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Review

Physiology of the European brown bear (*Ursus arctos arctos*)

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Although the American black (*Ursus americanus*) and American brown (grizzly) (*Ursus arctos horribilis*) bears have been the subjects of intensive physiological research, very little so far, has been done to understand the physiology of the European brown bear (*Ursus arctos arctos*). In our laboratory the research on the physiology of European brown bears started 10 years ago. Between 1987 and 1997, the seasonal changes in physiological indicators such as body temperature (Tb) and blood chemistry were studied in 12 bears under captive conditions in the Zoological Garden of the Department of Biology, University of Oulu, Finland. Changes in the Tb were measured either by using telemetric transmitters or dataloggers implanted into the abdominal cavity under anesthesia. The results show that the Tb decreases during the winter sleep to 3–5°C below the normal level or 37.0–37.5°C. Our bears only slept for just over three months per winter. The winter sleep started in late November/early December and ended in late February/early March. Our results do not support the theory of universal hibernation induction. Plasma from a winter-sleeping bear after injection either into Djungarian hamster (*Phodopus sungorus*) or laboratory rat did not affect body temperature or diurnal activity of the recipient. The urea concentration during the denning period is only half of that observed in the summer. This decrease during the denning period is in agreement with studies performed on American black and grizzly bears. In sharp contrast to earlier studies performed on the American black bear, our results reveal increases and decreases in the concentration of amino acids supporting the view that the denning bear may be even able to synthesize essential amino acids. The analysis of the total plasma fatty acids show that there may be a trend towards a greater need of unsaturated fatty acids in the denning bear as is the case of true hibernators.

1. Introduction

Although intensive research has been focused on understanding the physiology of the American black bear (*Ursus americanus*) and the American brown (grizzly) bear (*Ursus arctos horribilis*) (e.g. Hock 1960, Erickson & Youatt 1961, Seal *et al.* 1967, Pearson & Halloran 1972, Nelson *et al.*

1973, 1975, Lundberg *et al*. 1976, Matula *et al.* 1980, Brannon 1985ab, Schroeder 1987, Franzmann & Schwartz 1988, Storm *et al.* 1988, DelGiudice *et al*. 1991, Hellgren *et al*. 1993), only a limited amount of data were reported on the physiology of the European brown bear (*Ursus arctos arctos*) (Couturier 1954, Seal *et al*. 1967, Bush *et al.* 1980, Jamnicky *et al*. 1987, Hissa *et al*. 1992, 1994, Mominoki *et al*. 1996). So far, no physiological studies have been carried out on the seasonal changes in the metabolism, heart rate and respiratory rate of the European brown bear either.

In Finland the denning period of the European brown bear starts in October–November and lasts until April–May. Recording of the respiratory quotient (RQ) has shown that fat is the only substrate utilized to fulfil the energy requirement in the maximally 6–7-month long winter sleep. An RQ as low as 0.62 was measured in the American black bear (Nelson *et al*. 1973). The bear do not experience a change in lean body mass while utilizing fat reserves (Nelson *et al*. 1975, Lundberg *et al.* 1976). In late summer the feeding rate is two to three times above the normal level. As a result the body weight may increase 30–35% above the normal level. Just prior to entering the winter sleep bears become anorectic, ie. they stop feeding, empty their stomach and intestine. The loss of body weight during the winter sleep due to utilization of fat reserves is 250–500 g per day. Winter-sleeping bears do not eat, drink, defecate or urinate.

In our study we endeavored to use the same captive bears year after year (e.g. Hissa *et al.* 1992, 1994 and R. Hissa, M. Puukka & E. Hohtola unpubl., R. Hissa, E. Hohtola, T. Tuomola-Saramäki, T. Laine & H. Kallio unpubl.). The resulting variability should thus be taken into account when evaluating the data for age and season. In studies on black and grizzly bears in the United States, bears were raised in captivity or captured from the wild. Wild animals were either stunned by a shot from a helicopter or caught in different traps (e.g. Pearson & Halloran 1972, Matula *et al.* 1980, Nelson *et al.* 1984, Brannon 1985ab, Schroeder 1987). It is clear that the blood values of a bear under heavy stress may differ from those of captive bears (Matula *et al.* 1980). A captive bear is used to being close to humans, so anesthetizing such an animal and handling it from time to time are not particularly stressful events. That is why research using bears that live continuously in a standard captive environment and whose age structure is well known will most likely give more accurate information about the basic physiology of the bear than research dealing with wild, stressed animals. In studies on winter sleep of captive bears it is also essential that the animals are able to spend the winter in dens where the conditions follow the changes in temperature of the environment.

2. Material and methods

2.1. Animals and anesthesia

In our investigations, altogether 12 European brown bears were studied under captive conditions in the years between 1988 and 1997 in the Zoological Garden of the Department of Biology, University of Oulu (65°N, 25°24´E). Some of the bears were obtained from the Zoological Garden in Ranua, 150 km NE of Oulu, and some animals were raised in Oulu, having been found as cubs after the mother had been shot. The age and weight of the bears used in our studies are presented in Table 1. The bears were denned either in a concrete cylinder (0.9 × 1.4 m) or in a wooden den (0.8 × 1.5 m), both without windows. Loose hay was offered for the bedding material. One of the bears (No. 7) gave birth to three cubs in January 1995.

For operations and for the purpose of collecting blood samples two different delivery methods were used for intramuscular injections to immobilize the animals. The first method used an injection rifle (Paxarms, Timaru, New Zealand, Mark 24A, calibre 0.527) with 5 to 6.5-ml syringes and 12 gauge needles (45 mm) fired at a distance of 2–15 m. The maximum drug volume used was 4.5–5.0 ml for a single shot. This system was later replaced by a Dan-inject CO_2-pneumatically operated injection rifle (Model IM, Börkop, Denmark) equipped with 5.0-ml syringes and collared needles (2.0 × 40 mm) (Hissa *et al*. 1994). The latter rifle proved to be a more gentle way of immobilizing the animal at relatively short distances. Before injection and during the induction phase, disturbance to the bear was minimized and the lowest possible pressures were used when darting. During the induction phase, the bear remained calm and peaceful in the enclosures (20 × 25 m) after the immediate response to shot.

The anesthetics used were ketamine hydrochloride (Ketalar^R, Parke Davis) and medetomidine hydrochloride (Domitor^R, Lääkefarmos, Finland). The doses are shown in Table 1. The immobilization time usually lasted 30–50 min, after which additional doses were needed. Atipamezol (Lääkefarmos, Finland) was used to achieve a more rapid reversal. Half of the antagonist was given intramuscularly and the other half as a subcutaneous injection. In general,

Table 1. Age, body weight (BW), sampling dates and drug concentrations of the European brown bear (Hissa *et al*. 1994 and present study). Abbreviations: De = denning bear, P = pregnant, N = nursing period, D = domitor, and K = Ketalar. For further information, see text.

