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Post-feeding carbohydrate and ketone bodies metabolism in brain and liver of Atlantic salmon

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A study of several pathways of carbohydrate and ketone bodies metabolism was carried out in Atlantic salmon (*Salmo salar*) to assess the basal metabolism of the brain, and the possible existence of post-feeding changes in brain and liver metabolism. The main results obtained in brain of Atlantic salmon indicate a use of exogenous glucose as a main fuel source since important hexokinase activities were noticed, and brain glycogen levels were usually very low. Several post-feeding changes were observed in brain including an apparent decrease in glycolytic potential, as well as a decreased use of ketone bodies. In contrast, no major post-feeding changes were detected in liver metabolism. A role for ketone bodies as a metabolic fuel in brain of Atlantic salmon is supported by both the high levels of acetoacetate found in brain, and the presence of an active β-hydroxybutyrate dehydrogenase.

Key words: Brain, Post-feeding metabolism, Glucose, Ketone bodies, *Salmo salar.*

Glucose is accepted as the sole metabolic fuel for the brain and nervous system in mammals, with its levels varying directly with blood glucose concentration (6). Under normal conditions, glycolysis is regulated by hexokinase (HK) activity and not by glucose transport into the brain, and its metabolism is therefore adapted for rapid carbohydrate use for energy needs (6, 37). There is also a rapid

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and constant breakdown and synthesis of glycogen in mammalian brains.

In fish, brain metabolism accounts probably for only a small percentage of the overall metabolic demand (16). Recently, in lamprey *Petromyzon marinus*, carbohydrate metabolism in the brain of a non-mammalian chordate was studied for the first time showing that glucose is oxidized and converted into glycogen (12), and that lactate is metabolized by adult lamprey at a rate greater than glucose.

Organisms that normally experience hypoglycemia periods often show high brain glycogen concentrations. Thus, significant glycogen content in lower fish brain and nervous system indicate that fish brain may not rely on exogenous carbohydrates, i.e. that some fish brains may be partially autonomous through blood supplied glucose (26, 28). Thus, in spiny dogfish *Squalus acanthias*, DEROOS (7) has suggested that brain glycogen stores rather than circulation may be the proximate glucose source. However, the exact nature of the process by which brain glycogen is mobilized remains to be explained (26). In other elasmobranchs, exogenous glucose may represent an important metabolic fuel for the brain, such as in little skate *Raja erinacea* (28), although (judged on HK activity) only for short-term purposes. In contrast to elasmobranchs, no conclusive studies have been made to date in teleosts concerning brain glucose and glycogen metabolism.

Ketone bodies are the primary brain fuel during fasting or starvation in mammals (31), accounting for about 50 % of the oxygen uptake by a starved rat brain (37). In frog ketones, as well as possibly protein lipids, may also act as energy sources for normal brain function (13). In fish, the role of ketone bodies in brain metabolism is still poorly understood (8). ZAMMITT and NEWSHOLME (41) suggested that during starvation ketone bodies may not be an important fuel in teleosts, but no conclusive results have been obtained to date concerning this topic (5, 29).

What emerges from this introduction is a lack of knowledge regarding carbohydrate and ketone bodies metabolism in the brain of teleostean species. Therefore, the aim of this study was twofold when using the Atlantic salmon *Salmo salar* as a teleost model. First: to characterize brain metabolism by establishing basal levels of several metabolic pathways. Second: to evaluate changes in those metabolic pathways occurring several hours post-feeding.

Materials and Methods

Animals and experimental design.– Atlantic salmon (*Salmo salar*) used in this study belonged to a stock of 2000 parr (13 months old) from a fish farm in Vilagudín (Ordes, Galicia, Spain), held in a 10,000-liter tank under constant conditions for approximately 30 days prior to the start of the experiment. Fish used in the experiment weighed 113.8 ± 3.1 g (20.3 ± 0.2 cm length). The fish were fed once daily before the experiment with commercial dry pellets (Sterling Silver Cup, Spain). Approximate analysis: 50 % crude protein, 20 % fat, 21 % carbohydrate, and 9 % ash in the dry matter), with a ration equivalent to 2 % b.w. day^{-1}. The fish handling and care were maintained unchanged throughout the pre-sampling period, under natural conditions of temperature (11 ± 1 °C), pH (7.1 ± 0.2) and photoperiod. Common water quality criteria (pH, hardness, and the oxygen levels, carbon dioxide, hydrogen sulphide, nitrite, nitrate, ammonia, calcium, chlorine and suspended solids) were assessed with no major changes being observed during one month before the experiment.

In order to establish post-feeding changes in parameters, fish were sampled from the 10,000 liter tank on November 29 (1994) using the following time schedule: zero time was established at 10.00 h, i.e. 1 h pre-feeding (using fish that normally fed the previous day at 11.00 h). One hour later (at 11.00 h), food was delivered to fish (a ration equivalent to 2 % b. w.). The next samplings were performed in six hour periods after the first sampling, i.e. at 16.00 h; 22.00 h; 04.00 h, and 10.00 h, which means 5, 11, 17 and 23 h post-feeding, respectively. The photoperiod used was the natural at that time of the year (dawn at 08.00 h and dusk at 18.00 h).

