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Effects of dietary fatty acid composition on metabolic rate and responses to hypoxia in the European eel (*Anguilla anguilla*)

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Abstract

European eels (*Anguilla anguilla*, L.) were fed on a commercial diet supplemented either with 15% by dry feed weight of menhaden oil (MO), an oil rich in highly unsaturated fatty acids of the n-3 series (n-3 HUFA), or with 15% by dry feed weight of coconut oil (CO), an oil composed primarily of saturated fatty acids (SFA). Following 90 days of feeding, the mean final masses of eels fed the two different oil supplements were similar, and higher than the mean final mass of a group fed the commercial diet alone. The diets created two distinct phenotypes of eels, distinguished by the fatty acid (FA) composition of their tissue lipids. Eels fed MO had significantly more total n-3 FA and n-3 HUFA in muscle and liver lipids than did eels fed CO, leading to higher n-3/n-6 and eicosapentaenoic acid/arachidonic acid ratios in the MO group. Measurements of O_2 uptake (M_{O_2}) revealed that the MO group had a significantly lower routine metabolic rate (RMR) than the CO group. When exposed to progressive hypoxia, both groups regulated M_{O_2} at routine normoxic levels until critical water O_2 partial pressures that were statistically similar (9.62 ± 1.08 kPa in MO versus 7.57 ± 1.07 kPa in CO), beyond which they showed a reduction in M_{O_2} below RMR. The MO group exhibited a significantly lower M_{O_2} than the CO group throughout hypoxic exposure, but the percentage reductions in M_{O_2} below their relative RMR were equal in both groups. During recovery to normoxia, both groups exhibited an increase in M_{O_2} to rates significantly higher than their RMR. Throughout recovery, M_{O_2} was significantly lower in the MO group compared with the CO group, but the percentage increases in M_{O_2} relative to RMR were equal in both. During progressive hypoxia, neither group exhibited a marked ventilatory reflex response, both showed similar reductions in blood O_2 partial pressure and content, and similar increases in plasma lactate. The results indicate that, although the n-3 HUFA-enriched MO group had a significantly lower routine metabolic rate than the CO group, the difference in aerobic metabolism did not influence the European eel's homeostatic regulation of M_{O_2} in hypoxia.

Abbreviations: AA – arachidonic acid; Ca_{O_2} – arterial blood O_2 content; CO – coconut oil; COD – coconut oil diet; COVD – coconut oil and vitamin E diet; DHA – docosahexaenoic acid; EPA – eicosapentaenoic acid; FA – fatty acids; f_G – gill ventilation rate; HUFA – highly unsaturated fatty acids; M_{O_2} – oxygen uptake; MO – menhaden oil; MOD – menhaden oil diet; MOVD – menhaden oil and vitamin E diet; MUFA – mono-unsaturated fatty acids; Pa_{O_2} – arterial plasma oxygen partial pressure; $P_{O_2}c$ – critical partial pressure for O_2 uptake; P_{OP} – opercular pressure amplitude; PUFA – polyunsaturated fatty acids; Pw_{O_2} – water O_2 partial pressure; RMR – routine metabolic rate; SFA – saturated fatty acids; SMR – standard metabolic rate; U.I. – unsaturation index.

Introduction

In fish, the FA composition of tissue lipids reflects that of their diet (Watanabe 1982; Henderson and Tocher 1987) so that tissue lipid composition can be manipulated in farmed fish by diets which contain oils with different FA compositions. The respiratory and cardiovascular physiology of farmed fish is influenced by such manipulation of the FA composition of the tissues, with fish having tissue lipids rich in n-3 HUFA such as eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), appearing to have improved tolerance of physiological stresses such as live transport, hypoxia, or exhaustive exercise (Bell et al. 1991, 1993; Randall et al. 1992; McKenzie et al. 1995, 1996, 1997; Agnisola et al. 1996).

In particular, studies on the Adriatic sturgeon (*Acipenser naccarii*), a primitive chondrostean fish, found that those fed a commercial diet supplemented with n-3 HUFA (as menhaden oil at 15% of dry feed weight) had significantly lower metabolic rates (measured as M_{O2}) than sturgeon fed a diet supplemented with SFA (as 15% dry feed weight of coconut oil), although both groups grew equally well (McKenzie et al. 1994, 1997, 1999). The two groups exhibited differences in their responses to hypoxia; degrees of hypoxia that caused hyperventilation, depression of spontaneous swimming activity and depression of M_{O_2} in sturgeon fed SFA had no such effects on fish fed n-3 HUFA (McKenzie et al. 1995, 1997). Overall, the results indicated that, when compared to sturgeon fed SFA, those fed n-3 HUFA were more tolerant of hypoxia by virtue of their lower metabolic rate (McKenzie et al. 1999).

The present study investigated the hypothesis that increased n-3 HUFA levels in tissue lipids would cause reduced metabolic rates in a teleost, the European eel (*Anguilla anguilla*), and that the reduced metabolic rate would be linked to improved tolerance of hypoxia. Groups of eels were fed diets with supplements of either menhaden oil or coconut oil to elicit differences in the content of total n-3 FA and n-3 HUFA in their tissues, and the effects on metabolic rate assessed by measurements of O_2 uptake. Eels were then exposed to progressive hypoxia to determine whether differences in metabolic rate affected the critical P_{O_2} for maintenance of O_2 uptake ($P_{O_2}c$) or caused differences in ventilatory reflex responses and the regulation of blood O_2 content. The effects of adding vitamin E to the diets was also investigated, as previous studies have indicated that this lipid anti-oxidant can interact with tissue fatty acid manipulation to influence metabolic rate and responses to hypoxia in sturgeon (Randall et al. 1992; McKenzie et al. 1995, 1997; Agnisola et al. 1996).

Materials and methods

Animals and diets

European eels, *Anguilla anguilla* Linnaeus ($n = 250$) with a mean ($\pm$ SE) initial mass of 249 ± 4 g were divided into 10 groups of 25 animals, with no significant differences in mean mass amongst the groups. The eels were maintained indoors in 1 m^2 tanks with a continuous supply of biofiltered freshwater at $23 \pm 1\,^{\circ}C$ and pH 7.9, at the Experimental Thermal Aquaculture Plant 'La Casella' [via Argine del Ballottino, 29010, Sarmato (PC), Italy]. The animals were exposed through skylights to a natural photoperiod. One of four experimental diets was fed to two replicate groups. The first diet (MOD), comprised 150 g menhaden (*Brevoortia tyrannus*) oil supplements for every 850 g of a commercial eel feed (Farina Agridea, Agridea, Verona, Italy); the second diet (MOVD) comprised MOD + 500 Mg kg^{-1} vitamin E (α-tocopherol acetate). The third diet (COD) comprised 150 g coconut (*Cocos nucifera*) oil supplements for every 850 g of the commmercial feed; the fourth diet (COVD) comprised COD + 500 Mg kg^{-1} vitamin E. The remaining two groups were fed the commercial diet without oil or vitamin E supplements. All diets were also supplemented with 50 g kg^{-1}lecithin and 500 Mg kg^{-1} ascorbic acid.

The Farina Agridea commercial feed was composed of fishmeal, herring meal, fish oil, soya flour, yeast flour, yeast and etoxiquin, and the manufacturer provided the proximal composition carried in Table 1. The oils were obtained from ICN Biomedicals, the α-tocopherol acetate and ascorbic acid from Hoffman La Roche. The FA composition of the oils has been reported previously (McKenzie et al. 1995). The commercial feed contained 21% by weight of lipids (largely derived from fish oil), such that addition of further 15% by weight of oil supplements led to diets with approximately 36% lipid by dry weight. This is a higher lipid content than normal commercial diets, but was used in order to permit comparison with previous studies on sturgeon, where commercial diets with identical menhaden and coconut oil supplements (15% by dry weight) were employed (McKenzie et al.

Table 1. Composition of the commercial Farina Agridea fish feed (as supplied by the manufacturer, Agridea, Verona, Italy)

Moisture (%)	9
Protein (%)	44
Fat (%)	21
Fibre (%)	1.5
Ash (%)	9
Vitamin A	10,000
Vitamin D3	2,000
Vitamin PP (*sic*)	150
Vitamin C	200
α-tocopherol acetate (91% vitamin E)	200
Sodium menadione (51% vitamin K3)	10
Vitamin B1	25
Vitamin B2	40
Vitamin B6	25
Vitamin B12	0.05
Choline (mg)	1000
Pantothenic acid	60
Folic acid	5
Biotin	0.5
Inositol	150
Iron sulfate	40
Zinc oxide	50
Copper sulfate	5
Manganese sulfate	35
Cobalt carbonate	0.8
Iodine (as calcium iodide)	5
Selenium (sodium salt)	15

All micronutrients are given as Mg kg^{-1} diet, except vitamins A and D3 that are given as IU kg^{-1} diet.

