Ann. Zool. Fennici 35: 205–213
Helsinki 17 December 1998
ISSN 0003-455X

Seasonal changes in plasma nitrogenous compounds of the European brown bear (*Ursus arctos arctos*)

Raimo Hissa, Matti Puukka, Esa Hohtola, Mirja-Liisa Sassi & Juha Risteli

Hissa, R. & Hohtola, E., Department of Biology, University of Oulu, P.O. Box 333, FIN-90571 Oulu, Finland
Puukka, M., Laboratory, Oulu University Hospital, FIN-90220 Oulu, Finland
Sassi, M.-L. & Risteli, J., Department of Clinical Chemistry, University of Oulu, FIN-90220 Oulu, Finland

Received 15 May 1998, accepted 8 October 1998

The object of this study was to obtain quantitative information about the seasonal changes in plasma amino acids and other nitrogen compounds of the captive European brown bears (*Ursus arctos arctos*) studied between May 1993 and May 1997. Altogether 56 blood samples were analyzed by ion-exchange chromatography. Analysis of plasma revealed that the urea concentration during the denning period was only half of that measured in summer, whereas ammonia and α-amino-butyrate concentrations did not change. The concentrations of taurine and arginine were significantly ($p < 0.001$ and $p \approx 0.003$, respectively) lower, while ornithine significantly ($p < 0.001$) and citrulline somewhat higher in winter as compared with the other seasons. These changes indicate active urea recycling, and the synthesis of non-essential amino acids with ammonium and glycerol as precursors. Of essential amino acids, the levels of phenylalanine, lysine and methionine and of non-essential ones, alanine and histidine were significantly elevated during the denning period. No consistent changes occurred in other amino acids. One indication of the degradation of muscle protein as a source of amino acids in winter was the significant increase of 3-methylhistidine concentration in plasma. However, the degradation of type I collagen also increased, since the concentration of the cross-linked carboxyterminal telopeptide of type I collagen (ICTP) was about 3.5-fold higher in winter than in summer. These changes indicate that *de novo* synthesis of essential amino acids is not necessary in winter sleeping bears.

1. Introduction

The denning period of the European brown bear starts in Finland in October–November and lasts until April–May. In captive conditions, the winter dormancy normally lasts only 100–120 days. Denning bears do not drink, eat, urinate or defecate, the body temperature (Tb) decreases from 37–37.5°C to 32–33°C (Hissa *et al.* 1994, Hissa 1997), and fat is the only energy source. As shown by Nelson (1980), no ketosis develops despite the reliance on adipose tissue. The bears do not ex-

perience any change in lean body mass (BM), i.e. no nitrogen is lost while utilizing fat reserves as shown in the American black bear (*Ursus americanus*) by Nelson *et al.* (1975), and Lundberg *et al*. (1976). However, protein turnover increases 3–5-fold (Lundberg *et al.* 1976). This implies an increased activity of both the protein anabolic and the catabolic reactions. Paradoxically, the bear shows a net increase in plasma protein in winter (Nelson 1980, Hissa *et al.* 1994).

Bears are unique animals since they give birth, and suckle cubs during the winter sleep, i.e., under heavy starvation. This means that they must have a net amino acid, and protein anabolism while starving. Successful pregnancy and lactation are, e.g., in human, impossible under starvation. Also, in many other animal species, starvation and pregnancy are mutually exclusive, and lactation is impossible without extra food and water.

An important physiological and biochemical adaptation of the bear to starvation during the denning period is the control of urea metabolism, so that uremia and dehydration do not occur (Nelson *et al*. 1973). In fact, a decrease in blood urea nitrogen was observed in the winter-sleeping bear by Brown *et al.* (1971), Halloran and Pearson (1972), Nelson *et al*. (1973), and Franzmann and Schwartz (1988). In parallel with the decrease of urea, an increase in plasma creatinine level is observed (Nelson *et al*. 1973, 1984, Ramsay *et al.* 1991, Hissa *et al*. 1994). Since the plasma creatinine level is an indicator of renal function (Searcy 1969, Ensrud *et al*. 1986), the change in urea to creatinine ratio may signify lowered renal activity.

In general, if urination is prevented, an animal may die of prerenal azotemia (Nelson 1989). The data presented by Nelson *et al*. (1973) show that reabsorption of urine from the bladder occurs in the denning bear. When ^{14}C-labelled urea was injected, the turnover rate of urea in plasma was shown to be 2–10-fold slower in winter than in summer (Nelson *et al.* 1975). Between 2 and 4 g of urea per day is produced by a denning bear (Nelson *et al*. 1973). Prior to denning, bears are in hyperphagic condition and produce at least 90 g of urea daily (Nelson *et al.* 1975).

