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The effects of starvation on plasma free amino acid and glucose concentrations in lake sturgeon

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The effect of starvation on the metabolism of the lake sturgeon *Acipenser fulvescens* was examined by measuring haematocrit, plasma glucose concentrations, and plasma free amino acids. Plasma was sampled on day 0, 10, 20, 45 and 60 of a 60-day starvation period. Haematocrit was observed to decrease with starvation indicating a decreased oxygen carrying capacity of the blood. Plasma glucose levels differed only at day 10, with a decrease in blood glucose level in the starved group. No differences were detected between groups for alanine, aspartate, and serine, while elevated levels were observed for glutamine throughout the experiment. An increase in arginine, tyrosine, valine, methionine, tryptophan, phenylalanine, glutamate, glycine, isoleucine, histidine and leucine, concentrations were observed after 45 days of starvation. The maintenance, or increased plasma levels, of glucogenic amino acids in combination with the maintenance of blood glucose concentrations indicates active gluconeogenic processes in the liver supported by muscle proteolysis.

Key words: amino acids; starvation; fasting; glucose; lake sturgeon; *Acipenser fulvescens*; plasma.

INTRODUCTION

Fish deal with periods of low food abundance in a species-specific way, which will be affected also by environmental factors. However, the generally accepted model of metabolic reorganization in a teleost, during starvation, is a multiphasic, selective consumption of stored energy which can involve the progressive degradation of expendable tissues (Love & Black, 1990). This translates into the mobilization of lipids, carbohydrates, and amino acids, for the production of ATP by the peripheral tissues. Liver glycogen is usually the first substrate to be mobilized during starvation (Navarro & Gutiérrez, 1995). However, tissue glycogen levels (Mommsen *et al.*, 1980; Love & Black, 1990; Gerrits, 1994) and plasma glucose concentrations are usually defended during starvation (Blasco *et al.*, 1992; Navarro & Gutiérrez, 1995). To accomplish this, the rate of gluconeogenesis increases in the liver and kidney (Morata *et al.*, 1982; Foster & Moon, 1991). Gluconeogenesis occurs primarily from amino acids, mobilized from the peripheral tissues (Machado *et al.*, 1988; Blasco *et al.*, 1992).

Lipid reserves are thought to be utilized preferentially over protein stores in the early stages of starvation for the production of ATP (Black & Love, 1986; Love & Black, 1990; Navarro & Gutiérrez, 1995). Although lipids cannot be used to synthesize carbohydrates, their oxidation can provide much of the ATP needed for carbohydrate synthesis and other functions. As lipid stores become

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depleted, amino acids released through the proteolysis of body tissues become more important as energy sources (Navarro & Gutiérrez, 1995). Amino acids are mobilized primarily from white muscle during starvation in fish (Love, 1970; Love & Lavety, 1977; Lowery & Somero, 1990).

Although starvation has been examined extensively in teleost fishes especially salmonids, little is known of its metabolic consequences in other fish groups. The chondrostean lake sturgeon *Acipenser fulvescens* Rafinesque, like many other temperate fish species, regularly experiences periods of starvation as a result of natural seasonal fluctuations in prey species and temperature. Survival during such periods of starvation depends on an ability to adapt metabolic processes to re-allocate reserves to supply the various needs of different tissues. The nature of this metabolic reorganization in sturgeon is not well understood.

Sturgeon, as primitive osteichthyans, represent an important evolutionary link between the primitive elasmobranchs and the more advanced teleosts. The metabolic organization of *A. fulvescens* may reflect this intermediate position. Singer *et al.* (1990) have demonstrated similarities between sturgeon metabolism and that of elasmobranchs. This includes a reduced ability for extrahepatic lipid catabolism compared to that of the teleosts. This suggests a more important role of amino acids in oxidative metabolism of sturgeon relative to the teleosts and more in line with the elasmobranch model (Chamberlin & Ballantyne, 1992).

