Acute Handling Stress Alters Hepatic Glycogen Metabolism in Food-Deprived Rainbow Trout (*Oncorhynchus mykiss*)

M. M. Vijayan and T. W. Moon

Department of Biology, University of Ottawa, Ottawa, Ont. K1N 6N5, Canada

Vijayan, M. M., and T. W. Moon. 1992. Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 49: 2260–2266.

Acute handling stress resulted in significant elevation of plasma cortisol and lactate concentrations within 30 min in both fed and food-deprived rainbow trout (*Oncorhynchus mykiss*), indicating a typical stress response. Plasma glucose levels rose immediately (30 min) poststress in the food-deprived group, while there was a delayed response (2 h) in the fed group. The low liver glycogen content and total glycogen phosphorylase (GPase) and glycogen synthase (GSase) activities in the food-deprived group indicated an overall depression in glycogen metabolism. Acute handling stress maintained liver glycogen stores for up to 4 h in the food-deprived group, but the combined effects of limited substrate and increased energy demand necessitated mobilization of liver glycogen in the food-deprived group, but not in the fed group. This increased glycogen mobilization in the food-deprived group coincided with a secondary elevation in plasma cortisol concentration 4 h poststress. The results indicated that food-deprived rainbow trout were more sensitive to stress of handling and mobilized glycogen stores to meet the energy demand imposed by the stressor. The elevated plasma cortisol levels noted during acute handling stress could play an important role in energy partitioning, metabolically adapting the fish to handling stress.

Le stress aigu causé par la manipulation se traduit par une augmentation significative des concentrations plasmatiques de cortisol et de lactate dans les 30 min qui suivent chez la truite arc-en-ciel (*Oncorhynchus mykiss*) tant alimentée que privée de nourriture, ce qui est une réaction caractéristique du stress. Les concentrations plasmatiques de glucose ont augmenté immédiatement (30 min) après le stress dans le groupe privé de nourriture, tandis qu'on a observé un délai (2 h) dans le réponse du groupe de poissons alimentés. La faible teneur en glycogène hépatique et l'activité totale de la glycogène phosphorylase (GPase) et de la glycogène synthétase (GSase) dans le groupe privé de nourriture indiquaient un déclin global du métabolisme du glycogène. Le stress aigu causé par la manipulation a maintenu les réserves de glycogène hépatique pendant un maximum de 4 h dans le groupe privé de nourriture, mais les effets combinés du substrat limité et de la demande énergétique accrue ont nécessité la mobilisation du glycogène hépatique dans le groupe privé de nourriture, mais pas dans le groupe alimenté. Cette mobilisation accrue du glycogène dans le groupe privé de nourriture a coïncidé avec une augmentation secondaire de la concentration plasmatique de cortisol, 4 h après le stress. Les résultats indiquent que la truite arc-en-ciel privée de nourriture a été plus sensible au stress de la manipulation et a mobilisé ses réserves de glycogène pour répondre à la demande énergétique imposée par la source de stress. Les valeurs élevées de la concentration plasmatique de cortisol observées durant un stress aigu causé par la manipulation pourraient jouer un rôle important dans le fractionnement de l'énergie en permettant au poisson de s'adapter, sur le plant métabolique, au stress de manipulation.

Received January 13, 1992
Accepted May 21, 1992
(JB366)

Reçu le 13 janvier 1992
Accepté le 21 mai 1992

Handling is a common stress in fish aquaculture operations. Such a stressor elicits a primary stress response, which includes elevations in plasma cortisol and catecholamine levels (for reviews, see Mazeaud and Mazeaud 1981; Leatherland and Sonstegard 1984; Barton and Iwama 1991). These hormonal responses initiate a series of secondary stress responses, including alteration in glycogen and/or glucose metabolism, which enable the fish to avoid or cope with the maladaptive effects of the stressor (Mazeaud and Mazeaud 1981; Vijayan et al. 1991).

An important metabolic function of cortisol in teleosts appears to be the regulation of gluconeogenesis and/or glyconeogenesis (Vijayan et al. 1991). Elevated cortisol levels favour energy mobilization (Sheridan 1986; Vijayan et al. 1991), providing the fish with energy to respond to the immediate threat (Schreck 1981). The increased energy demand during stress must be met at the expense of anabolic processes, including growth (Pickering 1990; Vijayan et al. 1990).

Several studies have shown previously that stress affects the growth and performance of fish (for references, see Schreck 1981; Vijayan et al. 1990; Barton and Iwama 1991). Yet, little is known about the metabolic adaptation(s) of fish to stress. When brook trout (*Salvelinus fontinalis*) were stocked at high densities (chronic stressor), they exhibited lower growth rates and energy stores, indicating an increased energy demand (Vijayan et al. 1990). The ability to compensate for an additional stressor (such as acute handling) during chronic stress is impaired if the chronic stress had decreased food intake, thus leading to lower energy reserves (Vijayan et al. 1990).

Liver glycogen stores represent an important energy reserve, but to date, little is known about the temporal changes in glycogen metabolism during stress in fish. Previous studies have shown that hepatic glycogen levels decreased in stressed fish (Rush and Umminger 1978; Paxton et al. 1984; Vijayan et al. 1990). Since chronic cortisol treatment also decreased liver glycogen levels in teleosts (Davis et al. 1985; Barton et al. 1987;

Andersen et al. 1991; Vijayan et al. 1991), cortisol may exert an effect on stress-induced glycogen mobilization.

