Effects of changes in dietary fatty acids on isolated skeletal muscle function in rats

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Ayre, Kerry J., and A. J. Hulbert. Effects of changes in dietary fatty acids on isolated skeletal muscle function in rats. *J. Appl. Physiol.* 80(2): 464–471, 1996.—The effects of manipulating dietary levels of essential polyunsaturated fatty acids on the function of isolated skeletal muscles in male Wistar rats were examined. Three isoenergetic diets were used: an essential fatty acid-deficient diet (EFAD), a diet high in essential (n-6) fatty acids [High (n-6)], and a diet enriched with essential (n-3) fatty acids [High (n-3)]. After 9 wk, groups of rats on each test diet were fed a stock diet of laboratory chow for a further 6 wk. Muscle function was examined by using a battery of five tests for soleus (slow twitch) and extensor digitorum longus (EDL; fast twitch). Tests included single muscle twitches, sustained tetanic contractions, posttetanic potentiation, sustained high-frequency stimulation, and intermittent low-frequency stimulation. Results for muscles from the High (n-6) and High (n-3) groups were very similar. However, the EFAD diet resulted in significantly lower muscular tensions and reduced response times compared with the High (n-6) and High (n-3) diets. Peak twitch tension in soleus muscles was 16–21% less in the EFAD group than in the High (n-6) and High (n-3) groups, respectively [analysis of variance (ANOVA), $P < 0.01$]. Also, twitch contraction and half-relaxation times were significantly 5–7% reduced in the EFAD group (ANOVA, $P < 0.01$). During high-frequency stimulation, EDL muscles from the EFAD rats fatigued 32% more quickly (ANOVA, $P < 0.01$). During intermittent low-frequency stimulation, soleus muscles from the EFAD group generated 25–28% less tension than did the other groups (ANOVA, $P < 0.01$), but in EDL muscles from the EFAD group, endurance was 20% greater than in the High (n-6) group (ANOVA, $P < 0.05$). After 6 wk on the stock diet, there were no longer any differences between the dietary groups. Manipulation of dietary fatty acids results in significant, but reversible, effects in muscles of rats fed an EFAD diet.

essential fatty acid deficiency; (n-3) polyunsaturated fatty acids; soleus; extensor digitorum longus; muscle fatigue

SINCE THE CLASSIC STUDIES of Bergstrom et al. (4), it has become widely accepted that dietary carbohydrate loading can influence muscle performance. The converse of this is that it is generally believed that dietary fats are not of great importance in determining the exercise performance of animals, and little is known about the effects of different types of fatty acids on skeletal muscle function. Recently, however, a number of studies have shown that a high-fat diet can dramatically increase exercise performance in rats (22, 28), humans (18, 23), and dogs (30). In all of these studies, the effect of dietary fat enrichment on performance was greater than the effect of dietary carbohydrate enrichment.

Dietary fatty acids can be *1*) directly metabolized to provide energy, *2*) stored for later use, or *3*) incorporated into membranes. There are several types of fatty acids, many of which can be synthesized endogenously. However, vertebrates are unable to synthesize either the (n-6) or (n-3) classes of polyunsaturated fatty acids. These must be ingested in the diet and are called essential fatty acids (EFAs). In the past, as long as dietary requirements for the EFAs have been met, fats have largely been regarded as being similar in their influence on metabolism. It is now obvious, however, that not all dietary fats should be treated equally. The dietary lipid profile can influence the metabolic physiology of animals, and it has been suggested that one possible mechanism is by changing the fatty acid composition of cellular membranes and thus the activity of membrane-associated processes (25).

Previously, we have reported that manipulating the ratio of polyunsaturated fatty acids in the diet of rats produces marked changes in the fatty acid composition of skeletal muscle phospholipids (3). Similar effects have also been reported in cardiac muscle of both rats (7) and marmosets (8). Moreover, such dietary and compositional changes have been correlated with changes in cardiac muscle function (9, 19). The aim of this study was to determine whether the compositional profile of dietary fatty acids fed to rats could also influence the contractile properties of isolated skeletal muscles. Because fast-twitch and slow-twitch muscles differ markedly in their functional properties, we determined the effects of the same dietary fatty acid manipulations on a range of skeletal muscle contractile functions using in vitro preparations of extensor digitorum longus (EDL; fast twitch) and soleus (slow twitch) muscles. Finally, we investigated the extent to which dietary effects on muscle performance paralleled changes in muscle phospholipid composition (3) after returning animals to a single stock diet.

MATERIALS AND METHODS

Two dietary manipulations were performed. First, for 9 wk, rats were fed one of three test diets (each contained 10% wt/wt fat), which differed only in their fatty acid composition. Second, some rats from each dietary group were fed a common stock diet of laboratory chow for 6 wk to determine the reversibility of any dietary effects on muscle function.