1994, 1995, 1997, 1999; Agnisola et al. 1996). The diets were prepared as moist paste by adding 30% w/w water, divided into daily aliquots of 3% of the total weight of each replicate in the dietary groups, and stored at $-20\,^{\circ}C$ until use. Animals were weighed at 30 day intervals during the feeding period, and the weight of daily aliquots adjusted accordingly. Eels were maintained on the non-supplemented diet to provide information about the effects of the dietary oil supplements on final mass and the FA composition of the tissue lipids following 90 days' feeding.

Analysis of fatty acid composition of dietary and tissue lipids

The FA composition of the dietary lipids was measured on samples of freshly prepared diets, and the FA composition of tissue lipids was measured in liver and muscle of freshly killed eels from each dietary group ($n = 5$), after 90 days' feeding. Muscle samples were collected from a section of the same portion of each animal, immediately caudal to the pectoral girdle. Diet and tissue samples were frozen on dry ice and stored at $-20\,^{\circ}C$ for a maximum of two weeks prior to analysis. Samples were defrosted, homogenised, and total lipids were extracted with 20 ml g^{-1} of chloroform/methanol 2:1 (v/v), according to Folch et al. (1957), with 5 Mg l^{-1} butylated hydroxytoluene as an anti-oxidant, and extracted lipids dissolved in a known volume of chloroform/methanol (2:1 v/v). Acidic transmethylation (3N HCl in methanol) was used to prepare methylesters from 400 μg of the lipid extracts. These were then analysed using a Dani 8510 gas-chromatograph with a programmable temperature vapouriser injector and a column (Supelcowax 30 m, 0.30 Mm diameter, 0.27 μm film thickness) with temperature programming (150 to 220 °C at 2.5° min^{-1} increments). The content of individual FA was quantified as μg Mg^{-1} tissue lipids by reference to an internal standard. The percentage composition in FA was determined and the total percentages of n-3 FA, n-6 FA, SFA, mono-unsaturated FA (MUFA) and polyunsaturated FA (PUFA) calculated. The n-3/n-6 and eicosapentaenoic acid/arachidonic acid (EPA/AA) ratios were calculated, as was the unsaturation index (U.I.), this latter being the sum of the products: percentage content of FA x number of double bonds, as calculated for each FA in the mixture and describes the average number of double bonds ($\times 100$) of the FA mixture.

Oxygen uptake and routine metabolic rate

Analysis of the effects of the diets on O_2 uptake was initiated following 90 days' feeding on the experimental diets, and limited to a comparison between the four groups fed the experimental diets containing oil supplements, and hence diets with similar total lipid contents. Oxygen uptake was measured on 9 eels from each group, with the automated technique described by Steffensen et al. (1994) and McKenzie et al. (1995, 1997). Briefly, eels that had previously been starved for 24 h were placed in 10 l darkened Plexiglass respirometer chambers and allowed to recover for at least 12 h. The respirometer chamber was immersed beneath 25 cm of water in a 1 m^2 tank. Water in the sealed respirometer was mixed by the action of a pump (Eheim 1048) in a closed external circuit. The water O_2 partial pressure (Pw_{O_2}) in the respirometer was measured by a Radiometer E-5046

O_2 electrode in a thermostatted cuvette, with the signal displayed on a Radiometer PHM 73 Acid-Base analyzer and recorded by a PC with a Data translation DT2801 data acquisition interface board, driven by the program Labtech Notebook. Data were collected over a 10-min cycle, where a 6-min period of closed-cycle water circulation alternated with a 4 min period when the respirometer was flushed, by an Eheim 1060 pump, with fresh water from the surrounding tank. Variations in Pw_{O_2} were collected and stored every second by Labtech Notebook, then transferred every 10 min into a spread-sheet program (Lotus 1-2-3). Linear regressions between Pw_{O_2} and time during the 6 min period of recirculation were calculated automatically and the slopes used to quantify O_2 uptake with the following formula:

$$M_{O_2} = \text{slope} \times V_{\text{resp}} \times \text{mass}^{-1} \times \alpha, \qquad (1)$$

where M_{O_2} = O_2 uptake in MgO_2 kg^{-1} h^{-1}, V_{resp} = volume of the respirometer in liters, mass = fish mass in kg and α = solubility of O_2 in water at the experimental temperature (Cruz-Neto and Steffensen 1997). Only slopes derived from equations with a $r^2 > 0.9$ were used in the calculations. The water in the outer tank was circulated through a gas-exchange column counter-current to a flow of compressed air to maintain normoxia and through a biofilter to remove wastes. Water in the outer tank could be made hypoxic by opening a flow of 100% nitrogen through the gas-exchange column, with Pw_{O_2} of the water monitored by another Radiometer O_2 electrode and PHM 73 blood gas analyser.

Oxygen uptake rate was measured every ten minutes for 8h in normoxia with all measurements made between 03:00 and 11:00, with the fish left undisturbed by the experimenter during this period. A frequency distribution of O_2 uptake rates was described for each animal, by calculating the percentage of the M_{O_2} measurements that fell within given intervals of O_2 uptake: 0–20 Mg Kg^{-1} h^{-1}, 20–40, 40–60 and so on at intervals of 20 until 200 Mg Kg^{-1} h^{-1}. The mean frequencies were then calculated for each dietary group and used to develop a frequency distribution of rates of O_2 uptake for each group. This permitted a general description of overall 'patterns' of M_{O_2} in resting fish from the four dietary groups. The mean rates of O_2 uptakes over the entire 8 h measurement period were calculated for each individual animal, and were considered to represent routine metabolic rate (RMR), inasmuch as they comprised the natural range of M_{O_2} values of a routinely active (i.e., not exercised) eel. The mean of the six lowest rates of O_2 uptake (i.e., representing a total cumulative time of 1 h during the 8-h period) was also calculated for each animal and averaged across the dietary groups. This then allowed analysis of whether progressive hypoxia cause a decline in M_{O_2} below the lowest rates measured in normoxia.

Effects of progressive hypoxia on oxygen uptake

To investigate the effects of gradual hypoxia on M_{O_2} in the eels, measurements were made every 10 min during a 2-h period in which Pw_{O_2} was gradually lowered to approximately 3 kPa (= 20 MmHg) and also collected for 2.5 h during which Pw_{O_2} was allowed to return to normoxia (19.5 kPa; approx. 145 MmHg). The critical water O_2 partial pressure required for maintenance of O_2 uptake in hypoxia ($P_{O_2}c$) was calculated empirically with reference to RMR by identifying the Pw_{O_2} at which each animal initiated a consistent reduction in M_{O_2} below RMR, and then calculating the mean of this P_{O_2} value within each group. This empirical method of calculating $P_{O_2}c$ was used in order to establish a measure of 'hypoxia tolerance' that could be compared between the dietary groups. The degree of hypoxic depression of M_{O_2} for each individual eel was evaluated as the maximum percentage decline in O_2 uptake below RMR, which was almost always at the deepest level of hypoxia tested. Hypoxic depression of M_{O_2} was also evaluated against the lowest rates of O_2 uptake measured in normoxia (see above), to estimate whether progressive hypoxia caused a depression of M_{O_2} below the entire range of normoxic rates in the eel and hence a depression of aerobic metabolism. During recovery, the extent of the increase in M_{O_2} possibly associated with repayment of an 'oxygen debt' was evaluated by identifying the maximum (peak) M_{O_2} observed for each fish during recovery, whenever this might have occurred, and averaging this over the groups. The percentage increase of this peak rate over RMR was calculated.

Effects of progressive hypoxia on ventilatory and haematological variables

Following a period of at least 105 days' feeding on the experimental diets, eels from each group ($n = 6$, except for MOD, where $n = 5$) were starved for 24 h, anaesthetised in a 1:5,000 w/v buffered solution of tricaine methane sulphonate (MS 222), then transferred to a surgical table where they were artificially ventilated with an MS 222 solution at 1:10,000

w/v. A cannula (PE 50 Intramedic) was implanted, via the roof of the mouth, into the dorsal aorta (Soivio et al. 1972). An opercular cannula was fitted using heat-flared cannula tubing (PE 50 Intramedic) passed through a small hole in the operculum and secured with a cuff and sutures. Care was taken to ensure that the opercular cannula was placed in the same position, in the centre of the operculum, on each fish. Following surgery, fish were transferred to individual darkened plexiglass chambers (vol. 11 l) and allowed to recover for 48 h in a continuous flow of aerated water (normoxic $Pw_{O_2} = 19.2 \pm 0.5$ kPa). The dorsal aortic cannula was flushed twice daily with heparinised (50 IU l^{-1}) Cortland's saline (Wolf 1963).