Nutritional state is known to modify glycogen metabolism in teleosts (see Sheridan and Mommsen 1991). Since the nutritional state also has a profund influence on both the stress response (Barton et al. 1988) and cortisol-induced changes in metabolism (Leach and Taylor 1982) of fish, any alteration in feeding (e.g. due to chronic stress) could modify the stress response and the resulting metabolic adaptation of the fish to an additional stressor.

The purpose of the present study, therefore, was to test two hypotheses: (1) that acute handling stress alters glycogen metabolism in rainbow trout and (2) that food deprivation modifies the primary stress response and glycogen metabolism to an acute handling stress in rainbow trout. Liver glycogen content and the activities of the hepatic enzymes, glycogen phosphorylase (GPase) and glycogen synthase (GSase), were used as indicators of changes in glycogen metabolism.

Materials and Methods

Experimental Animals

Rainbow trout (*Oncorhynchus mykiss*), obtained from Linwood Acres Trout Farm (Campbellcroft, Ontario), were maintained in indoor tanks (300 L) supplied with running, dechlorinated, and well-aerated city water at 7 ± 1°C. The fish were maintained under a 12 h:12 h photoperiod and fed to satiation twice daily with commercial trout pellets (Purina Trout Chow). After 2 wk of acclimation (February 1991), all fish in this stock tank were weighed (in groups of five) and placed in experimental tanks.

Experimental Design

Groups of 20 fish (21 in one tank) (initial body weight 58.9 ± 3 g) were randomly assigned to four experimental tanks (200 L capacity) (two treatments each with a replicate) maintained under identical conditions to the stock tank. Two tanks were randomly chosen for the fed group and food was provided as before (feeding stopped 24 h prior to sampling), and the fish in the other two tanks were deprived of food during the 30-d experimental period.

Six fish were selected at random from each treatment for sampling (three fish from each tank) at the end of the 30-d period. The fish were quickly dip-netted and anaesthetized in a 1:10 000 (w/v) solution of neutralized aminobenzoic acid ethyl ester (MS222, Sigma Chemical Co.). Fish were bled rapidly by caudal puncture into heparinized syringes and the plasma, which was separated by centrifugation, was stored frozen at −80°C. The length and mass of each fish were established to determine condition factor. The liver was removed quickly, weighed (to determine hepatosomatic index, HSI), and freeze-clamped with Wollenberger tongs cooled in liquid nitrogen. Livers remained frozen for a few weeks at −80°C until analysed for glycogen content and GPase and GSase activities.

The remaining fish in the tanks were then subjected to a 3-min handling stress, which included chasing and scooping them in a net. The fish were sampled as above at 0.5 and 24 h poststress. At the other sampling times (1, 2, 4 and 8 h), five or six fish were removed from only one tank (no replicate), thereby alternating sampling among the duplicate tanks. This gave at least a few hours before the next sampling in each tank and prevented consecutive sampling from one tank.

The growth rate ([mean increase in body weight per day/body weight] × 100), condition factor ([body weight in g)/(length in cm)^3] × 100), and HSI ([(liver weight in g)/(body weight in g)] × 100) were calculated. After 30 d, the food-deprived group showed negative growth rate and significantly lower condition factor and HSI than the fed group, evidence that this group was fasted (Table 1).

TABLE 1. Effect of food-deprivation on growth rate, condition factor, and HSI in rainbow trout; *significantly different from the fed group ($p < 0.05$, ANOVA).

	Fed	Food-deprived
Growth rate (% wt./day)[a]	0.6 ± 0.1 (2)	−0.38 ± 0.04 (2)*
Condition factor [b]	1.13 ± 0.01 (41)	0.94 ± 0.01 (40)*
HSI[b]	1.33 ± 0.03 (41)	0.87 ± 0.02 (40)*

[a]Values represent mean ± SEM (n = total weight of all fish from two tanks).

[b]Values represent mean ± SEM (n = 40 or 41; values were pooled because there were no significant differences between the time periods).

Plasma cortisol concentrations were measured using a radioimmunoassay kit (ICN Biomedicals, Carson, CA) according to Andersen et al. (1991), while plasma glucose and liver glycogen contents were analysed enzymatically according to Foster and Moon (1989). Plasma lactate was enzymatically determined according to Bergmeyer (1983).

Hepatic enzyme activities were estimated according to Moon et al. (1989). Briefly, frozen liver pieces were sonicated (Kontes cell disrupter) in four volumes of a phosphorylation–dephosphorylation "stopping buffer" (Foster and Moon 1989) and centrifuged for 10 min at 12 000 × g. The supernatant was applied to a Sephadex G-25 column (Moon et al. 1989), and the eluant was used for measuring enzyme activity at 10°C using a Beckman DU-65 recording spectrophotometer.

GPase Assay

A two-buffer system was used to distinguish GPase a from GPase total (a + b). The ratio of GPase activities with and without caffeine, but in the presence of AMP, represents the percentage of total GPase (a + b) in the active form (a) (% GPase a) (Moon et al. 1989). Enzyme activity is expressed in units (micromoles glucosyl units per minute) per gram tissue.

GSase Assay

Glycogen synthase activity was measured according to the method of Thomas et al. (1968). Briefly, the activity was measured at 15°C in the presence of 5 mM UDP-[U-^{14}C]glucose (UDPG), 1% (w/v) glycogen, 1 mM EDTA, 20 mM glycylglycine, pH 7.4, and 10 mM Na_2SO_4 without (GSase a) or with 9 mM glucose 6-phosphate (GSase a + b). Enzyme activity is expressed in milliunits (nanomoles UDPG transformed to glycogen per minute under the assay conditions) per gram tissue. Results are presented as the percentage of total GSase (a + b) in the active form (a) (% GSase a).