Sex	Age (yr + mth)	BW (kg)	Date	Drugs
1. Male	1 + 11	122	25.11.1988	D 82 µg/kg + K 2.7 mg/kg
	2 + 3	155	18.04.1989	D 70 µg/kg + K 2.8 mg/kg
	2 + 10	185	29.11.1989	D 119 µg/kg + K 2.2 mg/kg
	3 + 2	139	16.03.1990 (De)	D 94 µg/kg + K 2.5 mg/kg
	3 + 4	155	10.05.1990	D 103 µg/kg + K 2.0 mg/kg
2. Female	10 + 10	168	07.11.1990	D 86 µg/kg + K 2.0 mg/kg
3. Female	1 + 11	108	10.12.1990	D 102 µg/kg + K 2.9 mg/kg
4. Female	1 + 11	109	17.12.1990	D 102 µg/kg + K 2.4 mg/kg
5. Male	1 + 2	90	17.01.1991 (De)	D 78 µg/kg + K 2.0 mg/kg
6. Female	1 + 2	88	17.01.1991 (De)	D 80 µg/kg + K 2.0 mg/kg
7. Female	2 + 2	80	21.03.1991 (De)	D 113 µg/kg + K 3.4 mg/kg
	2 + 8	117	03.09.1991	D 93 µg/kg + K 2.4 mg/kg
	2 + 10	131	05.11.1991	D 99 µg/kg + K 2.8 mg/kg
	2 + 10	132	25.11.1991	D 95 µg/kg + K 2.9 mg/kg
	3 + 1	110	12.02.1992 (De)	D 89 µg/kg + K 2.7 mg/kg
	3 + 2	103	10.03.1992 (De)	D 117 µg/kg + K 3.3 mg/kg
	3 + 4	113	12.05.1992	D 117 µg/kg + K 3.1 mg/kg
	4 + 6	120	08.06.1993	D 98 µg/kg + K 3.1 mg/kg
	4 + 11	141	13.12.1993	D 94 µg/kg + K2.8 mg/kg
	5 + 2	117	08.03.1994 (De)	D 120 µg/kg + K 3.3 mg/kg
	5 + 3	131	28.04.1994	D 111 µg/kg + K 3.3 mg/kg
	5 + 6	123	13.07.1994 (P)	D 134 µg/kg + K 4.0 mg/kg
	5 + 9	164	06.10.1994 (P)	D 104 µg/kg + K 3.1 mg/kg
	5 + 10	160	22.11.1994 (P)	D 109 µg/kg + K 3.3 mg/kg
	6 + 2	115	22.03.1995 (De)	D144 µg/kg + K 3.6 mg/kg
	6 + 5	133	06.06.1995 (N)	D 125 µg/kg + K 3.2 mg/kg
	6 + 8	142	29.09.1995	D 120 µg/kg + K 3.1 mg/kg
	6 + 10	151	30.11.1995	D 119 µg/kg + K 3.3 mg/kg
	7 + 1	–	29.02.1996 (De)	D 136 µg/kg + K 3.6 mg/kg
	7 + 4	113	28.05.1996	D 158 µg/kg + K 4.0 mg/kg
	7 + 8	145	04.09.1996	D 117 µg/kg + K 3.1 mg/kg
	7 + 10	147	04.12.1996	D 112 µg/kg + K 3.4 mg/kg
	8 + 1	122	26.02.1997 (De)	D 139 µg/kg + K 3.7 mg/kg
	8 + 4	131	15.05.1997	D 125 µg/kg + K 3.6 mg/kg
8. Male	1 + 4	87	24.05.1993	D 78 µg/kg + K 2.3 mg/kg
	1 + 10	127	25.11.1993	D 94 µg/kg + K 3.1 mg/kg
	2 + 1	109	22.02.1994 (De)	D 114 µg/kg + K 2.8 mg/kg
	2 + 2	105	14.03.1994 (De)	D 114 µg/kg + K 2.9 mg/kg
	2 + 4	132	03.05.1994	D 80 µg/kg + K 2.0 mg/kg
	2 + 6	155	13.07.1994	D 84 µg/kg + K 2.1 mg/kg
	2 + 9	168	06.10.1994	D 101 µg/kg + K 2.5 mg/kg
	2 + 10	163	22.11.1994	D 107 µg/kg + K 3.2 mg/kg
	3 + 1	127	23.02.1995 (De)	D 124 µg/kg + K 3.3 mg/kg
	3 + 3	143	13.04.1995	D 101 µg/kg + K 2.7 mg/kg

(Continues …)

Table 1. Continued.

Sex	Age (yr + mth)	BW (kg)	Date	Drugs
9. Male	1 + 4	104	24.05.1993	D 73 µg/kg + K 2.2 mg/kg
	1 + 11	156	09.12.1993	D 83 µg/kg + K 2.5 mg/kg
	2 + 1	131	22.02.1994 (De)	D 121 µg/kg + K 2.8 mg/kg
	2 + 2	127	14.03.1994 (De)	D 114 µg/kg + K 2.9 mg/kg
	2 + 4	150	03.05.1994	D 167 µg/kg + K 2.4 mg/kg
	2 + 6	160	13.07.1994	D 133 µg/kg + K 3.9 mg/kg
	2 + 9	193	06.10.1994	D 140 µg/kg + K 3.4 mg/kg
	2 + 10	178	22.11.1994	D 103 µg/kg + K 3.1 mg/kg
	3 + 3	162	13.04.1995	D 103 µg/kg + K 2.8 mg/kg
10. Male	0 + 8	63	29.09.1995	D 87 µg/kg + K 2.6 mg/kg
	0 + 10	85	30.11.1995	D 106 µg/kg + K 2.8 mg/kg
	1 + 1	64	29.02.1996 (De)	D 114 µg/kg + K 3.3 mg/kg
	1 + 4	99	28.05.1996	D 110 µg/kg + K 3.4 mg/kg
	1 + 8	139	04.09.1996	D 131 µg/kg + K 4.0 mg/kg
	1 + 11	145	04.12.1996	D 134 µg/kg + K 3.0 mg/kg
	2 + 1	127	26.02.1997 (De)	D 110 µg/kg + K 3.0 mg/kg
	2 + 4	154	15.05.1997	D 97 µg/kg + K 2.9 mg/kg
11. Female	0 + 8	55	29.09.1995	D 45 µg/kg + K 1.4 mg/kg
	0 + 10	63	30.11.1995	D 79 µg/kg + K 2.4 mg/kg
	1 + 1	47	29.02.1996 (De)	D 91 µg/kg + K 2.7 mg/kg
	1 + 4	55	28.05.1996	D 100 µg/kg + K 3.0 mg/kg
	1 + 8	89	11.09.1996	D 90 µg/kg + K 2.7 mg/kg
	1 + 11	92	04.12.1996	D 103 µg/kg + K 3.2 mg/kg
	2 + 1	75	26.02.1997 (De)	D 113 µg/kg + K 3.3 mg/kg
	2 + 4	86	15.05.1997	D 116 µg/kg + K 3.1 mg/kg
12. Female	0 + 8	55	29.09.1995	D 90 µg/kg + K 2.7 mg/kg
	0 + 10	62	30.11.1995	D 81 µg/kg + K 2.4 mg/kg
	1 + 1	49	29.02.1996 (De)	D 90 µg/kg + K 2.5 mg/kg
	1 + 4	62	29.05.1996	D 97 µg/kg + K 2.7 mg/kg
	1 + 8	91	11.09.1996	D 88 µg/kg + K 2.6 mg/kg
	1 + 11	95	04.12.1996	D 100 µg/kg + K 3.0 mg/kg
	2 + 1	82	26.02.1997 (De)	D 128 µg/kg + K 3.4 mg/kg
	2 + 4	93	15.05.1997	D 97 µg/kg + K 2.9 mg/kg

the average time necessary to regain coordinated walking was 15–20 min at an average total dosage of 277 mg/kg of atipamezol.

2.2. Recording the body temperature

The body temperature (Tb) of the bear was measured either by using telemetric transmitters (CTT85-LA or L-M-disc or VHF-T-1, Mini-Mitter Co., Sunriver, Oregon) or by using dataloggers (Hobo-Temp, thermistor accuracy ± 0.25°C or StowAway™ XTI, Onset Computer Corporation, MA, USA). The data of the transmitters were collected using portable receivers (CTR86 or TRC-220, Radio Shack or Telonics, AZ, USA). The CTR86-receiver was connected to a data-acquisition system (Dataquest III, DataSciences Inc., St. Paul, Minnesota) on an IBM XT computer. Launching and reading out of the Hobo-loggers were done using BoxCar® or LogBook® programs for Windows (Onset Computer Corp., MA, USA). The telemetric transmitters were calibrated in water bath before implantation. The transmitters or loggers were covered by a paraffin and Elvax coating. The devices were implanted into the abdominal cavity. The den and air temperatures were measured using Hobo-Temp dataloggers. The microphone inside the den was connected to a video taperecorder to record the noises from the den and to record and store all movements.

2.3. Hibernating induction trigger (HIT)

To study whether the denning bear has a HIT as suggested e.g. by Ruit *et al*. (1987) and Bruce *et al.* (1990) plasma from winter-sleeping bear was injected into the Djungarian

hamster and laboratory rat. Body temperature and activity were measured using intraperitoneally implanted miniature transmitters (Model VM-FH in hamsters and Model XM-FH in rats, Mini-Mitter Co., Sunriver, Oregon). The receivers (Model RA-1000-THA, Mini-Mitter Co.) were placed underneath the cages. The temperature and activity data from the receivers were collected on an IBM XT computer using a special interplace board and data-acquisition software (Dataquest, Data Sci. Inc. St. Paul, MN). The sampling interval was 5 minutes. (For more details see Karjalainen *et al.* 1994.)

2.4. Sampling and analytical methods

Blood samples were collected from the jugular vein into evacuated tubes containing either EDTA or Lithium heparin or into clot tubes. Blood samples were immediately centrifuged and the plasma stored at – 70°C until being analyzed. Clotted samples were centrifuged within one hour after collection.

Whole blood samples for leukocyte count (WBC), erythrocyte count (RBC), and mean corpuscular hemoglobin (MHC) were measured by a hematological cell counter (Technicon H). RBC and MHC values were used to calculate packed cell volume (Hct %), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and corpuscular hemoglobin. Since bear RBC volumes are not the same as in humans (about 10–15% smaller), the method developed for humans may lead to an error of 5% difference between calculated and real Hct%. Our results, however, are not corrected accordingly (Hissa *et al.* 1994). A list of hematological and biochemical analysis is given in Table 2.

