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# EFFECT OF GRADED LEVEL OF INSULIN ON BLOOD SUGAR AND LIPID DISTRIBUTION IN A HIBERNATING LIZARD

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The effects of intraperitoneal administration of insulin, in graded doses, was studied on blood sugar and lipid distribution in a hibernating lizard, *Uromastix hardwickii.* The lizards were found to be quite resistant to insulin, they tolerated up to 25 units of insulin, in 5 divided doses, without any insulin shock. The blood sugar was insignificantly below the normal level in the insulin treated animals. Plasma glyceride level was slightly lowered and liver glyceride was little elevated after insulin administration. The most prominent effect was observed in the case of adipose tissue glycerides. At lower doses the glyceride level of adipose tissue depressed insignificantly but highly significant depression was noted as soon as a dose higher than five units was injected. It appears that insulin induces a dose dependent lipolysis in adipose tissue. No marked variation in the total cholesterol content was observed in plasma, liver and adipose tissue after insulin administration. Plasma phospholipid level was gradually decreased reaching a minimum value at a dose of 10 units of insulin. At further higher doses the phospholipid level again started to increase, but still remained below the normal level. The reverse was true in the case of liver phospholipid. The major fatty acids of adipose tissue were palmitic, stearic and oleic acids. Myristic, palmitoleic, linoleic and arachidic acids were also present in appreciable amounts. The level of palmitoleic, oleic, linoleic and arachidic acid of adipose tissue was increased significantly with a concomitant decrease in the levels of palmitic and steric acids in the insulin treated animals.

## INTRODUCTION

Deficiency of insulin in men and experimental animals results in *Diabetes mellitus.* This disease is accompanied by severe changes in carbohydrate and lipid metabolism.

Similarly effect of excess insulin on metabolic set up has been studied in normal and diseased subjects. Such studies have resulted in an accumulation of contradictory information which was considered to be due to marked variation in insulin sensitivity from lower to higher animals. Such observations have opened a new avenue of investigations in comparative biochemistry.

In the present study, the effect of insulin administration in various graded doses on lipid and carbohydrate metabolism has been studied in 32 lizards, *Uromastix hardwickii* and the results are compared with other experimental animals belonging to different classes of the animal kingdom.

## MATERIALS AND METHODS

All the chemicals, reagents and solvents used were of reagent grade (E. Merck). Insulin (40 units per ml) was supplied by Burroughs Wellcome & Co. Limited, (Pakistan).

*Uromastix hardwickii,* were collected from the deserted area of Karachi and Sind region and were kept in wooden cages without any food or water throughout the period of study. 32 animals weighing about 200 – 280 g were selected for the study and were used irrespective of their age and sex. They were divided into 8 groups, each comprising of four animals. Group No. 1 was treated as control.

Insulin was diluted in physiological saline and 0.5 ml of diluted insulin containing 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5 units was injected intraperitoneally to 7 groups (28 animals) respectively for 5 consecutive days. 0.5 ml of physiological saline was injected intraperitoneally in each animal of the control group daily for 5 days.

Exactly after 120 hr of the first injection of insulin and 24 hr after the last injection, animals were sacrificed by cutting their neck and the blood was collected in a tube

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containing potassium oxalate as an anti-coagulant. Glucose was estimated from the whole blood by the method of Folin and Wu [1] and the remaining blood was centifuged at 5000 r.p.m. for 15 min. Plasma was transferred to small stoppered sample tube and kept in a refrigerator at $4^{o}$ until the estimations of the individual lipid components were performed.

The animals were then dissected ventrally. Liver and adipose tissues were removed, washed in Saline, dried with filter paper and weighed. Lipid was extracted with Chloroform: Methanol mixture (2:1 V/V) from liver, adipose tissue and plasma. Total glycerides were determined by the the method of Stern and Shapiro [2], Cholesterol by the method of Chiamori and Henry [3] and phospholipids by Zilversmit and Davis method [4].

