Dietary Fatty Acid Profile Influences the Composition of Skeletal Muscle Phospholipids in Rats[1,2]

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ABSTRACT Changes in dietary fatty acid composition alter phospholipid fatty acid composition in a variety of tissues, but little attention has been paid to skeletal muscle. In this study, rats were fed for 9 wk one of three isoenergetic diets: an essential fatty acid–deficient diet, a diet high in (*n*-6) fatty acids, and a diet enriched with (*n*-3) fatty acids. Some rats from each group were then fed a nonpurified diet for a further 2 or 6 wk. Neither body mass nor food consumption varied among the dietary groups at any stage. Analyses of total phospholipids in soleus (a "slow" twitch muscle) and extensor digitorum longus (a "fast" twitch muscle) revealed that after 9 wk of test diet consumption, muscle phospholipids from rats fed the essential fatty acid–deficient diet were deficient in essential polyunsaturated fatty acids (triene:tetraene ratio >0.5), whereas the polyunsaturated fatty acids in muscle phospholipids from rats fed the high (*n*-6) fatty acid and high (*n*-3) fatty acid diets reflected the compositions of their respective diets. Nevertheless, phospholipid fatty acid composition seemed to be selectively dynamic. After recovery, although the phospholipid fatty acid compositions of all groups were similar, they all contained a much higher proportion of (*n*-3) fatty acids than provided in the diets. Overall, these results demonstrate that in rats, the fatty acid profile of skeletal muscle phospholipids is strongly influenced by dietary changes, with most effects being reversible after short periods of adequate dietary intake. J. Nutr. 126: 653–662, 1996.

INDEXING KEY WORDS:

- *essential fatty acid deficiency*
- *(n-3) polyunsaturated fatty acids* • *soleus*
- *extensor digitorum longus* • *rats*

Studies using primates fed isoenergetic diets have shown that manipulation of dietary fatty acid profile can modify the phospholipid fatty acid composition in a variety of tissues, including liver, kidney, brain, heart, skeletal muscle and blood (Charnock et al. 1992). Modifications can occur rapidly (within days) and may affect the relative proportions of particular fatty acids as well as ratios of unsaturated fatty acid classes, e.g., the ratio of (*n*-6) to (*n*-3) polyunsaturated fatty acids. These dietary modifications in turn have been shown to dramatically alter a wide range of cellular activities through alteration of the physical properties of membranes, such as fluidity (McMurchie 1988). Such changes have generally been attributed to changes in levels of unsaturated fatty acids, but relatively little is known about the effects of variations in individual fatty acids, including the essential dietary fats (*n*-3) and (*n*-6).

A great deal of attention has been focused on the relationship between cardiac muscle composition and function. Several studies have shown that the phospholipid composition of cardiac muscle responds dramatically to dietary manipulation of polyunsaturated fatty acids (e.g., Abeywardena et al. 1987, Charnock et al. 1985a and 1985b, Gudbjarnason 1989). In rats, increased levels of dietary polyunsaturated fatty acids produce increased levels of polyunsaturated fatty acids in cardiac muscle phospholipids. In addition, polyunsaturated fatty acids afford protection from arrhythmia and infarction, whereas saturated fatty acids increase arrhythmogenesis (McLennan et al. 1989 and 1990). In contrast to the number of studies performed using cardiac muscle, little is known about the effects of variation in dietary fatty acid intake on the composition of skeletal muscle. Important exceptions include studies of the effects of dietary polyunsaturated fatty acids on general hindlimb muscles in marmosets (Charnock et al. 1989 and 1992), in which phospholipid fatty acid

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composition of marmoset cardiac and skeletal muscle was shown to be very similar, even after manipulation of dietary fatty acids. Also, Storlien et al. (1991) and Pan and Storlien (1993) have shown the effects of dietary lipid profile on skeletal muscle phospholipid composition and metabolism.

Most fatty acids can be derived from stored fatty acids (either in adipose tissue or intracellularly as triglycerides or free fatty acids) or synthesized de novo from standard precursor fatty acids. In contrast, the "essential" (*n*-6) and (*n*-3) polyunsaturated fatty acids, which are important components of muscle membranes (50–60% of total fatty acids, personal observation), are not believed to be synthesized de novo and must be ingested as part of the diet. This is an important point to consider when dealing with cases of abnormal nutritional status such as dietary disorders and starvation, as well as during fetal development and active growth periods.

The present study describes the effects of two manipulations of dietary fatty acid profile on the composition of skeletal muscle phospholipid, the major ubiquitous structural lipid of membranes. First, for 9 wk rats were fed one of three test diets that differed only in their fatty acid composition. The diets were *1*) an essential fatty acid–deficient diet (EFAD)[4] containing mainly saturated fatty acids, *2*) a diet high in (*n*-6) fatty acids [High (*n*-6)], and 3) a diet enriched with (*n*-3) fatty acids [High (*n*-3)]. Second, all groups were fed a nonpurified diet for 0, 2 or 6 wk to determine the rapidity of phospholipid fatty acid turnover in muscle. Dietary fatty acids were manipulated during the post-weaning growth phase, which is likely to be the most sensitive to dietary changes due to the manufacture of new membrane.

The aims of this study were *1*) to determine whether the phospholipid fatty acid composition of skeletal muscle is altered by deficiency and/or enrichment of dietary polyunsaturated fatty acid composition, and *2*) if effects are detected, to determine whether further changes are detected following subsequent periods of consumption of a nutritionally adequate nonpurified diet. The effects of change in dietary fatty acids on representatives of two major types of skeletal muscle ("slow-twitch" and "fast-twitch") were investigated. Soleus and extensor digitorum longus (EDL) muscles were chosen as the best examples of these muscle types. Soleus contains 87% type IA (slow-twitch oxidative) and 13% type IIA (fast-twitch oxidative) fibers, and EDL contains 2% type IA, 42% type IIA and 56% type IIB (fast-twitch glycolytic) fibers (Ariano et al. 1973).

MATERIALS AND METHODS

Animals and diets. All experiments were approved by the University of Wollongong Animal Experimentation Ethics Committee. Male weanling Wistar rats, bred at the University of Wollongong (housed individually at 22 ± 2°C and 57 ± 2% relative humidity, age 21–23 d, mean weight 54 ± 1 g), were assigned at random, one per litter, to each of three dietary groups. Groups of 12 rats were fed the three diets for 9 wk. Following this test period, subgroups of four rats fed each diet were randomly allocated to each of three treatments and killed immediately, killed after 2 wk of consuming a nonpurified diet (Allied Rat and Mouse Cubes, containing approximately 22% protein, 60% carbohydrate and 5% fat; Fielders' Agricultural Products, Tamworth, Australia) or killed after 6 wk of consuming the nonpurified diet. Another group of four rats was analyzed at weaning to give an indication of muscle phospholipid fatty acid composition prior to dietary intervention. Rats were killed by decapitation, and muscles were removed and stored at −80°C pending analyses. All rats were given free access to food and water, and food intake and body mass were recorded throughout the study.