Plasma amino acids and other nitrogen compound contents were measured by using ion-exchange chromatography (Biochrom20 amino acid Analyzer, Cambridge, England) as described by R. Hissa, M. Puukka and E. Hohtola (unpubl.). The annual changes in the major plasma fatty acids were analyzed gas chromatographically as described by R. Hissa, E. Hohtola, T. Tuomola-Saramäki, T. Laine and H. Kallio (unpubl.).

3. Changes of body temperature (Tb) and body weight (BW) during denning and nursing

According to Hock (1957), Folk (1967), and Watts *et al.* (1981), the Tb of black and grizzly bears decreases 5–7°C during the winter sleep. The heart rate declines by 80% and the metabolic rate by as much as 35–75% (Folk 1967, Folk *et al.* 1972, Nelson 1973).

As described by Hissa *et al.* (1994), during the denning period the Tb of the European brown bear decreases on the average as much as in the black bear, or from the normal Tb of + 37–37.5°C by 3–5°C (see Fig. 1A). When the Tb of a female bear giving birth was monitored an interesting and different result was seen. The Tb of the pregnant female was abnormally high before it gave birth to three cubs in 1995 (Fig. 1B). However, just prior to delivery (26 January 1995) the Tb was already on a decline, and the decline continued for some time after the bear had given birth, but the decline stopped at about 34.5–35.0°C. The Tb of a nursing black bear also does not fall as low as the Tb of non-nursing black bears (Maxwell *et al.* 1988, Hellgren *et al.* 1990). It is evident that a higher Tb together with nursing use up a large

Table 2. List of hematological and biochemical analysis of the European brown bear (present work and Hissa *et al.* 1994).

Hematology
White blood cell count (WBC)
WBC differential
Red cell count (RBC)
Hemoglobin (Hb)
Hematocrit (Hct%)
Mean cellular volume (MCV)
Mean cellular hemoglobin (MCH)
Mean cellular hemoglobin concentration (MCHC)
Red cell width (RDW)
Platelet concentration
Blood chemistry
Aldosterone
Alanine aminotransferase (ALAT)
Amino acids
Aspartate aminotransferase (ASAT)
Calcium
Catecholamines
Cholesterol
Creatinine
Creatinine kinase
Free fatty acid (FFA)
Glucose
Glycerol
High density lipoproteins (HDL)
Melatonin
Major fatty acids
Opiates
Parathormone (PTH)
Phosphorus
Thyroid hormones
Very low density lipoproteins (VLDL)
Vitamin D_3
Urea
Uric acid

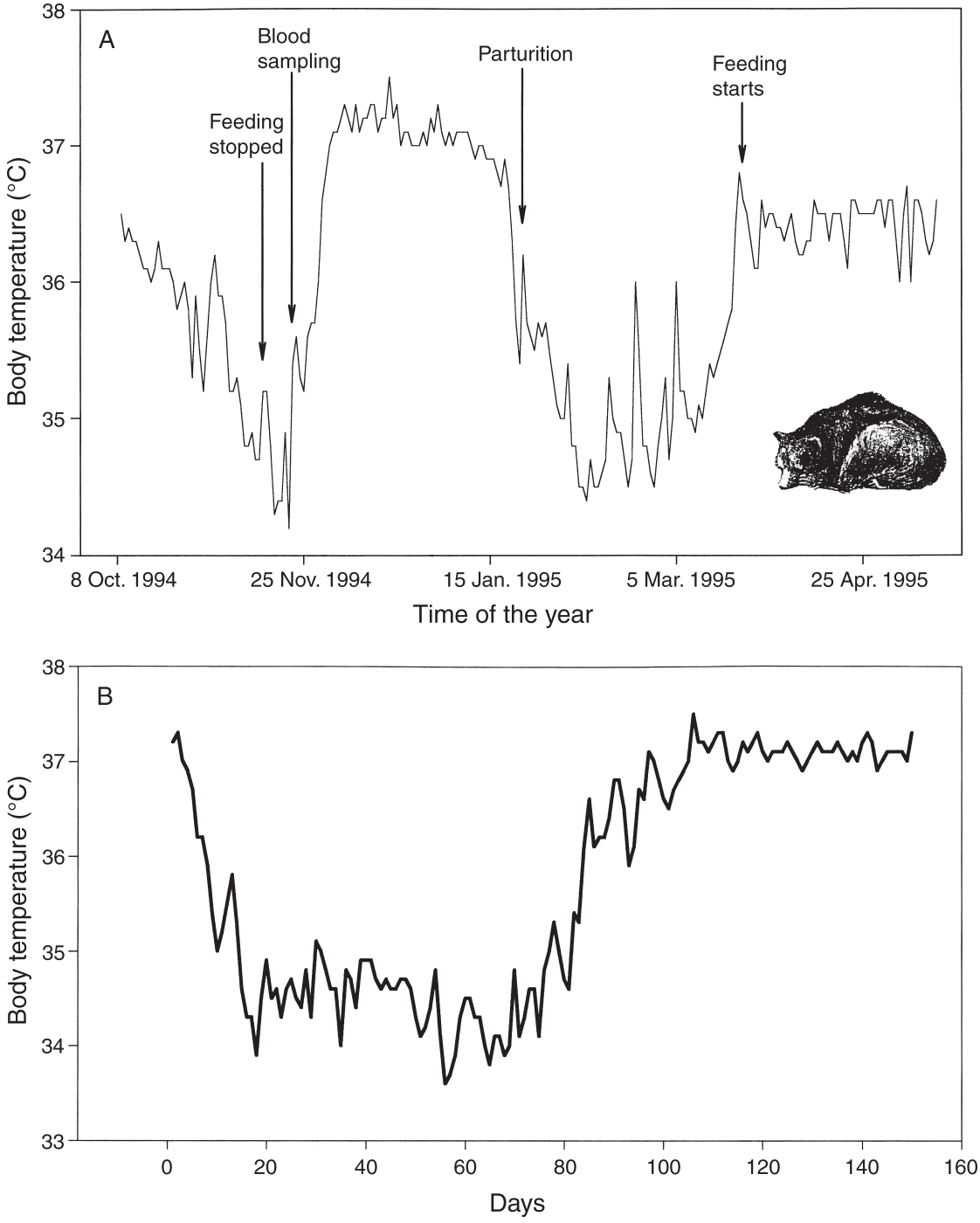

Fig. 1. Body temperature changes in one female (No. 7) before, during, and after parturition (winter 1994/95) (A) and one year later (winter 1995/96: Time scale started on 1 Dec. 1995 and ended on 28 April 1996) (B). (J. Siekkinen & R. Hissa, unpubl.)

part of the energy reserve of the female bear during the winter. Due to these factors a female bear after it had given birth and nursed her cubs lost 45 kg of its BW in 119 days (378 g/d on average). In winter 1993/94 it had lost 24 kg in 84 days (286 g/day).

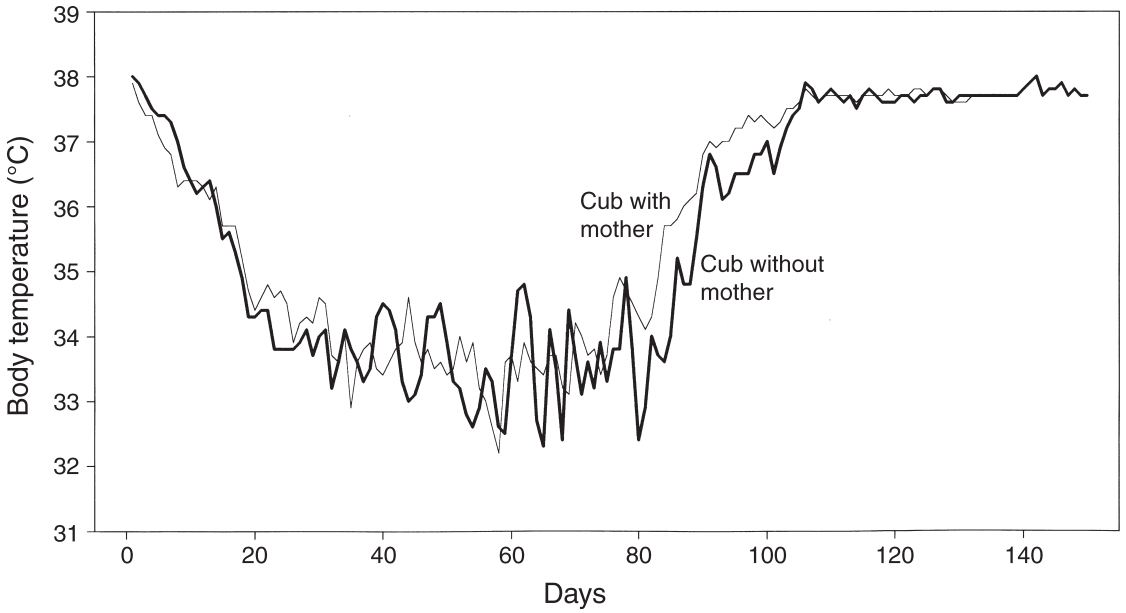

Fig. 2. Body temperature changes of cubs (< 1 year old) denned without the shelter given by mother, and cub denned with mother. Recording started on 1 Dec. 1995 and ended on 28 April 1996). (J. Siekkinen & R. Hissa, unpubl.)

