

Dietary fish oil reduces skeletal muscle oxygen consumption, provides fatigue resistance and improves contractile recovery in the rat *in vivo* hindlimb

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Dietary fish oil modulates skeletal muscle membrane fatty acid composition. Similar changes in heart membrane composition modulate myocardial oxygen consumption and enhance mechanical performance. The rat *in vivo* autologous perfused hindlimb was used to investigate the influence of membrane composition on skeletal muscle function. Male Wistar rats were fed either saturated fat (SF), *n*-6 PUFA (linoleic acid rich) or *n*-3 PUFA (fish oil) diets for 8 weeks. Hindlimb skeletal muscle perfused using the animal's own blood was stimulated via the sciatic nerve (1 Hz, 6–12 V, 0.05 ms) to contract in repeated 10 min bouts. The *n*-3 PUFA diet markedly increased 22:6*n*-3 DHA, total *n*-3 PUFA and decreased the *n*-6:*n*-3 PUFA ratio ($P < 0.05$) in red and white skeletal muscle membranes. There was no difference in initial twitch tension but the *n*-3 PUFA group maintained greater twitch tension within all contraction bouts and recovered better during rest to produce greater twitch tension throughout the final contraction bout ($P < 0.05$). Hindlimb oxygen consumption during contraction was significantly lower in the *n*-3 PUFA group compared with the SF group, producing a significantly higher O₂ efficiency index compared with both SF and *n*-6 PUFA groups ($P < 0.05$). Resting oxygen consumption was increased in recovery in the SF group ($P < 0.05$) but did not change in the *n*-3 PUFA group. Membrane incorporation of *n*-3 PUFA DHA following fish oil feeding was associated with increased efficiency of muscle O₂ consumption and promoted resistance to muscle fatigue.

Fish oil: *n*-3 PUFA: Oxygen consumption: Muscle fatigue: DHA

Dietary fatty acids affect structural membrane lipid composition, which in turn is strongly linked to cellular function⁽¹⁾, including myocardial cell function⁽²⁾. Although tissue fatty acid composition reflects the types of fatty acids in the diet^(3–6), patterns of incorporation vary according to the tissue. In particular, heart myocardial membranes incorporate the *n*-3 PUFA DHA, from the diet, but do so in concentrations well above the circulating concentrations⁽⁶⁾. Although less studied, skeletal muscle appears to share this property of membrane sensitivity to incorporation of DHA with fish oil diet intervention^(6–8). Furthermore, skeletal muscle membrane composition is influenced by exercise training^(9–11). In skeletal muscle, the incorporation of membrane *n*-3 PUFA may even be fibre-type specific and possibly be related to the high oxidative capacity of type I and IIa muscle cells where high levels of PUFA have been measured^(12,13).

There is extensive evidence that incorporation of dietary *n*-3 PUFA into myocardial membranes has cellular⁽²⁾ and direct effects on heart function. Fish oil feeding to increase dietary *n*-3 PUFA has been demonstrated to enhance mechanical performance of the heart in the marmoset monkey^(14,15) and rat^(16,17), decrease heart rate in exercising horses⁽¹⁸⁾ and in rats⁽⁵⁾, stabilise membranes to reduce arrhythmia vulnerability

in rats⁽⁴⁾ and marmosets⁽¹⁹⁾ and modulate myocardial O₂ consumption in rats⁽¹⁷⁾. In humans, the direct effects of dietary fish oil in the myocardium include reduced fatal arrhythmia⁽²⁰⁾ and reduced resting^(21,22) and exercising heart rate⁽²³⁾. The small but consistent reduction in heart rate has also been distilled from many clinical trials through meta-analysis⁽²⁴⁾ and indirectly supports the notion that myocardial O₂ consumption is reduced when fish is in the diet. Furthermore, there is evidence for reduction in whole body O₂ consumption in well-trained cyclists following dietary fish oil intervention⁽²³⁾, perhaps reflecting lower O₂ consumption by the working skeletal muscle. Reduced O₂ demand might produce fatigue resistance but study of isolated muscle has produced contradictory results and none have measured O₂ consumption. In view of limitations in O₂ delivery to isolated muscle bundles, an autologous, blood-perfused hindlimb method was developed⁽²⁵⁾ to investigate the effects of dietary *n*-3 PUFA on the skeletal muscle O₂ consumption and contractile function, measuring both short-term fatigue during repeated contractions and twitch contractile recovery between contraction bouts. The premise of the present study was the confirmed changes in skeletal muscle membrane fatty acid composition through dietary intervention would directly modify skeletal muscle O₂ consumption parallel to changes previously

Abbreviation: SF, saturated fat.

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reported for heart, which may be expressed as resistance to muscle fatigue.

Methods

Eighteen male Wistar rats (400–500 g) were used for the study (six per dietary intervention). Experiments were assessed and approved by the Animal Ethics Committee from the University of Wollongong, and all national and institutional guidelines were followed. Animals were housed two per cage in the University of Wollongong's animal facility with a room temperature maintained at 23–25°C and a 12 h light–dark cycle.

Diet composition

Three diets were prepared for the present study: saturated fat (SF); *n*-6 PUFA; *n*-3 PUFA. The diets all contained 10% fat by weight. The proportions of fat sources were selected to deliver similar total PUFA in the *n*-6 PUFA and *n*-3 PUFA diets, similar *n*-6 PUFA in the *n*-3 PUFA and SF diets and similar *n*-3 PUFA in the *n*-6 PUFA and SF diets. The final composition of each diet was established using GC against the known standards (Table 1). Olive oil was provided as light (refined) olive oil, therefore free of confounding sources of natural antioxidants; SF as beef tallow; *n*-6 PUFA as safflower oil; *n*-3 PUFA as high-DHA tuna fish oil (28.9%

DHA and 9% EPA) (Clover Corporation, Altona North, VIC, Australia). Diets were prepared from purified ingredients and stored at –20°C⁽²⁶⁾.