Animals and Diets

All experiments were approved by the University of Wollongong's Animal Experimentation Ethics Committee. Male weanling Wistar rats were bred at the University of Wollongong and housed individually at 22 ± 2°C and 57 ± 2% relative humidity. They were aged 21–23 days at weaning and had a mean weight of 54 ± 1 g. Littermates were randomly assigned (1 per litter) to each of the three dietary groups. Groups of 13 rats were maintained on each of three test diets for 9 wk. The test diets were *1*) deficient in the

essential fatty acids (EFAD), *2*) high in (n-6) fatty acids [High (n-6)], or *3*) enriched with (n-3) fatty acids [High (n-3)]. After this test period, rats on each diet were randomly allocated to each of two treatments and were killed either *1*) immediately (seven rats per dietary group) or *2*) after 6 wk on the stock diet (Allied Rat and Mouse Cubes (containing ~22% protein, 60% carbohydrate, 6% fat; Fielders' Agricultural Products, Tamworth, Australia); 6 rats per dietary group; Table 1). All rats were given free access to food and water, and food intake and body mass were recorded throughout the study.

All test diets were identical except for their lipid composition. Each diet contained 10% (wt/wt) fat but varied in the type of fat (Table 2). The EFAD diet contained 10% coconut oil (ETA Food Services, Wollongong, Australia) and was therefore lacking both (n-6) and (n-3) essential polyunsaturated fatty acids because coconut oil is extremely high (92%) in saturated fatty acids. The High (n-6) diet contained 10% sesame oil (Meadowlea Foods, Sydney, Australia), which is high in (n-6) fatty acids (43%). The High (n-3) diet contained 7% sesame oil and 3% Max EPA oil (R. P. Scherer, Melbourne, Australia; containing 30% (n-3) fatty acids. Therefore, it was enriched with (n-3) polyunsaturated fatty acids. The fatty acid content of each of the test diets and the stock diet is summarized in Table 3.

After the 9-wk period, rats fed the EFAD diet were verified to be deficient in essential fatty acids from the triene-to-tetraene ratio for both soleus and EDL muscles. This ratio is used as an indicator of essential fatty acid deficiency and is the ratio of the fatty acids 20:3 (n-9) to 20:4 (n-6) (15). 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-to-tetraene ratios >0.4 are considered indicative of essential fatty acid deficiency (15). EFAD rats used in this study had triene-to-tetraene ratios of 0.56 and 0.54 for soleus and EDL muscles, respectively (3). Values for the other dietary groups ranged from 0.000 to 0.004.

Final body weights of the rats were not significantly affected by diet, either after 9 wk on the test diets or after the additional 6 wk on the stock diet [analysis of variance (ANOVA), $P > 0.08$)]. Similarly, muscle mass, length, and cross-sectional surface area (CSSA) of soleus or EDL were unaffected by diet (ANOVA, $P > 0.07$).

Muscle Preparation

Soleus and EDL muscles were chosen to represent the best examples of slow- and fast-twitch muscles, respectively. Rat soleus contains 87% type Ia (slow-twitch oxidative) and 13% type IIa (fast-twitch oxidative) fibers, and rat EDL contains 2% type Ia, 42% type IIa, and 56% type IIb (fast-twitch glycolytic) fibers (1).

One rat per litter from each dietary group was tested on each testing day to ensure that all groups were exposed to similar day-to-day environmental changes. Rats were anesthetized with halothane (Fluothane, Imperial Chemicals, Sydney, Australia) in oxygen (delivered via a mask at an initial concentration of 5% and thereafter at a level of 1–2% as necessary). A complete soleus muscle was carefully removed. The hindlimb was then lightly covered with a mineral oil-petroleum jelly mixture and plastic film to prevent desicca-

Table 1. *Composition of stock diet*

Ingredient	Amount
Cereals, bran, and pollard	55–60 (2–2.5)
Vegetable protein (cottonseed, canola, and soybean meals)	15–20 (2–2.5)
Meat and bone meal	15 (10)
Beef tallow	1.5–2.0 (99)
Whey powder	4–5 (0.5)
Salts, vitamins, and minerals	3 (0)

Values are given in % by wt. Fat content of each ingredient is in parentheses. Information provided by Fielders' Agricultural Products (Tamworth, Australia).

