.

There does not seem to be a significant correlation between blood flow and ambient temperature, i.e. there is no significant straight line correlation of the blood flow between the different temperatures, p-value = 0,313 (table 4). But pairwise comparison between the temperatures shows that there is a statistical difference between mean blood flow at T_a -10°C and -30°C (table 5). F-tests for equal variance also show that the variances of blood flow mean at T_a -10C and 20°C is not statistically different, while both are significantly different from the variance at -30°C, p-value = 0,009 (testing equality of variance of blood flow at -10°C and -30°C) and p-value = 0,033 (testing equality of variance of blood flow at T_a 20°C and -30°C).

Table 3: **Summarized statistical data from the brachial blood flow experiments.**

Ambient temperature	N	mean brachial blood flow	SD	mean heart rate	SD
20°C	7	100,2921	21,96711	73,1429	17,17556
-10°C	7	120,226	18,91217	72,7755	10,33454
-30°C	7	81,7281	4,05248	79,7143	13,3104

Table 4: **Correlations between ambient temperature and mean brachial blood flow in two reindeer, controlling for individual effect and from which experiment the measurement came from.**

		Mean blood flow	Range of blood flow
Ambient temp	**Correlation**	-0,238	-0,454
	Sig.	0,313	0,044
Mean blood flow	**Correlation**	-	0,520
	Sig.	-	0,019

Table 5: **T-tests for equality of the mean blood flow at the three ambient temperatures, pairwise comparisons.**

T-test for equality of means of blood flow

Ambient temperature (°C)	t	sig	95% Confidence interval	
			lower	Upper
20 and -10 (equal variance)	-1,819	0,094	-43,80472	3,93705
20 and -30 (unequal variance)	2,199	0,067	-1,78033	38,90839
-10 and -30 (unequal variance)	5,266	0,001	20,96774	56,02799

When looking at the plot of the mean flows (fig 23) it is quite clear that there is an unequal variance. There seems to be a trend of flow being higher at -10°C than at the two other temperatures. Something that is further supported by the total mean blood flow for all measurements at the three ambient temperatures (table 5).

The range of blood flow (maximum value - minimum value) was significantly linearly correlated to the ambient temperature (table 3), what more is that the range is also significantly positively correlated to the mean (p=0,019). Which means that the higher the

mean, the higher the range. The flow traces from the experiments also presented the opportunity to look more closely at the heart rate at different ambient temperatures (fig 24).

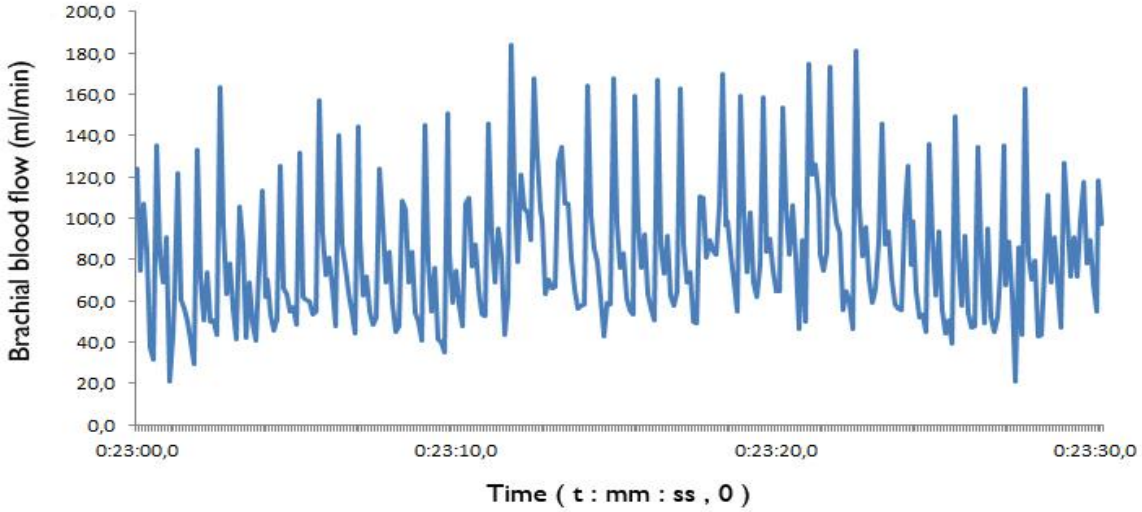

Fig 24: Typical example of pattern of brachial blood flow at T_a -30°C. The heart rate is easilly calculated from similar 30 second intervals.

