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Changes in plasma glucagon and insulin associated with fasting in sea bass (*Dicentrarchus labrax*)

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Abstract

Juvenile sea bass (*Dicentrarchus labrax*) were fasted for 22 days and changes in plasma insulin, glucagon and glucose levels, as well as glycogen and protein content in liver and muscle were analyzed. Glucagon increased initially on the 4th day of fasting. The glucagon/insulin ratio (G/I) increased from a value of 0.11 ± 0.02 (24h of fasting) to 0.21 ± 0.05 (4th day of fasting). Thereafter, both glucagon and insulin levels decreased and remained at low concentrations until the 22nd day of fasting. Plasma glucose levels fell at the beginning of fasting, stabilized between the 4th and 8th day, and gradually declined during the rest of the experiment. There was a body weight loss of 15% and a significant decrease in both the hepatosomatic index and mesenteric fat. The decrease in the percentage of muscle proteins was not significant, while liver glycogen content showed a sharp decline.

Introduction

Many species of teleosts are well adapted to survival during long periods of fasting. However, endocrine control of fish metabolism under these conditions of food deprivation is not well understood at present. In mammals, the maintenance of a continuous supply of blood glucose is very important and glucose homeostasis is mainly controlled by pancreatic hormones. There is a two-fold rise in plasma glucagon concentrations after three days of fasting in man, followed by a gradual decline towards postabsortive levels in the ensuing weeks (Marliss *et al.* 1970; Fisher *et al.* 1976). Insulin levels decline or remain unchanged on the first days of fasting in humans or rats respectively (Cahill *et al.* 1966; Seitz *et al.* 1977). Thus, the altered relationship of glucagon and insulin concentrations at the beginning of fasting activate the glycogenolysis and gluconeogenesis pathways, mainly in the liver, in order to maintain glucose homeostasis.

Little is known about circulating levels of pancreatic hormones during fasting in fish and most of the experiments have been carried out in ciprinids and salmonids. Plasma insulin levels decreased during fasting in goldfish (Patent and Foa 1971), trout (Thorpe and Ince 1976), and coho salmon (Plisetskaya *et al.* 1986). Recently, Moon and co-authors (1989) observed that a six-week fast in rainbow trout produced a decrease in plasma insulin and glucagon family peptides levels. The relative hormone decline results in an increase in the glucagon/insulin and especially GLP/insulin molar ratios, which seems to be related to an activation of gluconeogenesis in the liver. Although results of many studies on the control of storage and mobili-

zation of body energy reserves in fish have been reported (see Love 1980), these are often contradictory and complicated by inter- and intra-species variability.

Sea bass (*Dicentrarchus labrax*) is a very active species with high levels of several plasma metabolites, especially glucose, the average value of which is approximately 150 mg/100 ml during the annual cycle (Gutiérrez *et al.* 1987). The aim of this study was to investigate the changes in plasma insulin and glucagon levels in sea bass during a 22-day period of fasting and the relationship between these patterns and energy reserves in liver and muscle.

Material and methods

Animals and experimental procedure

Juvenile sea bass weighing 40–60 g, all belonging to the same brood, were kept indoors in tanks supplied with a constant flow of aerated sea water. The animals were maintained under natural conditions of light and temperature (22°–24°C) during the months of June and July.

Groups of 8–10 fish (randomly distributed) were sampled after of 2, 4, 8, 15, and 22 days of fasting. Control animals were fed *ad libitum* with natural food (chopped filleted fish, *Boops boops*) and were sampled at 1, 15, and 22 days of experiment. These fed groups were fasted for 24h before sampling. Blood was taken from the caudal vein into heparinized syringes. Following centrifugation, aliquots of plasma were frozen and stored at −30°C until assay. Liver and white muscle samples were quickly removed and frozen in liquid N_2, from control groups at 1, 15 and 22 days and from fish fasted for 15 and 22 days.

Analytical methods

Plasma insulin levels were measured by radioimmunoassay using bonito insulin as standard and a rabbit anti-bonito insulin as antiserum (Gutiérrez *et al.* 1984). Glucagon levels were determined by an adaptation of mammalian radioimmunoassay to fish (Gutiérrez *et al.* 1986). A protease inhibitor, Trasylol, Bayer (final concentration: 0.13 mg/ml), was added to the aliquots of plasma for glucagon analysis before freezing.

Plasma glucose levels were analyzed by the glucose-oxidase method (Hugget and Nixon 1957). Tissue glycogen and protein concentrations were measured by the anthrone reaction (Fraga *et al.* 1956) and Lowry method (Lowry *et al.* 1951), respectively. DNA and RNA content were analyzed by a spectrofluorimetric method (Buckley and Bullow 1987).