Table 2. *Composition of experimental diets*

Ingredient	EFAD	High (n-6)	High (n-3)
Protein (casein)	22	22	22
Sesame oil	0	10	7
Coconut oil	10	0	0
Max EPA oil	0	0	3
Salts mixture	5	5	5
Vitamin mixture	1	1	1
Cellulose	1	1	1
Water	5	5	5
Sucrose	56	56	56

Values are amounts given in % by wt. All diets were identical except for type of fat. Essential fatty acid-deficient (EFAD) diet contained 10% coconut oil; diet high in essential (n-6) fatty acids [High (n-6)] contained 10% sesame oil; and diet enriched with essential (n-3) fatty acids [High (n-3)] contained 7% sesame oil and 3% Max EPA oil. Salts mixture consisted of (in g) 139.3 NaCl, 0.79 KI, 389 KH_2PO_4, 57.3 $MgSO_4 \cdot 7H_2O$; 381.4 $CaCO_3$, 27.0 $FeSO_4 \cdot 7H_2O$, and 0.023 $CoCl_2$. Vitamin mixture consisted of (in g) 0.0175 menadione (vitamin K), 7 choline Cl, 0.3500 *p*-aminobenzoic acid, 0.3500 inositol, 0.1400 nicotinic acid, 0.1400 Ca pantothenate, 0.0280 riboflavine, 0.0175 thiamine·HCl, 0.0175 pyridoxine·HCl, 0.0070 folic acid, 0.0014 biotin, 0.000105 vitamin B_{12}, and up to 35 dextrose. Vitamins A, D, and E were added separately to provide 2000 IU vitamin A, 200 IU vitamin D, and 30 IU vitamin E per 100 g diet.

Table 3. *Fatty acid composition of EFAD diet, High (n-6) diet, and High (n-3) diet and stock diet of laboratory chow*

	Dietary Group			
Fatty Acid	EFAD	High (n-6)	High (n-3)	Stock diet
8:0	4.10 ± 1.3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10:0	7.20 ± 0.3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12:0	56.90 ± 0.6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
14:0	16.90 ± 0.4	0.00 ± 0.00	2.80 ± 0.1	1.71 ± 0.0
15:0	0.46 ± 0.04	1.38 ± 0.05	1.40 ± 0.1	1.37 ± 0.01
16:0	6.90 ± 0.2	10.30 ± 0.05	12.90 ± 0.1	20.30 ± 0.2
16:1 (n-9)	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 0.05	1.58 ± 0.03
18:0	2.40 ± 0.10	4.09 ± 0.02	3.70 ± 0.1	8.60 ± 0.2
18:1 (n-9)	4.10 ± 0.2	28.60 ± 0.20	22.10 ± 0.2	31.30 ± 0.3
18:1 (n-7)	0.00 ± 0.00	1.01 ± 0.02	1.69 ± 0.02	1.30 ± 0.04
18:2 (n-6)	1.15 ± 0.07	50.12 ± 0.04	35.20 ± 0.1	29.80 ± 0.3
18:3 (n-3)	0.00 ± 0.00	3.94 ± 0.04	3.00 ± 0.03	2.15 ± 0.02
18:4 (n-3)	0.00 ± 0.00	0.00 ± 0.00	1.34 ± 0.02	0.00 ± 0.00
20:5 (n-3)	0.00 ± 0.00	0.00 ± 0.00	6.23 ± 0.08	0.00 ± 0.00
22:6 (n-3)	0.00 ± 0.00	0.00 ± 0.00	3.45 ± 0.03	0.00 ± 0.00
%Saturated	94.90	16.20	21.30	33.20
Monounsaturated % (n-9)	4.10	28.60	22.50	31.60
Polyunsaturated % (n-6)	1.20	50.10	35.40	30.00
% (n-3)	0.00	4.10	15.50	2.20

Values are means ± SE given in % of 3 determinations. Only fatty acids >1% of total fatty acids are listed. EFAD diet contained 10% coconut oil, High (n-6) diet contained 10% sesame oil, and High (n-3) diet contained 7% sesame oil and 3% Max EPA oil. Stock diet consisted of standard laboratory chow.

tion while the soleus muscle was tested. The soleus muscle was initially incubated in 100 ml of freshly prepared and carbogenated (95% O_2-5% CO_2) Krebs solution (in mM: 4.74 KCl, 1.19 KH_2PO_4, 120 NaCl, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.3 $CaCl_2$, and 5 glucose), adjusted to 7.4 pH and containing 2.9×10^{-2} mM tubocurarine chloride, for 15 min at room temperature to ensure that neuromuscular transmission was blocked by the tubocurarine chloride. After evaluation of soleus function, the EDL muscle was removed from the anesthetized rat and treated in the same way as was the soleus.

To stimulate the muscles, a modified version of the method described by Carlsen and Walsh (6) was used. Each curarized muscle was attached to a force-displacement transducer (model FT 03 C, Grass, Quincy, MA) by the tendon of insertion by using no. 2.0 silk suture thread. The tendon of origin was held fixed by a modified hemostat. The whole muscle was lowered into a double-sided glass chamber containing curarized Krebs solution maintained at 33–34°C by a temperature-controlled circulating water bath. The solution in the chamber was continuously bubbled with carbogen and was replaced at the rate of one drop per second. The chamber contained two platinum sheet electrodes (3-cm long by 1-cm wide). These extended along the entire length of the muscle on two sides and produced a transverse electrical field when stimulated by a Grass stimulator and constant-current supplier (type SD 9).