Chemicals

Unless otherwise indicated, all biochemicals were obtained from Sigma Chemical Co., St. Louis, MO, or Boehringer Mannheim Co., Montreal, Que. All other reagents were purchased from local suppliers and were of the highest available purity.

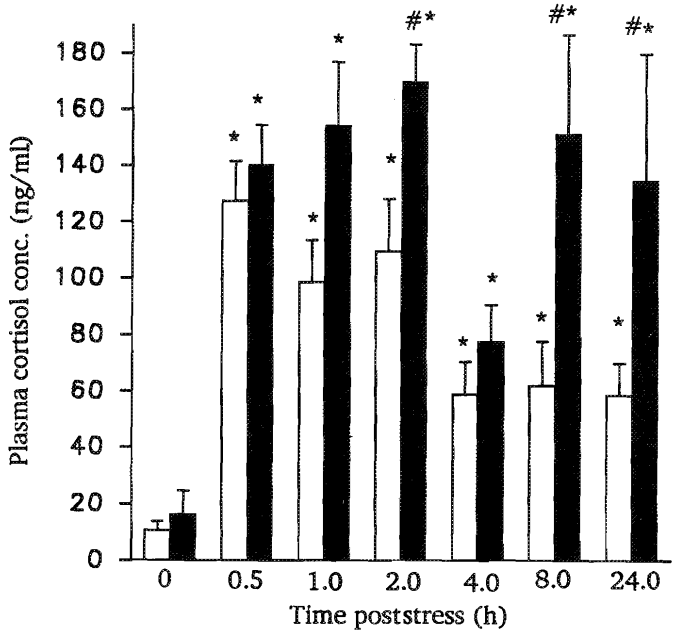

FIG. 1. Temporal changes in plasma cortisol concentrations after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean ± SEM (n = 4–6); *significantly different from the 0-h sampling period ($p < 0.05$, ANOVA); #significantly different from the fed group ($p < 0.05$, ANOVA).

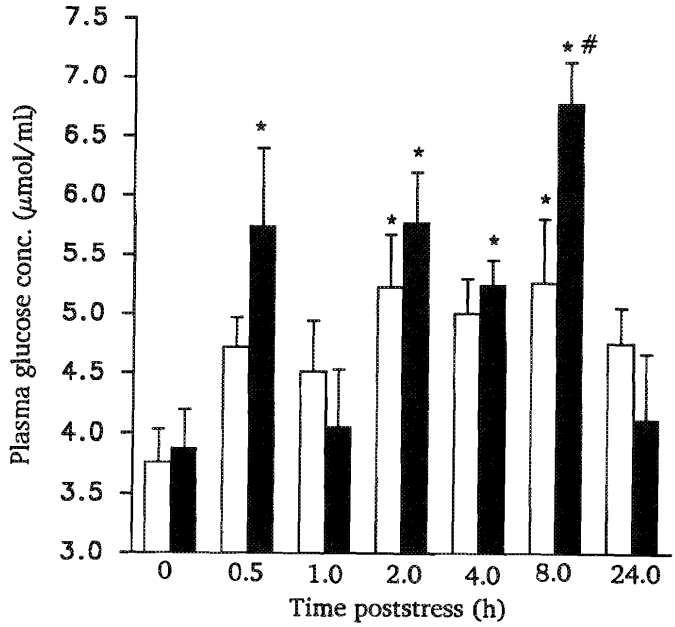

FIG. 2. Temporal changes in plasma glucose concentrations after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean ± SEM (n = 4–6); symbols as in Fig. 1.

Statistical Analysis

Data obtained were analysed statistically using two-way analysis of variance with treatment and time as factors. Where F values indicated significance ($p < 0.05$), means were compared using Tukey's test. There were no tank effects on any of the parameters tested at replicate sampling periods (0, 0.5, and 24 h poststress). Log-transformed data were used wherever necessary to satisfy homogeneity of variance, although non-transformed data are shown in the text.

Results

Plasma Parameters

There were no significant differences between the fed and food-deprived groups in any of the plasma parameters measured before acute handling stress (0 h sample; Fig. 1–3). Acute handling stress resulted in significant elevation of plasma cortisol levels within 30 min in both the fed and food-deprived groups. Plasma cortisol levels dropped at 4 h in both groups, but remained significantly elevated above prestress levels (Fig. 1). After 4 h, plasma cortisol levels remained stable in the fed group for the rest of the sampling times, while the levels rose again in the food-deprived group and were significantly higher than in the fed group at 8 and 24 h poststress (Fig. 1). Two-way analysis of variance showed a significant treatment effect, with the food-deprived group having significantly higher cortisol levels than the fed group.

