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Seasonal changes in fatty acids and leptin contents in the plasma of the European brown bear (*Ursus arctos arctos*)

Raimo Hissa, Esa Hohtola, Terhi Tuomala-Saramäki, Tommi Laine & Heikki Kallio

Hissa, R. & Hohtola, E., Department of Biology, University of Oulu, P.O. Box 333, FIN-90571 Oulu, Finland
Tuomala-Saramäki, T., Laine, T. & Kallio, H., Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 Turku, Finland

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The goal of this study was to examine whether or not the preparation of the bear to winter sleep requires changes in the composition of plasma fatty acids similar to those observed in deep hibernators. Seasonal changes in plasma fatty acids of six captive European brown bears (*Ursus arctos arctos*) were investigated between 1991 and 1996. Altogether fifty plasma samples were analysed chromatographically. The weight percentage of unsaturated to saturated fatty acids was 37.1% in winter but only 32.3% in summer. The proportions of palmitic (16:0), octadec-11-enoic (18:1n-7), arachidonic (20:4n-6), and docosahexaenoic (22:6n-3) acids increased significantly in the total fatty acid pool in winter. At the same time, a significant decrease in concentrations of heptadecanoic (17:0), stearic (18:0), oleic (18:1n-9), γ-linolenic (18:3n-6), α-linolenic (18:3n-3) and eicosapentaenoic (20:5n-3) acids was observed. Some fatty acids also act as precursors in the synthesis of specific tissue hormones. The leptin level reached its maximum just prior to winter sleep, i.e. when fat reserves were greatest.

1. Introduction

Recordings of the respiratory quotient (RQ) have shown that fat is the only substrate utilised to fulfil the energy requirement in the maximally 6–7-month-long winter sleep of the bear. An RQ as low as 0.62 was recorded in the American black bear (*Ursus americanus*) (Nelson *et al.* 1973, Ahlquist *et al.* 1976). Bears do not experience any major change in lean body mass while utilising fat reserves (Nelson *et al.* 1975, Lundberg *et al.* 1976). In late summer, the feeding rate is more than doubled. In preparation for winter sleep, the average consumption of 8 000 kcal per day increases up to 15 000–20 000 kcal per day (Nelson 1980). As a result of overeating (hyperphagia) a bear's body mass (BM) may rise 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. Their metabolic rate decreases and during the winter sleep the body

temperature (Tb) is reduced from 37.5°C to 32–33°C (Hissa *et al.* 1994). The loss of BM, mainly due to the consumption of fat reserves, varies from 250 to 500 g per day, depending on the size of the animal and ambient temperature. Winter-sleeping bears do not eat, drink, defecate or urinate.

In deep hibernators, the autumn fattening is associated with hyperinsulinaemia, hypertriglyceridemia, peripheral insulin resistance, and increased lipoprotein lipase activity in the adipose tissue (Florant *et al.* 1985, Feist *et al.* 1986, Bauman *et al.* 1987). In preparation for hibernation, the incorporation of ^{14}C-glucose into adipose tissue lipids may increase up to 88–108-fold (Bintz & Strand 1983). The incorporation of glucose into lipids is a result of preferential activation of the pentose shunt, which favours fatty acid synthesis.

There is still very little information about the hormonal, and neural factors that regulate the fat accumulation and lipolysis in the bear. The recently found protein leptin, a product of *ob* gene (147 amino acids, 16 kDa) released from adipocytes, has been suggested to be involved in the control of body mass in rodents and humans (for review see Remesar *et al.* 1997). Leptin is presumed to provide an afferent signal to hypothalamus which results in the loss of appetite and body fat (Zhang *et al.* 1994, Halaas *et al.* 1995, Pelleymounter *et al.* 1995). Consequently, the plasma-leptin level is supposed to decrease after a major reduction in adipose mass, and increase with increasing mass of white adipose tissue (Considine *et al.* 1995, Maffei *et al.* 1995). A functional, still unknown, relation prevails between insulin and leptin under conditions of hyperglycemia or high energy availability (e.g., Saladin *et al.* 1996).

It has been suggested that polyunsaturated fatty acids (PUFAs) are required in the diet for hibernation (Fawcett & Lyman 1954, Geiser & Kenagy 1987, Frank 1991, 1994, Florant *et al.* 1993, Frank & Storey 1996). Increasing the amount of linoleic (18:2n-6), and α-linolenic acids (18:3n-3) in the diet increases the hibernation ability and bouts (Florant *et al.* 1990, 1993, Frank 1991, Geiser 1991, Geiser & Kenagy 1987, 1994, Frank & Storey 1996). On the other hand, Florant *et al.* (1990) showed that saturated fatty acids are preferentially released in deep hibernation and PUFAs are selectively retained in the fat reserves.

The variation of the fatty acid composition in the plasma of the bear before, during and after the winter denning has received virtually no previous considerations. Käkelä and Hyvärinen (1996) studied the fatty acid composition of the adipose tissue in the European brown bear killed in the autumn.

The goal of this study was to examine whether the preparation of the European brown bear to winter sleep requires changes in the compisition of fatty acid similar to those observed in deep hibernators. This type of information did not exist before our study. As a first step, relative proportions of plasma fatty acids were analysed from total lipids without fractionation. We also decided to analyse the changes in ratios of individual fatty acids around the year to see whether or not there are any trends to be investigated later in more detail. Preliminary results on seasonal changes in the plasma-leptin level of the European brown bear are also presented.