After injection of ^{14}C-labelled urea in the ground squirrel, the appearance of $^{14}CO_2$ in expired air was considered to be a proof of urea degradation in the gut lumen where urease enzymes of bacteria split it into ammonia and carbon dioxide (Steffen *et al*. 1980). In the bear, at least part of the liberated ammonia is supposed to enter the liver for transamination reactions, producing amino acids using glycerol as carbon skeleton (Nelson *et al.* 1975, Lundberg *et al.* 1976, Wolfe *et al.* 1982, Ahlquist *et al*. 1984).

Nelson *et al*. (1973) and Nelson (1987) did not show any consistent changes in the concentrations of individual essential or non-essential amino acids in the denning American black bear. A *de novo* synthesis of essential amino acids was suggested. In humans and in deep hibernators like the hedgehog, a dramatic reduction in the levels of all amino acids occurs during prolonged starvation (Kristofferson & Broberg 1968, Felig *et al.* 1969, Al-Badry & Taha 1983).

Whether or not the denning bear is able to synthesise also essential amino acids is, however, still an open question. It is possible that essential amino acids are, at least partly, released from the existing proteins like collagens as a result of their degradation. The object of the present study was to obtain quantitative information about the seasonal changes in levels of plasma amino acids and other nitrogenous compounds in bears. It was also of interest to assay the changes in the cross-linked carboxyterminal telopeptide of type I collagen (ICTP) which indicate the degradation of mature, fibrillar type I collagen.

2. Materials and methods

This study was carried out on six European brown bears (*Ursus arctos arctos* L.) kept in the bear enclosures of the University of Oulu (65°N, 25°24´E) between May 1993 and May 1997. The age, body mass (BM), sampling dates, details on anaesthesia and handling of the bears are given elsewhere (Hissa *et al.* 1994, Hissa 1997). In short, the anaesthetics used were ketamine hydrochloride (Ketalar, Parke Davis) in concentrations of 2.0–5.0 mg kg$^{-1,}$ and medetomidine hydrochloride (Domitor, Lääkefarmos, Finland) in concentrations of 80–160 mg kg^{-1}. Winter sleep normally started in the beginning of December and lasted to the end of February, or the beginning of March. On the average, the bears lost 20%–23% of their BM during this period.

The body temperature (Tb) of the winter-sleeping bears was measured with dataloggers either with HOBO-Temp or StowAway™XTI (Onset Computer Corp., USA) (accuracy ± 0.25°C) chronically implanted in the body cavity. Tb varied between 33–35°C in the denning bear (for more

details *see* Hissa 1997).

A total of 56 blood samples during the 5 years of this study were collected from the jugular vein into evacuated glass tubes containing either EDTA or Lithium heparin. The blood samples were immediately centrifuged, and the plasma samples stored at –70°C until being analyzed.

Concentrations of amino acids in plasma were determined by ion-exchange chromatography (Biochrom 20 Amino Acid Analyzer, Pharmacia Biotech Ltd, England).

The ICTP (type I collagen) assay developed for human antigen (commercially available from Orion Diagnostica, Finland) could be used, since it has shown enough cross-reactivity with the bear serum samples. The original sample size (Risteli *et al.* 1993) was halved to 50 µl because of the high concentration of ICTP in bear blood. Aliquots of 100 µl of standards or 1:2 diluted serum samples were incubated with 200 µl of the iodinated tracer solution (about 50 000 counts min^{-1}), and 200 µl of diluted antiserum (in 0.5% normal rabbit serum) for 2h at 37°C. Thereafter, 500 µl of the solid-phase second antibody suspension (20 ml of goat anti-rabbit immunoglobulin antiserum and 150 g of PEG, mol. weight 6 000, in 1 litre of PBS containing 0.04% Tween 20) was added to each tube and mixed with vortex. After 30 min at 4°C, the bound fraction was separated by centrifugation (2 000 × g for 30 min at 4°C). The supernatant containing the unbound tracer was decanted, and the radioactivity of the precipitate containing the bound tracer was counted (Clinigamma 1272, LKB Wallac, Finland). The intra-assay variation of the ICTP assay was about 5% and interassay variation about 6%.

2.1. Statistics

For statistical analyses, all the data were combined in four groups, summer (June–September), autumn (October–November), denning period (December–February), and spring (March–May). For some bears, several samples from the same seasons were available from different years. In such cases, the mean value was used. For testing the statistical significance of the changes in nitrogen and amino acid concentrations, a repeated measures ANOVA with season as within-class variable was used as implemented in SPSS Software (SPSS Inc.). No groupwise *a posteriori* comparisons were made, so the *p*-values shown indicate the general effect of a season.

