Dietary Flaxseed Protects against Ventricular Fibrillation Induced by Ischemia-Reperfusion in Normal and Hypercholesterolemic Rabbits

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ABSTRACT Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the (n-3) PUFA found in fish oils, exert antiarrhythmic effects during ischemia. Flaxseed is the richest plant source of another (n-3) PUFA, α-linolenic acid (ALA), yet its effects remain largely unknown. Our objective was to determine whether a flaxseed-rich diet is antiarrhythmic in normal and hypercholesterolemic rabbits. Male New Zealand White (NZW) rabbits (n = 14–16) were fed as follows: regular diet (REG group); diet containing 10% flaxseed (FLX group); 0.5% cholesterol (CHL group); or 0.5% cholesterol + 10% flaxseed (CHL/FLX group) for up to 16 wk. Plasma cholesterol was significantly elevated in the CHL and CHL/FLX groups. Plasma triglycerides were unchanged. ALA levels increased significantly in plasma and hearts of the FLX and CHL/FLX groups. After the feeding period, rabbit hearts were isolated and subjected to global ischemia (30 min) and reperfusion (45 min). Ventricular fibrillation (VF) occurred during ischemia in 33% of REG but in none of FLX hearts, and 28% of CHL but only 6% of CHL/FLX hearts. VF incidence during reperfusion was 28% and 26% in REG and FLX hearts, respectively. The incidence significantly increased to 64% in CHL hearts, and was significantly attenuated (18%) in CHL/FLX hearts. CHL markedly prolonged the QT interval, whereas FLX significantly shortened the QT interval and reduced arrhythmias in the FLX and CHL/FLX hearts. In vitro application of (n-3) PUFA shortened the action potential duration, an effect consistent with the QT data. This study demonstrates that dietary flaxseed exerts antiarrhythmic effects during ischemia-reperfusion in rabbit hearts, possibly through shortening of the action potential.

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KEY WORDS: • (n-3) PUFA • α-linolenic acid • ion channels • arrhythmias • action potentials

Sudden death resulting from acute myocardial infarction accounts for the majority of deaths from cardiovascular disease in the developed world (1). Ischemia-induced arrhythmias, ventricular fibrillation in particular, are a serious and often fatal consequence of coronary heart disease. However, mortality and morbidity from cardiovascular disease have declined over the last decade, in part due to antiarrhythmic drugs, education of health care professionals, and increased public awareness of risk factors. Dietary interventions, in particular, have recently received attention as effective antiarrhythmic strategies.

Epidemiologic surveys, clinical trials, and laboratory studies [see (2–4) for reviews] demonstrate that (n-3) PUFA reduce the incidence and severity of cardiovascular disease. To date, most studies have focused on eicosapentaenoic acid [EPA,4 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)], which are the primary (n-3) PUFA found in fish. Fish oil supplements are an effective means of increasing (n-3) intake, but they may not be acceptable for vegetarians, and individuals consuming higher doses of fish oils often complain of the unpleasant aftertaste (5–7). Alternative sources of (n-3) PUFA exist in plants. Flaxseed, for example, is the richest known plant source of the (n-3) PUFA, α-linolenic acid [ALA, 18:3(n-3)] (8). Ground flaxseed has a pleasant nutty taste and may represent an ideal modality for the introduction of more (n-3) PUFA into the human diet. Unfortunately, relatively little is known about the cardiovascular effects of ALA because few studies have tested its effects as a lone dietary modification. This clearly warrants examination, espe-

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4 Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; APD, action potential duration; CHL, group fed the 0.5% cholesterol diet; CHL/FLX, group fed the 0.5% cholesterol + 10% flaxseed diet; DHA docosahexaenoic acid; ECG, electrocardiogram; EPA, eicosapentaenoic acid; FLX, group fed the 10% flaxseed diet; HBS, HEPES buffered saline; MAP, monophasic action potential; MAPD, action potential duration; MUFA, monounsaturated fatty acid; NZW, New Zealand White; OA, oleic acid; REG, group fed the regular diet; VF, ventricular fibrillation; VT, ventricular tachycardia.

