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EFFECT OF CORTISONE ON THE LIPID COMPOSITION OF ADIPOSE TISSUE, PLASMA AND LIVER IN A LIZARD

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The effect of Cortisone on the lipid composition of adipose tissue, plasma and liver was studied in a lizard, *Uromastix hardwickii*. The total lipid was analysed for triglycerides, cholesterol and phospholipid content spectrophotometrically. The fatty acids of adipose tissue (free and esterified) were determined by a combined technique of thin-layer and gas liquid chromatography.

In the adipose tissue a significant reduction in the concentration of both triglycerides and free fatty acids fractions was observed after cortisone administration indicating the mobilization of fat from the tissue. The reduced level of glycerides and FFA in the adipose tissue and simultaneously increased level of triglycerides in liver and plasma indicates that the mobilized fat is transported to the liver where it is re-esterified. There seems to be very little effect on the hepatic cholesterol level. The hepatic phospholipid turn over rate is, however, increased due to the increased lipid transportation process. The major fatty acids in the adipose tissue were palmitic, stearic and oleic acid. The changes in the fatty acid pattern of the adipose tissue after cortisone treatment revealed the fatty acid specificity in the process of mobilization.

Key words: Lipids, Cortisone, Lizard.

INTRODUCTION

Cortisone is one of the ACTH dependent glucocorticosteroidal hormone which is secreted by adrenal cortex. Many research groups in the past few decades attempted to study the effects of adrenocortical hormones on lipid metabolism but conclusions were found to be contradictory. It has been reported that the administration of cortisone inhibits the conversion of carbohydrates into lipids and decreases free fatty acids (FFA) re-esterification in the adipose tissue of rabbits and rats [1, 2]. Moreover it enhances the lipolytic activity in the epididymal fat pads [3, 4] and consequently increases the levels of plasma and liver glycerides and FFA [4-6]. The lipolytic effect was found to be even greater than adrenaline [3]. On the other hand it has also been reported that cortisone acetate given subcutaneously (s.c.) to rats did not produce any change in the weight and fat cell content of adipose tissue [7]. Furthermore the administration of hydrocortisone in rats has shown to decrease the concentration of lipids in the liver and stimulated lipogenesis from glucose in adipose tissue [8, 9]. The in vitro studies have, however, revealed that hydrocortisone incubated with isolated fat cells inhibits the incorporation of ^{14}C – labelled glucose into lipids [10] but this inhibitory effect was not observed with higher glucose concentration in the medium [11]. The incorporation of glucose into lipids and fatty acids and its oxidation to CO_2 was markedly decreased in the tissue isolated from rats 2 hours after intraperitoneal (i.p.) injection of cortisone or hydrocortisone, while no such depression was observed when the tissue was removed 6 hours after the injection [11]. All these reports have lead to conclude that the metabolic effect of the hormone vary with dosage, duration of treatment and species even strain of animals.

Although much work has been done on the higher animals, relatively less attention has been paid to establish the metabolic effects of hormone in lower animals. In the present study effort has been made to establish the effect of cortisone on the lipid composition of various tissues in hibernating lizards. Such studies may provide some information useful in elucidating the comparative status of the effect of hormone from lower to higher animals.

MATERIALS AND METHODS

All chemicals were of reagent grade and obtained from E. Merck. All solvents were anhydrous and of highest purity except ethanol which was first refluxed with zinc dust and potassium hydroxide and then redistilled prior to use. Cholesterol was recrystallized with ethanol before using as standard. Cortisone acetate of extra pure quality was obtained from Merck Sharp and Dohme of Pakistan Limited.

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During the present study 40 experimental lizards, *Uromastix hardwickii*, irrespective of their age and sex and weighing approximately 240 gm each were selected. These were collected from the deserted areas of Karachi and its suburbs and were kept in wooden boxes at room temperature (30°) without any food or water throughout the period of study. The animals were divided into 10 groups including one control group, each group comprising 4 animals. Cortisone acetate (2 mg/ml in physiological saline) was administered intraperitonially into each animal except the 4 animals of the control group in which only saline was administered. The animals were sacrificed by cutting their neck with razor blade after 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours after the administration of cortisone acetate. The blood was collected in a tube containing potassium oxalate as anticoagulant. The blood was then centrifuged at 5000 r.p.m. for 15 minutes. The plasma thus obtained was stored in small stoppered sample tubes in the refrigerator at 4° until analysed.