Following this recovery period, the water-filled opercular cannula was attached to a differential pressure transducer (Validyne 45DF) for measurement of gill ventilation rate (f_G, beats min^{-1}) and opercular pressure amplitude (P_{OP}, kPa), with these variables displayed and recorded on a chart recorder (Gould Windograf). Animals were exposed to 1 h of gradual progressive hypoxia, down to a Pw_{O_2} of 25 MmHg (approximately 3.4 kPa), and f_G and P_{OP} measured at 10 MmHg (approximately 1.3 kPa) intervals. Pw_{O_2} was then returned to normal over a 1-h period whilst measurements of ventilatory variables were collected. To quantify ventilation, rate was counted for 2 min, and P_{OP} averaged from 10 measurements of individual waveforms within that period. Opercular pressure amplitude was used as an index of ventilatory effort. Blood samples (300 μl) were withdrawn from the arterial cannula in normoxia and at hypoxic Pw_{O_2}'s of 80, 50, 40 and 35 MmHg (10.5, 5.2, 3.9 and 3.5 kPa, respectively) and following recovery in normoxia. Withdrawn blood was replaced with an equal volume of saline. Arterial blood O_2 partial pressure (Pa_{O_2}) was measured with a Radiometer O_2 electrode, thermostatted to the same water temperature as the fish and attached to a Radiometer PHM73 acid-base analyser. Arterial blood total O_2 content (Ca_{O_2}) was measured as described by Tucker (1967) with an Instrumentation Laboratories O_2 electrode and IL1302 blood-gas analyser thermostatted to 37 °C. Whole blood was centrifuged, plasma decanted and frozen in liquid nitrogen for subsequent analyses of lactate levels (Sigma assay; Lactate Method 735).

Statistical analyses

Comparisons between all four groups for any particular single variable (e.g., total tissue lipid content) were made by one-way ANOVA, with Tukey *post-hoc* tests to determine where any significant differences lay. T-tests were used to compare the pooled MO phenotype with the pooled CO phenotype. The effects of progressive hypoxia on O_2 uptake, ventilation and haematological variables were analysed within each dietary group by one-way ANOVA for repeated samples. Comparisons between groups for any particular measurement interval during progressive hypoxia or recovery were made either by one-way ANOVA and Tukey post-hoc tests (all four groups) or by T-test (comparing pooled MO group with pooled CO group). In those cases that responses were analysed as percentage change from the control value, data were arc-sine transformed before analysis by ANOVA or T-test (as appropriate). In all cases, $P<0.05$ was considered the fiducial level of significance.

Results

The eels from all of the dietary groups showed significant weight gains during the 90 days of feeding. The two replicate tanks fed the non-supplemented commercial feed had the lowest final mean masses, and all replicate tanks of the eels fed the oil-supplemented experimental diets, except one fed the MOVD, had significantly higher final mean masses than one or both of the replicate tanks fed the non-supplemented commercial feed. There were no significant differences in final mean mass between any two replicates of the same dietary group so the individual weights were pooled to provide a single mean value for each diet. Mean ($\pm$ SE) final masses were 338 ± 13 g ($n = 44$) for the MOD group; 312 ± 11 g ($n = 40$) for the MOVD group; 314 ± 10 g ($n = 40$) for the COD group; 326 ± 16 g ($n = 44$) for the COVD group, and 268 ± 13 g ($n = 45$) for the group fed the non-supplemented commercial diet. The final mean masses of the MOD and COVD groups were significantly higher than that of the group fed the non-supplemented commercial feed.

Fatty acid composition of dietary and tissue lipids

The FA compositions of the prepared diets are carried in Table 2. The diets supplemented with MO and CO had significantly higher lipid contents than the non-supplemented diet. As is visible in Table 2, for the MOD, this was linked to a significant increase in the percentage composition of PUFA, as a consequence

Table 2. Lipid content of diet and, within the dietary lipids, the content of individual fatty acids, percentage composition of selected fatty acid groups, and selected fatty acid ratios. Fatty acids that represented less than 0.5% of total lipids are not reported

	CON	MOD	COD
Total lipids	183.0 ± 06.0	298.6 ± 11.0[a]	269.6 ± 24.1[a]
14:0	21.7 ± 1.1	51.0 ± 4.3	127.3 ± 12.8[a,b]
16:0	169.3 ± 0.2	172.8 ± 11.3	151.8 ± 8.8
18:0	9.4 ± 0.03	16.9 ± 1.3[a]	37.0 ± 2.9[a,b]
16:1	51.6 ± 7.5	77.2 ± 4.5[a]	33.7 ± 4.5[b]
18:1n-9	216.0 ± 6.9	152.5 ± 6.6[a]	165.4 ± 14.9[a]
20:1n-9	22.8 ± 0.6	28.7 ± 1.7	20.0 ± 0.9
18:2n-6	81.4 ± 3.7	48.5 ± 2.0[a]	51.3 ± 1.4[a]
20:4n-6 (AA)	8.3 ± 0.4	8.8 ± 0.2	9.9 ± 2.0
18:3n-3	10.6 ± 0.6	9.9 ± 0.4	5.6 ± 0.3[a,b]
18:4n-3	7.5 ± 0.7	16.3 ± 1.5[a]	3.6 ± 0.4[a,b]
20:5n-3 (EPA)	34.4 ± 1.7	86.9 ± 3.9[a]	31.8 ± 3.7[b]
22:5n-3	8.5 ± 0.3	17.9 ± 0.9[a]	7.3 ± 0.6[b]
22:6n-3 (DHA)	67.8 ± 0.4	80.3 ± 4.2	58.8 ± 5.2[b]
n-3	17.6 ± 0.2	26.6 ± 0.9[a]	15.0 ± 1.6[b]
n-6	12.9 ± 0.2	8.0 ± 0.2[a]	9.4 ± 0.6[a]
n-3/n-6	1.4 ± 0.03	3.3 ± 0.1[a]	1.6 ± 0.1[b]
EPA/AA	4.1 ± 0.04	9.9 ± 0.4[a]	3.4 ± 0.3[b]
SFA	28.9 ± 0.9	32.1 ± 0.7	44.5 ± 1.0[a,b]
MUFA	40.1 ± 0.9	33.0 ± 0.7[a]	30.6 ± 1.9[a]
PUFA	31.0 ± 0.04	34.9 ± 1.1	24.9 ± 2.3[a,b]
U.I.	164.3 ± 1.3	192.5 ± 4.7[a]	136.4 ± 9.5[a,b]

Values are mean ± SE, $n = 5$ in all cases.
[a]Significantly different from CON group;
[b]Significantly different from MOD group ($P < 0.05$).
Total lipids are Mg g^{-1} wet weight of diet, individual FA are μg Mg^{-1} of total lipids. Total n-3, n-6, SFA, MUFA and PUFA are given as percentage of total FA.
CON, non-supplemented commercial feed; MOD, menhaden oil diet; COD, coconut oil diet; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U.I., unsaturation index.

of increased content of total n-3 FA, in particular n-3 HUFA such as EPA; for the COD, this was linked to a significant increase in the percentage composition of SFA and in the content of SFA such as 14:0.

Groups fed the experimental diets had higher lipid contents in muscle than did the group fed the non-supplemented feed, significantly higher in the MOVD, COD and COVD groups (Table 3). There were, however, no such differences between groups for the lipid content of the liver (Table 3). There were no differences in lipid content of skeletal muscle between the groups fed the supplemented diets (Table 3).