On each sampling time, 15 fish were quickly dip-netted and anesthetized with MS-222 (75 mg l^{-1}) buffered to pH 7.4 with sodium bicarbonate. Samples from three fish were pooled to have enough tissue to analyse all the parameters assessed in brain and plasma. The number of fish obtained per sampling was, therefore, 15, yielding five samples (N = 5). Liver and brain were quickly removed, weighed, frozen on dry ice and stored at –80 °C until further assay. Blood was obtained with ammonium-heparinized syringes from the caudal peduncle. Plasma samples were obtained after blood centrifugation (10 min at 2000 x g; Kubota KM 15200), and were immediately deproteinized (by using 6 % perchloric acid, final concentration) and neutralized (by using 1 mol • l^{-1} sodium bicarbonate) before freezing on dry ice and storage at –80 °C until assay. The time invested in processing samples from one pool of three fish was less than five minutes.

Analytical procedures.– Brain and liver samples were homogenized using a Potter-Elvejhem teflon-in-glass homogenizer held on ice with 10 vols of ice-cold stopping-buffer containing (in mmol • l^{-1}): 50, imidazole-HCl (pH 7.5); 15, 2-mercaptoethanol; 100, KF; 5, EDTA; 5, EGTA; and 0.1, PMSF (added as dry crystals to homogenization buffer immediately prior to homogenization). The homogenate was centrifuged (2 min at 9000 x g, Kubota microcentrifuge KM 15200) and the supernatant was used in enzyme assays.

Aliquots of the supernatant were deproteinized and neutralized with 6 % perchloric acid and 1 mol • l^{-1} sodium bicarbonate, respectively, to assay tissue metabolites. Acetoacetate (24) and lactate levels (15) were assessed in brains and in plasma following enzymatic methods. Brain and liver glycogen levels were assessed using the method of KEPPLER and DECKER (20). Glucose obtained after glycogen breakdown and plasma glucose levels were determined with a glucose oxidase-peroxidase method (Spinreact).

Enzyme activities were determined using a Uvikon 930 spectrophometer. Reaction rates of enzymes were determined by increase or decrease in absorbance of NADPH or NADH at 340 nm. The reactions were started by the addition of homogenates (0.05 ml), at a preestablished protein concentration, omitting the substrate in control cuvettes (final volume 1.1 ml), and allowing to proceed at 20 °C for preestablished times (data not shown). Protein was assayed per duplicate in homogenates (3), using bovine serum albumin (Sigma) as standard. Enzymatic analyses were all carried out at maximum rates in each tissue with the reaction mixtures set up in preliminary tests to render optimal activities. The specific conditions for enzyme assays were as follows:

Glycogen phosphorylase (EC 2.4.1.1.; GPase) was assayed as described by MOON *et al.* (27), and FOSTER *et al.* (12) with the following specific conditions: 50 mmol • l^{-1} potassium phosphate buffer (pH 7.0), 0.5 mmol • l^{-1} NADP, 5 mmol • l^{-1} glucose 1,6-bisphosphate, 2.5 mmol •

l^{-1} AMP, excess phosphoglucomutase, excess glucose 6-phosphate dehydrogenase (from bakers yeast), and 10 mg • ml^{-1} of glycogen (omitted for control). GPase *a* activities were measured in the presence of 10 mmol • l^{-1} caffeine, and total GPase activities were estimated without caffeine. The ratio of GPase activities with and without caffeine represents the percentage of total GPase (a+b) in the active form (% GPase *a*).

Glycogen synthetase (EC 2.4.1.11; GSase) was assayed spectrophotometrically through PK (12, 32). Specific assay conditions were: 50 mmol • l^{-1} imidazole-HCl (pH 7.5), 150 mmol • l^{-1} KCl, 15 mmol • l^{-1} $MgCl_2$, 5 mmol • l^{-1} PEP, 0.15 mmol • l^{-1} NADH, 2 mg • ml^{-1} glycogen, excess pyruvate kinase, excess lactate dehydrogenase, and 6 mmol • l^{-1} UDP-glucose (omitted for control). Total GSase activities were measured with 5 mmol • l^{-1} glucose 6-phosphate (G6P), and GSase *a* activities were estimated in the absence of G6P. The ratio of GSase activities without and with G6P represents the percentage of total GSase (a+b) in the active form (% GSase *a*).