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specialy because different PUFA have very different effects on the heart (9,10). One study compared the effects of emulsions of PUFA injected into the heart before coronary artery occlusion and found that ALA inhibited dangerous cardiac arrhythmias to an extent similar to that of EPA or DHA (11). However, the effects of dietary supplementations of ALA are relatively unknown. Dietary interventions may be more practical and effective way of delivering ALA to the body, but it is unclear whether sufficient ALA can be absorbed from dietary flaxseed to be cardioprotective. Also, it is possible that ALA is rapidly metabolized in the body after ingestion into longer (n-3) fatty acids, such as EPA and DHA, through a series of elongation and desaturation steps. Studies on the metabolic conversion of ALA to the longer (n-3) PUFA reported values ranging from 0.2 to 15% (12,13). Therefore, 2 critical questions remain to be answered. First, does a diet enriched in flaxseed provide antiarrhythmic effects during an ischemic/reperfusion challenge? Second, are the antiarrhythmic effects of flaxseed due to ALA or its in vivo conversion to the longer chain (n-3) PUFA, EPA, and/or DHA?

This study addresses these 2 questions using dietary flaxseed (containing high ALA) supplementation in rabbits. To further increase the pathological relevance of the study, we also studied the effects of dietary flaxseed to determine whether it remains antiarrhythmic in the presence of high circulating cholesterol levels. A rabbit model was selected to test 4 different diets, i.e., a regular control diet, a flaxseed-supplemented diet, a diet containing high cholesterol, and a diet containing both high cholesterol and flaxseed. We assessed the occurrence of ventricular tachycardia (VT) and fibrillation (VF) during a global ischemia-reperfusion protocol in hearts isolated from these rabbits after the dietary interventions. The acute effects of (n-3) PUFA were also examined to identify possible mechanisms of this antiarrhythmic action.

**MATERIALS AND METHODS**

**Diet and feeding.** All experiments conformed to the guidelines of the Canadian Council on Animal Care (14). Male New Zealand White (NZW) rabbits (2.8 ± 0.1 kg; Southern Rose Rabbitry) were assigned to receive 1 of the following 4 diets: regular (REG group), standard rabbit diet (CO-O P Complete Rabbit Ration, Federation Co-operatives); flaxseed (FLX group), standard diet containing 10% ground flaxseed (wt/wt, ALA comprises 70% total fatty acids, Pro-mega Flax from Polar Foods); cholesterol (CHL group), standard diet containing 0.5% cholesterol (wt/wt); and cholesterol plus flaxseed (CHL/FLX group), standard diet containing both 0.5% cholesterol and 10% ground flaxseed. The standard ration was ground and the appropriate dietary components were mixed in; flaxseed was ground just before mixing. The diets were then moistened, repelleted, and air-dried. Diets were refrigerated and protected from light. The diets were then raised to 225°C at 5°C/min and held for 10 min. The total temperature was held at 80°C for 1 min, raised to 140°C at 30°C/min, and then raised to 225°C at 5°C/min and held for 10 min. The total run time for each sample was 30 min. Components were identified by comparison with authentic standards (Nu-Chek-Prep).

**Perfusion and instrumentation of rabbit hearts.** Rabbits were anesthetized with isoflurane and hearts were rapidly excised and placed in Tyrode's solution containing (mmol/L): NaCl 115.0, NaHCO₃ 28.0, NaH₂PO₄ 0.5, glucose 20.0, KCl 4.0, CaCl₂ 2.0, and MgCl₂ 0.7 (pH 7.4). The aorta was tied onto the cannula of a custom-perfusion apparatus. Retrograde perfusion was started within 60 s. Hearts were perfused at 20 mL/min with Tyrode's solution maintained at 37.0 ± 0.5°C and bubbled with 95% O₂:5% CO₂. Hearts were instrumented as previously described (22). Briefly, the right atrium was removed, and the atrioventricular node was crushed to allow pacing of the heart. Electrodes were placed high into the right ventricle and hearts were paced at 2 Hz for the duration of the experiment.

**Monophasic action potentials (MAP)** were recorded from the endocardial and epicardial surfaces of the left ventricle as previously described (22). The instrumented heart was immersed in a circular acrylic bath (15-cm diameter) filled with Tyrode's solution (37°C) and gassed with 95% O₂:5% CO₂. Three Ag/AgCl electrodes, mounted in a simulated Einthoven configuration, were used to record “true” volume-conducted electrocardiograms (ECG) (22,23).