The most common FA in the muscle lipids from all groups were 18:1n-9 and 16:0, which reflects their predominance in the dietary lipids (Table 4). There were marked differences amongst the dietary groups in the content of other FA, differences that closely reflected those of the diet (Table 4). The MO supplements caused the MOD and MOVD groups to accumulate n-3 FA, particularly n-3 HUFA such as EPA and DHA, when compared to the eels fed the non-supplemented diets or those from the COD and COVD groups (Table 4). The CO supplements, on the other hand, caused the COD and COVD groups to accumulate SFA such as 14:0 when compared to the unsupplemented diet or the MOD and MOVD groups (Table 4). The differences in FA composition of the muscle lipids meant that the tissues of the MOD and MOVD groups had a

Table 3. Mean (± SE) lipid content of muscle and liver in eels fed the non-supplemented commercial diet and the four supplemented experimental diets

	CON	MOD	MOVD	COD	COVD
Muscle	105.6 ± 23.1	165.1 ± 14.1	206.3 ± 23.6[a]	190.9 ± 13.5[a]	184.6 ± 13.2[a]
Liver	68.4 ± 4.8	76.0 ± 14.4	99.0 ± 21.2	100.9 ± 13.9	117.0 ± 17.7

Units are Mg g^{-1} wet weight of tissue $n = 5$ in all cases.
[a]Significantly different from CON group.
CON, non-supplemented commercial feed; MOD, menhaden oil diet; MOVD, menhaden oil and vitamin E diet; COD, coconut oil diet; COVD, coconut oil and vitamin E diet;

Table 4. Mean (± SE) content of individual fatty acids, percentage composition of selected fatty acid groups, and selected fatty acid ratios, in lipids from the muscle of eels fed the non-supplemented commercial diet and the four supplemented experimental diets. Fatty acids that represented less than 0.5% of total lipids are not reported

	CON	MOD	MOVD	COD	COVD
14:0	36.5 ± 4.7	37.8 ± 3.1	38.5 ± 3.8	71.9 ± 15.0[a,b,c]	59.1 ± 4.9
16:0	112.3 ± 3.4	90.6 ± 18.2	113.1 ± 7.6	128.7 ± 34.9	107.3 ± 2.8
18:0	34.9 ± 12.5	18.4 ± 4.8	18.0 ± 4.4	19.3 ± 6.4	19.6 ± 1.2
16:1	42.2 ± 1.0	50.6 ± 2.7	53.5 ± 5.8	49.9 ± 9.6	37.3 ± 2.8
18:1n-9	171.5 ± 12.6	134.2 ± 9.5	136.0 ± 13.7	208.9 ± 39.1[b]	167.4 ± 5.7
20:1n-9	18.3 ± 1.9	12.6 ± 1.2	13.0 ± 0.9	16.0 ± 3.9	11.5 ± 0.7
18:2n-6	25.9 ± 2.2	21.7 ± 0.9	25.7 ± 2.1	30.0 ± 5.1	23.3 ± 1.5
20:4n-6 (AA)	4.4 ± 0.4	4.2 ± 0.2	4.5 ± 0.4	4.8 ± 0.9	3.5 ± 0.1
18:3n-3	3.4 ± 0.1	4.6 ± 0.2	5.3 ± 0.8	3.3 ± 0.5	2.8 ± 0.1[b,c]
18:4n-3	2.7 ± 0.1	5.1 ± 0.4[a]	5.4 ± 1.0[a]	2.6 ± 0.5[b,c]	2.0 ± 0.2[b,c]
20:5n-3 (EPA)	20.2 ± 0.7	34.2 ± 2.1[a]	36.3 ± 6.6[a]	20.5 ± 3.1[c]	16.4 ± 0.4[b,c]
22:5n-3	10.9 ± 0.9	15.4 ± 0.87	18.7 ± 3.1[a]	12.9 ± 2.3	10.2 ± 0.7[c]
22:6n-3 (DHA)	37.7 ± 1.6	50.57 ± 2.3[a]	55.9 ± 8.2[a]	43.7 ± 6.8	35.2 ± 1.1[c]
n-3	14.1 ± 0.4	22.5 ± 0.5[a]	22.2 ± 1.9[a]	13.8 ± 1.0[b,c]	13.2 ± 0.4[b,c]
n-6	6.4 ± 0.6	6.2 ± 0.2	6.5 ± 0.4	6.5 ± 0.6	5.9 ± 0.3
n-3/n-6	2.2 ± 0.1	3.6 ± 0.1[a]	3.5 ± 0.4[a]	2.1 ± 0.1[b,c]	2.2 ± 0.1[b,c]
EPA/AA	4.7 ± 0.4	8.1 ± 0.4[a]	7.8 ± 0.8[a]	4.4 ± 0.3[b,c]	4.7 ± 0.1[b,c]
SFA	35.1 ± 2.2	30.3 ± 1.9	32.7 ± 1.3	34.4 ± 3.3	37.5 ± 0.9
MUFA	43.9 ± 1.7	40.5 ± 1.6	38.1 ± 0.9	44.8 ± 1.9	42.9 ± 1.0
PUFA	21.0 ± 0.9	29.2 ± 0.7[a]	29.2 ± 1.7[a]	20.9 ± 1.5[b,c]	19.6 ± 0.4[b,c]
U.I.	137.0 ± 4.6	177.8 ± 3.9[a]	174.4 ± 9.0[a]	137.0 ± 8.4[b,c]	130.1 ± 1.9[b,c]

Individual FA are μg Mg^{-1} of total lipids. Total n-3, n-6, SFA, MUFA and PUFA are given as percentage of total FA.
$n = 5$ in all cases.
[a]Significantly different from CON group.
[b]Significantly different from MOD group.
[c]Significantly different from MOVD group ($P < 0.05$).
CON, non-supplemented commercial feed; MOD, menhaden oil diet; MOVD, menhaden oil and vitamin E diet; COD, coconut oil diet; COVD, coconut oil and vitamin E diet; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U.I., unsaturation index.

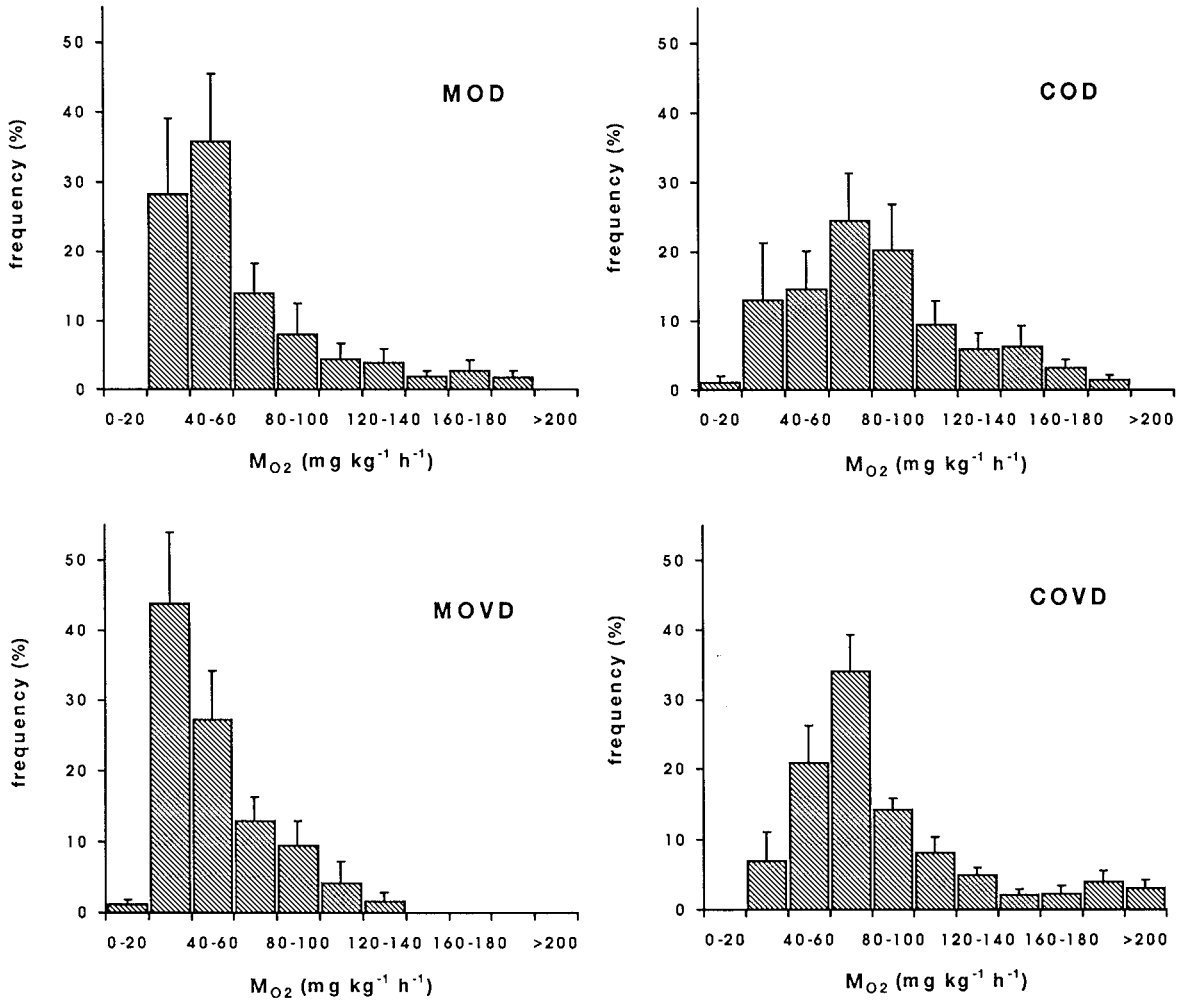

Figure 1. Distribution of mean (± SE) frequencies at which eels, fed the four oil-supplemented experimental diets, consumed O_2 within different rate intervals, as measured every 10 min for 8 h. n = approx. 330 observations on 9 animals from each group. MOD, menhaden oil diet; MOVD, menhaden oil plus vitamin E diet; COD, coconut oil diet; COVD, coconut oil plus vitamin E diet; M_{O_2}, O_2 uptake.