2. Materials and methods

2.1. Animals

The plasma of six European brown bears (*Ursus arctos arctos* L.) kept in captivity in the bear enclosures of the University of Oulu (65°N, 25°24´E) between autumn 1991 and spring 1996 was studied. The age, weight and anaesthesia of the bears and the sampling dates were presented earlier by Hissa *et al.* (1994), and Hissa (1997). Body temperatute (Tb) was measured with dataloggers, either with HOBO-Temp or StowAway™ XTI (Onset Computer Corp., USA; thermistor accuracy ± 0.25°C), chronically implanted in the body cavity. Tb varied between 33–35°C in the denning bear (Hissa *et al.* 1994).

During winter sleep, which normally started at the beginning of December and lasted to the end of February or beginning of March, the bears did not eat, drink, urinate or defecate. On average, the bears lost 20%–23% of the BM during the denning period.

Blood samples were collected from the jugular vein into evacuated glass tubes containing either EDTA or lithium heparin. Blood samples were immediatelly centrifuged and plasma stored at –70°C until being analysed.

2.2. Extraction of lipids

Lipids were extracted from 0.5–0.7 g of plasma with 6.0 ml chloroform–methanol (2:1, v/v) at room temperature according to a modified Folch method (Folch *et al.* 1957, Ways & Hanahan 1964). The solvents were evaporated under nitrogen stream to a volume of 2.0 ml, filtered with Minispike PUDF Bulk Acrodisc 13, 0.45 mm filters (Gleman Sciences Inc., USA) and evaporated to dryness. After weighing, the

lipids were dissolved in 1.0 ml of hexane and kept at –20°C until analysis took place. Extraction of each sample was carried out in duplicate.

2.3. Preparation of methyl esters

Hexane was evaporated and fatty acyl groups of lipids, mainly of acylglycerols, were transesterified with sodium methoxide (Christie 1982). The methyl esters were purified with a florisil column 20 × 5 mm (Fluka Chemie AG, Switzerland) and dissolved in 0.5 ml of hexane.

2.4. Gas chromatographic analyses

Gas chromatographic (GC) analyses of fatty-acid-methyl esters of fifty plasma samples of six bears were carried out with two instruments: (a) Varian 3300 (Limeric, Ireland) coupled with a Shimadzu C-R3A integrator, and (b) Perkin Elmer AutoSystem with an autosampler directed with a Turbochrom programme. The column and analytical conditions were identical in both systems. The fused silica columns (25 m × 0.32 mm, i.d.) had NB-351 liquid phase (d_f = 0.20 mm) (Nordion, Finland). Helium was used as the carrier gas (flow rate = 27 cm s^{-1} at 120°C), and its split ratio in the injector (225°C) was 40:1. The sample volume injected was 1.0 µl and each sample was analysed two or three times. The temperature programme was 2 min at 120°C, 3°C × min^{-1}, 20 min at 230°C. The temperature of the flame ionization detector was 240°C. No internal standards were used because only proportions of fatty acids were determined. Fatty acids were identified by commercial mixtures of reference fatty-acid-methyl esters: (a) branched 90-1051 BR 1 (Larodan Fine Chemicals AB, Sweden), and (b) straight chain saturated and unsaturated 68 A and 68 D (Nu Chek Prep, Inc., USA) and 90-1061 ME 61 (Larodan). The sum of the average 31 largest peaks were defined to be 100% in the quantitative analyses. The same peaks were taken into account in each chromatogram, the smallest compounds comprising ca. 0.1% proportion.

2.5. Assay of plasma leptin

Linco's multi-species leptin radioimmunoassay kit (Linco Research, Inc., USA) was used. The antibody was raised against human leptin but displays broad crossreactivity to leptin molecules of many species. Altogether 17 samples collected in 1996–1997 were analysed.

2.6. 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 fatty acid concentrations, Friedman's test for multiple dependent samples was used as implemented in SPSS Software (SPSS Inc.). No group-wise *a posteriori* comparisons were made so the *p*-values shown indicate a general effect of the season.

3. Results

Lipid content in plasma of the individual bear was shown to vary from as low as 0.25% (w/w) up to 1.89%, but there were no seasonal trends. The avearge plasma lipid content of six bears was 1.09 ± 0.15 (mean ± SE) in the summer, 1.10 ± 0.13 in the autumn, 0.91 ± 0.12 during the denning period, and 1.14 ± 0.11 later in the spring. None of the values differ statistically from each other.

As an example of the analyses, a chromatogram of the fatty-acid-methyl esters of plasma lipids collected in September 1995 from one male bear (Nr. 10; *see* Hissa 1997), is shown in Fig. 1. The indicated peaks were in every case included in the quantitative analyses. Repeatability of their GC analyses was good, with coefficients of variation (CV) from 1% to 3%.

As shown by the relative proportions, there was typically a statistical difference between the fatty acid levels of the denning period and other seasons (Fig. 2). The concentrations of palmitic (16:0), octadec-11-enoic (18:1n-7), arachidonic (20:4n-6) acids, and docosahexaenoic (DHA, 22:6n-3) in plasma in winter were typically higher, regardless of the sex, age, pregnancy or nursing period of the bears (Fig. 2). A slight decrease was observed in the concentrations of palmitoleic (16:1n-7), and dihomo-γ-linolenic (20:3n-6) acid. A significant drop in the levels of heptadecanoic (17:0), stearic (18:0), oleic (18:1n-9), γ-linolenic (18:3n-6), α-linolenic (18:3n-3), and eicosapentaenoic (EPA, 20:5n-3) acids was observed in the winter.

