Ann. Zool. Fennici 17:191-201. 1980

Seasonal changes in the fatty acid spectrum of the hedgehog's white and brown adipose tissue

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Laukola, S. 1980: Seasonal changes in the fatty acid spectrum of the hedgehog's white and brown adipose tissue. — Ann. Zool. Fennici 17:191—201.

In the hedgehog (*Erinaceus europaeus* L.) the main fatty acids in the white adipose tissue (WAT) and brown adipose tissue (BAT) are myristic, palmitic, palmitoleic, stearic, oleic and linoleic acids; WAT also contains one unsaturated unknown of the 16-carbon-group and one of the 18-carbon-group, while BAT contains a few percent of eicosenoic and docosahexaenoic acids. The proportion of long-chain acids is higher in BAT than in WAT. Of individual fatty acids, there is a higher proportion of palmitoleic acid in WAT, and of stearic acid in BAT. The fatty acid spectrum shows the greatest changes in autumn and at the onset of hibernation, and in spring when the animal begins to feed again. The even-numbered saturated fatty acids are lowest during the hibernation period, and their proportion increases in spring. The relative amounts of 15:0, 17:0, 17:1 and of the 14- and 16-unsaturated fatty acids are highest in autumn and winter. The proportion of 18:1 decreases in early autumn and increases before and during hibernation. The proportion of 18:2 is highest in early summer. In BAT the proportion of 22:6 decreases to a highly significant extent during the spring hibernation, and is lowest in early summer. The changes during the hibernating cycle are more striking in WAT than in BAT, in which several trends apparently compensate each other.

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1. Introduction

That energy metabolism during mammalian hibernation involves fats stored in the autumn has long been known (Kayser 1961). The brown fat is a producer of heat during arousals (e.g. Smalley & Dryer 1963, Joel et al. 1964, Edwards & Munday 1969), and has a powerful oxidative capacity (Chaffee et al. 1966 and Klain & Rogers 1970). But many details of the fat metabolism of hibernating animals are still poorly known, and there is no coherent picture of their fat economy. Different species may differ in basic functions, such as the autumnal storage of fats and the heat production mechanism of brown fat.

This study aims at describing the circannual changes in the fatty acids of the triglycerides in the adipose tissues of the hedgehog in greater detail than in the preliminary report (Laukola & Suomalainen 1971). Changes in the composition of fatty acids during the hibernating cycle were also studied in order to find out whether there is any selective consumption of the different fatty acids and whether any one acid dominates metabolism during hibernation.

2. Material and methods

Adult wild hedgehogs (*Erinaceus europaeus* L.) of both sexes were used. The care and handling of the animals was as described earlier (Kristoffersson & Soivio 1964a, Laukola & Suomalainen 1971).

In order to follow the variations during the autumn and the hibernating season, the hedgehogs were grouped as follows.

A. awake in August (20 to 29 Aug.)
B. awake in September (13 to 20 Sept.)
C. awake in October (25 to 29 Oct.)
D. at the start of hibernation (31 Oct. to 2 Nov.)
E. hibernating in November (15 to 16 Nov.)
F. hibernating in January (19 to 27 Jan.)
G. during spring hibernation (29 March to 5 April)
H. awake in May (23 to 31 May)

The body temperature of the hibernating hedgehogs was less than 1°C higher than the prevailing ambient temperature in the cold animal room. Active animals were killed after a 1-day fast, invariably before noon. They were anaesthetized with ether, administered for about 5 min before sampling.

For observation of the changes occurring in a single hibernation cycle (Kristoffersson & Soivio 1964a), the hedgehogs were grouped as follows.

1. about the middle of the hibernation cycle, i.e. deep hypothermia having lasted for 4 to 5 days. This group was the same as group F of the preceding list.

2. spontaneous arousal, T_B having reached 6°C.
3. spontaneous arousal, T_B having reached 15°C.
4. complete arousal, T_B at the level of an active hedgehog, having reached 15°C 2 1/2 to 3 h earlier.
5. re-entering hypothermia, T_B having fallen to 10°C.

Groups 1—5 were examined in January.