The only study of the effects of starvation on plasma free amino acids (FAA) in sturgeon, used recently captured, marine, white sturgeon *Acipenser transmontanus* Richardson, which were transferred immediately to fresh water (Swallow, 1985). The changes in plasma FAA may, therefore, not only be the result of starvation but may have resulted from freshwater acclimation. The fish were deprived of food for only 14–18 days, a relatively short period compared to most studies of fish starvation (Morata *et al.*, 1982; Foster & Moon, 1991; Blasco *et al.*, 1992; Kiessling *et al.*, 1992). Further studies are needed to establish the relationship between plasma FAA and plasma glucose during starvation in the sturgeon under more controlled circumstances. We, therefore, monitored alterations in the concentrations of plasma FAA and plasma glucose concentrations in cultured lake sturgeon during the course of a 60-day period of starvation. The plasma concentrations of amino acids and glucose reflect the net production, and utilization of these metabolites by the tissues. Changes in these processes may result in changes in the plasma concentrations of these metabolites.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Two-year-old lake sturgeon, raised in captivity from eggs, were obtained from the Deep River Research Station, Ontario, Canada, and held at the University of Guelph in an aerated 1000-l flow-through circular tank. Water flow rates were adjusted appropriately to maintain water quality characteristics. Fish were maintained under a 16 : 8 h L : D photoperiod (L=0700 to 2300 hours) throughout the study. Mean water temperature was $12{\cdot}0 \pm 0{\cdot}54^{\circ}$ C. Prior to the experimental period, all fish were fed a commercial salmonid diet daily (0·5% of live body weight per day). This value was determined previously by measuring the amount of feed that a fish would consume in a 10-min period. Eight fish were selected randomly and transferred to a 750-l tank for each of the

two groups; fed or food-deprived. Conditions in the tanks were identical to those described above. All fish were maintained under these conditions for a minimum of 30 days prior to the start of the experiment. Fish were selected randomly to ensure that the initial mean weights of each group were the same (Table I). The starvation period occurred during the months of October, November, and December.

Blood was taken before feeding the fed group. On day 1 of the experiment, a 1·0 ml blood sample (approximately 2% of blood volume) was taken from each non-anaesthetized fish by caudal puncture using a heparin-treated syringe (Harrington *et al.*, 1991). Each fish was then tagged and the length and weight measured. To minimize stress, length and weight were measured only at the beginning and end of the experiment. The maximum time the fish were out of water during the blood sampling procedure was 40 s. The condition index was calculated using the formula [mass (g) × 100] [length $(cm)^3]^{-1}$ (Maddock & Burton, 1994). Haematocrit values were measured from each whole blood sample. Plasma was separated from the blood samples and frozen at − 20° C until analysis (maximum 3 weeks). Fish in the fed group were fed for the remainder of the experimental period except 1 day prior to any blood sampling. Feed was given to the fish in the ratio described above. Feed was withheld from the food-deprived group for the 60-day duration of the experiment. Blood samples were taken from all fish on days 10, 20, 45, and 60 as described above. Blood was taken from the fish at the same time on each sampling day (3–4 h into photoperiod).

DETERMINATION OF PLASMA GLUCOSE CONCENTRATIONS

Plasma glucose concentrations were determined with a Sigma diagnostics glucose (HK) kit (Sigma Diagnostics, St Louis, MO, U.S.A.). Values reported are the mean values of duplicate measurements of each plasma sample.

DETERMINATION OF PLASMA FREE AMINO ACIDS

Plasma FAA concentrations were detected using an HPLC (Hewlett-Packard, HP 1090 series II/L liquid chromatograph) equipped with a UV-visible series II diode array detector. Amino acids were resolved on a narrow bore (200 × 2·1 mm) reversed-phase AminoQuant column (Hewlett-Packard, 79916AA-572) using the method described by Barton *et al.* (1995).