These calculated heart rates was the basis of the statistical analyses of the relationship between heart rate and ambient temperature, and to calculate cardiac output of the animal based on the found heart rate and previously known stroke volume in reindeer (Timisjärvi 1978). What we found was that there was a significantly different mean heart rate at -30°C and the two other ambient temperatures while there was no significant difference between the mean heart rate at -10°C and 20°C (fig 25, table 3 and table 6).

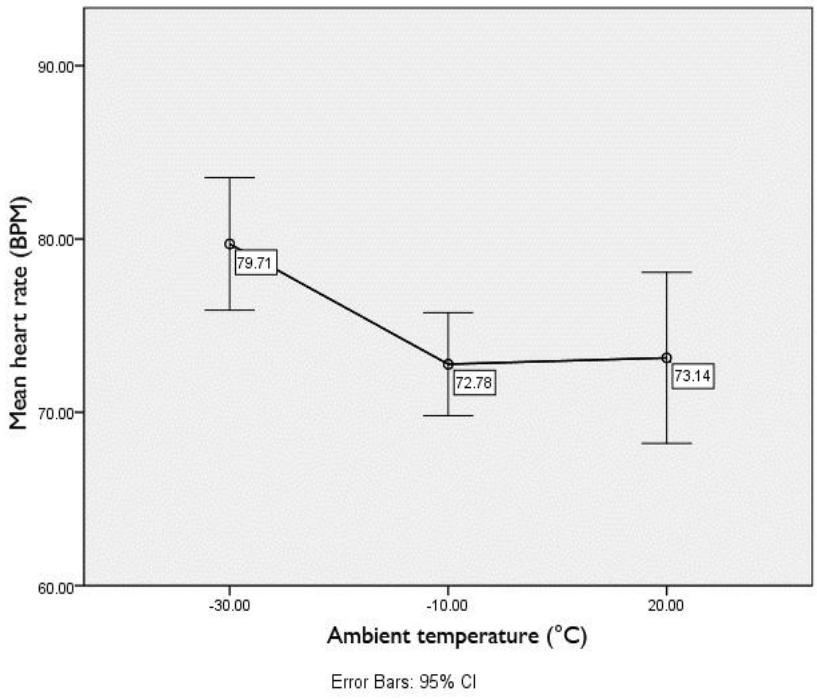

Fig 25: Mean heart rate of the two reindeer used in the brachial blood flow experiments at the three ambient temperatures.

Table 6: **T-tests for equality of mean heart rate at the three ambient temperatures, pairwise comparisons.**

T-test for equality of means of heart rate				
			95% Confidence interval	
Ambient temperature (°C)	**t**	**sig**	**lower**	**upper**
20 and -10 (unequal variance)	-0,128	0,898	-6,06745	5,33276
20 and -30 (unequal variance)	2,117	0,037	0,40474	12,73812
-10 and -30 (equal variance)	-2,822	0,005	-11,71731	-2,16024

A plot was made of the flow measurements averaged every 0,1 second versus time, including a trace of the ambient temperature versus time shows how the change in flow is delayed in relation to the change in ambient temperature (fig 26). The ambient temperature has already almost reached -30°C before the flow starts to drop. The larger variance at the two higher ambient temperatures are also clearly illustrated by this plot.