The tissue samples (subcutaneous lumbar adipose tissue and interscapular brown adipose tissue) were frozen in liquid nitrogen immediately after excision and stored in a deepfreeze at about —20°C until they were analysed. The tissues were dried over P_2O_5 under nitrogen in a desiccator for 24 h at 4°C. Drying was continued under a vacuum at room temperature for 4 h. The samples were crushed with quartz sand and extracted for 5 h with petroleum ether (boiling range 40—60°C, May & Baker Ltd.) in a Soxhlet apparatus under nitrogen. Most of the solvent was evaporated from the extract by gentle heating on an electric heater in a stream of nitrogen, and the remainder removed under reduced pressure at room temperature. The fat fraction was saponified at 85°C under nitrogen for 1 h, and refluxed with 20 ml of 0.7 N ethanolic KOH. After the mixture had cooled, 20 ml water and 5 ml of 10 % HCl were added. The mixture was shaken vigorously for about 1 min and then allowed to stand for 30 min. The lower aqueous phase was siphoned off and the fatty acids were washed twice with water and then extracted with two 10-ml volumes of petroleum ether. The solution was dried with anhydrous sodium sulphate. The solvent was removed from the filtered sample by gentle heating in a stream of nitrogen. The fatty acids were methylated by refluxing at 85°C with 4 ml of 1% H_2SO_4 in methanol for 2 h under nitrogen. After cooling, the solution was diluted with 10 ml of water and extracted twice with hexane (n-Hexane for chromatography, E. Merck AG). A mixture of anhydrous sodium carbonate and sodium sulphate (1:25) was added to dry the sample. The fatty acid esters were always dissolved in hexane and stored under nitrogen at 4°C. The methyl esters were submitted to gas liquid chromatography in a Perkin-Elmer F6 gas chromatograph with FID. The analyses were conducted under isothermal conditions as described earlier (Laukola & Suomalainen 1971). In addition, the methyl esters were analysed with temperature programming from 120 to 197°C at a rate of 10°C per min to resolve the short-chain ester peaks. The peaks were identified as follows: (1) Samples of authentic reference compounds were employed as both internal and external standards. (2) Graphic methods based on the relations between the logarithmic retention time, the number of carbon atoms and the number of double bonds. (3) The gas chromatogram was compared with that obtained from the hydrogenated sample. Hydrogenation was carried out in a test tube in 2—4 ml of methanol with about 5 mg of platinum oxide (Poukka et al. 1962). Hydrogen gas was passed through a capillary tube into the solution for 2—3 h; the contents were centrifuged and the supernatant was transferred to another tube. The solvent was then evaporated and the residue dissolved in hexane and analysed by gas chromatography. The proportions of methyl esters in the samples were assessed by multiplying the height of the peak found for each ester by its width, and calculating the percentage of the total. The mean value and its standard error are given for each group. The groups were compared, applying Student's *t* test.

3. Results

The fatty acids of triglycerides in the adipose tissues of the hedgehog were qualitatively similar. The percentage composition for 19 fatty acids is shown in Tables 1 and 2.

3.1. Saturated fatty acids

1. *Straight-chain even-numbered fatty acids.* From all samples the fatty acids from 6:0 to 22:0 could be identified. Palmitic acid (16:0) accounted for over 10% at all times. Myristic acid (14:0) and stearic acid (18:0) were next in quantity, and lauric acid (12:0) amounted to less than 1%. There were very small quantities of arachidic (eicosanic, 20:0) and behenic (docosanoic, 22:0) acids and of capric (10:0), caprylic (8:0) and caproic (6:0) acids.

2. *Straight-chain odd-numbered fatty acids.* The adipose tissues always contained a small amount (about 0.5 %) of pentadecanoic (15:0) acid and about 1% of heptadecanoic (17:0) acid. Nonadecanoic (19:0) acid could also be found in almost every sample, but in smaller quantities than the first two. It was not possible to obtain exact figures for this acid, since its peak overlaps with that of linoleic acid.

3. *Branched-chain fatty acids.* Small amounts of the branched forms of 14:0, 15:0, 16:0 and 17:0 acids were found in all samples. The identification was based on the observation that branched-chain esters elute before the corresponding straight-chain esters (Woodford & van Gent 1960) and that the respective peaks persisted in the hydrated samples. In the temperature-programmed gas chromtography runs one or several peaks could be noted after the solvent, the largest peak occurring just before caproic acid. This was not due to any impurity in the solvent, but the group of substances occurred in the samples themselves; they were perhaps derivatives of short-chain fatty acids.

3.2. Unsaturated fatty acids

1. *Even-numbered mono-unsaturated fatty acids.* The major monoenoic acid was oleic acid (octadecenoic acid, 18:1) with one double bond at position 9. In addition, particularly in WAT, there were varying amounts of an unsaturated 18-carbon acid of unknown structure, probably a positional isomer of oleic acid ($Unknown_3$ in the tables). In the chromatograms this appeared at a

Table 1. Percentages of triglyceride fatty acids in subcutaneous white and brown adipose tissue of the hedgehog at different times of year. Mean values ±*SE* and numbers of experimental animals (*N*) are given. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