There seems not to be any relationships in the proportions of saturated monoenoic, (n-3)-family or (n-6)-family fatty acids in the total fatty acid pool.

The ratio between the proportion of linoleic acid and arachidonic acid was the same both in summer and in winter (Table 1). The ratio between α-linolenic acid and EPA was 0.4 in the summer but in winter 1.2, i.e. the difference between winter and

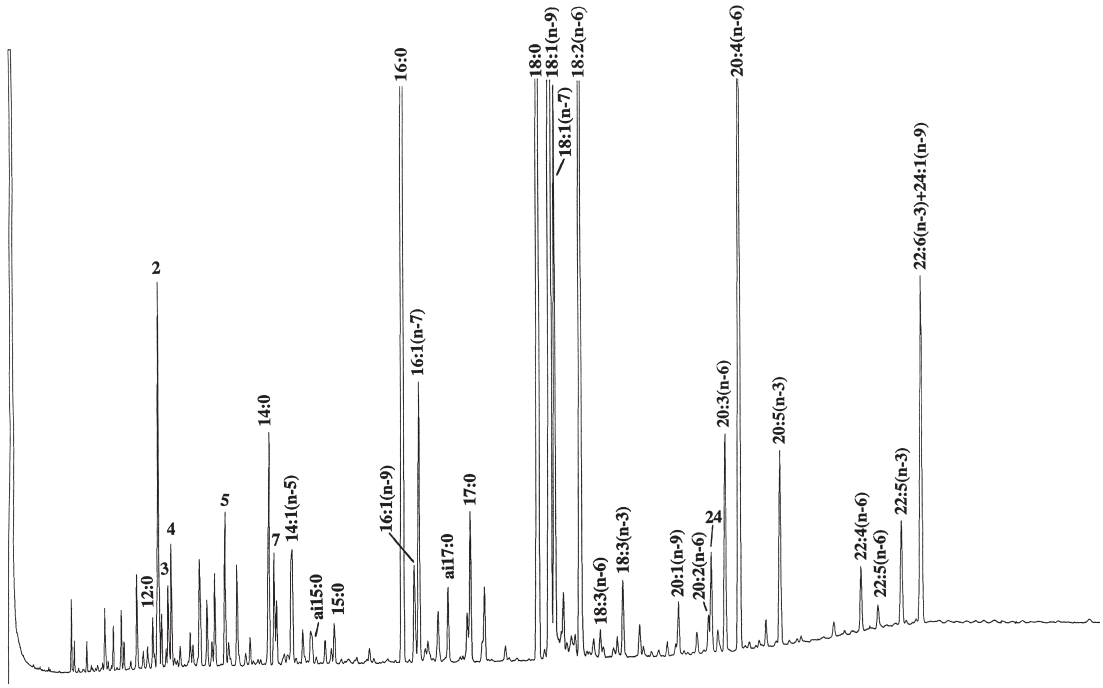

Fig. 1. An example chromatogram of fatty-acid-methyl esters of plasma lipids in one male bear (29 Sep. 1995).

summer was 4-fold (Table 1). The ratio between α-linolenic acid and DHA was unchanged (Table 1).

In the denning bear, the proportions of the (n-9)-family fatty acids (18:1n-9 and 20:1n-9) were only half of those observed in summer (11.8 ± 2.28 vs. 23.6 ± 5.50 and 0.1 ± 0.04 vs. 0.3 ± 0.09, respectively).

Differences between male and female bears as well as between pregnant and non-pregnant females were tested. All the differences observed were much less significant than those induced by the seasons. The ratio of the percentage amount of arachidonic acid (10.4 ± 0.53) to EPA (0.22 ± 0.09) was ca. 50 in winter but only 5 in summer (7.8 ± 1.86 vs. 1.6 ± 0.60). The ratio of DHA (4.1 ± 1.10) to EPA (0.22 ± 0.09) was 18.6 in winter compared with 1.4 in summer (2.2 ± 1.37 vs. 1.6 ± 0.60).

Averaged results of fatty acids of samples collected in autumn, together with reference to analysis of bear subcutaneous fat (Käkelä & Hyvärinen 1996) are presented in Table 2:

The plasma leptin level (data from only one bear), tend to increase in autumn thus correlating well with the increase in the BM, i.e., inreasing fat content (Fig. 3).

4. Discussion

Our study showed that the most dramatic changes in plasma fatty acids occured in the proportions of palmitic (16:0), stearic (18:0), octadecenoic

Table 1. Seasonal changes in the ratio of linoleic acid to the two eicosanoid precursors in the same (n-6)-family, dihomo-γ-linolenic acid and arachidonic acid; and the corresponding ratios of α-linolenic acid to EPA (also an eicosanoid precursor) and DHA in the European brown bear.