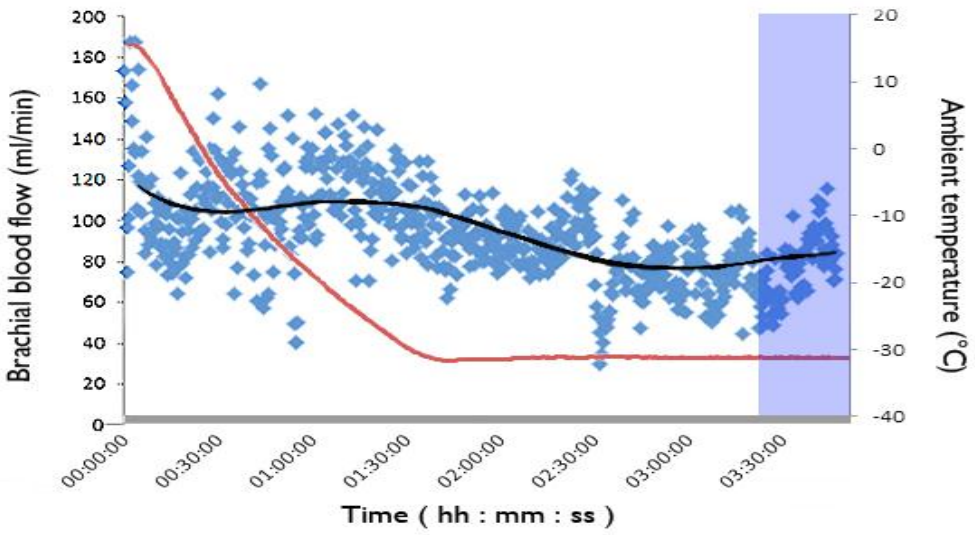

Fig 26: Brachial blood flow versus time and ambient temperature (T_a) versus time in relation to each other. The red line is the T_a and the black line is the trend line of the brachial blood flow. The blue box in the end indicates the measurements used as the basis of the statistics for this experiment.

It is worth noting that during the calibration with the brachial artery as calibration material, the distal part of the brachial artery, before the division into the *arteria radialis* and *arteria mediana*, was highly vasoactive. The first attempt to insert the silicone tube in the vessel, in the saline that held 18°C, led to a vasoconstriction of the vessel to such an extent that no opening in the vessel was observable. This did not happen when a more proximal portion of the vessel was used.

4. Discussion

Peripheral heterothermia is one of the important principles for thermoregulation in all endotherms, it is sometimes referred to as the core-shell concept (See for example Aschoff and Wever 1958). Heat is more readily lost from the extremities than the trunk of the body due to its larger area to volume ratio and poorer insulation (Johnsen *et al*, 1985), so to decrease this potential heat loss the reindeer allows its extremities to have a lower temperature than the rest of the body. Indeed it has several adaptations to low tissue temperature in the extremities, for example more unsaturated fatty acids in the bone marrow distally in the extremities (Meng *et al.* 1969). The peripheral heterothermia is accomplished by either reducing blood flow or by vascular counter current heat exchange, or a combination of both. As we have seen in the reindeer there is a relatively large area of possible heat exchange between the two counter current vessels, in spite of the lack of a more specialized structure. The lack of a more refined vascular arrangement could indicate that the regulation of blood flow is a very important mechanism for thermoregulation in the reindeer.

As seen in the results from the brachial blood flow experiments, there is a definite vasoconstriction at T_a -30°C, while there are higher flows at T_a -10°C and 20°C (fig 23). The highest mean blood flow was measured at T_a -10°C and still the same low subcutaneous temperature (6-8 °C) was observed (fig 20). At T_a -30°C on the other hand, it seems to be a combination of reduced blood flow and counter current heat exchange. It is remarkable that the flow is so much higher at T_a -10°C than at -30°C (approximately 50% higher), while the subcutaneous temperature is the same.

We must also keep in mind that the blood returning to the heart from the extremities has two alternatives. Either it takes the route through the superficial veins, *vena cephalica accessoria* and *vena cephalica*, and maximizes possible heat loss from the legs, or it flows back to the heart via the counter current vein, *vena mediana* (fig 15). When the animal is cold, a larger fraction, if not all, of the blood flow will presumably flow through the counter current vein.

It is feasible that the first line of defense, when ambient temperatures start to fall, is to decrease blood flow in the superficial veins, i.e. increase the efficiency of the counter current heat exchange system. This is done at the same time as the need for heat loss by panting is reduced, due to lower heat load and hence more blood can circulate the extremities instead of the tissue surrounding the airways. This is consistent with the difference in blood flow

measured in the carotid artery in reindeer at different ambient temperatures (unpublished observation, with permission, Blix, Walløe and Folkow). At T_a 0°C mean blood flow of approximately 5 ml·min^{-1}·kg^{-1} was observed in the left *arteria carotis*, while at 20°C the mean flow was approximately 7,5 ml·min^{-1}·kg^{-1}. The brain's metabolic need should not have increased, so the increase in blood flow is likely to go to the nose in order for the animal to loose heat by evaporation. In the calves used in the brachial blood flow experiments this is the equivalent of a change of approximately 130 ml·min^{-1} at that temperature interval. The difference in mean blood flow to one leg at T_a -10°C and 20°C is approximately 20 ml·min^{-1}, if we assume that the difference is the same in the hind legs as in the front legs, this adds up to 80 ml·min^{-1}, that no longer circulates the legs and instead can be used for evaporative heat loss in the airways. Although the lower T_a is not the same, it still illustrates the changes in blood flow to different parts of the body, and that there is reason to assume that the results from the brachial blood flow experiments are reasonable.