STATISTICS

Statistical comparisons were made among treatments at each sampling day and within treatments between sampling days for each individual amino acid concentrations, total amino acid concentration, total non-essential amino acid concentration, essential amino acid concentration, plasma glucose concentration, and haematocrit measurement using a two factor ANOVA followed by the Bonferroni *post hoc* test $P<0{\cdot}05$ (Steel & Torrie, 1980). Body weight, body length, and composition index data was analysed using a single factor ANOVA test and a Student's *t*-test with $P<0{\cdot}05$ (Steel & Torrie, 1980).

RESULTS

PHYSIOLOGICAL INDEXES

The food-deprived group had decreased in weight by 16% on day 60, although this difference was not statistically significant (Table I). The control group had increased significantly in body weight by 18% at day 60. The condition index was significantly different between the two treatment groups after 60 days of starvation. The condition index increased significantly in the fed group between sampling days 1 and 60 but there was no change in the starved group. The condition index was significantly lower in the starved group compared to the fed

TABLE I. Comparison of haematocrit measurements, body weight, body length, and condition index between fed and starved lake sturgeon, *Acipenser fulvescens*, at days 0, 10, 20, 45, and 60 of the experiment

Sampling day	Haematocrit (% packed cell volume)		Body weight (g)		Body length (cm)		Condition index [weight(100)] (length^3)$^{-2}$	
	Fed	ST	Fed	ST	Fed	ST	Fed	ST
0	24 ± 1	25 ± 1	447 ± 16	519 ± 35	48 ± 1	49 ± 1	0·40 ± 0·013	0·44 ± 0·024
10	22 ± 1	22 ± 1	nm	nm	nm	nm	—	—
20	22 ± 1	18 ± 1*	nm	nm	nm	nm	—	—
45	19 ± 1	16 ± 1*	nm	nm	nm	nm	—	—
60	20 ± 1	13 ± 1*†	550 ± 22*	464 ± 33†	50 ± 1	49 ± 1	0·43 ± 0·0090*	0·39 ± 0·018†

ST, starved.
* significant difference between measurements at sampling day 0 of the food starvation period and this measurement ($P<0·05$).
† significant difference between treatments at this sampling day ($P<0·05$).
Values are presented as means ± S.E. for eight determinations.
nm, not measured.

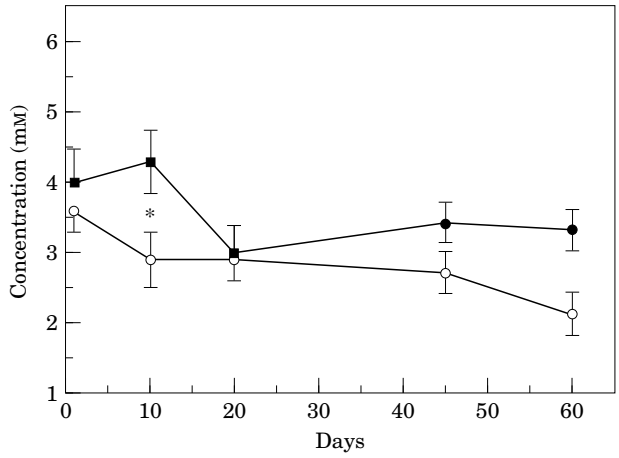

FIG. 1. Glucose concentrations in the plasma of starved (○) and fed (■) lake sturgeon *Acipenser fulvescens* over a 60-day period. Values are means ± S.E. for $n=8$. *Significant difference between treatments ($P<0{\cdot}05$).

group at day 60. The haematocrit of the food-deprived fish decreased steadily during the course of the starvation period (Table I). The haematocrit of food-deprived fish at day 60 was significantly lower (48% of day-0 fish). The haematocrit of the fed group was unchanged during the course of the experiment.

PLASMA GLUCOSE CONCENTRATION

Plasma glucose concentrations were significantly lower in the food-deprived sturgeon at sampling day 10 (Fig. 1). Throughout the remainder of the starvation period, there was no difference in the plasma glucose concentrations between the fed and food-deprived fish.

