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# The Effect of Insulin on the Blood and Liver Cholesterol in *Anabas Scandens* (BLOCH)

**PawlinVasanthi Joseph[1], Rajathi A.[2]**

[1]Department of Zoology, Nirmala College for Women, Redfields, Coimbatore-641018, Tamilnadu, India

[2]Department of Zoology, Holy Cross College, Tiruchirapalli-620002, Tamilnadu, India

**Abstract:** ***Fish requires lipids as a source of metabolic energy and to maintain the structure and integrity of cellular membranes.The reproductive tissues consume adequate amounts of cholesterol for the biosynthesis of various hormones needed for the process of reproduction. The information regarding the effect of insulin on the cholesterol level of the teleost fish is very limited. The present study aims at knowing the effect of insulin on the blood and liver cholesterol of the fresh water teleost Anabas scandens.Two series of experiments were carried out in the present study.In the first series of experiment, fishes were injected with 1,2,3 and 4 units of insulin and the blood and liver cholesterol was estimated after 30,60, 90 and 120 minutes. In the second series of experiment insulin was injected intermittently after 60 minutes. The result shows an increase in the liver cholesterol level and a decrease in the blood cholesterol.***

**Keywords:** cholesterol, insulin, liver cholesterol, blood cholesterol, intermittent injection

## 1. Introduction

The natural diet of most teleost fishes is a carnivorous one. Fish requires lipids as a source of metabolic energy and to maintain the structure and integrity of cellular membranes. The presence of large amount of oils in fish means that lipid rather than carbohydrates is the favoured energy reserve of most aquatic animals[1]. Fat plays an important role in maintaining the energy balance in the adaptation of animals to changing habitat conditions during the annual cycle[2]. The fat content of fish fluctuates considerably according to age, season, physiological conditions, food supply and other environmental conditions[3].

Cholesterol is a characteristic constituent of all living tissues, mainly associated with the unsaponifiable portion of the fat. The reproductive tissues consume adequate amounts of cholesterol for the biosynthesis of various hormones needed for the process of reproduction. Thereby it increases the demand on the liver. As a consequence during reproductively active phases, liver cholesterol remains elevated[4]. There are many reports on seasonal cyclic changes in serum and tissue cholesterol in migratory[5, 6, 7] and non-migratory species of catfish *Heteropneustesfossilis*. The elevated blood cholesterol during early post spawning is due mainly to cessation of vitellogenesis and conversion of cholesterol into gonadal steroids[8].

Insulin is the major hormone that promotes triglyceride synthesis. In the presence of adequate glucose, insulin greatly accelerates the deposition of fat in the body. But in the absence of glucose insulin has no effect on fat synthesis. Insulin provides a highly effective mechanism for controlling the normally reciprocal relationship between fat and carbohydrate metabolism[9]. It also has a dramatic effect on the incorporation of glycine into skeletal muscle proteins and to a lesser extent muscle lipid[10].

Literature regarding the effect of insulin on the carbohydrate and protein of fish is in plenty. But the information regarding the effect of insulin on the cholesterol level of the teleost fish is very limited. Considering this in mind the present study aims at knowing the effect of insulin on blood and liver cholesterol of the fresh water teleost *Anabas scandens*.

## 2. Materials and Methods

### 2.1 Collection and Maintenance

The animal used for this experimental study was the teleost fish *Anabas scandens*. They were collected from the river Cauvery at Tiruchirapalli. The diagnostic characteristics employed by Day[11] were helpful in the identification of the species. Fishes weighing between 8-10 gms were selected for experimentation. The fishes were acclimatized to the prevailing laboratory conditions.

### 2.2 Experimental Design

Two series of experiments were carried out in the present study. The first series of experiment included 16 groups of 4 fishes each. Each of the four groups was injected with 1 unit of insulin (Boots Company, India). The liver and blood cholesterol levels were estimated after 30, 60, 90 and 120 minutes of administration. The same procedure was repeated with 2, 3 and 4 units of insulin injection in the remaining 12 groups.