4. Results and discussion

4.1. Urea

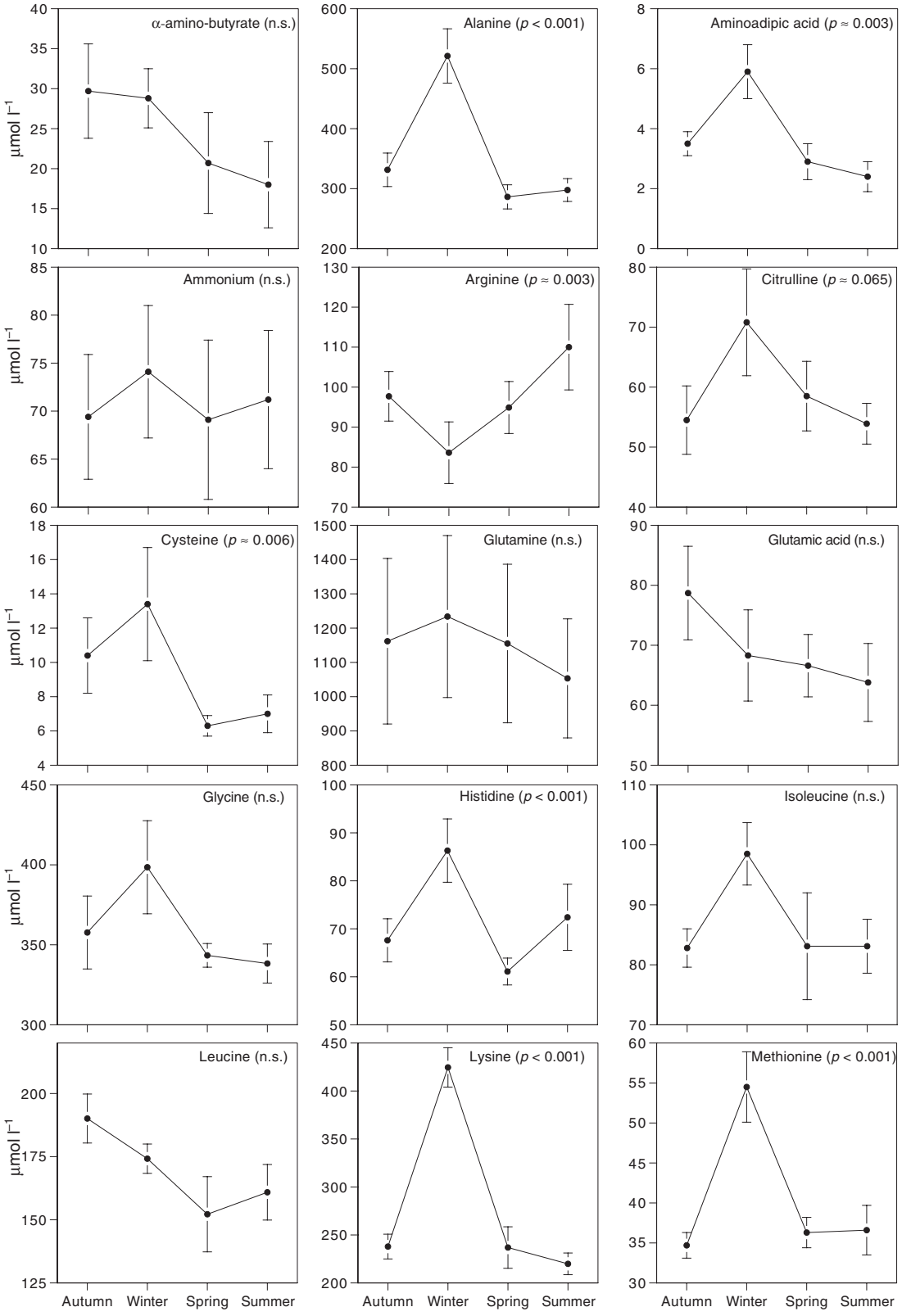
In the present study, urea concentration during the denning period was only half of that measured in summer ($p < 0.001$) (Fig. 1). This is in agreement with the results of the earlier studies of both the American black bear and the European brown bear (Nelson *et al.* 1973, Franzmann & Schwarz 1988, Hissa *et al.* 1994). At the same time, the levels of ammonia and α-amino-butyrate remained practically unchanged (Fig. 1).