Fatty acid	A. Awake Aug. (8)	A/B	B. Awake Sept. (8)	B/C	C. Awake Oct. (15)	C/D	D. Hib. Oct.-Nov. (12)	D/E	E. Hib. Nov. (8)	E/F	F. Hib. Jan. (7)	F/G	G. Hib. March-April (8)	G/H	H. Awake May (15)	A/H
White																
12:0	0.43±0.07		0.31±0.02		0.31±0.05		0.31±0.05		0.21±0.02		0.23±0.03		0.26±0.05		0.49±0.09	
14:0	3.28±0.39		4.00±0.38		3.45±0.21		3.38±0.38		2.49±0.18		2.97±0.26		2.71±0.30		3.55±0.33	
14:1	1.21±0.22		1.70±0.23		1.67±0.32		1.97±0.23		1.63±0.17		1.84±0.22	*	1.26±0.55	*	0.55±0.20	
$Unknown_{1}$	0.65±0.06		0.64±0.07		0.77±0.06		0.68±0.06		0.60±0.06	*	0.79±0.07		0.70±0.05	***	0.44±0.04	**
15:0	0.36±0.02	***	0.56±0.03		0.55±0.03		0.58±0.04		0.51±0.04		0.47±0.04		0.41±0.08		0.41±0.04	
16:0	14.51±1.36		14.46±1.24		13.20±0.98		12.32±1.39		11.45±0.80		10.80±0.47		11.56±1.06	*	15.52±1.10	
16:1	11.20±0.66	**	14.56±0.69		13.78±0.58		15.16±0.39	**	12.85±0.48		14.09±0.98	*	11.21±0.80	*	8.03±0.70	**
$Unknown_{2}$	2.73±0.48		4.01±0.41		4.00±0.79		5.32±0.55		4.29±0.42		5.17±0.57		3.58±0.58	***	1.32±0.28	*
17:0	0.65±0.06	**	1.13±0.12		0.97±0.07		0.99±0.07		1.11±0.10		0.97±0.08		0.88±0.07		0.69±0.06	
17:1	0.66±0.06	*	0.90±0.08		1.04±0.05		0.97±0.07	*	1.19±0.05	*	0.97±0.05		0.90±0.06	**	0.65±0.05	
18:0	2.28±0.20		2.14±0.16		2.22±0.22		1.70±0.21		1.88±0.12		1.64±0.11		2.01±0.25	*	3.73±0.46	*
18:1	36.66±2.08	**	28.44±1.38		31.19±1.95		27.21±1.20		29.26±1.58		27.87±1.90	*	33.65±1.71	*	42.17±2.20	
$Unknown_{3}$	15.11±3.42		13.46±2.81		13.65±2.34		17.18±2.17		20.11±2.22		18.67±1.40		17.85±2.86	*	7.64±1.59	
18:1 + $Unknown_{3}$	51.77		41.90		44.84		44.39		49.37		46.54		51.50		49.81	
18:2	5.34±0.28		5.19±0.49		6.31±0.37		5.40±0.24		5.39±0.39		6.04±0.35		6.69±0.49	**	8.76±0.44	***
18:3	1.25±0.11	*	1.95±0.18		2.09±0.19		1.78±0.11		2.19±0.60	*	1.61±0.07		1.55±0.18	*	1.01±0.11	
20:1	1.39±0.27		1.65±0.25		1.41±0.12		1.28±0.12		1.25±0.13		1.01±0.08		1.46±0.26	*	2.49±0.32	*
20:4	0.50±0.08		0.68±0.21		0.73±0.10		0.64±0.13		0.56±0.06		0.49±0.06		0.51±0.05		0.58±0.13	
20:5	0.79±0.15	*	2.13±0.54		1.37±0.15		1.72±0.30		1.40±0.13		1.53±0.34		0.95±0.19		0.83±0.18	
22:6	1.01±0.38	*	2.10±0.30	*	1.35±0.20		1.46±0.26		1.65±0.31		2.83±0.78		1.85±0.20		1.15±0.24	
Brown																
12:0	0.10±0.00	*	0.14±0.02		0.12±0.01		0.13±0.02		0.13±0.02		0.17±0.02		0.13±0.02		0.15±0.03	
14:0	2.45±0.19	*	3.89±0.56	**	2.50±0.15		2.38±0.24		1.74±0.15	*	2.29±0.21	**	1.44±0.11	**	2.44±0.23	
14:1	0.00±0.00		0.06±0.05		0.09±0.05		0.00±0.00		0.00±0.00		0.00±0.00		0.00±0.00		0.00±0.00	
$Unknown_{1}$	0.25±0.02	**	0.52±0.08		0.44±0.03		0.48±0.11		0.43±0.05		0.47±0.03	**	0.32±0.04		0.26±0.03	
15:0	0.23±0.02	*	0.39±0.06		0.33±0.03		0.35±0.05		0.28±0.02		0.27±0.03		0.31±0.04		0.27±0.02	
16:0	16.18±0.61		17.89±0.77	**	15.37±0.40		14.98±0.50	*	13.18±0.35		12.59±0.57		12.61±0.31	***	15.33±0.43	
16:1	4.74±0.37	***	7.42±0.42		6.03±0.56		5.68±0.30		5.26±0.38		5.96±0.65	*	4.01±0.32		3.84±0.26	
$Unknown_{2}$	0.31±0.01	***	0.63±0.07		0.82±0.13		0.56±0.06		0.63±0.06		0.61±0.06		0.64±0.04	***	0.46±0.02	***
17:0	0.63±0.04	***	0.89±0.04		0.93±0.05		1.00±0.07		1.15±0.09	*	0.81±0.12		0.73±0.05		0.68±0.05	
17:1	0.54±0.02		0.63±0.04	**	0.79±0.03		0.83±0.06	*	1.08±0.07	*	0.77±0.08		0.73±0.03		0.61±0.04	
18:0	5.61±0.32		5.96±0.57		5.76±0.30		5.98±0.32		6.01±0.34		5.71±0.35		4.89±0.36	***	6.96±0.31	*
18:1	50.65±1.92	**	42.32±1.13		43.09±2.00		44.26±1.17	*	47.90±1.22		48.60±2.31		52.88±0.52		50.45±0.88	
$Unknown_{3}$	0.00±0.00		0.41±0.43		2.08±0.75	*	0.00±0.00		0.00±0.00		0.00±0.00		0.00±0.00		0.00±0.00	
18:1 + Unknown	50.65		42.73		45.16		44.26		47.90		48.60		52.88		50.45	
18:2	8.30±0.56		7.19±0.75		8.53±0.44		9.10±0.52		8.60±0.66		9.33±0.41		9.24±0.50	*	10.55±0.34	**
18:3	1.26±0.13	*	1.76±0.13		1.93±0.13		1.88±0.19	*	2.55±0.24	*	1.69±0.19	*	1.18±0.13		0.91±0.09	*
20:1	3.29±0.99		3.33±0.20		2.94±0.20	**	3.84±0.24	*	3.08±0.17		3.19±0.09	***	4.46±0.20		4.37±0.22	
20:4	1.00±0.09		1.02±0.09	**	1.61±0.12		1.62±0.14		1.53±0.16	*	2.03±0.13		2.41±0.19	***	0.77±0.11	
20:5	1.79±0.35		1.58±0.17		2.18±0.22		2.49±0.23		2.25±0.24	**	1.33±0.12		2.01±0.37		1.19±0.23	
22:6	2.69±0.30	**	3.97±0.30		4.47±0.54		4.45±0.48		4.24±0.49		4.19±0.47	***	1.99±0.24	***	0.77±0.17	***