The animals were then dissected ventrally. Liver and adipose tissues were quantitatively removed, washed with saline, dried off extraneous water using a filter paper and weighed. Total lipid was extracted with chloroform: methanol mixture (2:1,v/v) from plasma, liver and adipose tissue. The extracts were concentrated and the constant weight of the total lipid was noted. From the total lipids of each tissue, glycerides were determined by the method of Stern and Shapiro [12], cholesterol by the method of Chiamori and Henery [13] and phospholipids by Zilversmit and Davis method [14].

Fatty acid composition (free and esterified) of adipose tissue was quantitatively determined by the combined technique of thin-layer and gas liquid chromatography. The lipid classes were separated on Silica gel G plates using petroleum ether (40°-60°): ether: acetic acid (80:20:1, v/v) as the solvent system. The free fatty acid and triglyceride fractions were quantitatively scrapped from the TLC plate and were converted into the methyl esters [15] with a known amount of heptadecanoic acid as internal standard. These methyl esters of fatty acids were resolved by gas liquid chromatography on H1-F1 Varian aerograph Model 600 D equipped with a hydrogen flame ionization detector. The column was packed with 20% diethylene glycol succinate coated over chromosorb W (30-100 mesh). Analysis was done at isothermal temperature (189°) with carrier gas (nitrogen) flow rate at 20 ml/minute. The peaks were identified by comparing their retention time with known standards and by plotting logarithmic retention time against carbon number [16]. The total fatty acids were calculated with the help of the internal standard.

RESULTS AND DISCUSSION

The total lipids and the levels of triglycerides, free fatty acids (FFA) and cholesterol fractions of adipose tissue before and after the administration of cortisone acetate has been illustrated in Fig. 1. A significant reduction in the total lipid level has been observed in the cortisone treated animals. The total lipid level was about 57% reduced within

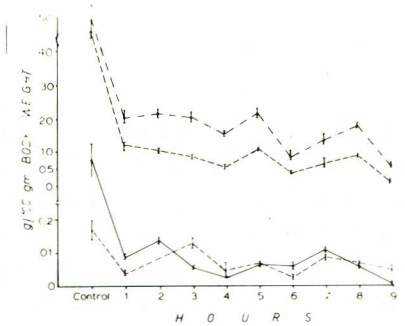

Fig. 1. Lipid composition of adipose tissue after cortisone treatment. _ ·· _ ·· _ = Total lipd, —— = Triglyceride, _ . _ . _ = Cholesterol, —— —— = Free fatty acid, I = S.E.

one hour of cortisone administration, followed by a gradual reduction upto nine hours. The triglyceride level in the abdominal adipose tissue has been dropped approximately upto 73% as compared to control after one hour of cortisone treatment. This level was further decreased gradually upto nine hours, reaching a minimum value of 0.15 gms, per 100 gms body weight, which is about 97% below the control value. The FFA level of adipose tissue has also been significantly reduced in the cortisone treated animals. The FFA level was reduced from 850 mg to 90 mg/100 gm body weight just after one hour of cortisone injection. As the duration of treatment increased, the depletion of FFA level became more significant, the lowest value obtained after the ninth hour of treatment was only 10 mg/100 gm body weight.The cholesterol level was also decreased from 170 mg to 40 mg per 100 gm body wight after one hour of cortisone treatment. In the subsequent hours, the cholesterol values fluctuated between 30 to 130 mg/100 gm body weight. Since the level of phospholipid in the adipose tissue was negligible, it could not be determined.

Fig. 2 represents the levels of total lipids, triglycerides and cholesterol in the liver. The total lipid and glyceride levels of liver are found to be increased in the cortisone treated animals. The cholesterol levels, however, did not exhibit any significant change after cortisone treatment. The phospholipids were only present in the control group but could not be found in detectable amount in any group of the cortisone treated animals.

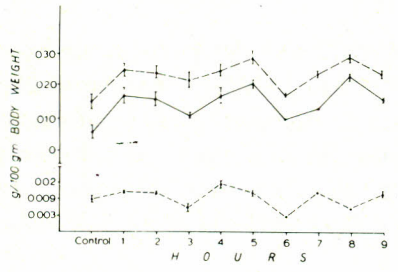

Fig. 2. Lipid composition of liver after cortisone treatment, — = Total lipids, ———— = Triglycerides, _ . _ . _ . _ = Cholesterol, I = S.E.