**Ischemia-reperfusion protocol for the isolated rabbit hearts.** Hearts were equilibrated for 50 min before a 30-min test ischemia.

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**Table 1.** Fatty acid composition of the experimental diets.

| Fatty acid  | REG  | FLX  | CHL  | CHL/FLX |
|------------|------|------|------|---------|
| g/100 g fatty acids |
| 14:0       | 0.8  | 0.6  | 0.7  | 0.5     |
| 16:0       | 13.0 | 14.2 | 17.6 | 13.4    |
| 18:0       | 6.2  | 5.9  | 5.7  | 5.2     |
| 16:1(n-7)  | 1.1  | 0.8  | 1.0  | 0.6     |
| 18:1(n-9)  | 30.0 | 28.5 | 28.6 | 23.4    |
| 18:1(n-7)  | 4.8  | 4.1  | 4.6  | 3.7     |
| 22:1(n-9)  | 0.1  | 0.4  | 0.3  | 0.1     |
| 18:2(n-6)  | 31.4 | 17.5 | 34.3 | 19.6    |
| 18:3(n-3)  | 5.6  | 29.9 | 5.9  | 32.2    |
| (n-6)/(n-3)  | 5.6 | 0.59 | 5.81 | 0.61    |

1 Fatty acids not shown represent <0.05 g/100 g fatty acids in the diet.

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| 16:0       | 13.0 | 14.2 | 17.6 | 13.4    |
| 18:0       | 6.2  | 5.9  | 5.7  | 5.2     |
| 16:1(n-7)  | 1.1  | 0.8  | 1.0  | 0.6     |
| 18:1(n-9)  | 30.0 | 28.5 | 28.6 | 23.4    |
| 18:1(n-7)  | 4.8  | 4.1  | 4.6  | 3.7     |
| 22:1(n-9)  | 0.1  | 0.4  | 0.3  | 0.1     |
| 18:2(n-6)  | 31.4 | 17.5 | 34.3 | 19.6    |
| 18:3(n-3)  | 5.6  | 29.9 | 5.9  | 32.2    |
| (n-6)/(n-3)  | 5.6 | 0.59 | 5.81 | 0.61    |

1 Fatty acids not shown represent <0.05 g/100 g fatty acids in the diet.
Global ischemia was initiated by bypassing flow to the heart and returning it to the buffer reservoir. Also, the solution in the intracellular bath was bubbled with 95% N₂/5% CO₂ during ischemia. Hearts were perfused for 45 min by reestablishing the flow with oxygenated Tyrode’s and the immersion bath was again gassed with 95% O₂/5% CO₂. An MP100 data acquisition system and AcqKnowledge 3.0 software (Biopac Systems) acquired the signals at 2 kHz. Arrhythmias were classified as VT or VF using the Lambeth Conventions (24). Electrical stimulation was not stopped during arrhythmic episodes.

**Cardiomyocyte isolation.** Hearts were excised from anesthetized male NZW rabbits (2.5–3.0 kg) and mounted on a Langendorff apparatus. Hearts were flushed at 20 ml/min with Ca²⁺-free Hepes-buffered saline (HBS) containing (mmol/L): NaCl 132.0, HEPES (Na⁺ salt) 20.0, MgSO₄ 1.2, NaH₂PO₄ 1.2, glucose 11.1, KCl 4.0, and CaCl₂ 2.0 (pH 7.4). Action potentials were recorded in current clamp mode with the perforated patch clamp technique (25). Before use, tips of glass pipettes (2–5 ΩM resistances) were filled with pipette solution containing (mmol/L): l-aspartic acid (monopotassium salt) 130.0, KCl 15.0, HEPES (free acid) 5.0, NaCl 10.0, CaCl₂ 0.5, and MgCl₂ 1.0 (pH 7.3). The rest of the pipette was back-filled with pipette solution containing amphotericin B (Sigma), which had been prepared as a 30 g/L stock in dimethyl sulfoxide and diluted to 0.12 g/L. Pipettes were connected to the headstage of an Axopatch-1D amplifier (Axon Instruments) and grounded through a Ag-AgCl wire in an agar bridge. Upon gigaseal formation, amphotericin B was allowed to partition across the cell membrane, and the membrane potential was recorded in current clamp mode. Cells were stimulated by intracellular injection of 3-ms current pulses at 0.5 Hz, and action potentials were recorded before and after the addition of 25 μmol/L DHA, ALA, or oleic acid (OA) directly into the bath. All experiments were performed at 23 ± 2°C. Action potentials were acquired and analyzed using pClamp 7.0 software (Axon Instruments). Action potential durations were calculated for 50% (APD₅₀) and 90% (APD₉₀) repolarization.