6-Phosphofructo 1-kinase (EC 2.7.1.11; PFK) was assessed two days after obtaining samples according to MOON *et al.*, (27), and SU and STOREY (39), using 50 mmol • l^{-1} imidazole-HCl (pH 7.8), 175 mmol • l^{-1} KCl, 0.25 mmol • l^{-1} NADH, 2 mmol • l^{-1} ATP, 17.5 mmol • l^{-1} $MgCl_2$, excess aldolase, excess triose phosphate isomerase, and excess a-glycerol phosphate dehydrogenase. Activities were determined at low (0.1 mmol • l^{-1} and 0.05 mmol • l^{-1}, for brain and liver, respectively) and high (10 mmol • l^{-1} and 2 mmol • l^{-1}, for brain and liver, respectively) fructose 6-phosphate concentrations (omitted for control). An activity ratio was calculated in each tissue as the activity at low [fructose 6P]/high [fructose 6P]. Similarly, a fructose 2,6-bisphosphate (F-2, 6-P_2) activation ratio was determined using low (1 µmol • l^{-1}) and high (5 µmol • l^{-1}) fructose 2,6-bisphosphate concentrations, and 0.1 mmol • l^{-1} (brain) or 0.05 mmol • l^{-1} (liver) fructose 6-phosphate concentrations.

Hexokinase (*EC* 2.7.1.1.; HK) was assessed in brain and liver mainly following the method of FIDEU *et al.*, (11), using 50 mmol • l^{-1} imidazole-HCl (pH 8), 0.16 mmol • l^{-1} NADP, 5 mmol • l^{-1} $MgCl_2$, 1 mmol • l^{-1} ATP, excess glucose 6-phosphate dehydrogenase (from bakers yeast), and 5 mmol • l^{-1} glucose (omitted for control).

β-Hydroxybutyrate dehydrogenase (EC 1.1.1.30; β-HBDH) was assessed mainly following the method of MOON and MOMMSEN (28), using 50 mmol • l^{-1} phosphate buffer (pH 6.8), 0.15 mmol • l^{-1} NADH, and 1 mmol • l^{-1} acetoacetate (omitted for control).

Lactate dehydrogenase (EC 1.1.1.27; LDH) was assessed essentially using the method of MOMMSEN *et al.*, (25), using 50 mmol • l^{-1} imidazole-HCl (pH 7.4), 0.15 mmol • l^{-1} NADH and 1 mmol • l^{-1} pyruvate (omitted for control).

Fructose 1,6-bisphosphatase (*EC* 3.1.3.11; FBPase) was assessed mainly following the method of MOMMSEN *et al.* (25). Specific assay conditions were: 50 mmol • l^{-1} imidazole-HCl (pH 7.2), 0.4 mmol • l^{-1} NADP, 6 mmol • l^{-1} $MgCl_2$, excess phosphoglucose isomerase, excess glucose 6-phosphate dehydrogenase (from bakers yeast), and 0.03 mmol • l^{-1} fructose 1,6-bisphosphate (omitted for control).

Data analyses.– The normal distribution of variables was tested using the Kolmogorov-Smirnov test, and group variance homogeneity was assessed using the Cochran's C test. In those cases where the normality or homoscedasticity conditions of the analysis of variance were fulfilled

(logarithmic transformations of the data were performed in some cases but data are shown in their decimal values), statistical differences were tested using a one-way analysis of variance, followed by a Student-Newman-Keuls multiple range test. In contrast, in those cases (GPase, GSase, PFK and LDH activities in liver) where the normality or homoscedasticity conditions were not reached, the differences were tested using a Kruskal-Wallis one way Anova on ranks, followed by a Student-Newman-Keuls multiple range test. The differences were considered statistically significant at $P < 0.05$.

Results

No changes were detected in brain and liver protein levels comparing fish sampled at different times (data not shown). Therefore, all the enzyme activities are expressed in terms of protein levels. In addition, no changes were noticed in any group of fish either in body weight/length or in liver weight (data not shown). No detectable FBPase was noticed in brain. At 17 h post-feeding brain glycogen levels and acetoacetate levels increased and decreased, respectively (fig. 1). No significant changes were detected in brain lactate levels.

Table I shows that in brain no post-feeding changes were detected after evaluation of total GPase activity. However, a slight decrease in the % GPase *a* was noticed at 5, 11 and 23 h post-feeding. As for GSase activity, no significant changes were detected in total activity, but the % GSase *a* increased sharply at 17 h post-feeding, with the values being significantly different in the first sampling. The GSase *a*/GPase *a* activity ratio increased post-feeding, with the highest values being found at 17 h. Significant changes were noticed in brain optimal PFK activi-

