Journal of Fish Biology (2000) **57,** 631–646
doi:10.1006/jfbi.2000.1341, available online at http://www.idealibrary.com on IDEAL®

Effects of food deprivation on 24 h-changes in brain and liver carbohydrate and ketone body metabolism of rainbow trout

R. I. Figueroa*, R. Rodríguez-Sabarís*, M. Aldegunde† and J. L. Soengas*‡

**Laboratorio de Fisioloxía Animal, Facultade de Ciencias, Universidade de Vigo, E-36200 Vigo, Spain and †Laboratorio de Fisioloxía Animal, Facultade de Bioloxía, Universidade de Santiago de Compostela, Spain*

(*Received 23 December 1999, Accepted 13 April 2000*)

Plasma glucose, lactate and acetoacetate, brain glycogen and acetoacetate, and liver acetoacetate, glycogen and lactate in fed rainbow trout exhibited daily changes. However, no daily changes were observed in the activities of the brain enzymes glycogen synthetase, 6-phosphofructo 1-kinase, and lactate dehydrogenase. Depending on the length of the previous fasting period most daily changes observed in the metabolic parameters of fed fish disappeared, except for liver acetoacetate levels, which displayed daily changes in both fed and fasted fish. These results suggest that feeding is an important factor regulating most daily changes in the brain and liver carbohydrate and ketone body metabolism of rainbow trout.

Key words: rainbow trout; daily changes; brain; food deprivation; glucose; ketone body.

INTRODUCTION

Biological rhythms play an important adaptive role in the temporal organization of living organisms, because they allow animals to adjust physiological processes to the chronological arrangement of the external world. Body temperature, storage or mobilization of energy reserves and enzymatic activity, have to be adjusted to synchronize with environmental changes. Numerous investigations have shown that photoperiod and feeding act as zeitgebers in fish and therefore can induce changes in the time course of physiological variables (Boujard & Leatherland, 1992*a*). Thus, plasma GH, cortisol, thyroxine and glucose concentrations of rainbow trout *Oncorhynchus mykiss* (Walbaum) display diel changes, and time of feeding has a marked effect on the pattern of these changes (Reddy & Leatherland, 1995). Only a few reports describe daily changes in fish held under controlled environments. When food is available at all times, feeding rhythm is synchronized by the light/dark cycle but can be influenced by restricted feeding time such as under fish farming conditions, where food usually is distributed at fixed time intervals each day (Boujard & Leatherland, 1992*a*).

There are very few data in the literature regarding fuel transport or utilization in fish brain (Foster *et al.*, 1993; de Roos, 1994). Glucose transport has been

‡Author to whom correspondence should be addressed. Tel.: +34 986 812564; fax: +34 986 812556; email: jsoengas@uvigo.es

0022–1112/00/090631+16 $35.00/0

demonstrated into rainbow trout brain (Aldegunde *et al.*, 2000), and the brain of rainbow trout oxidizes primarily glucose and lactate as fuels (Soengas *et al.*, 1998*a*). Also, brain carbohydrate and ketone bodies of rainbow trout (Soengas *et al.*, 1998*a*) and Atlantic salmon *Salmo salar* L. (Soengas *et al.*, 1996*b*) change during short- and long-term food deprivation, and preliminary data indicate post-feeding changes in brain metabolic parameters in Atlantic salmon (Soengas *et al.*, 1996*a*).

During fasting, fish appear to use catabolic energy conservation strategies which meet their caloric needs but minimize their tissue energy loss (Navarro & Gutiérrez, 1995). In most studies of the effects of fasting on plasma hormone and metabolite levels, samples have been obtained only at one time of the day. The effect of fasting on daily changes of plasma and tissue metabolite levels has been largely unexplored (Holloway *et al.*, 1994) and no studies exist regarding brain energy metabolism.

Circadian rhythms are considered to originate in the central nervous system. When external periodic factors are suppressed (de Pedro *et al.*, 1998) exogenous rhythms may lose their periodicity, whereas endogenous rhythms are self-sustained over several days with a period close to 24 h. Since feeding time is recognized as a major synchronizer of many physiological changes in fish, daily patterns in several parameters of carbohydrate and ketone body metabolism were examined in the brain and liver of rainbow trout subjected to progressive periods of fasting, to determine whether these daily changes depend on feeding as an exogenous factor.

MATERIALS AND METHODS

Immature rainbow trout, weighing 108 ± 9 g, were obtained in November 1998 from a fish farm in Soutorredondo (Noia, Galicia, Spain). Fish were acclimated, five per tank, for 4 weeks in 40 tanks (50 l) supplied with constantly running and aerated well water at 15° C (daily temperature change $<0{\cdot}2$° C), $7{\cdot}2 \pm 0{\cdot}04$ pH, and under an artificial photoperiod similar to the natural photoperiod at that time of the year (lights on at 0800 hours, lights off at 1900 hours) using a dimmer to simulate a 30 min dusk and dawn (included in the scotophase). The fish were fed to satiety once daily at 1100 hours with commercial dry pellets (Ewos, Spain, proximate analysis: 50% crude protein, 20% fat, 21% carbohydrate, and 9% ash in the dry matter). The handling and care of fish remained the same throughout the acclimation period. The common water quality criteria (hardness, and the levels of oxygen, carbon dioxide, hydrogen sulphide, nitrite, nitrate, ammonia, calcium, chlorine and suspended solids) were assessed throughout the experiment with no changes being observed. Replicate tanks were established for each sampling. To aid in sampling during the scotophase, a single low intensity red light was switched on for the entire period of the experiment.

Tanks were allocated randomly to one of four independent treatment groups: fed, fasted 1 day, fasted 2 days, and fasted 5 days. The fish in the fed group were fed once daily during the whole acclimation period as well as on the day of sampling. Fish in fasted groups had not received food for periods of 1, 2 or 5 days before the sampling day. Fish were sampled from each treatment group (two replicate tanks each with five fish) using the following time schedule. One hour after the first sampling at 1000 hours, food was delivered only to the fed group, and the next samplings were performed at 6-hourly intervals after the first sampling: at 1600, 2200, 0400 and 1000 hours (i.e. 5, 11, 17 and 23 h post-feeding, respectively). The number of fish used per sampling time was eight (four fish from each of the two replicate tanks). Fish of the groups which had been deprived of food for 1, 2 and 5 days were sampled on the same day as the fed fish.

At each sampling time, fish were removed quickly from the holding tanks with a dipnet and anaesthetized with MS-222 (75 mg l^{-1}) buffered to pH 7·4 with sodium bicarbonate. No effect on the measured parameters were observed due to the effect of anaesthesic (data not shown). The brain and liver were removed within 40 s for each fish, weighed, frozen on dry ice and stored at −80° C until assayed. Blood was obtained with ammonium-heparinized syringes from the caudal peduncle. Plasma samples were obtained after centrifuged of blood (1 min at 9000 ***g***; Kubota KM 152000), and were deproteinized immediately (using 6% perchloric acid) and neutralized (using 1 mol l^{-1} sodium bicarbonate) before freezing on dry ice and storage at −80° C until further assay.

The experiments described comply with the Guidelines of the European Union Council (86/609/EU) for the use of laboratory animals.

ANALYTICAL PROCEDURES

Brain and liver samples were minced on a chilled petri dish and divided into two different aliquots to assess enzyme activities and metabolite levels, respectively.