Study design

Animals were fed a wash-out diet containing olive oil for 14 d to standardise muscle membrane fatty acid composition. They were then randomly allocated into three groups and fed *ad libitum* the SF, safflower oil (*n*-6 PUFA) or the tuna fish oil (*n*-3 PUFA) diet for 8 weeks. Fresh food was provided twice per week and daily consumption (g) was estimated by weighing the remainder from the previous feeding. Animal weight was measured once a week.

Surgical preparation for ventilation, muscle perfusion and stimulation

The autologous pump-perfused hindlimb has been previously described in both dogs⁽²⁷⁾ and rats⁽²⁵⁾. In brief, rats (18–20 weeks) were anaesthetised (sodium pentobarbital 6 mg/100 g intraperitoneally) with supplementary injections (2 mg/100 intraperitoneally) as required. Experiments were performed with the rat and perfusion system enclosed in a heated perspex chamber (32.2 ± 0.3°C) with animal body temperature maintained at 37°C (rectal temperature). The rats were prepared for artificial ventilation and blood pressure recording by cannulation of the trachea and left carotid artery, respectively. Rats were ventilated (1 ml/100 g body weight) to ensure constant high arterial O₂ (Rodent Ventilator 7025, Ugo Basile, Italy).

All cannulae were fluid filled with saline containing 6% dextran (w/v) and 5000 IU heparin/100 ml (Sigma-Aldrich, Castle Hill, NSW, Australia). Arterial (oxygenated) blood was accessed via a cannula inserted into the right femoral artery (non-perfused leg) towards the heart and was connected to a re-sealing flexi-tube, passed through a peristaltic roller pump (Gilson Minipuls 3, Middleton, WI, USA). Arterial blood was then passed through a cannula that was inserted into the left femoral artery (perfused leg) and positioned to deliver blood directly to muscle groups below the knee. A T-junction inserted in the blood flow line on the perfused hindlimb side of the pump was connected to a pressure transducer (Argon CDXIII; Maxim Medical, Athens, TX, USA) for measurement of hindlimb perfusion pressure. Venous blood (de-oxygenated) return to the heart and lungs was achieved via cannula inserted into the left femoral vein of the perfused leg and right jugular vein at the other end for passive flow.

Hindlimb muscle contraction was achieved via sciatic nerve stimulation. A bipolar electrode (F-B5EI; Grass Instrument Division, West Warwick, RI, USA) was placed under the nerves for direct electrical stimulation of the trunk supplying the muscles of the gastrocnemius–plantaris–soleus muscle bundle. The nerve was crushed above the stimulation point to prevent retrograde conduction. The gastrocnemius–plantaris–soleus muscle group tendons were tied with non-compliant silk and connected to a force transducer (FT03C; Grass Instrument Division). Saline-soaked gauze was placed over the muscles to prevent them from drying.

Table 1. Fat sources and concentration of major fatty acids of experimental diets (g/kg diet)*

	Diet		
	SF	<i>n</i> -6 PUFA	<i>n</i> -3 PUFA
Fat source			
Olive oil	30	50	30
Beef tallow	70		
Safflower oil		50	
Fish oil			70
Fatty acid			
14:0	2.59	ND	2.12
16:0 Palmitic acid	20.56	8.08	17.06
16:1 Palmitoleic acid	3.16	0.40	3.38
18:0 Stearic acid	14.08	3.63	4.50
18:1 Oleic acid	47.94	49.97	33.52
18:2 (<i>n</i> -6) Linoleic acid	4.67	36.00	3.50
18:3 (<i>n</i> -3) α -Linolenic acid	0.58	0.49	0.56
20:4 (<i>n</i> -6) Arachidonic acid	0.04	0.06	1.30
20:5 (<i>n</i> -3) EPA	ND	ND	4.87
22:5 (<i>n</i> -3)	ND	ND	0.77
22:6 (<i>n</i> -3) DHA	ND	ND	20.20
EPA + DHA	0	0	25.07
Total SFA	38.06	12.59	25.48
Total MUFA	51.39	50.59	39.15
Total PUFA	5.34	36.65	31.40
<i>n</i> -6 PUFA	4.77	36.16	5.01
<i>n</i> -3 PUFA	0.58	0.49	26.39
<i>n</i> -6: <i>n</i> -3 ratio	8.27	73.80	0.19
P/S ratio	0.14	2.91	1.23
UI	62.84	124.71	203.10

SF, saturated fat; P/S ratio, ratio of PUFA/SFA; *n*-6:*n*-3 ratio, ratio of *n*-6 PUFA to *n*-3 PUFA; UI, unsaturated index; ND, not detectable.

*Fatty acid concentrations as determined by GC.

Hindlimb perfusion and stimulation protocol

All hindlimbs were perfused for 30 min at 1 ml/min to allow for perfusion pressure to reach steady state (approximately 100 mmHg). Baseline arterial and venous blood samples (400 μ l) were drawn after 30 min of the initial perfusion (flow = 1 ml/min) with no stimulation. Three separate 10 min stimulation bouts (1 Hz, 7–12 V, 0.05 ms) were delivered to the sciatic nerve separated by 30 min of muscle recovery. The flow rate was 1.5 ml/min during contraction periods and returned to 1 ml/min during the recovery periods. Arterial and venous samples were collected between the ninth and tenth minute of each contraction bout and between 25 and 30 min of recovery between bouts.