Fatty acid composition of adipose tissue (free and esterified) was quantitatively determined by GLC using Varian Aerograph Model 600 D with a hydrogen flame ionization detector. The column (175.26 cm and 0.33 cm I.D.) was packed with 20 % diethylene glycol succinate coated over chromosorb W (80 – 100 mesh). Analysis was done at isothermal temperature ($189^{o}$) with a carrier gas (nitrogen) flow rate at 20 ml/min. The peaks of the samples were identified by comparison with the retention time of known standard and by plotting a logarithmic retention time against carbon number. Heptadecanoic acid was used as internal standard for quantitative evaluation.

## RESULTS

The weights of the adipose tissue and liver and their total lipid contents are represented in Table 1. The tissue weights were found to be increased in all the insulin treated animals. Statistically significant increase was, however, noted in the group of animals, receiving 5, 7.5, 10 and 25 units of insulin. There was, however, no significant difference in the percent total lipid content in any of the group. This indicates that the increase in fat pad weight is due to the deposition of lipids in the existing fat pads and not due to the increased proliferation of new fat cells.

Fig. 1 represents the blood glucose levels after intraperitoneal injection of insulin to the animals under study. The blood glucose level was found to be only slightly below

Table 1. Weight of adipose tissue and liver and their total lipid content after intraperitoneal administration of graded insulin in the lizard, *Uromastix hardwickii.*

| *Group | Doses of Insulin | | Adipose Tissue | | | Liver | |
|---|---|---|---|---|---|---|---|
| | Total units in 5 days | Units/kg/day | Wt of the animal (g) | Wt. of the adipose tissue (g) | Total lipid (% Wet tissue) | Wt. of liver (g) | Total lipid (% Wet tissue) |
| 1. | 0** | 0 | 249.5 ± 15.6 | 7.3 ± 0.9 | 68.6 ± 7.3 | 6.0 ± 0.8 | 5.8 ± 0.3 |
| 2. | 2.5 | 1.95 ± 0.04 | 257.0 ± 4.4 (NS) | 8.2 ± 0.7 (NS) | 61.6 ± 5.3 (NS) | 6.5 ± 0.3 (NS) | 5.0 ± 0.6 (NS) |
| 3. | 5.0 | 3.80 ± 0.24 | 266.0 ± 15.7 (NS) | 11.2 ± 0.9 (P > 0.025) | 69.7 ± 5.0 (NS) | 8.1 ± 0.4 (P < 0.02) | 5.9 ± 0.1 (NS) |
| 4. | 7.5 | 5.81 ± 0.18 | 258.5 ± 6.7 (NS) | 11.6 ± 1.2 (P < 0.05) | 69.7 ± 1.9 (NS) | 9.0 ± 1.4 (NS) | 6.2 ± 0.6 (NS) |
| 5. | 10.0 | 7.29 ± 0.40 | 276.0 ± 12.2 (NS) | 14.8 ± 1.6 (P < 0.01) | 69.5 ± 6.4 (NS) | 9.3 ± 0.5 (P < 0.005) | 6.5 ± 0.3 (NS) |
| 6. | 15.0 | 11.35 ± 0.57 | 266.0 ± 12.0 (NS) | 9.6 ± 1.8 (NS) | 58.5 ± 7.3 (NS) | 7.0 ± 1.1 (NS) | 5.4 ± 0.5 (NS) |
| 7. | 20.0 | 14.66 ± 0.79 | 274.5 ± 12.0 (NS) | 10.4 ± 2.8 (NS) | 53.8 ± 2.9 (NS) | 7.5 ± 0.5 (NS) | 6.3 ± 0.6 (NS) |
| 8. | 25.0 | 19.73 ± 1.03 | 255.0 ± 11.4 (NS) | 12.1 ± 0.7 (P < 0.01) | 49.3 ± 5.8 (NS) | 9.0 ± 1.0 (P < 0.05) | 5.1 ± 0.4 (NS) |

= Each group comprises of 4 animals.
** 0.5 ml of Physiological saline was injected (i.p) daily for a total period of 5 days (control group).
P Probable level of significance.
NS Not significant.