We also wanted to find out whether a cub under the age of one year can manage alone in the next winter. The normal behavior at northern latitudes is denning of 1-year-old cubs with their mothers. The Tb of a cub that had denned alone did not differ from the Tb of the cub who had spent the winter 1995/96 with its mother (Fig. 2). Despite differences in changes of mass (170 g/day versus 230 g/d), the relative (%) changes were of the same order of magnitude, 24.6% and 24.7%. The body weight (BW) of the bears were 85 kg and 63 kg before winter sleep. On the basis of these results, we can assume that at least such a cub that spent the summer with its mother and thus was able to store enough fat will make it through winter alone. However, it should be remembered that these bears slept for only 91 days. Winter sleep can be considerably longer in natural conditions, even twice as long.

Our captive bears only slept for just over three months per winter on an average. Their winter sleep started in late November/early December, and ended in late February/early March. The bears never soiled the den, neither did they show any signs of thirst right after awakening, even though they willingly sampled a mouthful of snow. The average weight loss in percentage terms by awakening was 20–25%, which is about the same as that observed in captive black bears (Hock 1960) or in free-ranging black bears (Nelson *et al.* 1973). Since the bear do not go to sleep immediately after the last weighing, it should be pointed out that there are not yet absolutely correct data available about the changes in mass during winter sleep.

4. HIT, hibernation induction trigger: Does it exist?

The proximate mechanism that induces hibernation or winter sleep is still unknown. A special blood-borne "trigger" chemical was suggested (Dawe & Spurrier 1969, 1972, Spurrier *et al.* 1976, Bruce *et al.* 1984). When blood from hibernating 13-lined ground squirrels (*Spermophilus tricemlineatus*) was injected into summer active squirrel, the recipient started to hibernate. The existence of a blood-borne trigger was also reported in other hibernators (Dawe & Spurrier 1972). Trigger substances were also reported in the black bear (Ruit *et al.* 1987) and polar bear (*Ursus maritimus*) (Bruce *et al.* 1990). The trigger was characterized as a small protein, the structure and effects being similar across species lines (Oeltgen &

Spurrier 1981). It was also suggested that it is possible to induce a hibernation-like condition even in species which do not normally hibernate (Meeker *et al*. 1980, Myers *et al*. 1981).

In our study (Karjalainen *et al*. 1994) plasma from winter-sleeping European brown bear was injected into Djungarian hamster (*Phodopus sungorus*) and laboratory rats. Blood samples of the brown bear were collected between December 10 and February 12. In control injections plasma from a summer-active bear was used (for further details of injections, see Hohtola *et al*. 1991 and Karjalainen *et al.* 1994).

The study did not show any evidence for a universal HIT substance in the blood of winter-sleeping brown bear (see Fig. 3). Similar plasma transfusions were reported to be ineffective in induction of hibernation in the golden hamster (*Mesocricetus auratus*) (Minor *et al*. 1978), arctic ground squirrel (*Spermophilus undulatus kennicottii*), and Richardson's ground squirrel (*Spermophilus richardsonii*)(Abbots *et al.* 1979, Wang *et al.* 1988).

Deep hibernation and winter sleep are complex phenomena which include alterations in many physiological parameters. Consequently, it is most questionable whether only one single factor or chemical substance exists that regulates hibernation. Krueger and Shoham (1986) and Wang and Lee (1989) postulated that because hibernation is most likely a case of convergence of evolution as already suggested by Lyman (1963), a single type of HIT should not be expected to promote hibernation or winter sleep. Of course it is possible that a HIT chemical exists in the brown bear, but it may induce winter sleep only in other brown bears and/or in late fall. This, however, remains to be shown.

5. Why do bears give birth in the winter?

The most striking observation is that the female bears give birth to the cubs while fasting completely in winter. It has to be noted that pregnancy and lactation is absolutely impossible for other carnivores under starvation. They are mutually exclusive and lactation is impossible without food and water (Nelson 1989).

The fact that bears give birth in the middle of winter during winter sleep has puzzled researches. Even though bears are in heat in June, the fertilized egg (blastocyst) is not implanted in the uterus until the female retires to her winter den (delayed implantation) (Wimsatt 1963, Asdell 1964, Hensel *et al*. 1969). In order for an implantation to be successful it is essential for the mother to be in good shape.

Because of the short duration of gestation, only ca. 2 months, the new-born cubs weigh only 250–400 g. If the mother weighs 150 kg, the cub's proportional mass is only 0.2–0.3% of that. The period of lactation is on the other hand relatively long, extending at least for 4–5 months.

It is clear that the mother must make it through winter with the help of her fat stores, and she must also provide milk for her usually 1–4 cubs. How can she do this, and why does she give birth in the middle of winter? Why isn't the pregnancy prolonged until spring, up to the time when the female leaves her winter den?

There may be several reasons for the short duration of gestation and the small size of the cubs. A fetus needs a lot of carbohydrates, mainly glucose, for its development. A female bear cannot, however, store large amounts of carbohydrates in her body, she must in fact manufacture them during winter sleep, mainly from amino acids, by utilizing biochemical synthesis reactions. The unavailability of carbohydrates is thus a limiting factor in longer fetal development. From a developmental point of view it is, therefore, advantageous for the cub to switch over to milk as early as possible. Milk contains everything a cub needs for its development. The milk fat level of the black bear female in the middle of lactation is 25% and of the grizzly bear 19% (Jenness *et al.* 1972, Gittleman & Oftedal 1987, Oftedal *et al*. 1993). Fat content of polar bear milk varies from 25.5 to 35.8% (Derocher *et al.* 1993). The carbohydrate concentration is 1–3% (Farley & Robbins 1995).

Because a bear does not drink during winter sleep, it must be extremely careful with its utilization of water. For this reason it cannot let the volume of the uterus (liquid volume) grow too large, which would be necessary in the case of a longer gestation period. The growth of the larger uterus would also require an efficient protein synthesis. Because bears do not eat in the winter, getting the amino acids necessary for the protein synthesis would be a problem. Thus there is a lot