Measurements

Blood pressure and twitch force

Data were referenced to ground and amplified (Onspot Australia, Barrack Heights, NSW, Australia). The data acquisition software, Labview for Windows (National Instruments, Austin, TX, USA), was used to collect both pressure and twitch force simultaneously during the perfusion trials at a sampling rate of 200 Hz.

Blood samples

Arterial and venous blood samples (400 μ l) were collected via the re-sealing silicone sections of the cannulae located proximal and distal to the hindlimb. Of the 400 μ l blood sampled, 240 μ l was presented to the laboratory blood gas and electrolyte machine (BGElectrolytes; Instrumentation Laboratory, Lexington, MA, USA) for measurement of PO₂, PCO₂, Na⁺, K⁺, pH and Hb. The remaining 150–200 μ l was spun down on a bench-top micro centrifuge (Milipore, North Ryde, NSW, Australia), the plasma was removed and frozen for later analysis. Erythrocytes from the plasma collections were re-suspended in an equal volume of saline and re-injected into the venous side of the perfusion. Hb levels were maintained above 12.5 g/100 ml whole blood.

Muscle samples

Control muscle samples (gastrocnemius, plantaris and soleus) were taken from the right (non-perfused) leg immediately before the right femoral artery cannulation, to be used as within-animal control tissue. At the completion of each experiment and while the blood was still flowing, left leg (perfused and contracted) muscle samples (gastrocnemius, plantaris and soleus) were rapidly extracted. All muscles were freeze-clamped and stored (–80°C) for later analysis. Soleus was used for red/oxidative tissue and gastrocnemius portion was used for white/glycolytic tissue. Additional muscles (deep posterior tibial, extensor digitorum longus and tibialis anterior) from the perfused hindlimb were collected to establish total weight of the muscle involved in the perfusion.

Skeletal muscle membrane fatty acid analysis

The method of membrane fatty acid analysis has been described previously⁽⁷⁾. In brief, 100–150 mg of skeletal

muscle tissue were weighed and homogenised in a chloroform–methanol mixture (2:1, v/v) (analytical grade Fluka; Sigma-Aldrich). Steps were taken for total lipid extraction using 1 M-H₂SO₄ (Sigma-Aldrich), phospholipid separation via silica seppack columns (Waters, Sutton, MA, USA), methylation of phospholipid fatty acids using 14 % boron trifluoride in methanol ((Sigma-Aldrich) stored at 0–4°C) heated at 85°C for 1 h, purification via florisil Seppack columns (Waters) using diethyl ether (5 %) (Fluka; Sigma-Aldrich) in petroleum spirit (7 ml) (Fluka; Sigma-Aldrich) and finally GC (Shimadzu GC-17A, 30 m \times 0.25 mm internal diameter capillary column, total run time 23 min). All solvents were freshly prepared at the time of analysis and contained 0.01 % (w/v) butylated hydroxy toluene (Sigma-Aldrich). Individual fatty acids were identified by comparison with the known standards in the laboratory. The relative amount of each fatty acid was determined by integrating the area under the peak and dividing by the result for all fatty acids detected. Unsaturation index, which represents the number of double bonds per hundred fatty acid molecules, was calculated by summing the products of the proportion (mol%) of each fatty acid multiplied by the number of double bonds.

Statistical analysis

Computer software, Statistix, version 8 (Tallahassee, FL, USA), was used to analyse the data. One- (diet) and two-way (diet and time) ANOVA with individual means were compared using a corrected Bonferroni's *post hoc* analysis to indicate the significant difference. α was set at $P < 0.05$. Data were expressed as means with their standard errors.

Results

Food consumption and body weight

There was no significant difference in the quantity of food consumed between dietary groups at either the start, the end or as a mean weekly intake over the 8-week feeding period (SF: 6.85 (SEM 0.12); *n*-6 PUFA: 6.68 (SEM 0.15); *n*-3 PUFA: 6.70 (SEM 0.11) g/100 g body weight per week). Mean body weight was lower in the *n*-6 PUFA group (SF: 491 (SEM 27); *n*-6 PUFA: 427 (SEM 9); *n*-3 PUFA: 479 (SEM 15) g ($P < 0.05$ *n*-6) PUFA *v.* SF and *n*-3 PUFA). Despite the differences in mean body weight, there were no significant differences between dietary groups in mean total hindlimb muscle (SF: 4.48 (SEM 0.14); (*n*-6) PUFA: 4.23 (SEM 0.17); *n*-3 PUFA: 4.36 (SEM 0.15) g wet weight) or gastrocnemius–plantaris–soleus muscle bundle (SF: 2.42 (SEM 0.19); *n*-6 PUFA: 2.34 (SEM 0.11); (*n*-3) PUFA: 2.20 (SEM 0.11) g wet weight).