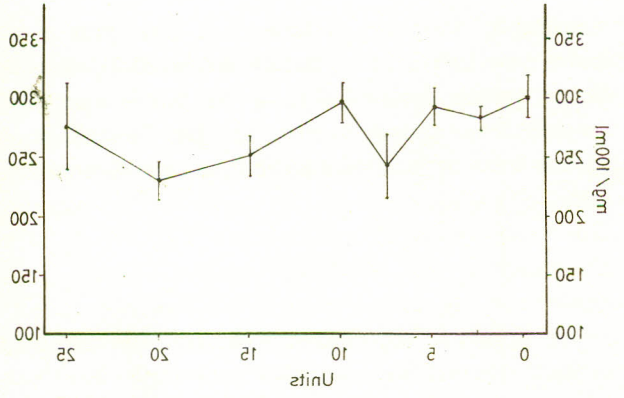

Fig. 1. Blood glucose level after intraperitoneal administration of insulin.

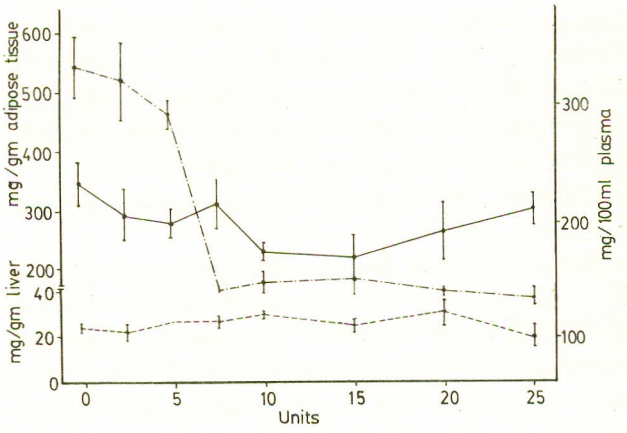


Fig. 2. Total glyceride level of plasma, liver and adipose tissue after intraperitoneal administration of insulin.

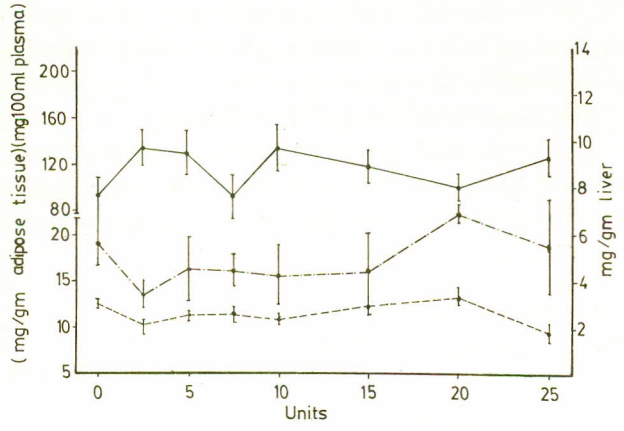


Fig. 3. Cholesterol level of plasma, liver and adipose tissue after intraperitoneal administration of insulin.

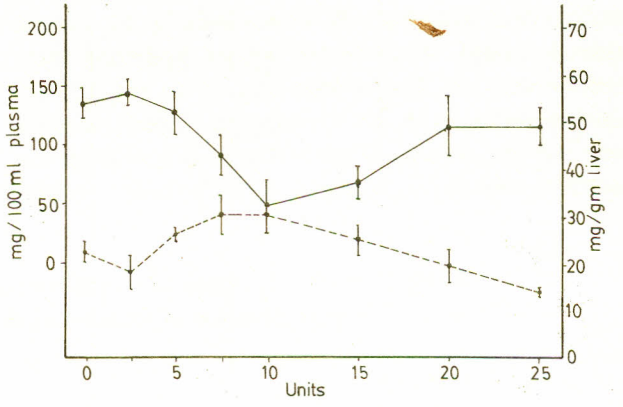


Fig. 4. Phospholipid level of plasma and liver after intraperitoneal administration of insulin.

the normal level up to a dose of 15 units of insulin administration. At a dose of 20 units the sugar level was depressed significantly ($P < 0.05$), but at a higher dose of 25 units the level was increased instead of further decreasing and the difference between the control and the treated animals again became insiginificant.