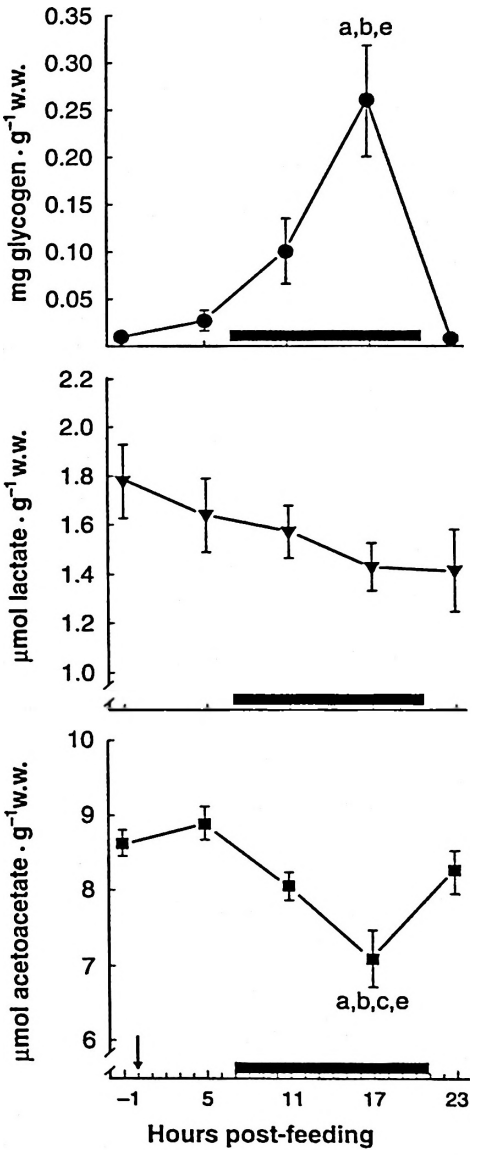

Fig. 1. *Post-feeding changes in the levels of glycogen, lactate, and acetoacetate in brain of Atlantic salmon.* The filled horizontal bar represents the dark period. Fish were fed at 11.00 h (arrow), and were sacrificed every 6 h during the day: 1 h pre-feeding (10.00 h), and 5 (16.00 h), 11 (22.00 h), 17 (04.00 h) and 23 h (10.00 h) post-feeding. Data are shown as mean ± S.E.M. (N = 5, each sample comes from 3 pooled fish). a, b, c, d, e, significantly different ($P < 0.05$) from the samplings performed 1 h pre-feeding and 5, 11, 17 and 23 h post-feeding, respectively.

Table I. *Post-feeding changes in the activities of potential regulatory enzymes of glycogenolysis, glycogenesis and glycolysis assayed in brain and liver of Atlantic salmon.*

Fish were fed at 11.00 h, and were sacrificed every 6 h during the day: 1 h pre-feeding (10.00 h), and 5 (16.00 h), 11 (22.00 h), 17 (04.00 h) and 23 h (10.00 h) post-feeding. One unit of enzyme activity is defined as that which produces 1 mmol NADPH · min^{-1} for GPase, as that which uses 1 mmol NADH · min^{-1} for GSase, and as that which uses 1 mmol fructose 6-phosphate · min^{-1} for PFK. % GPase *a*, percentage of total GPase (a + b) in the active form (a). % GSase *a*, percentage of total GSase (a + b) in the active form (a). GSase *a*/GPase *a* activity ratio is calculated using data from table. The activity ratio of PFK is defined as activity at low (0.1 mmol · l^{-1} and 0.05 mmol · l^{-1}, for brain and liver, respectively)/high (10 mmol · l^{-1} and 2 mmol · l^{-1}, for brain and liver, respectively) substrate fructose 6P concentration. Similarly, a F-2, 6-P2 activation ratio was determined using low (1 µmol · l^{-1}) and high (5 µmol · l^{-1}) F-2, 6-P2 concentrations, and 0.1 mmol · l^{-1} (brain) or 0.05 mmol · l^{-1} (liver) fructose 6-phosphate concentrations. Data are shown as mean ± S.E.M. (N = 5, each sample comes from 3 pooled fish). a, b, c significantly different ($P < 0.05$) from the samplings performed 1 h prefeeding and 5 and 11 h post-feeding, respectively.