Skeletal muscle membrane fatty acids

The fish oil fed group showed several marked membrane fatty acid concentration differences compared with the SF and *n*-6 PUFA groups, which did not differ from each other in any significant way (Table 2). In red and white portions of skeletal muscle, the *n*-3 PUFA group had significantly lower 18:2*n*-6c (linoleic acid), 20:4*n*-6 (arachidonic acid) and total *n*-6 PUFA ($P < 0.05$) and significantly higher 20:5*n*-3 (EPA), 22:6*n*-3

Table 2. Skeletal muscle (red and white) membrane phospholipid fatty acid profiles (*n* 6)*
(Mean values with their standard errors)

	SF				<i>n</i> -6 PUFA				<i>n</i> -3 PUFA			
	Red		White		Red		White		Red		White	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:0	0.25	0.03	0.26	0.02	0.16	0.04	0.18	0.02	0.27	0.04	0.24	0.01
16:0	17.38	3.76	20.63	0.72	18.69	1.95	21.15	0.52	19.17	4.21	22.97	0.83
18:0	11.54	2.34	11.93	0.36	13.44	1.57	12.10	0.49	12.82	0.68	11.21	0.45
18:1 <i>n</i> -9	8.64	2.17	9.60	1.25	8.86	1.35	7.42	0.78	8.29	0.55	7.26	0.32
18:2 <i>n</i> -6c	13.75 ^b	3.04	14.59 ^b	0.32	14.80 ^b	1.58	15.06 ^b	0.19	10.06 ^a	0.67	9.63 ^a	0.7
20:4 <i>n</i> -6	15.30 ^b	0.86	16.63 ^b	0.68	17.24 ^b	2.29	20.81 ^b	1.47	10.37 ^a	0.960	9.43 ^a	0.84
20:5 <i>n</i> -3	0.11 ^b	0.07	0.04 ^b	0.03	nd	nd	nd	nd	1.03 ^a	0.06	1.11 ^a	0.07
22:5 <i>n</i> -3	1.68	0.51	2.47	0.30	1.47	0.22	1.77	0.14	1.18	0.14	1.08	0.08
22:6 <i>n</i> -3	13.03 ^b	3.36	9.98 ^b	1.05	8.93 ^b	0.98	9.10 ^b	0.31	24.36 ^a	0.68	25.16 ^a	1.06
ΣSFA	29.17	6.0	32.81	1.0	32.30	3.42	33.43	0.95	36.26	1.29	34.42	1.13
ΣPUFA	43.90	2.58	44.26	1.82	45.63	1.79	45.30	3.27	47.19	1.57	46.68	3.47
Σ(<i>n</i> -6)	30.10 ^b	3.44	33.89 ^b	1.74	35.37 ^b	1.79	36.67 ^b	1.47	20.06 ^a	1.13	20.53 ^a	0.70
Σ(<i>n</i> -3)	14.84 ^b	3.12	12.50 ^b	1.18	10.41 ^b	1.17	10.88 ^b	0.36	26.49 ^a	0.75	27.47 ^a	1.19
<i>n</i> -6: <i>n</i> -3	2.75 ^b	0.53	2.79 ^b	0.24	3.69 ^b	0.65	3.38 ^b	0.16	0.76 ^a	0.04	0.75 ^a	0.0-4
UI	187 ^b	12	186 ^b	10	178 ^b	9	186 ^b	6	224 ^a	7	232 ^a	8

SF, saturated fat; ΣSFA, sum of SFA; ΣPUFA, sum of PUFA; Σ(*n*-6), sum of (*n*-6) PUFA; Σ(*n*-3), sum of (*n*-3) PUFA; UI, unsaturation index; *n*-6:*n*-3, ratio of *n*-6 PUFA to *n*-3 PUFA; nd, not detectable.

^{a,b} Mean values within a row with unlike superscript letters were significantly different (*P*<0.05).

* Fatty acid concentrations as determined by GC.

(DHA) (Fig. 1(A), *P*<0.05), and total *n*-3 PUFA (Fig. 1(B), *P*<0.05) compared with both the SF and (*n*-6) PUFA groups. This was reflected by the significantly lower *n*-6:*n*-3 PUFA ratio for the *n*-3 PUFA muscles (Table 2, *P*<0.05). There were no significant differences that could be attributed to the red (soleus) or white (gastrocnemius) portion of muscle for these diets. The unsaturation index was significantly elevated in *n*-3 PUFA muscles compared with the SF and *n*-6 PUFA groups, which were not different from each other. There were no significant differences between groups in either total SFA or total PUFA.

Baseline perfusion

There was no significant difference in rectal temperature between dietary groups monitored during the procedure (SF: 37.0 (SEM 0.9); *n*-6 PUFA: 36.7 (SEM 0.6); *n*-3 PUFA: 36.0 ± 0.7°C). Hindlimb perfusion pressure after 30 min (1 ml/min) was significantly higher in the SF group compared with the *n*-3 PUFA group (SF: 126 (SEM 10); *n*-6 PUFA: 102 (SEM 14); *n*-3 PUFA: 93 (SEM 10) mmHg) (*P*<0.05). There were no significant differences between dietary groups in the baseline arterial blood samples (Table 3). In all groups, artificial ventilation and resulting PaO₂ were sufficient to achieve approximately 98% O₂ saturation of the erythrocytes and corresponding high content of arterial O₂ supply (approximately 19–20 ml/100 ml). Baseline venous samples demonstrated a significant drop in PO₂ compared with arterial blood (*P*<0.05) and uptake of O₂ across the resting muscle bundle. Although NS, the *n*-3 PUFA group demonstrated a trend for lower (*a* - *v*)O₂ difference (ml/100 per ml) and basal O₂ consumption (μmol/g per min) (Table 3) compared with both the *n*-6 PUFA (*P*=0.13) and SF (*P*=0.09) groups.