The second series of experiment consisted of 4 groups of 4 fishes each. 1 unit of insulin was administered intermittently at 60 minutes interval to a group of 4 fishes and were sacrificed for cholesterol estimation at the end of the experimental period. The same procedure was carried out with an intermittent injection of 2, 3 and 4 units of insulin to the remaining 3 groups of 4 fishes each. A group of 4 fishes served as control and received an injection of physiological saline.

### 2.3 Method

Estimation of cholesterol by Ferric chloride and sulphuric acid method

### 2.4 Statistical Analysis

The Arithmetic mean, standard deviation and standard error of the mean of different samples were calculated. 't' test was used to find the significance of difference between means obtained in this investigation. Simple regression analysis was used to plot the standard graph for cholesterol.

## 3. Results and Discussion

### 3.1 Blood Cholesterol

Table 1 gives an indication of the cholesterol level of the blood while injecting varied doses of insulin during the period of investigation. The normal cholesterol level was 452.5± 4.3 mg/100ml. injection of one unit of insulin had a depressing effect on the cholesterol after 30 minutes. However, decrease in cholesterol after 30 minutes was followed by an increase after 60, 90 and 120 minutes of injection.

The administration of 2, 3 and 4 units of insulin caused significant decreases after 30, 60 and 90 minutes. After 120 minutes there was an upward trend in the same parameter. There was a recovery trend in the cholesterol level after 120 minutes of injection of different units. An intermittent injection of different units of insulin at an interval of 60 minutes induced a highly significant decrease in the cholesterol level (Table 2).

### 3.2 Liver Cholesterol

The normal level of cholesterol in the liver was 33± 2.3 mg/gm. A statistically significant increase in the cholesterol level was noted after 30 and 60 minutes of injection of 1 unit of insulin (Table 3). But after 90 and 120 minutes of injection, the cholesterol level was lowered to a more or less normal value. There was a recovery after 90 and 120 minutes of injection since the difference between the normal and injected were insignificant. However the injection of 2, 3 and 4 units of insulin caused an increase in cholesterol level after 30, 60 and 90 minutes of injection. Subsequently there were decreases after 120 minutes indicating the possibility of recovery.

The administration of different doses of insulin with a subsequent interval of 60 minutes induced an increase in the cholesterol level. The decreases were significant for 1 and 4 units and insignificant for 2 and 3 units (Table 4). The results obtained in this study clearly reveal that injection of insulin increases the liver cholesterol level in *Anabas scandens*. The liver is able to synthesize all the cholesterol required from Acetyl CoA by multistage series of condensation reactions. The enzyme B-hydroxy-B-methyl glutaryl CoA reductase acts as important regulator of cholesterol biosynthesis. The administration of insulin is said to increase the enzyme B-hydroxy-B-methyl glutaryl CoA reductase[12]. Insulin is also said to increase the rate of regeneration of NADPH and acetate units which are the precursors of cholesterol synthesis.

Very low density lipoprotein is the major vehicle for transport of endogenously synthesized triacylglycerol. It has been suggested that the rate of triacylglycerol secretion by the liver might be an important determinant of hepatic cholesterol balance[13]. Insulin directly stimulates very low density lipoprotein triacylglycerol secretion[14]. The half-life of circulating insulin is about 7-15 minutes[12, 15]. Thus, any observable effect could be measured within half an hour of insulin administration[16].

The following reasons could be attributed to the decrease in the blood cholesterol noted in the present study. The blood cholesterol could be transported to the tissue[17]since insulin can potentiate the steroidogenic responses in Bovine adrenal cortical cells. Insulin might increase the number of high affinity low density lipoprotein receptors in the receptor cells. These receptors in their turn might increase the delivery of cholesterol from low density lipoprotein to the cells[8]. Decrease might also be due to increased biliary secretion since the absorption and secretion of cholesterol into the bile are under the control of different hormones including insulin Williams[18].

## 4. Conclusion

The present study reveals that insulin has a definite role in the maintenance of blood and liver cholesterol levels of the fish. But the mechanism by which this hormone influences the cholesterol level in fish needs further studies about the control mechanism. Studies on the separation of different lipids and lipoprotein component of blood and liver after insulin administration might throw light on the underlying mechanism.