Hours post-feeding:	−1	5	11	17	23
			Brain		
Glycogen phosphorylase					
Total activity (U • mg^{-1} protein)	2.14 ± 0.07	2.11 ± 0.03	2.45 ± 0.14	2.33 ± 0.07	2.18 ± 0.06
% GPase *a*	99.5 ± 1.1	93.1 ± 1.2[a]	92.1 ± 0.8[a]	95.9 ± 1.1	95.5 ± 0.9
Glycogen synthetase					
Total activity (U • mg^{-1} protein)	0.22 ± 0.04	0.15 ± 0.01	0.18 ± 0.02	0.26 ± 0.04	0.23 ± 0.01
% GSase *a*	34.4 ± 4.9	60.5 ± 7.8	87.2 ± 7.5[a]	97.6 ± 7.6[a]	73.2 ± 8.3[a]
GSase *a*/GPase *a* activity ratio	0.036	0.061	0.069	0.114	0.067
6-Phosphofructo 1-kinase					
Optimal activity (U • mg^{-1} protein)	1.27 ± 0.01	1.16 ± 0.03	1.08 ± 0.10	0.41 ± 0.04[a,b,c]	0.48 ± 0.05[a,b,c]
Activity ratio	0.48 ± 0.06	0.33 ± 0.04	0.31 ± 0.05	0.26 ± 0.01[a]	0.25 ± 0.05[a]
F-2,6-P_2 activation ratio	0.92 ± 0.17	0.78 ± 0.09	0.69 ± 0.05	0.93 ± 0.02	0.96 ± 0.02
			Liver		
Glycogen phosphorylase					
Total activity (U • mg^{-1} protein)	2.06 ± 0.19	1.83 ± 0.03	1.60 ± 0.07	1.69 ± 0.06	1.68 ± 0.03
% GPase *a*	88.1 ± 2.9	79.9 ± 1.5	80.4 ± 1.4	83.4 ± 1.5	82.1 ± 1.3
Glycogen synthetase					
Total activity (U • mg^{-1} protein)	0.36 ± 0.04	0.40 ± 0.01	0.37 ± 0.03	0.33 ± 0.01	0.33 ± 0.02
% GSase *a*	46.8 ± 0.45	43.9 ± 1.2	43.6 ± 1.7	53.2 ± 4.6	52.6 ± 2.5
GSase *a*/GPase *a* activity ratio	0.093	0.121	0.125	0.125	0.126
6-Phosphofructo 1-kinase					
Optimal activity (U • mg^{-1} protein)	0.44 ± 0.01	0.39 ± 0.02	0.49 ± 0.03	0.49 ± 0.07	0.61 ± 0.02[b]
Activity ratio	0.36 ± 0.01	0.39 ± 0.01	0.32 ± 0.03	0.26 ± 0.04[b,e]	0.40 ± 0.04
F-2,6-P_2 activation ratio	0.25 ± 0.01	0.28 ± 0.02	0.31 ± 0.09	0.57 ± 0.10[a,b,c]	0.47 ± 0.04

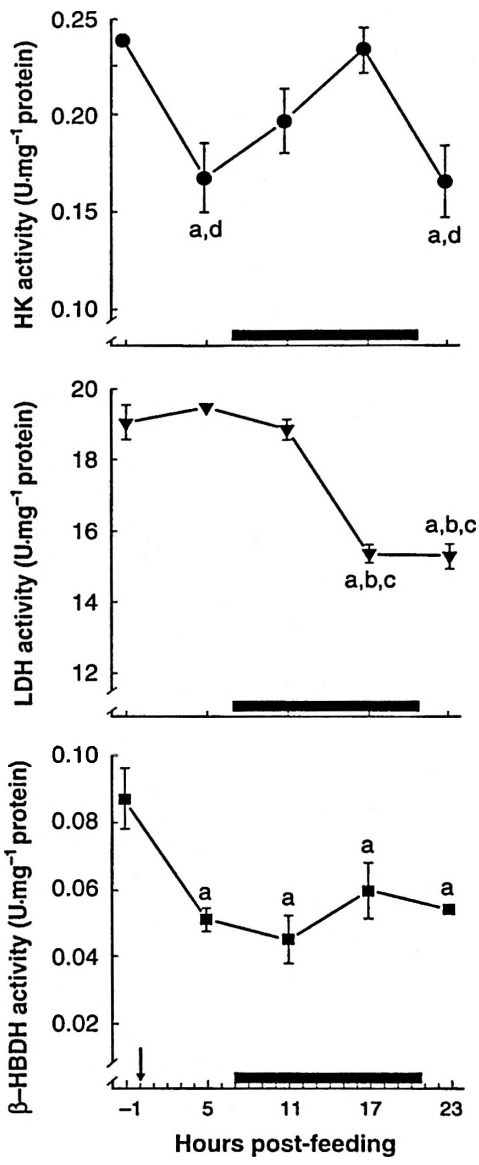

Fig. 2. *Post-feeding changes in the activities of hexokinase (HK), lactate dehydrogenase (LDH), and β-hydroxybutyrate dehydrogenase (β-HBDH) in Atlantic salmon brain.*

One unit of enzyme activity is defined as that which uses 1 µmol glucose • min^{-1} for hexokinase, as that which uses 1 mmol pyruvate • min^{-1} for lactate dehydrogenase, and as that which uses 1 µmol acetoacetate • min^{-1} for β-hydroxybutyrate dehydrogenase. Further details as in legend to fig. 1.