The aliquots of brain and liver used for the assessment of metabolite levels were homogenized immediately using a Potter-Elvejhem teflon-in-glass homogenizer held on ice with 10 vols of ice-cooled 6% perchloric acid, neutralized (using 1 mol l^{-1} sodium bicarbonate), centrifuged (2 min at 9000 ***g***, Kubota microcentrifuge KM 15200), and the supernatant used to assay tissue metabolites. Acetoacetate levels were assessed in brain, liver, and plasma following the enzymatic method of Mellanby & Williamson (1974). Plasma, liver and brain lactate levels were determined using the enzymatic method of Guttman & Wahlefeld (1974). Brain and liver glycogen levels were assessed using the method of Keppler & Decker (1974). Glucose obtained after glycogen breakdown (brain and liver), as well as plasma glucose levels, were determined with a glucose oxidase-peroxidase method (Biomérieux, Spain).

The aliquots of brain used for the assessment of enzyme activities were homogenized using a Potter-Elvejhem teflon-in-glass homogenizer held on ice with 10 vols of ice-cold stopping-buffer containing: 50 mmol l^{-1} imidazole-HCl (pH 7·5), 15 mmol l^{-1} 2-mercaptoethanol, 100 mmol l^{-1} KF, 5 mmol l^{-1} EDTA, 5 mmol l^{-1} EGTA and 0·1 mmol l^{-1} PMSF (the last added as dry crystals immediately before homogenization). The homogenate was centrifuged (2 min at 9000 ***g***, Kubota microcentrifuge KM 15200) and the supernatant used in enzyme assays.

Enzyme activities were determined using a Unicam UV6-220 spectrophotometer. Reaction rates of enzymes were determined by the decrease in absorbance of NADH at 340 nm. The reactions were started by the addition of homogenates (0·05 ml), at a pre-established protein concentration, omitting the substrate in control cuvettes (final volume 1·1 ml), and allowing the reactions to proceed at 15° C for pre-established times (5–15 min). Protein was assayed in triplicate in homogenates as detailed by Bradford (1976), using bovine serum albumin (Sigma, U.S.A.) 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 enzymes assays were described previously (Soengas *et al*., 1996*b*, 1998*a*), and were as follows.

Glycogen synthetase (*EC* 2.4.1.11.; GSase) was assayed in brain spectrophotometrically through PK, with the following specific assay conditions: 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, 3 U ml^{-1} pyruvate kinase, 3 U ml^{-1} 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) present, and GSase *a* activities were estimated from incubations without G6P. The ratio of GSase activities without and with G6P multiplied by 100 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 in brain 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} $MgCl_2$, 1 U ml^{-1} aldolase, 4 U ml^{-1} triose phosphate isomerase, and 1 U ml^{-1} α-glycerol phosphate dehydrogenase. Activities were determined at low (0·1 mmol l^{-1}) and high (10 mmol l^{-1}) 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} fructose 6-phosphate concentration.

Lactate dehydrogenase (*EC* 1.1.1.27.; LDH) was assessed in brain 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).

DATA ANALYSES

The normal distribution of variables was tested using the Kolmogorov–Smirnov test, and group variance homogeneity was assessed using Cochrans' C test. Logarithmic transformations of the data were made where necessary, but data are shown in their decimal values for simplicity. The possible existence of a tank effect was tested, for each parameter, and treatment assessed, using a Student *t*-test. As no differences were obtained between replicate tanks for any of the parameters assessed (data not shown) the values used in further statistical testing were from fish of two pooled tanks ($n=8$). Statistical differences were tested using a two-way analysis of variance, with treatment (fed, fasted 1 day, fasted 2 days, and fasted 5 days) and time (1000, 1600, 2200, 0400, and 1000 hours) being the main factors. In those cases where a significant effect for a factor was obtained in the ANOVA, values were compared using a Student–Newman–Keuls multiple range test. The differences were considered statistically significant at $P<0·05$.

RESULTS

No significant differences were found for any size measurements (body weight, body length, liver weight, and brain weight) in the groups assessed (data not shown), so that differences between fed and food deprived fish are considered not due to changes in those parameters. Also, no changes were found in brain protein levels by brain weight in any of the groups studied (data not shown), and therefore all the enzyme activities are expressed in terms of mg protein.

Plasma glucose levels increased in fed animals with peak levels observed at 2200 hours (11 h post-feeding) (Fig. 1). This peak in plasma glucose levels was observed also in fish fasted for 1 day but not in fish fasted for 2 and 5 days (Fig. 1). Plasma glucose levels after 5 days of fasting were significantly lower, at most sampling times, than those of fed fish (Fig. 1).

A similar increase to that described for plasma glucose was observed for brain glycogen levels in fed fish (Fig. 2) which displayed daily variations with peak values at 2200 hours (11 h post-feeding). In fish fasted for 1 day, the highest brain glycogen levels were observed at 1600 hours, but these levels were not statistically different from those at 2200 hours (Fig. 2). The daily increase in brain glycogen levels disappeared after 2 and 5 days of fasting (Fig. 2). Compared with fed fish, brain glycogen levels were lower at most sampling times after 5 days of fasting (Fig. 2).

Total GSase activity did not vary in fed or fasted fish (Table I). However, in fed fish the %GSase *a* was increased at 2200 hours (11 h post-feeding) but not in fasted fish. No significant changes were observed for LDH or PFK activities either considering fed or fasted fish, in contrast to changes in brain lactate levels (see below).

Liver glycogen levels in fed fish were highest at 2200 hours (11 h post-feeding) but less elevated with progressive food deprivation (Fig. 3). Liver glycogen levels