Table 2. Percentages of triglyceride fatty acids in subcutaneous white and brown adipose tissue of the hedgehog in different phases of the hibernation cycle. Mean values ±*SE* and numbers of experimental animals (*N*) are given. * = $P<0.05$, ** = $P<0.01$ and *** = $P<0.002$.

Fatty acid	1. Deep hypothermia		2. Arousing T_B 6°		3. Arousing T_B 15°		4. Fully awake		5. Entering hypothermia T_B 10°		
	(7)	1/2	(6)	2/3	(7)	3/4	(7)	4/5	(7)	1/5	1/4
White											
12:0	0.23±0.03	*	0.35±0.04		0.33±0.05		0.37±0.04		0.37±0.08		*
14:0	2.97±0.26		3.50±0.66		3.54±0.64		4.03±0.27	*	3.34±0.17		*
14:1	1.84±0.22		1.82±0.20		1.94±0.37	*	0.71±0.22	**	1.79±0.21		**
Unknown$_1$	0.79±0.07	**	0.50±0.03	*	0.60±0.03	*	0.77±0.05	**	0.59±0.03	*	
15:0	0.47±0.04		0.48±0.06		0.56±0.04		0.49±0.03		0.56±0.03		
16:0	10.80±0.47		11.98±1.37		12.47±2.03		14.83±0.78	*	12.27±0.67		***
16:1	14.09±0.98		13.45±1.31		14.79±0.84		13.71±0.79		11.94±1.39		
Unknown$_2$	5.17±0.57		4.33±0.79		5.43±1.33	*	2.50±0.23		4.16±0.85		**
17:0	0.97±0.08		1.03±0.08		1.13±0.08		1.03±0.07		1.04±0.04		
17:1	0.97±0.05		1.05±0.06		1.00±0.06		1.01±0.05		1.04±0.04		
18:0	1.64±0.11		1.85±0.33		2.01±0.34		2.31±0.15		2.11±0.14	**	**
18:1	27.87±1.90		30.02±2.59		27.71±2.20	*	35.81±2.08		31.43±3.38		*
Unknown$_3$	18.67±1.40		18.23±2.86		16.14±3.77		7.89±2.08		13.77±2.38		**
18:1 + Unknown$_3$	46.54		48.25		43.85		43.70		45.20		
18:2	6.04±0.35		4.20±1.32		4.40±0.89		6.00±0.43		7.80±1.31		
18:3	1.61±0.07		1.75±0.06		1.91±0.20		2.07±0.11		1.96±0.07	**	**
20:1	1.01±0.08		1.25±0.16		1.51±0.18		1.39±0.13		1.53±0.15	*	*
20:4	0.49±0.06		0.68±0.12		0.60±0.11		0.47±0.07	**	0.99±0.15	**	
20:5	1.53±0.34		1.17±0.12		1.47±0.21		1.50±0.10		1.39±0.13		
22:6	2.83±0.78		2.35±0.53		2.44±0.60		3.10±0.34		1.93±0.44		
Brown											
12:0	0.17±0.02		0.20±0.05		0.26±0.04		0.19±0.03		0.16±0.03		
14:0	2.29±0.21		2.08±0.28		2.20±0.23		1.91±0.17		1.83±0.26		
14:1	0.00±0.00		0.00±0.00		0.00±0.00		0.00±0.00		0.00±0.00		
Unknown$_1$	0.47±0.03		0.42±0.04	**	0.57±0.07	***	0.40±0.02		0.44±0.04		
15:0	0.27±0.03		0.35±0.08		0.44±0.08	*	0.30±0.02		0.29±0.04		
16:0	12.59±0.57		13.30±0.61		12.81±0.46		12.99±0.38		12.51±0.86		
16:1	5.96±0.65		5.67±0.53		6.74±0.41	*	5.44±0.31		4.74±0.62		
Unknown$_2$	0.61±0.06		0.77±0.06		0.84±0.07	*	0.64±0.03		0.64±0.08		
17:0	0.81±0.12		0.80±0.04		0.93±0.07		0.89±0.04		0.74±0.09		
17:1	0.77±0.08		0.82±0.02		0.87±0.06		0.77±0.04		0.79±0.08		
18:0	5.71±0.35	**	4.13±0.40		4.06±0.18	*	5.06±0.37		4.99±0.39		
18:1	48.60±2.31		50.30±1.92		48.67±0.58		49.40±2.08		51.37±2.61		
Unknown$_3$	0.00±0.00		0.00±0.00		0.00±0.00		0.00±0.00		0.00±0.00		
18:2	9.33±0.41		8.92±0.62		8.41±0.56		8.64±0.66		9.80±0.64		
18:3	1.69±0.19		1.85±0.13		2.04±0.17	*	1.53±0.05		1.61±0.18		
20:1	3.19±0.09		3.40±0.13		3.26±0.20		3.43±0.30		3.70±0.29		
20:4	2.03±0.13		2.12±0.26		2.10±0.22		1.89±0.23		2.16±0.18		
20:5	1.33±0.12		1.40±0.14		1.64±0.33		1.70±0.20		1.34±0.22		
22:6	4.19±0.47		3.48±0.39		4.14±0.61		4.83±0.75	*	2.90±0.40		