Plasma glucose levels increased significantly within 30 min poststress in the food-deprived group and remained elevated for 8 h, with the exception of a drop at 1 h poststress (Fig. 2). In the fed group, the rise in plasma glucose levels was more gradual and it was significantly above the prestress level only at 2 and 8 h poststress. Plasma glucose levels in the food-deprived group were significantly higher than in the fed group

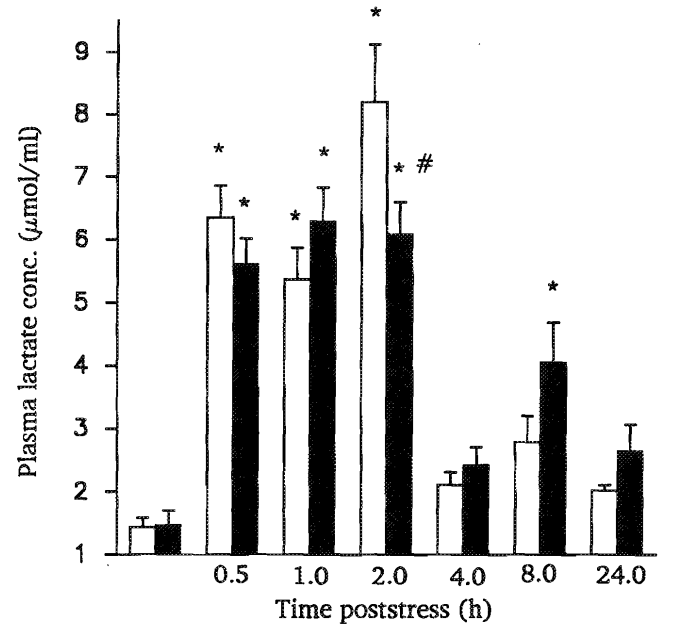

FIG. 3. Temporal changes in plasma lactate concentrations after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean ± SEM (n = 5–6); symbols as in Fig. 1.

at 8 h poststress after which the levels in both groups fell to levels which did not differ from prestress values.

Acute handling stress resulted in significant elevation of plasma lactate in both groups between 0.5 and 2 h poststress (Fig. 3). Plasma lactate levels dropped thereafter in both groups and were not significantly different from the prestress level in the fed group for the rest of the recovery period, but increased significantly in the food-deprived group at 8 h poststress. The

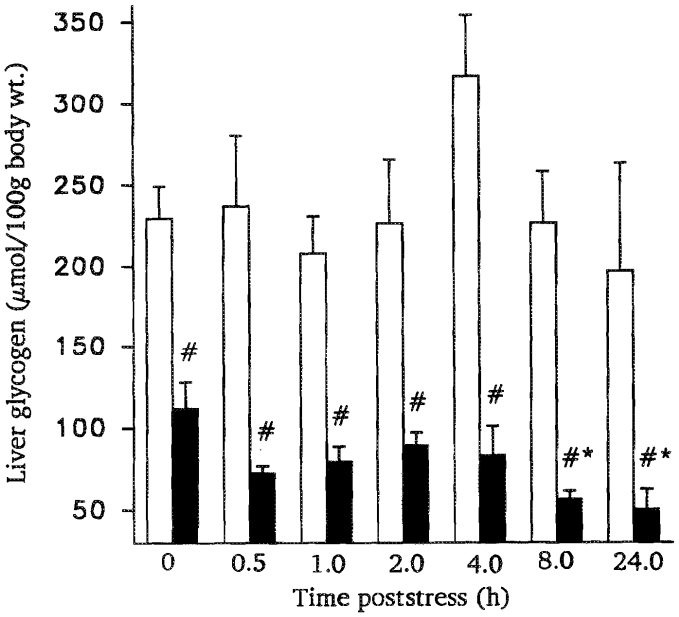

FIG. 4. Temporal changes in liver glycogen content as a function of body mass after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean ± SEM (n = 4–6); symbols as in Fig. 1.

food-deprived group had significantly lower plasma lactate than the fed group at 2 h poststress.

Liver Parameters

Food deprivation significantly lowered liver glycogen content in rainbow trout and this persisted at all time periods after the acute stress (Fig. 4). Handling stress did not alter liver glycogen content in the fed group over the 24 h period, while liver glycogen content in the food-deprived group dropped significantly at 8 and 24 h poststress.

The total hepatic activities of GPase and GSase were significantly lower in the food-deprived group than in the fed group (Table 2). There was a significant drop in total GPase activity at 0.5 h poststress in the fed group and at 8 h in both fed and food-deprived groups. Acute handling stress resulted in significant lowering of total GSase activity at 0.5 h in both treatment groups.

On the one hand, there was no significant difference in % GPase *a* (0-h sample; Fig. 5) between the fed and food-deprived groups prestress. On the other hand, % GSase *a* (0-h sample; Fig. 6) was significantly higher in the food-deprived group, suggesting an increased glyconeogenic potential.

Acute handling stress resulted in a significant decrease in hepatic % GPase *a* in the fed group at 4 and 24 h poststress (Fig. 5). In the food-deprived group, % GPase *a* was significantly higher than the prestress value only at 8 h poststress and remained consistent throughout the other sampling times. When compared with the fed group, the food-deprived group had significantly lower % GPase *a* at 1 h and significantly higher % GPase *a* at 4, 8, and 24 h poststress, although these differences are due primarily to a change in the fed rather than the food-deprived group.

Percent GSase *a* showed a gradual increase post-stress in the fed group achieving significantly higher levels at 8 and 24 h poststress (Fig. 6). In the food-deprived group, % GSase *a* activity decreased significantly compared with the prestress level at 0.5 and 1 h poststress, after which the value showed an increasing trend reaching a significantly higher value at 4 h poststress and falling back to prestressed levels thereafter. At 4 h poststress, the food-deprived group had significantly higher % GSase *a* than the fed group (Fig. 6).

