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Plasma insulin, glucagon, glucagon-like peptide and glucose levels in response to feeding, starvation and life long restricted feed ration in salmonids

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Abstract

Plasma insulin, glucagon, glucagon-like peptide (GLP) and glucose were measured in samples taken from rainbow trout, *Oncorhynchus mykiss*, (1.5 years of age) before feeding and at selected times up to 9 days after feeding. The feed contained 21.7% carbohydrate (65% digestible) in the dry matter. The fish responded to feeding with an elevated plasma insulin level ($p < 0.005$) 0.5 h post-feeding, which may account for the unchanged plasma glucose levels. Twentyfour hours after feeding, plasma insulin level had returned to pre-feeding levels, while 4–9 days after feeding, a significant reduction compared to pre-feeding levels was observed ($p < 0.001$). During this period plasma glucose levels remained unchanged. The corresponding plasma glucagon or GLP levels showed no significant elevation in response to starvation, the plasma GLP concentration was even significantly reduced on days 4–9 post-feeding ($p < 0.01-0.001$).

Atlantic salmon, *Salmo salar*, (3.5 years of age) fed a calculated satiation ration (RL = 100) throughout their lifetime had, in addition to a higher body weight, significantly higher plasma insulin ($p < 0.005$) glucagon ($p < 0.0001$) and GLP levels ($p < 0.0001$) than fish fed half the satiation ration. The plasma glucose levels were, however, not significant different between the groups.

Introduction

The rates of removal of administered glucose from blood in fish (Tashima and Cahill 1968; Epple 1969; Moen 1975; Yone 1979) is much slower than in mammals. This has been attributed to an insufficient insulin increment in response to glucose loading in fish (Furuichi and Yone 1981). In healthy humans, plasma glucose and insulin levels increase to a maximum within 0.5–1.5 h following a meal. Three hours after the meal both plasma glucose and insulin have reached pre-feeding levels (Vaaler 1986). Both the time and degree of response will vary with the type of food consumed. In carp, (*Cyprinus carpio*), red sea bream (*Chrysophrys major*) and yellowtail (*Seriola quinqueradiata*) elevated plasma insulin levels were measured 2 h after an oral glucose administration (Yone 1979). In rainbow trout, *Oncorhynchus mykiss*, a high carbohydrate or a high protein meal resulted in a elevated plasma insulin level 3 h post-feeding (Hilton *et al.* 1987). Starvation for 1–2 weeks in cod, *Gadus morhua* (Thorpe and Ince 1976) and in rainbow trout (Plisetskaya *et al.* 1986a) resulted in a significantly reduced plasma insulin level. Reduced plasma insulin levels in rainbow trout was also reported after 6 weeks of starvation (Moon *et al.* 1989).

Glucagon counteracts insulin action, both in mammals and fish, elevation of hepatic glucagon level activates glycogenolysis and gluconeogenesis (Suarez and Mommsen 1987; Mommsen and Moon 1989). In man and geese, elevated plasma glucagon is evident after 48 h of starvation (Aguiler-Parada *et al.* 1969; Felig 1979; Sitbon and Miahle 1979; Aoki 1981; Cherel *et al.* 1988). However, in rainbow trout after 6 weeks of starvation, Moon *et al.* (1989) observed a depression in plasma glucagon and glucagon-like peptid (GLP) levels, although less pronounced than the concomitant reduction in plasma insulin.

No direct metabolic role is demonstrated for GLP in mammals (Ørskov and Holst 1987), but this hormone may act in teleosts on the same metabolic processes as glucagon, and according to Mommsen *et al.* (1987) and Mommsen and Moon (1989) may be even more important than glucagon in controlling hepatic gluconeogenesis.

In the present investigation, plasma insulin, glucagon, GLP and glucose levels in rainbow trout from 0.5 h after feeding to 9 days without further feeding were studied.

Fish exposed to lifelong restricted feeding should be in a situation of prolonged hunger. Groups of 3.5 year-old Atlantic salmon, *Salmo salar*, following lifelong exposure to optimal and restricted feeding were included in order to study the effects of different feed ration levels upon plasma insulin, glucagon, GLP and glucose levels.