site between oleic acid (18:1) and linoleic acid (18:2). The acid was not elaidic acid, since the *cis* and *trans* configurations were not separated in the runs. For positional isomers in columns of various types, e.g. Apiezon L, ethylene glycol succinate (EGS) and diethylene glycol succinate (DEGS), the retention time is usually greater for the isomer having longer proximal end or the smaller ω value (Holman & Rahm 1966). This suggests that the unknown isomer is vaccenic acid, which has a double bond farther from the proximal end (at position 11) than oleic acid. Vaccenic acid has been encountered in mammals in adipose tissue and milk triglycerides and in the lipids of some tissues (Masoro 1968). Palmitoleic acid (hexadecenoic acid, 16:1) was always present in abundance (4—15%) in the samples. Another regular component was eicosenoic acid (20:1), 1—4%, and in WAT 1—2% tetradecenoic acid (14:1). Two unknown unsaturated acids (Unknown$_1$ and Unknown$_2$ in the tables) were present in small amounts. The logarithms of the retention times of these unknown components 1, 2 and 3, plotted against carbon chain length, gave a line running parallel with the lines of known monoenoic and saturated acids. This may be evidence of structural similarity between these unknown components.

2. *Odd-numbered mono-unsaturated fatty acids.* Heptadecenoic acid (17:1) was regularly present

(0.5—1.2%). Pentadecenoic acid (15:1) appeared sporadically in exceedingly small amounts.

3. *Polyunsaturated fatty acids.* The polyenoic fatty acids of BAT and WAT have 18, 20 or 22 carbon atoms with 2 to 6 double bonds. Linoleic acid (18:2) constituted 5—10%, and linolenic acid (18:3) only 1—2% of the total quantity of fatty acids. Interesting additions to the preliminary list (Laukola & Suomalainen 1971) were the eicosatetraenoic (arachidonic, 20:4), eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids. These acids were regularly present in the samples in measurable amounts, although their elution peaks were broad and flat, owing to their high retention times. The composition of these acids was verified by comparison with the methyl esters of the following acids: 5,8,11,14-eicosatetraenoic, 5,8,11,14,17-eicosapentaenoic and 4,7,10,13,16,19-docosahexaenoic acid (Applied Science Laboratories, Inc.). The retention time of methyl arachidonate was shorter than expected and the peak appeared at a point where, according to calculations, there should have been a 20-carbon-chain methyl ester with three double bonds. A similar phenomenon has also been observed by Orr & Callen (1959), who used a different polyester column: Reoplex 400.

4. Discussion

4.1. White adipose tissue

In fatty acid composition the hedgehog's WAT differs somewhat from the reserve fats of other mammals (e.g. Hilditch & Williams 1964, Jeanrenaud 1965, Gunstone 1967, Masoro 1968). The quantity of palmitic acid (16:0) was less (10—15%) than in mammals in general (25—32%), but there was more palmitoleic acid (16:1), 8—15% (usually far below 10% in mammals). Ewing et al. (1970) observed a similar phenomenon in three species of *Myotis*. The amount of stearic acid (18:0) varies markedly in different mammals. In small mammals it is a few percent only (in the hedgehog 2%). In species whose WAT has a low relative stearic acid content, linoleic acid is often very abundant. The percentage of linoleic acid is high in hibernating species such as hamster, ground-squirrel, woodchuck and dormouse, but lower in the hedgehog (Table 1 and Åkesson 1972). The longer-chain fatty acids receive little attention in reports on reserve fat analyses performed with mammals, perhaps owing to their low proportion in terrestrial animals and to their cumbersome analysis. Åkesson (1972), however, determined 20- and 22-carbon-chain mono-unsaturated acids from hedgehog's WAT.

The composition of WAT appears to vary with species and nutrition and to a lesser extent with season, ambient temperature and hibernation. The genus *Erinaceus* is known to be of great evolutionary age. Among several primitive traits preserved in the hedgehog one might also include the heterogeneity of the reserve fat. The general trend in the evolution of fats has been a reduction in the number of fatty acids from a broad spectrum to a few, especially to the saturated palmitic acid and the mono-unsaturated oleic acid and a reduction in the degree of unsaturation. Palmitoleic acid and the long-chain polyunsaturated fatty acids are typical of fats of aquatic species and gradually disappear in terrestrial animals. Within the limits imposed by species, nutrition also influences reserve fat composition (e.g. Tove & Smith 1960, Ostwald et al. 1962, Davis & McCarthy 1965, West & Coady 1974). In the present study the hedgehogs were fed on milk and Baltic herring, whereas the animals studied by Åkesson (1972) were given table scraps. Even though in main features the results of the two studies agree, the present analyses showed higher proportions of the acids 16:1, 18:2, 18:3, 20:5 and 22:6, whereas Åkesson found more 16:0, 18:0 and 20:1. These differences may be partly attributable to the differences in nutrition. Linko (1967) showed that in Baltic herring the acids 16:0, 16:1, 18:1, 20:5 and 22:6 constitute two-thirds of all fatty acids. This would explain the occurrence in our hedgehogs of fatty acids that are uncommon in mammals. Milk, which has a high content of short-chain saturated fatty acids (e.g. Antila 1966), may have influenced the amounts of myristic (14:0) and lauric (12:0) acids and of fatty acids with even shorter chains (Dobiášová et al. 1964).

