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SEASONAL CHANGES IN PLASMA LIPID DISTRIBUTION OF A HIBERNATING LIZARD *(UROMASTIX HARDWICKII)*

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Plasma lipid concentrations were studied in the hibernating lizard *Uromastix hardwickii* during hibernation, arousal and activity. The highest concentration of esterified fatty acids was only noted during prehibernation period. Plasma free fatty acid concentration was significantly increased upon arousal from hibernation suggesting an intensive lipolysis for thermogenesis. Both plasma cholesterol and phospholipid concentrations were lowest during prehibernation as compared to the other phases of the hibernating cycle. Except for cholesteryl ester there were more saturated fatty acids during all three phases of lizard's life cycle.

INTRODUCTION

Hibernation, a physiological adaptation of the animal, is markedly associated with changes in the distribution of lipid in body tissues since the energy supply of the animal is believed to be met by the body fat. A few reptiles possess large adipose tissues [1,2] which are prominent in the spring and summer, but decrease in the autumn and disappear in the winter, which suggests that they are used as a source of energy and water during hibernation and the periods of food deficiency. Adipose tissue and its function has been studied in several mammalian hiberantors such as the hedgehog [3], hamster [4] and golden-mantled ground squirrel [5] during hibernation and activity. However, little attention has been paid to the role of lipid when the animal arouse from hibernation. Since arousal represents a dramatic alteration in body physiology, the energy required is met only by the fat stored in adipose tissue [6]. Glycogen stores are unable to provide substantial energy for arousal from hibernation.

Little information is available on lipid concentration in plasma or serum for the various phases of hibernation. There are only a few reports available regarding the composition of fat in the serum of some mammalian hibernators such as bat [7], woodchuck [8] and hedgehog [3,9]. Relatively little is known about hibernating reptiles and amphibians. The present study describes the fatty acid composition of the plasma lipid classes in the hibernating lizard *Uromastix hardwickii* during hibernation, arousal and activity. The concentration of esterified fatty acids (EFA), free fatty acids (FFA), cholesterol and phospholipids (PL) have also been measured.

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MATERIALS AND METHODS

Lizards, *Uromastix hardwickii,* were collected on the 27th day of each month from the deserted area of Sind (Pakistan) and six animals weighing between 384–453 g were killed onthe same day irrespective of their age and sex. Blood was collected separately from the jugular vein of each animal directly in a centrifuge tube containing potassium oxalate as an anticoagulant. Plasma samples, obtained by centrifugation for 15 min at 5,000 rev/min were stored below 4° till further analysis.

Plasma (2 ml) was added dropwise into a 25-ml volumetric flask containing 15 ml ethanol – diethyl ether (3:1. by vol). The volume was made up to 25 ml with the same solvent and left for 3 – 4 hr at room temperature. The precipitate was filtered through methanol-washed glass wool and the solvent evaporated under a slow stream of nitrogen at 50°.

EFA, total cholesterol and phospholipid were estimated spectrophotometrically using a Unicam SP 500 spectrophotometer. The methods used for the estimation of EFA [10], total cholesterol [11], phospholipids [12] and FFA [13] were slightly modified.

The concentrations of FFAs were estimated by a combined technique of GLC and TLC using heptadecanoate as an internal standard. The fatty acid composition of the class lipids were determined as follows: 25 mg of plasma lipid was applied on an activated, methanol-washed silica gel G plate (20×20 cm). The individual lipid classes were resolved in a solvent system: light ether (b.p.40–60°) diethyl ether – acetic acid (80: 20: 1, by vol.) and located by exposure to iodine vapour. Each lipid component was quantitatively scraped from the plate and converted to their

methyl esters [14] with 20% (v/v) methanolic sulphuric acid for subsequent GLC analysis. A Varian aerograph model 600 D equipped with a hydrogen-flame ionization detector was used. The column (5 ft 9 in and 1/8 in I.D.) was packed with 20% (w/w) diethylene glycol succinate coated over Chromosorb W (80–100 mesh). The individual fatty acids were identified by comparing their retention times with known standards and by plotting logarithmic retention time against carbon number [15]. Unsaturated fatty acids were further identified by bromination [16] and by potassium permagnate oxidation [17].