Materials and methods

The experiments on 1.5 year old rainbow trout (body weight 100 g) were performed indoors at Oslomarkas Fiskeadministrasjon (OFA), Trout Enhancement Station Oslo, Norway on September 12 1986 (50 fish) and repeated on June 1–10 1987 (106 fish). The water temperatures varied between 7.5–9.0°C during the experimental periods.

Only plasma insulin and glucose concentrations were measured in the one day experiment in September, while in addition plasma glucagon and GLP concentrations were measured in the June experiment. As all the experimental conditions were identical and the plasma insulin and glucose values were not significantly different during the two experimental periods, the data were combined.

Prior to the experiment, the rainbow trout were fed a commercial diet; Tess Elite (manufactured by T. Skretting A/S, Norway) twice a day at 09.00 h and 16.00 h to satiation. The feed contained 50% crude protein (85% digestible), 19.4% fat (87% digestible), 21.7% carbohydrate (65% digestible) and 8.9% ash in the dry matter. Prior to blood sampling, groups of 10–12 trout were anesthetized in 0.03% chlorbutol at the following points in time related to feeding; 1 hour before morning feeding (16 h after evening feeding) then 0.5 h, 2, 4, 7 h after feeding. When the experiments was repeated in June, blood was also drawn 1, 4, 7 and 9 days after the feeding.

Observations during feeding, and examination of the stomach and intestinal contents at sampling the first hours after feeding, indicated that all the fish examined had eaten.

The Atlantic salmon, were raised in fresh water at AKVAFORSK, Sunndalsøra, until smoltification and then transferred to seawater at AKVAFORSK, Averøy.

During the period in seawater and until the termination of the experiment the Atlantic salmon were fed a diet; Tess Edel® (manufactured by T. Skretting, A/S Norway) which contained 49.2% crude protein (88% digestible), 24,8% fat (87% digestible), 16.0% carbohydrate (65% digestible) and 9.9% ash in the dry matter. The food was delivered by automatic feeding every 15 min during the light period. One group was fed a calculated satiation ration level, (RL = 100) according to an estimate of expected growthrate based on temperature and bodyweight (Storebakken and Austreng 1987; Austreng *et al.* 1987). The ration was 0.85% of the bodyweight the last 24 h before sampling. A second and a third group were given restricted rations amounting to 75% (RL = 75) and 50% (RL = 50) of the satiation ration. At 3.5 years of age (June 1987) blood was drawn from 5 fish in each group. The fish were anesthetized in 0.03% chlorbutol and weighed before blood was collected from the caudal vein into heparinized vacutainers, containing a trypsin inhibitor (0.1 mg/ml, Aprotinin, Novo).

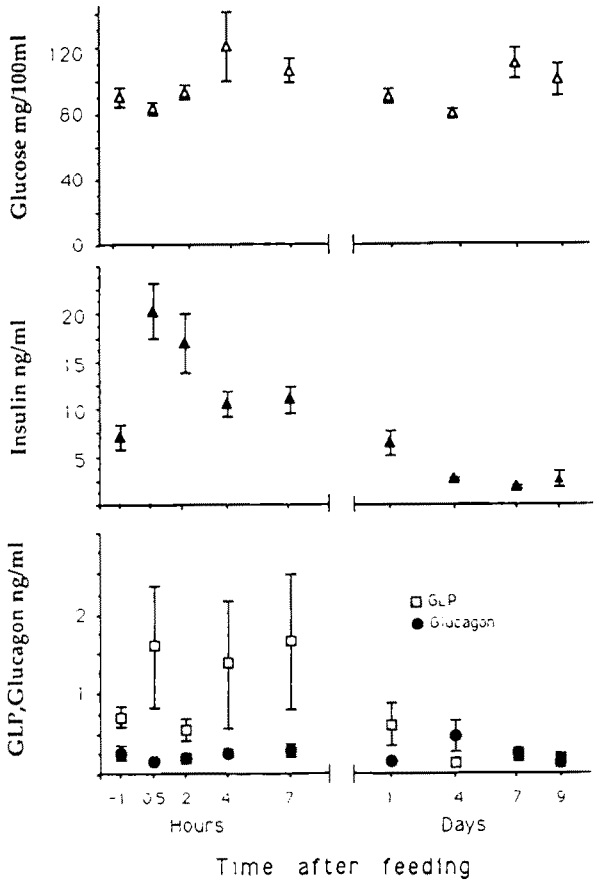

Fig. 1. Plasma level of glucose, insulin, glucagon and GLP before feeding and at various times afterwards in 1.5 year old (100 g) rainbow trout. Values are mean ± SEM (n = 12–21).