All diets were identical except for their lipid component. Each diet contained 10 g fat/100 g diet, but the type of fat varied (**Table 1**). The EFAD diet contained 10 g coconut oil/100 g (ETA Food Services, Wollongong, Australia) and was therefore lacking both (*n*-6) and (*n*-3) essential polyunsaturated fatty acids because coco-

[4] Abbreviations used: EDL, extensor digitorum longus; EFAD, essential fatty acid–deficient; High (*n*-6), diet high in (*n*-6) fatty acids; High (*n*-3), diet high in (*n*-3) fatty acids.

TABLE 1

Composition of experimental diets[1]

Ingredient	Diet		
	High (*n*-6)	EFAD	High (*n*-3)
	g/kg diet		
Protein (casein)	220	220	220
Sesame oil	100	—	70
Coconut oil	—	100	—
MaxEPA oil	—	—	30
Salt mix[2]	50	50	50
Vitamin mix[3]	10	10	10
Cellulose	10	10	10
Water	50	50	50
Sucrose	560	560	560

[1] All diets were identical except for the type of fat. The EFAD diet contained 10% coconut oil, the High (*n*-6) diet contained 10% sesame oil and the High (*n*-3) diet contained 7% sesame oil and 3% MaxEPA oil. Dashes denote zero amount.

[2] The salt mixture consisted of NaCl (139.3 g); KI (0.79 g); KH_2PO_4 (389.0 g); $MgSO_4 \cdot 7H_2O$ (57.3 g); $CaCO_3$ (381.4 g); $FeSO_4 \cdot 7H_2O$ (27.0 g); $CoCl_2$ (0.023 g).

[3] The vitamin mixture consisted of menadione (0.0175 g); choline Cl (7 g); *p*-aminobenzoic acid (0.3500 g); inositol (0.3500 g); nicotinic acid (0.1400 g); calcium pantothenate (0.1400 g); riboflavin (0.0280 g); thiamine HCl (0.0175 g); pyridoxine HCl (0.0175 g); folic acid (0.0070 g); biotin (0.0014 g); vitamin B-12 (0.000105 g); dextrose (up to 35 g). Vitamins A, D and E were added separately to provide 11 mg retinyl palmitate, 50 μg cholecalciferol and 58 mg *RRR*-α-tocopherol per kilogram of diet, respectively.

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TABLE 2

Fatty acid composition of experimental diets and nonpurified diet[1]

Fatty acid[2]	Diet			
	High (*n*-6)	EFAD	High (*n*-3)	Nonpurified
	g/100 g fatty acids			
8:0	—	4.10 ± 1.3	—	—
10:0	—	7.20 ± 0.3	—	—
12:0	—	56.90 ± 0.6	—	—
14:0	—	16.90 ± 0.4	2.80 ± 0.1	1.71 ± 0.0
15:0	1.38 ± 0.05	0.46 ± 0.04	1.40 ± 0.1	1.37 ± 0.01
16:0	10.30 ± 0.05	6.90 ± 0.2	12.90 ± 0.1	20.30 ± 0.2
16:1(*n*-9)	—	—	3.33 ± 0.05	1.58 ± 0.03
18:0	4.09 ± 0.02	2.40 ± 0.1	3.70 ± 0.1	8.60 ± 0.2
18:1(*n*-9)	28.60 ± 0.2	4.10 ± 0.2	22.10 ± 0.2	31.30 ± 0.3
18:1(*n*-7)	1.01 ± 0.02	—	1.69 ± 0.02	1.30 ± 0.04
18:2(*n*-6)	50.12 ± 0.04	1.15 ± 0.07	35.20 ± 0.1	29.80 ± 0.3
18:3(*n*-3)	3.94 ± 0.04	—	3.00 ± 0.03	2.15 ± 0.02
18:4(*n*-3)	—	—	1.34 ± 0.02	—
20:5(*n*-3)	—	—	6.23 ± 0.08	—
22:6(*n*-3)	—	—	3.45 ± 0.03	—
% Saturated	16.20	94.90	21.30	33.20
% Unsaturated				
% (*n*-9)	28.60	4.10	22.50	31.60
% (*n*-6)	50.10	1.20	35.40	30.00
% (*n*-3)	4.10	—	15.50	2.20
(*n*-3):(*n*-6)	0.08	—	0.44	0.07

[1] The diet high in (*n*-6) fatty acids [High (*n*-6) diet] contained 10% sesame oil, the essential fatty acid–deficient (EFAD) diet contained 10% coconut oil, and the diet high in (*n*-3) fatty acids [High (*n*-3) diet] contained 7% sesame oil and 3% MaxEPA oil. The nonpurified diet consisted of standard rat pellets. Values are means ± SEM of three determinations.

[2] Only fatty acids detected at greater than 1 g/100 g total fatty acids are listed.

nut oil is extremely high in saturated fatty acids (92%). This diet was chosen because a deficiency of essential fatty acids is likely to occur in conditions of malnutrition, including those due to eating disorders. The High (*n*-3) diet contained 7 g sesame oil/100 g (Meadowlea Foods, Sydney, Australia) [containing 43% (*n*-6) fatty acids] and 3% MaxEPA oil (R. P. Scherer, Pty. Ltd., Melbourne, Australia) [containing 30% (*n*-3) fatty acids]. It was therefore enriched with (*n*-3) polyunsaturated fatty acids. This diet was investigated because, for humans, increased levels of (*n*-3) fatty acids are increasingly recommended by health professionals due to their beneficial effects on the cardiovascular system. Also, recent surveys have shown documented shifts in fat consumption away from saturated fats towards polyunsaturated fats (Castles 1993). The High (*n*-6) diet contained sesame oil (10 g/100 g), which is high in (*n*-6) fatty acids (43%). Such a diet is most similar to a typical human diet. A listing of fatty acids in the test diets and the nonpurified diet is shown in **Table 2**. Initial trial studies (Ayre 1994) were conducted to establish diets that were isoenergetic and equally palatable and thus equally consumed.

Analyses of phospholipids. Diet and muscle lipid samples were analyzed in a blind trial in which the dietary group of each sample was concealed.

Total lipids were extracted with chloroform-methanol (2:1) using the method of Folch et al. (1957). Neutral lipids were separated from phospholipids by silicic acid chromatography using the method of Borgstrom (1952). Phospholipid fatty acids were methylated using boron trifluoride in methanol, and the methyl esters formed were evaporated to approximately 3 mL under nitrogen (Morrison and Smith 1964). Fatty acid methyl esters were purified on Florisil chromatography columns using the method of Carrol (1961). They were then separated on an SGE capillary column (25QC2) in a gas chromatograph (Varian model 3300, Varian Australia Pty. Ltd., Sydney, Australia) and a flame ionization detector. The output from the gas chromatograph was integrated using an electronic integrator (Shimadzu Chromatopac CR-3, Shimadzu Corporation, Kyoto, Japan). Individual fatty acids were identified by comparison of retention times with standard methyl esters (Sigma Chemical, St. Louis, MO; Larodan, Sweden and Activon, Sydney, Australia).

