Seasonal Changes in the Lipids of Adipose Tissue in a Hibernating Lizard (*Uromastix hardwickii*) (35389)

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(Introduced by E. L. Becker)
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Earlier investigations of hibernation and arousal in mammalian species have indicated some significant alterations in the lipid distribution of the body tissues (1–4). In most animals, arousal resulted in a marked reduction in adipose tissue fat. In some species such as the hamster (5) and in bats (6), the fat was found to be desaturated during hibernation, the golden mantled squirrel (*Citellus lateralis*) however showed no such change in the level of unsaturation (1).

Reports on reptiles and amphibians are limited and contradictory and so it is not known whether the changes in lipid metabolism in these animals during different physiologic states are comparable to those in mammals. Histochemical studies on a toad (*Bufo melanostictus*) (7), and a lizard (*Anolis carolinesis*) (8), have indicated a rise in the size and lipid content of fat depot during prehibernation period. On the other hand, another lizard (*Varanus griseus*) had fully developed fat pads during spring and summer which decreased or disappeared during the fall and winter (9). In view of these contradictory results, further studies in lower animals are needed.

A preliminary report (10) on a hibernating *Uromastix hardwickii* has indicated a high fat content both in the adipose tissue and liver. The present report describes the seasonal changes in individual lipid classes in adipose tissue over a period of 11 months as well as the fatty acid composition during hibernation, arousal, and activity.

Methods and Materials. Lizards (*Uromastix hardwickii*) were collected from the Sind area (Pakistan) and kept in wooden boxes at room temperature. In spite of several attempts, they never ate or drank.

Observations were made on a total of 66 lizards. They were collected from the fields on the 27th day of each month and six weighing between 384 and 453 g, were sacrificed that day for study without regard to age or sex.

The two abdominal adipose pads, which are localized entities, were removed quantitatively by dissection from each animal and dried with filter paper. They were homogenized and extracted with chloroform–methanol (2:1; v/v), reduced to a minimum volume by evaporation under a slow stream of nitrogen, and stored at 4° until analyzed.

The tissue weight, total lipid content, total esterified fatty acids (EFA), and total cholesterol were measured monthly from August to the following June. The amount of phospholipid is not reported as it was found to remain between 0.1–0.5% of total fat during hibernation, arousal, and activity. The fatty acid composition of each lipid class was determined in November, April, and June by gas–liquid chromatography. These months represented the periods of hibernation, arousal, and activity.

Total EFA was determined by the method of Stern and Shapiro (11), total cholesterol by the method of Chiamori and Henry (12), and phospholipids by the Zilversmit and Davis procedure (13). Quantitative analysis of glycerides and free fatty acids was carried out by combined thin-layer and gas–liquid chromatography (TLC-GLC). Individual lipid constituents were separated on silica gel G (Merck) coated plates using a petroleum ether–ether–acetic acid (80:20:1; v/v) solvent system (14).

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TABLE I. Seasonal Variation in the Tissue Weight and Lipid Content of Abdominal Adipose Tissue in *Uromastix hardwickii.*

Months	Tissue wt (g ± SD)[a]	Total lipid (g ± SD)[a]	EFA[b] (g ± SD)[a]	Cholesterol (mg ± SD)[a]
Aug.	10.6 ± 2.7	9.6 ± 2.5	6.4 ± 1.7	380 ± 200
Sept.	14.3 ± 4.1	13.5 ± 3.9	11.8 ± 4.4	908 ± 240
Oct.	11.6 ± 4.1	11.0 ± 4.1	9.4 ± 4.4	760 ± 350
Nov.	13.3 ± 0.4	11.4 ± 0.4	11.0 ± 0.5	437 ± 148
Dec.	11.5 ± 0.5	10.4 ± 0.5	9.6 ± 0.5	382 ± 425
Jan.	9.4 ± 0.7	7.9 ± 0.6	6.8 ± 0.6	389 ± 288
Feb.	11.8 ± 0.4	11.6 ± 0.3	10.3 ± 0.3	571 ± 151
Mar.	14.9 ± 0.5	12.8 ± 0.4	11.6 ± 0.4	642 ± 206
Apr.	5.7 ± 0.3	4.7 ± 0.3	4.3 ± 0.3	186 ± 97
May	5.5 ± 0.4	4.7 ± 0.4	3.9 ± 0.3	156 ± 63
June	8.3 ± 0.7	7.3 ± 0.7	5.8 ± 0.5	235 ± 15

[a] SD = standard deviation.