Results are given as mean ± SEM and compared using the Duncan test.

Results

Fasting provoked clear changes in body parameters in sea bass. A 15% decrease in body weight was observed at the end of the experiment. At 15 and 22 days of fasting a significant decrease in the hepatosomatic index and mesenteric fat was found (Table 1).

In control fish, plasma insulin and glucagon levels remained constant throughout the experiment (average values were 10.7 ± 0.25 ng/ml and 681.9 ± 166.8 pg/ml, respectively). Fasting caused a significant decrease in plasma insulin at 15 and 22 days (Fig. 1). A sharp increase in glucagon plasma levels was observed on the 4th day of fasting (1311 ± 309 pg/ml). Glucagon concentrations declined gradually from the 8th day of food deprivation to below control levels and reached a minimum value of 305 ± 54 pg/ml at 22 days (Fig. 2).

The changes in the glucagon/insulin (G/I) molar ratio are shown in Fig. 3. A substantial increase in the G/I ratio was found on the 4th day of fasting, mostly due to the elevated levels of plasma glucagon.

Plasma glucose values in control fish remained constant. In fasted fish, glucose fell on the 2nd day and was significantly lower on the 4th day. A relative stabilization took place between the 4th and 8th day. Thereafter, glucose levels decreased progressively and reached a minimum value of 60.6 ± 6.4 mg/100 ml at 22 days (Fig. 4).

Table 1. Differences in body parameters and tissue composition of fed and fasted sea bass

Days	Fed			Fasted	
	0	15	22	15	22
HSI (%)	1.20 ± 0.12^{c}	1.04 ± 0.06^{b}	1.08 ± 0.05^{b}	0.79 ± 0.05^{a}	0.81 ± 0.04^{a}
MF (%)	3.27 ± 0.15^{b}	3.61 ± 0.14^{b}	3.24 ± 0.18^{b}	2.12 ± 0.20^{a}	2.02 ± 0.45^{a}
Δ weight (%)		+ 8.72 ± 1.55	+ 11.31 ± 3.05	− 9.71 ± 0.74	− 15.26 ± 1.61
Liver					
Water (%)	64.82 ± 0.93^{b}	65.07 ± 0.50^{b}	65.40 ± 1.03^{b}	65.65 ± 1.57^{b}	65.43 ± 1.11^{b}
Glycogen (%)	3.83 ± 0.78^{b}	1.84 ± 0.79^{b}	2.71 ± 0.75^{b}	0.10 ± 0.05^{a}	0.04 ± 0.02^{a}
Proteins (%)	11.38 ± 0.39^{b}	11.03 ± 0.70^{b}	11.53 ± 0.37^{b}	12.88 ± 0.74^{b}	12.87 ± 0.62^{b}
DNA (mg/100 g)	242.33 ± 34.22^{b}	224.60 ± 50.84^{b}	235.61 ± 42.16^{b}	295.63 ± 75.48^{b}	341.08 ± 73.50^{b}
RNA (mg/100 g)	1221.04 ± 91.00^{b}	1179.03 ± 101.23^{b}	1358.30 ± 76.04^{b}	1266.42 ± 121.12^{b}	1350.11 ± 128.28^{b}
RNA/DNA	4.24 ± 9.46^{b}	5.68 ± 0.71^{b}	5.46 ± 0.74^{b}	4.17 ± 0.61^{b}	4.00 ± 0.75^{b}
Muscle					
Water (%)	77.04 ± 0.57^{a}	76.57 ± 0.63^{a}	76.78 ± 0.48^{a}	78.54 ± 0.22^{a}	79.02 ± 0.57^{b}
Proteins (%)	14.75 ± 0.95^{b}	14.28 ± 0.87^{b}	14.35 ± 0.78^{b}	12.43 ± 0.98^{b}	12.60 ± 1.13^{b}
DNA (mg/100 g)	50.26 ± 7.01^{b}	72.18 ± 6.08^{b}	66.07 ± 5.03^{b}	88.68 ± 6.29^{b}	74.98 ± 6.70^{b}
RNA (mg/100 g)	167.36 ± 6.08^{b}	191.44 ± 21.45^{b}	180.11 ± 16.62^{b}	152.75 ± 20.92^{b}	114.67 ± 6.74^{a}
RNA/DNA	3.67 ± 0.46^{b}	3.01 ± 0.32^{b}	2.81 ± 0.30^{b}	1.72 ± 0.18^{a}	1.57 ± 0.11^{a}

HSI = Hepatosomatic index (% body weight); MF = Mesenteric fat (% body weight). Results are expressed as mean ± SEM (n = 8–10). Within a row, values followed by a different letter superscript are significantly different ($P < 0.05$).