higher ratio between total n-3/n-6 FA content, a higher EPA/AA ratio and a higher unsaturation index than the muscle of the eels fed the non-supplemented diet, the COD and the COVD (Table 4). The n-3/n-6 ratio, EPA/AA ratio and unsaturation index were quite similar between the group fed the non-supplemented diet and those fed the coconut oil supplements (COD and COVD groups). Addition of vitamin E to the supplemented diets had no effect on FA composition of muscle lipids, the MOD and MOVD groups could not be distinguished on the basis of their FA composition, and neither could the COD and COVD groups (Table 4).

The liver of all groups exhibited increased contents of HUFA such as AA and DHA when compared to the muscle (Table 5). Nonetheless, the most common FA in the liver were 16:0 and 18:1n-9, reflecting their predominance in the diet (Table 5). The effects of the MO and CO supplements on liver FA content were similar to the effects on the muscle, although the differences in, e.g., DHA levels were less marked (Table 5). As seen with the muscle lipids, addition of vitamin E to the supplemented diets had no effect on FA composition of liver lipids, the MOD and MOVD groups could not be distinguished on the basis of their FA composition, and neither could the COD and COVD groups (Table 5). Given that the addition of vitamin E to the diet had no effect on tissue FA composition, the MOD and MOVD groups could be considered a single MO phenotype and the CO and COVD groups a single CO

Table 5. Mean (± SE) content of individual fatty acids, percentage composition of selected fatty acid groups and selected fatty acid ratios, in lipids from the liver of eels fed the non-supplemented commercial diet and the four supplemented experimental diets. Fatty acids that represented less than 0.5% of total lipids are not reported

	CON	MOD	MOVD	COD	COVD
14:0	32.8 ± 7.1	30.5 ± 6.0	37.3 ± 6.3	60.8 ± 4.8[a,b,c]	50.6 ± 1.5[a,b]
16:0	120.2 ± 9.5	135.3 ± 11.2	130.3 ± 8.2	167.7 ± 14.1	104.9 ± 6.7
18:0	49.7 ± 3.2	43.9 ± 4.4	48.3 ± 4.9	48.4 ± 3.1	46.2 ± 4.3
16:1	25.2 ± 1.2	35.1 ± 6.3	37.0 ± 4.2	37.5 ± 3.7	23.0 ± 2.3
18:1n-9	189.7 ± 14.6	102.6 ± 12.8	141.6 ± 17.1	195.9 ± 24.6	148.9 ± 10.4
20:1n-9	9.1 ± 2.0	7.9 ± 1.4	11.9 ± 2.0	16.5 ± 1.3	15.5 ± 0.8
18:2 n-6	25.3 ± 3.7	22.9 ± 3.4	26.8 ± 2.5	32.5 ± 2.3	24.4 ± 1.3
20:4 n-6 (AA)	14.0 ± 1.3	14.9 ± 1.7	11.6 ± 1.9	11.9 ± 1.5	8.7 ± 1.2
18:3 n-3	2.2 ± 0.2	3.5 ± 0.8	4.0 ± 0.7	2.8 ± 0.4	2.0 ± 0.2
18:4 n-3	0.6 ± 0.2	2.3 ± 0.9[a]	2.3 ± 0.5[a]	0.8 ± 0.2[b,c]	0.5 ± 0.1[b,c]
20:5 n-3 (EPA)	16.8 ± 1.8	37.1 ± 7.8[a]	30.9 ± 4.8[a]	22.2 ± 2.6	13.3 ± 1.3[b,c]
22:5 n-3	11.7 ± 1.1	17.5 ± 5.5	21.3 ± 3.8	14.9 ± 2.0	9.5 ± 1.5[c]
22:6 n-3 (DHA)	80.5 ± 6.2	109.4 ± 13.1	97.4 ± 10.0	78.4 ± 5.0	54.1 ± 5.7[b,c]
n-3	18.9 ± 1.6	29.0 ± 2.8[a]	25.5 ± 2.8	17.0 ± 1.3[b,c]	15.4 ± 1.2[b,c]
n-6	7.7 ± 0.8	8.0 ± 0.6	7.4 ± 0.4	7.4 ± 0.4	7.4 ± 0.2
n-3/n-6	2.5 ± 0.4	3.7 ± 0.5	3.5 ± 0.4	2.3 ± 0.1[b]	2.1 ± 0.2[b,c]
EPA/AA	1.2 ± 0.1	2.6 ± 0.8	2.8 ± 0.5	1.9 ± 0.3	1.6 ± 0.2
SFA	34.3 ± 2.2	37.1 ± 2.9	35.2 ± 1.2	39.3 ± 1.2	39.4 ± 1.0
MUFA	38.2 ± 2.8	25.2 ± 1.0[a]	31.1 ± 2.2	35.3 ± 2.3[b]	36.7 ± 1.5[b]
PUFA	27.5 ± 1.7	37.7 ± 2.4[a]	33.7 ± 3.0	25.4 ± 1.5[b,c]	23.9 ± 1.2[b,c]
U.I.	170.3 ± 8.9	213.7 ± 15.2[a]	195.9 ± 14.5	153.7 ± 5.7[b,c]	146.4 ± 6.3[b,c]

Individual FA are μg Mg^{-1} of total lipids. Total n-3, n-6, SFA, MUFA and PUFA are given as percentage of total FA.
$n = 5$ in all cases.
[a]Significantly different from CON group.
[b]Significantly different from MOD group.
[c]Significantly different from MOVD group ($P < 0.05$).
CON, non-supplemented commercial feed; MOD, menhaden oil diet; MOVD, menhaden oil and vitamin E diet; COD, coconut oil diet; COVD, coconut oil and vitamin E diet; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U.I., unsaturation index.

phenotype, the two phenotypes being distinguished by the FA composition of their tissue lipids.

Oxygen uptake and routine metabolic rate

Figure 1 shows the frequency distributions of rates of O_2 uptake in the four groups fed the dietary oil supplements. The MOD and MOVD groups were very similar in their pattern of M_{O_2}, exhibiting a narrow distribution of low rates. They differed from the COD and COVD groups, which both exhibited a wider range of more elevated rates. Mean (± SE) RMR, as MgO_2 Kg^{-1} h^{-1}, was 66.2 ± 9.1 for MOD; 51.2 ± 5.7 for MOVD; 82.6 ± 9.9 for COD, and 84.2 ± 6.2 for COVD ($n = 9$). The MOVD group had a significantly lower RMR than the COD and COVD groups, with no other significant differences between the groups. The lowest rates of O_2 uptake measured in normoxia were 32.3 ± 4.7 for MOD; 28.2 ± 2.5 for MOVD; 56.4 ± 7.4 for COD, and 47.0 ± 4.6 for COVD (mean ± SE, $n = 9$). The MOVD group had a significantly lower mean rate than the COD group, with no other significant differences between the groups.