RESULTS

Esterified fatty acids, cholesterol and phospholipid concentrations were quantitatively estimated spectrophotometrically during the whole year in order to study the seasonal variations of the plasma lipids. The values (mg/100 ml) are shown in Fig. 1 and the plasma lipid distribution in each physiological state of the animal is given in Table 1. The prehibernation period starts in August, with hibernation beginning by about November. Arousal from hibernation occurs by the end of April, after which the animals remain active till the prehibernation period. The concentration of FFA was only estimated during the months of November (hibernation), April (arousal) and June (activity). The fatty acid composition of each lipid class was determined by combined TLC–GLC analysis of four individual samples from each representative month of hibernation, arousal and activity.

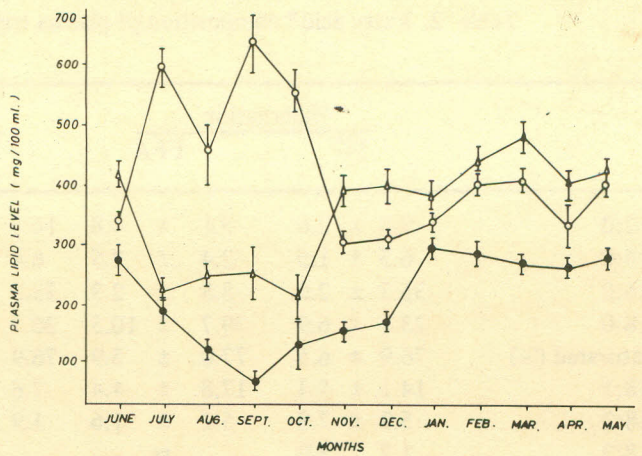

Fig. 1. Seasonal variations in plasma lipid concentration in the lizard *Uromestix hardwickii* (each value is an average of 6 animals ± standard error).

(0 Esterified fatty acids, Δ cholesterol, phospholipid, I standard error.)

The concentration of EFA in plasma was highest during prehibernation and lowest during arousal, whereas only slight variations were noted in hibernating and in active animals. During prehibernation, the concentration of cholesterol and of phospholipids were depressed compared to the rest of the life cycle. These concentrations were significantly increased during hibernation and activity. The maximum concentration of cholesterol was observed in March, and was depressed in April when the animal aroused

Table 1. Plasma lipid distribution in *Uromastix hardwickii.*

State of animal	EFA (mg/100 ml)	FFA ** (μ eq / l)	Cholesterol (mg/100 ml)	Phospholipid (mg/100 ml)
Prehibernation (July – Oct.)	*556.3 ± 46.9 (24)† (P<0.001)	–	231.8 ± 12.1 (24) (P<0.001)	123.6 ± 30.1 (24) (P<0.05)
Hibernation (Nov – Mar)	349.7 ± 24.9 (30) NS	924.3 ± 12.4 (6) (P<0.001)	416.6 ± 22.0 (30) NS	234.6 ± 36.1 (30) NS
Arousal (April)	330.0 ± 33.0 (6) NS	2187.9 ± 18.5 (6) (P<0.001)	396.0 ± 25.4 (6) NS	263.1 ± 15.5 (6) NS
Activity (May – June)	368.5 ± 11.4 (12) (P<0.001)	1318.3 ± 18.8 (6) (P<0.001)	415.0 ± 20.6 (12) (P<0.001)	276.0 ± 11.1 (12) (P<0.001)

* Mean ± SE

† The number in parenthesis indicates the number of animals used.

** FFA concentration were determined in 6 samples taken from 6 individual lizard, in each representative month of hibernation (Nov.), arousal (April) and activity (June). P = Probable level of significance (1 *v* 2 *v* 3 *v* 4 *v* 1) NS = non significant

Table 2. Fatty acid* composition of plasma triglyceride (TG) and free fatty acid (FFA) (weight %)