AMINO ACIDS

The concentration of total plasma FAA increased significantly in the food-deprived fish after sampling day 45 [Fig. 2(a)]. Similar increases were observed in the concentrations of essential amino acids [Fig. 2(b)] and non-essential amino acids [Fig. 2(c)]. The concentrations of 12 of the 19 amino acids resolved (glutamate, asparagine, glycine, histidine, arginine, tyrosine, valine, methionine, tryptophan, phenylalanine, isoleucine, and leucine) increased significantly in concentration in the experimental treatment group compared to the control group at sample day 60, as well as in comparison to all sampling days within the treatment. There were no differences in the concentrations of alanine [Fig. 3(a)], aspartate [Fig. 3(b)], serine [Fig. 3(c)] and taurine between the treatment and control groups at any of the sampling days. Glutamine was significantly higher in concentration in the experimental treatment group at sampling days 10, 20, and 60 [Fig. 3(d)]. Lysine was significantly higher in concentration in the experimental treatment groups at sampling days 10 and 20 [Fig. 3(e)].

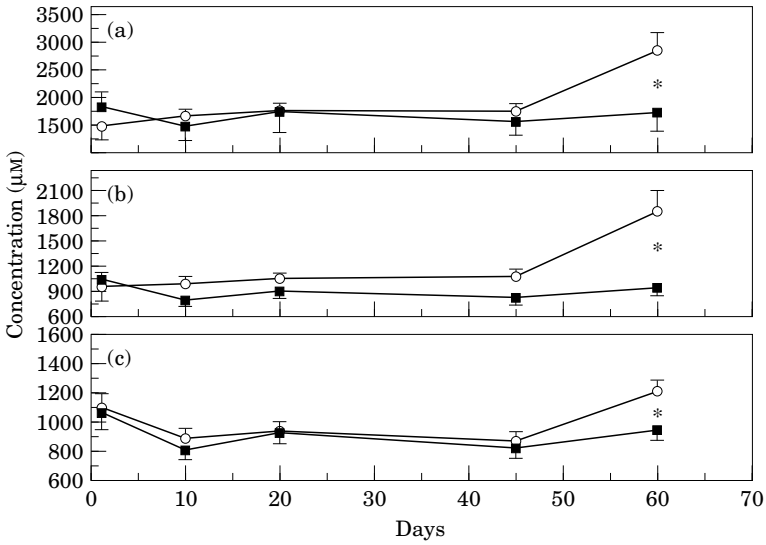

FIG. 2. Concentrations of the total free amino acids (a), essential amino acids (b), and non-essential amino acids (c) in the plasma of starved (○) and fed (■) lake sturgeon *Acipenser fulvescens* over a 60-day period. Values are means ± S.E. for $n=8$. *Significant difference between treatments ($P<0{\cdot}05$).

DISCUSSION

Reorganization of metabolic processes to utilize stored energy efficiently is an important strategy during starvation. The observed decrease in haematocrit signifies a reduced oxygen carrying capacity of the blood reflecting a reduced oxygen requirement of the sturgeon as a result of starvation. Although repeated blood sampling has been demonstrated to decrease haematocrit in some species (Hoffman & Lommel, 1983), the constancy of the haematocrit in the fed fish in the present study indicates the sampling volume was appropriately low.

The lack of significant weight loss observed in the sturgeon after 60 days of starvation is not indicative of the extent of depletion of body energy reserves. An increase in water content has been observed in tissues of various teleost species during starvation (Stirling, 1976; Black & Love, 1986; Maddock & Burton, 1994). A replacement by water of constituents removed from the tissues would act to maintain the volume and mass of the starved fish (Navarro & Gutiérrez, 1995).

