Effects of Diet on Responses to Hypoxia in Sturgeon (Acipenser Naccarii)

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EFFECTS OF DIET ON RESPONSES TO HYPOXIA IN STURGEON (*ACIPENSER NACCARII*)

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Summary

We investigated the effects of a diet enriched in omega-3 (ω3) polyunsaturated fatty acids (PUFA) and vitamin E on responses of sturgeon (*Acipenser naccarii*) to hypoxia. After 3 months of feeding, there were significant increases in ω3 PUFA in liver and muscle, and of vitamin E in muscle, of fish fed the enriched diet (ED) compared with fish on a standard diet (SD), indicating that tissue composition is influenced by diet. Acute exposure to hypoxia (10 min at 10.8 kPa water O_2 tension, $P_{W_{O_2}}$) had no effect on oxygen consumption ($\dot{V}_{O_2}$), increased gill ventilation frequency (f_G) and reduced arterial blood O_2 content ($C_{a_{O_2}}$) in both dietary groups, but ED sturgeon exhibited a significantly smaller decrease in $C_{a_{O_2}}$ than did SD animals. Progressive hypoxic exposure ($P_{W_{O_2}}$ decreasing gradually from 20.5 to 3.6 kPa within 45–60 min) led to a significant increase in $\dot{V}_{O_2}$ at intermediate levels of $P_{W_{O_2}}$ in SD sturgeon that was not seen in ED animals. Furthermore, ED sturgeon showed no significant reduction in arterial plasma pH (pHa) and $C_{a_{O_2}}$ at $P_{W_{O_2}}$ levels that caused significant reductions in these variables in SD sturgeon. ED sturgeon exhibited a smaller increase in plasma lactate level than did SD fish. We suggest that PUFA and/or vitamin E contribute significantly to regulation of metabolism in hypoxia.

Introduction

Although species differences are important factors in determining the characteristics of stress responses in animals, the nutritional status of the animal also plays a primary role (Parke and Ioannides, 1981; Vergroesen and Crawford, 1989; Packer,

Key words: *Acipenser naccarii*, fish, hypoxia, oxygen consumption, polyunsaturated fatty acids, sturgeon, vitamin E.

1991). Indeed, the nutritional status of laboratory animals may account for differences in responses to stress when comparing data from different studies.

Increases in the relative amount of ω3 polyunsaturated fatty acids (PUFA) in the diet are reported to protect against the effects of oxidative stress, decreasing the likelihood of chronic degenerative cardiovascular disease in humans and increasing the resistance of the mammalian heart and brain to ischaemic damage (see Hornstra, 1989, for a review). Vitamin E, a lipid-soluble anti-oxidant, is also reported to protect against cardiovascular disease and ischaemic damage (Crary and McCarty, 1984; Budowski and Sklan, 1989; Downey, 1990; Packer, 1991). Thus, there is evidence to suggest that variations in the quality of the diet, particularly the ω3 fatty acid and vitamin E content, may lead to changes in whole-animal responses to hypoxic stress.

Because fish health and physiological processes are strongly dependent on dietary ω3 fatty acid and anti-oxidant status (Ackman, 1980; Bell *et al.* 1986; Cowey, 1986; Raynard *et al.* 1991), we investigated the effects of a combined pretreatment with dietary ω3 PUFA and vitamin E on the responses of Cobice sturgeon (*Acipenser naccarii*) to hypoxia. Dietary composition is shown to have a marked effect on the regulation of metabolism during hypoxia.

Materials and methods

Animals

Cobice sturgeon, *Acipenser naccarii* (Bonaparte), were maintained at *La Casella* experimental thermal fish farm [Via Argine del Ballottino, 29010, Sarmato (PC), Italy] in large fibreglass tanks with a continuous water supply (25°C, pH 7.9).