The total glyceride content of plasma, liver and adipose tissue after intraperitoneal administration of insulin are provided in Fig. 2. Compared to the control group decreased plasma glyceride levels were noted in each group of animals receiving various doses (2.5 – 25 units) of insulin, but the statistically significant depression ($P < 0.05$) was only observed in those groups of animals in which 10 and 15 units of insulin were injected. Contrary to the plasma glyceride levels, the liver glycerides were insiginificantly higher in the insulin treated animals when compared with the control group. Drastic changes were noted in the glyceride content of adipose tissue. At lower doses (2.5 and 5.0 units) the glyceride level was insiginificantly decreased, but highly significant depression ($P < 0.001$) was observed as soon as a dose of 7.5 units of insulin was injected and this level was maintained up to the dose of 25 units.

As indicated from the Fig. 3, no marked variation in the cholesterol content of plasma, liver and adipose tissue was noted after various doses of insulin administration. Compared to control, the plasma cholesterol level was slightly higher and the adipose tissue and liver cholesterol levels were lower in the insulin treated animals. Only the animal receiving 20 units of insulin showed a slight depression in their plasma cholesterol level and a little elevation in their tissue cholesterol. These changes were, however, statistically insignificant. Liver cholesterol, only at a dose of 25 units of insulin exhibited a significant depression ($P < 0.05$) when compared with the control level.

The phospholipid levels of plasma and liver after intraperitoneal administration of insulin are provided in Fig. 4. No detectable amount of this component was found in the adipose tissue. The plasma phospholipid level exhibit

a linear decline after insulin administration reaching a minimum value ($P < 0.01$) when 10 units of insulin were injected. The phospholipid level of plasma was again started to increase at higher doses of insulin, but still significantly below ($P < 0.02$) the normal level upto a dose of 15 units. The level of plasma phospholipids, inspite of its fluctuations, remained always depleted in the insulin treated animals as compared to control. Contrary to plasma phospholipids, the liver phospholipid level was gradually increased after insulin administration. At lower doses this increase was not significant but it became significant as soon as 7.5 units of insulin were administered. Highly significant value ($P < 0.01$) was obtained when 10 units of insulin were injected. The level was then gradually decreased and was only significant ($P < 0.05$) up to a dose of 15 units.

Since insulin administration depleted the glyceride content of the adipose tissue, detailed composition of total fatty acids was performed for each animal which is represented in Table 2. The major fatty acids of adipose tissue were palmitic, stearic and oleic acid. Myristic, palmitoleic, linoleic and arachidic acids were also present in appreciable amounts. Myristic acid which was found in small quantities in the control animals showed a significant increase after a dose of 7.5 and remained high up to 20 units of insulin. After 25 units the level returned to its normal value. Palmitic acid level was depressed after insulin administration and a significant depression was noted at a dose of 2.5 and 25 units of insulin. Stearic acid concentration was decreased significantly after insulin injection in any dose ranging from 2.5 to 25 units, but the most effective dose was found to be 10 units where the depression in the stearic acid level was maximum. Contrary to stearic acid, the level of arachidic acid showed significant increase after the administration of insulin in various doses (2.5 – 25 units).

Significantly increased levels of palmitoleic acids were observed after a dose of 5 to 25 units of insulin. At a lower dose of 2.5 units the increase in the level of palmitoleic acid was not significant. The level of linoleic acid was also increased after insulin injections, but the only significant increase was noted after a dose of 7.5 units. Oleic acid level was initially decreased with the injection of small doses of insulin (2.5 and 5 units) and then increased at higher doses.