4.2. Brown adipose tissue

In BAT the lipid content is considerably lower, but that of proteins and water higher, than in WAT (Smith & Horwitz 1969). In the hedgehog lipids amount to 20—30% in BAT, and 80% in WAT (Laukola & Suomalainen 1971). The fats in WAT are 99% triglycerides, whereas in BAT cholesterol and phospholipids account for a small percentage of the lipid quantity.

In the hedgehog's BAT the short-chain fatty acids 12:0, 14:0, 14:1, 15:0 and the unknowns 1, 2 and 3 were present in clearly lower proportions than in WAT. Further, there is only 3—7% palmitoleic acid (16:1) in BAT, but 8—15% in WAT, while the amount of stearic acid (18:0) in BAT is twice that found in WAT. The proportions of 18:2 and 20:1 were significantly greater in BAT throughout the year. The proportions of 16:0, 20:4 and 22:6 were higher in this tissue than in WAT, except in the early summer group (H) and, with regard to 22:6, also during the spring hibernation (G). No significant differences were noted between samples from different anatomical sites (neck or circumscapular area).

Brown fat has been found in most mammals. It is particularly abundant in neonates and very young individuals, and in hibernators of any age. Previous comparisons between the triglyceride fatty acid compositions of BAT and WAT have been made in the hedgehog (Laukola & Suomalainen 1971, Åkesson 1972), ground-squirrel (Spencer et al. 1966), woodchuck (Bigelow et al. 1964, Davis & McCarthy 1965), hamster (Williams & Platner 1967, Smalley et al. 1970), lemming (West & Coady 1974), man and rabbit (Dawkins & Stevens 1966), mouse (Spencer & Dempster 1962) and rat (Chalvardjian 1964, Dobiášová et al. 1964, Moriya & Itoh 1969). In all these species the proportion of stearic acid is higher in BAT than in WAT. Almost as common a feature is the lower proportion of palmitoleic acid in BAT, although on this point Dobiášová et al. (1964) and Smalley et al. (1970) dissent. It also seems that the proportion of long-chain fatty acids is higher in BAT. The differences between BAT and WAT are believed to be due to enzymatic differences. BAT has been shown to contain very large amounts of the various enzymes required in metabolism of fatty acids (Joel 1965, Smalley & Dryer 1967, Smith & Horwitz 1969, Williamson et al. 1970). The local heat production of BAT and the powerful respiratory activity have long been known (Smalley & Dryer 1963, Smith & Hock 1963, Dawkins & Hull 1964, Joel et al. 1964, Haywood et al. 1965, Horwitz et al. 1968, Edwards & Munday 1969). Of the fatty acids liberated in lipolysis by no means all pass into the bloodstream greater part remains in the BAT cells. There the fatty acids are either re-esterified or oxidized. The hypothesis that the rapid local consumption of fats favours certain fatty acid types gains no support from comparison of the triglycerides in BAT and WAT.

4.3. Effect of seasons and of hibernation on the fatty acid composition of white and brown adipose tissue

The autumn preparation for hibernation involves a marked and rapid increase in weight, mainly due to increase reserves of fat (Jameson & Mead 1964, Kristoffersson & Suomalainen 1964). In the hibernating period the animal constitutes a metabolically closed system and consumes the bulk of its stored fat. The present study was designed to clarify what changes, if any, take place in the triglycerides of the hedgehog's adipose tissue during the various phases of the annual cycle. The results of chromatographic analyses show the figures associated with the annual rhythm (Table 1), and with the different stages of the hibernation cycle (Table 2). Figs. 1 and 2 show the seasonal changes in the principal fatty acids. The values for oleic acid and for the unknown$_3$ structurally associated with it have been combined in the tables and figures, because both are 18-carbon mono-unsaturated acids. The changes are greatest in the autumn and when hibernation begins. The hibernation season is a time of slower change as regards the fatty acid spectrum of total fats. This certainly does not exclude the possibility that there may be selective consumption during periodic arousals, but it is probable that independently of these a rapid levelling of the changes takes place by metabolic means.

The differences between the spring hibernation group and the early summer group are naturally great, because during hibernation the fat reserves have been almost used up and fats are once again obtained from the food. In WAT palmitic acid reaches a peak early in spring, and the values then decline steadily during the autumn. In BAT the proportion of 16:0 increases from spring to autumn and only begins to decrease in late autumn. It would seem that during the summer this fatty acid is transported from WAT to BAT, or that it is converted into other fatty acids, first in WAT and later in BAT. In these respects the present results differ from those of Åkesson (1972), who found that the proportion of palmitic acid was fairly constant throughout the year. The increment in linoleic acid in early summer in both tissues reflects the nutrition, since the animal itself is unable to synthesize this acid. Linoleic acid is important as a precursor of arachidonic acid. It appears that 18:2 and 20:4 are hardly consumed at all during hibernation. The decrease in 22:6 in BAT during