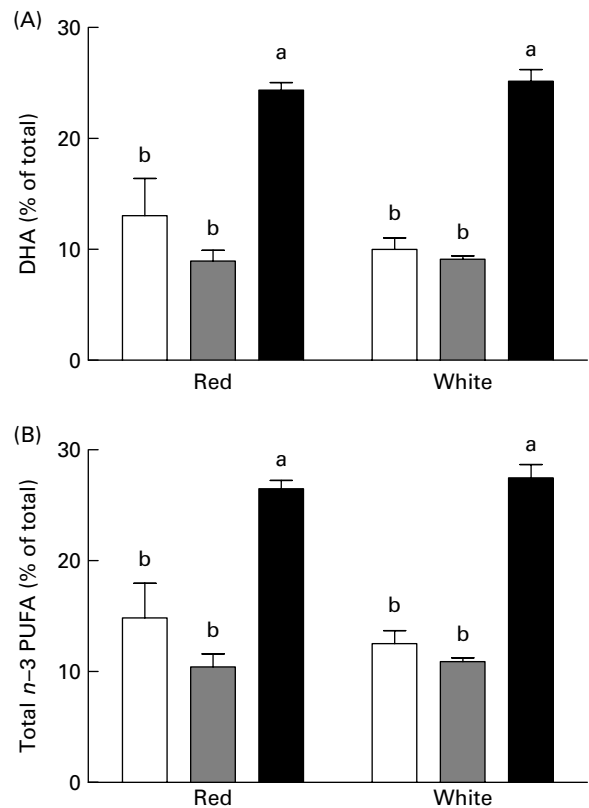


Fig. 1. Skeletal muscle PUFA phospholipid concentrations for saturated fat (SF), safflower oil (*n*-6 PUFA) and DHA-rich fish oil (*n*-3 PUFA) after 8 weeks feeding. (A) Total *n*-3 PUFA; (B) DHA concentration. Mean values with unlike letters between diet groups were significantly different (*P*<0.05). Mean values with their standard errors. □, SF; ■, *n*-6 PUFA; ■, *n*-3 PUFA.

Table 3. Baseline measurements of systemic arterial and venous hindlimb samples at a constant flow rate of 1 ml/min (*n* 6) (Mean values with their standard errors)

	SF		<i>n</i> -6 PUFA		<i>n</i> -3 PUFA	
	Mean	SEM	Mean	SEM	Mean	SEM
Arterial blood gas and metabolite						
pH	7.53	0.03	7.5	0.01	7.55	0.02
PaO ₂ (mmHg)	87	2	90	4	93	2
PaCO ₂ (mmHg)	31	2	34	2	33	2
Hb (g/100 ml)	13.3	0.3	13.9	0.5	14.3	0.5
CaO ₂ (ml/100 ml)	18.96	0.7	19.61	0.5	19.93	0.8
HCO ₃ ⁻ (mm)	25.8 ^a	1.1	32.1	3.0	29.2	2.3
Lactate (mm)	2.92	0.17	2.68	0.12	2.76	0.24
Venous blood gas and metabolite						
pH	7.41	0.02	7.45	0.02	7.41	0.03
PvO ₂ (mmHg)	40 ^b	2	43 ^{a,b}	3	47 ^a	2
PvCO ₂ (mmHg)	42	3	46	3	48	4
Hb (g/100 ml)	13.75	0.5	14.16	0.4	14.4	0.5
CvO ₂ (ml/100 ml)	14.38	1.0	15.63	0.7	16.53	0.9
HCO ₃ ⁻ (mm)	26.2 ^a	1.2	32.8 ^b	2.8	29.9 ^{a,b}	2.5
Lactate (mm)	2.62	0.2	3.66	0.19	3.12	0.5
Hindlimb resting oxygen consumption						
(<i>a</i> - <i>v</i>)O ₂ (ml/100 ml)	4.57	0.73	3.97	0.77	3.39	0.45
VO ₂ (μmol/g per min)	0.41	0.07	0.37	0.07	0.31	0.04

SF, saturated fat.

^{a,b} Mean values within a row with unlike superscript letters were significantly different (*P* < 0.05).

Muscle contraction

There was a significant decline in initial and maximum tensions between Bout 1 and Bout 3 independent of diet (*P* < 0.05) (Fig. 2). The decline was smallest in the *n*-3 PUFA group for both initial and maximum tension (*P* = 0.07 and 0.09, respectively). There was no significant difference in the final tension between Bout 1 and Bout 3 independent of diet; however, final tension in the *n*-3 PUFA group was significantly higher compared with the SF group (*P* < 0.05). As hindlimb blood flow increased, perfusion pressure significantly increased on each occasion independent of dietary group (*P* < 0.05). There were no significant effects of diet on hindlimb perfusion pressure during twitch contractions.

The recovery of initial, maximum and final twitch tension in Bout 3 (1.5 ml/min) was compared to Bout 1 (1 ml/min) (Fig. 3(A)). The *n*-3 PUFA group demonstrated a greater recovery of twitch tension in contraction Bout 3 (*P* < 0.05) compared with the SF and *n*-6 PUFA groups. Additionally, when final twitch tension was expressed as a percentage of maximum twitch tension (Fig. 3(B)), in bouts 1, 2 and 3, the *n*-3 PUFA group demonstrated greater maintenance of twitch tension over the 10 min (*P* < 0.05). Twitch characteristics including maximum rate of contraction (+*dT/dt*) and maximum rate of relaxation (-*dT/dt*) decreased across Bouts 1, 2 and 3 independent of diet. There was no significant difference between diets at the points of initial, maximum and final twitch tension (data not shown).

During contraction, the (*a* - *v*)O₂ difference (ml/100 per ml) and O₂ consumption (μmol/g per min) (Fig. 4(B)) were significantly lower in the *n*-3 PUFA group compared with the SF group. The efficiency index, calculated as the twitch tension developed relative to muscle mass for a given amount of O₂ consumed (N/g per μmol per min), was significantly higher in the *n*-3 PUFA group across all exercise bouts compared with both SF and *n*-6 PUFA groups (*P* < 0.05) (Fig. 4(C)).