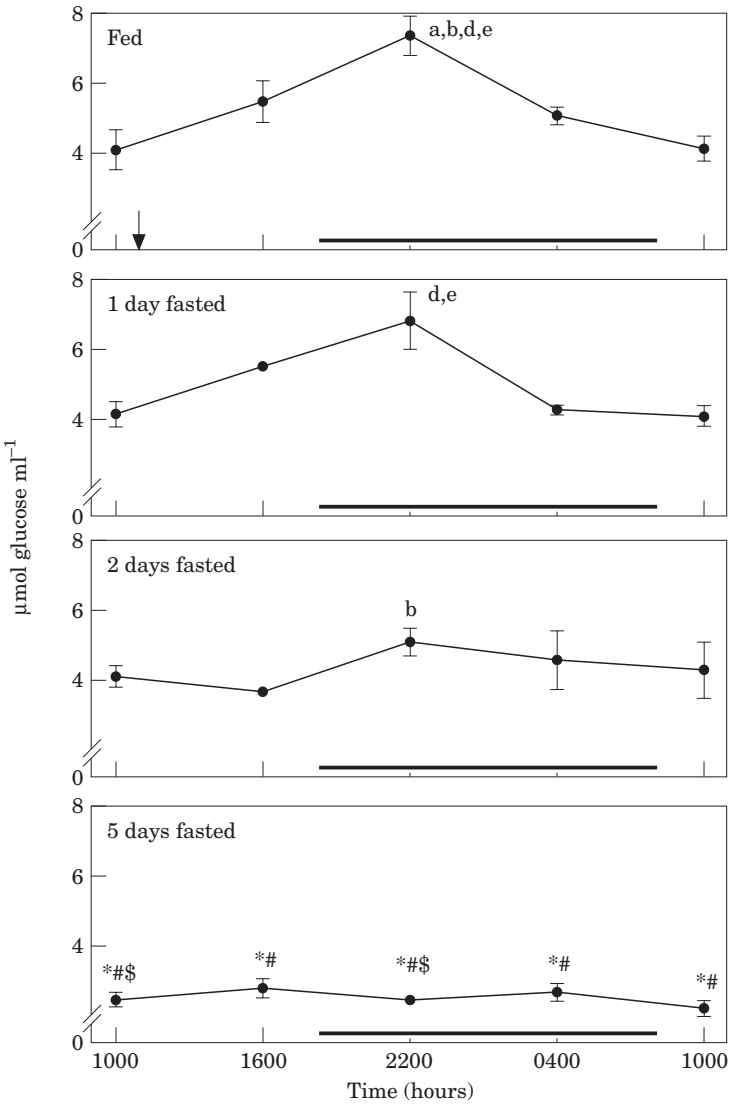

FIG. 1. Effect of progressive periods of fasting for up to 5 days on 24 h changes in plasma glucose levels in rainbow trout. The filled horizontal bar represents the dark period. The fed group was given a single daily meal at 1100 hours (indicated by the arrow), and all groups were sampled at 1000, 1600, 2200, 0400, and 1000 hours. The fasted groups were sampled the same day following 1, 2 or 5 days of food deprivation. Data are shown as mean ± S.E. and were analysed by two-way ANOVA (n=8). *, Significantly different (P<0·05) from the fed fish sampled at the same time of the day; #, significantly different (P<0·05) from the fish fasted 1 day and sampled at the same time of the day; $, significantly different (P<0·05) from the fish fasted 2 days and sampled at the same time of the day; a, b, c, d, e, significantly different (P<0·05) from the fish of the same treatment sampled at 1000, 1600, 2200, 0400, and 1000 hours, respectively.

appeared to decrease following food deprivation, and values of fish fasted 5 days were significantly lower, at most sampling times, than those of fed fish and fish fasted 1 day (Fig. 3).

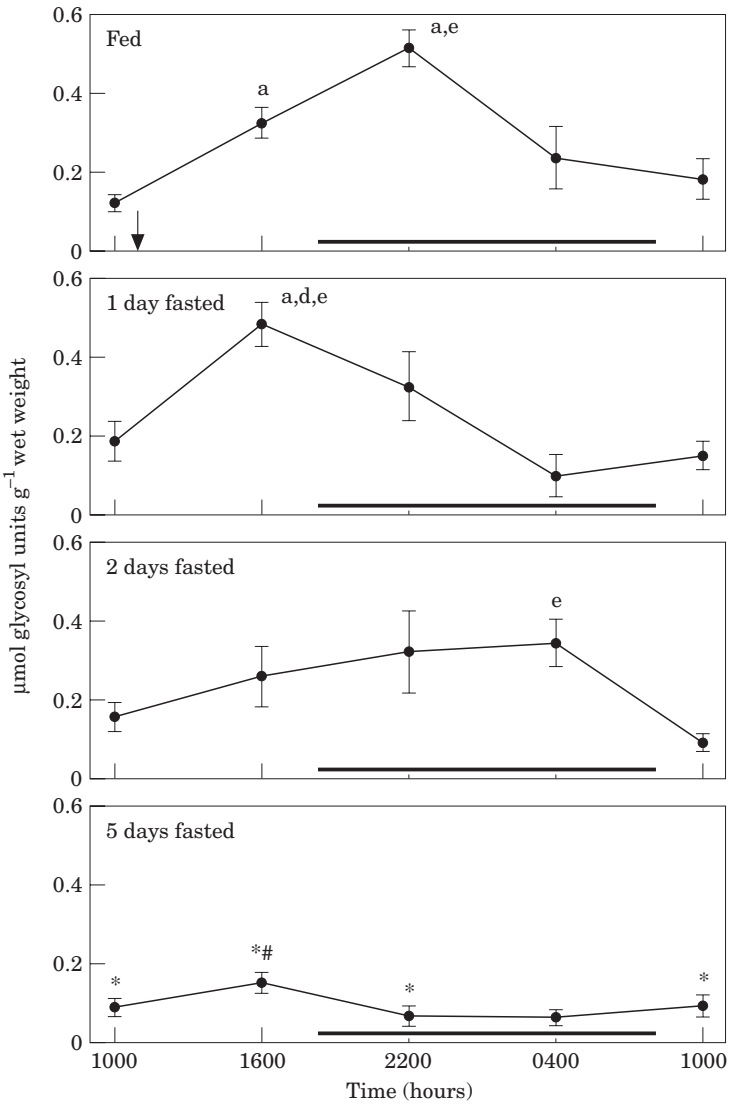

FIG. 2. 24 h changes in brain glycogen levels in rainbow trout either fed or following 1, 2 or 5 days of food deprivation. Further details as in legend to Fig. 1.

Plasma lactate levels fell in fed fish at 2200 hours (11 h post-feeding), but not in fasted fish (Fig. 4). Overall plasma lactate levels of fish fasted for 5 days tended to be lower than those of fed fish and fish fasted for 1 day (Fig. 4). Liver lactate levels in fed fish were lowest at 2200 hours (11 h post-feeding) whereas levels in fasted fish were more stable (Fig. 5).

Plasma acetoacetate levels increased to a maximum of 0400 hours (17 h post-feeding) in fed fish but not in fasted fish (Fig. 6). Values of plasma acetoacetate levels in 5 days food-deprived fish were lower than those of fed fish for most of the sampling times studied (Fig. 6). Brain acetoacetate levels of fed

TABLE I. Effect of progressive fasting for up to 5 days on daily changes in the activities of potential regulatory enzymes of glycogenesis (glycogen synthetase, GSase) and glycolysis (6-phosphofructo 1-kinase, PFK; and lactate dehydrogenase, LDH) assayed in brain of rainbow trout. The fed group was given a single daily meal at 1100 hours, and were sampled every 6 h during the day. The fasted groups were sampled every 6 h after 1, 2 or 5 days of food deprivation[1]