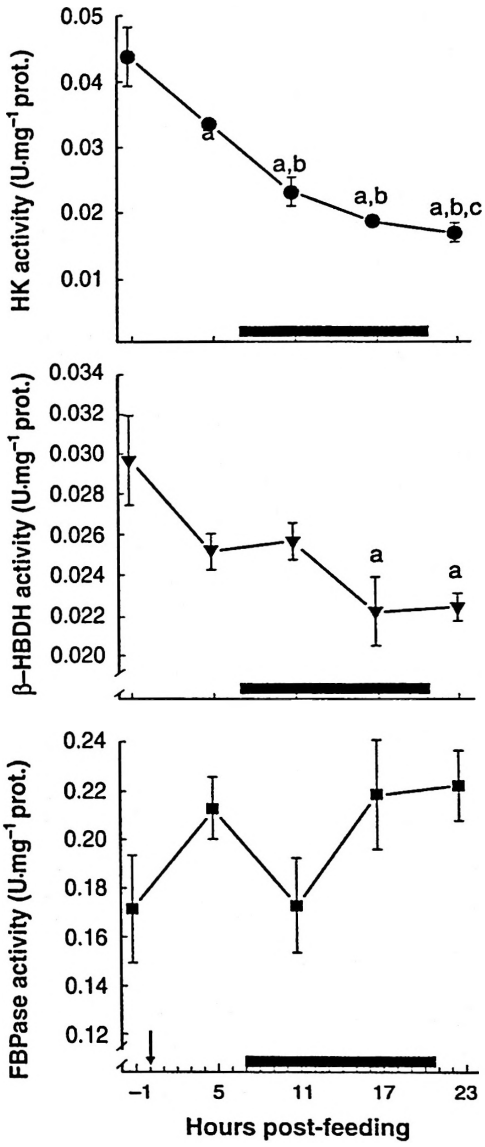

Fig. 3. *Post-feeding changes in the activities of hexokinase (HK), β-hydroxybutyrate dehydrogenase β-HBDH) and fructose 1,6-bisphosphatase (FBPase) in Atlantic salmon liver.*

One unit of enzyme activity is defined as that which uses 1 µmol glucose • min^{-1} for hexokinase, as that which uses 1 µmol acetoacetate • min^{-1} for β-hydroxybutyrate dehydrogenase, and as that which utilizes 1 µmol fructose 1,6-bisphosphate • min^{-1} for fructose 1,6-bisphosphatase. Further details as in legen to fig. 1.

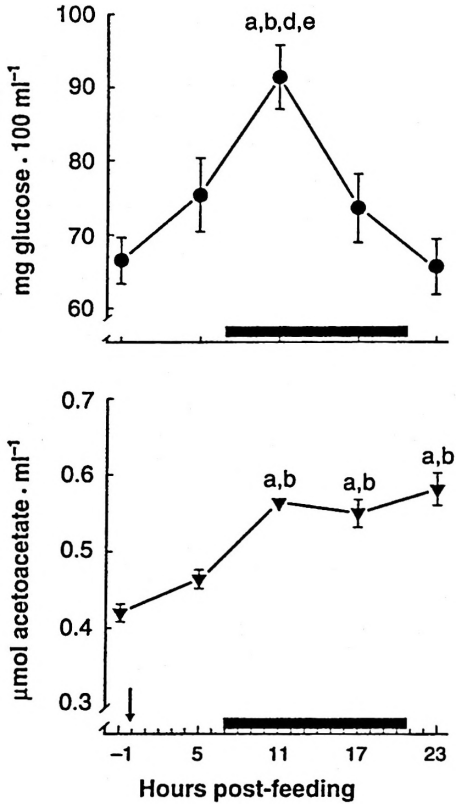

Fig. 4. *Post-feeding changes in the levels of glucose and acetoacetate in Atlantic salmon plasma.* Further details as in legend to Figure 1.

ty, which decreased post-feeding throughout samplings being significantly different at 17 and 23 h. Also, significant changes were detected in PFK activity ratio, reflecting a continuous decrease post-feeding, which was significantly different at 17 and 23 h (table I). No significant changes were detected in the PFK F-2,6-P_2 activation ratio. Two significant falls were detected in HK activity in brain (fig. 2) at 5 and 23 h post-feeding. Moreover, a decrease was observed in the activity of brain LDH, very similar to that observed in PFK activity, at 17 and 23 h post-feeding, which was significantly different from the other samplings. Finally, brain β-HBDH activity decreased significantly at 5, 11, 17 and 23 h post-feeding (fig. 2).

Glycogen is the only metabolite assayed in liver in this experiment, and no significant post-feeding changes were observed with the values ranged form 10 to 30 $mg.g^{-1}$ w w (data not shown). No significant post-feeding changes were detected in the activity of liver GPase either evaluating the total activity of the enzyme or the % GPase *a* (table I). Similar results were obtained in liver for GSase activity, and GSase *a*/GPase a activity ratio. When evaluating the optimal activity of liver PFK, an increase was only observed at 23 h post-feeding, which was significantly different from the sampling performed at 5 h post-feeding (table I). As for the activity ratio of this enzyme in liver, a significant decrease was found at 17 h post-feeding coinciding with a similar increase found in the F-2,6-P_2 activation ratio, which was significantly different from those values observed at 5, 11, and 17 h post-feeding. Liver HK activity significantly decreased throughout samplings with the lowest levels being detected at 23 h post-feeding (fig. 3). No significant changes were detected for liver LDH activity (data not shown), while liver β-HBDH activity decreased significantly at 17 and 23 h post-feeding. In contrast, no significant changes were noticed for liver FBPase activity (fig. 3).