Plasma was immediately frozen and stored at −20°C until assayed. Insulin, glucagon and GLP were measured by homologous salmonid RIAs based on coho salmon purified pancreatic hormones and rabbit antisalmon pancreatic hormone serum as described by Plisetskaya *et al.* (1985, 1986a, 1989) and Sundby *et al.* (1991). Plasma glucose concentration was measured by a hexokinase method on a Technicon RA 1000 autoanalyser.

Statistical evaluation was preformed on log values from individual fish. The log values fulfilled the requirement of normal distribution. ANOVA and Bonferroni tests were used to evaluate the variance (Jennrich and Sampson 1983).

Results

Response to feeding and starvation in rainbow trout

Plasma concentrations of glucose, insulin, glucagon and GLP in the 1.5 year old rainbow trout at the selected sampling times are given in Fig. 1.

Significantly increased plasma insulin levels were found in rainbow trout 0.5 h after feeding ($p < 0.005$) and the level was still significantly elevated 2 h after feeding. Four to 9 days after feeding, the insulin levels were significantly lower than 1 h before feeding ($p < 0.001$).

No significant variation in plasma glucose concentration were measured during the 9 days post feeding period.

Plasma glucagon levels showed no significant variation during the sampling period, while plasma GLP concentration was significantly lower on days 4–9 post feeding than in the period 0.5 to 7 h after feeding ($p < 0.01–0.001$).

In rainbow trout, glucagon/insulin and the GLP/insulin molar ratio were significantly reduced at 0.5 and 2 h after feeding compared to the pre-feeding values. Glucagon/insulin molar ratio was elevated on the 4th and 7th day post feeding compared to the values pre-feeding and the first hours post-feeding, while no such elevation was observed in the GLP/insulin ratio (Table 1).

Response to life long restricted feed ration in Atlantic salmon

In 3.5 year old Atlantic salmon, body weight, plasma insulin, glucagon and GLP concentrations varied with the feed ration levels, while plasma glucose concentration did not (Fig. 2). Atlantic salmon fed the calculated satiation ration level (RL = 100) throughout their lifetime were 3.4 times heavier than those fed 75% of the satiation ration level (RL = 75). The salmon on the RL = 75 were 2.7 times heavier than those fed fed 50% of the satiation ration level (RL = 50).

Plasma insulin level was not significantly different in salmon fed the RL = 100 ration compared to

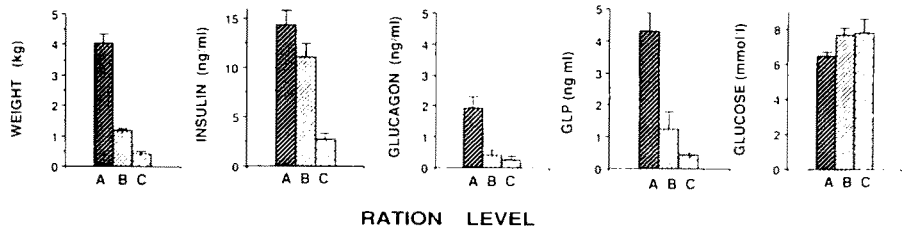

Fig. 2. Body weight and plasma insulin, glucagon, GLP and glucose levels in 3.5 year old Atlantic salmon kept on different feed ration levels (RL) throughout their entire lives; (A) a calculated satiation ration for maximal growth; RL = 100, (B) RL = 75 and (C) RL = 50 of the satiation ration. Values are mean ± SEM (n = 5).

Table 1. Glucagon/insulin and GLP/insulin molar ratios in rainbow trout before feeding and various times after feeding.