The patterns of M_{O_2} did not differ between the MOD and MOVD groups, and they could be considered a single MO phenotype in terms of the FA composition of their tissue lipids. The same was true of patterns of M_{O_2} and tissue FA composition in the COD and COVD groups. Therefore, the MOD and MOVD groups were pooled to form a single MO group (phenotype), and the COD and COVD groups were pooled to form a single CO group (phenotype). Each group, therefore, contained an identical propor-

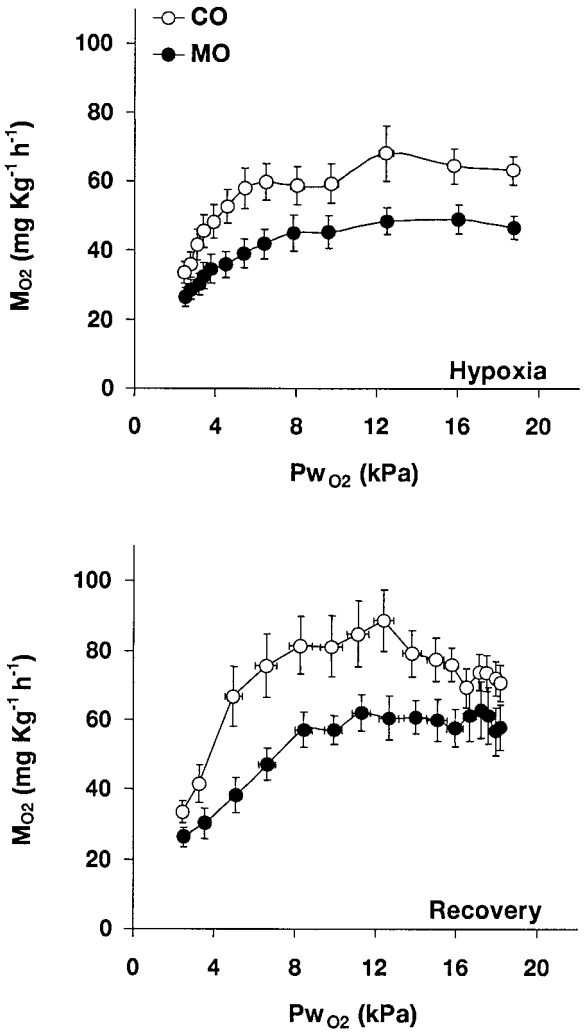

Figure 2. Mean (± SE) O_2 uptake in eels of the MO and CO groups when exposed to progressive hypoxia (upper panel) or during recovery to normoxia (lower panel). $n = 18$ in both cases. MO, menhaden oil; CO, coconut oil; M_{O_2}, O_2 uptake; Pw_{O_2}, water O_2 partial pressure.

tion (50%) of eels fed the vitamin E supplements. The frequency distributions of rates of M_{O_2} in these MO and CO groups were similar to those of their component groups; the MO animals consumed O_2 within a narrow range of low rates while the CO animals exhibited a wider range of more elevated rates. The MO group had a mean RMR that was significantly lower than that of the CO animals (Table 6). As regards the lowest rates measured in normoxia, these were 35.3 ± 3.1 MgO_2 kg^{-1} h^{-1} in the MO group, significantly lower than 51.7 ± 4.4 in the CO group (mean ± SE, $n = 18$).

Effects of progressive hypoxia on oxygen uptake

Analysis of the effects of progressive hypoxia on M_{O_2} have been confined to a comparison between MO and CO groups (Figure 2). In both groups, hypoxia caused a significant depression of M_{O_2}, followed by a significant increase in M_{O_2} during recovery, this latter presumably associated with repayment of an 'oxygen debt' (Figure 2). Overall, Figure 2 reveals that the MO group had a lower mean M_{O_2} than the CO group throughout hypoxia and recovery, but the pattern of change in M_{O_2} with P_{O_2} was similar in both groups. There were no differences in $P_{O_2}c$ between the two groups (Table 6). Both groups showed a significant depression of M_{O_2} compared to their RMR at the most profound degree of hypoxia but, although the M_{O_2} values measured at that point were significantly lower in the MO as compared with the CO group, the mean percentage decreases in M_{O_2} from RMR were similar in both (Table 6). Both groups also showed a significant depression in M_{O_2} below the lowest rates measured in normoxia, by $33.6 \pm 5.4\%$ in the MO group and $36.2 \pm 5.3\%$ in the CO group (mean ± SE, $n = 18$). With regard to the increased M_{O_2} observed during recovery, the MO group showed a mean peak in M_{O_2} that was significantly lower than the mean peak rate measured in the CO group, but the mean percentage increases in M_{O_2} over RMR were similar in both groups (Table 6).

Effects of progressive hypoxia on ventilatory and haematological variables

As was the case for the analyses of FA composition and O_2 uptake, there were no differences in any ventilatory or blood gas variable between MOD and MOVD groups, nor between COD and COVD groups, at any level of hypoxia or recovery (data not shown). Thus, analysis of the effects of progressive hypoxia on ventilatory and blood gas variables is limited to a comparison between eels fed MO supplements (pooled MOD and MOVD) versus those fed CO supplements (COD and COVD). The MO group did not exhibit any reflex ventilatory responses to progressive hypoxia, with no significant changes in either f_G or P_{OP} (Figure 3). Indeed, Figure 3 shows some evidence of a reduction in P_{OP} at the most profound levels of hypoxia, although this was not significant. The CO group showed a modest but significant increase in f_G at intermediate levels of hypoxia and in P_{OP} at the most profound level of hypoxia (Figure 3). Overall, however, the patterns of response in the MO and CO

Table 6. Routine metabolic rate, critical water O_2 partial pressure for maintenance of routine metabolic rate, lowest rates of O_2 uptake measured in progressive hypoxia and peak rates of O_2 uptake measured during normoxic recovery in the MO and CO groups, and their percentage changes relative to routine metabolic rate

	MO group	CO group
RMR ($mgO_2\ kg^{-1}\ h^{-1}$)	58.3 ± 5.4	83.3 ± 5.7[a]
$P_{O_2}c$ (kPa)	9.62 ± 1.08	7.57 ± 1.07
Lowest M_{O_2} in hypoxia ($mgO_2\ kg^{-1}\ h^{-1}$)	23.6 ± 2.5[b]	32.6 ± 3.4[a,b]
% decline below RMR	59.6 ± 3.8	59.3 ± 3.6
Peak M_{O_2} in recovery ($mgO_2\ kg^{-1}\ h^{-1}$)	79.1 ± 8.6[b]	108 ± 9.9[a,b]
% increase above RMR	36.4 ± 11.3	38.5 ± 10.4

Values = mean ± SE, $n = 18$ in all cases.
[a]Significantly different from MO group.
[b]Significantly different from RMR in the same group.
RMR, routine metabolic rate; $P_{O_2}c$, critical water O_2 partial pressure for maintenance of routine metabolic rate; M_{O_2}, O_2 uptake.

were very similar, with no significant differences in ventilatory variables between them in normoxia or at any level of hypoxia (Figure 3). During recovery, the MO group showed a significant increase in f_G, followed by a significant decrease as Pw_{O_2} returned towards normoxia, but there were no effects on P_{OP} (Figure 3). The CO group also exhibited a significant hyperventilation during recovery, and in this group it was associated with a significant change in P_{OP}, which declined from the elevated value measured in deep hypoxia as the water Pw_{O_2} returned to normoxic values (Figure 3). As observed during progressive hypoxia, the response patterns of both groups were very similar and there were no significant differences between the two for either ventilatory variable at any measurement interval of recovery.

Progressive hypoxia elicited a significant reduction in Pa_{O_2} and Ca_{O_2} in both groups, and there were no differences between them for either of these variables at any measurement interval (Figure 4). The MO group had a significantly lower plasma lactate concentration than the CO one in normoxia, but both showed similar significant increases during progressive hypoxia (Figure 4). The CO group, however, exhibited a higher concentration of plasma lactate following recovery in normoxia (Figure 4).

Discussion

Lipid content in the eel muscle was similar to that previously reported for farmed individuals of this species fed on similar diets, and was higher than that found in wild-caught eels (Abrami et al. 1992). As is sometimes the case in freshwater fish, the eels in the present study had higher lipid contents in muscle than in liver (Henderson and Tocher 1987), indicating that the muscle is a lipid storage organ in the eel. This assumption is borne out by the fact that the increased dietary lipid provided by the MO and CO supplements was linked to increased lipid content in the muscle but not in the liver.

The differences in RMR between the MO and CO groups were presumably a result of the differences in FA composition of their dietary and tissue lipids, the major differences being in their total n-3 FA content, n-3/n-6 ratio and EPA/AA ratio. The fact that the groups fed the oil supplements had similar mean final masses following the feeding trial, and higher final masses than eels fed the non-supplemented commercial feed, indicates that the lower metabolic rate in the MO group was not linked to reduced growth. Similar effects on metabolic rate and growth of n-3 HUFA versus SFA have been reported previously for a primitive chondrostean fish, the Adriatic sturgeon (McKenzie et al. 1994, 1997, 1999), which may indicate that dietary and consequent tissue FA composition exerts profound effects on metabolic rate that are similar in very different fish species.