Since glucose is an essential fuel for a number of tissues, it is particularly important that glucose levels be maintained throughout starvation. In the present study, the concentration of plasma glucose in the starved sturgeon remained constant with a single exception at day 10. The difference observed at this time between the two groups appears to be as a result of an increase in plasma glucose in the fed fish in combination with a decrease in plasma glucose concentrations in the starved fish. In general, however, plasma glucose concentrations were maintained during starvation. Since dietary sources of carbohydrates were not available to the food-deprived group, enhanced rates of gluconeogenesis may have been required. Increased rates of gluconeogenesis during starvation have been observed in many species of teleosts (Zammit &

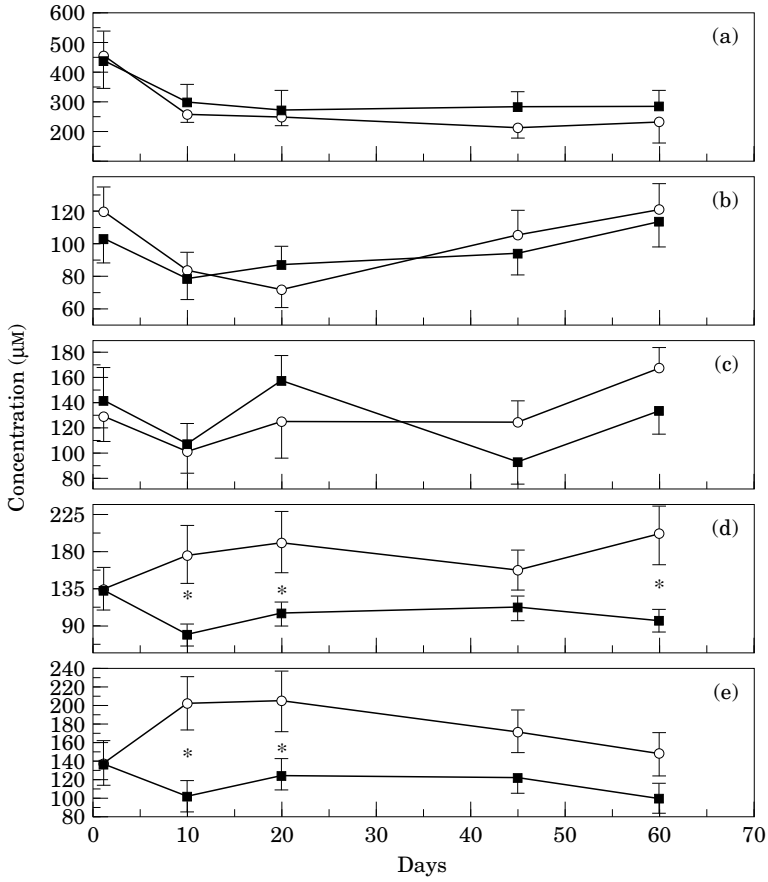

FIG. 3. Concentrations of the free amino acids: alanine (a), aspartate (b), serine (c), glutamine (d), and lysine (e) in the plasma of starved (○) and fed (■) lake sturgeon *Acipenser fulvescens* over a 60-day period. Values are means ± S.E. for $n=8$. *Significant difference between treatments ($P<0{\cdot}05$).

Newsholme, 1979; Moon & Johnston, 1980; Morata *et al.*, 1982; Moon *et al.*, 1989; Foster & Moon, 1991).

Certain amino acids, specifically alanine, serine, and aspartate, have been illustrated to be glucogenic in teleosts (French *et al.*, 1983; Moon *et al.*, 1985; Suarez & Mommsen, 1987; Hansen & Abraham, 1989) as well as in the more primitive elasmobranchs (Leech *et al.*, 1979). The results of our study indicate a similar role for these amino acids in sturgeon. Alanine, serine and aspartate concentrations in the plasma of the starved sturgeon remained constant while most other amino acids changed in concentration during the starvation period. This suggests that throughout the starvation period, a steady state existed between the release of these glucogenic FAA from the muscle by proteolysis and utilization for gluconeogenesis in the liver. This is congruent with the relatively constant concentration of glucose in the plasma throughout the starvation period.