	Hibernation		Arousal		Activity	
	TG	FFA	TG	FFA	TG	FFA
$C_{12:0}$	9.8 ± 2.6	5.1 ± 4.8	16.5 ± 10.9	6.8 ± 5.4	24.5 ± 7.9	6.7 ± 2.6
$C_{14:0}$	6.5 ± 1.5	2.4 ± 2.5	6.4 ± 2.4	3.0 ± 1.6	7.9 ± 1.2	4.7 ± 2.5
$C_{16:0}$	35.3 ± 2.6	28.8 ± 2.3	33.3 ± 9.3	37.7 ± 2.3	34.5 ± 6.5	38.3 ± 4.8
$C_{18:0}$	23.1 ± 6.9	39.7 ± 10.5	20.7 ± 10.7	38.6 ± 5.2	18.2 ± 1.4	33.4 ± 7.9
Staturated (%)	76.9 ± 6.5	77.0 ± 5.9	76.9 ± 14.9	86.1 ± 4.8	85.1 ± 8.3	83.3 ± 6.9
$C_{18:1}$	14.1 ± 5.4	17.8 ± 4.4	7.6 ± 3.4	9.5 ± 1.6	10.7 ± 3.6	13.3 ± 4.8
$C_{18:2}$	5.9 ± 7.8	5.2 ± 1.6	1.9 ± 3.2	3.9 ± 1.8	tr	3.5 ± 3.3
$C_{18:3}$	1.7 ± 3.0	tr	tr	0.5 ± 0.5	tr	tr
$C_{20:uns}$	1.4 ± 2.4	–	7.9 ± 3.9	–	4.2 ± 5.7	–
$C_{22:2}$	tr	–	5.7 ± 5.5	–	tr	–
Unsaturated (%)	23.1 ± 6.5	23.0 ± 5.9	23.1 ± 14.9	13.9 ± 4.8	14.9 ± 8.3	16.7 ± 6.9

* The values indicated are the mean of 4 individual samples from each representative month of hibernation (Nov.) arousal (April) and activity (June) ± Standard error. tr = found only in traces.

Table 3. Fatty acid* composition of plasma phospholipid (PL) and steryl esters (SE).

Fatty acid	Hibernation		Arousal		Activity	
	PL	SE	PL	SE	PL	SE
$C_{10:0}$	–	tr	–	4.8 ± 5.2	–	–
$C_{12:0}$	0.2 ± 0.4	2.0 ± 0.8	0.8 ± 1.4	16.7 ± 9.8	0.5 ± 0.8	2.7 ± 0.8
$C_{14:0}$	1.3 ± 1.1	1.2 ± 0.2	1.4 ± 1.6	8.6 ± 4.5	1.9 ± 1.4	1.0 ± 0.5
$C_{16:0}$	43.7 ± 11.3	7.9 ± 2.8	36.7 ± 10.4	20.6 ± 7.5	33.4 ± 9.3	10.8 ± 5.7
$C_{18:0}$	26.3 ± 13.0	4.6 ± 1.8	36.0 ± 12.1	9.4 ± 4.6	43.7 ± 6.1	9.5 ± 7.1
Saturated (%)	71.5 ± 5.4	15.7 ± 3.9	75.5 ± 11.1	60.1 ± 22.3	79.5 ± 8.9	24.0 ± 8.0
$C_{16:1}$	–	0.2 ± 0.3	–	0.7 ± 1.2	–	–
$C_{18:1}$	9.3 ± 3.2	27.6 ± 18.5	13.0 ± 10.9	19.8 ± 12.8	9.0 ± 2.3	16.8 ± 13.6
$C_{18:2}$	9.7 ± 2.9	12.0 ± 7.8	11.5 ± 6.5	6.2 ± 8.4	5.3 ± 2.8	2.6 ± 2.6
$C_{18:3}$	6.1 ± 2.7	0.4 ± 0.7	tr	3.6 ± 2.4	tr	3.5 ± 2.9
$C_{20:2}$	3.4 ± 5.8	5.1 ± 3.7	tr	tr	6.2 ± 4.7	5.6 ± 4.8
$C_{20:3}$	–	tr	–	5.8 ± 6.8	–	13.0 ± 6.3
$C_{22:3?}$	–	23.6 ± 12.8	–	tr	–	tr
$C_{22:4?}$	–	15.4 ± 9.2	–	3.8 ± 6.4	–	34.5 ± 6.0
Unsaturated (%)	28.5 ± 5.4	84.3 ± 3.9	24.5 ± 11.1	39.0 ± 22.3	20.5 ± 8.9	76.0 ± 6.0