In the denning black bear, the volume of urine appearing in the bladder has been shown to be reduced by 95% in winter (Nelson *et al.* 1973). Since the denning bear does not urinate, the produced urine must be reabsorbed from the kidney and bladder. The bladder of all mammals seems virtually permeable to various nitrogen compounds (Regdeczi *et al*. 1965, Nelson *et al*. 1975, Nelson 1980, Harlow 1987). After absorption, urea is transported via the circulatory system into the gut, excreted into the lumen where bacteria hydrolyse urea to ammonia. Ammonia is then carried to the liver, and nitrogen is combined to glycerol to produce non-essential amino acids (Mason 1984, Egan *et al.* 1986). A decreased glycerol level in the denning brown bear (Hissa *et al.* 1994), also supports this assumption.

Glycerol, a metabolite of triglycerides, however, cannot serve as a carbon skeleton for the essential amino acids, since once deaminated, they can be replaced only from exogenous sources. Therefore, the mechanism of the significant ($p < 0.001$) increase in concentrations of phenylalanine, lysine and methionine in winter in the present work (Fig. 1) remains unclear.

In all mammals, urea is produced by a special group of enzymes, which together constitute the urea cycle in the liver. The cycle is composed of five reactions each catalysed by a different enzyme. An immediate source of nitrogen is ammonia and the amino group of aspartate. Carbamyl phosphate formed from CO_2, NH_3 and ATP is condensed with ornithine to form citrulline. Once ornithine is formed, the reactions to arginine occur via the urea cycle. Ornithine belongs to the nutritional essentials, mammals are incapable of its synthesis. Small amounts of ornithine are, however, formed in the liver through reversal reactions from arginine.

Citrulline is condensed with aspartate to yield argininosuccinate, which is then converted to arginine and fumarate. Thus, the key step in the urea cycle is the hydrolysis of arginine resulting in ornithine after releasing urea.


α-amino-butyrate (n.s.)
Alanine (p < 0.001)
Aminoadipic acid (p ≈ 0.003)
Ammonium (n.s.)
Arginine (p ≈ 0.003)
Citrulline (p ≈ 0.065)
Cysteine (p ≈ 0.006)
Glutamine (n.s.)
Glutamic acid (n.s.)
Glycine (n.s.)
Histidine (p < 0.001)
Isoleucine (n.s.)
Leucine (n.s.)
Lysine (p < 0.001)
Methionine (p < 0.001)
µmol l⁻¹
Autumn
Winter
Spring
Summer