Time after feeding	Glu/ins	GLP/ins
1 h	0.11 ± 0.05	0.35 ± 0.11
0.5 h	0.01 ± 0.002	0.10 ± 0.04
2 h	0.02 ± 0.005	0.06 ± 0.01
4 h	0.05 ± 0.01	0.22 ± 0.12
7 h	0.05 ± 0.01	0.17 ± 0.03
1 day	0.03 ± 0.005	0.10 ± 0.04
4 day	0.33 ± 0.15	0.05 ± 0.02
7 day	0.21 ± 0.06	0.16 ± 0.06
9 day	0.06 ± 0.02	0.10 ± 0.07

Values are means ± SEM (n = 10–12).

those on the RL = 75 ration, which were significantly higher ($p < 0.005$) than those obtained in salmon fed the RL = 50 ration.

For glucagon and GLP, the plasma levels were lower in salmon on the RL = 75 ration than on the RL = 100 ration ($p < 0.005$, $p < 0.001$, respectively), however, no further significant decrease was observed in fish fed a RL = 50 ration.

Glucagon/insulin and GLP/insulin molar ratios for the Atlantic salmon are given in Table 2. The only significant difference observed is a lower glucagon/insulin ratio for the fish on the RL = 75 than for those on the RL = 100 ration ($p < 0.05$).

Discussion

Response to feeding and starvation in rainbow trout

As in mammals (Hove and Blom 1971; Trenkle 1971; Hara and Saito 1980; Vaaler 1986), rainbow trout respond to feeding with a marked increase in the plasma insulin levels. The elevated plasma insulin level observed 0.5 and 2 h after feeding in this study, is in accordance with the elevation of plasma insulin levels 2 h after oral glucose administration in carp, red sea bream, and yellowtail, reported by Yone (1979). The magnitude of response was similar to that measured by Yone (1979), but the insulin levels in the present investigation were about 10 times higher. The levels were, however, lower than those observed in fed rainbow trout, reported by Hilton *et al.* (1987).

The lack of increase in plasma glucose level following food intake in our study are in contrast to the findings in various fish species 1–8 h after food or carbohydrate administration (Palmer and Ryman 1972; Moen 1975; Yone 1979 and Hilton *et al.* 1987). This discrepancy may be due to differences in the initial absorptive state, in the amount and type of carbohydrates and the amount of amino acids in the diet, differences in insulin responsiveness, gastric evacuation rate, temperature or other experimental conditions.

Our results indicate that rainbow trout may be able to regulate plasma glucose levels following a meal containing at least 21.7% carbohydrate (65% digestible).

The unaltered plasma glucose level 7 and 9 days post-feeding is in accordance with our unpublished results on plasma levels after 7 weeks of starvation in Atlantic salmon and after 3.5 months of natural starvation at spawning in rainbow trout. The present results also correspond with those observed after 1 month starvation in northern pike (*Esox lucius*) reported by Ince and Thorpe (1976). They found, however, after 3 months of starvation a

reduced plasma glucose level. A decline in plasma glucose was also reported in rainbow trout following 6 weeks of starvation (Moon *et al.* 1989), in cod after 16 days–4 weeks of starvation (Kamara 1966; Hemre *et al.* 1990) and in catfish (*Rhamdia hilarii*) following 30 days of starvation (Machado *et al.* 1988). The discrepancies might be species – dependent and also depend on the nutrional state prior to the starvation period, energy expenditure during starvation, life stage or environmental factors.

Insulin injection into rainbow trout reduces plasma glucose concentrations (Cowey *et al.* 1977; Plisetskaya *et al.* 1985). The initial rise in plasma insulin observed 0.5 h post feeding in the present experiment, may be one of the main factors responsible for keeping glucose at a constant level during the first hour after feeding. Insulin exerts anabolic effects in fish (for review see Sundby 1989). The elevated insulin level after a meal, as seen after 0.5 h in the present experiment, may to some extent contribute to the increased protein synthesis measured 3–4 h after refeeding in rainbow trout (McMillan and Houlihan 1988) and in cod (Lied *et al.* 1983). The low level of plasma insulin measured 9 days after starvation will contribute to stabilizing the plasma glucose level during the short term starvation. The finding of reduced plasma insulin level following starvation is in accordance with earlier observations in rainbow trout and cod (Thorpe and Ince 1976; Plisetskaya *et al.* 1986a; Moon *et al.* 1989).