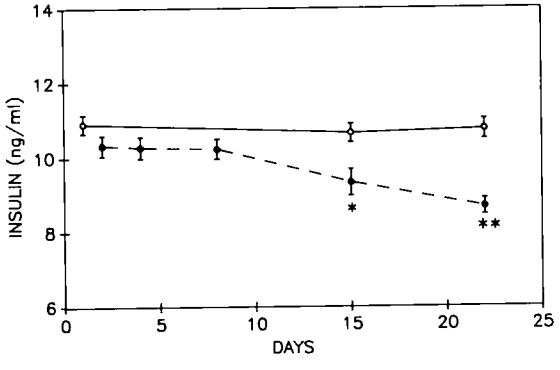

Fig. 1. Plasma insulin levels in control (——) and fasted fish (– – –). Values shown are means ± SEM for 8–10 animals in each group and compared using the Duncan test, ** = $P < 0.01$, * = $P < 0.05$.

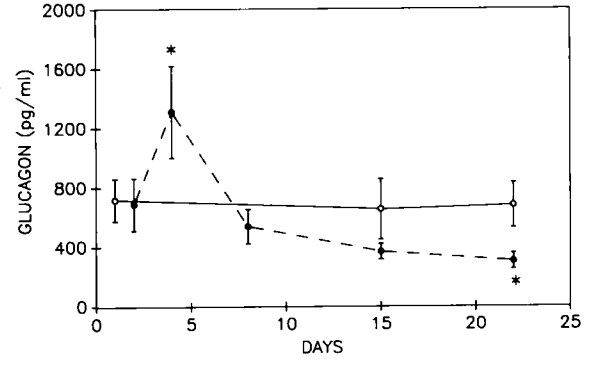

Fig. 2. Plasma glucagon levels in control (——) and fasted fish (– – –). Values and statistical significance are given as in Fig. 1.

The differences between fasted and fed animals in the tissue energy reserves are shown in Table 1. The main effect of fasting was a significant decrease in liver glycogen (from 3.8% to 0.1% at 15 days), and a fall in the percentage of mesenteric fat, while liver protein content and the RNA/DNA ratio did not change significantly (Table 1). There were no significant changes in muscle protein content, but there was a clear decrease in the RNA content and RNA/DNA ratio.

Discussion

Plasma insulin concentrations found in control sea bass coincided with those described previously in

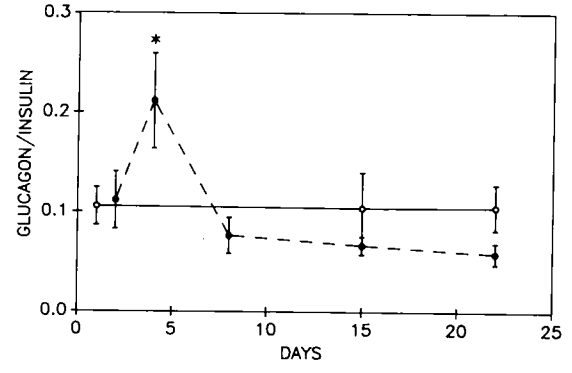

Fig. 3. Plasma glucagon: insulin molar ratio in control (——) and fasted fish (– – –). Values and statistical significance are given as in Fig. 1.

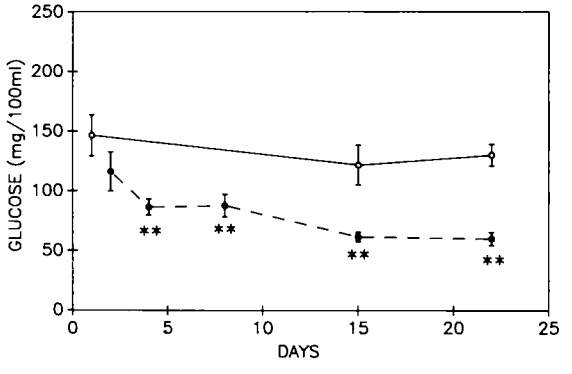

Fig. 4. Plasma glucose levels in control (——) and fasted fish (– – –). Values and statistical significance are given as in Fig. 1.