If the ambient temperature continues to fall, below T_a -10°C, a fall in brachial blood flow was observed. Perhaps the counter current heat exchange simply is not efficient enough to prevent excessive heat loss at lower T_a, and hence the blood flow to the front leg is decreased by vasoconstriction of *arteria brachialis*. The intense vasoconstriction observed in said artery during the calibration of the flow probe seems to indicate that this is an important part of the maintenance of the peripheral heterothermia, and hence the reduction of heat loss.

During the subcutaneous temperature measurements at T_a -30°C a phenomenon previously described for human skin blood flow "Lewis vasodilatory waves" (Lewis 1930), can be observed (fig 22). These waves are usually explained as consequences of rhythmical changes in blood flow and might be a mechanism to prevent freezing of the distal parts of the extremities. Although tissue temperatures measured during the experiments in this study was not even close to freezing, one must keep in mind that the temperature out in the distal part of the hoof might be several degrees lower than at the top of the hoof (see fig 10 for position of TC 1), and tissue freezing might be a potential risk.

From the temperature and anatomical results it seems implausible that the vascular bundle in the muscles on the posterior side of the front leg could be a *rete* for counter current heat exchange. There is no indication that the blood that flow through this area would continue down the leg to the hoof, and a counter current *rete* just for the muscle circulation seems unlikely. When looking at the artery to vein ratio, it seems like the veins

are outnumbering the arteries by at least 5 to 1, and the veins are not closely associated with the arteries, unlike the arrangement seen in heat exchange retia of for example marine mammals (Scholander and Schevill 1955). Instead I propose that the vascular bundle probably has more of a draining function, enabling heat to efficiently leave the working muscle during high levels of activity. Diverting this warm blood back to the body instead of out to the skin and distal parts of the extremities might seem to be counterproductive, but considering that the legs are rather well insulated and the high capacity of reindeer to pant (Blix and Johnsen 1983), it might be more efficient to direct the blood to the airways instead to enable heat loss through evaporation. Actually about 90% of heat loss in exercising winter insulated reindeer is through evaporation and convection combined and in winter insulated reindeer at rest at high ambient temperatures evaporative heat loss is >40% of total heat loss (Folkow and Mercer 1986). This is also consistent with the lower blood flow in the legs at T_a 20°C than at -10°C.

When looking at the tissue composition of the leg, one thing stands out; very little tissue with high metabolic needs are found, especially on the lower half of the leg. There are predominantly tissues with low metabolic needs such as tendons, cartilage, bone and skin. (fig 17 and table 2). This might indicate that instead of evolving an effective heat exchange system enabling continuous relatively high blood flow to tissues in the extremities, the amount of tissue demanding high blood flow has been reduced, allowing for low blood flow rates to be maintained. In addition, tissues that have lower temperature also have lower metabolic rate, this effect is called the Q10 effect (Schmidt-Nielsen 1990). So by first reducing tissue temperature by means of vascular counter current heat exchange, the metabolic needs of the tissue is reduced and hence the blood flow to the tissue can be reduced. This corresponds well to observed changes in subcutaneous temperature and brachial blood flow seen in the experiments.

When measuring blood flow in the extremities of a homeotherm, one has to keep in mind that stress or disturbances in any form greatly influences blood flow. Even in humand asignificant fall in blood flow was observed in a test person when he observed another person coming into the room next to his (Thoresen and Walløe 1980). In other words the stimulus does not even have to be particularly stressful in order for it to affect the blood flow. The typical response to alertness in man is however vasoconstriction in arteries in the extremities and this is found to be the case for the reindeer as well (unpublished observation, with permission, Blix, Walløe and Folkow). Disturbances of the animal at T_a -

30°C, when it is expected to be fully vasoconstricted, should not change the blood flow (fig 23). At the other two ambient temperatures on the other hand, this might affect the results, and it is therefore possible that the blood flow values at 20°C and -10°C in some instances may be low. This might also explain the differences seen in the range and variance of blood flow at T_a -10°C and 20°C compared with the range and variance of blood flow at -30°C.