Diets

Groups of 30 sturgeon in separate tanks were maintained on two different diets. Standard-diet animals (SD sturgeon) were fed a commercial sturgeon feed (Alma Storioni, Agros, Bolzano, Italy) with ascorbic acid and lecithin supplements, as pellets. Experimental-diet animals (ED sturgeon) were fed pellets of the same feed but with additional fish oil and vitamin E supplements. Feeds were pelleted freshly every 2–3 days and stored at 4°C. The two diets had the composition reported in Table 1. Following 90 days of feeding to satiation, samples of liver and

Table 1. *Composition of control and experimental diets*

	Control	Experimental
Total lipids ($g\,kg^{-1}$)	120	200
ω3 fatty acid ($g\,kg^{-1}$)	26	48
ω6 fatty acid ($g\,kg^{-1}$)	11	12
Arachidonic acid ($g\,kg^{-1}$)	0	0
Eicosapentaenoic acid ($g\,kg^{-1}$)	9.0	26.8
Vitamin E ($g\,kg^{-1}$)	0.056	0.556

muscle were obtained from five freshly killed fish from each group and immediately frozen on dry ice for subsequent analysis of fatty acid and vitamin E content. Lipids were extracted from 1 g of a homogenate of the whole liver or half of the myotome muscle mass with chloroform:methanol (2:1 v/v), as described by Folch *et al.* (1957), with 5 mg l^{-1} butylated hydroxytoluene as an anti-oxidant. Total fatty acid levels were measured by gas chromatography using a Dani gas chromatograph with a programmable temperature vapouriser injector and a column (Supelcowax 30 m, 0.30 mm inner diameter, 0.27 μm film thickness) with temperature programming (150–220°C at 2.5° min^{-1} increments). Vitamin E was extracted from 1 g of the homogenate from each tissue, as described by Weber (1987). Vitamin E was measured by HPLC with fluorescence detection (Jasco 880 intelligent pump; Speri-5 C18 reverse-phase column and Jasco 821 FP intelligent detector) with a methanol mobile phase, as described by Weber (1987). Fatty acid and vitamin E levels in the two tissues were expressed as absolute amounts per 100 g tissue.

Following 150 days of feeding to satiation with the diets, responses to two hypoxic challenges were measured in nine SD animals (mean mass±S.E.= 783.8±40.49 g) and six ED animals (mean mass±S.E.=867.8±71.7 g).

Surgical procedures

Sturgeon were anaesthetised in a 1:10 000 buffered solution of tricainemethanesulphonate (MS 222) and then transferred to a surgical table and artificially ventilated with an MS 222 solution at 1:20 000. A dorsal aortic cannula (PE 50, Intramedic) was implanted using the technique of Soivio *et al.* (1972). Animals were allowed to recover for 24–48 h in a Plexiglas respirometer chamber (10 l volume) with a continuous water supply. The chamber was darkened on all sides except for a small space on the upper surface, which permitted video recording of ventilation frequency (see below). The cannula was flushed with heparinised Cortland's saline (Wolf, 1963) every 24 h.

Measurement of respiratory and blood variables

Water flow through the Plexiglas chamber could be stopped by two three-way valves which caused the water to be recirculated through an external loop with an Eheim model 1034 pump (Eheim, Germany). A Yellow Springs O_2 electrode (model YSI 5331) placed at the outflow of the chamber and attached to an Amel 321 O_2-meter (Amel, Italy) monitored the decline in water O_2 levels during the period of recirculation, with data registered on a Philips PM 8252 potentiometric recorder. This subsequently allowed calculation of O_2 consumption ($\dot{V}_{O_2}$, as mg O_2 kg^{-1} h^{-1}) from the volume of the recirculating system and the mass and volume of the fish. Gill ventilation frequency (f_G, as beats min^{-1}) was recorded by a Sony video recorder placed above the respirometer chamber. Blood samples could be withdrawn anaerobically from the dorsal aortic cannula (which passed through an airtight hole in the respirometer). Arterial plasma pH (pHa) was measured using an IL System-1302 pH-blood gas analyser (Instrumentation Laboratories) regulated at 37°C. Whole blood samples were centrifuged in sealed