The mechanism(s) underlying the effects of FA on metabolic rate are not known. FA have at least three roles in animal cells, as fuels (triacylglycerols) as membrane components (phosphoglycerides), and, for some C_{20} HUFA (20:3n-6; AA; EPA), as precursors of eicosanoids required for cellular regulatory processes. Aerobic metabolism in fish is primarily fuelled by FA oxidation (Hochachka and Somero 1984),

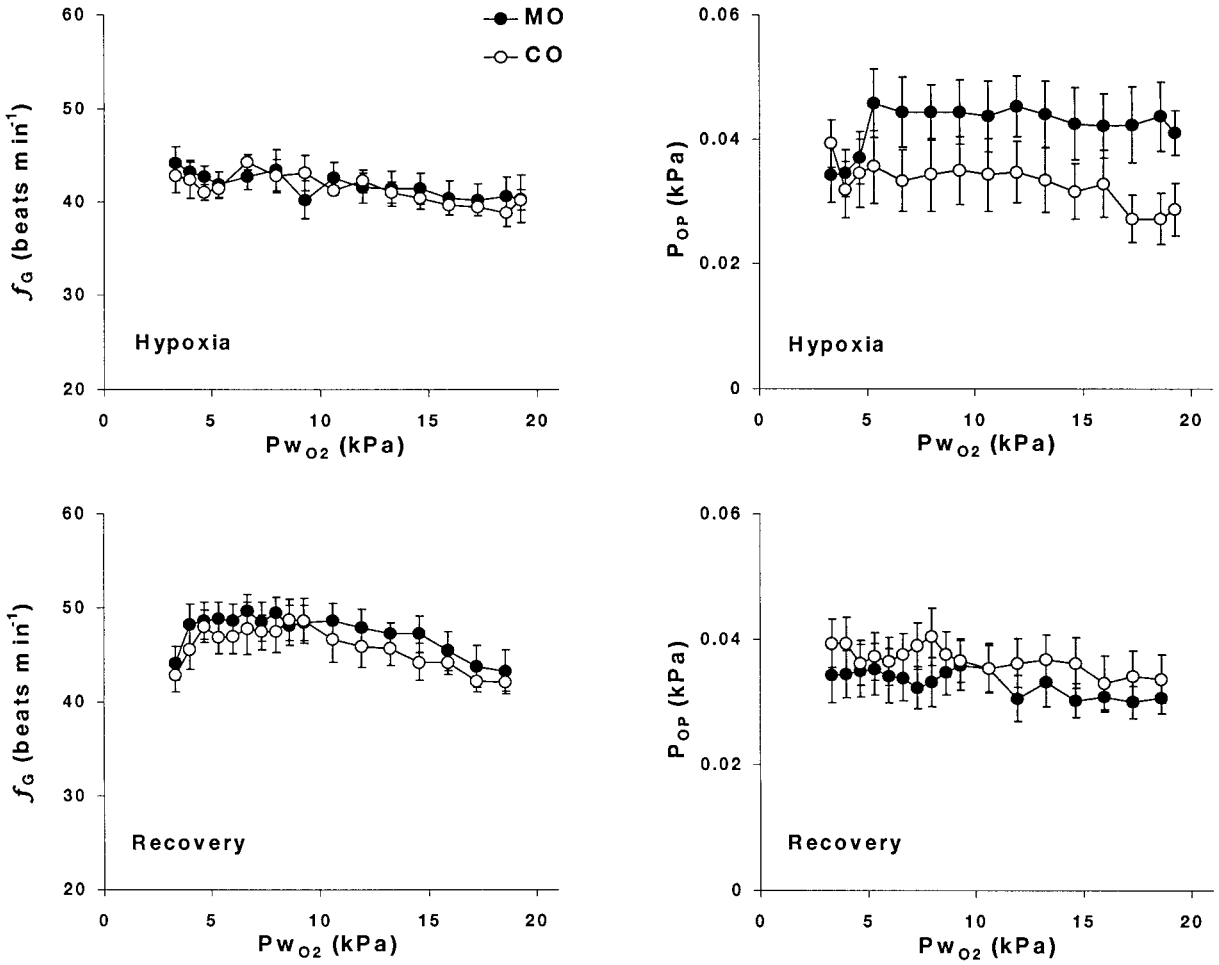

Figure 3. Mean (± SE) gill ventilation frequency and opercular pressure amplitude in eels of the MO and CO groups when exposed to progressive hypoxia (upper panel) or during recovery to normoxia (lower panel). $n = 11$ for MO and 12 for CO. MO, menhaden oil; CO, coconut oil; f_G, gill ventilation frequency; P_{OP}, opercular pressure amplitude; Pw_{O_2}, water O_2 partial pressure.

and there is much *in vitro* evidence indicating that the liver, heart and red muscle of fish exhibit differences in their ability to oxidise FA, with MUFA being preferred over SFA which, in turn, are preferred over PUFA (and therefore HUFA) as substrates (Henderson and Sargent 1985; Sidell and Driedzic 1985; Egginton 1996). The reduced metabolic rate of sturgeon and eels with tissues rich in n-3 HUFA might, therefore, have been a result of such substrate preferences causing sub-optimal FA oxidation. This, however, seems unlikely because in the present study the mono-unsaturate 18:1n-9 and saturate 16:0 were the predominant FA in muscle and liver of both the MO and CO groups, with similar tissue contents in both groups. Furthermore, it might be expected that if fish fed n-3 HUFA had lower metabolic rate as a consequence of sub-optimal β-oxidation they would exhibit low growth rates, but this does not appear to be the case in either eels (this study) or sturgeon (McKenzie et al. 1994).

Both n-3 HUFA and SFA could influence aspects of cell energy production and utilisation through effects on membrane-associated processes. In mammals, the degree of coupling between M_{O_2} and ATP production (P/O ratios) in mitochondria can influence standard metabolic rate (SMR; Rolfe and Brown 1997), and both n-3 HUFA and SFA are reported to affect P/O ratios in mammalian tissues *in vitro* (Piquet et al. 1996; Hermesh et al. 1998). Activity of the Na^+,K^+-ATPase and Ca^{2+}-ATPase represent a significant proportion of mammalian cell energy consumption and SMR (Rolfe and Brown 1997) and, therefore, differ-

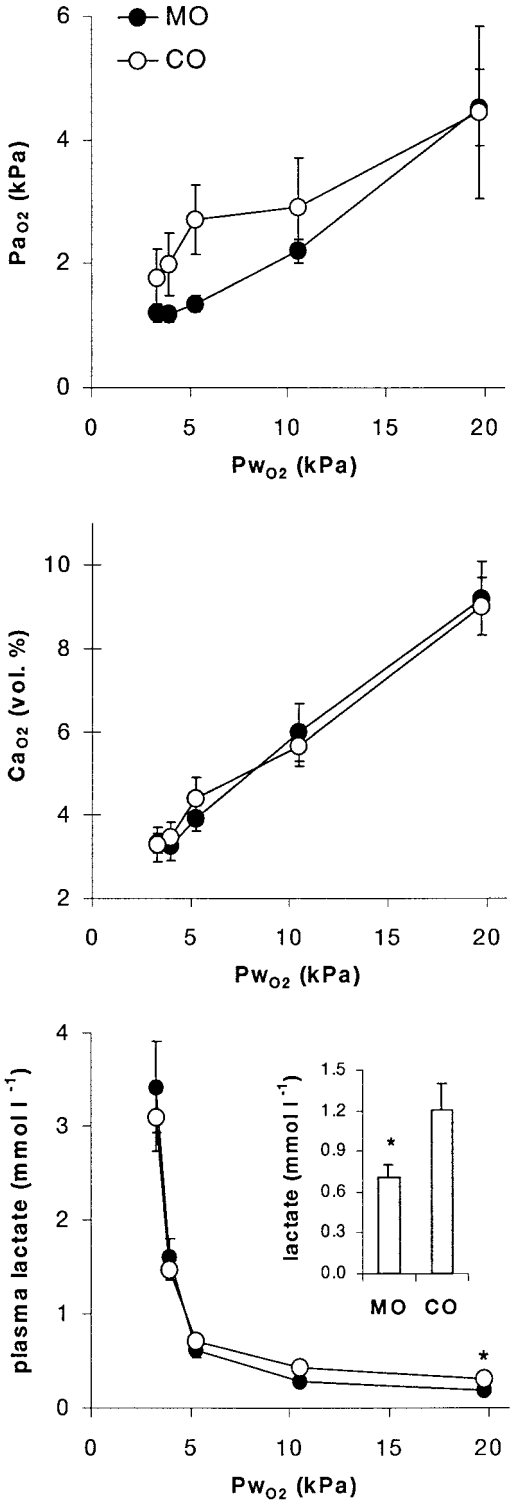

Figure 4. Mean (± SE) arterial blood O_2 partial pressure; O_2 content, and plasma lactate concentration in eels of the MO and CO groups when exposed to progressive hypoxia. Inset shows plasma lactate following 30 min recovery in normoxia. $n = 11$ for MO and 12 for CO. * = significantly lower than CO group. MO, menhaden oil; CO, coconut oil; Pa_{O_2}, arterial blood O_2 partial pressure; Ca_{O_2}, arterial blood O_2 content; Pw_{O_2}, water O_2 partial pressure.

ences in energy consumption by membrane-bound ATPases could lead to differences in tissue O_2 demands and SMR in fish. Studies on mammals and fish indicate that tissue n-3 HUFA levels can influence activity of the Na^+,K^+-ATPase and Ca^{2+}-ATPase (Gerbi et al. 1994; Ushio et al. 1997). Paige et al. (1996) reported that increased dietary n-3 HUFA levels were linked to reduced sarcoplasmic reticulum Ca^{2+}-ATPase activity in trout hearts, even though such hearts produced greater contractile force.