The plasma triglycerides, cholesterol and phospholipid levels are represented in Fig. 3. Although the levels of plasma triglycerides fluctuated between one and nine hours of cortisone treatment, nevertheless the concentration of this fraction was always significantly higher than that of control except after six and eight hours of treatment where the levels were approximately same as control. The plasma cholesterol level were gradually decreased with the increase in the duration of treatment except after the 5th hour when the values were found to be as high as control. No significant effect of cortisone was noticed on the plasma phospholipid concentration. The values remained below the normal level in all the treated animals except after the 9th hour when the phospholipid level was significantly increased.

In order to study the release of specific fatty acids from the adipose tissue during lipolysis, the free and esterified fatty acids were determined by the combined technique of thin layer and gas liquid chromatography. Table 1 represents the free fatty acid (FFA) composition of adipose tissue in normal and cortisone treated animals. A considerable

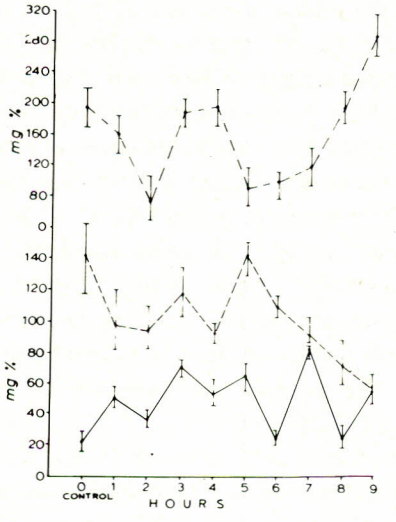

Fig. 3. Lipid composition of plasma after cortisone treatment, ——— = Triglycerides, — = Cholesterol, _ .. _ .. _ = Phospholipid, I = S.E.

decrease in the concentration of palmitic and oleic acids was observed after one hour of cortisone treatment. The level of palmitic and oleic acids were, however, returned to the normal value after the third and the first hour of cortisone injection respectively. Linoleic acid which was present in minor amount in the control animals showed a significant increase after one hour of cortisone administration. Its level was, however, reached to the normal level in the subsequent hours of treatment. Other components were comparatively minor and showed greater variations.

Table 2 illustrates the fatty acid composition of adipose tissue triglycerides before and after cortisone administration. The major fatty acids in this fraction were palmitic and oleic acids. Oleic acid was found to be more than 50% of the total fatty acids present. No definite effect of corti-

Table 1. Free fatty acid (FFA) composition of adipose tissue in control and cortisone treated lizards (weight %)

Fatty acid components	Control	Cortisone acetate treated animals (hours)								
		1	2	3	4	5	6	7	8	9
C12:0	—	5.89	—	2.22	3.51	—	—	—	—	3.76
C14:0	7.30	4.76	5.35	12.37	5.42	2.86	3.07	2.85	1.78	4.94
C14:1	—	—	—	—	3.48	—	—	4.74	1.53	—
C16:0	30.15	23.77	26.13	22.28	28.40	29.65	26.68	25.32	26.40	31.69
C16:1	6.54	8.76	10.35	7.18	7.04	8.61	7.83	5.96	5.18	6.86
C18:0	13.03	10.36	15.23	11.88	11.33	20.34	20.29	15.41	12.47	16.62
C18:1	35.97	25.32	32.79	35.76	34.03	34.25	36.42	36.24	39.96	32.05
C18:2	7.01	21.14	10.15	4.97	6.88	9.01	7.88	5.69	8.45	4.08
C18:3	—	—	—	3.35	—	—	—	3.79	4.23	—
Saturated (%)	50.48	44.78	46.71	48.74	48.57	52.85	50.04	43.58	40.65	57.01
Unsaturated (%)	49.52	55.22	53.29	51.26	51.43	47.15	49.96	56.42	59.35	42.99

Table 2. Free fatty acid composition of adipose tissue triglycerides after cortisone treatment (weight %)