All chemicals were analytical grade; solvents were nanograde purity. Methanol, NaCl, Na_2SO_4, florisil and petroleum ether were from BDH (Melbourne, Australia), chloroform and diethyl ether from Mallinckrodt (Sydney, Australia), BHT (2,6-ditert-butyl-*p*-cresol) and silicic acid from Sigma Chemical (St. Louis, MO), boron

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trifluoride from Merck (Darmstadt, Germany) and hexane from AJAX (Sydney, Australia).

To verify that the rats fed the EFAD diet were deficient in essential fatty acids, the triene:tetraene ratio was calculated for both soleus and EDL muscles at each of the three testing stages. This ratio [20:3(*n*-9) to 20:4(*n*-6)] is used as an indicator of essential fatty acid deficiency. When the supply of (*n*-6) fatty acids is sufficient, the ratio is low, but in conditions of essential fatty deficiency, the ratio increases. Triene:tetraene ratios greater than 0.4 are considered indicative of essential fatty acid deficiency (Holman 1960).

Statistical methods. All results are expressed as means ± SEM. The level of significance chosen was $P < 0.05$. A one-way ANOVA was used to test for heterogeneity of food intake and rat weights. To test for significance of the effects of the fixed factors diet and time on muscle phospholipid fatty acid composition, two-way ANOVA were used. Because the primary interest was in differences among the dietary groups at each time, one-way ANOVA were performed in cases where the two-way ANOVA revealed a significant dietary effect. Scheffé's F tests were applied a posteriori to determine which dietary groups differed significantly (Zar 1984).

RESULTS

Food consumption and rat weights. Rats fed the three diets did not differ significantly in their patterns of food consumption throughout the 9-wk test period. Furthermore, total food consumption over the 9 wk did not vary significantly among dietary groups and were 967 ± 42 g [High (*n*-6) diet], 990 ± 31 g (EFAD diet) and 1004 ± 34 g [High (*n*-3) diet]. Body mass did not differ significantly among the three dietary groups after 9 wk of consuming the diets or during the 2- and 6-wk recovery periods when rats were fed a nonpurified diet. Mean body mass increased from 54 ± 1 g at weaning to 375 ± 6 g during the 9-wk test diet period and to 427 ± 9 g during the 6-wk recovery period.

Muscle phospholipid fatty acid composition at weaning. For their first 3 wk, rats obtained their dietary supply of fatty acids indirectly from mothers fed the nonpurified diet. Baseline values at weaning showed that both types of skeletal muscle had a similar phospholipid fatty acid composition (**Tables 3** and **4**), including 38% essential (*n*-6) and 7% essential (*n*-3) fatty acids, giving a ratio of (*n*-3):(*n*-6) fatty acids of 0.2 in both muscles.

Effects of the test diets on muscle phospholipid fatty acid composition. The phospholipid fatty acid composition of skeletal muscles was influenced by, but did not directly reflect, the fatty acid composition of the diet. After the 9-wk test period, the proportions of total saturated and unsaturated fatty acids were remarkably similar for all three dietary groups in both soleus (Table 3) and EDL (Table 4) muscles, despite large differences in the balance of dietary saturated and unsaturated fatty acids. For example, the diets varied substantially in the relative concentrations of saturated fatty acids (from 16 to 95%), but the muscle phospholipids contained 32–38% saturated fatty acids regardless of the diet. Although the relative proportions of the major classes of unsaturated fatty acids differed significantly among the three dietary groups, the unsaturated fatty acid composition of the muscle phospholipids reflected the dietary fatty acid composition for rats fed the High (*n*-6) diet and for those fed the High (*n*-3) diet and to a lesser extent for rats fed the EFAD diet. There was little variation among animals within groups and little difference between soleus and EDL muscles (Tables 3 and 4, respectively).

Phospholipids from both soleus and EDL muscles of rats fed the EFAD diet contained a significantly higher proportion of (*n*-9) fatty acids than those from either the High (*n*-6) or High (*n*-3) group (Tables 3 and 4, respectively). The levels of all (*n*-9) fatty acids, 16:1, 18:1 and the unusual polyunsaturate 20:3 were significantly greater than in the other groups. In fact, the muscle phospholipids of rats fed the EFAD diet contained 60 to 100 times more 20:3(*n*-9) than those of the High (*n*-6) and High (*n*-3) groups. These effects of the EFAD diet were reflected by a significantly elevated triene:tetraene ratio (>0.5) compared with ratios for the High (*n*-6) and High (*n*-3) groups (**Table 5**), and the EFAD rats were therefore judged essential fatty acid deficient (triene:tetraene ratio > 0.4; Holman 1960).

In contrast to the effects of the EFAD diet, the unsaturated fatty acid compositions of muscle phospholipids of rats fed the High (*n*-6) and High (*n*-3) diets more closely reflected the respective dietary fatty acid compositions. In addition to a low level of (*n*-9) fatty acids, both soleus and EDL phospholipids from rats fed the High (*n*-6) diet contained a significantly higher proportion of (*n*-6) fatty acids than those from the EFAD and High (*n*-3) groups and a correspondingly lower proportion of (*n*-3) fatty acids than those from rats fed the High (*n*-3) diet (Tables 3 and 4). This was reflected by ratios of (*n*-3):(*n*-6) fatty acids in both muscles in the High (*n*-3) group, which were significantly greater than ratios in muscles of the other two groups.

Effects of the nonpurified diet on muscle phospholipid fatty acid composition. The rate of change of phospholipid fatty acids, following the switch from the test diets to the nonpurified diet, differed markedly between the two muscle types and varied among the classes of fatty acids. Although rats in all three dietary groups showed changes in their muscle phospholipid fatty acid composition, the changes were more rapid for soleus.

In the EFAD group, there was a rapid decrease in (*n*-9) fatty acids in both soleus and EDL muscles (Tables 3 and 4). The rate of change seemed to be slower in

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TABLE 3

Effects of dietary fatty acid profile on phospholipid fatty acid composition of soleus muscles of Wistar rats[1]