Plasma glucose levels significantly peaked at 11 h post-feeding, and the levels returned to basal values at 23 h post-feeding (fig. 4). However, no significant trend was observed for plasma lactate levels (data not shown). Finally, plasma acetoacetate levels significantly increased from 11 to 23 h post-feeding.

Discussion

Glucose levels increased in plasma of Atlantic salmon at 11 h post-feeding, returning to initial values at 23 h post-feeding. These changes are similar to those previously described in several teleost species such as in sea bass *Dicentrarchus labrax* (14), rainbow trout *Oncorhynchus mykiss* (19, 22), and brook trout *Salvelinus fontinalis* (1), although peak glucose occurred at different times. Contrariwise no post-feeding increase was found in glycemia in other cases such as rainbow trout (34, 40) and cod *Gadus morhua* (17) and a post-feeding decrease was even found in other cases such as sea bass (33) and brown trout *Salmo trutta* (30). Plasma glucose peaks may reveal terminal digestion since the gall bladder depletion observed by NAVARRO *et al.* (30) in brown trout occurs 11 h post-feeding, which coincides with the glycemia peak observed in Atlantic salmon. Nevertheless, the glycemia peak may reflect the gluconeogenic effects of postprandial elevations in plasma cortisol, as suggested for rainbow trout (2), or a possible increase related to the night period.

In this study, very low brain glycogen levels were found in Atlantic salmon until 17 h post-feeding when about 0.25 mg • g^{-1} wet wt levels were reached. This value is considerably lower than the glycogen levels previously noticed in some teleosts, such as goldfish *Carassius auratus* (4), rainbow trout (8), and European eel *Anguilla anguilla* (10), and in cyclostomes, e.g. in lamprey (12). Glycogen levels changes coincided with the increased % GSase *a*, whereas no major changes were detected either in the total GPase activity or in the % GPase *a* in brain of Atlantic salmon. Therefore, it is suggested that the rise in glycogen levels, and the increase at 17 h post-feeding in the GSase *a*/GPase *a* activity ratio (a balance favouring glycogen synthesis), may reflect an increased glycogenesis. The percentage of GPase activity in the active form in Atlantic salmon brain, around 90 %, is actually more similar to the values found in mammals (6) than to those previously obtained from other fish species, such as goldfish (38) and lamprey (12) where values around 45-70 % were found. Thus, the potential for glycogen breakdown is very high in Atlantic salmon brain possibly resulting in lower glycogen content. As for GSase activity, in what may be the only up to the present study in fish, FOSTER *et al.* (12) reported in lamprey an about 22 % of GSase *a*, which is comparable to the observed one 1 h pre-feeding in Atlantic salmon.

Fish brain tissue appears to use glucose for energy both under normoxic and hypoxic conditions (8). Its use has been measured both in catfish *Ictalurus nebulosus* where it was always lower than half of the lactate produced (16), and in lamprey (12), where glucose could be metabolized either to glycogen or to oxidation. In this study, HK activity in brain has been measured, since it has been greatly used to estimate maximum rates of glucose uptake by tissue. Thus, after transforming HK activity into U g^{-1} wet wt. an about 20 value is obtained, which is even higher than those previously reported in other teleost species, such as 9.9 U g^{-1} wet wt. in rainbow trout and 16.2 U g^{-1} wet wt. in goldfish (40). Therefore, Atlantic salmon brain may be hypothesized to rely on exogenous glucose for energy requirements, based on the average high activity noticed in brain HK, in contrast to other lower fish where brain glycogen stores are more important than the exogenous source of glucose, though this hypothesis should be thoroughly tested.

Atlantic salmon brain has a higher glycolytic potential than that of the liver, as

judged by PFK and LDH activities, coinciding with other studies in rainbow trout (9, 21), and goldfish (39). In this study, brain glycolytic potential has been found to decrease at 17 and 23 h post-feeding as judged by the decrease observed in both LDH and PFK activities. For this latter enzyme a decrease was also seen in its activity ratio, which means that there is a decreased enzyme affinity for its substrate. No significant changes were observed in lactate brain levels, revealing that the important glycolytic potential of this tissue is mostly directed through the aerobic rather than the anaerobic pathway. On the other hand, lactate can be metabolized by the adult lamprey brain (12).It cannot therefore, be affirmed, that the slight decrease found in brain lactate levels is due to metabolism.

No detectable FBPase activity was observed in Atlantic salmon brain, suggesting a low or no gluconeogenic potential in its brain, in agreement with another study carried out on rainbow trout (21). However, recent studies performed in brains of both rainbow trout (9) and lamprey (12) have indicated significant FBPase, and PEPCK activities accompanied by a net production of glucose from lactate, respectively. Thus, further studies are needed to elucidate a possible gluconeogenesis involvement in Atlantic salmon brain, although it is suggested that the importance of this pathway in fish brain may be species-specific.