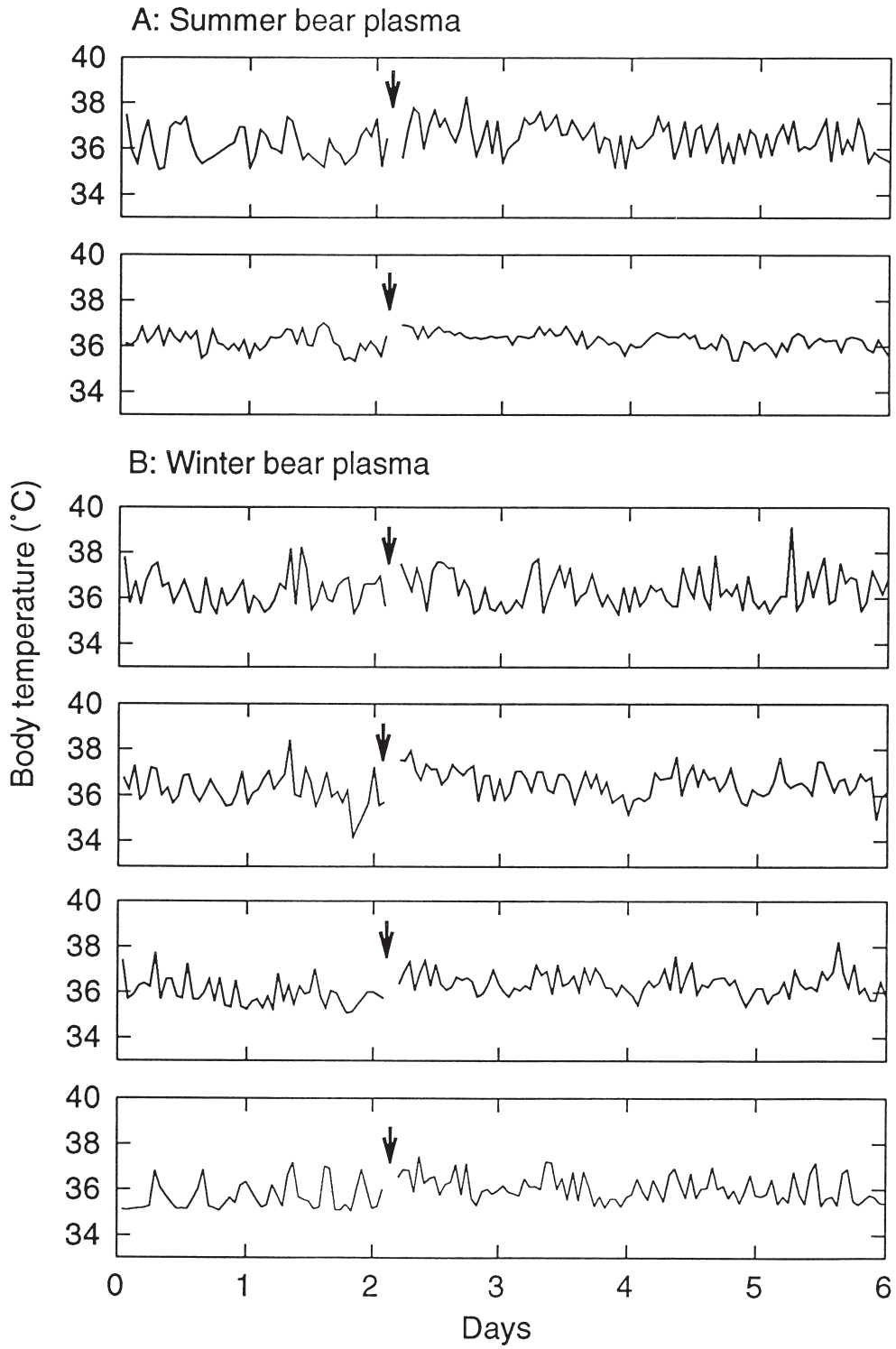

Fig. 3. The effect of intraperitoneal injection of brown bear plasma (0.4 ml) on the body temperature of Djungarian hamster (*Phodopus sungorus*). — A: Plasma from summer-active bear. — B: Plasma from winter-sleeping bear. The injections are given at the time indicated by arrows. Ambient temperature was 2°C. (Karjalainen *et al.* 1994, with permission.)

that speaks for a gestation period that only lasts a few months.

6. Hematological characteristics and blood chemistry

Data on the hematology of the family Ursidae are still few. Reported data involve both captive and free-ranging bears. Hematological findings were presented for captive black bears (Svihla *et al.* 1955, Youatt & Erickson 1958, King *et al*. 1960, Hock 1966, Seal *et al.* 1967), free-ranging black bears (Matula *et al.* 1980, Beeman 1981, Schroeder 1987, Franzmann & Schwartz 1988, DelGiudice *et al*. 1991, Hellgren *et al.* 1993), captive brown (grizzly) bears (Seal *et al*. 1967), free-ranging grizzly bears (Halloran & Pearson 1972, Pearson & Halloran 1972, Brannon 1983, 1985a), captive European brown bear (Seal *et al*. 1967, Jamnicky *et al.* 1987, Hissa *et al.* 1994), captive Japanese brown bear (*Ursus arctos yesoensis*) (Seal *et al*. 1967), and of free-ranging polar bear by Lee *et al*. (1977).

RBC-count varies from 5.20×10^6/µl to 8.5×10^6/µl in both grizzly and European brown bears (Table 3). These values are parallel to those obtained in the black bear (Youatt & Erickson 1958, Erickson & Youatt 1961, Seal *et al.* 1967). In general, RBC-count in bears is higher than in humans (Guyton 1981), perhaps due to lower MCV of RBC (see Henry 1964).

Matula (1976) presented reference of blood values for black bears during predenning (July–

Table 3. A comparison of hematological values of grizzly (*Ursus arctos horribilis*) and European brown (*Ursus arctos arctos*) bears.

Species	Season	RBC (10^6/µl)	WBC (10^3/µl)	Hb (g/dl)	Hct (l/l)	MCV (fl)	MCH (pg)	Author
U. a. horribilis	May–July	6.1	8.0	15.5	49	81.6	25.5	Brannon (1985a)
	May–July	6.3	10.0	16.3	51	81.0	25.8	
	May–July	5.9	7.3	15.0	49	82.0	25.3	
U. a. arctos	Nov.	7.8	9.3	19.5	55	70.0	25.0	Hissa *et al.* (1994)
	April	6.6	8.5	16.5	47	71.4	25.0	
	Nov.	7.3	11.0	17.8	48	66.1	24.4	
	March	7.2	10.0	17.9	48	66.6	24.9	
	Nov.	6.6	10.9	16.8	45	67.2	25.2	
	March	7.9	7.7	17.5	55	70.0	22.2	
	Sept.	8.1	6.1	20.0	54	67.0	24.7	
	Nov.	8.3	8.7	21.0	58	70.4	25.4	
	Nov.	8.3	6.4	21.4	57	68.9	25.7	
	Feb.	8.5	5.3	21.1	58	68.6	24.8	
	March	8.4	6.9	20.2	58	70.0	24.2	
	May	7.5	8.3	19.9	51	68.6	26.5	
U. a. horribilis	April–May	7.2	–	15.9	49	69	22.0	Pearson & Halloran (1972)
	July–Aug.	5.9	–	15.0	44	75	25	
	Oct.	8.5	–	18.8	56	66	22	
U. a. arctos								
♀	April	5.2	12.2	17.5	51	98	35	Seal *et al.* (1967)
♂	April	5.8	11.4	18.4	52	89	36	
U. a. horribilis								
♀	April	5.8	9.5	18.8	54	93	35	Seal *et al.* (1967)
♂	April	5.6	8.6	18.3	52	93	35	
♂	Nov.	5.7	8.3	18.4	50	88	36	
♀	Nov.	5.4	10.4	17.6	48	88	36	
♀	Dec.	5.2	12.0	14.9	42	80	36	
♀	Jan.	5.5	16.6	17.0	47	86	36	

December) and postdenning (April–July) periods. Storm *et al*. (1988) presented data on the blood chemistry of the black bear during the denning period. Erickson and Youatt (1961) and Hellgren *et al*. (1993) noted an increase in red cell corpuscles (RBC), hemoglobin (Hb), packed cell volume (hematocrit, Hct%), and plasma protein content during the denning period in the captive black bear. Matula *et al.* (1980) also noted significantly higher Hb, Hct%, and RBC prior to denning than after denning in the free-ranging black bears. Seasonal changes in these parameters and also in mean corpuscle volume (MCV), and mean corpuscle hemoglobin (MCH) have been shown in the studies of Seal *et al.* (1967), Pearson and Halloran (1972), Franzmann and Schwartz (1988) and Hissa *et al.* (1994) (Table 3).

Since in most studies MCV was shown to be smaller and RBC higher in winter conditions, it was assumed that erythrocytes are smaller, containing less Hb by weight (Matula *et al*. 1980). On the other hand cells are more numerous. The results of Pearson and Halloran (1972) support this view. They observed that the erythrocyte diameter was only 6.2 µm immediately after the denning period but later in the summer 7.2 µm. Similar observations were also made in typical hibernators (Sealander 1962, Hock 1964). The smaller size and larger number of erythrocytes may facilitate oxygen exchange as suggested by Matula *et al*. (1980). Couturier (1954) described the range of erythrocyte diameter for the European brown bear as 5.8 to 10.2 µm for the male and 5.1 to 8.5 µm for the female.

Differences in blood parameters between the sexes of the black bear for MHC and MCHC were observed by Matula *et al.* (1980), Beeman (1981) and Schroeder (1987). Male black bears have smaller MCH values than females. Brannon (1985a) noted higher Hct% for males than for females of free-ranging grizzly bears. However, no marked differences were observed in hematological values between sexes by Pearson and Halloran (1972), Lee *et al*. (1977), Matula *et al.* (1980), Beeman (1981) and Storm *et al*. (1988). It was noted by Hellgren *et al*. (1993) that there are differences in the amplitude of seasonal pattern in hematological characteristics such as RBC, Hb, and Hct, which are dependent on the geographical latitudes. For instance, the highest variation of Hct occurs in the far north (Alaska).

Data on the seasonal functions of the immune system of the family of Ursidae are still scarce. In smaller hibernators, the lymphoid system undergoes involution during hibernation (Svihla & Bowman 1952, Lyman & Chatfield 1955, Suomalainen & Granström 1955). A total WBC-count decreases during the denning period both in the American black bear (Erickson & Youatt 1961), and in the European brown bear (Hissa *et al*. 1994). Additionally, the total number of leukocytes is greater in the fall than in the spring after the denning period in the black bear (Seal *et al*. 1967) and in the grizzly bear (Pearson & Halloran 1972). The decline of total white cell count has been observed in all hibernating species studied (Lyman & Chatfield 1955).