0.3 ml plastic micro-test-tubes containing no air within 2 min of collection. Plasma pH at 25 °C was then calculated using a pH:temperature coefficient of 0.013 pH units per degree, as given for rainbow trout plasma by Heisler (1984). Although the absolute values for pHa obtained by this method may not be reliable, because all samples were treated identically comparisons between samples were valid. Arterial blood O_2 content (Ca_{O_2}) was measured using the technique of Tucker (1967) and an Instrumentation Laboratories P_{O_2} electrode (model 68653) regulated at 37 °C. Sturgeon in the ED group had a slightly higher mean mass than sturgeon in the SD group and, presumably, larger blood volumes. Arterial blood O_2 content measurements in both groups were corrected for blood volume loss as a result of sampling, assuming a blood volume of 5 % body volume, in order to correct for apparent differences in Ca_{O_2} between the groups that might have resulted from the sampling regime. Plasma from centrifuged blood samples was frozen in liquid N_2 within 1 min of collection for subsequent measurement of plasma circulating catecholamine and lactate levels. Plasma catecholamines were measured on alumina-extracted samples using HPLC with electrochemical detection, with a mobile phase consisting of: 0.08 mol l^{-1} citric acid, 0.04 mol l^{-1} Na_2HPO_4, 0.1 mmol l^{-1} sodium EDTA, 0.64 mmol l^{-1} sodium octyl sulphate, 10 % (v/v) methanol, at pH 3.2, a Shimadzu LC6A solvent delivery pump, a Shimadzu SIL 6B/9A sample injector, a Nucleosil C_{18} RP 7 μm particle size reverse-phase column (Macherey-Nagel, Germany), a Coulochem 5100A electrochemical detector (Environmental Science Associates, USA) and a Shimadzu C-R4A integrator, according to the method of Bondiolotti *et al.* (1987). Plasma lactate was measured using a Sigma ultraviolet colorimetric assay.

Protocol

All experiments were conducted at *La Casella* experimental fish farm. Sturgeon from both dietary groups were exposed to two different hypoxic challenges.

Acute hypoxic exposure

Control measurements of $\dot{V}_{O_2}$ and f_G were made under normoxic conditions (Pw_{O_2}=20.5 kPa), during a 10 min recirculation period. A 1.4 ml blood sample was withdrawn (and replaced with an equal volume of heparinised saline) for measurement of normoxic pHa, Ca_{O_2} and plasma catecholamine titre. The respirometer system was then flushed with hypoxic water until the water was approximately half saturated with oxygen (Pw_{O_2}=10.8±0.3 kPa). Water was then recirculated for 10 min to allow measurement of $\dot{V}_{O_2}$ and f_G under hypoxic conditions. A further 1.4 ml blood sample was withdrawn at the end of the hypoxic exposure period for measurement of pHa, Ca_{O_2} and catecholamines. Normoxic water flow through the system was then resumed, and the animal was allowed 1–1.5 h to recover.

Progressive hypoxic exposure

Following recovery from acute hypoxia, a 1.4 ml blood sample was withdrawn

for measurement of pHa, Ca_{O_2} and plasma lactate levels. Water was then recirculated so that the sturgeon created a gradual progressive hypoxia within the respirometer as it consumed the oxygen. Thus, $\dot{V}_{O_2}$ and f_G were measured as Pw_{O_2} decreased to 3.6 kPa. The duration of progressive hypoxia was between 45 and 60 min. Blood samples (0.4 ml) were withdrawn at 15.7, 9.7 and 6.0 kPa Pw_{O_2} and pHa and Ca_{O_2} were measured. At 3.6 kPa, a 1.4 ml blood sample was withdrawn for measurement of pHa, Ca_{O_2} and plasma lactate levels. Normoxic water flow through the respirometer was then resumed for 10 min, after which it was recirculated again for measurement of $\dot{V}_{O_2}$ and f_G during 10 min of recovery in normoxia. Following this, normoxic flow was again resumed.

Data analysis

Levels of PUFA and vitamin E in liver and muscle of SD and ED sturgeon groups were compared using unpaired *t*-tests. Respiratory and internal variables measured during normoxia were compared with those in acute hypoxia using a paired *t*-test. During gradual hypoxia, variables under normoxic conditions were compared with measurements taken at each level of hypoxia or during recovery using a paired *t*-test. Variables measured at a given Pw_{O_2} in fish fed the standard diet were compared with those measured in animals fed the experimental diet using unpaired *t*-tests. Differences with $P<0.05$ were considered statistically significant.