The voltage output from the force transducer was converted to digital signal (MacLab, Analog Digital Instruments, Sydney, Australia) and recorded on Macintosh SE computer by using the software package Chart (Analog Digital Instruments, ver. 3.1.3, 1991).

All chemicals were analytic grade. Tubocurarine chloride came from Sigma Chemical (St. Louis, MO), and all other chemicals came from BDH Chemicals (Melbourne, Australia).

Electrical Stimulation

Each muscle was adjusted to optimal length (the length at which peak twitch tension is maximal (10) by using a micromanipulator (no. 1241, Norishige, Japan). The stimulus intensity was also increased incrementally until maximal twitch force was obtained; stimulation intensity was then set above this level to ensure supramaximal stimulation (generally 24 V).

Tests Conducted

A six-step protocol was used for evaluating muscle function. Except for the high-frequency fatigue test, all stimulus pulses were 0.2 ms in duration. The following responses were elicited.

Single muscle twitches (single pulse stimuli were given at 0.1 Hz for 10 s). Maximum isometric twitch tension (g), contraction time (ms), and half-relaxation time (time to relax to half-peak tension, ms) were measured (11).

Force-frequency relationship (muscles were stimulated for 500 ms, once every 100 s, at frequencies from 50 to 130 Hz). Optimal stimulus frequency was assigned to the lowest stimulus frequency giving a maximal response (10).

Sustained tetanic contraction (tetanus was induced at 300 Hz for 500 ms). Maximum isometric tetanic tension (g) and tetanic relaxation time (time to relax to half-peak tension, ms) during repetitive stimulation were measured (11).

Posttetanic potentiation (%) of single twitches (EDL muscles only). Initial twitch responses were elicited by stimulating with interrupted tetanic trains (30 Hz for 330 ms, repeated 1/s) for 15 s (150 stimuli). Posttrain twitch responses were elicited at 0.10 Hz beginning 10 s after the last train (10). Because posttetanic potentiation is a phenomenon normally found only in adult fast muscles (11), it was tested only in EDL muscles.

Fatigue during high-frequency stimulation (i.e., sustained tetanic contraction). Pulses of 0.02-ms duration were delivered at 300 Hz for 20 s (soleus) and 10 s (EDL), followed by pulses of 0.2-ms duration at 300 Hz for 20 s (soleus) and 10 s (EDL) (16). Peak tension (g) during high-frequency stimulation and fatigue time (time taken to fatigue to half-peak tension, s) were measured.

Fatigue during intermittent low-frequency stimulation (interrupted tetanic stimulation). Pulses were delivered at 60% of the optimal tetanic frequency (see *Force-frequency relationship*) for 330 ms, each 1 s for 4 min (5, 6). Stimulation frequency rates ranged from 60 to 67 Hz for all groups. Peak tension (g) during low-frequency stimulation and endurance (time to reach half-peak tension, s) were measured.

After testing, the length and mass of each muscle were determined and CSSA was estimated by dividing muscle mass by muscle length.

Statistical Methods

To test for differences in isolated muscle function between the dietary groups, the statistical package SAS (SAS Institute, 1979), was used to perform model III two-factor ANOVAs without replication; the fixed factor was diet and the random factor was litter and/or day of testing. Because only one rat per litter was allocated to each treatment and each litter was tested on a different day, this design cannot be used to determine whether significant effects of this factor are litter effects per se, or the product of day-to-day variation in experimental conditions. However, this design ensures that variation among litters and days of testing does not obscure effects of the diets. Two separate two-factor ANOVAs were performed: after 9 wk on the test diets and after 6 wk on the stock diet. Wherever ANOVA revealed significant effects of diet, Tukey's studentized range test was used to determine which dietary groups produced significantly different responses (31).

All analyses of force were normalized by adjusting values for estimated muscle CSSA. All values are presented as means ± SE after either 9 wk on one of the three test diets [EFAD, High (n-6), or High (n-3); $n = 7$ rats for all groups] or 9 wk of test diet followed by 6 wk on the common stock diet ($n = 6$ rats for all dietary groups). The level of statistical significance chosen was $P < 0.05$.

RESULTS

In this study, we found no significant effect of diet on final body mass, which ranged from 390 ± 9 to 438 ± 13 g for all dietary groups. In addition, there were no significant dietary effects on muscle mass, length, or CSSA.

Contractile Properties

Single muscle twitches. TWITCH TENSION. After 9 wk on the test diets, the soleus muscles of the EFAD group generated 16–21% less peak twitch tension than did either the High (n-6) group or the High (n-3) group, respectively (Fig. 1*A*). However, after 6 wk on the stock diet, all groups generated similar twitch tensions. There was no effect of diet on twitch tension in EDL muscles.