* The values indicated are the mean of 4 individual samples from each representative month of hibernation (Nov), arousal (April) and activity (June) ± Standard error tr = found only in traces.

from hibernation. The higher concentrations of PL which were maintained from January to June decreased successively from July onward and the lowest value was obtained in September. The most prominent change was noticed in the FFA fraction. The increase in the concentration of plasma FFA was highly significant. The concentration of FFA was then depressed significantly during activity. The data in Table 2 shows the fatty acid composition of triglycerides (TG) and of free fatty acid (FFA) during hibernation, arousal and activity. No change in the total unsaturation in TG fatty acids could be seen during arousal from hibernation, although this fraction became highly saturated during activity which was mainly due to the increased proportion of lauric acid in triglyceride. The major fatty acids were lauric, palmitic, stearic, oleic and linoleic acid. The FFAs were more saturated during arousal and activity as compared to hibernation which was mainly due to the decreased concentration of palmitic acid in the hibernating lizard.

Table 3 gives the fatty acid composition of plasma

phospholipids (PL) and of cholesteryl esters (SE). Here again the fatty acid composition of PL was slightly more saturated during arousal and activity than during hibernation. This increase was mainly associated with the enhanced proportion of stearic acid. Drastic changes occurred in the fatty acid composition of the SE fraction during three periods of lizard's physiological state. The acids were highly unsaturated during hibernation and activity, mainly due to the accumulation of polyunsaturated C_{22} acids.

DISCUSSION

An increased concentration of EFA was only noted during prehibernation, which is the period of excessive intake and storage of exogenous food. This suggests that the plasma EFA might partly have originated from the diet and partly from synthesis in the liver. The sudden decline in the concentration of EFA as soon as the animal enters into hibernation and the concomitant increase in the weight and fat content of adipose tissue [6] indicate that the circulating EFA are quickly taken up by the abdominal fat pads. However, some of them may also be used as an energy source.

Adipose tissue lipids are used for winter survival in many hibernating species [2, 18–20]. By contrast the spiny tail lizard *Uromastix hardwickii* maintains the size, weight and glyceride content of its adipose tissue throughout the period of dormancy [6, 21]. This agrees with the previous results obtained using ground squirrels [5] and hibernating bats [22]. The increased concentration of fat in the adipose tissue during hibernation suggests that this lizard might use lipids stored elsewhere for maintenance. It may also be possible that thermogenesis in fat pads might result from the hydrolysis and resynthesis of triglycerides in the tissue with no apparent overall loss. This would seem to be supported by the accelerated triglyceride cycling in the brown fat of cold-acclimated rats [23] and of new-born rabbits [24]. There is, however, a sudden decline in the fat-content of adipose tissue immediately after arousal from hibernation as that of the ground squirrel [5] and the hedgehog [25]. The increased concentration of plasma FFA and the corresponding decrease in the concentration of fat in adipose tissue [6] suggest intensive lipolysis of fat pads in order to meet the energy required for such a drastic physiological change.

The cholesterol data for lizards during arousal agreed with the values observed in arousing hedghog [3]. However, the hibernating lizards showed much higher concentration of plasma cholesterol as compared to the hibernating hedgehog [3] and ground squirrels [26]. Contrary to this, lower concentration of plasma phospholipids were observed compared to the corresponding concentration of serum phospholipids in the hedgehog and the ground squirrel. The marked elevation in concentration of plasma cholesterol and phospholipid during hibernation and activity might be due to increased synthesis and decreased turnover rates.

Large variations were also noted in the fatty acid pattern of individual classes of plasma lipids during the hibernating cycle. The elevated concentration of saturated fatty acids in the plasma TG, PL and FFA fractions suggest that the unsaturated fatty acids are quickly utilized either for energy or are stored in the fat pads or liver during hibernation. This is also supported by our previous results which indicated the higher values of unsaturated fatty acid in the adipose tissue [6] and liver (unpublished data) during hibernation. The only exception noted was in the cholesteryl ester fraction where the concentration of unsaturated fatty acid was more than the saturated counterparts during active and hibernating lizards. A significant reduction of unsaturated fatty acids upon arousal from hibernation revealed a general phenomenon of desaturation.

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