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VARIATION IN LIPID COMPOSITION BY OVINE GROWTH HORMONE IN *ANABAS TESTUDINEUS* (BLOCH).

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SUMMARY

The hormonal control of lipid metabolism in heterotherms has received scant attention. The aim of this study was to evaluate the alterations in lipid components by ovine growth hormone (oGH) in a teleost *Anabas testudineus*. To study the role of oGH, three doses of oGH were given as intraperitoneal injections (0.1, 0.2, 0.5µg/g body wt/ day). Ovine GH at the higher doses significantly reduced serum cholesterol and LDL+VLDL, while increasing HDL. The concentrations of liver free fatty acids, triglycerides and cholesterol decreased and phospholipids increased in hormone treated specimens. Muscle triglycerides and cholesterol were increased after GH treatment. The results suggest the ability of oGH to break down neutral fat or oGH stimulates hepatic lipid mobilization in *A. testudineus*. Increased concentration of RNA suggests the ability of oGH to increase protein synthesis. Growth hormone appears to be antilipogenic and anabolic in *A. testudineus*.

Key words : *Anabas*; cholesterol; free fatty acids; growth hormone; phospholipids; RNA; triglycerides.

INTRODUCTION

Lipid metabolism in fish can no longer be considered simply a matter of dietary fatty acids. The interrelationship among dietary fatty acids, membrane integrity and metabolic pathways are particularly evident in poikilotherms as these are in constant flux in this group. The interdependence of environmental factors and lipid metabolism in fish is clearly reviewed(1). The hormonal regulation of lipid metabolism in mammals has been well studied. However, it has received scant attention in heterotherms. Virtually nothing is known about the control of lipid synthesis and deposition in member groups. As in the case of mammals, lipid metabolism in these groups is regulated by hormones, but the diversity of life history patterns complicate

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regulation of lipid storage. Growth Hormone (GH) from all zoological origins are active in fish. Exogenous GH can accelerate growth in fish as in the case of mammals. This is irrespective of whether mammalian, avian or porcine GH is administered and whether GH is given by regular intraperitoneal or intramuscular injections or by sustained release implants(2). The biological activity of vertebrate GH on fish is confirmed by radioreceptor assays and all the GH tested were capable of binding to the salmonid receptor (3,4). In salmonids, human GH, bovine GH, ovine and chicken GH have comparable activities (5,6). Growth hormone is known to exert a number of important regulatory functions in the control of metabolism, in addition to its well known effects on normal growth (7-11). Unfortunately, no unified picture has emerged on the role of GH on lipid turnover in fish. GH has varied conflicting effects upon lipid metabolism in fish and in lower vertebrates. Generally, the length of exposure and dosage of GH are complications which arises when assessing GH action. Long exposure to GH results in lipolytic effect, whereas short exposure periods usually result in lipogenic action (12). Since biological systems are very sensitive to the quantity of available chemical regulators, small changes in concentrations may have profound biological effects on physiology and behaviour. Growth hormone dosage in this experiment was selected on the basis of pilot experiments designed to determine the minimum dose of GH required to induce significant change on parameters studied. Fish system forms the best model for studies due to the presumed simplicity and phylogenetic position of the taxon (13). The aim of this study was to evaluate the role of ovine GH (OGH) on lipid turnover in a fresh water teleost, *Anabas testudineus*. To analyze the changes in the lipid reserves under altered hormonal conditions, various lipid classes in liver, muscle and serum were estimated.

MATERIALS AND METHODS

Experimental Animals.

Anabas testudineus collected from local suppliers were kept in large aerated storage tanks under natural photoperoid at least for one month. The fish were fed *ad libitum* with 40% protein feed. One week prior to experiment, male fish of body weight 30 ± 5 g were transferred to experimental aquaria, maintained in conditions identical to those of the stock tanks. Fish were starved for 24 hr before sampling in each experiment.

Experimental Procedures

The acclimated fish were divided into four groups. Fish of group one served as control and received 0.9% NaCl (saline). Other groups received, 0.1, 0.2 and 0.5 µg oGH per g body weight per day as intraperitoneal injections between 7.00 and 8.00h. Animals were sacrificed after 10 days and liver, muscle and serum were taken for lipid analysis. White muscle samples were standardized by taking all samples from the area below the dorsal fin. Data were collected from six animals in each group.

Total serum cholesterol (14) and LDL+VLDL (15) were estimated. For the analysis of lipids in the liver and muscle, total lipid was extracted from the tissue (16). Total cholesterol, triglycerides

(17), phospholipids (18) and free fatty acids (19) were determined. To analyze the changes in response to oGH, total protein (20) and RNA (21) concentrations were also determined.