Table. II Fatty Acid Composition (Free and Esterified) of Adipose Tissue After interaperitoneal injections of insulin in *Uromastix harwickii.*

| Doses (Units) | Fatty :Acids | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $C_{14:0}$ | $C_{16:0}$ | $C_{16:1}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{20:0}$ | $C_{21:1}$ | $C_{20:2}$ |
| 0 | 0.8 ± 0.1 | 24.8 ± 1.5 | 3.1 ± 0.1 | 12.1 ± 0.5 | 43.0 ± 1.9 | 3.9 ± 1.3 | 1.0 ± 0.6*** | 0.6 ± 0.0* | 9.1 ± 1.3 |
| 2.5 | 0.7 ± 0.1 (NS) | 17.9 ± 1.0 (P < 0.02) | 3.8 ± 0.4 (NS) | 10.4 ± 0.4 (P < 0.05) | 37.7 ± 1.2 (P < 0.05) | 6.6 ± 0.6 (NS) | 4.5 ± 0.5 (P< 0.005) | 2.6 ± 0.0 | 14.1 ± 1.3 (P< 0.05) |
| 5.0 | 0.8 ± 0.2 (NS) | 22.4 ± 0.3 (NS) | 6.0 ± 0.2 (P< 0.001) | 9.3 ± 0.2 (P < 0.005) | 41.6 ± 1.6 (NS) | 6.5 ± 0.4 (NS) | 5.2 ± 0.5 (P < 0.001) | – – | 4.1 ± 0.7 (P < 0.05) |
| 7.5 | 2.1 ± 0.5 (P < 0.05) | 21.7 ± 0.5 (NS–) | 6.4 ± 0.1 (P < 0.001) | 9.4 ± 0.2 (P < 0.005) | 46.3 ± 0.8 (NS) | 7.3 ± 0.5 (P < 0.05) | 6.6 ± 0.5 (P < 0.001) | – | – |
| 10.0 | 1.3 ± 0.2 (P < 0.05) | 22.7 ± 0.5 (NS) | 5.8 ± 0.3 (P < 0.001) | 8.4 ± 0.2 (P < 0.001) | 51.4 ± 0.6 (P < 0.005) | 5.5 ± 0.2 (NS) | 5.0 ± 1.0 (P < 0.02) | – | – |
| 15.0 | 1.2 ± 0.2 (P < 0.01) | 23.2 ± 0.6 (NS) | 5.4 ± 0.4 (P < 0.005) | 9.7 ± 0.6 (P < 0.05) | 46.6 ± 2.3 (NS) | 5.1 ± 0.9 (NS) | 7.3 ± 1.6 (P < 0.01) | 1.0 ± 0.0* | 1.5 ± 0.5 |
| 20.0 | 1.3 ± 0.1 (P < 0.05) | 23.9 ± 1.2 (NS) | 5.9 ± 0.4 (P < 0.001) | 9.7 ± 0.3 (P < 0.05) | 50.3 ± 1.0 (P < 0.02) | 5.0 ± 0.4 (NS) | 6.1 ± 1.0 (P < 0.005) | – | – |
| 25.0 | 0.8 ± 0.1 (NS) | 20.7 ± 0.7 (P < 0.05) | 5.4 ± 0.1 (P < 0.005) | 9.2 ± 0.1 (P < 0.005) | 52.8 ± 1.0 (P < 0.005) | 4.3 9b 0.1 (NS) | 5.8 ± 0.3 (P < 0.001) | – | 1.2 ± 0.5 |

* One animal out of our contained this component.
** Two animals out of four contained this component.
*** Three animals out of four contained this component.
P Probable level of significant.
NS Not significant.

The significant changes were, however, noted after the administration of 2.5, 10, 20 and 25 units of insulin. The $C_{20:2}$ was a major component in the normal animals. Its level was slightly increased when 2.5 units of insulin were administered. However, on subsequent doses (from 5 to 25 units) this components was found to be either absent or reduced to a very low level ranging from 1.2 to 4.1 %. Other components being minor in concentration showed greater variation in their levels after insulin treatment. It can be concluded that the insulin increases the concentration of mono and dienoic acids (palmitoleic, oleic and linoleic acids) and arachidic acid with a concomitant decrease in the level of palmitic and stearic acids.