**Statistical analysis.** Statistical analyses of data were performed using SigmaStat software (version 2.03, SPSS). Differences in incidence of arrhythmias between groups were assessed with a Fisher Exact test. QT intervals, lipids, and fatty acids for each diet were compared using 1-way ANOVA and Student-Newman-Keuls post hoc test. Interactions in the CHL/FLX group were evaluated by a two-way ANOVA. An ANOVA on ranks was used if variances were not equalized by other transformations. The association between cardiac ALA and the incidence of arrhythmias was assessed by Pearson product moment correlation. The percentage changes in APD were compared using a t test (two-sided). The significance level was set at P < 0.05. All results are expressed as means ± SEM.

**RESULTS**

Animal health was monitored throughout the study. One REG and 2 CHL rabbits were removed from the trial due to complications from loss of appetite. Results from 8 and 16 wk did not differ; therefore, the arrhythmia and QT data from the 2 groups were pooled. Final group sizes for the study were 15, 16, 14, and 16 for the REG, FLX, CHL, and CHL/FLX groups, respectively. Food was consumed daily in its entirety and rabbits gained weight at the same rate in all groups. Weights did not differ among the groups at any time during the feeding trial. Body weight after 16 wk was (kg) 3.9 ± 0.1, 3.9 ± 0.1, 3.8 ± 0.2, and 3.8 ± 0.1 for the REG, FLX, CHL, and CHL/FLX groups. This result confirmed the lack of a difference among the diets with respect to their energy content.

**Plasma and tissue fatty acids.** None of the dietary interventions affected triglyceride concentrations in this study. Consumption of the 0.5% cholesterol diet elevated the relative abundance of palmitoleic acid (P < 0.05). The CHL/FLX group also had significant elevations in ALA, with levels equal to the FLX group. Notably, total fatty acids were significantly increased (>9-fold) in both the CHL and CHL/FLX groups compared with the REG and FLX groups. This caused the absolute amounts of nearly all fatty acid species to be significantly elevated in these groups. The concentration of the longer-chain (n-3) PUFA, EPA, increased slightly in both the CHL and CHL/FLX groups, but DHA made up <0.05 g/100 g fatty acids in all groups.

Dietary flaxseed increased cardiac ALA levels by 3- to 4-fold in the FLX and CHL/FLX groups (Table 2). Levels of EPA were doubled in these groups compared with controls, but DHA levels only rose slightly. The (n-6) PUFA, AA, decreased significantly in the FLX and CHL/FLX groups compared with control. The overall ratios of (n-6):(n-3) fatty acids were also lowered in the flax-supplemented groups.

**Arrhythmogenesis.** Hearts isolated from rabbits fed different diets exhibited a 19–38% incidence of VT during ischemia, compared with 33% in controls (Fig. 2). Fibrillation was observed during ischemia in 27% of REG hearts and this was similar to the CHL group (29%). In contrast, the flaxseed diet prevented VF when given alone (P < 0.05) and also reduced VF in the CHL/FLX group to 6% (P = 0.11). There was a significant negative correlation (r = −0.865, P < 0.05) between the cardiac levels of ALA and the incidence of arrhythmias.

![FIGURE 1](https://academic.oup.com/jn/article-abstract/134/12/3250/4688617?middle)
Reperfusion induced a greater incidence of tachyarrhythmias than ischemia. Upon reperfusion, VT occurred in about half of control hearts and this was not affected by diet. Reperfusion resulted in VF in 27% of control hearts, and this value was not reduced by flaxseed. However, CHL hearts exhibited a significantly greater incidence of VF (64%), and this was decreased to 19% by including flaxseed in the diet (P < 0.05).