Results

Following 90 days of feeding to satiation, there were significant differences in tissue fatty acid composition and vitamin E content between the two dietary groups. ED sturgeon had significantly more total $\omega 3$ PUFA in liver and muscle than did SD fish, and they also had significantly higher $\omega 6$ fatty acid levels in their livers (Table 2). Despite this accumulation of $\omega 6$ fatty acids in the liver, both the $\omega 3/\omega 6$ and eicosapentaenoic acid/arachidonic acid ratios were significantly higher

Table 2. *Liver and muscle mean fatty acid and vitamin E concentrations and $\omega 3/\omega 6$ and EPA/AA ratios in SD and ED* Acipenser naccarii

	Liver		Muscle	
	SD	ED	SD	ED
$\omega 3$	1.52±0.14	8.66±0.20*	1.18±0.06	2.18±0.11*
$\omega 6$	0.75±0.06	2.08±0.16*	0.40±0.03	0.56±0.04
$\omega 3/\omega 6$	1.98±0.07	4.16±0.08*	3.12±0.13	3.86±0.08*
EPA/AA	2.72±0.10	6.28±0.38*	7.39±0.30	9.34±0.39*
Vitamin E	0.17±0.004	0.13±0.001*	0.009±0.0003	0.013±0.0001*

Values are mean±S.E. ($N=5$).

EPA, eicosapentaenoic acid; AA, arachidonic acid; SD, standard diet; ED, experimental diet; *significantly different from SD value ($P<0.05$).

Table 3. *The effects of acute hypoxic exposure on mean* $\dot{V}_{O_2}$, f_G, *pHa,* Ca_{O_2}, *noradrenaline and adrenaline, and of progressive hypoxia on plasma lactate, in SD and ED* Acipenser naccarii

	SD		ED	
	Normoxia	Hypoxia	Normoxia	Hypoxia
$\dot{V}_{O_2}$ (mg O_2 kg^{-1} h^{-1})	151.8±21.4	139.4±4.8	141.7±19.1	128.5±11.5
f_G (beats min^{-1})	98.2±6.9	137.0±2.8*	99.0±11.7	143.0±4.45*
pHa	7.75±0.01	7.77±0.02	7.76±0.02	7.72±0.02
Ca_{O_2} (vol%)	7.70±0.56	2.79±0.43*	7.69±0.64	5.25±0.62*†
NA (nmol l^{-1})	4.3±0.6	45.1±8.6*	3.7±1.1	54.1±19.2*
A (nmol l^{-1})	5.2±1.6	29.9±6.8*	5.7±1.7	60.2±17.3*
Lactate (mmol l^{-1})	0.30±0.19	3.65±0.76*	1.18±0.81†	2.52±0.44*
Δ[Lactate] (mmol l^{-1})	–	3.67±1.56	–	1.37±0.74†

Values are mean±S.E.

For SD animals, N=9 for $\dot{V}_{O_2}$, pHa and Ca_{O_2}, N=6 for f_G, N=7 for NA and A and N=6 for lactate measurements. For ED animals, N=6 in all cases.

SD, standard diet; ED, experimental diet; NA, noradrenaline; A, adrenaline; Δ[Lactate], increase in plasma lactate concentration during progressive hypoxia; *significantly different from normoxic value (P<0.05); †significantly different from value in SD sturgeon (P<0.05).

in liver and muscle of ED sturgeon, the differences being particularly marked in the liver (Table 2). Vitamin E levels were significantly elevated in muscle of ED sturgeon when compared with SD fish, but were significantly lower in the liver of ED fishes (Table 2). These differences in tissue composition were still present in SD and ED sturgeon following 1 year on the diets (E. Agradi, G. Abrami, G. Serrini, D. McKenzie, L. Bolis and P. Bronzi, in preparation) and are, therefore, representative of the tissue composition of those animals exposed to hypoxia in this study.

Under normoxic conditions, the values for $\dot{V}_{O_2}$, f_G, pHa, Ca_{O_2}, noradrenaline and adrenaline in the SD and ED animals did not differ (Table 3). Acute hypoxic exposure had no effect on $\dot{V}_{O_2}$ or pHa in either dietary group but gill ventilation frequency increased significantly and there was a significant decrease in Ca_{O_2}. ED sturgeon, however, maintained Ca_{O_2} at levels significantly higher than those measured in SD animals during acute hypoxic exposure (Table 3). Under normoxic conditions, plasma catecholamine levels in both dietary groups were similar to those of teleosts (Perry *et al.* 1989); both SD and ED fish showed a similar and statistically significant increase in plasma noradrenaline and adrenaline concentrations during hypoxic exposure (Table 3).