The high HK, PFK and LDH activities found in Atlantic salmon brain lend support to the idea of a primarily carbohydrate-based metabolism, similar to the way previously suggested for little skate (28). On the other hand, the glycolytic potential decrease at 17 and 23 h post-feeding may indicate an energetic requirement decrease in brain, probably associated with decreased brain activity during the night period.

This study shows the existence of detectable brain and liver β-HBDH activity of Atlantic salmon, in agreement with data from teleost species (23) and elasmobranchs (28, 36). The decreased brain ß-HBDH activity post-feeding may be related to the simultaneously increased glucose levels found in plasma, making the catabolism of metabolites other than glucose, such as ketone bodies, unnecessary. The decrease in brain acetoacetate levels 17 h post-feeding could be the result of decreased acetoacetate uptake into brain rather than resulting from changes in its endogenous metabolism. In addition, the parallel increases of glucose and acetoacetate levels in plasma in the first hours post-feeding also point to a fall in the metabolic use of ketone bodies in tissues like the brain, which uses ketone bodies as fuel. This parallel increase of acetoacetate and glucose in plasma has been already described in sea bass (5, 41). Moreover, lipid levels in liver are known to increase in fish post-feeding (35), and part of these lipids are probably directed to ketone bodies synthesis in Atlantic salmon liver. This increased synthesis of ketone bodies could result in increased levels in plasma, just as in Atlantic salmon. On the other hand, the acetoacetate levels in Atlantic salmon plasma are more similar to those in elasmobranchs (7) than in teleosts (29, 41).

Glycogen levels did not change post-feeding in liver, which is in agreement with previous studies carried out in rainbow trout (18, 34), and cod (17). However a non-significant decrease was seen at 11 and 17 h post-feeding, i.e. at 22.00 h and 04.00 h, in agreement with BOUJARD and LEATHERLAND (2) who found higher glycogen levels during the day than during the night period in rainbow trout. Also, LAIDLEY and LEATHERLAND (8) observed in rainbow trout that liver glycogen peaks varied with season and

could not be readily correlated with time of feeding. Moreover, a continuous decrease was found in liver HK activity of Atlantic salmon, revealing that most increased glucose plasma levels were directed to other tissues, as carbohydrates are not an important fuel for liver metabolism in fish. Furthermore, no significant changes were detected in liver FBPase, LDH and PFK activities, reflecting the absence of major changes in the glycolytic/gluconeogenic potential of Atlantic salmon liver in post-feeding conditions.

In summary, Atlantic salmon brain possesses important HK activities while brain glycogen levels are usually very low, thus suggesting the use of exogenous glucose as a main fuel source. Several post-feeding changes were noticed in brain metabolism including an apparent decrease in glycolytic potential, and a decrease in the use of ketone bodies. In contrast, no major post-feeding changes were detected in liver carbohydrate metabolism. A role for ketone bodies in Atlantic salmon brain as a metabolic fuel is also supported by the high acetoacetate levels found in brain, as well as by the presence of an active β-HBDH. As a whole, these findings suggest that the brain ketone body metabolism of Atlantic salmon may be more similar to that of elasmobranchs than to that up to date described for teleosts, as both elasmobranchs and Atlantic salmon experience natural periods of fasting although not to the same extent.

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J. L. SOENGAS, E. F. STRONG, J. FUENTES, M. ALDEGUNDE and M. D. ANDRÉS. *Metabolismo post-ingesta de carbohidratos y cuerpos cetónicos en cerebro e hígado de salmón del Atlántico.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (3), 131-142, 1996.

Se estudian varias rutas del metabolismo de carbohidratos y de cuerpos cetónicos en cerebro y en hígado del salmón del Atlántico (*Salmo salar*), con el objeto de caracterizar tanto sus niveles basales de actividad como los cambios que ocurran, después de la ingesta. Los resultados obtenidos en cerebro indican que este tejido utliza glucosa exógena como fuente principal de energía, dado que se detecta una elevada actividad hexoquinasa, mientras que los niveles de glucógeno cerebral son relativamente bajos. Se demuestra la existencia de cambios en el metabolismo cerebral después de la ingesta, que incluyen un descenso en el potencial glucolítico del tejido y en el uso de cuerpos cetónicos. En contraste, no se encuentran cambios importantes en el metabolismo hepático. Se sugiere, que los cuerpos cetónicos pueden ser una fuente de energía para el cerebro del salmón del Atlántico debido a la existencia de niveles elevados de acetoacetato en el cerebro así como por la presencia en ese tejido de una importante actividad β-hidroxibutirato deshidrogenasa.

Palabras clave: Cerebro, Ingesta, Metabolismo, Glucosa, Cuerpos cetónicos, *Salmo salar.*

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