**Monophasic action potentials.** Epicardial and endocardial MAP durations (MAPD at 50 and 90% repolarization) were monitored throughout the experiment. There was no difference in MAPD dispersion between epicardium and endocardium (an index of heterogeneity) among the different groups of hearts during ischemia or reperfusion (data not shown).

**QT interval.** The QT intervals of the ECGs were examined to relate the characteristics of electrical activity with arrhythmogenesis. The QT intervals were measured in the paced hearts before the ischemia-reperfusion protocol in the Langendorff perfused hearts. Rabbits fed flaxseed exhibited a shorter QT interval than the controls, whereas the longest QT intervals were measured in the cholesterol-fed group (Table 3). The addition of flaxseed to the cholesterol-supplemented diet significantly shortened the QT interval in these hearts. Shortening of the QT interval was associated with a marked reduction in arrhythmias in the FLX and CHL/FLX groups.

**Acute effects of fatty acids on the cardiac action potential.** The duration of the action potential can influence arrhythmogenesis. To further delineate the potential mechanism for the antiarrhythmic effects of flaxseed, we examined the acute effects of ALA, as well as other fatty acids on action potentials in cardiomyocytes isolated from control rabbits. The monounsaturated fatty acid (MUFA), OA, had no effect on APD50 at any concentration tested (Table 4), but did shorten APD90 (P < 0.05). In contrast, the (n-3) PUFA tested, ALA and DHA, shortened both APD50 and APD90 (P < 0.05) (Fig. 3).

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**TABLE 2**

Relative concentrations of total fatty acids in plasma and cardiac muscle of rabbits after feeding of diets with or without cholesterol and flaxseed1,2

| Fatty acid | Plasma | Heart |
|-----------|--------|-------|
|           | REG    | FLX   | CHL   | CHL/FLX | REG    | FLX   | CHL   | CHL/FLX |
| 14:0      | 0.6 ± 0.2 | 0.7 ± 0.3 | 0.4 ± 0.1 | 0.26 ± 0.03 | 1.6 ± 0.1 | 2.1 ± 0.2 | 2.3 ± 0.3 | 2.0 ± 0.2 |
| 16:0      | 24.1 ± 0.8a | 21.9 ± 0.5b | 20.8 ± 0.2bc | 18.6 ± 1.2c | 17.0 ± 1.1 | 18.3 ± 2.4 | 19.7 ± 2.7 | 19.7 ± 1.9 |
| 18:0      | 10.4 ± 0.8a | 10.0 ± 1.1a | 7.0 ± 0.1b | 6.7 ± 0.4b | 11.1 ± 0.4 | 9.1 ± 1.1 | 9.2 ± 1.0 | 8.4 ± 0.6 |
| 20:0      | tr² | tr | 0.09 ± 0.01 | 0.05 ± 0.01 | 0.2 ± 0.1b | 0.5 ± 0.1a | 0.2 ± 0.1b | 0.41 ± 0.04a |
| 22:0      | tr | tr | 0.18 ± 0.03a | 0.27 ± 0.01b | 0.6 ± 0.2 | 1.1 ± 0.3 | 1.2 ± 0.2 | 0.7 ± 0.1* |
| 16:1(n-7) | 1.3 ± 0.3c | 0.9 ± 0.4c | 4.3 ± 0.4a | 2.9 ± 0.3b | 2.3 ± 0.2b | 2.7 ± 0.3b | 3.9 ± 0.6a | 2.8 ± 0.4b |
| 18:1(n-9) | 33.0 ± 1.4a | 28.4 ± 1.6b | 37.3 ± 0.5a | 32.5 ± 1.3a | 17.6 ± 1.3 | 18.6 ± 2.2 | 18.8 ± 2.1 | 18.9 ± 1.7 |
| 20:1(n-9) | tr | tr | 0.08 ± 0.02a | 0.02 ± 0.01b | tr | tr | 0.2 ± 0.1 | tr |
| 18:2(n-6) | 25.6 ± 1.7a | 22.0 ± 1.8a | 21.4 ± 0.5a | 18.8 ± 0.7b | 26.2 ± 0.8a | 21.6 ± 1.6b | 22.8 ± 1.5ab | 21.5 ± 1.3b |
| 20:2(n-6) | tr | tr | 0.11 ± 0.02 | 0.06 ± 0.02 | 0.6 ± 0.2 | 1.0 ± 0.2 | 1.2 ± 0.2 | 0.6 ± 0.1* |
| 18:3(n-3) | 1.8 ± 0.3b | 14.4 ± 0.9a | 2.8 ± 0.1b | 15.4 ± 0.9a | 2.5 ± 0.3b | 8.8 ± 1.2a | 2.2 ± 0.4b | 11.0 ± 1.1a |
| 20:3(n-6) | tr | tr | 0.06 ± 0.04 | tr | 13.1 ± 1.1a | 7.8 ± 2.0b | 9.8 ± 2.3ab | 7.3 ± 1.2b |
| 20:4(n-6) | 1.2 ± 0.2a | 0.6 ± 0.3b | 1.3 ± 0.1a | 0.9 ± 0.1ab | 0.9 ± 0.2b | 2.0 ± 0.5a | 0.7 ± 0.3b | 1.6 ± 0.3ab |
| 20:5(n-3) | tr | tr | 0.11 ± 0.01b | 0.22 ± 0.01a | 1.7 ± 0.4 | 1.7 ± 0.4 | 2.0 ± 0.5 | 1.0 ± 0.1 |
| 22:6(n-3) | tr | tr | 0.05 ± 0.01 | tr | 8.0 ± 0.7a | 2.5 ± 0.4b | 6.9 ± 0.8a | 2.2 ± 0.2b |
| (n-6):(n-3) | 14.0 ± 2.2a | 1.6 ± 0.3c | 7.7 ± 0.3b | 1.3 ± 0.1c | 16.3 ± 1.6a | 16.3 ± 1.0b | 28.4 ± 2.9 | 47.2 ± 11.8 |