There were marked differences in the responses to progressive hypoxia between SD and ED sturgeon. SD sturgeon became extremely agitated during exposure to progressive hypoxia, whereas ED sturgeon did not. During progressive hypoxic exposure, $\dot{V}_{O_2}$ was significantly elevated at Pw_{O_2} values between 15.7 and 7.2 kPa in SD animals (Fig. 1). Below a Pw_{O_2} of 10.8 kPa, $\dot{V}_{O_2}$ began to decline in SD

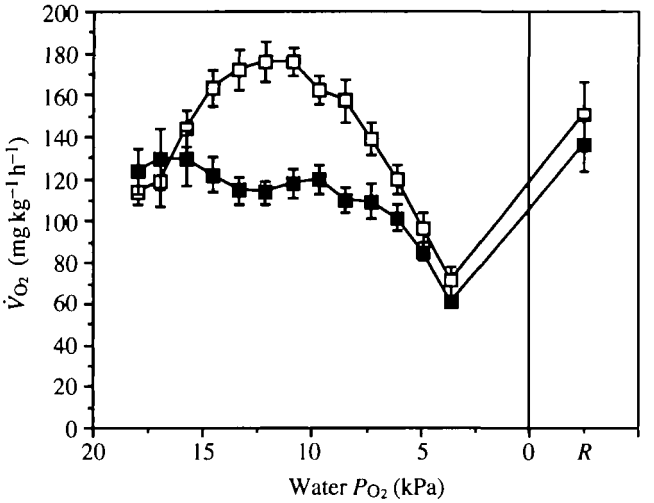

Fig. 1. The effects of progressive hypoxia on the mean (±S.E.) rate of oxygen uptake ($\dot{V}_{O_2}$) in ED (filled symbols) and SD (open symbols) *Acipenser naccarii*. N=6 for ED and 9 for SD fish. *R*, recovery; SD, standard diet; ED, experimental diet.

sturgeon and at 3.6 kPa Pw_{O_2}, $\dot{V}_{O_2}$ was significantly decreased when compared with normoxia. Following 10 min of recovery in normoxic water, $\dot{V}_{O_2}$ increased significantly when compared with that at 3.6 kPa Pw_{O_2}, to a value not significantly different from that in pre-hypoxic exposure (Fig. 1). Animals given the experimental diet maintained $\dot{V}_{O_2}$ statistically unchanged down to a Pw_{O_2} of 4.8 kPa during progressive hypoxic exposure and, between 14.5 and 7.2 kPa Pw_{O_2}, $\dot{V}_{O_2}$ in ED sturgeon was significantly lower than that of SD fish. At Pw_{O_2} below 4.8 kPa, $\dot{V}_{O_2}$ was significantly reduced compared with control values (Fig. 1). Following 10 min of recovery in normoxia, ED animals showed a significant increase in $\dot{V}_{O_2}$ compared with that at 3.6 kPa Pw_{O_2}. Oxygen consumption by ED sturgeon during recovery did not differ from that seen in SD fish during recovery (Fig. 1).

Progressive hypoxia led to a significant increase in f_G in both dietary groups (Fig. 2). In SD sturgeon, f_G was significantly increased at a Pw_{O_2} of 13.3 kPa, and reached a maximum increase of 57 % above normoxic values at a Pw_{O_2} of 8.5 kPa. At 3.6 kPa, SD sturgeon showed a significant decrease in f_G when compared with f_G at 6.0 kPa. Following 10 min of recovery in normoxic water, ventilation was significantly elevated when compared with either pre-hypoxic exposure values or those seen at 3.6 kPa Pw_{O_2} (Fig. 2). In ED sturgeon, there was no significant increase in f_G during progressive hypoxic exposure until 10.8 kPa Pw_{O_2} and, at 13.3 kPa Pw_{O_2}, ED sturgeon had a significantly lower f_G than SD animals. The maximum increase in f_G was also 57 % above normoxic values at a Pw_{O_2} of 6.0 kPa. The hyperventilation was maintained down to the lowest level of Pw_{O_2}, so that at 3.6 kPa Pw_{O_2} ED sturgeon had a significantly higher f_G than did SD animals (Fig. 2). Following 10 min of recovery in normoxia, ED fish showed a hyperventilation that was no different from that seen in SD sturgeon (Fig. 2).