Fatty acid	At weaning	After 9 wk of test diet			After 2 wk of recovery			After 6 wk of recovery			Two-way ANOVA Significance of treatment		
		High (n-6)	EFAD	High (n-3)	High (n-6)	EFAD	High (n-3)	High (n-6)	EFAD	High (n-3)	Diet (D)	Time (T)	D × T
	g/100 g fatty acids												
16:0	21.5 ± 0.6	13.2 ± 0.6	10.2 ± 1.8	14.2 ± 1.0	10.2 ± 1.8	8.3 ± 1.5	13.8 ± 0.1	16.2 ± 0.5	15.8 ± 0.6	15.2 ± 0.8	0.01	0.0001	NS
16:1(n-9)	0.8 ± 0.1	0.9 ± 0.4^{b}	5.3 ± 0.5^{a}	0.7 ± 0.4^{b}	—	—	—	1.4 ± 0.3	1.9 ± 0.2	1.7 ± 0.4	0.0001	0.0001	0.0001
18:0	19.0 ± 0.4	19.4 ± 1.6	21.9 ± 1.8	20.0 ± 1.3	24.0 ± 2.0	25.8 ± 0.8	22.1 ± 0.8	19.4 ± 0.6	19.7 ± 0.2	19.5 ± 0.5	NS	0.0004	NS
18:1(n-9)	10.8 ± 0.7	8.4 ± 1.7^{b}	15.6 ± 1.4^{a}	6.0 ± 0.5^{b}	6.4 ± 0.9^{a}	5.1 ± 0.3^{b}	5.2 ± 0.2^{b}	8.9 ± 1.5	8.7 ± 0.8	8.4 ± 1.3	0.002	0.0001	0.0003
18:1(n-7)	2.5 ± 0.1	3.1 ± 0.2	4.2 ± 0.7	2.9 ± 0.3	2.6 ± 0.4	2.6 ± 0.4	3.2 ± 0.3	2.7 ± 0.3	2.4 ± 0.8	2.1 ± 0.7	NS	NS	NS
18:2(n-6)	19.7 ± 0.5	20.1 ± 1.4^{a}	9.2 ± 1.0^{c}	15.4 ± 1.0^{b}	14.7 ± 1.0	13.7 ± 0.5	16.4 ± 1.1	14.8 ± 0.5	14.3 ± 0.3	14.6 ± 0.0	0.0001	NS	0.0001
20:3(n-9)	0.4 ± 0.0	0.1 ± 0.1^{b}	9.6 ± 0.3^{a}	—^{b}	1.0 ± 1.0	2.1 ± 0.7	—	—^{b}	0.4 ± 0.1^{a}	—^{b}	0.0001	0.0001	0.0001
20:3(n-6)	0.9 ± 0.0	0.4 ± 0.2	0.9 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	0.4 ± 0.2	0.3 ± 0.3	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	NS	NS	NS
20:4(n-6)	14.1 ± 0.4	23.0 ± 1.2^{a}	17.7 ± 2.1^{b}	10.4 ± 0.4^{c}	21.1 ± 2.3	22.8 ± 1.1	20.4 ± 3.3	21.4 ± 0.7^{a}	20.1 ± 0.5^{a}	17.3 ± 0.8^{b}	0.0002	0.004	0.007
20:5(n-3)	0.2 ± 0.0	—^{b}	—^{b}	1.1 ± 0.4^{a}	0.3 ± 0.1	—	0.1 ± 0.2	0.2 ± 0.2	—	0.5 ± 0.5	0.01	NS	NS
22:4(n-6)	1.7 ± 0.1	2.4 ± 0.6^{a}	1.1 ± 0.2^{ab}	—^{b}	2.2 ± 0.8	2.4 ± 0.4	2.0 ± 1.0	1.5 ± 0.1^{a}	1.2 ± 0.1^{a}	0.4 ± 0.2^{b}	0.01	0.009	NS
22:5(n-6)	1.7 ± 0.1	3.8 ± 0.5^{a}	1.2 ± 0.7^{b}	—^{b}	3.7 ± 1.8	6.3 ± 0.3	2.8 ± 1.4	2.3 ± 0.0^{a}	2.3 ± 0.1^{a}	0.1 ± 0.1^{b}	0.0003	0.0001	0.02
22:5(n-3)	2.0 ± 0.1	1.6 ± 0.9^{ab}	0.2 ± 0.1^{b}	3.5 ± 0.2^{a}	2.7 ± 0.2	2.1 ± 0.1	1.8 ± 1.0	2.4 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	0.01	NS	0.002
22:6(n-3)	4.9 ± 0.1	3.7 ± 0.3^{b}	2.6 ± 0.5^{b}	25.5 ± 1.7^{a}	10.8 ± 3.2	8.5 ± 0.6	11.8 ± 6.1	8.6 ± 0.4^{c}	10.6 ± 0.3^{b}	17.3 ± 0.4^{a}	0.0001	NS	0.0001
Σ Saturated	40.6 ± 0.8	32.6 ± 1.3	32.1 ± 1.9	34.2 ± 0.5	34.2 ± 0.3	34.1 ± 0.9	35.9 ± 0.7	35.6 ± 0.9	35.4 ± 0.5	34.7 ± 0.7	NS	0.02	NS
Σ Unsaturated													
Σ (n-9)	14.6 ± 0.9	9.4 ± 2.0^{b}	30.5 ± 1.6^{a}	6.6 ± 0.9^{b}	7.1 ± 1.1	7.4 ± 0.9	5.2 ± 0.2	10.3 ± 1.6	11.0 ± 1.0	10.0 ± 1.7	0.0001	0.0001	0.0001
Σ (n-6)	37.7 ± 0.9	49.6 ± 0.8^{a}	30.0 ± 2.5^{b}	26.3 ± 1.1^{b}	42.5 ± 3.6	45.4 ± 0.9	41.9 ± 6.3	40.1 ± 0.5^{a}	38.2 ± 0.6^{a}	32.8 ± 1.2^{b}	0.0001	0.0007	0.0001
Σ (n-3)	7.2 ± 0.2	5.4 ± 0.9^{b}	2.9 ± 0.5^{b}	30.1 ± 1.6^{a}	13.7 ± 3.5	10.6 ± 0.7	13.7 ± 7.1	11.2 ± 0.4^{c}	13.0 ± 0.4^{b}	20.5 ± 0.2^{a}	0.0001	NS	0.0001
(n-3):(n-6)	0.2 ± 0.0	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	1.2 ± 0.1^{a}	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.3	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.6 ± 0.0^{a}	0.0001	NS	0.0001

[1] Phospholipid fatty acid composition of soleus muscles at weaning and after 9 wk of consumption of the three test diets—the diet high in (n-6) fatty acids [High (n-6) diet], the essential fatty acid–deficient (EFAD) diet, and the diet enriched with (n-3) fatty acids [High (n-3) diet]—followed by 2 wk and 6 wk of consumption of a nonpurified diet. Only fatty acids detected at levels greater than 0.1 g/100 g total fatty acids are listed. Values are means ± SEM, $n = 4$. The statistical significance of variation in mean phospholipid fatty acid levels among dietary treatments, among times, and due to interactions between diet and time were assessed by two-way ANOVA. Where there was a significant effect of diet, one-way ANOVA and Scheffé's F test were used to test for significant variation in the effects of diet at each time. At each time point within a row, significantly different treatment means are denoted by different superscripts. NS = not significant ($P > 0.05$).