Discussion

The results from the present study, based on plasma and liver metabolite levels, clearly indicate that food deprivation increased the sensitivity of these trout to the stress of handling compared with the fed controls. Plasma cortisol and glucose concentrations are commnly used as indicators of primary and secondary stress responses to an acute stress in fish (see the introduction for references). The immediate elevation in plasma cortisol concentration following an acute stress seen in the present study supports the above statement. The secondary elevation (after 4 h) in plasma parameters occurred only in the food-deprived group and could potentially be due to other factors, including circadian rhythms. Several studies have demonstrated that salmonids exhibit circadian rhythms in plasma cortisol concentrations (Rance et al. 1982; Pickering and Pottinger 1983; Nichols and Weisbart 1984). Since the secondary elevation in plasma cortisol concentration did not occur in the fed fish, the absence of feeding may alter these endogenous rhythms in trout.

In the present study, there were no differences in any plasma parameters studied at 30 d of food deprivation (0-h sample; Fig. 1–3). Plasma cortisol and lactate levels did not change with 30 d of food deprivation in brook trout (Vijayan et al. 1991). Plasma glucose levels, however, are variable with food deprivation, showing either no change (Sheridan and Mommsen

TABLE 2. Temporal changes in hepatic total GPase and GSase activities after an acute handling stress in fed and food-deprived (FD) rainbow trout. Values represent mean ± SEM (n = 4 or 6); enzyme activity is expressed as micromoles glucosyl units per minute per 100 g body weight (taking HSI into account); *significantly different from the 0-h sampling period ($p < 0.05$, ANOVA).

Time (h)	GPase		GSase	
	Fed	FD[a]	Fed	FD[a]
0	3.65 ± 0.35	2.34 ± 0.04	0.41 ± 0.07	0.38 ± 0.05
0.5	2.80 ± 0.37*	2.70 ± 0.24	0.22 ± 0.02*	0.18 ± 0.02*
1.0	3.13 ± 0.26	2.65 ± 0.29	0.37 ± 0.09	0.27 ± 0.02
2.0	3.05 ± 0.11	2.46 ± 0.16	0.46 ± 0.06	0.41 ± 0.08
4.0	3.58 ± 0.30	2.16 ± 0.15	0.28 ± 0.03	0.45 ± 0.06
8.0	1.96 ± 0.21*	1.82 ± 0.16*	0.52 ± 0.13	0.26 ± 0.02
24.0	3.13 ± 0.17	2.19 ± 0.12	0.54 ± 0.06	0.34 ± 0.11

[a]Significantly different from the fed group ($p < 0.05$) at all sampling times.

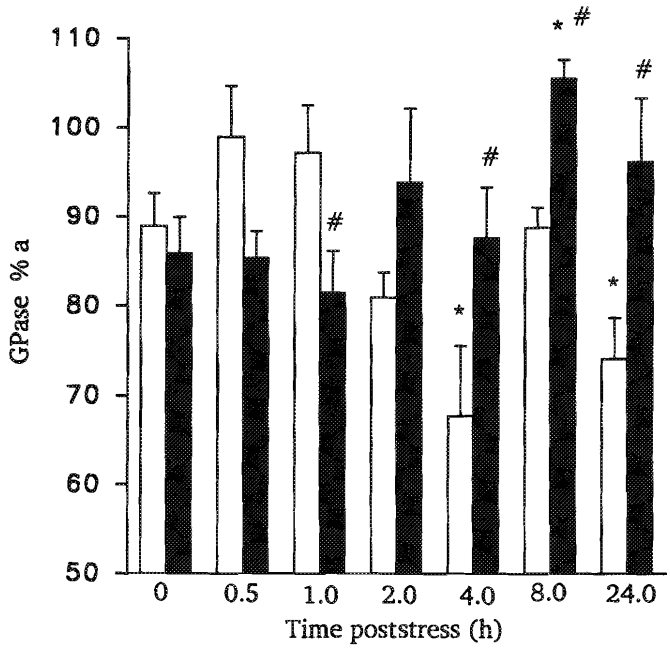

FIG. 5. Temporal changes in percent GPase *a* levels in liver after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean ± SEM (n = 5–6); symbols as in Fig. 1.

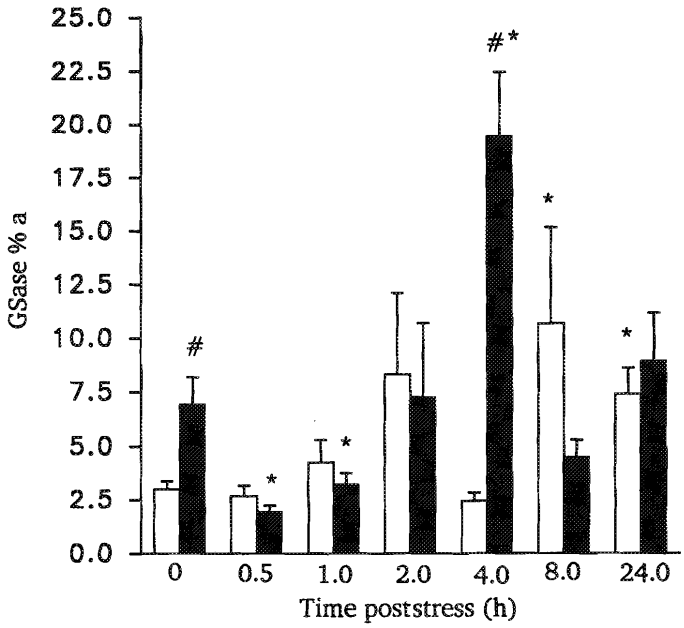

FIG. 6. Temporal changes in percent GSase *a* levels in liver after an acute handling stress in fed (open bar) and food-deprived (solid bar) rainbow trout. Values represent mean ± SEM (n = 5–6); symbols as in Fig. 1.