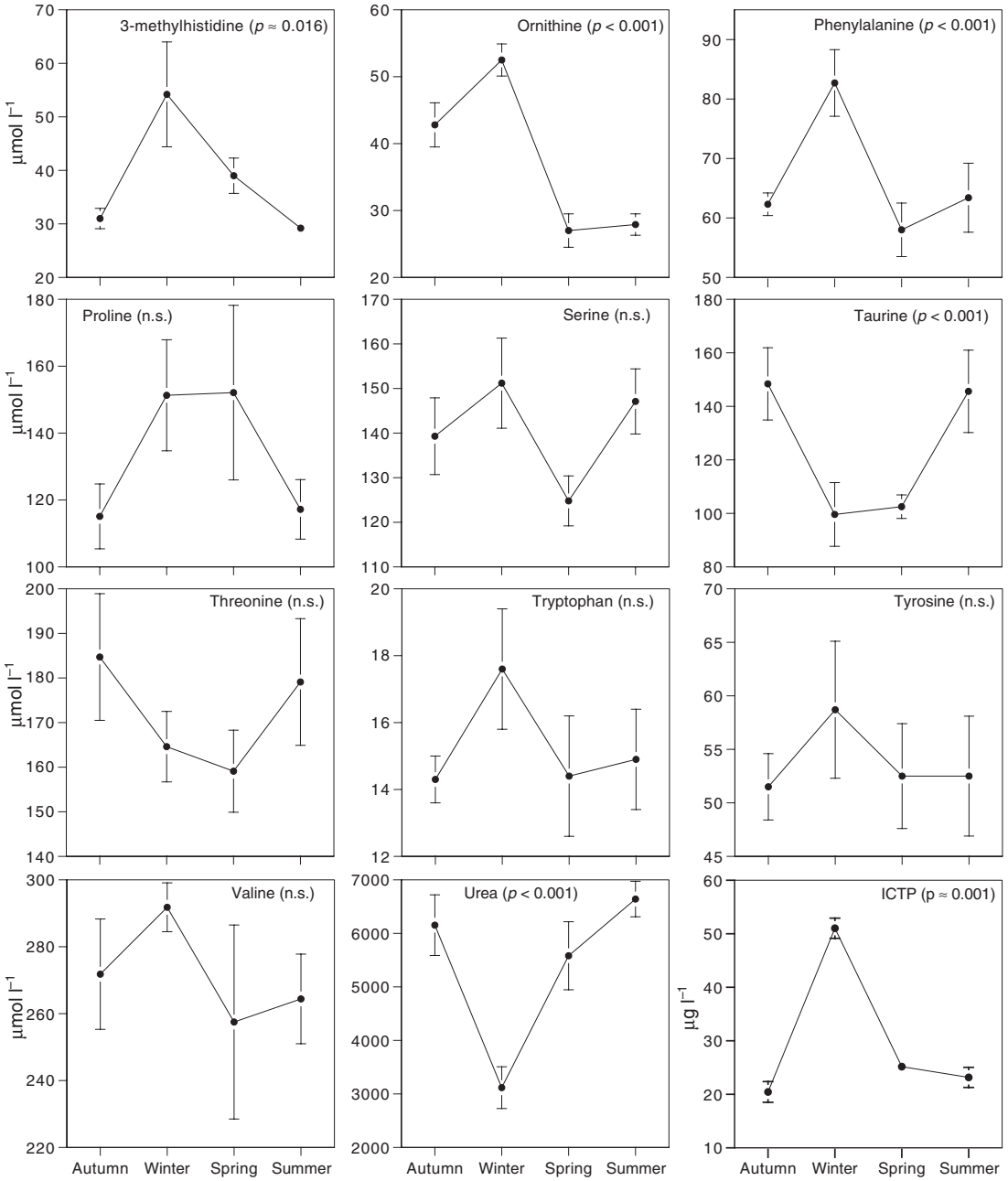


Fig. 1 (above and on the left). Seasonal changes in concentrations (mean ± SE) of plasma amino acids and other nitrogen compounds (May 1993–May 1997), as well as ICTP (Feb 1995–Dec 1997) in the European brown bear (*p*-values from ANOVA).

In the present study, the level of ornithine was significantly ($p < 0.001$) and citrulline somewhat elevated in winter (Fig. 1). On the other hand, the arginine concentration was at the same time significantly ($p = 0.003$) reduced, indicating an active urea cycle.

4.2. Amino acids

In our experiment, in the denning bears, there was a significant increase in the plasma concentrations of alanine ($p < 0.001$), aminoadipic acid ($p \approx 0.003$), cystine ($p \approx 0.006$), histidine ($p < 0.001$),

lysine ($p < 0.001$), methionine ($p < 0.001$), 3-methylhistidine ($p \approx 0.016$), ornithine ($p < 0.001$), phenylalanine ($p < 0.001$), and a slight increase in the concentrations of citrulline, glutamine, glysine, proline, isoleucine, valine and tryptophan. The level of arginine was significantly ($p \approx 0.003$) lower, and the concentrations of glutamic acid, serine and tyrosine, threonine and leucine remained unchanged (Fig. 1).

Survey of the literature showed that amino acid concentrations in plasma of the European brown bear are of about the same order of magnitude in summer as in other mammals, with the exception of glutamic acid which level is extremely high (63.8 ± 6.5 µmol l^{-1}; mean ± SE) in comparison with its concentrations in humans or hedgehogs (Kristofferson & Broberg, 1968).

According to Nelson *et al*. (1973) and Nelson (1987) there are no marked seasonal changes in plasma amino acid levels. However, in our experiment, a highly significant increase in concentrations of essential amino acids lysine, phenylalanine, methionine, and non-essential alanine, cystine, ornithine and histidine was found in winter (*see* Fig. 1). However, the arginine level decreased significanly (*see* Fig. 1). Although Nelson (1987) and Nelson & Jones (1987) did not find any substantial differences in concentrations of individual amino acids in the American black bear, they suggested a *de novo* synthesis of essential amino acids.

In contrast to our results, plasma amino acid concentrations decrease dramatically in starving humans or hedgehogs (Kristofferson & Broberg 1968, Felig *et al.* 1969). Comparisons with various deep hibernators yield, however, striking contrasts. Hedgehogs, for example, become uremic as hibernation continues, and arousal occurs as a result of the increased uremia (Kristofferson 1963). Ground squirrels, however, show no evidence of increased uremia during hibernation (Pengelley *et al*. 1971). These results suggest species-specific differences.