Parameter	Treatment	Time (hours)				
		1000	1600	2200	0400	1000
GSase activity						
Total activity	Fed	0·17 ± 0·09	0·17 ± 0·01	0·35 ± 0·11	0·22 ± 0·05	0·36 ± 0·10
(U mg^{-1} protein)	1 day fasted	0·34 ± 0·11	0·19 ± 0·04	0·38 ± 0·11	0·47 ± 0·17	0·22 ± 0·07
	2 days fasted	0·20 ± 0·10	0·38 ± 0·07	0·50 ± 0·08	0·26 ± 0·08	0·22 ± 0·06
	5 days fasted	0·39 ± 0·08	0·41 ± 0·14	0·47 ± 0·11	0·29 ± 0·05	0·46 ± 0·15
% GSase *a*	Fed	28·7 ± 7·7	29·6 ± 3·1	86·8 ± 4·1a,b	41·9 ± 5·6c	41·7 ± 3·9c
	1 day fasted	40·9 ± 4·2	19·2 ± 4·2	66·9 ± 19·5	54·7 ± 7·1	45·6 ± 11·0
	2 days fasted	44·9 ± 10·3	62·1 ± 9·9†	61·5 ± 9·2	67·0 ± 6·6	55·5 ± 14·9
	5 days fasted	40·4 ± 9·8	44·7 ± 9·1	44·1 ± 4·6*	33·7 ± 3·1‡	25·1 ± 7·8
LDH activity	Fed	11·2 ± 2·2	9·0 ± 2·6	10·7 ± 2·1	4·8 ± 1·1	5·6 ± 1·1
(U mg^{-1} protein)	1 day fasted	6·1 ± 1·6	8·4 ± 2·5	9·8 ± 4·0	9·8 ± 1·1	13·9 ± 2·5
	2 days fasted	14·5 ± 3·2	14·7 ± 3·8	15·2 ± 3·7	10·3 ± 4·4	9·2 ± 4·2
	5 days fasted	13·9 ± 3·4	11·1 ± 2·5	15·8 ± 2·2	12·8 ± 4·1	19·1 ± 4·9
PFK activity						
Optimal activity	Fed	0·28 ± 0·03	0·34 ± 0·01	0·35 ± 0·05	0·74 ± 0·09a	0·51 ± 0·07
(U mg^{-1} protein)	1 day fasted	0·45 ± 0·12	0·44 ± 0·11	0·23 ± 0·04	0·50 ± 0·19	0·38 ± 0·03
	2 days fasted	0·46 ± 0·06	0·49 ± 0·13	0·46 ± 0·12	0·51 ± 0·09	0·60 ± 0·22
	5 days fasted	0·73 ± 0·17*	0·42 ± 0·04	0·65 ± 0·15†	0·36 ± 0·04*	0·55 ± 0·17
Activity ratio	Fed	0·38 ± 0·07	0·34 ± 0·08	0·78 ± 0·11	0·52 ± 0·16	0·52 ± 0·10
	1 day fasted	0·56 ± 0·09	0·57 ± 0·06	0·40 ± 0·22	0·35 ± 0·14	0·35 ± 0·15
	2 days fasted	0·38 ± 0·06	0·45 ± 0·14	0·50 ± 0·20	0·69 ± 0·34	0·46 ± 0·10
	5 days fasted	0·31 ± 0·17	0·97 ± 0·25	0·41 ± 0·17	0·83 ± 0·12	0·48 ± 0·14
F 2,6-P_2 activation ratio	Fed	0·45 ± 0·08	0·47 ± 0·01	0·50 ± 0·04	0·60 ± 0·03	0·68 ± 0·06
	1 day fasted	0·49 ± 0·14	0·38 ± 0·04a	0·37 ± 0·03a	0·41 ± 0·06	0·45 ± 0·02
	2 days fasted	0·47 ± 0·13	0·92 ± 0·31	0·42 ± 0·04	0·47 ± 0·08	0·42 ± 0·12
	5 days fasted	0·80 ± 0·12	0·52 ± 0·01	0·40 ± 0·06	0·64 ± 0·07	0·57 ± 0·12

[1]One unit of enzyme activity is defined as that which utilizes 1 μmol NADH min^{-1}, 1 μmol pyruvate min^{-1}, and 1 μmol fructose 6-phosphate min^{-1}, for GSase, LDH, and PFK, respectively. % GSase *a*, percentage of total GSase (a+b) in the active form (a). PFK activity ratio is defined as activity at low (0·05 mM) : high (2 mM) fructose 6P concentration. Similarly, a fructose 2,6-bisphosphate activation ratio was determined using low (1 μM) and high (5 μM) fructose 2,6-bisphosphate concentrations, and 0·05 mM fructose 6P concentrations. Data are shown as mean ± S.E. and were analysed by two-way ANOVA (n=8). *, Significantly different (P<0·05) from the fed fish sampled at the same time of the day; †, significantly different (P<0·05) from the fish fasted 1 day at the same time of the day; ‡, significantly different (P<0·05) from the fish fasted 2 days at the same time of the day; a,b,c, significantly different (P<0·05) from the fish of the same treatment sampled at 1000, 1600 and 2200 hours, respectively.

fish increased from 0400 hours (17 h post-feeding) onwards, but not in fasted fish (Fig. 7). Strikingly, brain acetoacetate decreased to a minimum at 1600 hours for fish fasted 2 and 5 days (Fig. 7). Liver acetoacetate levels increased around 2200 hours in fasted but not in fed fish (Fig. 8).

DISCUSSION

Plasma glucose levels of rainbow trout were elevated after feeding, which is in agreement with most studies on teleosts including rainbow trout (Laidley & Leatherland, 1988; Boujard & Leatherland, 1992*b*; Boujard *et al.*, 1993;

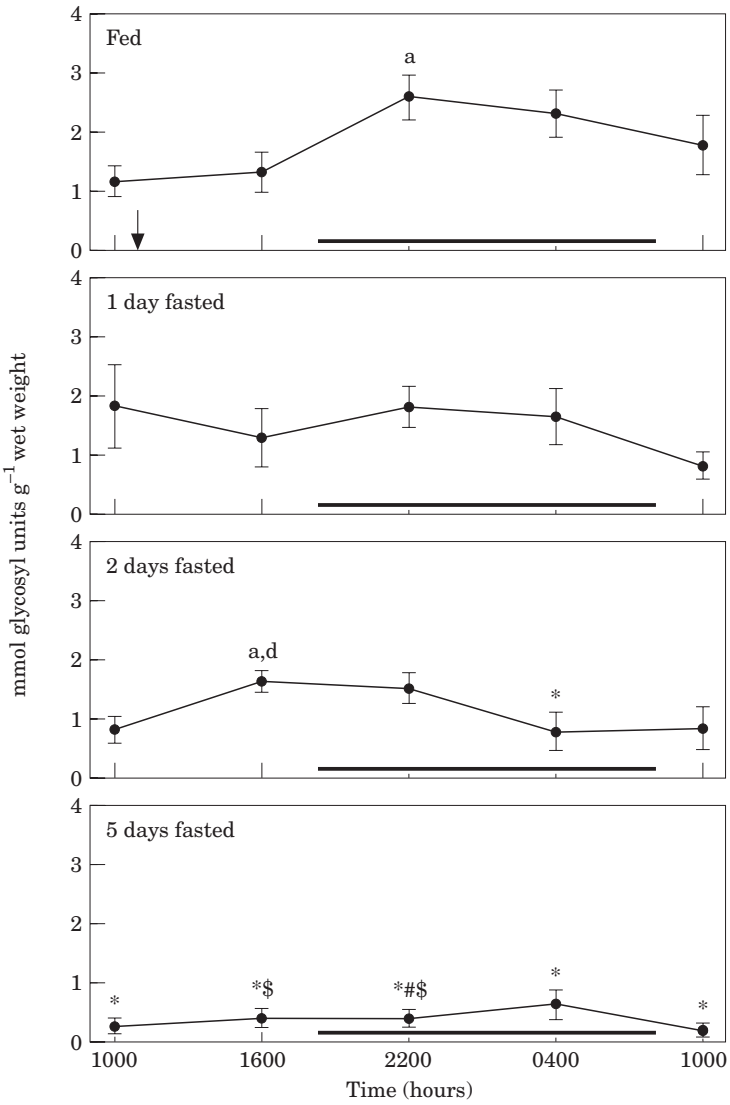

FIG. 3. 24 h changes in liver glycogen levels in rainbow trout either fed or following 1, 2 or 5 days of food deprivation. Further details as in legend to Fig. 1.