[b] EFA = esterified fatty acid.

Each lipid constituent was removed from the plate, and the glycerides and fatty acids were converted to their methyl esters (15). These esters were then analyzed by GLC using a Varian Aerograph Model 600 D with a hydrogen flame ionization detector. The column (5 ft, 9 in. and 1/8-in. I.D) was packed with 20% diethylene glycol succinate coated over Chromosorb W (80–100 mesh). Analysis was done at isothermal temperature (189°) with a carrier gas (nitrogen) flow rate of 20 ml/min. The peaks of the samples were identified by comparison with the retention times of known standards, and by plotting a logrithmic retention time against carbon number. Unsaturated fatty acids were further identified by bromination (16) and potassium permanganate oxidation (17). The quantitative estimations of individual lipid classes were made by the use of heptadecanoate as an internal standard. The calculations were based on the same principle as for phospholipid (18).

Results. The seasonal variation in the weight and lipid content of the abdominal adipose tissue is given in Table I. The lipid values are further illustrated (g/100 g of body wt) in Fig. 1. The prehibernation period starts in August with hibernation beginning by about November. In this period the

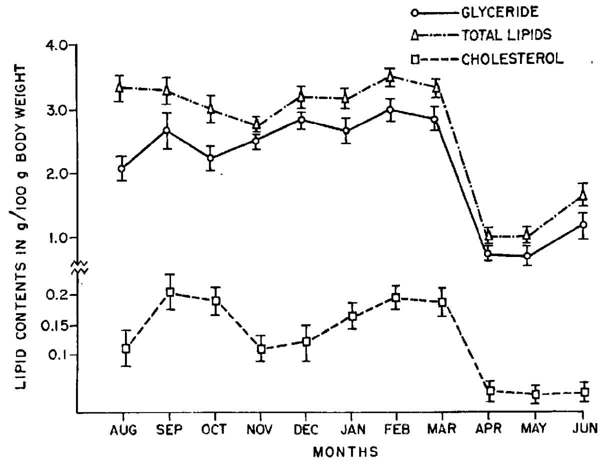

FIG. 1. Seasonal variation in lipids of abdominal adipose pads in *Uromastix hardwickii.*

TABLE II. Distribution of Class Lipids in the Adipose Tissue (% total lipid).

Period	Triglyceride[a]	Diglyceride[a]	Monoglyceride[a]	FFA[a,b]	Cholesterol[a]
Hibernation	92.7 ± 2.1	1.2 ± 1.1	1.3 ± 0.6	1.2 ± 0.8	3.6 ± 1.4
Arousal	89.1 ± 5.2	2.2 ± 2.6	2.2 ± 1.1	2.9 ± 1.7	3.8 ± 0.6
Activity	89.4 ± 3.2	2.5 ± 0.8	2.4 ± 2.0	2.1 ± 1.2	3.6 ± 0.8

[a] Mean ± SD (standard deviation).

[b] FFA = free fatty acid.

animals stored fat, but in each group there was considerable individual variation. Thereafter, the well developed abdominal fat bodies of the hibernating lizards varied little from animal to animal. Arousal occurred toward the end of April and was accompanied by a rapid fall to a half to one third the original level in the adipose tissue weight and in its glyceride content. The level of cholesterol also dropped during this period. These values remained low in May and June, the months of activity. Adipose tissue contained unusually high amounts of cholesterol during prehibernation and hibernation periods.

Table II shows the percentage composition of the adipose tissue from 18 animals determined by TLC-GLC: six were collected in November (hibernation), six in April (arousal) and six in June (activity). Despite the reduction in EFA and cholesterol during arousal and activity (Table I), the proportion of triglycerides (TG) and cholesterol remain similar. On the other hand there is a significant rise in diglycerides (DG), monoglycerides (MG), and free fatty acids (FFA) during these periods.