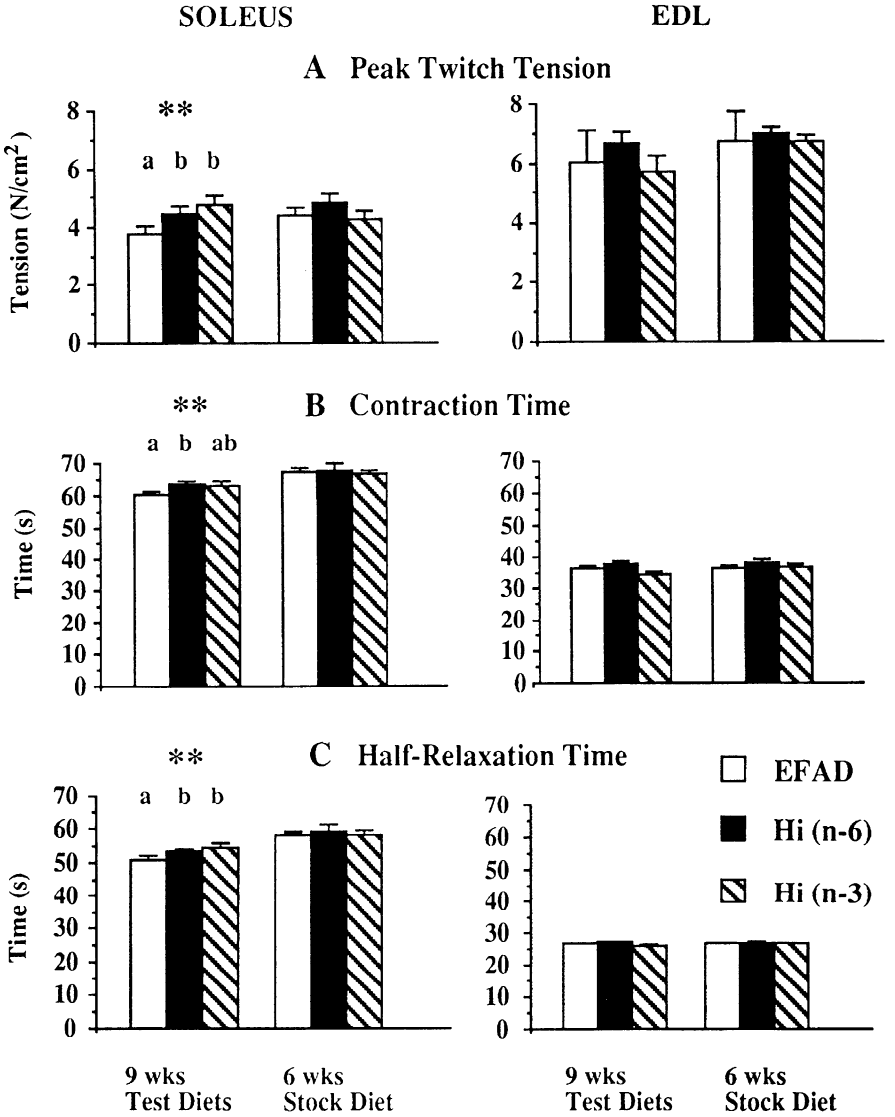

Fig. 1. Single-twitch responses responses of isolated soleus and extensor digitorum longus (EDL) muscles for groups of 6–7 male Wistar rats. Values are means ± SE. Stimuli were 0.2-ms square-wave pulses delivered at 0.1 Hz for 10 s. EFAD, diet deficient in essential fatty acids; High (n-6), diet high in essential (n-6) fatty acids; High (n-3), diet enriched with essential (n-3) fatty acids. Significantly different treatment means are denoted by different lowercase letters. $^{**}P < 0.01$.

TWITCH CONTRACTION AND HALF-RELAXATION TIMES. Twitch contraction times in soleus muscles after the 9-wk test period were affected by diet with EFAD rats contracting significantly 5% more quickly than in the High (n-6) group (Fig. 1*B*). Half-relaxation times for the EFAD group were also significantly 5–7% less than for the High (n-6) and High (n-3) groups (Fig. 1*C*), and these followed the lower tensions generated in the EFAD muscles during the muscle twitch (Fig. 1*A*). After 6 wk on the common stock diet, both contraction and half-relaxation times were similar for all three groups. Diet had no significant effect on either contraction time or half-relaxation time in EDL muscles at either stage (Fig. 1, *B* and *C*).

Sustained tetanic contractions. MAXIMUM TETANIC TENSION. Diet had no significant effect on maximum tetanic tension or tetanic relaxation time in either muscle.