1991; present study) or a decrease (Barton et al. 1988; Moon et al. 1989; Foster and Moon 1991; Vijayan et al. 1991) with food deprivation. Several factors might be responsible for this variable glucose response, including differences in species, age, season, temperature, length of fast, and prior rearing of the fish (Suarez and Mommsen 1987).

The higher plasma cortisol levels in the food-deprived group could be attributed to several possibilities. First, the interrenal tissue may be sensitized to the stressor in the food-deprived group, resulting in higher cortisol secretion rates. It has been shown previously in both brook trout and coho salmon (*Oncorhynchus kisutch*) that interrenal tissue from fish subjected to a stressor (high stocking density) had higher cortisol secretion rates (Patino et al. 1986; Vijayan and Leatherland 1990). Second, the clearance of cortisol may be attenuated by food deprivation, resulting in consistently higher levels of cortisol concentration (Fig. 1). This, however, seems unlikely, as the levels decreased at 4 h in both groups, suggesting a similar clearance mechanism. The explanation for the fall in plasma cortisol concentration at 4 h is not clear, but could be due to increased liver uptake and catabolism (Vijayan and Leatherland 1990). It seems, therefore, that with food deprivation the secretion of cortisol may far exceed the clearance, resulting in elevated plasma cortisol levels. Plasma cortisol levels remained elevated in both groups even at 24 h poststress (compared with the prestress levels). This result is consistent with that of Barton et al. (1987), where plasma cortisol levels remained elevated up to 24 h after a 30-s handling stress in juvenile rainbow trout. Another possible explanation for the elevated cortisol levels may be the density changes associated with fish removal after each sampling. Although the handling stress elevated plasma cortisol levels in both groups, the effects of stress on hepatic glycogen metabolism were evident only in the food-deprived group, suggesting that nutritional state of the animal modifies the stress response (Barton et al. 1988).

Plasma lactate increased with stress in both groups (Fig. 3), peaking at about 2 h, which is in agreement with studies on exercise in teleosts (Wood and Perry 1985). The transient increase in plasma lactate at 8 h in the food-deprived group indicates an increased stress response, in concert with both plasma cortisol (Fig. 1) and glucose (Fig. 2) increases. However, it is yet to be ascertained if cortisol has any direct role in this secondary lactate release. Cortisol may also modulate the rate of utilization of lactate. Earlier studies have indicated both an increased oxidation in fish treated with cortisol (Chan and Woo 1978) and an increase in the rate of gluconeogenesis from lactate in hepatocytes incubated with cortisol (Renaud and Moon 1980).

The increased plasma glucose level in rainbow trout after an acute handling stress could potentially be due to the action of cortisol. It has been shown that plasma cortisol and glucose levels increase with acute stress in fish (for references, see Barton and Iwama 1991). Moreover, several studies have shown that cortisol increased the gluconeogenic capacity in teleosts (for references, see Vijayan et al. 1991), although plasma glucose levels are variable and are not solely indicative of an increased cortisol concentration. The reason for this discrepancy between plasma cortisol and glucose may be related to an altered glucose turnover, and if so, plasma glucose levels are not a good indicator of long-term glucose metabolism (Suarez and Mommsen 1987).

Glucose elevation in the food-deprived group was immediate after handling stress (within 30 min) as opposed to a delayed response (2 h) in the fed group (Fig. 2). This rapid elevation in plasma glucose in the food-deprived group could be due to the action of catecholamines. Under acute stress, catecholamines are released rapidly, directing the phosphorylation of the inactive form of GPase, resulting in increased glycogenolysis in fish (Perry and Wood 1989; Wright et al. 1989). Surprisingly, there was no effect on % GPase *a* in the food-deprived group at 30 min. One possible explanation may be that % GPase *a* was already under maximum or near maximum stimulation (>80%) (Fig. 5) and was adequate to provide the necessary

plasma glucose. The changes in plasma glucose in the absence of changes in % GPase *a* at 30 min suggest that some other mechanism(s) may be regulating glucose availability in this species during acute stress. It may also be that the phosphorylation and dephosphorylation of GSase plays an important regulating role in the shift in metabolic flux. The significant lowering of % GSase *a* at 30 min in the food-deprived group implies a reduction in the glyconeogenic capacity poststress. This response, however, was not seen in the fed group, implying that food deprivation may modify the response of liver to catecholamine action. Reid et al. (1992) have recently demonstrated that chronic cortisol treatment alters β-receptor dynamics in rainbow trout hepatocytes, making them more sensitive to catecholamine action. From the present result, it appears that the nutritional state may either directly influence catecholamine responsiveness or indirectly favour catecholamine sensitivity through the elevation of cortisol concentration. Further work is necessary before the process can be clearly explained.

This study is the first to examine the temporal changes in glycogen metabolism in response to an acute handling stress in a teleost. The results clearly indicate that handling stress mobilizes hepatic glycogen stores to a greater extent in food-deprived rainbow trout than in fed fish.