	Summer	Autumn	Winter	Spring
[linoleic acid (18:2n-6)]:[dihomo-γ-linolenic acid (20:3n-6)]	9.1	7.0	13.9	9.0
[linoleic acid]:[arachidonic acid (20:4n-6)]	1.6	1.4	1.6	1.8
[α-linolenic acid (18:3n-3)]:[eicosapentaenoic acid (20:5n-3)]	0.3	0.4	1.2	0.3
[α-linolenic acid]:[docosahexaenoic acid (22:6n-3)]	0.2	0.2	0.2	0.2

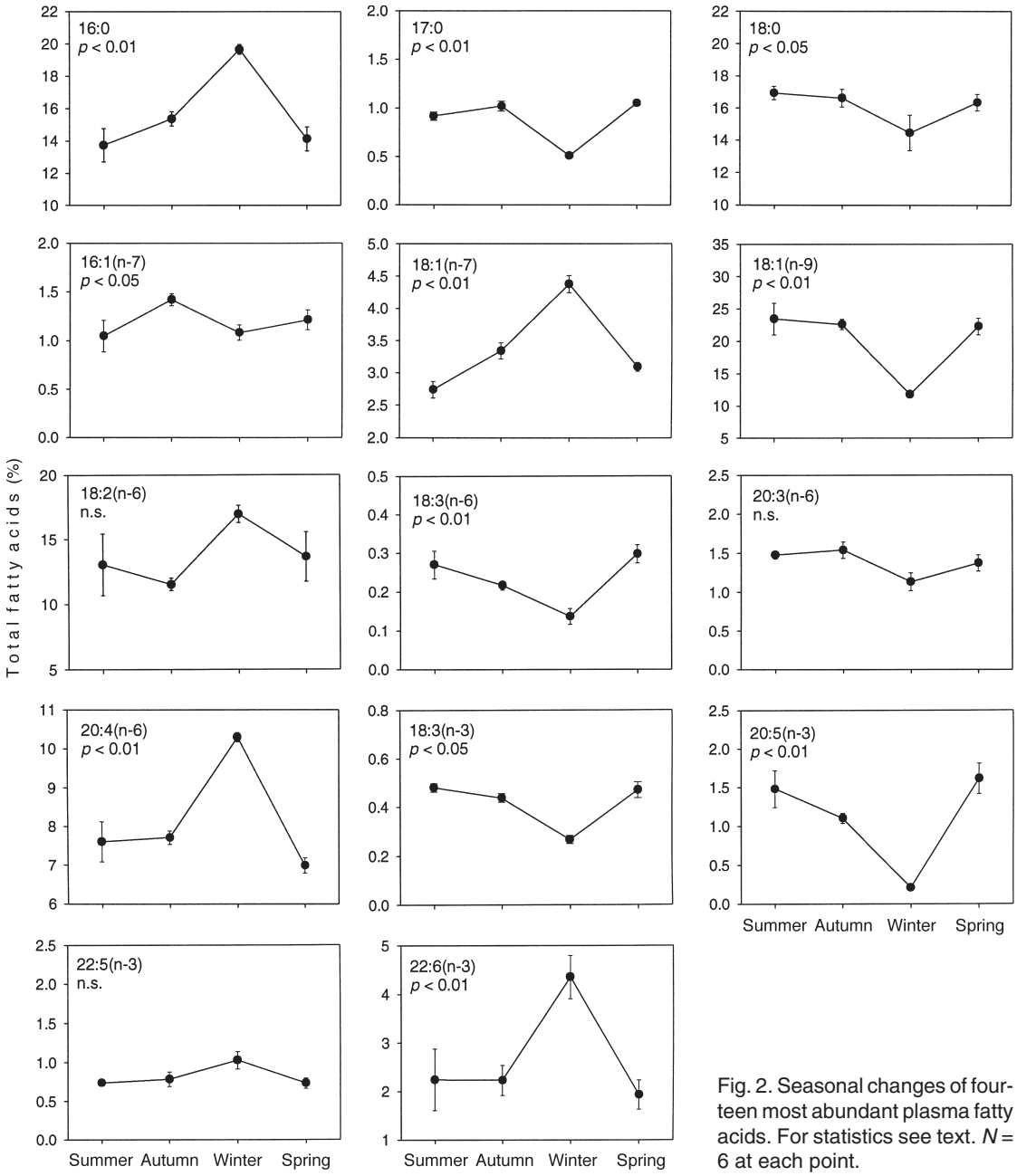

Fig. 2. Seasonal changes of fourteen most abundant plasma fatty acids. For statistics see text. $N = 6$ at each point.

(18:1n-7), oleic (18:1n-9), heptadecenoic (17:0), arachidonic (20:4n-6), linoleic (18:2n-6), α-linolenic (18:3n-3), γ-linolenic (18:3n-6), EPA and DHA acids in the total fatty acid pool during the denning period. It was of interest to note that EPA and DHA showed absolute out-of-phase profiles during winter, without any exceptions. These pure analytical data do not give, however, any explanations as to why it was so, but the profiles (which are not the only interesting ones in our investigation) are clear indications of the still unknown details of lipid metabolism.

It is well known that the increase in the concentration of unsaturated fatty acids (UFA) and PUFA, especially in the cell membranes, is typical to ectothermic animals acclimated to cold. In

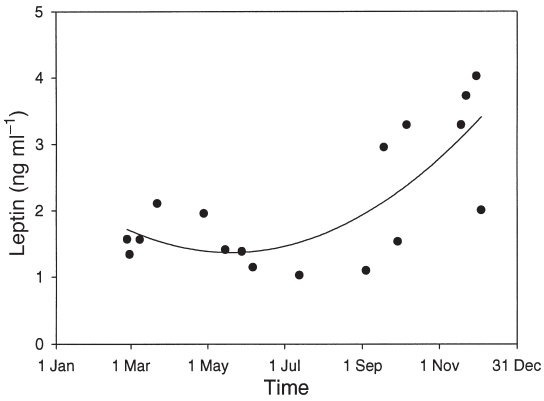

Fig. 3. An example of seasonal changes in the plasma leptin contentration (ng ml^{-1}) of one bear (22 Feb 1994–18 Nov 1997).