POSTTETANIC POTENTIATION. Peak twitch tension in EDL muscles after intermittent tetanic stimulation was increased by 23–30% after the 9-wk test diet period in all dietary groups, but this posttetanic potentiation was not affected by diet.

Resistance to Fatigue

High-frequency stimulation. SHORT PULSES (0.02-MS DURATION). During high-frequency stimulation (with short pulses) of both soleus and EDL muscles, neither peak tension nor fatigue time was significantly affected by diet at any stage, but the results were highly variable.

LONG PULSES (0.2-MS DURATION). During high-frequency stimulation with longer pulses, peak tension was unaffected by diet in both soleus and EDL. However, EDL muscles from the EFAD group fatigued to half-peak tension 32% more quickly than did both the High (n-6) and High (n-3) groups (Fig. 2).

Low-frequency stimulation. PEAK TENSION. For soleus, there was a highly significant effect of diet on peak tension generated during intermittent low-frequency stimulation after 9 wk on the test diets. The EFAD group generated 25–28% less tension than the High (n-6) and High (n-3) groups (Fig. 3*A*). However, after 6 wk on the stock diet, there was no difference between the groups. For EDL, there was no effect of diet on peak tension.

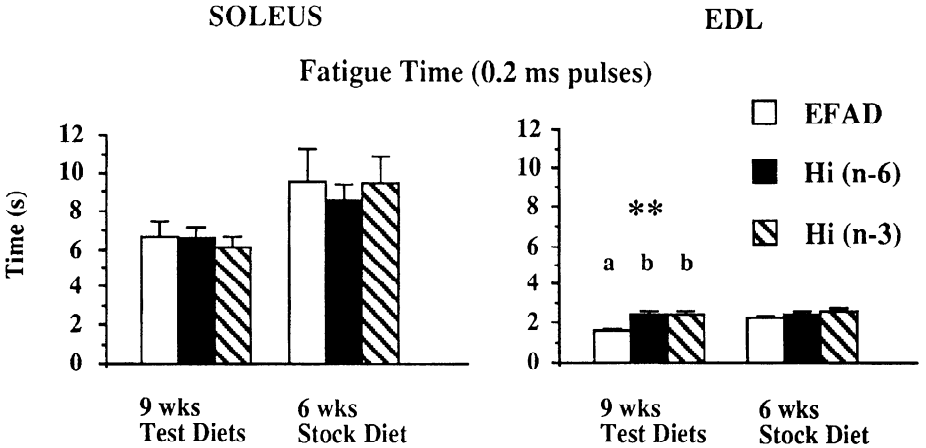

Fig. 2. Fatigue time during high-frequency stimulation of isolated soleus and EDL muscles for groups of 6–7 male Wistar rats. Values are means ± SE. Stimuli were 0.2-ms square-wave pulses for 20 s (soleus) or 10 s (EDL) delivered at 300 Hz. Fatigue time was measured as time to fatigue to half-peak tension. Significantly different treatment means are denoted by different lowercase letters. $^{**}P < 0.01$.

ENDURANCE. Diet did not affect endurance in soleus (measured as time to fatigue to half-peak tension; Fig 3*B*). However, for EDL, endurance was significantly 20% greater for the EFAD rats compared with the High (n-6) rats (Fig. 3*B*).

After 6 wk on the common stock diet, there were no longer any differences between the groups in either peak tension or endurance for both muscles.

DISCUSSION

Our study provides evidence that essential fatty acid deficiency can affect skeletal muscle function in rats. The EFAD rats, which were deficient in both (n-6) and (n-3) essential fatty acids (3), showed reduced performance in muscle twitches and during high- and low-frequency stimulation. In rats on the EFAD diet, soleus muscles generated less tension during both muscle twitches and intermittent low-frequency stimulation. The muscle twitches also had shorter contraction and half-relaxation times. Although EDL muscles from the EFAD rats fatigued more quickly during high-frequency stimulation, in contrast with soleus, they showed increased endurance during low-frequency stimulation. Despite having different proportions of (n-6) and (n-3) essential fatty acids (3), the performances of muscles from rats fed the polyunsaturated fatty acid-adequate High (n-6) and High (n-3) diets were generally similar to those reported for age-matched rats of various strains, none of which was essential fatty acid deficient (10, 12, 26). Our study also showed that recovery from the effects of the essential fatty acid deficient diet occurred within 6 wk on a stock diet.