The suppression of the humoral antibody production and cellular immune reactions in the fall is typical for smaller hibernators (for review see Shivatcheva 1989). As shown by Karjalainen *et al*. (1995) the relative number of monocytes was found to decrease during the denning period in the brown bear, whereas the relative number of lymphocytes showed no seasonal variations. Furthermore, the ratio of T to B cells appeared to be relatively constant. The decreased monocyte number was suggested to be associated with a reduced T-cell reactivity against an antigenic stimulus. Also other reasons were suggested (see Karjalainen *et al.* 1995). The decline in cellular immune reaction in the bear is not due to impaired function of T-lymphocytes because the response to antigenic phytohemagglutinin stimulus was not lowered in the denning bear. As a summary, it can be concluded that the small changes in the function of the immune system of the denning bear are not as dramatic as in small hibernators.

7. Recycling of urea: amino acid and protein synthesis

Although the urea turnover rate increases 2–10 fold during the denning period (Nelson 1973, Nelson *et al*. 1975) the available data indicate that the blood urea level decreased during the denning period in black, brown and polar bears (Brown *et al.* 1971, Halloran & Pearson 1972, Nelson *et al*. 1973, Franzmann & Schwartz 1988, Hissa *et al*.

1994). Associated with this change is an increase in blood creatinine (Nelson *et al*. 1973, 1984, Ramsay *et al*. 1991, Hissa *et al*. 1994) (Table 4). Nelson *et al.* (1984) showed that the ratio of serum urea to serum creatinine (U/C) was reduced in the black bear from a normal value of 25 or more to a value below 10 during the denning period. A similar reduction of the U/C ratio was also observed in the polar bear and European brown bear (Derocher *et al*. 1990, Ramsay *et al*. 1991, Hissa *et al*. 1994). This is a unique characteristic and is specific only for denning bears. Other hibernators or starving mammals show either no change or an increase in the U/C ratio (for review see Ramsay *et al*. 1991). Bears starving during the summer are unable to reduce the U/C-ratio and become uraemic (Nelson *et al*. 1975).

Available data clearly indicate that nitrogen from urea is recycled through amino acids and plasma proteins back to urea in the denning bear. Calculations based on winter measurements show that black bears may even have a small net increase in lean body weight (BW) during the denning period (Nelson 1980, Nelson *et al*. 1983). Nelson *et al.* (1975) suggested that in the black bear urea moves passively from the bladder into blood circulation and gut lumen. As a result of enzyme activity ammonia is released and carried to the liver and used as a nitrogen substrate for amino acid synthesis. Released nitrogen is combined in the liver to glycerol, producing amino acids like alanine and serine and new proteins (Nelson *et al*. 1975, Lundberg *et al*. 1976, Wolfe *et al*. 1982ab, Nelson 1987). Consequently it was suggested that the decline of urea level in the winter, at least partly, is a result of an increased use of amino acids as precursors for neurotransmitters or hormones as well as in protein synthesis

Table 4. Seasonal changes in plasma amino acids and other nitrogen compounds (μmol l^{-1}) of the European brown bear (Hissa *et al.* 1998a).

Compounds	Summer	*N*	Winter	*N*	*P*
Alanine	319.2 ± 20.8	6	559.6 ± 35.8	6	< 0.001
Aminoadipic acid	2.9 ± 0.4	6	5.8 ± 1.0	6	< 0.05
α-Aminobutyrate	18.7 ± 4.0	6	31.6 ± 3.8	6	< 0.01
Ammonium	74.0 ± 6.3	6	71.3 ± 6.9	6	n.s.
Arginine	101.7 ± 5.7	6	83.4 ± 5.7	6	< 0.05
Asparagine	60.6 ± 4.6	6	42.4 ± 4.9	6	< 0.05
Asparagine acid	48.6 ± 9.4	6	31.5 ± 6.1	6	n.s.
Citrulline	58.7 ± 3.5	6	69.9 ± 6.5	6	n.s.
Cystine	6.9 ± 0.9	6	13.5 ± 2.7	6	< 0.05
Cystationine	6.2 ± 1.3	6	7.6 ± 1.7	6	n.s.
Glutamine	1 012 ± 152.4	6	1 322 ± 168.4	6	n.s.
Glutamic acid	66.9 ± 5.2	6	60.1 ± 6.9	6	n.s.
Glycine	309.0 ± 27.9	6	416.1 ± 33.7	6	< 0.05
Histidine	79.6 ± 7.4	6	88.2 ± 9.2	6	n.s.
Isoleucine	81.0 ± 4.7	6	99.5 ± 7.8	6	n.s.
Leucine	160.5 ± 11.8	6	184.2 ± 15.6	6	n.s.
Lysine	220.9 ± 9.9	6	411.6 ± 19.5	6	< 0.001
Methionine	36.6 ± 1.9	6	54.2 ± 3.8	6	< 0.01
Methylhistidine	36.0 ± 2.4	6	54.7 ± 2.6	6	< 0.001
Ornithine	29.7 ± 1.8	6	52.1 ± 2.7	6	< 0.001
Phenylalanine	63.6 ± 4.7	6	72.9 ± 6.5	6	n.s.
Proline	108.9 ± 13.1	6	145.8 ± 15.1	6	n.s.
Serine	146.2 ± 8.1	6	146.4 ± 7.3	6	n.s.
Taurine	159.7 ± 12.53	6	104.6 ± 12.0	6	< 0.01
Threonine	188.8 ± 6.6	6	159.9 ± 10.4	6	n.s.
Tyrosine	52.4 ± 3.8	6	54.4 ± 5.9	6	n.s.
Valine	300.8 ± 13.8	6	307.0 ± 19.6	6	n.s.
Urea	9 814 ± 501.5	6	4 973 ± 368.3	6	< 0.001
Uric acid	44.9 ± 9.53	3	62.0 ± 8.81	6	n.s.

(Nelson 1989). The experiments performed by Wolfe *et al.* (1982ab) also showed that injection of ^{15}N-labeled urea in denning black bear causes a rapid appearance of nitrogen in amino acids like ornithine, arginine, glycine, tyrosine, phenylalanine and threonine. Therefore, it had been assumed that the bear is able to synthesize also essential amino acids like leucine.

Injection of ^{14}C-labeled alanine during winter sleep yielded pyruvate, lactate and glucose (Nelson *et al*. 1975, Ahlquist *et al*. 1984, Nelson 1989). Part of the amino acids synthesized are thus also used in gluconeogenesis for the production of new glucose molecules. The bear could, in principle, manage a long fast with the help of the fat it has stored, but since the central nervous system needs carbohydrates which cannot be stored for very long, they must be produced either from glycerol or amino acids. The amino acid level in humans always decreases in fasting (Felig *et al*. 1969). The same phenomenon can be observed in hibernators like in the hedgehog (Kristofferson & Broberg 1968). Since the bear does not eat during winter, one would naturally expect a marked decline in the level of amino acids, particularly essential ones. However, this is not the case. Nelson *et al*. (1973) and Nelson (1987) noted that the concentration of unessential and essential amino acids remained constant throughout the denning period. *De novo* synthesis of essential amino acids was suggested. Unessential amino acids such as alanine and serine were shown to be synthesized using glycerol derived from triglycerides as a precursor (Ahlquist *et al.* 1984).

On the other hand it was also questioned whether essential amino acids can escape deamination during winter sleep. The study performed by Nelson and Jones (1987) shows unequivocally that the metabolism of labeled leucine is independent of season. Leucine was quickly cleared from plasma, appearing in protein. Meredith *et al*. (1988) suggested that essential threonine is likewise synthesized in the denning bear and used in protein synthesis. As found by R. Hissa, M. Purkka and E. Hohtola (unpubl.), the concentrations of cystine, glycine, methionine, lysine and alanine are significantly elevated during the denning period in the European brown bear (Table 4). On the other hand, the amount of taurine, arginine, and asparagine are significantly decreased. No significant changes in the concentration of other essential amino acids such as leucine, isoleucine, threonine and valine were detected. The slight increase in the concentration of essential histidine and phenylalanine during the denning of the brown bear supports the view that the synthesis of essential amino acids may be possible, as also suggested by Nelson *et al*. (1973, 1975) and Nelson and Jones (1987).

The significantly elevated output of 3-methylhistidine (Table 4) in the winter reflects quantitatively the rate of muscle degradation (Haverberg *et al.* 1975). This shows that existing proteins are broken down, thus releasing essential amino acids for other purposes. In fact, Lundberg *et al*. (1976) have reported a three- to five-fold increase in the protein turn-over-rate in the denning black bear. It must, however, be borne in mind that the source of essential amino acids contained in proteins does have a limit, so that their synthesis cannot be ruled out.