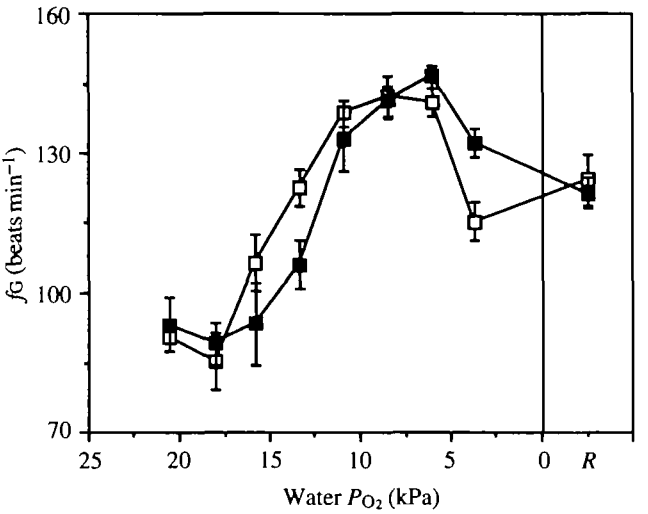

Fig. 2. The effects of progressive hypoxia on mean (±S.E.) gill ventilation frequency (f_G) in ED (filled symbols) and SD (open symbols) *Acipenser naccarii*. N=6 for ED and SD fish. *R*, recovery; SD, standard diet; ED, experimental diet.

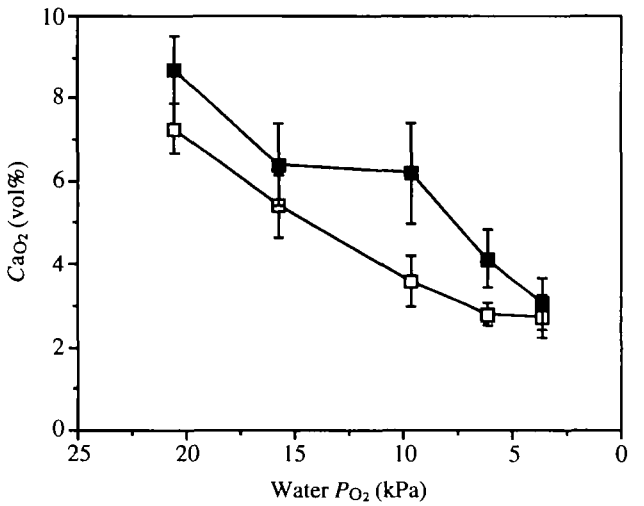

Fig. 3. The effects of progressive hypoxia on mean (±S.E.) arterial blood oxygen content (Ca_{O_2}) in ED (filled symbols) and SD (open symbols) *Acipenser naccarii*. N=6 for ED and SD fish. SD, standard diet; ED, experimental diet.

In both SD and ED fish, Ca_{O_2} showed a progressive reduction as Pw_{O_2} decreased during progressive hypoxic exposure (Fig. 3). In SD sturgeon, however, Ca_{O_2} was significantly reduced below 9.7 kPa Pw_{O_2}, whereas ED sturgeon did not exhibit a significant hypoxaemia until 6.0 kPa Pw_{O_2} (Fig. 3).

Temperature-corrected arterial plasma pH also decreased significantly as Pw_{O_2}