## DISCUSSION

Unlike rats [5] domestic fowls [6,7], fish [8], eel [9] and water snake [10], the lizards [11, 12, 13] have been reported to be quite resistant to the hypoglycemic effect of insulin. 40 units/kg/day for four days produced moderate hypoglycemia inthe lizard, *Varanus monitor* [13], while the symptoms of insulin shock appears only on the 5th day Similarly 40 units/kg/day for 4 days produced hypoglycemic effects in the lizard *Calotes versicolor* [14] and *Uromastic hardwickii* [15] without any insulin shock. As reported by Miller and Doris [11] a minimum shocking does of insulin is approximately 1000 units/kg in the lizards. Larger doses of insluin 2000 – 10500 units/kg did not increase the degree of shock but prolonged its duration.

Our observations are in confirmation to the above reported results. No insulin shock was noted even after the administration of 25 units of insulin in 5 divided doses. An insignificant decrease in the blood sugar level was, however, observed with a minimum dose of 7.5 units.

Present data clearly indicates that insulin administration caused mobilization of fat from the adipose tissue. The fat might have been transported to the liver via blood. Since free fatty acids are released from the adipose tissue during mobilization which normally results in the elevation of plasma free fatty acids, the estimation of which might have been an extra evidence for such insulin induced mobilization. Unfortunately such estimations could not been done in the present study.

Our results are contradictory to the previous findings [16, 17, 18, 19] that insulin inhibits lipolysis in adipose tissue by lowering thecellular level of cyclic AMP elevated by the lipolytic agents. On the other hand some reports are also available [20, 21] which indicate that insulin especially at low physiological concentrations, does not detectably decrease the cellular level of cyclic AMP and under certain selected conditions produced a biphasic or a stimulatory effect [22, 23] on lipolysis. Further studies on the effect of insulin [24] on cellular level of cyclic AMP and the rate of lipolysis under various conditions in isolated rat epididymal fat cells, lead to conclude the insulin induces multiple effects onthe level of cyclic AMP and lipolysis depending on its concentration and the nature and concentration of the liploytic agents present. The same might be true in lizards also. As glucose lowering effect of insluin has not been observed in our experimental animals, it seems probable that these animals are utilizing fat or protein for glucose synthesis.

We did not notice any appreciable effect of insulin on cholesterol level in various tissues. Insulin even at a dose as high as 40 units/kg/day for 4 days, did not effect the plasma cholesterol level in this lizard [15]. Apparently exogeneous insulin has neither a direct nor indirect effect on cholesterol metabolism. It has been observed that insulin increased the phospholipid level in the liver with a concomitant decrease in plasma phospholipid. The most effective dose was found to be 10 units. This effect of insulin might be due to the involvement of phospholipid in the fat transport from liver to the blood and vice versa.

The increase in the concentration of unsaturated fatty acids with the concomitant decrease in their saturated counterparts in the adipose tissue of our lizards indicates that mono- and dienolic acids are synthesised from their saturated analogue after insulin administration. The accumulation of arachidic acid ($C_{20:0}$) in the treated animals and the decrease of its unsaturated analogue ($C_{20:0}$) however, reveals that the formation of the latter from its saturated analogue has been inhibited during insulin treatement. The actual mechanism through which these transformations are taking place instill unknown. It may also be possible that due to the different turn over rates, the unsaturated fatty acids are slowly lipolysed and released into the blood as compared to their saturated analogue. The increase in the level of arachidic acid after insulin treatment may also be due to its larger retaining capacity in the tissue. It can not be exactly pointed out whether the transformation of unsaturated fatty acid from the saturated analogue or the decrease rate of oxidation and their greater retaining capacity or both are responsible for maintaining the level of unsaturated fatty acids higher then the saturated acids.

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