The other disturbance that was observed for some of the reindeer during the measurement of subcutaneous temperature was that they maintained a high subcutaneous temperature regardless of the ambient temperature. These animals did not stand quietly in the restraining box but showed signs of restlessness and on occasion tried to get out of the restraining box. During the experiments however, the behavior of the animals was closely observed and if the animal was not standing quietly, the experiment was ended and the data were discarded. The high subcutaneous temperature observed during these experiments can thus be explained by the increased physical activity of the animals, which increases the need of heat loss and therefore increases the blood flow to the skin causing the skin temperature to remain high to prevent hyperthermia.

Timisjärvi *et al* (1984) have shown that the stroke volume in calves, ages 5-18 months and body weights of 18-44 kg, is on average 2,6 $ml \cdot kg^{-1}$and their mean heart rate was 50 beats per minute (bpm) with a range of 40-260 bpm. This data can be compared with the results from the 9-10 months old calves where mean heart rate were about 73 bpm at -10°C and 20°C and approximately 80 bpm at -30°C. The blood volume of the animals calculated from the data of Timisjärvi *et al* (1984) was found to be about 5,5 liters, and stroke volume about 135 ml. The average blood flow in the brachial artery was found to be 100 $ml \cdot min^{-1}$ (20°C), 120 $ml \cdot min^{-1}$ (-10°C) and 82 $ml \cdot min^{-1}$ (-30°C). Cardiac output for the reindeer used in the experiment should according to this be 9,9-10,8 $liters \cdot min^{-1}$. This seems rather much but is consistent with the findings of Timisjärvi *et al*. So assuming they were correct, only approximately 3-5% of the cardiac output circulates the distal parts of the four legs even at high ambient temperatures. Used for the unpublished data of Blix, Walløe and Folkow (with permission), the equivalent percentage of cardiac output to the head of the animal at T_a 20°C is almost 8%, while at T_a 0°C it is 5%. The increased blood flow to the head at high ambient temperatures, while blood flow decreases to the legs, yet again emphasizes the significance of the evaporative and convective heat loss from the airways.

The change in heart rate (fig 25) might be an indication that the reindeer used in the flow experiments were at their lower critical temperature, or even outside of their

thermoneutral zone. The lower critical of adult Eurasian tundra reindeer (*Rangifer tarandus tarandus*) has been found to be -30°C (Nilssen *et al* 1984), and considering that the reindeer used for the brachial blood flow experiments in this study were less than 1 year old, they may actually be below their lower critical temperature at T_a -30°C, something that corresponds well to the increased heart rate seen at this ambient temperature. This might also verify that the vasoconstriction observed in the reindeer at this ambient temperature is maximal, and the flow is determined by the combination of metabolic needs of the tissue and the need to prevent freezing.

5. Conclusion

When the winter insulated reindeer is experiencing the relatively high ambient temperature of 20°C, it is likely that it will circulate the superficial veins (*vena cephalica* and *vena cephalica accessoria*). As the ambient temperature decreases, and the animal is experiencing an ambient temperature somewhere in the lower part of the thermoneutral zone of the animal, -10°C, the heat loss seems to be reduced mainly by peripheral heterothermia through changing circulatory pattern towards the counter current vein (*vena mediana*), since blood flow at this temperature is high and the skin temperature is low. When temperatures drop further, to -30°C, flow starts to decrease, but the skin temperature remains at the same low level found at -10°C. The initial drop in subcutaneous temperature seen in the reindeer seems to be primarily an effect of the changed circulatory pattern enabling effective vascular heat exchange, while decreasing blood flow appears to be a secondary mechanism that does not contribute to reducing heat loss until the subcutaneous temperature is already decreased. This means that the peripheral heterothermia is achieved by in the first step, vascular counter current heat exchange and is then upheld and augmented by low blood flow.

References

Anonymous, (1997). CardioMed, medical volume flowmeter, physiological TraCe system, Medi.Stim AS,Oslo, Norway

Aschoff, J. and Wever, R. (1958). "Kern und Schale im Wärmehaushalt des Menschen." Naturwissenschaften **45**(20): 477-485.

Blix, A. S. (1989). Arctic resignation: Winter dormancy without hypothermia. Living in the cold II. Malan, A. and Canguilhem, B. Montrouge, John Libbey Eurotext. Ldt.**:** 117-119.

Blix, A. S. and Johnsen, H. K. (1983). "Aspects of nasal heat exchange in resting reindeer." Journal of Physiology **340**: 445-454.