Glucagon has been found to have a major effect upon glycogenolysis in coho salmon liver slices (Plisetskaya *et al.* 1987). GLP is an even more potent activator of gluconeogenesis than glucagon in hepatocytes isolated from trout and salmon (Mommsen *et al.* 1987; Mommsen and Moon 1989). As glucose remains unaltered during starvation, one would expect an increase in plasma glucagon and GLP levels to account for part of the necessary glucose production. This seems to be the case at least for glucagon in man and geese as elevated levels are measured after 48 h of starvation. (Aguiler-Parada *et al.* 1969; Felig 1979; Sitbon and Miahle 1979; Aoki 1981; Cherel *et al.* 1988). However, elevated glucagon and GLP levels could not be demonstrated in rainbow trout even after 9 days of starvation.

The generally low levels of GLP, glucagon and insulin after 9 days of starvation raise the question whether the concentrations of other hormones participating in metabolic regulation are low as well. If so, this may indicate that a general regulatory mechanism used by the salmonid to meet starvation may be to turn down the total metabolism to a minimum. This assumption is supported by the findings of reduced plasma thyroxine levels in starved rainbow trout (Eales 1988) and by the depressed metabolic rate in starving eel *Anquilla rostrata* (Walsh *et al.* 1983). In addition, Suarez and Moon (1987) suggest that in some fish species metabolic depression, resulting in a decrease in the rate of glucose utilization, may be one of the mechanisms to avoid severe hypoglycemia during starvation. However, Moon *et al.* (1989) reported increased activities by some gluconeogenetic liver enzymes after 6 weeks starvation in rainbow trout.

GLP and glucagon are processed from the same precursor. The large variation in GLP in the rainbow trout after feeding and the small variation in glucagon may be caused by the observed different clearance rate reported by Oshima *et al.* (1988).

Response to life long restricted feed ration in Atlantic salmon

In the 3.5 year old Atlantic salmon, our results clearly showed the impact of feed supply not only on body weight, but also on plasma insulin, glucagon and GLP levels. Plasma glucose level was not influenced by the prolonged reduced feed supply. This is similar to observations of the starved rainbow trout in the present study. It seems reasonable that reduced ration level will result in reduced weight and reduced plasma insulin level. The unexplainable lack of elevation in plasma glucagon and GLP levels in the Atlantic salmon on life long restricted diet is, however, in accordance with the results for the 9 days starved rainbow trout discussed above and with the data on the 6 week starved rainbow trout reported by Moon *et al.* (1989).

Table 2. Glucagon/insulin and GLP/insulin molar ratios in Atlantic salmon at different feeding levels.

Feed ration level	Glu/ins	GLP/ins
100	0.2 ± 0.06	0.5 ± 0.07
75	0.08 ± 0.04	0.25 ± 0.15
50	0.31 ± 0.16	0.42 ± 0.15

Values are mean ± SEM (n = 5).

Glucagon/insulin and GLP/insulin molar ratio

As glucagon-family peptides and insulin have antagonistic effects on hepatic metabolism in salmonids, the molar ratio between these hormones may be important for the net catabolic/anabolic flux. The molar ratio for glucagon-family peptides should be low in an anabolic situation and high in a catabolic situation. In the present study, the molar hormone ratio in the rainbow trout indicates an anabolic situation 0.5 and 2 h after feeding. During the starvation period, the molar ratio values were confusing and further investigation is needed. In the experiment with restricted feeding in Atlantic salmon the molar ratio values were also difficult to interpret, the molar ratio for the salmon on the RL = 100 ration being surprisingly high compared to that found by Moon *et al.* (1989). However, the ratios obtained for the fish fed the RL = 75 and RL = 50 ration correspond to those reported by Moon *et al.* (1989) in fed and starved fish, respectively. The discrepancies between the present results and those reported by Moon *et al.* (1989) may be due to species differences, different feeding regimes and feed ration levels.

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