Dietary n-3 HUFA levels also influence patterns of eicosanoid production in fish tissues, possibly as a consequence of changes in the EPA/AA ratio in tissue lipids (Bell et al. 1993, 1997). Eicosanoids have many effects on cell metabolism (Lands 1991, Rowley et al. 1995), and differences in constitutive eicosanoid production could conceivably, therefore, be linked to differences in autocrine stimulation of cell metabolic activity. Clearly, the differences in metabolic rate between the MO and CO phenotypes could be a result of a combination of the various potential effects of FA on cell energy production and utilisation.

In the present study, vitamin E supplements of 500 Mg kg^{-1} dry feed weight had no effect on growth or aerobic metabolism. This contrasts with sturgeon, where the same dose of vitamin E led to poor growth and condition factor (D.J. McKenzie, unpublished observations) and elevated rates of routine M_{O_2} (McKenzie et al. 1995, 1997). This difference in the effects of vitamin E on aerobic metabolism between eels (no effect) and sturgeon (negative effect) may reflect the fact that in sturgeon combinations of α-tocopherol and ascorbic acid can have pro-oxidant effects, resulting in increased lipid peroxidation in Lake sturgeon (*Acipenser fulvescens*) liver (Moreau et al. 1999).

In sturgeon, the differences in RMR between animals fed n-3 HUFA versus SFA were associated with differences in ventilatory activity; ventilatory effort being greater in sturgeon with high RMR fed SFA (McKenzie et al. 1997). This was not the case in the eel, where differences in RMR had no effect on ventilation. Eels exhibit much lower mass-specific rates of O_2 uptake compared with sturgeon at the same temperature (McKenzie et al. 1997), and the relatively elevated O_2 demand of the CO animals may not, therefore, have been sufficient to stimulate increased ventilatory drive. It is also possible that the increased demand for O_2 was met in part by cutaneous respiration (Berg and Steen 1965; Le Moigne et al. 1986) or adaptive cardiovascular responses.

The values of $P_{O_2}c$ for a decline in M_{O_2} below RMR found at 23 °C in the present study (9.6 kPa in MO fish and 7.6 kPa in CO fish), are similar to the value of 9.2 kPa reported by Le Moigne et al. (1986) for *A. anguilla* at 18 °C and the values of 11.5 kPa at 10 °C and 16.8 kPa at 32 °C reported by Chan and Woo (1978) for *A. japonica.* However, they are much higher than the value of 3.4 kPa obtained by Cruz-Neto and Steffensen (1997) for *A. anguilla* at 25 °C using an automated respirometry system identical to that employed in the current study. The relatively low $P_{O_2}c$ reported by Cruz-Neto and Steffensen (1997) may be because these authors identified the critical P_{O_2} for maintenance of so-called SMR (estimated as the lowest rates measured over 24 h in normoxia), rather than RMR. In the present study if $P_{O_2}c$ is estimated with reference to the lowest rates of O_2 uptake measured in normoxia, by the same empirical method as used to calculate $P_{O_2}c$ relative to RMR (i.e., the water P_{O_2} is identified beyond which the eels exhibit a consistent decline in M_{O_2}), then the values obtained are similar to those reported by Cruz-Neto and Steffensen (1997), being 4.3 ± 0.2 kPa in the MO group and 5.1 ± 0.7 kPa in the CO group (mean $\pm$ SE, $n = 18$). These values are not significantly different.

The fact that the difference in metabolic rate between the MO and CO groups was not reflected in any differences in $P_{O_2}c$ in hypoxia might be considered counter-intuitive. That is, it would seem axiomatic that increased M_{O_2} in the CO group should be linked to increased sensitivity to reduced O_2 supply and therefore increased $P_{O_2}c$ (Herreid, 1980). It is interesting that although the absolute values of M_{O_2} in hypoxia and recovery were significantly lower in the MO group, the percentage changes in M_{O_2} relative to RMR during hypoxia and recovery were similar in both groups. The results clearly indicate that the increased metabolic rate of the eels from the CO group did not influence their homeostatic regulation of O_2 uptake in hypoxia.

Herreid (1980) has argued that in animals that regulate O_2 uptake during hypoxia, $P_{O_2}c$ will depend on the ratio between M_{O_2} and the conductance of O_2 to the tissues. That is, if M_{O_2} increases but $P_{O_2}c$ does not change, then the expectation would be that conductance of O_2 to the tissues has increased (Herreid 1980). It might be expected, therefore, that the CO group would show a more marked ventilatory reflex response than the MO group during progressive hypoxia, but this was not the case. Indeed, the fact that neither of the groups exhibited any marked hyperventilation despite the profound aquatic hypoxia and associated severe reductions in Ca_{O_2}, is unusual when compared to other teleosts. Current understanding of cardioventilatory control in teleosts considers that O_2 uptake is regulated in hypoxia through reflex changes in cardiac and ventilatory activity, with reflex hyperventilation being stimulated both by externally-oriented O_2 chemoreceptors sensitive to reductions in PwO_2 and internally-oriented receptors sensitive to reductions in Ca_{O_2} (Randall 1982; Smatresk 1990). The eels did not appear to follow this model with respect to the ventilatory responses.

Tissue O_2 conductance may have been sustained during hypoxia by adaptive cardiovascular responses and increased cutaneous O_2 uptake (Berg and Steen 1965; Le Moigne et al. 1986), thereby obviating the need for hyperventilation. It is difficult to envisage how cutaneous respiration (Berg and Steen 1965; Le Moigne et al. 1986) would sustain O_2 uptake in hypoxia better than increased counter-current ventilation/perfusion of the gills. There may have been differences between the dietary groups in perfusion of the gills and skin during hypoxia, permitting the CO group to regulate their more elevated O_2 uptake without any marked changes in ventilation. However, there were no differences in heart rate or blood pressure between the CO and MO groups during progressive hypoxia, both groups exhibiting a progressive bradycardia, no significant change in mean arterial pressure, but an increase in pulse pressure (D.J. McKenzie, unpublished observations).

Thus, the differences in metabolic rate elicited by the diets did not influence the animal's rather unusual response to hypoxia, a response characterised by an absence of any marked hyperventilation despite a profound decline in Ca_{O_2} and M_{O_2}, and no relationship between M_{O_2} and $P_{O_2}c$ (Herreid, 1980). The eels therefore differ from the Adriatic sturgeon, where the relatively elevated M_{O_2} of sturgeon fed SFA was linked to more profound hypoxaemia, greater hypoxic hyperventilation, and more profound hypoxic depression of O_2 uptake than in sturgeon with low M_{O_2} fed n-3 HUFA (McKenzie et al. 1995, 1997). The only difference between the MO and CO groups in the present study was that the CO group released more lactate into the plasma during recovery, indicating that there may have been a greater reliance on anaerobic respiration during deep hypoxia and therefore maintenance of an elevated anaerobic metabolic rate in that group.

In conclusion, these results have confirmed the hypothesis that the FA composition of dietary and tissue lipids would influence patterns of O_2 uptake in the

European eel, with routine metabolic rate being significantly lower in animals fed n-3 HUFA supplements (MO group) when compared to those fed SFA supplements (CO group). However, this difference in the rate of normoxic O_2 uptake between MO and CO animals was not associated with a difference in their regulation of aerobic metabolism in progressive hypoxia, a result that is not in agreement with the original hypothesis.

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