Fatty acid components	Control	Cortisone acetate treated animals (hours)								
		1	2	3	4	5	6	7	8	9
C12:0	0.80	2.60	—	—	1.86	—	—	—	4.85	1.45
C14:0	1.47	1.58	1.83	2.07	2.49	1.78	2.19	1.86	2.81	1.81
C16:0	21.53	24.50	27.78	20.65	23.36	22.58	27.04	25.44	33.66	30.00
C16:1	6.96	6.83	6.28	6.97	7.03	5.95	8.14	7.19	5.29	14.37
C18:0	10.66	10.79	15.62	10.77	8.72	15.22	11.25	10.24	10.67	9.41
C18:1	57.06	49.00	48.49	53.53	53.90	54.47	51.38	48.37	40.24	38.13
C18:2	1.52	4.70	—	6.01	2.64	—	—	6.9	2.48	4.83
Saturated (%)	34.46	39.47	45.23	33.49	36.43	39.58	40.48	37.54	51.99	42.67
Unsaturated (%)	65.54	60.53	54.77	66.51	63.57	60.42	59.52	62.46	48.01	57.33

sone was noticed on the esterified fatty acid composition. The palmitic acid level was slightly increased and oleic level was slightly decreased in the cortisone treated animals as compared to control, but these variations were insignificant. The percent composition of unsaturated fatty acids in this fraction was found to be higher than the saturated fatty acids in both normal and treated animals.

The data presented in this report clearly indicates that cortisone acetate administered i.p. into the lizards stimulates the lipolytic process in the adipose tissue. Previously [17-21, 25], it has been reported that the lipolytic hormones increase the activity of adenylate cyclase and thus the concentration of cyclic AMP. The increased cellular concentration of cAMP activates the cAMP-dependent protein kinase which in turn leads to phosphorylation and stimulation of triacylglycerol lipase. A steady state relation between the activation state of cAMP- dependent protein Kinase and lipolysis has been described quantitatively by Honnor *et. al.* [22]. Similar type of mechanism may also be responsible for the stimulation of lipolysis by cortisone acetate in our experimental animals.

A decreased concentration of total lipids and triglycerides in the adipose tissue after cortisone administration and at the same time increased level of glycerides in the liver and plasma indicates, that the lipid is extensively mobilized from the adipose tissue and the mobilized fat is transported to the liver via blood in the form of lipoprotein, where it is re-esterified. The enhanced re-esterification of mobilized FFA as well as lipogenesis from acetate and lactate leads to the accumulation of fat in the liver, as the rate of its transportation seems to be slower than its synthesis. In adipose tissue significant reduction in the concentration of both triglycerides and FFA was noticed after cortisone injection. Since cortisone activates the hydrolysis of triglycerides in the fat depots, increased level of FFA would have been expected in the tissue if they were not being taken up by the blood immediately . As the FFA level in the tissue is significantly decreased, it is suggested that lipolysis of fat from the depot have been associated with its enhanced uptake by the blood.

Our findings of increased mobilization of fat from adipose tissue during cortisone therapy are in confirmation to the results obtained by other workers [3, 6]. The onset of lipolysis, however, depends upon the dose, duration of treatment, species and strains of animals as well as the presence of lipolytic and antilipolytic agents in the tissue [4, 11, 22, 23]. In our experimental lizards a dose of 2 mg/animal was sufficient for the stimulation of lipolysis.

Cortisone has caused hyperlipemia and hypertriglyceroderia in our experimental animals but has not acted as hypercholesterolemic agent as reported earlier [24]. Instead the blood cholesterol levels were found to be depressed. There is, however, no significant effect on the hepatic cholesterol level which is in confirmation to the findings of Friedman *et al.* [4]. These authors [4] have suggested that cortisone induced hypercholesterolemia only indirectly as a result of sufficient degree of preceeding hypertriglyceridemia which stems in part from a relative inability of liver to remove triglycerides from plasma.

No significant effect of cortisone was observed on the plasma phospholipid level. The levels are however, found to be elevated as compared to control. In contrast the liver phospholipids could not be found in detectable amount after cortisone treatment. Previous report [24] have indicated that phospholipid is synthesised several time faster in the cortisone treated animals as compared to control. It appears that cortisone has highly increased the phospholipid turnover rate in our experimental animals which may be due to its increased utilization in the transportation process.

Considerable variations have been noted in the fatty acid composition of adipose tissue during cortisone induced liplysis. The levels of palmitic and oleic acids which were the major fatty acids of the tissue, were decreased while the linoleic acid which was present in minor amount in the control animals was significantly increased in the first hour of cortisone treatment. This indicates that the rate of release of palmitic and oleic acid is slow as compared to linoleic acid. These changes, however, became insignificant with increase in the duration of treatment. There seems to be very little effect on the fatty acid composition of triglyceride fraction. This fraction has been found to contain more unsaturated fatty acids as compared to saturated fatty acids while the ratio of saturated and unsaturated fatty acids in FFA fraction is about 1:1.

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