**Table 1:** Effect of different dosage of insulin on blood cholesterol (mg/100ml) level at different time intervals in the fish *Anabas scandens*.

| *Insulin dosage* | *Control* | *Experimental* | | | |
|---|---|---|---|---|---|
| | | *Time in minutes* | | | |
| | | *30* | *60* | *90* | *120* |
| 1 unit | 452.5± 4.3 | 220±14.7 P<0.01 | 327±29.2 P<0.05 | 340±2.5 P<0.01 | 365±18.4 P<0.05 |
| 2 units | 452.5± 4.3 | 387.5±11.08 P<0.05 | 377.5±10.3 P<0.05 | 247.5±2.5 P<0.01 | 300±28.4 P<0.01 |
| 3 units | 452.5± 4.3 | 345±27.53 P<0.05 | 250±4.08 P<0.01 | 207.5± 11.08 P<0.01 | 280±7.5 P<0.01 |
| 4 units | 452.5± 4.3 | 207±5.7 P<0.01 | 190±13.22 P<0.01 | 147±5.7 P<0.01 | 183.3±2.8 P<0.01 |

The values are the mean ± S.E. of 4 observations.

**Table 2:** Effect of an intermittent injection of different dosages of insulin at an interval of 60 minutes on the blood cholesterol (mg/100ml) level of *Anabasscandens*

| Insulin dosage | Control | Experimental | % of increase |
|---|---|---|---|
| 1 unit | 452.5±4.3 | 240±0P<0.01 | 43.6 |
| 2 units | 452.5±4.3 | 160±0P<0.01 | 64.6 |
| 3 units | 452.5±4.3 | 145±5P<0.01 | 67.9 |
| 4 units | 452.5±4.3 | 212±11.54 P<0.01 | 52.9 |

The values are the mean ± S.E. of 4 observations.

**Table 3:** Effect of different dosage of insulin on liver cholesterol (mg/gm of tissue) level at different time intervals in the fish *Anabas scandens*

| *Insulin dosage* | *Control* | *Experimental* | | | |
|---|---|---|---|---|---|
| | | *Time in minutes* | | | |
| | | *30* | *60* | *90* | *120* |
| 1 unit | 33±2.3 | 44±1.2 P<0.05 | 54±4.9 P<0.01 | 34±1.3 P>0.05 | 32±1.1 P>0.05 |
| 2 units | 33±2.3 | 51.7±1.8 P<0.01 | 60±3 P<0.01 | 69.4±0.6 P<0.01 | 52.5±3.4 P<0.01 |
| 3 units | 33±2.3 | 53±4.1 P<0.01 | 56±2.7 P<0.01 | 63.6±4.7 P<0.01 | 62±2.1 P<0.01 |
| 4 units | 33±2.3 | 48±2.2 P<0.01 | 53±2.1 P<0.01 | 68 ±1.4 P<0.01 | 63±1.5 P<0.01 |

The values are the mean ± S.E. of 4 observations.

**Table 4:** Effect of an intermittent injection of different dosages of insulin at an interval of 60 minutes on the liver cholesterol (mg/gm) levelof*Anabasscandens*

| *Insulin dosage* | *Control* | *Experimental* | *% of increase* |
|---|---|---|---|
| 1 unit | 33±2.3 | 47±3.4 P<0.05 | 42.4 |
| 2 units | 33±2.3 | 37.5±2.0 P>0.05 | 13.6 |
| 3 units | 33±2.3 | 35±0.7 P>0.05 | 6.6 |
| 4 units | 33±2.3 | 53.2±0.6 P<0.01 | 61.2 |

The values are the mean ± S.E. of 4 observations.

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## Author Profile

**Dr Pawlin Vasanthi Josephis** an Associate Professor in theDepartment of Zoology, Nirmala College for women, Coimbatore. Her work is on Epidemiological and spatial statisticalapproaches in the study of Acute Gastroenteritis in Coimbatore district and has also worked in the field of Endocrinology. She has published seven papers in National and International journals.

**Dr Rajathi A** has presently retired as Associate Professor, Department of Zoology, Holy Cross College, Tiruchirapalli. She specializes in Endocrinology. She has guided M.Phil and Ph.D students and has published more than 30 papers in refereed journals.