A significantly lowered urea level in the winter and a rise of the level of many amino acids, even essential ones, shows that the bear has no problems with its nitrogen metabolism in winter. This also ensures that the protein content, and likewise muscle fitness, of a bear waking up after winter sleep is as good as in the fall. In support of this assumption Koebel *et al*. (1991) found that muscle glycogen and triglycerides were relatively unchanged throughout the season in the black bear. Muscle oxidation capacity, likewise, as reflected by citrate synthesis activity was unchanged. Consequently, muscle energy metabolism seems to maintain full capacity throughout the denning period. However, muscle protein concentration was observed to decline by 10%. On the other hand, muscle DNA concentration increased significantly suggesting there was no loss of muscle fibers. The biochemistry of muscle cells is, however, still poorly understood, i.e. it is not known whether a bear that has just woken up from winter sleep is as capable of effective power production as in the fall.

It was assumed that certain types of plasma proteins are somehow related to the hibernation of ground squirrels and chipmunks (see e.g. Srere *et al*. 1992, 1995, Kondo & Kondo 1992). Kondo and Kondo (1992) showed that plasma levels of certain proteins were low before and during the hibernation period. At least one of these proteins

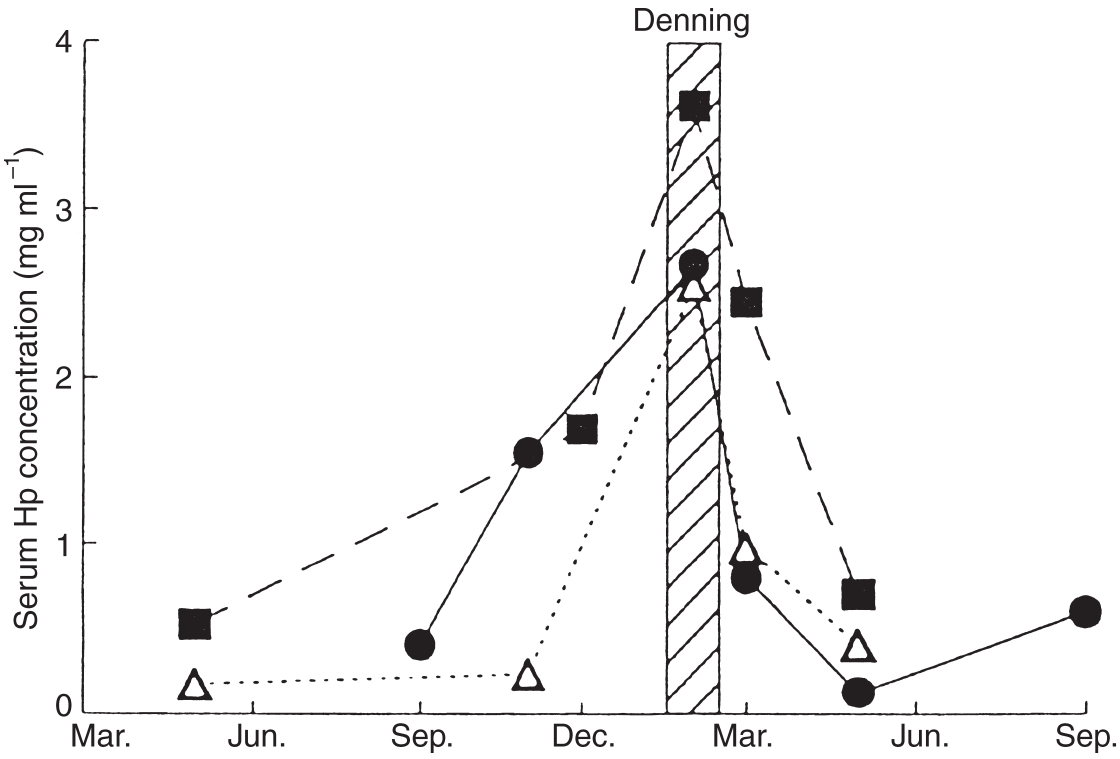

Fig. 4. Seasonal changes of serum haptoglobin level in the European brown bear. (Mominoki *et al.* 1996, with permission.)

was homologous with α_1-antitrypsin. Srere *et al.* (1992, 1995) noted an increase of α_2-macroglobulins in deep hibernators like Richardson's and Columbian ground squirrels.

Haptoglobin is known to be an acute-phase protein which is also known to participate in hemoglobin transport from blood to the liver and the recycling of iron (see e.g. Bowman & Kurosky 1982, Eckersell & Conner 1988). In our study (Mominoki *et al.* 1996) blood samples from Japanese and European brown bears were collected both in Japan and in Oulu. Haptoglobin level showed a peak during the denning period (Fig. 4). It was suggested that the increase of haptoglobin in winter is based on a specific effect of season. Similar seasonal changes in the acute phase proteins were also observed in rodent hibernators (Kondo & Kondo 1992). The observed increase in haptoglobin was, however, not related to the denning period itself. It was suggested that the increase is most probably related to some extrinsic factors, like temperature, light or starvation. Haptoglobin may also act as a reservoir of amino acids.

As discussed above, it is now quite clear that the denning bears are capable of synthesizing the amino acids and proteins in spite of heavy starvation. The linkage of increase of haptoglobins and other macroproteins to the turnover of urea degradation and amino acid synthesis however needs further research.

8. Fat metabolism during the denning period

Mammals, such as chipmunks which fall into deep hibernation need and use a particular type of fatty acid in order to prepare themselves for hibernation and to wake up after it (Frank 1991, 1994, Geiser 1991, Florant *et al.* 1993, Frank & Storey 1996). It was even shown that hibernation will not succeed unless a mammal has access to polyunsaturated amino acids of a certain kind (Frank & Storey 1996). But what about the bear? So far no one has studied precisely what fatty acids out

of tens of different types the bear needs during its winter sleep, or the changes involved in their use at the onset of winter sleep, or upon awaking.

A particularly interesting question is whether certain polyunsaturated fatty acids have a special significance, and how the bear can ensure the availability of essential fatty acids during winter sleep? Ketosis was not reported in the denning black bear (Nelson 1980). Only a slight increase in β-hydroxybutyrate (BUT) was detected in the denning European brown bear. BUT varied from 0.1 to 0.3 mmol/l indicating unchanged production and/or rapid metabolism of ketones (Hissa *et al*. 1994).

Polyunsaturated fatty acids can also act as precursors of tissue hormones classified as prostaglandins, prostacyclins, tromboxanes and leucotrienes. They are substances with special local physiological activity. They can regulate e.g. blood vessel lining and smooth muscle function as well as blood pressure (Boissoneault & Hayek 1992, Bruckner 1992). Cells also have a very limited life span, and since cell membrane is mostly made up of different fatty acids and their combinations, there is an obvious need for fatty acids throughout winter. It is of course known that the bear stores an imposing amount of fat under its skin and around its internal organs in late summer, thanks to its enormous appetite. White fat can make up as much as 30–35% of the total body mass of the bear. The composition of the subcutaneous fat stored in the European brown bear in the fall was clarified by Käkelä and Hyvärinen (1996).

Blood concentrations of cholesterol, triglycerides and phospholipids increase in the black bear during the denning period (Nelson *et al*. 1973, Ahlquist *et al*. 1984, Franzmann & Schwartz 1988, Hellgren *et al*. 1989). On the other hand, Matula *et al.* (1980), Schroeder (1987) and Hissa *et al*. (1994, Table 5) did not see any changes in these parameters. The mean cholesterol level during the winter sleep was shown to be, however, significantly higher in black and brown bears (Matula *et al*. 1980, Franzmann & Schwartz 1988, Storm *et al.* 1988).

But what about the fatty acid composition of blood plasma in the fall, during winter sleep and in the spring when the bears wake up? Are there seasonal changes in the composition? In studies on fatty acid composition it was shown that radical changes occur in the relative amounts of certain fatty acids, particularly polyunsaturated ones (R. Hissa, E. Hohtola, T. Tuomola-Saramäki, T. Laine & H. Kallio unpubl.). The most dramatic changes in the plasma fatty acids during the denning period occurred in the proportions of palmitic (16:0), oleic (18:1n-9), arachidonic (20:4n-6), eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids. In denning bear, the proportion of the (n-9)-family fatty acids (18:1n-9 and 20:1n-9) were only half of what was observed in summer. Differences between male and female bears as well as between pregnant and non-pregnant females were tested. All differences observed were much less significant than seasonal differences.