Table III shows the fatty acid pattern of triglycerides. The major fatty acids were oleic and palmitic comprising 50–53% and 20–24%, respectively. The main changes occurring in the three physiological states were a slight fall in palmitic and palmitoleic levels, and a concomitant rise in stearic and linolenic acid levels in arousal and activity. Oleic acid levels were relatively constant throughout and linoleic acid fell slightly during activity. At all times the proportion of unsaturated acids (65%) was greater than saturated ones and they did not change during hibernation, arousal, and activity.

TABLE III. Fatty Acid Composition of Triglycerides (wt %).[a]

Acid	State of animal		
	Hibernation	Arousal	Activity
14:0	0.6 ± 0.4	0.8 ± 0.6	0.8 ± 0.1
16:0	24.1 ± 3.4	20.3 ± 1.6	22.8 ± 3.9
16:1	5.2 ± 1.1	2.9 ± 1.4	3.5 ± 0.8
18:0	9.6 ± 1.0	13.1 ± 3.6	12.8 ± 2.6
18:1	50.3 ± 8.1	52.7 ± 4.8	50.1 ± 8.4
18:2	6.7 ± 2.2	7.9 ± 2.8	4.7 ± 0.5
18:3	1.6 ± 1.3	2.3 ± 1.1	3.2 ± 1.5
20:2	1.9 ± 1.8	Trace	2.0 ± 1.9

[a] Weight % indicated with standard deviation (±).

Table IV gives the fatty acid composition of FFA, MG, and DG. These fractions are presumably the lipolytic products of triglycerides. The fatty acid pattern showed a marked variation in the levels of palmitic, oleic, stearic, linoleic, and linolenic acids. During arousal and activity stearic and especially linoleic acids were increased in all fractions, whereas oleic acid levels were always decreased significantly. Linolenic acid was increased in all fractions in arousal and activity except in DG in arousal, and palmitic was decreased during these periods in all but DG in arousal. The occurrence of odd chain length and branched fatty acids was variable in arousal. The occurrence of odd chain fatty acids, eicosadienoic acid was only present in significant amounts in hibernation.

Discussion. It has been shown (3) that the increased weight of adipose tissue in cold-induced hibernation in ground squirrels is due to the accumulation of brown fat and fluid, and not to an increase in the cellular component of the tissue. It is likely that this is the case in all hibernating animals. It has also been noted that adipose tissue then remains in large amounts throughout hibernation (1, 10) similar change being described in cold acclimatized animals (19). Many mechanisms have been suggested to explain

TABLE IV. Fatty Acid Composition of FFA, Mono- and Diglyceride (wt %).[a]

	Hibernation			Arousal			Activity		
Fatty acid	MG	DG	FFA	MG	DG	FFA	MG	DG	FFA
12:0	0.7	1.9	1.4	—	—	—	—	—	—
14:0	2.3	1.9	4.4	6.8	3.2	6.0	4.5	1.3	5.5
15:0	0.2	0.2	1.3	2.0	0.1	1.5	2.2	0.8	2.1
16:0	29.6	22.3	30.5	19.1	39.4	20.7	15.7	17.3	23.7
16:1	1.5	1.3	0.7	tr	tr	1.4	tr	1.2	tr
18:Obr	—	—	—	—	—	4.2	4.8	1.1	—
18:0	13.2	15.0	12.7	15.5	19.2	19.5	20.6	22.7	19.7
18:1	35.7	30.9	32.5	17.9	19.1	12.0	14.9	18.9	12.8
18:2	2.6	1.8	1.5	18.3	9.6	16.1	17.3	16.2	12.8
18:3	4.9	6.4	3.4	10.0	5.5	8.9	12.1	11.7	11.5
20:2	6.0	11.7	8.0	3.6	tr	tr	tr	tr	tr
21:0	—	—	—	7.1	tr	5.9	5.6	tr	8.3
22:0	2.1	2.6	tr	2.7	0.3	3.8	2.2	3.1	1.8
22:uns	1.2	4.3	3.6	0.6	tr	tr	tr	tr	tr