Recovery between contraction bouts

The hindlimb O₂ consumption in the SF group was increased during recovery periods 1 and 2 compared with its own baseline (Fig. 4(A)) (*P* < 0.05). In comparison, O₂ consumption returned towards original baseline levels in both the *n*-3 PUFA and *n*-6 PUFA groups. Notably, within each recovery period, O₂ consumption for the *n*-3 PUFA group was significantly lower than that for the SF group (*P* < 0.05).

Discussion

In rats fed fish oil, skeletal muscle was resistant to fatigue during continuous muscle twitch contractions and recovered contractile force better between repeat bouts. Importantly, the improved contractile performance in the fish oil-fed animals was achieved with reduced skeletal muscle O₂ consumption both during contraction bouts and during recovery. These changes in muscle function were associated with the incorporation of *n*-3 PUFA into skeletal muscle membrane which reflected the well-documented membrane phospholipid responses to the dietary fish oil in excitable tissue⁽²⁸⁾ including heart and skeletal muscle in the marmoset monkey⁽⁶⁾, rat⁽⁸⁾ and human subjects⁽²⁹⁾.

Skeletal muscle membrane fatty acid composition

Large differences in skeletal muscle membrane fatty acid composition were achieved with 8 weeks of dietary manipulation; however, there was little indication in the present study that this was specific to muscle fibre type as previously suggested^(12,30). There was some reduction in the concentration of SFA palmitic acid (16:0) of red fibres compared with that of the white fibres, which has been inversely related to insulin sensitivity⁽³¹⁾. In addition, when provision of essential fatty acids was low (SF feeding), the concentration of

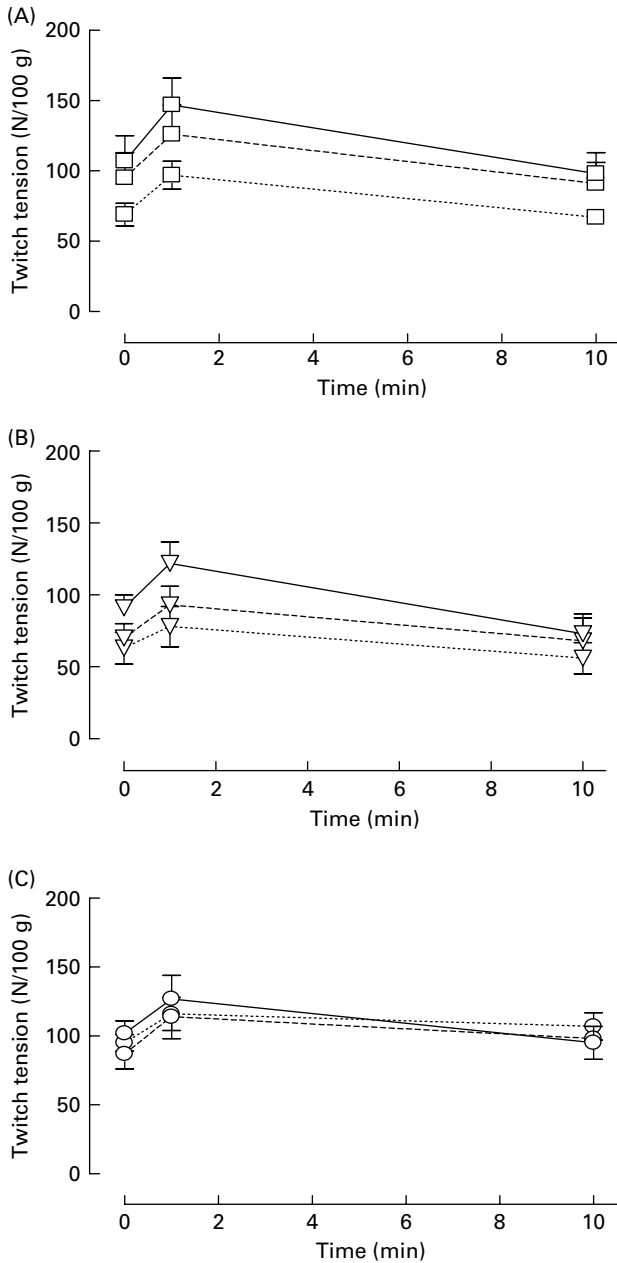


Fig. 2. Twitch tension development (N/g wet weight) for Bout 1, Bout 2 and Bout 3 for (A) saturated fat (SF), $-\square-$, Bout 1; $--\square--$, Bout 2; $\cdots\square\cdots$, Bout 3; (B) *n*-6 PUFA (safflower oil), $-\nabla-$, Bout 1; $--\nabla--$, Bout 2; $\cdots\nabla\cdots$, Bout 3 and (C) *n*-3 PUFA (DHA-rich fish oil) groups, $-\circ-$, Bout 1; $--\circ--$, Bout 2; $\cdots\circ\cdots$, Bout 3. Flow = 1.5 ml/min. Initial tension (time = 0), maximum tension (time = 60 s) and final tension (time = 10 min) are shown (*n* 6). Values displayed as means with their standard errors.

DHA was retained at higher levels in the red muscle compared with that of the white muscle. That is, the high oxidative fibres retained higher DHA concentrations compared with the low oxidative fibres⁽¹²⁾.