Holloway *et al.*, 1994; Reddy & Leatherland, 1995), brown trout *Salmo trutta* L. (Navarro *et al.*, 1993), Atlantic salmon (Soengas *et al.*, 1996*a*), sea bass *Dicentrarchus labrax* L. (Pérez *et al.*, 1988) and tench *Tinca tinca* L. (de Pedro *et al.*, 1998), but not with others such as in rainbow trout (Sundby *et al.*, 1991) or brook trout *Salvelinus fontinalis* Mitchill (Biron & Benfey, 1994). The glucose elevation may reflect the end of digestion as suggested by Navarro *et al.* (1993), but also may indicate gluconeogenic effects of postprandial elevations in plasma cortisol levels as suggested previously for rainbow trout (Boujard & Leatherland, 1992*b*). Fish fasted for 1 and 2 days still showed a daily increase in plasma glucose levels at 2200 hours which is comparable with the increase observed in

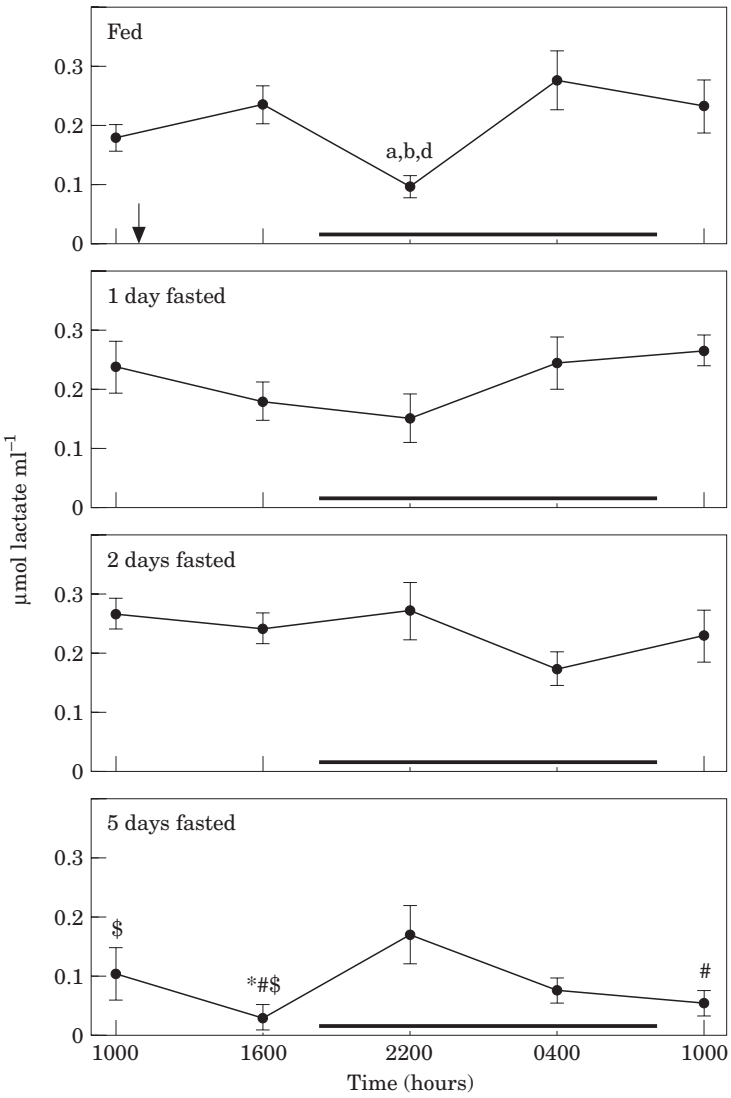

FIG. 4. 24 h changes in plasma lactate levels in rainbow trout either fed or following 1, 2 or 5 days of food deprivation. Further details as in legend to Fig. 1.

fasted sea bass (Pavlidis *et al.*, 1997; Cerdá-Reverter *et al.*, 1998), sea bream *Sparus auratus* L. (Pavlidis *et al.*, 1997), and dentex *Dentex dentex* L. (Pavlidis *et al.*, 1999). Thus, the tendency of plasma glucose to increase was retained in trout fasted for up to 2 days, despite the decline in mean plasma glucose concentrations. In fish fasted for 5 days two different processes were evident. First, levels at most sampling items were lower than those of fed fish, which is in agreement with studies in which samples were obtained at only one sampling time (Tranulis *et al.*, 1991; Holloway *et al.*, 1994; Soengas *et al.*, 1998*a* in rainbow trout, and Soengas *et al.*, 1996*b* in Atlantic salmon). Second, the increase in plasma glucose levels disappeared completely in fish fasted for 5 days

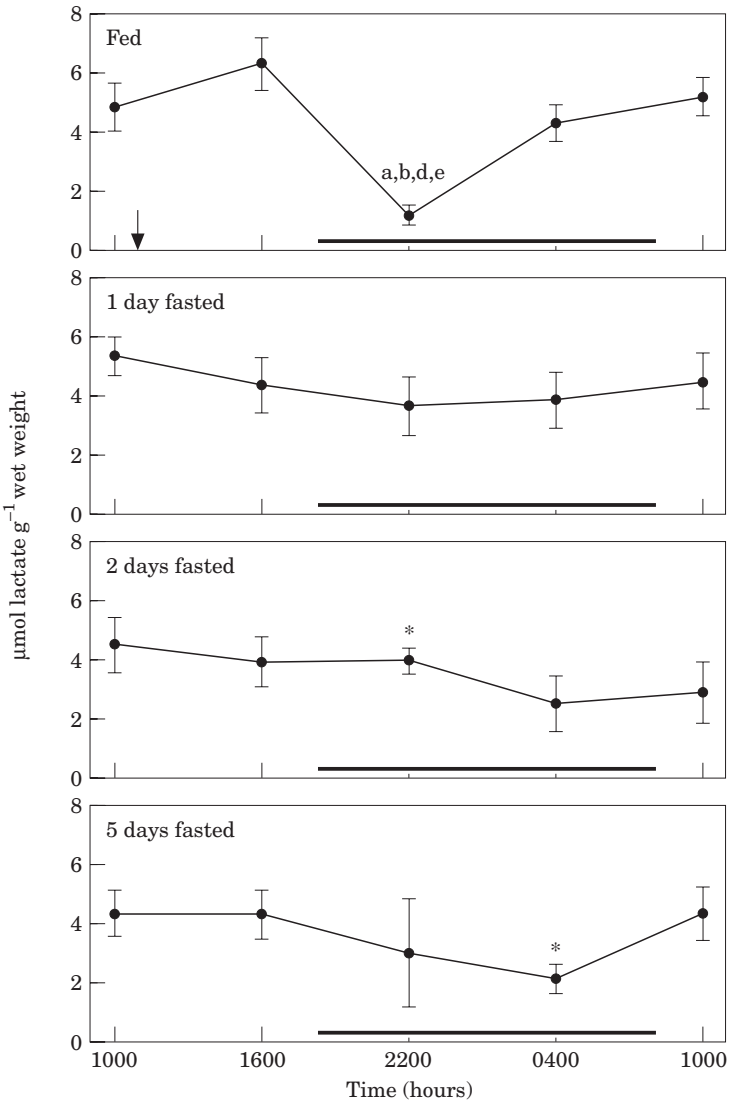

FIG. 5. 24 h changes in liver lactate levels in rainbow trout either fed or following 1, 2 or 5 days of food deprivation. Further details as in legend to Fig. 1.

suggesting that variations of plasma glucose levels were dependent on feeding. However, Holloway *et al.* (1994) using a similar experimental design found daily increases in glycemia in rainbow trout fasted for 3 or 7 weeks. We have no explanation for this rhythm after longer food deprivation, but it is probably related to the increased necessity for glucose synthesis during prolonged starvation and may be controlled by gluconeogenic hormones like glucagon (Navarro *et al.*, 1992) or cortisol (Holloway *et al.*, 1994).