No marked changes were observed in the plasma tryptophan, threonine, valine, leucine or isoleucine concentrations (essential amino acids in humans) in the denning brown bear (*see* Fig. 1). This supports the results of Nelson and Jones (1987) who obtained similar results in the American black bear. Most of the ^{14}C-labelled leucine was quickly removed from plasma, reappearing in the plasma proteins (Nelson & Jones 1987). A continuous turnover of leucine was also observed throughout winter. Only a small part of leucine was shown to be oxidised into carbohydrate intermediates, which points to the fact that a *de novo* synthesis of leucine might be possible.

In the hibernating hedgehog, the amounts of valine, leucine and isoleucine in plasma were relatively high in comparison with the other amino acids, although all were greatly decreased (Kristofferson & Broberg 1968); however, the arginine concentration was exceptionally high, 3-fold higher in the hibernating then in the awake hedgehogs. As shown in the present work, the arginine level was significantly lower in the denning bear (*see* Fig. 1).

The glycine level was slightly but not significantly elevated in winter (*see* Fig. 1). Interestingly, every third amino acid in collagen is glycine. Glycine is also needed during the denning period for the synthesis of the porphyrin moiety of haemoglobin, myoglobin and cytochromes (Munro 1970). Nitrogen in each pyrrole ring is derived from glycine. The relationship of glycine to haeme synthesis is obvious in the succinate-glycine cycle, in which, glycine is linked to the citric acid cycle forming aminoadipic acid as a precursor leading to a purine ring. In our study, the concentration of aminoadipic acid was considerably elevated in the denning bears (*see* Fig. 1). Glycine is also known to conjugate with bile acids forming cholic acid; creatine can also be derived from glycine.

Alanine is not known to exert any other specific function except serving as a component of protein structures. However, it may be degradated to acetate. The metabolic reactions of labelled alanine during the winter sleep of the American black bear has been shown to yield pyruvate, lactate and glucose (Nelson *et al.* 1975, Ahlquist *et al*. 1984). In our study, the significantly increased alanine concentration in winter (*see* Fig. 1), may also be linked to the decreased alanine aminotransferase activity, which was found in the denning brown bear by Hissa *et al.* (1994). Alanine and valine are also necessary for the production of pantothenic acid, and for the biosynthesis of coenzyme A, which is an active element in the citric acid cycle. Thus the synthesis of pantothenic acid plays an important role in the catabolism of car-

bohydrates, fats (beta oxidation) and proteins. In addition, pantothenic acid is needed in the synthesis of cholesterol and steroid hormones.

Aminoadipic acid serves as a precursor in the biosynthesis of essential lysine (at least in humans). The lysine concentration was highly elevated in the denning bear (*see* Fig. 1). This amino acid does not participate in the reversible deamination or transamination reactions in mammals. It is also known that lysine is both glycogenic and ketogenic, and consequently, it may serve as a precursor for the synthesis of glycogen and triglyserides.

Using ^{14}C- labelled essential threonine, Meredith *et al*. (1988) were able to collect ^{14}C continuously from the expired air. However, most of the threonine was incorporated into plasma proteins which agrees with our results; no seasonal changes in the plasma concentration of threonine were observed (*see* Fig. 1).

There is a close metabolic relationship between phenylalanine (essential amino acid in humans) and tyrosine, but biochemically the only way to produce tyrosine is to transform phenylalanine into tyrosine; tyrosine does not belong to the essential amino acids like phenylalanine. Tyrosine is important as a precursor of thyroid hormones and various neurotransmitters, like catecholamines.

The cystine concentration was highly elevated during in the denning bears (*see* Fig. 1). This amino acid is a source of sulfur in the biosynthesis of methionine. The carbon skeleton of methionine originates from aspartic acid. Since animals are unable to convert cystine to methionine, methionine belongs to the essential amino acids. Why then the methionine level is highly elevated in the denning brown bear, remains an open question. However, it is well known that methionine is one of the principal methyl donors in the body. Cystine does not belong to the essential amino acids since methionine can be converted to cystine in the animal body.

Taurine is found in a high concentration in the mammalian heart muscle, where it makes half of the total pool of free amino acids (Jacobsen & Smith 1968). Numerous functions have been ascribed to cardiac taurine in hibernating species (Hilton *et al.* 1990). Hilton *et al*. (1990) saw no changes in the plasma or heart taurine concentrations of hibernating or awake brown bats. Taurine is derived from cysteine, and as a cholic acid conjugate is involved in forming of taurocholic acid (bile acid). As found in the present study, taurine level fell during winter starvation (*see* Fig. 1), which was probably a consequence of the decreased need for bile acids. In awake hedgehogs, taurine level was 406 μmol l^{-1}, i.e., 3–4-fold higher than in our experiment, but in deep hypothermia it was only 103 μmol l^{-1} (Kristofferson & Broberg 1968). The change in taurine in the denning brown bear is in agreement with these results (*see* Fig. 1).