Table 2. Comparison between the weight percentage of plasma fatty acids of seventeen samples collected from six brown bears during autumns 1991–95 and subcutaneous fatty acids (Käkelä & Hyvärinen 1996) of the brown bear in autumn.

Fatty acid	Plasma* (P)	Subcutaneous fat (S)	P:S
12:0	0.2 (29.6)		
14:0	1.3 (27.6)	1.8	0.7
14:1(n-5)	0.6 (30.6)		
ai15:0	0.6 (74.7)		
15:0	0.2 (20.0)	0.5	0.4
16:0	15.3 (13.0)	24.7	0.6
16:1(n-9)	0.6 (29.6)	0.6	1.0
16:1(n-7)	1.3 (28.2)	6.5	0.2
ai17:0	0.3 (16.9)		
17:0	1.1 (22.2)		
18:0	17.8 (7.4)	8.0	2.2
18:1(n-9)	23.0 (12.1)	42.7	0.5
18:1(n-7)	3.3 (10.4)		
18:2(n-6)	13.8 (25.2)	4.1	1.2
18:3(n-6)	0.3 (27.7)		
18:3(n-3)	0.5 (21.4)	1.4	0.4
20:1(n-9)	0.3 (32.3)	0.5	0.6
20:2(n-6)	0.3 (70.4)		
20:3(n-6)	1.8 (13.4)	0.1	18
20:4(n-6)	8.1 (9.6)	0.3	27
20:5(n-3)	1.4 (37.4)	0.1	14
22:4(n-6)	0.4 (19.8)	0.1	4
22:5(n-6)	0.1 (27.3)		
22:5(n-3)	0.9 (25.3)	0.2	4.5
22:6(n-3)	2.4 (40.8)	0.1	24
Sn-6 PUFA	24.8	4.6	5.4
Sn-3 PUFA	5.2	1.8	2.9

*Results are means with RSD% in parentheses.

typical hibernators, however, these seasonal shifts are far less pronounced (e.g., Geiser *et al.* 1984, Aloia 1988). The ratio of UFA to saturated fatty acids show only a slight increase during the hibernation period and PUFAs are predominantly conserved (Aloia 1988, Florant *et al*. 1990).

Most of the gain of BM in the bear in late summer is in the form of fat. At this time of year, bears favour berries like bilberry (*Vaccinium myrtillus*), lingonberry (*V. vitis-idaea*), bogwhortleberry (*V. ulicinosum*), cranberry (*V. oxycoccos*), crowberry (*Empetrum nigrum*), cloudberry (*Rubus chamaeomorus*); and other parts of herbaceous plants (Haglund 1968, Mysterud 1977, Danilov 1983, Pulliainen 1986). The carbohydrate content of berries is relatively high and sugars are biochemically transformed into lipids for storage in the adipose tissue. According to Shapiro (1977), all vertebrates can synthesise fatty acids containing either no (saturated) or one double bond (monosaturates), but they are incapable of synthesising polyunsaturated fatty acids (PUFAs). PUFAs of eaten plants are incorporated into the fat reserves in mammals (Mead *et al.* 1986, Frank & Storey 1996)). Bears as well derive the essential PUFAs primarily from berries. They do not, however, neglect the use of animal food if available. Haglund (1968) observed that in Sweden the number of reindeers killed by bears is more than doubled in autumn.

Composition of the PUFA pool in animals is the net result of a complex interrelationship of a number of factors. These include the dietary intake of various fatty acids, the rates of their desaturation-elongation processes, the rates of their oxidation for energy production, and other biosynthetic reactions (Lee *et al.* 1992, Colby & Pond 1993, Cherian & Sim 1995, Klingenberg *et al.* 1995). So far, the fat composition of the food preferred by bears before the winter sleep has not been studied.

The ratio of the fatty acid contents of the (n-6)- and (n-3)-families was five in winter and four in summer. The variations were, however, not analogous within the two families. There was only a small drop in the concentration of dihomo-γ-linolenic acid (20:3n-6) during denning period, but the level of metabolically neighbouring arachidonic acid (20:4n-6) was at the same time at its maximum (Fig. 2).

Oleic acid is a typical major fatty acid in plant lipids. Linoleic and α-linolenic acids are also abundant components of most vegetable oils and belong to the essential fatty acids of all vertebrates (Robbins 1983, Mead *et al.* 1986). Actually, together with arachidonic acid they form the most common PUFAs in animal adipose tissue.

Saturated fatty acids as well as unsaturated members of the (n-7)- and (n-9)-families can be synthesised *de novo* by mammals to supplement the dietary intake. The sequences of reactions taking place in linoleic and α-linolenic acids are analogous. Linoleic acid is the dietary precursor of dihomo-γ-linolenic, and arachidonic acids of the (n-6)-family and α-linolenic acid is the precursor of EPA of the (n-3)-family. These three products, again, are precursors of certain eicosanoids of different types of prostanoids and leucotrienes with various physiological functions (Sprecher 1989).

The content of EPA in plasma was very low in winter, whereas the concentrations of docosapentaenoic acid (DPA, 22:5n-3) and DHA were at their maxima. All these polyunsaturated fatty acids, except α-linolenic acid, were enriched in plasma when compared with the subcutaneous fat of the bear (*see* Table 2). Particularly the differences in the levels of arachidonic acid and DHA were notable. Biologically, the balance between arachidonic acid and EPA is very important, because it affects the amounts and ratios of eicosanoid biosynthesis (Hwang 1992).