An obstacle to evaluating the effects of a particular treatment on muscle function is the interdependence of

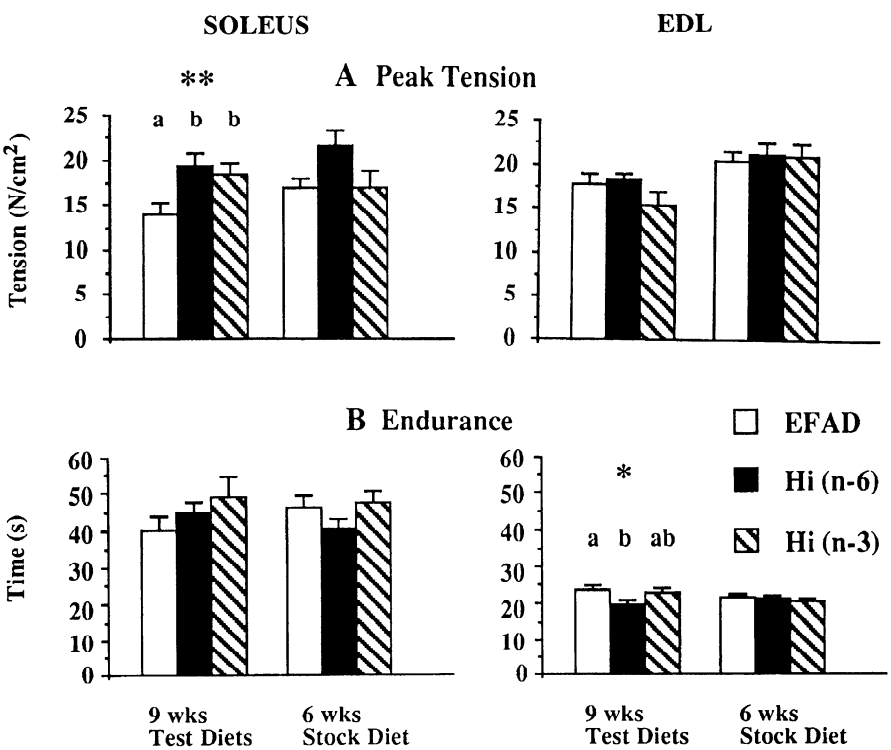

Fig. 3. Low-frequency stimulation responses of isolated soleus and EDL muscles for groups of 6–7 male Wistar rats. Values are means ± SE. Stimuli were 0.2-ms square-wave pulses delivered at 60% of the optimal frequency of each muscle for 330 ms each second for 4 min. Endurance was measured as time to reach half-peak tension. Significantly different treatment means are denoted by different lowercase letters. $^{*}P < 0.05$. $^{**}P < 0.01$.

most aspects of muscle performance. This can make it difficult to decide if a treatment has enhanced or reduced the performance of a muscle. In isometric twitches and tetanic contractions, an increased speed of contraction might be considered to indicate improved performance, but if it is because peak tension is reduced, then is this fast poor response indicative of impaired or enhanced performance? The problem is compounded by the fact that most muscles have multiple functions and their performance must therefore represent a compromise. For these reasons, and because there have been few previous studies in this area of dietary fat effects on muscle performance, a number of tests were conducted to determine overall patterns of response. In this study, although several aspects of muscle function of the EFAD groups were impaired, all aspects of muscle function had returned to normal after 6 wk on the stock diet.

In interpreting the effects of diet on muscle performance, we assume that our in vitro assay is a realistic measure of the performance of these muscles in vivo. However, isolated muscles stimulated in this fashion can be hypoxic and devoid of glycogen in their core because they rely almost entirely on diffusion through the muscle. If so, then factors such as altered levels of $[H^+]$ and inorganic phosphate, which are known to be increased during fatigue and hypoxia (13), may have been responsible for some of the observed changes in muscle tension. In this study we were interested in effects of dietary changes on overall muscle function, so we used isolated whole incubated muscles. Studies of single fibers might provide more precise information about the responses of the different fiber types, but because almost all muscles are heterogeneous [and it is difficult to assign individual fibers to one of several fiber types (14)], they may prevent extrapolation to whole muscle function. Because all muscles were treated identically, significant differences observed between the dietary groups are due to the changes in dietary fatty acids.

Mechanisms of Dietary Effects

Almost all observed dietary effects in this study were attributable to differences in performance of the EFAD group compared with the other two groups. Because each of the diets contained the same amount of fat, the differences between the groups may reflect either direct effects of essential fatty acid deficiency or changes in the balance of their resulting eicosanoids, which mediate many cellular reactions related to secretory, cardiovascular, and immune functions (17, 27). Because phospholipids are the major structural lipid in membranes, changes in the dietary fatty acid profile could also have profound effects on membrane physical properties. For example, essential fatty acid deficiency can result in changes in the fluidity of membranes (20) and therefore possibly exert influence over specific membrane proteins, such as enzymes (24), or have a controlling influence on transport of small molecules across cell membranes (26).