Σ Fatty acids 1.6 ± 0.2c | 1.6 ± 0.5c | 21.3 ± 1.6a | 16.3 ± 1.0b | 28.4 ± 2.9 | 47.2 ± 11.8 | 42.5 ± 9.6 | 47.8 ± 11.1

1 Values are means ± SEM. Means in a row without a common superscript differ, P < 0.05. *Interaction of CHL × FLX, P < 0.05.
2 Plasma samples obtained after 16 wk feeding (n = 7–8). Cardiac muscle samples obtained following 8 wk feeding (n = 5).
3 tr, trace.

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**FIGURE 2** Incidence of spontaneous ventricular tachycardia and fibrillation during 30 min of global ischemia and 45 min of reperfusion in hearts isolated from rabbits fed diets with or without cholesterol and flaxseed supplementation. Data are combined from 8 and 16 wk feeding periods. n = 14–16. Different from REG, P < 0.05; †different from the CHL group, P < 0.05.
DISCUSSION

Dietary flaxseed caused a significant increase in plasma ALA in the rabbits from ~2 to ~15 g/100 g of total fatty acids. The FLX and CHL/FLX groups had significantly higher plasma ALA levels than controls. Although the relative amounts of ALA were equal, the absolute amounts were markedly higher (9-fold) in the CHL/FLX group than the FLX group, despite consumption of the same amount of flaxseed. This increase in total fatty acids may be the result of a cholesterol-facilitated uptake of fatty acids by the jejunum as reported previously (26,27). The increase in fatty acids may also reflect the increased demand for fatty acids required for the formation of cholesteryl esters with consumption of the high-cholesterol diets. Because total lipids and not specific fatty acid pools were examined, we cannot determine whether specific changes occurred in the amount or relative composition of FFA, phospholipids, triglycerides, or cholesteryl esters.

Dietary flaxseed did not increase the levels of other (n-3) PUFA in the plasma. EPA and DHA were unchanged and undetectable in plasma, making up <0.05 g/100 g fatty acids. In the heart, DHA levels were unchanged and EPA levels doubled in the FLX and CHL/FLX groups, but remained one of the least abundant fatty acids detected. The ALA levels were much higher than those of any other (n-3) fatty acid; thus, any effects seen in this study were not likely to have been due to the small increases in EPA or DHA. A comparison of the levels of the 20-carbon PUFA, AA and EPA, indicated that the lower ratio of (n-6):(n-3) fatty acids favored a greater metabolic production of (n-3) PUFA via Δ-6 desaturase. This finding highlights the relative importance of a lower (n-6):(n-3) ratio to increase the endogenous production of long-chain (n-3) PUFA.