It is known that eicosanoids affect blood pressure, stimulate the parasympathetic nervous system, act on smooth muscle fibers, platelet aggregation, and contraction/dilatation of glandular vessels (Hwang 1992). A deficiency of essential fatty acids and their metabolites may also result in inadequate formation and maintenance of phospholipids of cell membranes. DHA is also a key fatty acid in phopholipids, especially in neural membranes and the retinal tissue but it is not a precuror of eicosanoids.

Arachidonic, dihomo-γ-linolenic and EPA are precursors of tissue hormones classified as prostaglandins, prostacyclins, tromboxanes and leucotrienes (Bjerve *et al*. 1990). They are control substances with special, local physiological activity (Mead *et al.* 1986, Boissonneault & Hayek 1992, Bruckner 1992). Within the group of prostanoids, the prostacyclins and tromboxanes have many physiological effects also at the cellular level.

It was of interest to recognise the marked increase in palmitic acid, and the simultaneous decrease of stearic acid in the plasma of the bear in winter. Palmitic and stearic acids are the precursors of palmitoleic and oleic acids, respectively. Proportions of both of these unsaturated acids, produced by δ-9-desaturase enzyme, decreased in the fatty-acid pool in winter. No accumulation of palmitoleic or oleic acids in plasma occurred, neither from subcutaneous storages nor via a *de novo* synthesis. The concentration of positional isomer of oleic acid, octadec-11-enoic acid (18:1n-7), showed *vice versa* an increasing trend in the denning bear. Since the dietary input is non-existent during the winter sleep, all differences in the plasma fatty acid composition are due to changes in the release, elongation and desaturation of the depot fatty acids or, *de novo* synthesis of saturated, (n-7)- or (n-9)-fatty acids.

Whether the released fatty acids in the plasma are in a direct proportion to their content in the adipose tissue is still very much an open question (*see also* Raclot & Groscolas 1993). In some studies, the release of fatty acids was shown to be a random process (*see* e.g. Meinertz 1963, Hunter *et al.* 1970, Gavino & Gavino 1992). Comparison of the plasma fatty acid contents presented here with their composition in the adipose tissue (Käkelä & Hyvärinen 1996), however, indicates that the release of fatty acids from the adipose tissue of the bear to plasma is not a random but a highly selective process (Table 2). According to Raclot and Groscolas (1993), fatty acids are more readily mobilised when they are short and unsaturated.

Laboratory experiments with deep hibernators such as the yellow-bellyed marmot (*Marmota flaviventris*) (Florant *et al.* 1990), the yellow pine chipmunk (*Eutamias amoenus*) (Geiser 1993, Geiser & Kenagy 1987), and the ground squirrel (*Spermophilus belding*) (Frank 1991) showed that high levels of linoleic and α-linolenic acids are needed in the diet during fattening and preparation for proper hibernation. It can be assumed that under steady conditions during winter sleep of the bear, all saturated fatty acids must arise either from lipolysis or via a *de novo* synthesis of fatty acids. However, the *de novo* synthesis may not be a most probable option due to the 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). Therefore one would expect that bears also have a depressed fatty acid synthesis under heavy starvation during winter sleep.

The involvement of insulin and glucocorticoids in the process of fattening in the predenning bear is still unknown. In hibernators, both insulin and glucocorticoids were observed to be at peak levels prior to hibernation (Shivatcheva *et al.* 1988, Armitage 1991, Boswell *et al.* 1994). In recent studies, leptin was shown to be involved in the control of food intake in rodents (Campfield *et al.* 1995, Halaas *et al.* 1995, Ormseth *et al.* 1996, Pelleymounter *et al*. 1995), and in their preparation for hibernation (for a review see Boyer *et al*. 1996). Insulin was also shown to play a significant role in up-regulating of the leptin synthesis (for review *see* Boyer *et al.* 1996). The circulating level of leptin in the bear was at its highest at the beginning of winter sleep, i.e., when the fat content was highest (Fig. 3). A theory of the feedback regulatory system involving the circulating leptin, and its binding to hypothalamic receptors, however, is too simple. Certainly, also other still unknown variables are associated with the regulation of adiposity.

Summarising, the catabolic and anabolic control mechanisms that produce the observed changes in plasma fatty acids in the denning bear are still unknown, and beyond the scope of the present study. The analyses of plasma revealed that the weight percentage of unsaturated to saturated fatty acids was 37.1% in winter and 32.3% in summer. Consequently, it may be assumed that there is a trend towards greater use of, and need for, unsaturated fatty acids for the energy production during the denning period of the bear. However, the precise significance and the regulation of the observed alterations in individual plasma fatty acids during winter sleep remains to be elucidated. It is hard to believe that due to only a small drop in body temperature, PUFAs are needed for the preparation of cell membranes for functioning at decreased body temperature.

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References

Ahlquist, D. A., Nelson, R. A., Jones, J. D. & Ellefson, R. D. 1976: Glycerol and alanine metabolism in the hibernating black bear. — Physiologist 19: 107.

Aloia, R. C. 1988: Lipid, fluidity and functional studies of membranes of hibernating mammals. — In: Aloia, R. C., Curtain, C. C. & Gordon, L. M. (eds.), Physiological regulation of membrane fluidity: 1–39. Liss, New York.

Armitage, K. B. 1991: Factors affecting corticosteroid concentrations in yellow-bellied marmots. — Comp. Biochem. Physiol. 98A: 47–54.

Bauman, W. A., Meryn, S. & Florant, G. L. 1987: Pancreatic hormones in the nonhibernating and hibernating golden mantled ground squirrel. — Comp. Biochem. Physiol. 86A: 241–244.