4.3. Protein degradation

The measurement of the 3-methylhistidine level has been suggested to be a simple test allowing the estimation of skeletal-muscle metabolism (Burtis & Ashwood, 1994). Post-translational methylation of histidine residues in muscle fibers produces 3-methylhistidine which is secreted into the urine in amounts proportional to a total body-skeletal-muscle degradation (Haverberg *et al.* 1975, Burtis & Ashwood, 1994). Conditions like trauma, steroid administration or starvation have been shown to accelerate the excretion of 3-methylhistidine. In the present study, a highly elevated 3-methylhistidine (*see* Fig. 1) suggests a stimulated muscle-protein degradation in winter. Koebel *et al* (1991) showed that the skeletal muscles of the bears are well adapted to the inactivity in winter. Their energetic substrates, and oxidative metabolic capacity are maintained throughout the winter. However, the muscle-protein concentration was estimated to decline approximately by 10%–20% in winter.

One important source of amino acids seems to be the type I collagen of the connective tissues found all over the animal body. The dramatic, 3.5-fold, increase in the plasma ICTP concentration in winter in comparison with the level in autumn found in our study supports this view (*see* Fig. 1).

5. Conclusions

As a conclusion, we suggest that the elevation of essential amino acids in plasma in winter may be a result of the increased degradation of the type I collagen in the connective tissues, as indicated by the highly elevated plasma ICTP. Another source

of the essential amino acids may be muscle as indicated by the high 3-methylhistidine level. These findings favour the view of the release of essential amino acids from existing protein structures instead of their *de novo* synthesis. Non-essential amino acids are primarily synthetised as a result of urea recycling.

Acknowledgements: The authors express their appreciation to Mrs. M.-L. Martimo for her help in handling the samples. We thank the staff of the Zoological Garden for their technical help in handling the animals and Tiina Karvonen for performing the ICTP analyses. This work has been reviewed and accepted by the Committee of Animal Experimentation of the University of Oulu (31/93).

References

Ahlquist, D. A., Nelson, R. A., Steiger, D. L., Jones, J. D. & Ellefson, R. D. 1984: Glycerol metabolism in the hibernating black bear. — J. Comp. Physiol. B155: 75–79.

Al-Badry, K. S. & Taha, H. M. 1983: Hibernation — hypothermia and metabolism in hedgehogs. Changes in some organic components. — Comp. Biochem. Physiol. 74A: 143–148.

Brown, D. C., Mulhausen, R. O., Andrew, D. J. & Seal, U. S. 1971: Renal function in anesthetized dormant and active bears. — Am. J. Physiol. 220: 293–298.

Burtis, C. A. & Ashwood, E. R. 1994: Clinical Chemistry. — W. B. Saunders Co. Philadelphia.

Egan, A. R., Boda, K. & Varady, J. 1986: Regulation of nitrogen metabolism and recycling. — In: Miligan, L. P., Grovum, W. L. & Dobson, A. (eds.), Control of digestion and metabolism in ruminants: 386–402. Englewood Cliffs, Nj: Prentice-Hall.

Ensrud, E. R., Nelson, R. A., Alt, G., Beck, T., Matula, G. & Rogers, L. 1986: Seasonal variations in the ratio of serum urea to creatinine (U/C) in black bears. — Physiologist 29: 116.

Felig, P., Owen, O. E., Wahren, J. & Cahill, G. F. Jr. 1969: Amino acid metabolism in pronlonged starvation. — J. Clin. Invest. 48: 584–594.

Franzmann, A. W. & Schwartz, C. C. 1988: Evaluation condition of Alaskan black bears with blood profiles. — J. Wildl. Manage. 52: 63–70.

Halloran, D. W. & Pearson, A. M. 1972: Blood chemistry of the brown bear (Ursus arctos) from southwestern Yokon Territory, Canada. — Can. J. Zool. 50: 827–833.

Harlow, H. J. 1987: Urea-hydrolysis in euthermic hibernators and non-hibernators during periods of food availability and deprivation. — J. Thermal Biol. 12: 149–154.

Haverberg, L. N., Deckelbaum, L., Bilmazes, C., Munro, H. N. & Young, V. R. 1975: Myofibrillar protein turnover and urinary N-methylhistidine output. — Biochem. J. 152: 503–510.

Hilton, F. K., Bhatnagar, K. P., Hilton, M. A., Newkirk, M. W. & Montgomery, W. D. 1990: Taurine concentrations in hearts of big brown bats (Eptesicus fuscus) during hibernation, arousal and normothermic behavior. — Comp. Biochem. Physiol. 96A: 431–433.