The relevance of the observed increase in lysine concentrations in the starved fish during the early days of the starvation period is not known at this time.

Lysine can be used in the production of ketone bodies which have been demonstrated to be of importance in mammals during starvation (Newsholme & Leech, 1983). Enzymes involved in ketone body metabolism have been reported in tissues of *A. fluvescens* (Singer *et al.*, 1990). Further studies of the importance of ketone bodies in sturgeon are needed.

The low capacity for fatty acid oxidation in sturgeon demonstrated by Singer *et al.* (1990) suggests that amino acids may be the preferred oxidative substrate in these ancient fish. This is consistent with observations of the primitive fishes including elasmobranchs (Chamberlin & Ballantyne, 1992) and holosteans (Chamberlin *et al.*, 1991). Glutamine has been determined to be the most readily oxidized amino acid in red muscle mitochondria isolated from two species of elasmobranch (Chamberlin & Ballantyne, 1992) and the bowfin *Amia calva* L., a holostean (Chamberlin *et al.*, 1991). These primitive fish utilize glutamine in preference to other substrates, whereas mitochondria from the lake trout *Salvelinus namaycush* (Walbaum), a teleost, catabolize fatty acids at high rates. Based on this evidence, the authors proposed that amino acids in general, and glutamine specifically, are important metabolic substrates in nonteleost red muscle. The increased plasma glutamine concentration throughout the starvation period may indicate an enhanced metabolism of this amino acid during starvation. The capacity for glutamine synthesis is high in the liver of the white sturgeon (Webb & Brown, 1980) and this would help maintain high levels of this amino acid in the plasma.

The increase in total FAA by day 60 of starvation may be indicative of a critical point in the adaptation of metabolic processes to starvation. An enhanced proteolysis to supply amino acids is reflected by an increase in plasma FAA, and may signal the maximal duration for the maintenance of metabolic functions without significant reorganization. The extent of proteolysis may be so great as to include red muscle. A uniform decrease in the size of all types of muscle fibres, including red muscle, was observed by Kiessling *et al.* (1992) in *A. transmontanus* after 35 days of starvation. In teleosts starved for similar periods, red muscle integrity is typically defended (Johnston, 1981; Beardall & Johnston, 1983). The destruction of red muscle in sturgeon could have a significant long-term effect on the animal and demonstrate an important difference between sturgeon and teleosts with respect to starvation.

The results of this study differ from that by Swallow (1985) on starvation in *A. transmontanus*. The differences between these two studies may be due to the fact that the white sturgeon were transferred to fresh water at the onset of the starvation period. Comparisons were, therefore, made between the fish which were both food-deprived and freshwater acclimated and fish recently caught from a marine environment. Changes in plasma FAA have been associated with transfer from sea water to fresh water in numerous species (Sweeting *et al.*, 1985; Thoroed & Fugelli, 1993). It is, therefore, not possible to relate the changes observed by Swallow (1985) unequivocally to starvation.

The results of the present study suggest that, during starvation, the utilization of amino acid carbon, specifically serine, alanine, and aspartate for gluconeogenesis in sturgeon resembles that of other fish groups including teleosts and elasmobranchs. The dramatic increase in total plasma free amino acids by day 60 of the experiment may be indicative of a non-selective proteolysis of

muscle tissue. Additionally, based on the elevated plasma glutamine concentrations in the starved fish, it appears that these primitive fish have an increased reliance on amino acid metabolism for the production of energy. This may be a function of a reduced capability in sturgeon for lipid catabolism compared to teleosts (Singer *et al.*, 1990) and an ability to oxidize amino acids, specifically glutamine, similar to other primitive fish (Chamberlin *et al.*, 1991; Chamberlin & Ballantyne, 1992). These findings may reflect the evolutionary position occupied by sturgeon as the utilization of stored metabolites during starvation appears to be intermediate to that of the elasmobranchs and the teleosts.

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