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TABLE 4

Effects of dietary fatty acid profile on phospholipid fatty acid composition of extensor digitorum longus muscles of Wistar rats[1]

Fatty acid	At weaning	After 9 wk of test diet			After 2 wk of recovery			After 6 wk of recovery			Two-way ANOVA Significance of treatment		
		High (n-6)	EFAD	High (n-3)	High (n-6)	EFAD	High (n-3)	High (n-6)	EFAD	High (n-3)	Diet (D)	Time (T)	D × T
	g/100 g fatty acids												
16:0	26.5 ± 0.3	21.8 ± 1.0	23.9 ± 0.7	21.6 ± 2.2	17.4 ± 1.8	21.8 ± 2.0	21.0 ± 1.4	18.0 ± 1.3	19.8 ± 1.5	20.3 ± 0.8	NS	0.05	NS
16:1(n-9)	0.9 ± 0.0	0.2 ± 0.2[b]	6.4 ± 0.6[a]	0.3 ± 0.2[b]	0.3 ± 0.3	1.5 ± 0.2	0.9 ± 0.3	—	—	—	0.0001	0.0001	0.0001
18:0	14.8 ± 0.3	15.9 ± 0.3[ab]	13.6 ± 0.7[b]	17.6 ± 1.0[a]	20.5 ± 1.4	17.3 ± 1.4	16.6 ± 1.1	17.6 ± 1.3	16.0 ± 0.2	14.1 ± 1.4	0.02	0.02	0.03
18:1(n-9)	10.0 ± 0.0	5.9 ± 0.3[b]	21.6 ± 0.5[a]	6.2 ± 0.3[b]	8.1 ± 0.6[ab]	10.5 ± 0.4[a]	7.2 ± 0.9[b]	4.7 ± 0.2	5.7 ± 0.0	5.4 ± 0.4	0.0001	0.0001	0.0001
18:1(n-7)	2.5 ± 0.0	2.1 ± 0.1[b]	3.3 ± 0.1[a]	2.2 ± 0.2[b]	1.6 ± 0.1[b]	2.1 ± 0.0[a]	1.8 ± 0.1[ab]	1.8 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	0.0001	0.0001	0.0003
18:2(n-6)	19.0 ± 0.4	19.1 ± 1.3[a]	6.8 ± 0.5[b]	16.3 ± 0.6[a]	14.2 ± 0.8	15.9 ± 1.0	14.6 ± 0.5	13.7 ± 0.4[b]	18.1 ± 0.3[a]	14.2 ± 1.4[b]	0.02	NS	0.0001
20:3(n-9)	0.4 ± 0.0	0.1 ± 0.0[b]	6.1 ± 0.7[a]	—[b]	—[b]	4.2 ± 0.2[a]	0.2 ± 0.0[b]	—[b]	1.4 ± 0.2[a]	—[b]	0.0001	0.0001	0.0001
20:3(n-6)	1.1 ± 0.0	0.2 ± 0.2[ab]	0.5 ± 0.2[a]	—[b]	—[b]	—[b]	0.1 ± 0.0[a]	—[b]	0.3 ± 0.1[a]	—[b]	0.001	0.02	0.01
20:4(n-6)	14.0 ± 0.3	19.9 ± 2.0[a]	11.3 ± 0.7[b]	7.2 ± 0.4[c]	21.3 ± 0.8[a]	15.8 ± 1.0[b]	10.0 ± 0.3[c]	22.7 ± 1.1[a]	19.0 ± 0.5[b]	14.4 ± 0.8[c]	0.0001	0.0001	NS
20:5(n-3)	0.3 ± 0.0	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.4 ± 0.1	—	—	—	NS	NS	0.006
22:4(n-6)	1.7 ± 0.0	2.0 ± 0.6[a]	0.9 ± 0.1[ab]	—[b]	3.1 ± 0.3[a]	1.2 ± 0.1[b]	0.1 ± 0.1[c]	2.4 ± 0.2[a]	1.6 ± 0.1[b]	0.6 ± 0.1[c]	0.0001	0.007	NS
22:5(n-6)	1.6 ± 0.0	2.8 ± 0.8[a]	2.0 ± 0.1[ab]	—[b]	5.5 ± 0.6[a]	3.1 ± 0.6[b]	0.2 ± 0.1[c]	5.5 ± 0.3[a]	3.8 ± 0.3[b]	0.4 ± 0.0[c]	0.0001	0.0001	0.03
22:5(n-3)	2.1 ± 0.0	1.2 ± 0.3[b]	0.5 ± 0.0[b]	3.1 ± 0.2[a]	1.6 ± 0.1[b]	1.0 ± 0.1[b]	2.7 ± 0.2[a]	3.1 ± 0.3	2.5 ± 0.1	2.7 ± 0.2	0.0001	0.0001	0.0001
22:6(n-3)	5.1 ± 0.1	7.2 ± 3.9[b]	2.3 ± 0.2[b]	24.5 ± 1.3[a]	5.8 ± 0.4[b]	3.9 ± 0.6[b]	23.3 ± 1.1[a]	10.0 ± 0.9[a]	9.0 ± 0.8[a]	25.2 ± 1.7[b]	0.0001	0.02	NS
Σ Saturated	41.3 ± 0.6	37.7 ± 0.7	37.5 ± 1.1	39.1 ± 1.3	37.9 ± 0.7	39.1 ± 0.7	37.6 ± 0.6	36.6 ± 0.1	35.8 ± 13	34.3 ± 1.2	NS	0.0008	NS
Σ Unsaturated													
Σ (n-9)	13.8 ± 0.2	6.1 ± 0.5[b]	34.0 ± 1.4[a]	6.5 ± 0.5[b]	8.4 ± 0.7[b]	16.3 ± 0.7[a]	8.3 ± 1.2[b]	4.7 ± 0.2[b]	7.0 ± 0.1[a]	5.4 ± 0.4[b]	0.0001	0.0001	0.0001
Σ (n-6)	37.3 ± 0.6	43.9 ± 4.5[a]	21.5 ± 0.4[b]	23.5 ± 0.4[b]	44.1 ± 0.7[a]	36.1 ± 0.8[b]	25.1 ± 0.6[c]	44.2 ± 0.7[a]	42.9 ± 0.8[a]	29.5 ± 2.2[b]	0.0001	0.0001	0.0001
Σ (n-3)	7.6 ± 0.1	10.2 ± 4.1[b]	3.7 ± 0.3[b]	28.7 ± 1.6[a]	8.1 ± 0.8[b]	6.3 ± 0.6[b]	27.3 ± 1.1[a]	13.6 ± 1.2[b]	12.1 ± 1.1[b]	28.7 ± 1.8[a]	0.0001	0.01	NS
(n-3):(n-6)	0.2 ± 0.0	0.2 ± 0.1[b]	0.2 ± 0.0[b]	1.2 ± 0.9[a]	0.2 ± 0.0[b]	0.2 ± 0.0[b]	1.0 ± 0.1[a]	0.2 ± 0.0[b]	0.2 ± 0.0[b]	0.9 ± 0.1[a]	0.0001	NS	NS

1 Phospholipid fatty acid composition of extensor digitorum longus muscles at weaning and after 9 wk of consumption of the three test diets—the diet high in (n-6) fatty acids [High (n-6) diet], the essential fatty acid–deficient (EFAD) diet, and the diet enriched with (n-3) fatty acids [High (n-3) diet]—followed by 2 wk and 6 wk of consumption of a nonpurified diet. Only fatty acids detected at levels greater than 0.1 g/100 g total fatty acids are listed. Values are means ± SEM, $n = 4$. The statistical significance of variation in mean phospholipid fatty acid levels among dietary treatments, among times, and due to interactions between diet and time were assessed by two-way ANOVA. Where there was a significant effect of diet, one-way ANOVA and Scheffé's F test were used to test for significant variation in the effects of diet at each time. At each time point within a row, significantly different treatment means are denoted by different superscripts. NS = not significant ($P > 0.05$).