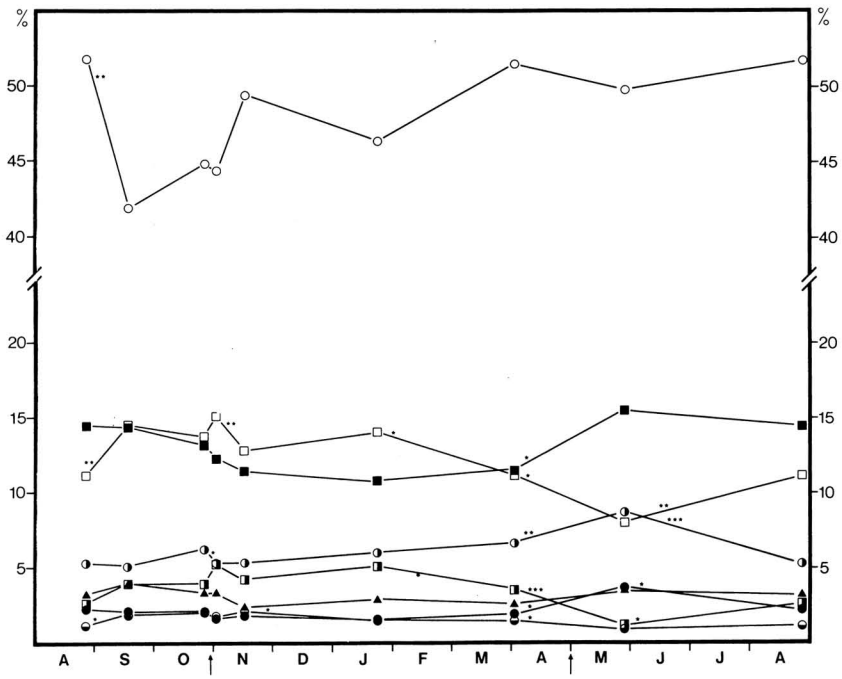

Fig. 1. Seasonal changes in triglyceride fatty acids of subcutaneous white adipose tissue. Letters denote calendar months. Arrows indicate the beginning and end of hibernation. The figures are percentages of the total amount of fatty acids.*, ** and *** indicate inter-group *P* values.

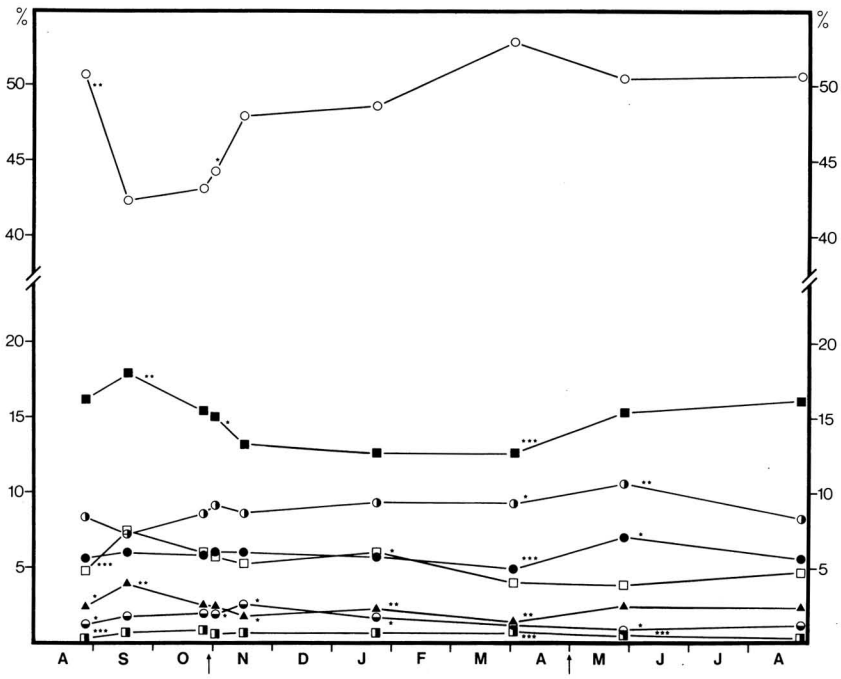

Fig. 2. Seasonal changes in triglyceride fatty acids of brown adipose tissue. Symbols as in Fig. 1.

the spring hibernation is highly significant. In early summer this fatty acid is present in exceedingly small quantities, and the deviation from all other groups is highly significant.

The spectrum of the individual fatty acids at different seasons has been studied not only in the hedgehog, but also in ground-squirrels (Spencer et al. 1966, Platner et al. 1972), bats (Rondinini et al. 1962, Wells et al. 1965, Paulsrud & Dryer 1968), the hamster (Williams & Platner 1967, Minor et al. 1973), the dormouse (Kaufmann et al. 1966) and woodchucks (Bigelow et al. 1964, Davis & McCarthy 1965). Taxonomically, hibernators vary so widely that they cannot be expected to be metabolically uniform. So far, however, the results of experiments are conflicting even for one and the same species. This is at least partly due to inaccurate determination of the animal's state. In woodchucks and in the dormouse so significant seasonal changes were observed, although the relative amounts of 18:2 and the polyunsaturated fatty acids appear to increase towards the end of hibernation, while 18:0 and 18:1 acids decrease somewhat. In BAT of the dormouse the quantities of 18:2 and 20:4 increase more strongly than in WAT, and Kaufmann et al. (1966) therefore assume that BAT serves as a kind of storage tissue with regard to these essential fatty acids. An equivalent phenomenon is observable in the present study. The percentage increase in oleic acid in the cold and during hibernation and the corresponding decrease in palmitic acid appear to be typical of hamsters, as they are of hedgehogs. A certain likeness to the hedgehog is also evident regarding the principal fatty acids in the bat *Eptesicus fuscus* (Paulsrud & Dryer 1968), but the seasonal variations in the fatty acid spectrum of various bat species differ considerably. Likewise the fat analyses of various species of ground-squirrel differed considerably, and the fatty acid composition appeared to bear no correlation either to season or to diet.