The post-prandial increase in brain glycogen levels of fed rainbow trout agrees with data obtained in Atlantic salmon (Soengas *et al.*, 1996*a*). This increased

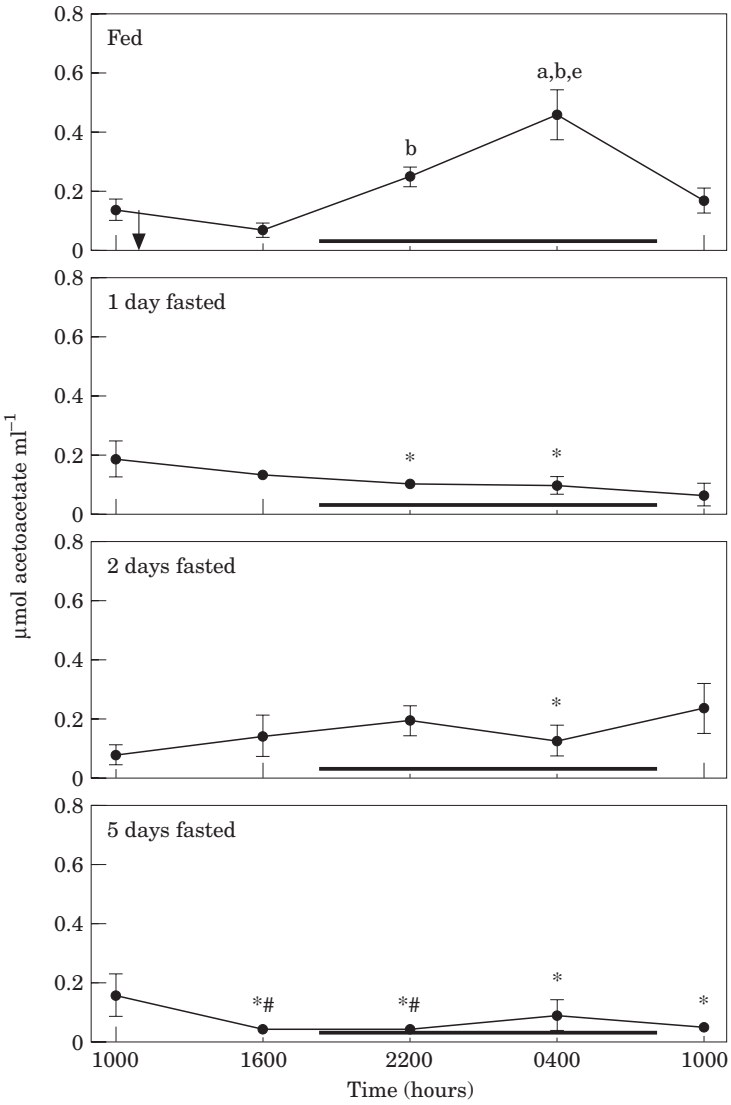

FIG. 6. 24 h changes in plasma acetoacetate levels in rainbow trout either fed or following 1, 2 or 5 days of food deprivation. Further details as in legend to Fig. 1.

brain glycogen level coincides with an increase in the %GSase *a* in this tissue, which might result in increased glycogenesis. The daily increase in brain glycogen levels and %GSase *a* tended to disappear in fasted fish indicating that daily changes observed in fed fish were dependent mostly on feeding. The lower brain glycogen levels in fasted compared with fed fish agree with studies in fasted rainbow trout (Soengas *et al.*, 1998*a*) and Atlantic salmon (Soengas *et al.*, 1996*b*) in which samples were obtained only at one sampling time in the morning.

Brain PFK and LDH activities did not change in any group suggesting feeding was unimportant as a synchronizer of brain glycolytic capacity. This contrasts

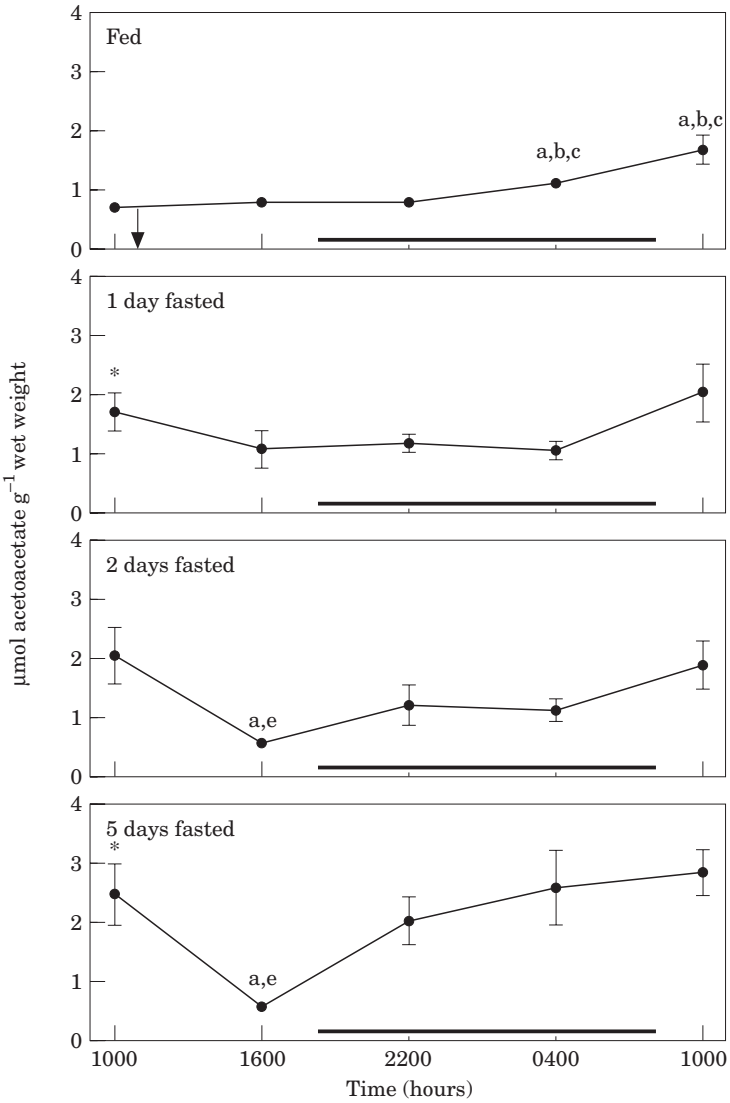

FIG. 7. 24 h changes in brain acetoacetate levels in rainbow trout either fed or following 1, 2 or 5 days of food deprivation. Further details as in legend to Fig. 1.

with the decrease in Atlantic salmon brain LDH activity in the morning (Soengas *et al.*, 1996*b*), but agrees with previous measures of brain PFK activity (Soengas *et al.*, 1996*b*, 1998*a*).