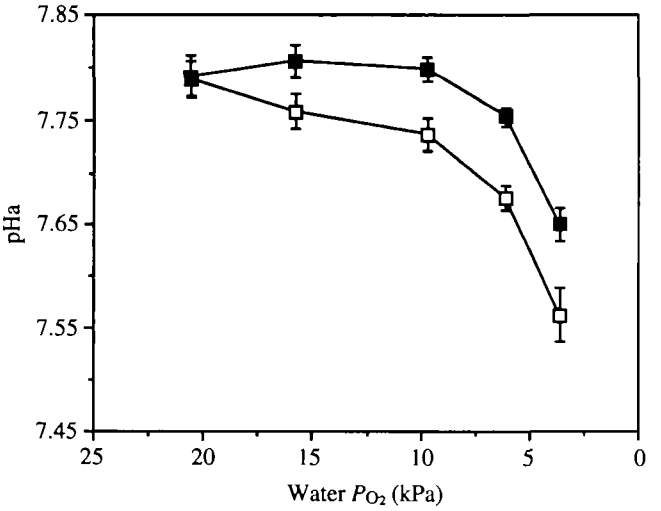

Fig. 4. The effects of progressive hypoxia on mean (±S.E.) arterial plasma pH (pHa) in ED (filled symbols) and SD (open symbols) *Acipenser naccarii*. N=6 for ED and SD fish. SD, standard diet; ED, experimental diet.

declined in both SD and ED animals (Fig. 4). In SD sturgeon, pHa was significantly reduced compared with normoxic values at 6.0 kPa Pw_{O_2} and below. ED fish did not exhibit a significant decrease in pHa until a Pw_{O_2} of 3.6 kPa. At 9.7 and 6.0 kPa Pw_{O_2}, the pHa values for ED animals were significantly higher than those of SD fish (Fig. 4). The acidosis was partially metabolic in origin, as both groups of sturgeon showed a significant increase in plasma lactate concentration following progressive hypoxic exposure (Table 3). Animals fed the experimental diet, however, had higher lactate levels in normoxia than did SD sturgeon and showed a significantly smaller increase in plasma lactate concentration during hypoxic exposure than did the SD animals (Table 3).

During exposure to progressive hypoxia, an accumulation of metabolic CO_2 within the respirometer would have created some degree of hypercapnia. It is likely that this contributed to the responses observed and it may have contributed a respiratory component to the plasma acidosis measured in both dietary groups.

Discussion

The results indicate that diet is a factor involved in the control of $\dot{V}_{O_2}$ during hypoxic stress. The sturgeon in the two dietary groups were of the same genetic stock, of the same age and mass and were maintained in the same environment, suggesting that physiological disparities during hypoxic exposure can be attributed to the differences in ω3 PUFA and vitamin E status, as indicated by tissue levels of PUFA and vitamin E (Maxwell and Marmer, 1983; Chow, 1985).

In normoxic conditions, there were no significant differences in Ca_{O_2} and pHa

measured in ED fish when compared with SD fish, indicating that the disparities in these variables between the dietary groups during hypoxia cannot be ascribed to differences under resting conditions. The differences in Ca_{O_2} during hypoxia may have been the result of differences in blood haemoglobin concentrations or blood O_2-affinity between the two groups and/or differences in cardiovascular function similar to those reported to occur in mammals (Hornstra, 1989; Budowski and Sklan, 1989; Downey, 1990). During progressive hypoxia, it is also possible, however, that differences in Ca_{O_2} and pHa may have been secondary to disparities in metabolic energy requirements between SD and ED sturgeon, as indicated by the increased $\dot{V}_{O_2}$ and larger accumulation of lactate in the plasma of the former. A higher tissue energy requirement in SD fish might have led to an increase in O_2 removal from the blood causing a greater reduction in Ca_{O_2}. An increased $\dot{V}_{O_2}$ in the SD fish would also lead to the production and accumulation within the respirometer of more CO_2 which, in concert with the larger increase in plasma lactate concentration seen in these animals, would lead to a greater reduction in pHa than that seen in ED sturgeon. In addition, the more severe acidosis might also cause further reductions in Ca_{O_2} as a result of Bohr and Root effects.

The higher $\dot{V}_{O_2}$ in the SD sturgeon may have been the result of the agitation observed in that dietary group during progressive hypoxia. During acute hypoxic exposure, there were no significant differences in $\dot{V}_{O_2}$ between the dietary groups, although ED fish showed a slightly reduced $\dot{V}_{O_2}$ and a slightly larger increase in f_G than did SD fish (Table 3). This indicates that the differences in $\dot{V}_{O_2}$ during progressive hypoxia may be related either to the pattern of hypoxic exposure or to an accumulation of metabolic CO_2 in the respirometer.