The objectives of this study were to determine whether dietary flaxseed exerts antiarrhythmic effects and whether these effects were mediated specifically through ALA. We found that dietary flaxseed exerts a potent antiarrhythmic effect in rabbits. Flaxseed supplementation completely suppressed the VF normally seen during ischemia. In addition, when added to a high-cholesterol diet, flaxseed moderately reduced VF during ischemia and significantly decreased VF during reperfusion. These effects were specific to VF because dietary flaxseed did not affect VT. In addition, no adverse effects were associated with the flaxseed-enriched diet in our study.

The antiarrhythmic action of flaxseed was clearly not due to the generation of substantial quantities of EPA or DHA. Several findings support the suggestion that dietary flaxseed exerts its antiarrhythmic action through an elevation of car-

### TABLE 3

| Dietary group | QT interval (ms) |
|---------------|------------------|
| REG           | 234 ± 2b         |
| FLX           | 226 ± 2c         |
| CHL           | 241 ± 2a         |
| CHL/FLX       | 225 ± 2c         |

1 All hearts were paced at 2 Hz.
2 Values are means ± SEM. Means in a row without a common superscript differ, \( P < 0.05 \). Data were pooled from 8 and 16 wk, \( n = 14–16 \).

### TABLE 4

| Fatty acid      | \( \text{APD}_{50} \) | \( \text{APD}_{90} \) |
|-----------------|----------------------|----------------------|
| MUFA            | 18:1(n-9)            | 100.4 ± 3.2          |
|                 | 18:3(n-3)            | 87.1 ± 3.3*          |
|                 | 22:6(n-3)            | 90.9 ± 2.4*          |
| PUFA            | 18:1(n-9)            | 92.2 ± 2.4*          |
|                 | 18:3(n-3)            | 93.3 ± 2.1*          |
|                 | 22:6(n-3)            | 92.8 ± 2.3*          |

1 Values are expressed as the mean percentage of APD before fatty acid addition normalized to 100% ± SEM, \( n = 6–10 \). * Different from before fatty acid application, \( P < 0.05 \).
cardiac [ALA]. First, (n-3) PUFA are antiarrhythmic (2–4,11) and ALA was present in much greater quantities in the heart than any other (n-3) PUFA. Second, there was a strong negative correlation ($r = -0.865$, $P < 0.05$) between cardiac levels of ALA and arrhythmia incidence. Dietary flaxseed increased cardiac ALA levels by 3-fold in the FLX and CHL/FLX groups vs. the REG and CHL groups. The CHL/FLX group had the highest cardiac ALA levels and lowest incidence of VF in our study. Conversely, the CHL and REG groups exhibited a higher incidence of VF and had lower tissue ALA levels. Third, emulsions of ALA delivered in vivo suppressed arrhythmias (11). Last, we showed that ALA can significantly alter electrical activity in the heart (Fig. 3).

To identify possible mechanisms for the antiarrhythmic effects of flaxseed, we measured QT intervals in the hearts as an index of cardiac APD. Dietary flaxseed was associated with a shortening of the QT interval. In contrast, the longest QT interval occurred in the cholesterol-fed group, which also had the highest incidence of VF. The addition of flaxseed to the high-cholesterol group resulted in a shortening of the QT interval and an antiarrhythmic effect. This suggests that dietary flaxseed may exert its protective effect through APD shortening. Indeed, pronounced QT prolongation (genetic or drug-induced) is often associated with polymorphic tachycardia. Indeed, pronounced QT prolongation (genetic or drug-induced) is often associated with polymorphic tachycardia. Importantly, arrhythmias including VF are often associated with polymorphic tachycardia.

Flaxseed is often associated with polymorphic tachycardia. Indeed, pronounced QT prolongation (genetic or drug-induced) is often associated with polymorphic tachycardia. The antiarrhythmic potential of dietary flaxseed may be more pronounced in vivo due to the availability of both circulating and endogenous tissue stores of ALA (48). Flaxseed also contains other components such as lignans (i.e., secoisolariciresinol diglucoside), which have notable free radical scavenging abilities and provide an important antiatherogenic component to dietary flaxseed (49). Thus, the possibility exists that these additional components in flaxseed may offer added protection against cardiovascular disease.

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