Hissa, R. 1997: Physiology of the European brown bear (Ursus arctos arctos). — Ann. Zool. Fennici 34: 267–287.

Hissa, R., Siekkinen, J., Hohtola, E., Saarela, S., Hakala, A. & Pudas, J. 1994: Seasonal patterns in the physiology of the European brown bear (Ursus arctos arctos) in Finland. — Comp. Biochem. Physiol. 109A: 781–791.

Jacobsen, J. G. & Smith, L. H. 1968: Biochemistry and physiology of taurine and taurine derivatives. — Physiol. Rev. 48: 424–511.

Koebel, D. A., Miers, P. G., Nelson, R. A. & Steffen, J. M. 1991: Biochemical changes in skeletal muscles of denning bears (Ursus americanus). — Comp. Biochem. Physiol. 100B: 377–380.

Kristofferson, R. 1963: Urea levels in blood and tissues of hibernating and non hibernating hedgehogs. — Nature 197: 402–403.

Kristofferson, R. & Broberg, S. 1968: Free amino acids in blood stream of hedgehogs in deep hypothermia and after spontaneous arousals. — Experientia 24: 148–150.

Lundberg, D. A., Nelson, R. A., Wahner, H. W. & Jones, J. D. 1976: Protein metabolism in the black bear before and during hibernation. — Mayo Clin. Proc. 51: 716–722.

Mason, V. C. 1984: Metabolism of nitrogenous compounds in the large gut. — Proc. Nutr. Soc. 43: 45–53.

Meredith, M., Layman, D. H., Baker, D. H. & Nelson, R. A. 1988: Threonine metabolism in black bears. — Faseb J. Abstract 2: 7166.

Munro, H. N. 1970: Mammalian protein metabolism,Vol. 4: 299–386. Academic Press.

Nelson, R. A. 1980: Protein and fat metabolism in hibernating bears. — Fed. Proceed. 39: 2955–2958.

Nelson, R. A. 1987: Black bears and polar bears — still metabolic marvels. — Mayo Clin. Proc. 62: 850–853.

Nelson, R. A. 1989: Nitrogen turnover and its conservation in hibernation. — In: Malan, A. & Canguilhem, B. (eds.), Living in the cold: 299–307. John Libbey, Eurotext Ltd.

Nelson, R. A. & Jones, J. D. 1987: Leucine metabolism in the black bear. — Int. Conf. Res. Manage. 7: 329–331.

Nelson, R. A., Beck, T . D. I. & Steiger, D. L. 1984: Ratio of serum urea to serum creatinine in wild black bears. — Science 226: 841–842.

Nelson, R. A., Wahner, H.W., Jones, J. D., Ellefson, R. D. & Zollman, P. E. 1973: Metabolism of bears before, during, and after winter sleep. — Am. J. Physiol. 224: 491–496.

Nelson, R. A., Jones, J. D., Wahner, H. W., McGill, D. B. & Code, C. F. 1975: Nitrogen metabolism in bears: urea metabolism in summer starvation and in winter sleep and role of urinary bladder in water and nitrogen conservation. — Mayo Clin. Proc. 50: 141–146.

Pengelley, E. T., Asmundson, S. J. & Uhlman, C. 1971:

Homeostasis during hibernation in the golden-mantled ground squirrel Citellus lateralis. — Comp. Biochem. Physiol. 38A: 645–653.

Ramsay, M. A., Nelson, R. A. & Stirling, I. 1991: Seasonal changes in the ratio of serum urea to creatinine in feeding and fasting polar bears. — Can. J. Zool. 69: 298–302.

Regdeczi, E., Irons, L., Koj, A. & McFarlane, A. S. 1965: Isotopic studies of urea metabolism in rabbits. — Biochem. J. 95: 521–527.

Risteli, J., Elomaa, I., Niemi, S., Novamo, A. & Risteli, L. 1993: Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: A new serum marker of bone collagen degradation. — Clin. Chem. 39: 635–640.

Searcy, R. L. 1969: Diagnostic Biochemistry. — McGraw Hill Book Co., New York.

Steffen, J. M., Rigler, G. L., Moore, A. K. & Riedesel, M. L. 1980: Urea recycling in active golden-mantled ground squirrels, Spermophilus lateralis. — Am. J. Physiol. 8: R168–R173.

Wolfe, R. R., Nelson, R. A., Wolfe, M. H. & Rogers, L. 1982: Nitrogen cycling in hibernating bears. — Proc. Soc. Mass Spectrom. 30: 426.