STATISTICAL ANALYSIS

SPSS setup was used for statistical analysis. Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test (22). Difference was considered significant when $P < 0.05$.

RESULTS

Liver protein concentration reduced significantly in one group which received the minimum dose. Total protein in muscle decreased in one group (0.1µg) and increased in another group (0.5µg) (Fig. 1). Liver and muscle RNA showed significant increase except in the first group (0.1µg) in which there was a decrease (Fig. 2). The minimum dose of GH (0.1µg) treatment significantly elevated serum cholesterol, LDL + VLDL and HDL cholesterol. GH at 0.2 µg and 0.5 µg doses reduced serum cholesterol and LDL+VLDL in gneral except HDL which increased in fish treated with 0.5 µg dose of GH (Table. 1). Concentrations of liver FFA showed significant reduction in all the treated groups and muscle FFA was not altered. Liver TG decreased in one group (0.2µg) and was not altered in other groups while muscle TG exhibits increased concentrations in one group (0.2 µg) and reduction in the third group (0.5µg) (Table

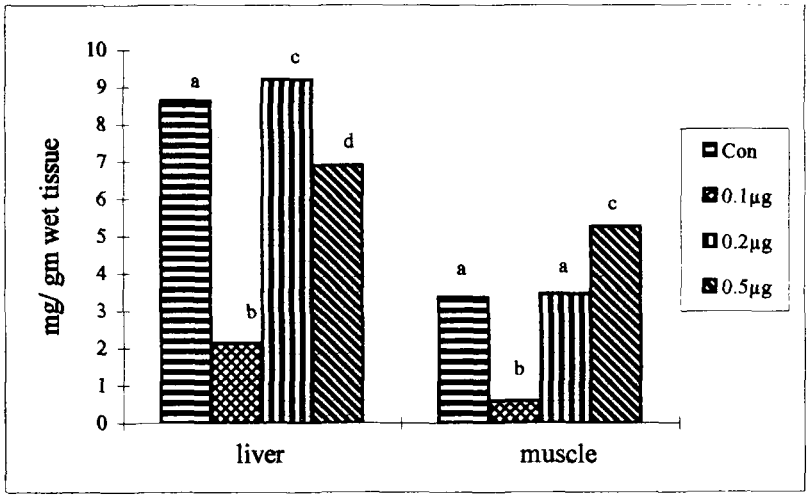

Figure. 1 : Effect of oGH on total protein in liver and muscle of *A. testudineus*. Groups with superscript are signiicantly different (p<0.05) from the control

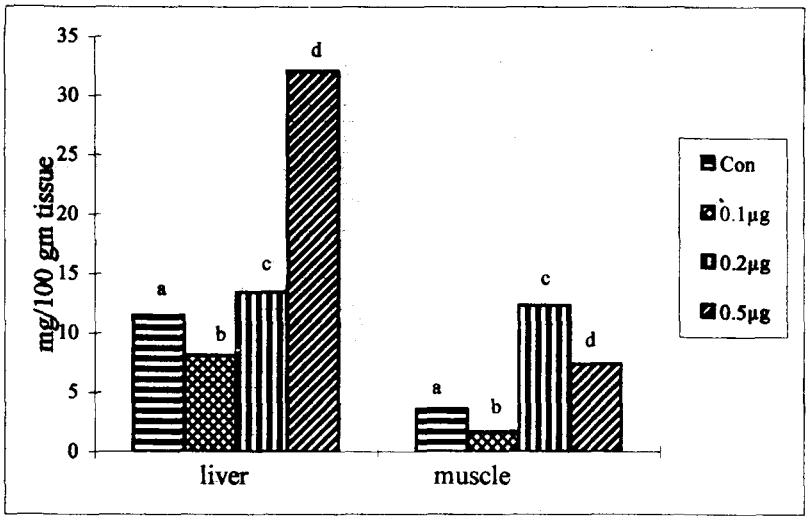

Figure 2 : Effect of oGH on RNA concentration in liver and muscle of *A. testudineus*. Groups with superscripts are signiicantly different (p<0.05)

Table 2. Effect of ovine GH on the concentrations of free fatty acids, triglycerides, cholesterol and phospholipids in *A. testudineus*.