The long-chain *n*-3 PUFA DHA was the major fatty acid incorporated into skeletal muscle membranes with fish oil feeding, and the changes were comparable to those seen in the heart after similar dietary interventions^(5,6,16). Although the present study used a DHA-rich tuna fish oil supplement, it is evident that DHA incorporation is always the primary

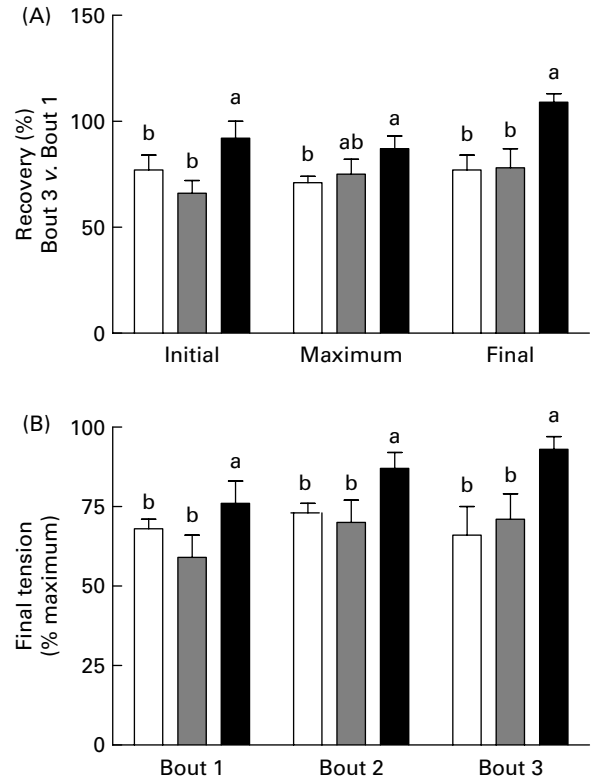


Fig. 3. (A) Recovery (%) for initial, maximum and final twitch tension Bout 3 v. Bout 1. (B) Final twitch tension as a percentage of maximum twitch tension (Bout 1, Bout 2 and Bout 3). \square , SF; \blacksquare , *n*-6 PUFA; \blacksquare , *n*-3 PUFA. Mean values with unlike superscript letters between diet groups were significantly different ($P < 0.05$) (*n* 6). Values displayed as mean with their standard errors.

response of excitable tissue to long-chain *n*-3 PUFA fatty acids in the diet, even when EPA-rich fish oils or purified EPA are consumed^(5,6). A systematic comparison of tissue fatty acid composition of the marmoset monkey demonstrated remarkably similar fatty acid profiles between heart and skeletal muscle⁽⁶⁾, whereas other tissues such as vascular smooth muscle, platelets or kidney do not reflect the same extent of DHA incorporation. The DHA concentration in skeletal muscle of the marmoset monkey is very similar to the red and white skeletal muscle of the rat in the present study and further similarities were seen in both SF and *n*-6 PUFA groups, where DHA concentrations were lower and arachidonic acid concentrations were higher⁽⁶⁾. The similarity in fatty acids of heart and skeletal muscle merits the consideration that skeletal muscle could be used as an indirect marker of the heart *n*-3 PUFA composition⁽⁶⁾. It is already established that the fatty acid composition of human skeletal muscle reflects the diet⁽³²⁾ and further use of the biopsy technique in skeletal muscle could allow for indirect monitoring of changes in the heart of human subjects.

Skeletal muscle function

This is the first study to investigate skeletal muscle function on the basis of the modulated O₂ consumption previously demonstrated in the heart⁽¹⁷⁾, and its success is largely dependent on properties of the *in vivo* autologous hindlimb model⁽²⁵⁾, adapted from canine muscle fatigue experiments⁽³³⁾. Only a

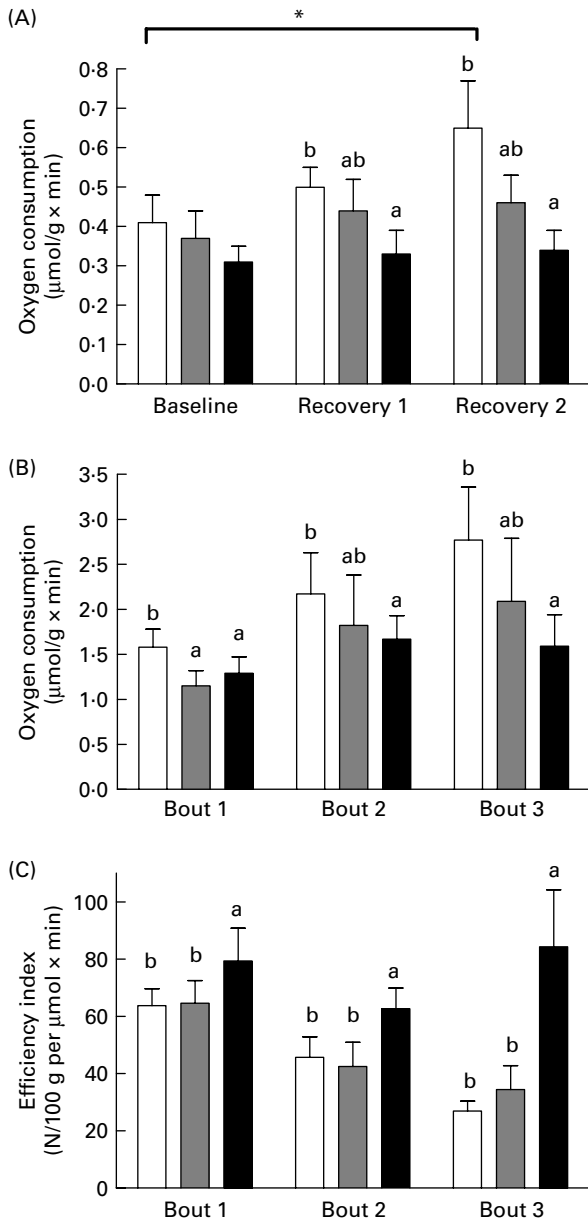


Fig. 4. (A) Oxygen consumption ($\mu\text{mol/g} \times \text{min}$) at baseline and recovery 1 and recovery 2 (following Bout 1 and 2 respectively). Flow rate = 1 ml/min, (B) contracting O_2 consumption ($\mu\text{mol/g} \times \text{min}$) and (C) efficiency index ($\text{N}/100 \text{g per } \mu\text{mol} \times \text{min}$) at flow rate 1.5 ml/min (Bout 1, Bout 2 and Bout 3). \square , SF; \blacksquare , n-6 PUFA; \blacksquare , n-3 PUFA. Mean values with unlike superscript letters between diet groups were significantly different ($P < 0.05$). *Mean values were significantly different within groups ($n = 6$) ($P < 0.05$). Values displayed as means with their standard errors.