Bintz, G. L. & Strand, C. E. 1983: Radioglucose metabolism by Richardson's ground squirrels in the natural environment. — Physiol. Zool. 56: 639–647.

Bjerve, K. S., Brekker, O.-L., Fougner, K. J. & Midthjell, K. 1990: Omega-3 and omega-6 fatty acids in serum lipids and their relationship to human disease. — In: Galli, C. & Simopoulos, A. P. (eds.), Dietary ϖ3 and ϖ6 fatty acids: 241–251. Plenum Press, New York, London.

Boissonneault, G. A. & Hayek, M. G. 1992: Dietary fat, immunity, and inflammatory disease. — In: Chow, C. K. (ed.), Fatty acids in foods and their health implications: 707–734. Marcel Dekker, New York.

Boswell, T., Woods, S. C. & Kenagy, G. J. 1994: Seasonal changes in body mass, insulin, and glucocorticoids of free-living golden-mantled ground squirrels. — J. Mammal. 53: 157–167.

Boyer, B. B., Ormseth, O. A., Nicolson, M. & Pelleymounter, M. A. 1996: Is leptin involved in the seasonal control of appetite in hibernating mammals? — In: Geiser, F., Hulbert, A. J. & Nicol, S. C. (eds.), Adaptations to the cold: Tenth Intern. Hibernation Symp: 237–244. Univ. New England Press, Armidale.

Bruckner, G. 1992: Biological effects of polyunsaturated fatty acids. — In: Chow, C. K. (ed.), Fatty acids in foods and their healthy implications: 631–646. Marcel Dekker, New York.

Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R. & Bum, P. 1995: Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. — Science 269: 546–549.

Cherian, G. & Sim, J. S. 1995: Dietary α-linolenic acid alters the fatty acid composition of lipid classes in swine tissues. — J. Agr. Food Chem. 43: 2911–2916.

Christie, W. W. 1982: Lipid analysis. — Pergamon, New York.

Colby, R. H. & Pond, M. A. 1993: Site-specific differences in the responses of guinea-pig adipose tissue to changes in the fatty acid composition of the diet. — Nutr. Res. 13: 1203–1212.

Considine, R. V., Considine, E. L., Williams, C. J., Nyce, M. R., Magosin, S. A., Bauer, T. L., Rosato, E. L., Colberg, J. & Caro, J. F. 1995: Evidence against either premature

stop codon or the absence of obese gene mRNA in human obesity — rapid publication. — J. Clin. Invest. 95: 2986–2988.

Danilov, P. I. 1983: The brown bear (Ursus arcos L.) as a predator in the European taiga. — Acta Zool. Fenn. 174: 159–160.

Fawcett, D. W. & Lyman, C. P. 1954: The effect of low environmental temperature on the composition of depot fat in relation to hibernation. — J. Physiol. Lond. 126: 235–247.

Feist, D., Florant, G., Greenwood, M. R. C. & Feist, C. 1986: Regulation of energy stores in arctic ground squirrels: Brown fat thermogenic capacity, lipopotein lipase and pancreatic hormones during fat deposition. — In: Heller, H. C., Musacchia, X. C. & Wang, L. C. H. (eds.), Living in the cold: 281–285. Elsevier, New York.

Florant, G. L., Lawrence, A. K., Williams, K. & Bauman, W. A. 1985: Seasonal changes in pancreatic B-cell function in euthermic yellow-bellied marmots. — Am. J. Physiol. 249: R159–R165.

Florant, G. L., Nuttle, L. C., Mullinex, D. E. & Rintoul, D. A. 1990: Plasma and white adipose tissue lipid composition in marmots. — Am. J. Physiol. 258: R1123–R1131.

Florant, G. L., Hester, L., Ameenuddin, S. & Rintoul, D. A. 1993: The effect of a low essential fatty acid diet on hibernation in marmots. — Am. J. Physiol. 264: R747–R753.

Folch, J., Lees, M. & Stanley, G. H. S. 1957: A simple method for the isolation and purification of total lipids from animal tissues. — J. Biol. Chem. 226: 497–509.

Frank, C. L. 1991: Adaptations for hibernation in the depot fats of a ground squirrel (Spermophilus beldingi). — Can. J. Zool. 69: 2707–2711.

Frank, C. L. 1994: Polyunsaturated content and diet selection by ground squirrels (Spermophilus lateralis). — Ecology 75: 458–463.

Frank, C. L. & Storey, K. B. 1996: The effect of total unsaturate content on hibernation. — In: Geiser, H. F. & Nicol, S. C. (eds.), Adaptation to the cold: 211–216. 10th Int. Hibern. Symp. University of New England Press, Armidale.

Gavino V. C. & Gavino, G. R. 1992: Adipose hormone-sensitive lipase preferentially releases polyunsaturated fatty acids from triglyserides. — Lipids 27: 950–954.

Geiser, F. 1991: The effect of unsaturated and saturated lipids on the pattern of the fatty acid composition of tissues and membranes of the deer mouse, Peromyscus maniculatus. — J. Comp. Biochem. Physiol. B 161: 590–597.

Geiser, F. 1993: Dietary lipids and thermal physiology. — In: Carey, C., Florant, G. L., Wunder, B.A. & Horwitz, B. (eds.), Life in the cold: Ecological, physiological and molecular mechanisms: 141–153. Westview Press, Boulder.

Geiser, F. & Kenagy, G. J. 1987: Polyunsaturated lipid diet lengthens torpor and reduce body temperature in a hibernator. — Am. J. Physiol. 252: R897–R901.