According to our results, it can be assumed that during winter sleep all saturated fatty acids must arise either from lipolysis or via *de novo* synthesis of fatty acids. However, the *de novo* synthesis may be the only and exceptional option due to high energy demand. In deep hibernators the activity of liver enzymes required for fatty acid synthesis from glucose is greatly depressed (Willis 1982, Florant *et al*. 1990). Consequently, it is most evident that also bears have a depressed fatty acid synthesis under heavy starvation during the winter sleep (R. Hissa, E. Hohtola, T. Tuomola-Saramäki, T. Laine & H. Kallio unpubl.).

In the fall the relative proportions of fatty acids in plasma differ greatly from the ones in subcutaneous fat (Käkelä & Hyvärinen 1996, R. Hissa, E. Hohtola, T. Tuomola-Saramäki, T. Laine & H. Kallio unpubl.). The relative differences in the composition of fatty acids in subcutaneous fat and blood plasma show that fatty acids are not passively transferred into circulation in unchanging proportions, but this occurs selectively, apparently regulated by what is needed at any particular time. Unlike typical mammals that are deep hibernators, the bear does not have brown fat tissue which acts as a "radiator", rapidly raising the body temperature of an animal as it awakes up from hibernation.

9. Why not osteoporosis?

If a person is forced to lie down immobile for months, like the bear in its winter den, it inevitably leads to osteoporosis, loss of bone tissue. Does the bear also suffer from osteoporosis? Parathyroid

hormone (PTH), calcitonin produced by the C cells of the thyroid and 1, 25-dihydroxyvitamin D_3 participate in the regulation of calcium metabolism. The function of PTH is to stimulate the action of bone-eating osteoclasts. As a result, bone becomes fragile and plasma calcium level increases. Calcitonin and vitamin D_3 are necessary for strengthening bone. In our studies we have been able to show that there is no seasonal variation in plasma PTH concentrations in the bear. On the other hand, vitamin D_3 content is significantly decreased from 48.7 ± 3.19 pg ml^{-1} to 23.3 ± 2.43 pg ml^{-1} in winter as shown in Table 5. This is at least partly a result of the fact that the bear does not eat. Vitamin D_3 is needed for transferring calcium through the intestinal wall into circulation. Since there are no seasonal changes in the blood plasma calcium levels it is fairly certain that the bone tissue of a bear in winter sleep remains unaltered and provides a firm insertion surface for the muscles in the spring. Our results support the study performed on the American black bear. No seasonal changes in the plasma calcium level, bony mass or bone structure were observed (Floyd *et al*. 1990).

As shown in our study, the level of phosphorus in the blood was clearly diminished in winter. Plasma aldosterone concentration was significantly elevated. The reason for the changes remain to be solved. It is probably that elevated aldosterone level is somehow involved in the changed activity of kidney tubuli in the absorption of sodium and potassium from the glomerular filtrate during the denning period. This however remains to be solved.

10. Neuroendocrine regulation

Some questions arise regarding fat storage and its use. What triggers the bear's enormous appetite in late summer and early fall? The stimuli controlling feeding appear to be very diverse and still poorly known (for references see Kupfermann 1994). In the past years, several ideas about the regulation of feeding have been suggested. In recent years, however, the important role of hypothalamus has received growing support. In late summer the bear eats two or three times as much food

Table 5. Annual changes in plasma characteristics of European brown bear (Hissa *et al.* 1994, and R. Hissa, E. Hohtola, H. Vierimaa, O. Vakkuri, S. Saarela, M. Laitinen & M. Puukka, unpubl.).

Characteristic	Summer	*N*	Winter	*N*	*P*
ALAT (U l^{-1})	24.6 ± 1.11	3	19.8 ± 3.63	6	n.s.
Aldosterone (pg ml^{-1})	35.2 ± 4.20	10	55.9 ± 5.31	11	< 0.001
ASAT (U l^{-1})	36.0 ± 2.97	6	40.6 ± 4.52	6	n.s.
Calcium (mmol l^{-1})	0.38 ± 0.005	9	0.38 ± 0.007	9	n.s.
Catecholamines					
Adrenaline (ng ml^{-1})	0.8 ± 0.30	7	0.04 ± 0.02	7	< 0.001
Noradrenaline (ng ml^{-1})	0.9 ± 0.32	7	0.3 ± 0.07	7	< 0.001
Cholesterol (mmol l^{-1})	8.5	1	7.0	2	–
Creatinine (μmol l^{-1})	110.9 ± 7.19	3	207.3 ± 21.56	6	< 0.01
Creatinine kinase (U l^{-1})	49.9 ± 8.23	3	54.0 ± 7.47	6	n.s.
FFA (mmol l^{-1})	0.348 ± 0.06	7	0.365 ± 0.06	7	n.s.
Glucose (mmol l^{-1})	8.6 ± 0.95	7	6.7 ± 0.51	7	n.s.
Hematocrit (%)	54.0 ± 0.93	9	53.4 ± 0.98	9	n.s.
Hemoglobin (g dl^{-1})	19.6 ± 0.46	9	19.4 ± 0.32	9	n.s.
Phosphorus (mmol l^{-1})	2.04 ± 0.13	12	1.21 ± 0.07	12	< 0.001
PTH (pg ml^{-1})	60.8 ± 10.79	12	57.3 ± 9.63	12	n.s.
Thyroid hormones					
T_4 (nmol l^{-1})	40.8 ± 3.06	7	41.1 ± 2.85	7	n.s.
T_3 (nmol l^{-1})	0.94 ± 0.13	7	0.86 ± 0.05	7	n.s.
Total proteins (g dl^{-1})	6.89 ± 0.21	11	7.44 ± 0.08	7	< 0.05
Triglycerides (mmol l^{-1})	3.1 ± 0.14	7	2.3 ± 0.19	7	n.s.
Vitamin D_3 (pg ml^{-1})	40.0 ± 3.89	5	23.7 ± 2.48	6	< 0.001

as the normal daily consumption. Berries play an important role as a food source. Their high carbohydrate content is necessary for the synthesis of fats. Sugars that end up in cells are rapidly processed biochemically into fat. It is unclear what role insulin and glucocorticoids play in the bear's fattening process. It is, however, known that in deep hibernators the concentrations of these hormones are at their highest just before the onset of hibernation (Armitage 1991, Boswell *et al*. 1994).

Leptin is a hormone-like substance found in the circulation, probably present in all mammals (Halaas *et al*. 1995, Pelleymounter *et al*. 1995, Hamann & Matthaei 1996, Ormseth *et al*. 1996, Weigle *et al*. 1997). It is derived from white fatty tissue and it affects the center controlling hunger sensation in the hypothalamus. The more leptin there is, the weaker the hunger sensation. It could be assumed that it is lack of leptin that causes the bear's appetite to increase in late summer. On the other hand, when leptin abounds, i.e. there is a lot of fat stored, the bear loses its appetite. However, it is still a mystery how the bear regulates the synthesis, storage and use of fat. Due to the dramatic seasonal changes in the loss of appetite and ability to store enormous amounts of fat the bear is an excellent experimental animal in studies of obesity.

In summary, we have very little information about the hormonal and neural factors that regulate fat storage, lipolysis and the settling down to winter sleep in general. Cravatt *et al.* (1995) have found that a certain fatty acid amid derivatives in cat brain, may participate in the regulation of sleep in one way or another. Whether this factor also participates in the regulation of winter sleep in the bear is still unclear. What function do leptin, insulin, glucocorticoids and catecholamines have in fat storage and its use? There is an abrupt and highly significant decrease in catecholamines in the fall (Table 5).

It is well known that normally thyroid gland activity is positively correlated with energy intake. It is interesting to note that although dramatic changes occur in the food intake and metabolic rate, in some studies no seasonal changes were measured in blood T_4 or T_3-concentration of the bears (Hellgren *et al.* 1993) (see also Table 5). On the other hand decreased thyroid gland activity and decreased T_4 and T_3 levels were demonstrated in the denning black bear (Azizi *et al*. 1979).

11. Bears as research animals and the future

From a physiologist's point of view the bear is a very interesting mammal for several reasons. It can, for example, be used as an experimental animal in studies concerning the regulation of adaptation to a long winter period with no food — a fast lasting as long as six months. It is also a good model for studying what regulates appetite and loss of appetite — states the bears go through in late fall before settling down to winter sleep. How can the bear manage without drinking, defecating or urinating for six months or even longer? How are these functions regulated? What causes the drowsiness in the bear, how is it regulated? Is the bear capable of synthesis of essential amino acids even during winter sleep? Being a large animal, the bear also offers good possibilities for sample collecting, and the same sample can be used for several different measurements.

During its evolution the bear has developed many physiological characteristics and skills that still offer a lot of scope for study. These data about the bear can yield information that may be helpful even in solving problems of human physiology. The bear is therefore in many ways an interesting experimental animal model.

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