few studies have considered the effects of dietary-induced changes in membrane composition on skeletal muscle function^(8,34,35). These studies used large muscle bundles *in vitro*, not reflective of *in vivo* O_2 delivery⁽³⁶⁾ or voluntary exercise *in vivo* and produced contradictory outcomes.

The progressive fatigue of the gastrocnemius–plantaris–soleus muscle bundle *in vivo* during repetitive twitch contractions was less evident in animals fed the n-3 PUFA diet, and fatigue resistance became even more apparent in repeat contraction bouts. Twitch force recovery between contraction bouts was also greater with n-3 PUFA feeding.

This is comparable to the enhanced cardiac contractile function^(14,15,37) and improved post-*ischaemic* cardiac recovery^(16,17) seen in association with fish oil feeding.

The inherently low resting O_2 consumption of perfused hindlimb was not affected by diet and concurs with findings from O_2 consumption of the K^+ -arrested, quiescent heart⁽¹⁷⁾. In contrast, O_2 consumption of the contracting hindlimb, evaluated during the final twitches of each contraction bout, increased in SF and n-6 PUFA hindlimbs, despite the decline in contractile function, but remained low in the n-3 PUFA group although muscle contraction was declining less. O_2 consumption and efficiency were modulated when the muscle was put under conditions of increased O_2 demand just as is observed in myocardium under stress such as increased filling pressure or acute myocardial *ischaemia* and reperfusion⁽¹⁷⁾. This represents a clear increase in efficiency of muscle O_2 use, again mimicking the effects observed in the heart⁽¹⁶⁾ where stress-induced increases in O_2 use is moderated by fish oil feeding.

Like fish oil feeding in the present study, endurance training promotes greater recovery from bouts of exercise⁽³⁸⁾. O_2 consumption, CO_2 production, minute ventilation and heart rate all show faster adjustment to relative and absolute exercise levels and recovery after exercise. Fish oil supplementation reduces whole body O_2 consumption and heart rate throughout prolonged exercise bouts in trained cyclists⁽²³⁾. The observations in the present study suggest that fish oil, like exercise training, may reduce fatigue and improve recovery after exercise.

The similarities between fish oil influences on skeletal muscle and myocardial composition and function suggest common underlying mechanisms. Identification of mechanisms of n-3 PUFA effects is more advanced for myocardium than for skeletal muscle. A vast array of n-3 PUFA-responsive cell signalling processes has been identified, including changes in eicosanoid and free-radical production⁽²⁾. Irrespective of the intracellular signalling, the n-3 PUFA are available through their prior incorporation into phospholipids of the cell membranes. Altered cellular Ca^{2+} handling is implicated in many of the effects of dietary fish oil in the heart⁽³⁹⁾, including appearing as a common endpoint or intermediate for effects on myocardial intracellular signalling⁽²⁾. Excessive O_2 consumption, cardiac arrhythmias and their modulation by n-3 PUFA have all been associated with conditions of cellular sarcoplasmic reticulum and mitochondrial Ca^{2+} overload^(17,40). Although skeletal muscle does not mimic the Ca-induced Ca-release of myocardium, it still makes use of the basic properties of Ca release from the sarcoplasmic reticulum into the cytosol and re-uptake which is implicated in muscle fatigue⁽⁴¹⁾. Therefore, Ca^{2+} cycling processes appear as one potential mechanism of action of fish oil fatty acids in excitable cell function that may translate to skeletal muscle.

The higher unsaturation index of skeletal muscle from fish oil-fed rats implies an increased risk of cellular oxidative damage, which might translate into poor contractile performance in line with the free-radical theory of fatigue⁽⁴²⁾. The paradoxical fatigue resistance provoked by fish oil feeding suggests that like *ischaemic* preconditioning⁽⁴³⁾, and nutritional preconditioning by n-3 PUFA in the heart⁽¹⁶⁾, or exercise training⁽⁴⁴⁾, the n-3 PUFA up-regulate the endogenous protective mechanisms in skeletal muscle.

In summary, using the rat *in vivo* autologous pump-perfused hindlimb, fish oil feeding delayed muscle fatigue and improved contractile recovery in association with changes in membrane phospholipid fatty acid composition. As previously observed in heart, there is evidence for the modulation of skeletal muscle O₂ consumption associated with increased membrane DHA levels. It is of primary interest to further investigate the levels of fatigue and the corresponding O₂ consumption at the commencement of contraction and establish that these findings are reproducible at all points of isometric fatigue. Additionally, it is of interest to establish if these observations also hold true when the skeletal muscle is subjected to greater levels of contractile fatigue (<50% maximum twitch tension) and longer duration of stimulation, which will also impact on the degree of fatigue. The present findings demonstrate the direct implications for dietary fats on skeletal muscle contractile performance and may have applications in cases of muscle fatigue and dysfunction such as heart failure and muscle dystrophy.

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