[a] Each value is mean of determination on 6 animals. MG = monoglyceride; DG = diglyceride; FFA = free fatty acid.

this phenomenon. First it has been proposed that fat accumulates during hibernation when females are not laying eggs and that this is then slowly utilized when eggs are formed following this period (20). However, as the same fat changes occur in males this explanation seems unlikely. Second, it may be that low metabolism during hibernation (21) allows fat accumulation. But the results reported here show that this accumulation occurs before hibernation, and remains constant during that period. In any case, it seems unlikely that the low metabolic activity of hibernation would allow of significant lipogenesis. The present results suggested that lipid accumulates before hibernation and then remains constant until there is a sudden reduction at the time of arousal which suggests that the lipid is being used for thermogenesis at that time. This latter mechanism has been suggested for the cold-induced hibernation of ground squirrels (3).

The amount of cholesterol in the adipose tissue is very high especially during the prehibernation and hibernation periods. This has been noticed by others (10) in *U. hardwickii*. Comparison of the level of cholesterol in the hibernating lizard with that of the hibernating hedgehog (22) shows that the former is 20 times higher than the latter. Whether such high levels of cholesterol are specific for lizards as a group is not known. It may be that during the prehibernation period most of the cholesterol came from exogenous sources, as the cholesterol content of liver was also high during this period (unpublished data). It remained high during hibernation presumably because of the reduced physiologic and metabolic activity of the animal. Thus the cholesterol might not have been catabolized to its major products, the bile acids which are eventually excreted in the feces. The last step is the major mechanism for the elimination of cholesterol from the body. Such an explanation lacks factual support at this time, and requires further investigation.

Arousal represents a dramatic alteration in body physiology which may be the result of hormonal changes such as increased thyroid activity which occurs at this time (23). This would uncouple oxidative phosphorylation thereby providing increased energy (24). Arousal has been shown to have features in common with the "alarm reaction" (25) and hormones such as noradrenaline, which are released in these conditions, are known to cause lipolysis (2). The present results, showing increased FFA, MG, and DG, support the idea that lipolysis is occurring, which would explain the reduction of noradrenaline in brown fat during arousal in hedge hog (26).

Little has been done on the fatty acid changes in hibernation, arousal, and activity. Most reports have referred to changes in the amount of unsaturation of fatty acids or total fat such as those of Turchetto *et al.* (27) in the bat, and Fawcett and Layman (5) in the hamster, who reported increases in hibernation; and Spencer *et al.* (1), who found no consistent changes in *Citellus lateralis.* We found that during arousal and activity palmitic and palmitoleic acids were reduced in triglycerides, MG, DG, and FFA perhaps indicating a specially high ability to metabolize these fatty acids. This may also be the case for oleic acid as it decreased in the lipolytic products of triglycerides in the same period. On the other hand lipolytic products containing stearic, linoleic, and linolenic acids, and particularly the latter two, all increased, which suggest that they are not so readily metabolized. This may be related to a conservation of the essential fatty acids in starvation in rats, a suggestion made by Chalvardjian (28).

Summary. Studies were made on the changes in the lipid pattern of adipose tissue in a lizard during hibernation, arousal, and activity. A relation appears to exist between the fat composition of the adipose tissue and the lizards seasonal physiological state. Adipose tissue shows a marked increase in size and lipid content during the prehibernation and hibernation periods. Arousal resulted in a two- to threefold reduction in the tissue weight and its fat content, and this remained low during activity. Triglycerides always represented the bulk of the lipid fraction. During arousal and activity the levels of EFA and cholesterol were reduced. Despite the alterations in the fatty acid pattern of triglycerides, especially in the levels of palmitic, palmitoleic, stearic, and linolenic acids, there was no change in the total unsaturation during hibernation, arousal, and activity. The relative proportions of the lipolytic products (FFA, DG, and MG) were increased during arousal and activity, and were associated with marked variations in their fatty acid composition which may be related to variable metabolic rates for these acids.

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