Folkow, L. P. and Mercer, J. B. (1986). "Partition of heat loss in resting and exercising winter- and summer-insulated reindeer." American Journal of Physiology **251**. 32-40

Henttonen, H. and Tikhonov, A. (2008). "Rangifer tarandus. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4.". Retrieved 22 March, 2011.

Irving, L. and Krog, J. (1955). "Temperature of skin in the arctic as a regulator of heat." Journal of Applied Physiology **7**(4): 355-364.

Johnsen, H. K., Blix, A. S., Jorgensen, L. and Mercer, J. B. (1985b). "Vascular basis for regulation of nasal heat exchange in reindeer." Am J Physiol Regulatory Integrative Comp Physiol **249**: 617-623.

Johnsen, H. K., Rognmo, A., Nilssen, K. J. and Blix, A. S. (1985a). "Seasonal changes in the relative importance of different avenues of heat loss in resting and running reindeer." Acta physiol. scand. **123**: 73-79.

Larsen, T. S., Nilsson, N. Ö. and Blix, A. S. (1985). "Seasonal changes in lipogenesis and lipolysis in isolated adipocytes from Svalbard and Norwegian reindeer." Acta Physiologica Scandinavica **123**(1): 97-104.

Lewis, T. (1930). "Observations upon the reactions of the vessels of the human skin to cold." Heart **15**: 177-208.

Mathiesen, S. D., Mackie, R. I., Aschfalk, A., Ringø, E. and Sundset, M. A. (2005). Microbial ecology of the digestive tract in reindeer: seasonal changes. Microbial Ecology in Growing Animals. Holzapfel, W. H. and Naughton, P. J., Elsevier Limited. **2:** 75-102.

McDowell, E. M. and Trump, B. F. (1976). "Histologic Fixatives suitable for diagnosticlight and electron microscopy." Arch Pathol Lab Med **100**.

Meng, M. S., West, G. C. and Irving, L. (1969). "Fatty acid composition of caribou bone marrow." Comparative Biochemical Physiology **30**: 187-191.

Midtgård, U. (1980). "Blood vessels in the hind limb of the mallard (*Anas platyrhynchos*): anatomical evidence for a sphincteric action of shunt vessels in connection with the arterio-venous heat exchange system." Acta Zool. **61**: 39-49.

Mootie, I. (1955). "The thermal insulation of caribou pelts." Textile Research Journal **25**: 832-837.

Nilssen, K. J., Sundsfjord, J. A. and Blix, A. S. (1984). "Regulation of metabolic rate in Svalbard and Norwegian reindeer." American Journal of Physiology **247**: 837-841.

Olsson, E. A. M. (2008). Countercurrent vascular heat exchange in the extremities of reindeer. Department of Arctic Biology. Tromsø, University of Tromsø. **Bachelor**.

Pabst, D. A., Rommel, S. A., McLellan, W. A., Williams, T. M. and Rowles, T. K. (1995). "Thermoregulation of the intra-abdominal testes of the bottlenose dolphin (Tursiops truncatus) during exercise." Journal of Experimental Biology **198**(1): 221-226.

Rommel, S. A. and Caplan, H. (2003). "Vascular adaptations for heat conservation in the tail of Florida manatees (Trichechus manatus latirostris)." Journal of Anatomy **202**(4): 343-353.

Røed, K. H. (2007). "Taksonomi og opprinnelse til rein." Rangifer **12**: 17-20.

Schmidt-Nielsen, K. (1990). Animal Physiology. Cambridge, Cambridge University Press.

Scholander, P. F. and Krog, J. (1956). "Countercurrent heat exchange and vascular bundles in sloths." Journal of Applied Physiology **10**: 405-411.

Scholander, P. F. and Schevill, W. E. (1955). "Countercurrent vascular heat exchange in the fins of whales." Journal of Applied Physiology **8**: 279-282.

Thoresen, M. and Walløe, L. (1980). "Skin blood flow in humans as a function of environmental temperature measured by ultrasound." Acta Physiol. Scand. **109**: 333-341.

Timisjärvi, J. (1978). "Left ventricular volumes and functioning of the reindeer heart." Basic Research in Cardiology **73**(4): 355-364.

Timisjärvi, J., Nieminen, M. and Sippola, A.-L. (1984). "The structure and insulation properties of the reindeer fur." Comparative Biochemistry and Physiology Part A: Physiology **79**(4): 601-609.