Hibernation is not a continuous state: the animals awaken periodically during the hibernating period (Lyman 1958, Pengelley & Fisher 1961, Kristoffersson & Soivio 1964a). The hedgehog arouses spontaneously during the hibernation period about once a week. During this process BAT produces metabolic heat, and at an ambient temperature of 5°C the animal reaches its normal active temperature in about 3 h. Although the periodic arousals constitute hardly 10% of the whole period of hibernation, the bulk of the stored energy is consumed during this time. According to Kayser (1961), carbohydrates, in addition to lipids, are consumed during awakening, since the R.Q. value, which in hibernation approximates 0.7, increases to 0.8—1.0 during arousal. At this time the glycogen stores in liver and muscles decrease (Lyman & Leduc 1953, Kayser 1961). Studies with radioglucose (Tashima et al. 1970) have demonstrated, however, that during hibernation and the first stages of arousal the primary sources of oxidative energy are compounds other than carbohydrates.

The changes in fatty acid composition during the hibernating cycle are different in WAT and BAT (see Table 2). In WAT the group 4 (fully awake) differs most of all from the other groups, but there are no significant differences in BAT between groups 1 and 4. In BAT stearic acid decreases significantly at the beginning of arousal. In WAT mono-unsaturated fatty acids were consumed first, and at the time of entering into hibernation they were replaced with saturated fatty acids, in particular 16:0, by desaturation. It is natural that the changes in WAT become apparent later than those in BAT, which is highly active, starting with the first phases of awakening, and even in hibernation metabolically more active than WAT. In addition, WAT warms up more slowly owing to its location and poorly developed vascularization. WAT is gradually used up during the hibernation period, but the fat content of BAT remains fairly constant over the hibernation period as a whole (Laukola & Suomalainen 1971). In BAT rapid depletion of neutral fats has been observed during arousals (Joël et al. 1964, Joel 1965, Spencer et al. 1966, Burlington et al. 1969). The fats consumed are replaced between arousals. It is obvious that the changes in BAT are so rapid during the hibernation cycle that they are not revealed by an analysis of this kind. Moreover, local consumption of fatty acids takes place in BAT as well as their structural conversion and simultaneous release into the bloodstream. During these processes the changes in composition are compensated and are thus not visible in the total fatty acid composition.

The round-the-year rhytmic changes in the fatty acid composition are due partly to the fact that the animal does not eat during the hibernation period, whereas at other seasons there is an influence of nutrition. Even apparently the same food varies with the seasons. Linko & Karinkanta (1970) observed that Baltic herrings have a higher content of long-chain fatty acids in the autumn than in spring and that these are in part different (see 20:5 and 22:6 in Table 1).

Therefore on this basis alone, the year is divided into two, and perhaps even more different periods. Apart from the potential effects of nutrition, the annual variations also indicate the rhythmicity caused by natural hibernation. However, this can be observed only by following the fatty acid composition all the year round. This may possibly explain why some investigators have noted no differences whatsoever. The variation observed among hibernators is further augmented by the fact that some species feed during the periodic arousals.

A fairly general phenomenon, however, could be held to be the increase in the proportion of oleic acid during hibernation and the relative increase in palmitic and palmitoleic acids during the autumn, and their gradual decrease during the hibernating period. The proportion of linoleic acid remains fairly constant throughout hibernation, increasing only as a result of feeding in the in the spring. The unsaturation of reserve fats increases with cold (Fawcett & Lyman 1954, Mefferd et al. 1958, Kodama & Pace 1964, Williams & Platner 1967, Moriya & Itoh 1969, Minor et al. 1973). The reserve fats of hibernators in general appear to contain a high proportion of unsaturated fatty acids. A direct effect of cold cannot be involved in the autumn, but in hibernating animals low temperature can also be considered a cause of the high degree of unsaturation. Paulsrud & Dryer (1968) and Dryer et al. (1970) report that in BAT *in vitro* lower temperature favours the oxidation of 1—^{14}C-palmitic acid as compared with 1—^{14}C-oleic acid. This reveals a certain ability of the tissue to adapt to low temperature. The position of the fatty acid residues in the glyceride molecules may also have an effect on the rate at which these acids transform into transportable form. The internal arrangement of fatty acids in a triglyceride molecule is affected by the length of the carbon chain and the number of double bonds. Moreover, the origin of the fatty acids affects their affinity for various positions. It is possible that under the influence of cold or of some physiological changes the selectivity of triglyceride lipase undergoes a change. A considerable number of enzymatic studies will be needed, however, before it will be possible to explain the changes in fatty acid composition. Another step that is essential for our understanding of the interactions of BAT and WAT is to clarify the role played by the liver and by the blood lipids in fat metabolism during hibernation.

Acknowledgements. This work was begun under the late Prof. Paavo Suomalainen and completed after his retirement in 1973 and his death on 16 October 1976. My thanks are due to Prof. Henrik Wallgren, Ph.D., Head of the Department of Zoology, for working facilities in the laboratory, and to Assoc. Prof. Rolf Kristoffersson, Ph.D., for encouragement during the work and for constructive discussions concerning the manuscript.

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Received 25. III. 1980
Printed 29. X. 1980