The lowest value of plasma lactate levels of fed fish occurred at 2200 hours, i.e. the time at which the highest levels of plasma glucose were noticed. A similar decline was observed for liver lactate levels suggesting an increased use of lactate at this time. Since post-prandial peaks of gluconeogenic hormones such as glucagon (Navarro *et al.*, 1992) or cortisol (Holloway *et al.*, 1994) occur in salmonids, the decreased plasma lactate levels may be related to an increased use

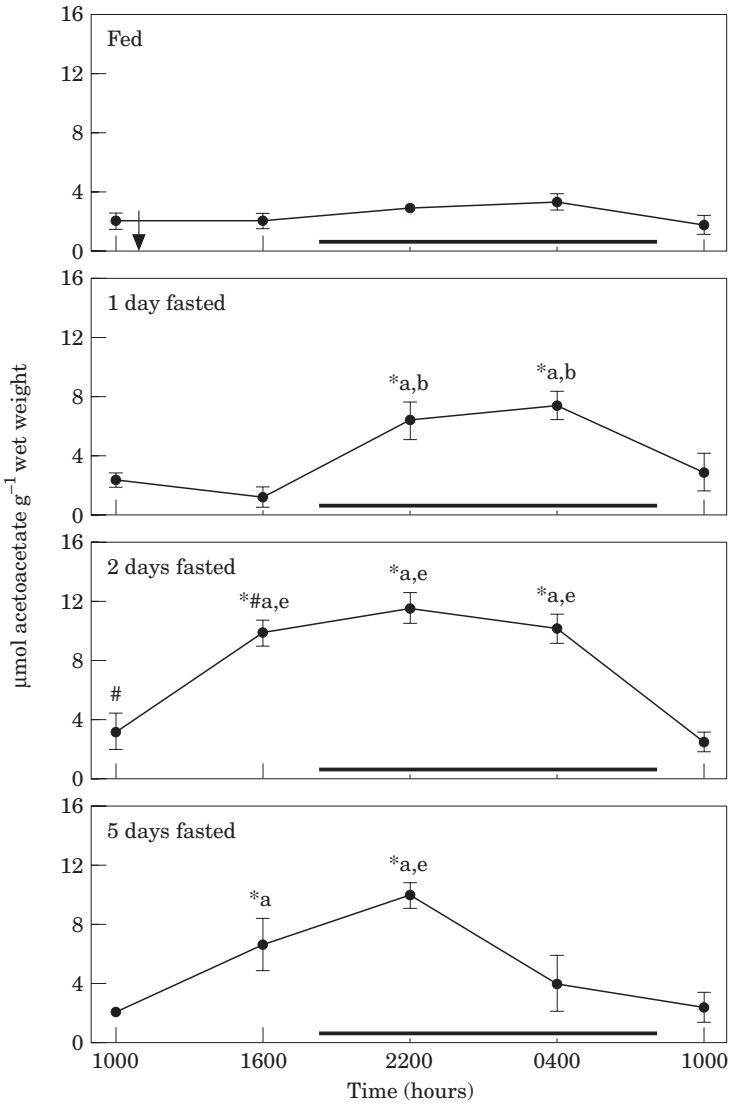

FIG. 8. 24 h changes in liver acetoacetate levels in rainbow trout either fed or following 1, 2 or 5 days of food deprivation. Further details as in legend to Fig. 1.

of a gluconeogenic precursor at that time period. The fall of lactate level at 2200 hours in plasma and liver did not occur in fasted fish, suggesting a dependency on feeding.

Liver glycogen levels increased during the scotophase and decreased during the photophase in fed rainbow trout, in agreement with other studies on rainbow trout (Laidley & Leatherland, 1988), sea bass (Pérez *et al.*, 1988) and tench (de Pedro *et al.*, 1998) but in contrast to other studies on rainbow trout (Hilton *et al.*, 1987; Reddy & Leatherland, 1994) and Atlantic salmon (Soengas *et al.*, 1996*a*). These increased glycogen levels may reflect that at least part of the increased plasma glucose levels post-feeding were directed to the liver to be stored as

glycogen or, alternatively, that part of the stored glycogen may be released as plasma glucose. Overall liver glycogen levels in the fasted were lower than those in fed fish in agreement with other studies in which samples were obtained at one time of day (Navarro *et al.*, 1992; Vijayan & Moon, 1992; Soengas *et al.*, 1996*b*, 1998*a*).

There are few reports on ketone body metabolism in fish (de Roos, 1994; Segner *et al.*, 1997; Soengas *et al.*, 1998*a*). The increase in plasma and brain acetoacetate levels of fed fish during the scotophase probably reflected a fall in the metabolic use of ketone bodies in the brain after feeding. A comparable post-prandial daily increase in plasma acetoacetate levels was observed in sea bass (Carrillo *et al.*, 1982). These changes disappeared in the fasted fish, suggesting the dependence on feeding for the changes observed. Strikingly, decreased acetoacetate levels in the brain were observed at 1600 hours in rainbow trout fasted for 2 and 5 days. The higher brain and lower plasma acetoacetate levels in fasted *v.* fed rainbow trout agrees with results from Atlantic salmon sampled in the morning (Soengas *et al.*, 1996*b*).

Liver ketogenesis is caused by an imbalance between the catabolism of carbohydrates and lipids (Segner *et al.*, 1997). High nocturnal levels of acetoacetate were observed only in liver of fasted fish. Since increased levels of non-esterified fatty acids (NEFA) have been reported to occur at dusk in rainbow trout (Boujard *et al.*, 1993), it may be that ketone body production by the liver is maximal when the highest levels of ketone precursor (NEFA) are available, i.e. at night. Considering that the use of ketone bodies by fish brain is known to increase with fasting (Soengas *et al.*, 1998*a*), it is not surprising to see the increased nocturnal production observed in fasted fish. Since daily changes in liver acetoacetate levels appeared in fasted fish, they may be of a circadian nature not strictly dependent on feeding, and may be related to the effect of hormones like melatonin (Soengas *et al.*, 1998*b*) or cortisol (Reddy & Leatherland, 1995).

In summary, there were marked patterns over 24 h in most metabolic parameters assessed in brain, liver and plasma of fed rainbow trout. Fasting changed the pattern of 24 h changes in a way similar to that described by Holloway *et al.* (1994) regarding hormone concentration, suggesting that feeding is an important factor responsible for these 24 h changes.

This study was supported by research grants from the Xunta de Galicia (PGIDT99PXI30106A) and the Universidade de Vigo (64502C926) to JLS.

References

Aldegunde, M., Andrés, M. D. & Soengas, J. L. (2000). Uptake of 3-*O*-methyl-D-[U-^{14}C] glucose into brain of rainbow trout. Possible effects of melatonin. *Journal of Comparative Physiology B* **170,** 237–243.

Biron, M. & Benfey, T. J. (1994). Cortisol, glucose and hematocrit changes during acute stress, cohort sampling, and the diel cycle in diploid and triploid brook trout (*Salvelinus fontinalis* Mitchill). *Fish Physiology and Biochemistry* **13,** 153–160.

Boujard, T. & Leatherland, J. F. (1992*a*). Circadian rhythms and feeding time in fishes. *Environmental Biology of Fishes* **35,** 109–131.

Boujard, T. & Leatherland, J. F. (1992*b*). Circadian pattern of hepatosomatic index, liver glycogen and lipid content, plasma non-esterified fatty acid, glucose, T_3, T_4,

growth hormone and cortisol concentrations in *Oncorhynchus mykiss* held under different photoperiod regimes and fed using demand-feeders. *Fish Physiology and Biochemistry* **10,** 111–122.