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TABLE 5

Effects of dietary fatty acid profile on the triene:tetraene ratio in phospholipid fatty acids of soleus and extensor digitorum longus muscles of Wistar rats[1,2]

	Soleus				Extensor digitorum longus			
	High (*n*-6)	EFAD	High (*n*-3)	*P*	High (*n*-6)	EFAD	High (*n*-3)	*P*
9 wk of test diet	—[b]	0.56 ± 0.06[a]	—[b]	0.001	—[b]	0.54 ± 0.08[a]	—[b]	0.001
2 wk of recovery	0.05 ± 0.05	0.10 ± 0.03	—	NS	—[b]	0.27 ± 0.02[a]	0.02 ± 0.0[b]	0.001
6 wk of recovery	0.01 ± 0.00	0.02 ± 0.01	—	NS	—[b]	0.07 ± 0.01[a]	—[b]	0.001

[1] The triene:tetraene ratio is the ratio of the amount of 20:3(*n*-9) to 20:4(*n*-6). It provides an indication of the level of adequacy of essential fatty acids. If the ratio is >0.4, the tissue is considered to be essential fatty acid deficient (Holman 1960).

[2] Rats were fed one of three test diets for 9 wk—the diet high in (*n*-6) fatty acids [High (*n*-6) diet], the essential fatty acid-deficient (EFAD) diet, and the diet enriched with (*n*-3) fatty acids [High (*n*-3) diet]—followed by 2 wk and 6 wk of consumption of a nonpurified diet. Ratios are based only on fatty acids detected at levels greater than 0.1 g/100 g of total fatty acids. Values are means ± SEM, $n = 4$. The statistical significance of variation in mean phospholipid levels among dietary treatments was assessed by one-way ANOVA. Within muscles, significantly different treatment means are denoted by different superscripts. NS = not significant ($P > 0.05$).

EDL because the triene:tetraene ratio in these muscles was still significantly higher in the EFAD group than in the High (*n*-6) and High (*n*-3) groups after 6 wk, whereas in soleus muscle there was no difference among the dietary groups in the triene:tetraene ratios after 2 wk (Table 5). Concurrent with the decrease in (*n*-9) fatty acids in the nonpurified diet–fed EFAD group was a dramatic increase in the levels of various (*n*-6) and (*n*-3) fatty acids because the precursors of these were now available in the diet (Tables 3 and 4).

Rats in the High (*n*-6) group showed an overall increase in (*n*-3) fatty acids in both soleus and EDL during the recovery period. In soleus there was a concurrent compensatory decrease in (*n*-6) fatty acids, but there was no change in (*n*-6) fatty acids in EDL.

The levels of (*n*-3) fatty acids showed the most resistance to change in response to dietary changes. Both soleus and EDL muscles from rats fed the High (*n*-3) diet contained a significantly greater proportion of (*n*-3) fatty acids after the 9-wk test period, and this trend continued during recovery. Although the level of (*n*-3) fatty acids decreased (soleus) or stayed constant (EDL) in the High (*n*-3) group during recovery, this group still retained a significantly higher level at 6 wk than in the other two groups and a correspondingly higher ratio of (*n*-3):(*n*-6) fatty acids. Although in soleus the levels of total (*n*-3) fatty acids were not different among the groups after 2 wk (Table 3), there was a significant difference after 6 wk, as there was in EDL at both times (Table 4).

DISCUSSION

This study demonstrates that the phospholipid fatty acid composition of both fast and slow skeletal muscles is influenced by the fatty acid profile of the diet. Indeed, after 9 wk, all three test diets produced striking and essentially predictable changes in phospholipid fatty acids. The finding that phospholipid unsaturated fatty acid composition in muscle may reflect the fatty acid composition of the diet is supported by the limited set of earlier studies that show separately the dietary induction of similarly increased (*n*-3) polyunsaturated fatty acid levels in unspecified skeletal muscles of turkeys (Neudoerffer and Lea 1967) and marmosets (Charnock et al. 1989 and 1992), sartorius muscles of chicks (Olomu and Baracos 1991), and quadriceps (a mixed fiber muscle) in essential fatty acid–deficient weanling rats (Alling et al. 1972). Nevertheless, this study showed that phospholipid fatty acid composition does not always reflect dietary composition. This was most noticeable in the (*n*-3) fatty acids. Furthermore, there was substantial variation among muscles and fatty acids in the rate of return to pre-diet levels for rats switched to a nonpurified diet. These findings suggest that the consequences of changing the dietary composition depend upon the initial composition of the phospholipids and the types of fatty acids available in the diet.

The finding that changes can occur so rapidly may have important implications for future studies of the relationship between diet and the contractile properties of muscle. In addition, skeletal muscle is the primary site of insulin action, and muscle phospholipid fatty acid composition is associated with insulin sensitivity in both rats (Storlien et al. 1991) and humans (Borkman et al. 1993). It is therefore possible that hyperinsulinemia and insulin resistance, which are features of disorders such as obesity and noninsulin-dependent diabetes mellitus, may be altered by changes in dietary fatty acids.

In this study, the overall effects of changes in dietary fatty acids were similar in both soleus and EDL muscles. The ratio of saturated to unsaturated fatty acids remained stable despite dietary changes, but the proportions of the different classes of unsaturated fatty

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acids differed from the dietary fatty acid composition. The high levels of (n-9) fatty acids in the EFAD group are most likely to have come from endogenous conversion of other fatty acids, because these fatty acids were not high in the EFAD diet. However, the high levels of (n-6) and appreciable levels of (n-3) fatty acids in muscles from this group are more difficult to explain. Although the EFAD diet contained no measurable levels of (n-3) fatty acids and only 1% (n-6) fatty acids, after 9 wk the muscle phospholipids of rats consuming this diet contained 3% (n-3) and nearly 30% (n-6) fatty acids. It is widely accepted that these fatty acids are "essential" and therefore must be included in the diet. In other words, they cannot be converted from other fatty acids. If this is so and because the levels of these fatty acids were negligible in the diet, virtually all of these fatty acids must have been retained from the preweaning or in utero periods.