Parameter	Control	Doses of oGH (µg/g body weight)		
		0.1 µg/g	0.2 µg/g	0.5 µg/g
Free fatty acids	$_1$ 430.23 ± 14.2	296.53 ± 6.21[a]	393.6 ± 20.5[a]	316.30 ± 42[a]
	$_2$ 156.84 ± 12.84	149.94 ± 6.21[a]	172.42 ± 17.37[a]	144.88 ± 9.05[a]
Tryglycerides	$_1$ 23.93 ± 3.45	22.91 ± 4.38[a]	14.99 ± 1.49[a]	23.04 ± 1.70[a]
	$_2$ 10.17 ± 1.78	11.87 ± 1.61[a]	15.87 ± 1.70[a]	7.49 ± 0.58[a]
Cholesterol	$_1$ 548.47 ± 7.73	438.8 ± 46.79[a]	585.81 ± 68.60[a]	376.89 ± 17.15[a]
	$_2$ 77.15 ± 5.47	131.18 ± 13.04[a]	75.27 ± 7.28[a]	94.75 ± 3.66[a]
Phospholipids	$_1$ 133.15 ± 4.94	153.54 ± 8.48[a]	249.21 ± 15.08[a]	138.81 ± 4.5[a]
	$_2$ 58.69 ± 2.23	66.74 ± 5.15[a]	61.5 ± 4.18[a]	60.47 ± 2.97[a]

Concentrations are expressed as mg/ 100g tissue. Results are expressed as mean ± SD of 6 animals (n=6). The significant difference between the groups was analyzed by one way analysis of variance, mean values of groups with superscript letters in a given row are significantly different (P<0.05) as determined by Duncan's multiple range test. 1. Liver 2. Muscle.

2). Cholesterol concentration in liver significantly decreased in treated groups, except for the group which received the medium dose but in the muscle,it was elevated in two groups

Table 1. Effect of ovine GH on serum cholesterol concentrations.

Parameters	Control	Doses of oGH (µg/g body weight)		
		0.1 µg	0.2 µg	0.5 µg
Total Cholesterol	426.9 ± 44.4	565.02 ± 31.66[a]	357.5 ± 22.31[a]	307.6 ± 18.54[a]
HDL	39.12 ± 6.85	104.58 ± 11.88[a]	36.27 ± 7.44[a]	52.21 ± 8.4[a]
LDL+ VLDL	387.78 ± 40.0	460.11 ± 36.0[a]	321.4 ± 21.55[a]	255.4 ± 12.93[a]

Concentrations are expressed as mg/100ml serum. Results are expressed as mean ± SD of 6 animals (n=6). The significant difference between the groups was analyzed by one way analysis of variance, mean values of groups with superscript letters in a given row are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

(0.1µg & 0.5 µg) (Table 2). Phospholipid concentration increased significantly in the liver of fish treated with 0.2µg and 0.5 µg oGH and in muscle of fish which received 0.1 µg GH (Table 2).

DISCUSSION

Growth hormone at the dose of 0.1 µg reduces RNA concentrations suggesting decreased rate of protein synthesis in response to the low dose of it. Though RNA concentration was decreased, protein concentration in the liver increased. This may be due to the break down of muscle protein to amino acids for further metabolic need in response to GH, as evinced by the low concentration of muscle protein in the GH treated group. Protein catabolism in muscle cells also provides amino acids for liver gluconeogenesis and thereby can contribute indirectly to blood glucose (23). The significant increase in RNA concentration shows the ability of GH to increase protein synthesis in 0.2 and 0.5 µg treated groups. The reduction in liver protein concentration in these groups may be due to enhanced amino acid metabolism and hence, the availability of amino acids for enzyme synthesis. Ovine GH significantly increased wholebody growth rate, wholebody protein accretion rates, stimulated tissue protein synthesis and tissue protein accretion rates in rainbow trout (7). Our experiments also reveal an anabolic effect of GH in *A. testudineus*, this effect may be due to GH enhancing amino acid transport and hence the availability of amino acid for protein synthesis.

The significant increase in serum cholesterol, LDL +VLDL and HDL cholesterol in the GH treated group (0.1 µg) may be due to the rapid mobilization of lipids in response to the lowest dose of oGH. This apparent lipogenic effect may be due to GH-induced inhibition of lipoprotein lipase activity, which would impair VLDL clearance. LDL receptors on the surface of cells are carefully regulated by metabolic state of the cell (24). Experiments with oGH indicate that there exists a short-loop negative feed back on GH secretion at the pituitary level in rainbow trout (25). The groups which received 0.2 and 0.5 µg oGH exhibit reduced serum cholesterol, LDL +VLDL and HDL in one group (0.5 µg). GH appears to have an inhibitory effect on cholesterol and lipoprotein (LDL) but stimulatory effect on HDL concentration, in the present study. Clinical observations support the

proposal that GH may have a protective role in prevention of hyperlipidemia (9, 30). Increase in HDL suggests extrahepatic mobilization of lipids. Lowering of LDL + VLDL cholesterol may be due to increase in the catabolism of apolipoprotein B-LDL by oGH. Ovine GH (0.5µg) may cause an increase in synthesis of apolipoprotein A-1 resulting in higher HDL cholesterol. Our findings also support the HDL increasing and LDL lowering action of GH.