Boujard, T., Brett, S., Lin, L. & Leatherland, J. F. (1993). Effect of restricted access to demand feeders on diurnal pattern of liver composition, plasma metabolites and hormone levels in *Oncorhynchus mykiss. Fish Physiology and Biochemistry* **11,** 337–344.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72,** 248–254.

Carrillo, M., Zanuy, S. & Herrera, E. (1982). Growth and diurnal variations in metabolic parameters in the starved bass, *Dicentrarchus labrax*, after experimental feeding. *Comparative Biochemistry and Physiology* **72A,** 11–16.

Cerdá-Reverter, J. M., Zanuy, S., Carrillo, M. & Madrid, J. (1998). Time-course studies on plasma glucose, insulin, and cortisol in sea bass (*Dicentrarchus labrax*) held under different photoperiodic regimes. *Physiology and Behavior* **64,** 245–250.

Foster, G. D., Youson, J. H. & Moon, T. W. (1993). Carbohydrate metabolism in the brain of the adult lamprey. *Journal of Experimental Zoology* **267,** 27–32.

Gutmann, I. & Wahlefeld, A. W. (1974). L-(+)-Lactate. Determination with lactate dehydrogenase and NAD. In *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1464–1472. New York: Academic Press.

Hilton, J. W., Plisetskaya, E. M. & Leatherland, J. F. (1987). Does oral 3,5,3′-triiodo-L-thyronine affect dietary glucose utilization and plasma insulin levels in rainbow trout (*Salmo gairdneri*)? *Fish Physiology and Biochemistry* **4,** 113–120.

Holloway, A. C., Reddy, P. K., Sheridan, M. A. & Leatherland, J. F. (1994). Diurnal rhythms of plasma growth hormone, somatostatin, thyroid hormones, cortisol and glucose concentrations in rainbow trout, *Oncorhynchus mykiss*, during progressive food deprivation. *Biological Rhythm Research* **25,** 415–432.

Keppler, D. & Decker, K. (1974). Glycogen. Determination with amyloglucosidase. In *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1127–1131. New York: Academic Press.

Laidley, C. W. & Leatherland, J. F. (1988). Circadian studies of plasma cortisol, thyroid hormone, protein, glucose and ion concentration, liver glycogen concentration and liver and spleen weight in rainbow trout, *Salmo gairdneri* Richardson. *Comparative Biochemistry and Physiology* **89A,** 495–502.

Mellanby, J. & Williamson, D. H. (1974). Acetoacetate. In *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1841–1843. New York: Academic Press.

Navarro, I. & Gutiérrez, J. (1995). Fasting and starvation. In *Biochemistry and Molecular Biology of Fishes*, Vol. 4 (Hochachka, P. W. & Mommsen, T. P., eds), pp. 393–434. Amsterdam: Elsevier.

Navarro, I., Gutiérrez, J. & Planas, J. (1992). Changes in plasma glucagon, insulin and tissue metabolites associated with prolonged fasting in brown trout (*Salmo trutta fario*). *Comparative Biochemistry and Physiology* **102A,** 401–407.

Navarro, I., Carneiro, N. M., Párrizas, M., Maestro, J. L., Planas, J. & Gutiérrez, J. (1993). Post-feeding levels of insulin and glucagon in trout (*Salmo trutta fario*). *Comparative Biochemistry and Physiology* **104A,** 389–393.

Pavlidis, M., Berry, M., Divanach, P. & Kentouri, M. (1997). Diel pattern of haematocrit, serum metabolites, osmotic pressure, electrolytes and thyroid hormones in sea bass and sea bream. *Aquaculture International* **5,** 237–247.

Pavlidis, M., Greenwood, L., Paalavuo, M., Mölsa, H. & Laitinen, J. T. (1999). The effect of photoperiod on diel rhythms in serum melatonin, cortisol, glucose, and electrolytes in the common dentex, *Dentex dentex. General and Comparative Endocrinology* **113,** 240–250.

Pedro, N., de Delgado, M. J., Pinnilos, M. L., Alonso-Gómez, A. L. & Alonso-Bedate, M. (1998). Daily rhythms in NAT activity, cortisol, glucose, glycogen and catecholamines in tench (*Tinca tinca* (L.)). *Polish Archives of Hydrobiology* **45,** 321–329.

Pérez, J., Zanuy, S. & Carrillo, M. (1988). Effects of diet and feeding time on daily variations in plasma insulin, hepatic c-AMP and other metabolites in a teleost fish, *Dicentrarchus labrax* L. *Fish Physiology and Biochemistry* **5,** 191–197.

Reddy, P. K. & Leatherland, J. F. (1994). Does the time of feeding affect the diurnal rhythms of plasma hormone and glucose concentration and hepatic glycogen content in rainbow trout? *Fish Physiology and Biochemistry* **13,** 133–140.

Reddy, P. K. & Leatherland, J. F. (1995). Influence of the combination of time of feeding and ration level on the diurnal hormone rhythms in rainbow trout. *Fish Physiology and Biochemistry* **14,** 26–36.

Roos, R. de (1994). Plasma ketone, glucose, lactate, and alanine levels in the vascular supply to and from the brain of the spiny dogfish shark (*Squalus acanthias*). *Journal of Experimental Zoology* **268,** 354–363.

Segner, H., Döle, A. & Böhm, R. (1997). Ketone body metabolism in the carp *Cyprinus carpio*: biochemical and ^{1}H NMR spectroscopical analysis. *Comparative Biochemistry and Physiology* **116B,** 257–262.

Soengas, J. L., Strong, E. F., Fuentes, J., Aldegunde, M. & Andrés, M. D. (1996*a*). Post-feeding carbohydrate and ketone bodies metabolism in brain and liver of Atlantic salmon. *Journal of Physiology and Biochemistry* **52,** 131–142.

Soengas, J. L., Strong, E. F., Fuentes, J., Veira, J. A. R. & Andrés, M. D. (1996*b*). Food deprivation and refeeding in Atlantic salmon, *Salmo salar*: effects on brain and liver carbohydrate and ketone bodies metabolism. *Fish Physiology and Biochemistry* **15,** 491–511.

Soengas, J. L., Strong, E. F. & Andrés, M. D. (1998*a*). Glucose, lactate, and β-hydroxybutyrate utilization by rainbow trout brain: changes during food deprivation. *Physiological Zoology* **71,** 285–293.

Soengas, J. L., Strong, E. F., Andrés, M. D. & Aldegunde, M. (1998*b*). Dose-dependent effects of acute melatonin treatments on brain carbohydrate metabolism of rainbow trout. *Fish Physiology and Biochemistry* **18,** 311–319.

Sundby, A., Eliassen, K., Blom, A. K. & Asgard, T. (1991). Plasma insulin, glucagon, glucagon-like peptide and glucose levels in response to feeding, starvation and life long restricted feed ration in salmonids. *Fish Physiology and Biochemistry* **9,** 253–259.

Tranulis, M. A., Chistophersen, B., Blom, A. K. & Borrebaek, B. (1991). Glucose dehydrogenase, glucose-6-phosphate dehydrogenase and hexokinase in liver of rainbow trout (*Salmo gairdneri*) effects of starvation and temperature variations. *Comparative Biochemistry and Physiology* **99B,** 687–691.

Vijayan, M. M. & Moon, T. W. (1992). Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* **49,** 2260–2266.