this species (Gutiérrez *et al.* 1984; Pérez *et al.* 1988a, 1988b). Fasting produces a decrease in circulating insulin in mammals (Ruderman 1975; Gelfand and Sherwin 1983) and in teleosts. Serum insulin values of fed goldfish were nearly double those of fish fasted for 5 days (Patent and Foa 1971). Some authors have reported a two to four-fold decrease in plasma insulin levels in trout and in coho salmon after 1–2 weeks of fasting (Thorpe and Ince 1976; Plisestkaya *et al.* 1986), and a six-fold decrease in rainbow trout deprived of food for 6 weeks (Moon *et al.* 1989). In all of the above studies there was a greater fall in insulin levels than the decrease found in this study. This difference could possibly be due to the physiological stage of the fish. Summer is a growing period in this species, when high plasma insulin levels are found (Gutiérrez *et al.* 1987). This fact could explain the reason for the delay in a decrease in insulin.

The sharp increase in plasma glucagon levels observed on the 4th day of fasting in sea bass has not yet been described in fish. In mammals, glucagon concentration rises at the beginning of fasting and reaches a maximum on the 3rd day followed by a decline (Marliss *et al.* 1970). It would be interesting to speculate that the response in this metabolically active species could be an analogy to the mammalian hormonal response. However, the G/I ratio found in mammals reaches values of 1 or 2 (Unger and Lefebvre 1972; Plisestkaya 1990), while in sea bass it was 0.21, similar to that reported in rainbow trout fasted for 2–4 days (Plisestkaya *et al.* 1987). In rainbow trout, this increase was mostly due to a lowering of insulin levels and not to an elevation of plasma glucagon. The highest G/I ratio observed in this experiment coincided with a stabilization of glucose levels between 4th and 8th days, followed by a gradual decrease in both parameters.

Glucagon and GLP levels have both been reported to decline in salmon and trout following a fast (Sheridan and Plisestkaya 1988; Moon *et al.* 1989). The temporal pattern reported in the present study indicates both a short-term and a long-term response. The short-term response (increase) is similar to mammals, while the long-term response (decrease) is similar to trout and salmon.

In a recent experiment on *Salmo trutta*, (unpublished data) we found a slight increase of glucagon at 3–5 days of fasting. In carp (*Cyprinus carpio*) deprived of food for 50 days different responses of glucagon were observed which depended on the age and physiological state of the animals (Gutiérrez *et al.* 1989). Therefore, the response of glucagon at the beginning of fasting seems to be at least partially dependent on species and condition.

As occurred in the present experiment, fasting causes a loss of weight due to the mobilization and utilization of reserves. Part of this weight loss could be due to the mesenteric fat, which significantly decreased at 15 and 22 days. It has been demonstrated that insulin inhibits lipid mobilization in fish (Minick and Chavin 1972; Ince and Thorpe 1974; Pérez *et al.* 1989), so the fall in insulin levels could favour a decrease in mesenteric fat. The drastic decrease in liver glycogen is in agreement with

previous data on fasted sea bass (Stirling 1976) as well as in other teleostean species (Love 1980). This decrease in glycogen could be due to the initial glycogenolysis stimulated by the rise in the G/I ratio. In fact, the glycogenolytic action of glucagon has been demonstrated in *in vivo* and *in vitro* studies of salmonids (Plisestkaya *et al.* 1989) and catfish (Ottolenghi *et al.* 1988).

In higher vertebrates, glucose homeostasis during the starvation period is achieved by the activation of glycogenolytic and also gluconeogenic pathways. In our case, the decline in the RNA/DNA ratio of white muscle suggests a decrease in protein synthesis. Furthermore, although there was no significant change in muscle protein content, since muscle represents 60–70% of fish body weight, small muscle changes may still provide an important supply of amino acids. The supply of gluconeogenic substrates from the muscle to the liver could be related to an enhancement of gluconeogenesis. An increase in gluconeogenic enzymes has been previously found in fasted sea bass (Zammit and Newsholme 1979), plaice (Moon and Johnston 1979), and salmonids (Moon 1988) and glucagon and glucagon-like peptides have an important role in the activation of the gluconeogenic pathway (Moon 1988; Moon *et al.* 1989). Thus, the changes in pancreatic hormone concentrations in fasted sea bass could explain liver glycogenolysis pathway in an attempt to maintain normoglycaemia.

In conclusion, sea bass fasted for 22 days showed a mobilization of liver glycogen, mesenteric fat and muscle proteins. This mobilization was associated with an initial increase in plasma glucagon and a decline in insulin levels.

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