Reduction in the concentrations of free fatty acids and triglycerides in one group (0.2 µg) may be due to enhanced degradation or reduction in the biosynthesis of fat. Triglyceride concentration in muscle increased significantly in two groups (0.1 and 0.2 µg). GH at the dose of 0.2 µg may enhance liver TG degradation to increase TG concentration in muscle. GH (0.5 µg) treatment may result in an increased usage of free fatty acids for metabolic fuel and increased amino acid availability for protein synthesis as evident from the low concentration of TG and high concentration of protein in muscle. This dose dependent lipid mobilization may be related to the number and kinds of hormone receptors present in the tissues. Liver cholesterol concentration was decreased by GH (0.1 & 0.5 µg) while increasing it in muscle. The increased concentrations of muscle cholesterol may be due to mobilization of lipids from mesenteric fats. The muscle of fish which received 0.1 µg GH exhibits significant increase in triglycerides, cholesterol and phospholipids. This may be due to the mobilization of lipid in response to the lowest dose of GH treatment as evinced by high concentrations of circulating lipoproteins. In fish, lipids are partitioned among mesenteric fat, liver and dark muscle (26). Adipose tissues respond quickly to metabolic and hormonal stimuli and takes part in an active interplay with the liver, skeletal muscles and the heart. Liver phospholipid concentrations are significantly increased in two groups (0.1 & 0.2 µg). The fatty acids released in response to GH may be directed towards phospholipid synthesis resulting in increased concentrations. Phospholipids are important constituents of membranes and serve to function both cell activity and cell permeability. Phospholipids form important intermediate substances in the transport of lipids from and to the liver and phospholipid formation in the liver seems to be an essential prerequisite for the rapid oxidation of fatty acids (27). Increase in the concentration of phospholipids may suggest the action of GH in regulating the activity of membrane integrity including receptors, transmembrane transport proteins and enzymes and its role on fatty acid oxidation.

GH directly stimulates hepatic lipid mobilization in rainbow trout by enhancing triacyl glycerol lipase activity (28). Direct stimulation of lipolysis by GH has been observed *in vitro* with adipose tissue from some species, for example rats and chickens. GH reduces the activity of both acetyl-Co A carboxylase and glucose-6-phosphate dehydrogenase, with also has some tendency for depressed fatty acid synthase, 6-phosphogluconate dehydrogenase and isocitrate dehydrogenase. The antilipogenic effect is one of the direct physiological actions of GH in view of its ability to reduce adiposity in pigs, sheeps and cattle (29). Recent report from the author's laboratory shows that oGH has antilipogenic effect by having inhibitory effect on lipogenic enzymes, such as malic enzyme, glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase *in vivo* and *in vitro* in *A. testudineus* (30), suggesting antilipogenic action of oGH in *Anabas*.

Interpretation of GH effects on lipid metabolism are complicated by length of exposure to GH, seasonal variation, diurnal rhythms and interaction with other hormones (31). Triiodothyronine (T_3) has a stimulatory effect on GH-receptor/GH binding protein (GHR/GHBP) gene transcription which is indirect and additive to the GH- induced changes (32). Mammalian GH has been shown to enhance TG lipase activity in Salmon parr (12). In our experiment, the overall concentrations of lipid components are decreased by hormone treatment. The findings of our study suggest antilipogenic and /or lipolytic effects of GH in *A. testudineus* and the effect on various lipid components depends on the dosage of hormone administered. Normal plasma GH level in female Silver eel is 5 ng/ml (33). It has been known that hormone doses higher than the circulating levels are necessary for eliciting the hormone mediated responses in fishes (34). Therefore, the present stimulatory effect observed after three concentrations of GH appears to be physiological. Although it is clear that GH has important effects on the regulation of hepatic lipoprotein metabolism, many questions however, remains to be answered. Future studies are needed to examine, in more detail, the mechanism of GH action, and its potential in the treatment of lipoprotein abnormalities.

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