Despite these differences in metabolic regulation at intermediate levels of O_2 availability, the ED animals were not more resistant to hypoxia: at the end of the progressive hypoxic exposure, Ca_{O_2}, pHa and $\dot{V}_{O_2}$ values of ED and SD sturgeon were not significantly different. In fact, ED fish exhibited a significant decrease in $\dot{V}_{O_2}$ at a Pw_{O_2} that did not cause a significant decrease in SD animals. Thus, an increase in tissue $\omega 3$ fatty acid and vitamin E levels does not increase overall resistance to progressive hypoxia, but only alters the characteristics of the hypoxic response.

It is not known why increases in the $\omega 3$ fatty acid and vitamin E content of the tissues of ED sturgeon lead to such differences in metabolic regulation during hypoxia. Changes in tissue $\omega 3$ fatty acid and vitamin E content may, however, exert modulatory effects at different levels of the oxidative transformations of lipids, leading to the differences in hypoxic responses observed.

The relative tissue levels of $\omega 3$ and $\omega 6$ PUFA and vitamin E have been shown to modulate the biological impact of processes regulated by prostaglandins (Lands, 1986; Hornstra, 1989; Budowski and Sklan, 1989). Prostaglandins are oxygenated metabolites of PUFA found in a very broad range of organisms including plants, prokaryotes, invertebrates and vertebrates (Christ and Van Dorp, 1972). We found significant differences in the $\omega 3/\omega 6$ and eicosapentaenoic acid/arachidonic acid ratios and vitamin E content of tissues from SD and ED sturgeon. Differences

in prostaglandin formation between SD and ED sturgeon may, thus, be one explanation for the differences in metabolic regulation observed during hypoxia. Furthermore, supplementing the diet with fish oil could affect O_2-dependent lipid catabolism, leading to changes in the relative activities of mitochondrial and peroxisomal pathways (Moyes *et al.* 1990).

The ability of vitamin E to inhibit peroxidative reactions of PUFA and to scavenge free radicals is known to reduce the oxidative stress caused by hypoxia in animal tissues (Budowski and Sklan, 1989; Park *et al.* 1991; Dhaliwal *et al.* 1991). Oxidative stress caused by progressive hypoxic exposure may have contributed to the agitation seen in the SD sturgeon, leading to an increase in $\dot{V}_{O_2}$, an effect that was inhibited in ED fish by the higher levels of vitamin E in their tissues. It is also possible, however, that the agitation was caused by the more profound hypoxaemia and acidosis measured in SD sturgeon when compared with ED fish.

A. naccarii fed both fish oil and vitamin E supplements showed better growth than those fed fish oil supplements alone (E. Agradi, G. Abrami, G. Serrini, D. McKenzie, L. Bolis, and P. Bronzi, in preparation), giving indirect evidence for a combined beneficial effect of ω3 PUFA and vitamin E.

It has been shown that enrichment of the diet with ω3 PUFA and vitamin E protects against cardiovascular disease and against acute cardiac and cerebral ischaemia in mammals (Vergroesen, 1989; Hornstra, 1989). Our data indicate that a reduced O_2 requirement of tissues during hypoxia in animals with increased tissue ω3 and vitamin E levels may contribute to the protective role of ω3 PUFA and antioxidants in mammalian cardiac and cerebral ischaemia.

It is of interest that *A. naccarii* did not show the reduction in metabolic rate during acute hypoxic exposure that has previously been observed in the related species *A. transmontanus* at 15°C (Burggren and Randall, 1978). Acclimation of SD *A. naccarii* to 15°C had no effect on the response to acute hypoxia (D. J. Randall and D. J. McKenzie, unpublished observations), and the short duration of the acute hypoxia trial in this study precludes the possibility that an accumulation of metabolic CO_2 within the respirometer could have compromised a metabolic depression. Thus, the disparities in the responses may be a result of species differences but, given the differences between SD and ED *A. naccarii* in metabolic regulation during hypoxia, it is tempting to speculate that disparities between *A. naccarii* and *A. transmontanus* (Burggren and Randall, 1978) may be at least partially a result of differences in nutritional status.

In conclusion, dietary and tissue ω3 fatty acid and vitamin E levels are clearly important variables in regulating metabolism during hypoxia in fish, and consideration of these factors may explain the variability in hypoxic responses measured by different investigators. Evaluation of physiological variables in conjunction with analysis of diet and tissue composition will increase our understanding of the impact of nutrients on whole-animal responses to environmental and pathological stresses.

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