The increased % GSase *a* in the food-deprived group prior to stress (0-h sample) suggests that glyconeogenesis is enhanced to replenish glycogen stores should adequate substrate be available. The negative growth rate and lower condition factor in the food-deprived group at 30 d (Table 1) support the idea that at least body protein mobilized in food-deprived rainbow trout is utilized to provide some glycogen synthesis in order to conserve the glycogen which does exist (Sheridan and Mommsen 1991). The low liver glycogen content together with decreased total activities of both GPase and GSase in the food-deprived group indicates a reduction in glycogen metabolism (Fig. 4; Table 2). Thus, their metabolic potential was severely compromised compared with the fed fish. Foster and Moon (1991) have shown an overall depression in the hepatic metabolic potential of yellow perch (*Perca flavescens*) during fasting. In such a situation the fish may not have been able to maintain glycogen stores at the expense of other substrates; any additional demand may have led to increased glycogen mobilization. Despite this, the food-deprived fish did maintain their glycogen stores initially at a level not significantly different from the prestress level, possibly by glyconeogenesis as the 4-h increase in % GSase *a* (Fig. 6) seems to indicate. Due to their reduced metabolic reserves (Table 1), however, the substrate available to fuel metabolic demands cannot be maintained by breakdown of proteins and lipids alone, resulting in enhanced liver glycogen mobilization at 8 and 24 h (Fig. 4). This increase in mobilization is associated with an increased % GPase *a* (Fig. 5). If another stress or a more substantial stress were to occur, one questions whether these fish could survive.

Acute handling stress had no effect on liver glycogen content in the fed group. Since the fed fish are in a positive energy balance, as shown by their growth rate and condition factor (Table 1), they have abundant energy reserves for mobilization. When exposed to a stressor, the fish probably rely primarily on lipids and proteins for their energy needs as well as for replenishing their glycogen stores. Elevated cortisol levels poststress might favour lipid and protein mobilization for energy and gluconeogenesis (Foster and Moon 1986; Vijayan et al. 1991), thereby allowing the fish to cope with the immediate stress without depleting glycogen stores (Sheridan and Mommsen 1991). In fact, decreases in % GPase *a* at 4 and 24 h (Fig. 5) and increases in % GSase *a* at 8 and 24 h (Fig. 6) would assist in the restoration of any glycogen which may have been used during early periods of the stress.

It is possible that cortisol is playing a direct role in glycogen mobilization under acute stress situations. Recently, treatment of brook trout with RU486 (a glucocorticoid antagonist) resulted in significant elevation of liver glycogen content, suggesting that cortisol might have a direct effect on hepatic glycogen metabolism (Vijayan and Leatherland 1992). Even in the present study, increased glycogen mobilization in the food-deprived group poststress coincided with a secondary elevation in plasma cortisol levels 4 h poststress (Fig. 1 and 4). The change in glycogen mobilization was observed only in the food-deprived group, indicating that the nutritional state of the animal influences the cortisol response in this species (Leach and Taylor 1982; Vijayan et al. 1991).

In conclusion, the results indicate that food deprivation in rainbow trout is associated with a more severe metabolic effect to acute handling stress than the fed group. Liver glycogen content may be maintained just after handling stress by enhanced glyconeogenesis in the food-deprived group. However, the recovery process in the food-deprived group requires far more energy than in the fed group because of its low metabolic reserves, resulting in glycogen depletion. Liver glycogen content may be an important factor regulating the performance of the fish because of its utilization for immediate energy requirement. Long-term depletion of hepatic glycogen stores might be detrimental to the fish because metabolically they are less capable of tolerating an additional stressor. Cortisol appears to be an important glucoregulatory hormone in rainbow trout, mobilizing energy stores to compensate for the demand imposed by the handling stress during food deprivation.

Acknowledgements

The glycogen synthase assay was carried out during a visit to the laboratory of Dr. Gerald van de Werve, Department of Nutrition, Université de Montréal, with the assistance of Mr. Glen Foster. The authors are indebted to Dr. van de Werve for his help with the assay. The authors thank Prof. Steve Perry for reviewing the manuscript. This study was supported by NSERC grant No. A6944 to T.W.M. and a NSERC postdoctoral fellowship to M.M.V.

References

ANDERSEN, D. E., S. D. REID, T. W. MOON, AND S. F. PERRY. 1991. Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1811–1817.

BARTON, B. A., AND G. K. IWAMA. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1: 3–26.

BARTON, B. A., C. B. SCHRECK, AND L. D. BARTON. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis. Aquat. Org. 2: 173–185.

BARTON, B. A., C. B. SCHRECK, AND L. G. FOWLER. 1988. Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile chinook salmon. Prog. Fish-Cult. 50: 16–22.

BERGMEYER, H. U. [ED.] 1983. Methods of enzymatic analysis. Vol. VI. 3rd ed. Verlag Chemie, Weinheim. p. 163–172 and 582–588.

CHAN, D. K. O., AND N. Y. S. WOO. 1978. Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. Gen. Comp. Endocrinol. 35: 205–215.

DAVIS, K. B., P. TORRANCE, N. C. PARKER, AND M. A. SUTTLE. 1985. Growth, body composition and hepatic tyrosine aminotransferase activity in cortisol-fed channel catfish, *Ictalurus punctatus* Rafinesque. J. Fish Biol. 27: 177–184.

FOSTER, G. D., AND T. W. MOON. 1986. Cortisol and liver metabolism of immature American eels, *Anguilla rostrata* (LeSueur). Fish Physiol. Biochem. 1: 113–124.

1989. Insulin and the regulation of glycogen metabolism and gluconeogenesis in American eel hepatocytes. Gen. Comp. Endocrinol. 73: 374–381.

1991. Hypometabolism with fasting in the yellow perch (*Perca flavescens*): a study of enzymes, hepatocyte metabolism, and tissue size. Physiol. Zool. 64: 259–275.