Geiser F. & Kenagy, G. J. 1994: Dietary fats and torpor patterns in hibernating ground squirrels. — Can. J. Zool. 71: 1182–1185.

Geiser, F., Augee, M. L. & Raison, J. K. 1984: Thermal response of liver mitochondrial membranes of the heterothermic bat (Miniopterus schreibersii) in summer and winter. — J. Thermal Biol. 9: 183–188.

Haglund, B. 1968: De stora rovdjurens vintervanor II. — Viltrevy 5: 217–361.

Halaas, J. L., Gajiwala, K. S., Maffei, M., Cohen, S. L., Chait, B. T., Rabinowitz, D., Lallone, R. L., Burley, S. K. & Friedman, J. M. 1995: Weight-reducing effects of the plasma protein encoded by the obese gene. — Science 269: 543–546.

Hissa, R. 1997: Physiology of the European brown bear (Ursus arctos arctos): a review. — Ann. Zool. Fennica 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. A 109: 781–791.

Hunter, J. D., Buchanan, H. & Nye E. R. 1970: The mobilization of free fatty acids in relation to adipose tissue triglyseride fatty acids in the rat. — J. Lipid Res. 11: 259–263.

Hwang, D. 1992: Dietary fatty acids and eicosanoids. — In: Chow, C. K. (ed.), Fatty acids in foods and their health implications: 545–557. Marcel Dekker, New York, Hong Kong.

Klingenberg, I. L., Knabe, D. A. & Smith, S. B. 1995: Lipid metabolism in pigs fed beef tallow or high-oleic acid sunflower oil. — Comp. Biochem. Physiol. 110B: 183–192.

Käkelä, R. & Hyvärinen, H. 1996: Site-specific fatty acid composition in adipose tissues of several northern aquatic and terrestrial mammals. — Comp. Biochem. Physiol. 115B: 501–514.

Lee, J. H., Ikeda, I. & Sugano, M. 1992: Effects of dietary n-6/n-3 polyunsaturated fatty acid balance on tissue lipid levels, fatty acid patterns, and eicosanoid production in rats. — Nutrition 8: 162–166.

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.

Maffei, M., Halaas, J., Ravissin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganathan, S., Keern, P.A. & Friedman, J. M. 1995: Leptin levels in human and rodent: Measurement of plasma leptin and ob mRNA in obese and weight-reduced subjects. — Nature Medicine 1: 1155–1161.

Mead, J. F., Alfin-Stater, R., Howton, D. & Popjak, G. 1986: Lipids: chemistry, biochemistry, and nutrition. — Plenum Press, New York.

Meinertz, H. 1963: Selective retentition of oleic acid by adipose tissue in vitro. — Fed. Proc. 22: 375A.

Mysterud, I. 1977: Problemer i forskning og forvaltning av bjørn in Norge. — Viltrapport 4: 19–51.

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

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.

Ormseth, O. A., Nicolson, M., Pelleymounter, M. A. & Boyer, B. B. 1996: Leptin inhibits prehibernation hyperphagia and reduces body weight in arctic ground squirrels. — Am. J. Physiol. 271: R1775–R1779.

Pelleymounter, M. A., Cullen, M., Baker, M., Hecht, R., Winters, D., Boone, T. & Collins, F. 1995: Effects of the obese gene product on body weight regulation in ob/ob mice. — Science 269: 540–543.

Pulliainen, E.1986: Habitat selection in the brown bear in Eastern Finland. — In: Proc. Grizzly Bear Habitat Symposium, Missoula, Montana, April 30–May 2, 1985. General Techn. Rep. INT-207. United States Department of Agriculture.

Raclot T. & Groscolas, R. 1993: Differential mobilization of white adipose tissue fatty acids according to chain length, unsaturation, and positional isomerism. — J. Lipid Res. 34: 1515–1526.

Remesar, X., Rafecas, I., Fernandez-Lopez , J. A. & Alemany, M. 1997: Leptin. — Med. Res. Rev. 17: 225–234.

Robbins, C. T. 1983: Wildlife feeding and nutrition. — Academic Press, New York.

Saladin, R. P. De Vos, Guerre-Millo. M., Leturque, A., Girard, J., Staels, D. & Anwertz, J. 1996: Transient increase in obese gene expression after food intake or insulin administration. — Nature 377: 527–529.

Shapiro, B. 1977: Adipose tissue. — In: Snyder, F. (ed.), Lipid metabolism in mammals: 287–316. Plenum Press, New York.

Shivatcheva, T. M., Ankov, V. K. & Hadjioloff, A. I. 1988: Circannual fluctuations of the serum cortisol in the European ground squirrel, Citellus citellus L. — Comp. Biochem. Physiol. 90A: 515–518.

Sprecher, H. 1989: (n-3) and (n-6) fatty acid metabolism. — In: Galli, C. & Simopoulos, A. P. (eds.), Dietary n3 and n6 fatty acids: Biological effects and nutritional essentiality: 69–80. Plenum Press, New York.

Ways, P. & Hanahan, D. J. 1964: Characterization and quantification of red cell lipids in normal man. — J. Lipid Res. 5: 318–328.

Willis, J. S. 1982: Intermediate metabolism in hibernation: — In: Lyman, C. P., Willis, J. S., Malan, A. & Wang, L. C. H. (eds.), Hibernation and torpor in mammals and birds: 124–139. Academic Press, New York.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, I. & Friedman, J. M. 1994: Positional cloning of the mouse obese gene and its human homologue. — Nature 372: 425–432.