The tenacity with which the (n-6) and (n-3) polyunsaturated fatty acids are retained in muscle phospholipids is remarkable. For example, the EFAD rats were deprived of any measurable amount of (n-3) in their diet for 9 wk from the time of weaning. During this time, they underwent a sevenfold increase in body mass and presumably a similar increase in the amount of body muscle. In adult rats, body musculature is approximately 43% of body mass (Hulbert and Else 1989). If we assume that weanling rats have the same percentage of their body mass as musculature and that the muscle phospholipid (n-3) fatty acids present in the adult rats were already present in the weanling rats, then the calculated percentages of (n-3) PUFA present in the muscles of weanling rats would have been 20–30%. This is approximately the same relative amount of (n-3) polyunsaturated fatty acids found in the muscles of adult rats fed the diet highly enriched with (n-3) fatty acids since weaning. Such calculations assume that there was no metabolism of these (n-3) fatty acids since weaning. If there were, the calculated values would be even greater. The tenacity of (n-3) retention is thus even more remarkable because Leyton et al. (1987) have demonstrated that dietary 18:3(n-3) is preferentially metabolized compared with both saturated fatty acids and many other unsaturated fatty acids. Similarly, the (n-6) polyunsaturated fatty acids were also strongly retained in the muscle phospholipids. In this case, however, there were small but measurable amounts of (n-6) fatty acids in the EFAD diet (1.2%), and thus the growing rats received small quantities in their diet. Although the EFAD rats received no (n-3) fatty acids and only a small amount of (n-6) fatty acids in their food, it is not known if they received any of these essential fatty acids from microbial synthesis in their gut. The role of the gut flora in essential fatty acid provision is an area worthy of study.

Although many studies of dietary manipulation have used experimental periods of several weeks, months or even years, the present study and the earlier work of Innis and Clandinin (1981) show clearly that major changes in membrane fatty acid composition can occur within a few days. In this study, it is likely that changes in muscle phospholipid composition during the 9-wk test period may reflect the creation of much new membrane because body mass increased about sevenfold in that time. However, although there was little additional growth during the period when rats were fed the nonpurified diet (only 10–12% in all dietary groups), the changes in fatty acid proportions were equally dramatic. For example, the total proportion of (n-9) fatty acids in soleus muscles from the EFAD groups decreased by 75% during the 2-wk recovery period. Also, the proportion of (n-3) fatty acids in soleus muscles from the High (n-6) group increased 150% during the same period. On this basis, total turnover of muscle phospholipid occurs in less than 14 d.

The results of transferring all rats to a nonpurified diet following 9 wk of consuming experimental diets show that phospholipid fatty acids turn over relatively rapidly in muscle. In liver, phospholipids of the endoplasmic reticulum seem to belong to two pools, one with a half-life of 15 h and the other with a half-life of 80 h (Finean et al. 1974). Although we did not measure the half-life of muscle phospholipids, our results from analyses of changes in muscle phospholipid fatty acid composition suggest half-lives of this magnitude may also be applicable to muscle.

In this study, none of the commonly reported symptoms of EFA deficiency (such as dry scaly skin, skin lesions and reduced weight gain) (Holman 1968) were present. Despite this, however, it is important to note that these rats were clearly deficient in essential fatty acids as judged by a triene:tetraene ratio greater than 0.4 (Holman 1960). It has been suggested (Mead 1984, Phinney et al. 1993) that these symptoms, all related to membrane function, may be alleviated in conditions of high relative humidity, which was the case in this study (57%).

The soleus and EDL muscle phospholipids showed some interesting differences with respect to changes in fatty acids. Although the different dietary lipids had similar effects on phospholipid composition in soleus and EDL, the muscles differed in their rates of recovery. During the recovery period, the EFAD rats received the essential precursor (n-6) and (n-3) fatty acids in the nonpurified diet, and their desaturase enzymes would have been preferentially producing the longer-chain fatty acids (Jeffcoat and James 1984). This is evidenced by the significantly decreased proportion of (n-9) fatty acids and the significantly increased proportions of (n-6) and (n-3) fatty acids in both muscles from EFAD rats relative to the 9-wk test period (Tables 3 and 4). Most differences in soleus muscle phospholipid fatty acid composition among the dietary groups were no longer apparent after 2 wk of recovery. Although EDL muscles took longer to reach the same levels, in most cases there was no difference among the dietary groups

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within 6 wk. The finding that the two types of muscles differed in their rates of recovery was not surprising because, in a comparison of several other tissues in mice, Burns et al. (1983) showed significant variation among tissues in the rate of response to dietary manipulation. This indicates that future investigations into the effects of dietary fatty acid manipulation on skeletal muscle structure and function need to specify the types of muscle fibers being tested.

Results from this study suggest that turnover of fatty acids in muscle phospholipids can be rapid (i.e., ≤ 2 wk). Innis and Clandinin (1981) reported similarly high rates of phospholipid turnover in cardiac mitochondrial lipids. Changes in (*n*-9) and (*n*-6) fatty acids were typically rapid in soleus muscles; however, these changes were not complete in EDL muscles even after 6 wk. Both muscles seemed resistant to the loss of (*n*-3) fatty acids, inasmuch as they still contained a high level after the 6-wk recovery period. This retention for at least 6 wk of high levels of (*n*-3) fatty acids in the phospholipids of rats in the High (*n*-3) group indicates that turnover rates vary markedly among classes of fatty acids. Apparently both EDL and soleus tenaciously retained their high levels of (*n*-3) phospholipid fatty acids, as has been demonstrated in chick brain and retina (Anderson et al. 1992), despite evidence that (*n*-3) fatty acids are oxidized more rapidly than (*n*-6) fatty acids (Leyton et al. 1987). This may be an adaptive response to maximize the availability of fatty acids that are essential for neural development and function.

The complex changes in muscle phospholipid composition observed in the present study may have many physiological consequences. In parallel studies, we found no significant effects of these diets on aspects of muscle membrane biochemistry that are believed to underlie muscle function, i.e., neither the concentration of Na^+,K^+-ATPase nor the activity of Na^+,K^+-ATPase or Ca^{2+}-ATPase varied significantly among the three dietary groups (Ayre 1994). However, we have described a set of changes in the performance of isolated soleus and EDL muscles that are well correlated with the changes in muscle phospholipid composition (Ayre and Hulbert 1996). Specifically, the muscles contralateral to the ones assessed in this study were analyzed for a wide variety of muscle contraction properties. Several aspects of muscle function were impaired following consumption of the EFAD diet (e.g., significantly lower tensions generated and reduced response times). However, the responses of the rats fed the High (*n*-6) and those of rats fed the High (*n*-3) diets were indistinguishable [despite the differing (*n*-3):(*n*-6) ratios]. The muscle responses of all dietary groups were similar after rats had been fed the nonpurified diet for 6 wk; however, for some properties the EDL muscles took longer to recover than the soleus muscles. It therefore remains to be determined whether EFAD rats are more strongly influenced by deprivation of (*n*-3) or (*n*-6) fatty acids. However, it is clear that changes in isolated muscle function may be functionally related to the changes in phospholipid fatty acid composition reported here.