LEACH, G. J., AND M. H. TAYLOR. 1982. The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 48: 76–83.

LEATHERLAND, J. F., AND R. A. SONSTEGARD. 1984. Pathobiological responses of feral teleosts to environmental stressors: interlake studies of the physiology of Great Lake salmon, p. 116–149. *In* J. O. Nriagu, V. Cairns, and P. Hodson [ed.] Monitoring contaminant effects on fisheries. Wiley, Toronto, Ont.

MAZEAUD, M. M., AND F. MAZEAUD. 1981. The role of catecholamines in the stress response of fish, p. 49–75. *In* A. D. Pickering [ed.] Stress and fish. Academic Press, London.

MOON, T. W., G. D. FOSTER, AND E. M. PLISETSKAYA. 1989. Changes in peptide hormones and liver enzymes in the rainbow trout deprived of food for 6 weeks. Can. J. Zool. 67: 2189–2193.

NICHOLS, D. J., AND M. WEISBART. 1984. Plasma cortisol concentrations in Atlantic salmon, *Salmo salar*: episodic variations, diurnal change, and short term response to adrenocorticotropic hormone. Gen. Comp. Endocrinol. 56: 169–176.

PATINO, R., C. B. SCHRECK, J. L. BLANKS, AND W. S. ZAUGG. 1986. Effects of rearing conditions on the developmental physiology of smolting coho salmon. Trans. Am. Fish. Soc. 115: 828–837.

PAXTON, R., D. H. GIST, AND B. L. UMMINGER. 1984. Serum cortisol levels in thermally-acclimated goldfish (*Carassius auratus*) and killifish (*Fundulus heteroclitus*): implications in control of hepatic glycogen metabolism. Comp. Biochem. Physiol. B 8: 813–816.

PERRY, S. F., AND C. M. WOOD. 1989. Control and coordination of gas transfer in fishes. Can. J. Zool. 67: 2961–2970.

PICKERING, A. D. 1990. Stress and the suppression of somatic growth in teleost fish, p. 473–479. *In* A. Epple, C. G. Scanes, and M. H. Stetson [ed.] Progress in comparative endocrinology. Wiley-Liss, Inc., New York, NY.

PICKERING, A. D., AND T. G. POTTINGER. 1983. Seasonal and diel changes in plasma cortisol levels of the brown trout, *Salmo trutta* L. Gen. Comp. Endocrinol. 49: 232–239.

RANCE, T. A., B. I. BAKER, AND G. WEBLEY. 1982. Variations in plasma cortisol concentrations over a 24-hour period in the rainbow trout *Salmo gairdneri*. Gen. Comp. Endocrinol. 48: 269–274.

REID, S. D., T. W. MOON, AND S. F. PERRY. 1992. Rainbow trout (*Oncorhynchus mykiss*) hepatocyte β-adrenoceptors, catecholamine responsiveness and the effects of cortisol. Am. J. Physiol. 262: R794–R799.

RENAUD, J. M., AND T. W. MOON. 1980. Characterization of gluconeogenesis in hepatocytes isolated from the American eel, *Anguilla rostrata* Lesueur. J. Comp. Physiol. B 135: 115–125.

RUSH, S. B., AND B. L. UMMINGER. 1978. Elimination of stress-induced changes in carbohydrate metabolism of goldfish (*Carassius auratus*) by training. Comp. Biochem. Physiol. A 60: 69–73.

SCHRECK, C. B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors, p. 295–321. *In* A. D. Pickering [ed.] Stress and fish. Academic Press, London.

SHERIDAN, M. A. 1986. Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. Gen. Comp. Endocrinol. 64: 220–238.

SHERIDAN, M. A., AND T. P. MOMMSEN. 1991. Effects of nutritional state on in vivo lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 81: 473–483.

SUAREZ, R. K., AND T. P. MOMMSEN. 1987. Gluconeogenesis in teleost fishes. Can. J. Zool. 65: 1869–1882.

THOMAS, S. A., K. K. SCHLENDER, AND J. LARNER. 1968. A rapid filter paper assay for UDPG glucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-^{14}C-glucose. Anal. Biochem. 25: 486–499.

VIJAYAN, M. M., J. S. BALLANTYNE, AND J. F. LEATHERLAND. 1990. High stocking density alters the energy metabolism of brook charr, *Salvelinus fontinalis*. Aquaculture 88: 371–381.

1991. Cortisol-induced changes in some aspects of the intermediary metabolism of *Salvelinus fontinalis*. Gen. Comp. Endocrinol. 82: 476–486.

VIJAYAN, M. M., AND J. F. LEATHERLAND. 1990. High stocking density affects cortisol secretion and tissue distribution in brook charr, *Salvelinus fontinalis*. J. Endocrinol. 124: 311–318.

1992. *In vivo* effects of the steroid analogue RU486 on some aspects of intermediary and thyroid metabolism of brook charr, *Salvelinus fontinalis*. J. Exp. Zool. 263: 265–271.

WOOD, C. M., AND S. F. PERRY. 1985. Respiratory, circulatory, and metabolic adjustments to exercise in fish, p. 2–22. *In* R. Gilles [ed.] Circulation, respiration, metabolism. Spring-Verlag, Berlin.

WRIGHT, P. A., S. F. PERRY, AND T. W. MOON. 1989. Regulation of hepatic gluconeogenesis and glycogenolysis by catecholamines in rainbow trout during environmental hypoxia. J. Exp. Biol. 147: 169–188.