In general, membrane fluidity increases with increased lipid unsaturation (15). It is difficult to use dietary manipulation to alter the ratio of saturated to unsaturated fatty acids in membrane phospholipids (and thus membrane fluidity) because of the biochemical preference for a saturated fatty acid in the *sn*-1 position and an unsaturated fatty acid in the *sn*-2 position in phospholipids (29). This means that one position almost always contains a saturated fatty acid while the other fatty acid is unsaturated. As we have shown (3), despite the EFAD diet containing 95% saturated fatty acids, the rats in this group had a saturated fatty acid composition in their muscle phospholipids of only 32–39%, as did the rats in the other two groups (Table 4). This homeostatic mechanism may therefore maintain a relatively constant level of membrane fluidity despite large fluctuations in dietary fatty acid profile. Although it is not possible to greatly alter the ratio of saturated to unsaturated fatty acids in muscle phospholipids, it is possible to change the proportions of the different classes of unsaturated fatty acids (Table 4).

Of interest in this study is the similar performance of rats in the High (n-6) and High (n-3) groups, despite differences in the fatty acid profile of the two diets. One possible explanation for the reduced performance in the EFAD group compared with these other two groups is that muscles from the EFAD rats had much higher levels of monounsaturated fatty acids. Alternatively, the EFAD group had both lower levels of (n-3) fatty acids than did the High (n-3) group and lower levels of (n-6) fatty acids than did the High (n-6) group. The High (n-6) and High (n-3) groups could generate similar results because either both diets contain sufficient (n-6) and (n-3) fatty acids to maintain normal muscle function or, alternatively, any detrimental effect of (n-3) deficiency in the High (n-6) diet or (n-6) deficiency in the High (n-3) diet must be compensated for by the presence of elevated levels of the other fatty acid.

Two membrane-bound enzymes, Na^+-K^+-adenosinetriphosphatase (ATPase) (of the sarcolemma) and Ca^{2+}-ATPase (of the sarcoplasmic reticulum) are vital components of muscle function. Both enzymes are known to be influenced by the composition of the surrounding fatty acids (24). Because the changes in composition of

Table 4. *Summary of fatty acid composition of soleus and EDL muscles after 9 wk on test diets*

	Soleus			EDL		
Fatty Acid Class	EFAD	High (n-6)	High (n-3)	EFAD	High (n-6)	High (n-3)
Saturated	32	33	34	38	38	39
Monounsaturated	30	9	7	34	6	6
Polyunsaturated						
% (n-6)	30	50	26	22	44	24
% (n-3)	3	5	30	4	10	29

Values are given in %. Only fatty acids >1% of total fatty acids are listed. EDL, extensor digitorum longus. Data are summarized from Ayre (2).

dietary fatty acids altered the phospholipid fatty acid composition of both muscles (3), the observed effects of essential fatty acid deficiency in decreasing tensions could be related to changes in sarcolemmal properties (such as propagation of action potentials and maintenance of ionic concentration gradients) and/or sarcoplasmic reticulum membrane properties (such as the rate of release and reuptake of calcium).

Conclusion

Whatever the mechanism of dietary effects, manipulation of dietary fatty acids did produce some marked effects on muscle function. Furthermore, these effects appear to be related to the phospholipid fatty acid composition of the muscle because the pattern of recovery of muscle performance on the stock diet mirrored the pattern of change in phospholipid composition documented in our earlier study (3). It remains to be determined, however, whether EFAD rats are more strongly influenced by deprivation of (n-3) or (n-6) fatty acids and whether other, more subtle, changes in phospholipid composition may also produce measurable changes in muscle function.

The present results, together with our earlier findings that these same dietary regimens affect muscle phospholipid composition (3) and whole animal physical performance (2), produce a complex pattern of dietary effects. Specifically, we found that the relative proportions of (n-9) and (n-6) unsaturated fatty acids in muscle phospholipids were similar to those of the diet but that (n-3) fatty acids were tenaciously retained by the muscles when dietary composition was altered (3). Moreover, we found in whole animals that while forelimb grip strength, basal metabolic rate, and oxygen consumption during strenuous exercise were unaffected by alterations in dietary fatty acids, consumption of a diet high in (n-3) fatty acids resulted in a dramatic reduction in treadmill-running endurance compared with rats fed a high (n-6) diet (2).

Because forelimb muscles contain mainly fast-twitch fibers, the lack of a dietary effect on forelimb grip strength was not surprising given that, in the current study, changing the composition of dietary fatty acids had little effect on the predominantly fast-twitch EDL muscle. However, the finding of reduced endurance in rats on the High (n-3) diet was unexpected because this diet did not affect isolated muscle function. Because endurance is a multifactorial phenomenon (in contrast to grip strength, which is probably more closely related to muscle function), the observed effects of dietary changes on endurance may reflect changes in the respiratory and/or cardiovascular systems rather than on the muscles themselves. To resolve the mechanism of such dietary effects would require further experimental study.

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