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LITERATURE CITED

Abeywardena, M. Y., McLennan, P. L. & Charnock, J. S. (1987) Long-term saturated fat feeding-induced changes in rat myocardial phospholipid fatty acids are reversed by crossover to polyunsaturated diets: differences between n-3 and n-6 lipid supplements. Nutr. Res. 7: 743–754.

Alling, C., Bruce, A., Karlsson, I., Sapia, O. & Svennerholm, L. (1972) Effect of maternal essential fatty acid supply on fatty acid composition of brain, liver, muscle and serum in 21-day-old rats. J. Nutr. 102: 773–782.

Anderson, G. J., van Winkle, S. & Connor, W. E. (1992) Reversibility of the effects of dietary fish oil on the fatty acid composition of the brain and retina of growing chicks. Biochim. Biophys. Acta 1126: 237–246.

Ariano, M. A., Armstrong, R. B. & Edgerton, V. R. (1973) Hindlimb muscle fiber populations of five mammals. J. Histochem. Cytochem. 21: 51–55.

Ayre, K. J. (1994) Dietary Fats and Exercise. Ph.D. Thesis. University of Wollongong, Wollongong, Australia.

Ayre, K. J. & Hulbert, A. J. (1996) Effects of changes in dietary fatty acids on isolated skeletal muscle function in rats. J. Appl. Physiol. 80(2), (in press).

Borgstrom, B. (1952) Investigation of lipid separation methods, separation of phospholipids from neutral fat and fatty acids. Acta Physiol. Scand. 25: 101–110.

Borkman, M., Storlien, L. H., Pan, D. A., Jenkins, A. B., Chisholm, D. J. & Campbell, L. V. (1993) The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N. Engl. J. Med. 328: 238–244.

Burns, C. P., Rosenberger, J. A. & Luttenegger, D. G. (1983) Selectivity in modification of the fatty acid composition of normal mouse tissues and membranes in vivo. Ann. Nutr. & Metab. 27: 268–277.

Carrol, K. K. (1961) Separation of lipid classes by chromatography on florisil. J. Lipid Res. 2: 135–141.

Castles, I. (1993) Apparent Consumption of Foodstuffs and Nutrients, Australia, 1990–1991. Australian Bureau of Statistics, Canberra, Australia.

Charnock, J. S., Abeywardena, M. Y. & McLennan, P. L. (1989) Tissue specific differences in the fatty acid composition of the marmoset monkey (Callithrix jacchus). Comp. Biochem. Physiol. 92A: 299–304.

Charnock, J. S., Abeywardena, M. Y., Poletti, V. M. & McLennan, P. L. (1992) Differences in fatty acid composition of various tissues of the marmoset monkey (Callithrix jacchus) after different lipid supplemented diets. Comp. Biochem. Physiol. 101A: 387–393.

Charnock, J. S., McIntosh, G. H., Abeywardena, M. Y. & Russell, G. R. (1985a) Changes in fatty acid composition of the cardiac

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phospholipids of the cotton-eared marmoset (Callithrix jacchus) after feeding different lipid supplements. Ann. Nutr. & Metab. 29: 83–94.

Charnock, J. S., McLennan, P. L., Abeywardena, M. Y. & Russell, G. R. (1985b) Altered levels of (n-6)/(n-3) fatty acids in rat heart and storage fat following variable dietary intake of linoleic acid. Ann. Nutr. & Metab. 29: 279–288.

Finean, J. B., Coleman, R. & Michell, R. H. (1974) Membranes and Their Functions. Blackwell Scientific Publications, Oxford, England.

Folch, J., Lees, M. & Sloane Stanley, G. H. (1957) A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226: 497–509.

Gudbjarnason, S. (1989) Dynamics of n-3 and n-6 fatty acids in phospholipids of heart muscle. J. Intern. Med. 225(S1): 117–128.

Holman, R. T. (1960) The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. J. Nutr. 70: 405–410.

Holman, R. T. (1968) Essential fatty acid deficiency. In: Progress in the Chemistry of Fats and Other Lipids (Holman, R. T., ed.), Vol. 9, pp. 275–348. Pergamon Press, New York, NY.

Hulbert, A. J. & Else, P. L. (1989) The evolution of mammalian endothermic metabolism: mitochondrial activity and changes in cellular composition. Am. J. Physiol. 256: R63–69.

Innis, S. M. & Clandinin, M. T. (1981) Dynamic modulation of mitochondrial inner-membrane lipids in rat heart by dietary fat. Biochem. J. 193: 155–167.

Jeffcoat, R. & James, A. T. (1984) The regulation of desaturation and elongation of fatty acids in mammals. In: Fatty Acid Metabolism and Its Regulation (Numa, S., ed.), pp. 8–112. Elsevier, Amsterdam, The Netherlands.

Leyton, J., Drury, P. J. & Crawford, M. A. (1987) Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. Br. J. Nutr. 57: 383–393.

McLennan, P. L., Abeywardena, M. Y. & Charnock, J. S. (1989) The influence of age and dietary fat in an animal model of sudden cardiac death. Aust. N.Z. J. Med. 19: 1–5.

McLennan, P. L., Abeywardena, M. Y. & Charnock, J. S. (1990) Reversal of the arrhythmogenic effects of long-term saturated fatty acid intake by dietary (n-3) and (n-6) polyunsaturated fatty acids. Am. J. Clin. Nutr. 51: 53–58.

McMurchie, E. J. (1988) Dietary lipids and the regulation of membrane fluidity and function. In: Physiological Regulation of Membrane Fluidity (Aloia, R. C., ed.), pp. 189–237. Alan R. Liss, New York, NY.

Mead, J. F. (1984) The non-eicosanoid functions of the essential fatty acids. J. Lipid Res. 25: 1517–1521.

Morrison, W. R. & Smith, L. M. (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5: 600–608.

Neudoerffer, T. S. & Lea, C. H. (1967) Effects of dietary polyunsaturated fatty acids on the composition of the individual lipids of turkey breast and leg muscle. Br. J. Nutr. 21: 691–714.

Olomu, J. M. & Baracos, V. E. (1991) Prostaglandin synthesis and fatty acid composition of phospholipids and triglycerides in skeletal muscle of chicks fed combinations of flaxseed oil and animal tallow. Lipids 26: 743–749.

Pan, D. & Storlien, L. H. (1993) Dietary lipid profile is a determinant of tissue phospholipid fatty acid composition and rate of weight gain in rats. J. Nutr. 123: 512–519.

Phinney, S. D., Clarke, S. D., Odin, R. S., Moldawer, L. L., Blackburn, G. L. & Bistrian, B. R. (1993) Thermogenesis secondary to transdermal water loss causes growth retardation in essential fatty acid–deficient rats. Metabolism 42: 1–5.

Storlien, L. H., Jenkins, A. B., Chisholm, D. J., Pascoe, W. S., Khouri, S. & Kraegen, E. W. (1991) Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and (n-3) fatty acids in muscle phospholipid. Diabetes 40: 280–289.

Zar, J. H. (1984) Biostatistical Analysis, 2nd ed. Prentice-Hall International, Englewood Cliffs, NJ.

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