

## $\alpha$ -Linolenic Acid and Risk of Nonfatal Acute Myocardial Infarction

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**Background**—Intake of long-chain n-3 fatty acids found in fish is low in many countries worldwide.  $\alpha$ -Linolenic acid could be a viable cardioprotective alternative to these fatty acids in these countries.

**Methods and Results**—Cases (n=1819) with a first nonfatal acute myocardial infarction and population-based controls (n=1819) living in Costa Rica matched for age, sex, and area of residence were studied. Fatty acids were assessed by gas chromatography in adipose tissue samples and by a validated food frequency questionnaire specifically designed for this population. Odds ratios and 95% confidence intervals were calculated from multivariate conditional logistic regression models.  $\alpha$ -Linolenic acid in adipose tissue ranged from 0.36% in the lowest decile to 1.04% in the highest decile. The corresponding median levels of intake were 0.42% and 0.86% energy. Greater  $\alpha$ -linolenic acid (assessed either in adipose or by questionnaire) was associated with lower risk of myocardial infarction. The odds ratios for nonfatal myocardial infarction for the highest compared with the lowest deciles were 0.41 (95% confidence interval, 0.25 to 0.67) for  $\alpha$ -linolenic acid in adipose tissue and 0.61 (95% confidence interval, 0.42 to 0.88) for dietary  $\alpha$ -linolenic acid. The relationship between  $\alpha$ -linolenic acid and myocardial infarction was nonlinear; risk did not decrease with intakes  $>\approx 0.65\%$  energy (1.79 g/d). Fish or eicosapentaenoic acid and docosahexaenoic acid intake at the levels found in this population did not modify the observed association.

**Conclusions**—Consumption of vegetable oils rich in  $\alpha$ -linolenic acid could confer important cardiovascular protection. The apparent protective effect of  $\alpha$ -linolenic acid is most evident among subjects with low intakes. (*Circulation*. 2008; 118:339-345.)

**Key Words:**  $\alpha$ -linolenic acid ■ adipose tissue ■ diet ■ epidemiology ■ fatty acids  
■ heart diseases ■ risk factors

Numerous clinical and epidemiological studies have shown that greater intake of long-chain n-3 fatty acids from fish, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), reduces all-cause mortality, cardiac and sudden death, and stroke.<sup>1</sup> However, the availability of fish and other marine products that are the main sources of long-chain fatty acids is limited, and it has been questioned whether sufficient resources exist to provide these fatty acids in the amounts needed worldwide.<sup>2</sup>

### Editorial p 323 Clinical Perspective p 345

$\alpha$ -Linolenic acid, an essential n-3 fatty acid found in vegetable cooking oils such as soybean and canola oils and other products of plant origin, has been proposed as a viable alternative to fish oils, but data to support this initiative are scarce.<sup>3</sup> In cohort studies, low  $\alpha$ -linolenic acid intake has been associated with risk of fatal coronary heart disease<sup>4,5</sup> and sudden cardiac death.<sup>6</sup> Some studies,<sup>7,8</sup> but not all,<sup>4,6</sup> suggest

a strong inverse association between  $\alpha$ -linolenic intake and nonfatal acute myocardial infarction (MI). A clinical trial<sup>9</sup> reported a protective effect of a Mediterranean diet high in  $\alpha$ -linolenic acid against cardiovascular mortality and the risk of recurrent MI, but this trial was not designed to specifically test the effects of  $\alpha$ -linolenic acid.

Coronary heart disease is generally high in countries where the estimated intake of EPA and DHA is extremely low (long-chain n-3 fatty acid intake  $\leq 0.07\%$  energy).<sup>10</sup> Because of price, availability, or cultural preference, many of these countries have little or no possibility of increasing fish intake.<sup>11</sup> European countries with extremely low EPA and DHA intakes such as Bulgaria and Romania<sup>10</sup> also can have low intakes of  $\alpha$ -linolenic acid from vegetable oils because they almost exclusively use sunflower oil, which does not contain  $\alpha$ -linolenic acid.<sup>12</sup> Similarly, low intakes of  $\alpha$ -linolenic acid can be found in developing countries where cardiovascular disease is on the rise<sup>13</sup> because they almost exclusively consume palm oil, another vegetable oil that lacks

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$\alpha$ -linolenic acid.<sup>14</sup> Because of the large number of people living in these countries, the potentially protective effects of  $\alpha$ -linolenic acid from vegetable oils need to be studied more extensively. Since the early 1980s, Costa Rica has experienced a decrease in the intake of palm oil and an increase the intake of soybean oil.<sup>14,15</sup> This transition has led to increased intake of  $\alpha$ -linolenic acid in the population. In a previous analysis, our data suggested that  $\alpha$ -linolenic acid in adipose tissue is associated with a decreased risk of MI in the Costa Rican population.<sup>7</sup> The proposed study expands this previous observation to test the hypotheses that  $\alpha$ -linolenic acid (assessed both by questionnaire and in adipose tissue) is associated with reduced risk of MI in a larger population, that the maximum benefit of  $\alpha$ -linolenic acid is obtained within a specific range of intake, and that the association between  $\alpha$ -linolenic acid and MI is independent of fish intake.

## Methods

### Study Population

The catchment area for this study, 34 counties in the Central Valley of Costa Rica, covered a full range of socioeconomic levels, as well as urban, peri-urban, and rural lifestyles. Eligible case subjects were adult residents who were diagnosed as survivors of a first acute MI by 2 independent cardiologists at any of the 6 recruiting hospitals in the catchment area between 1994 and 2004. All cases met the World Health Organization criteria for MI, which require typical symptoms plus either elevations in cardiac enzyme levels or diagnostic changes in the ECG. One free-living control subject for each case, matched for age ( $\pm 5$  years), sex, and area of residence (county), was randomly selected using the information available at the National Census and Statistics Bureau of Costa Rica. Because of the comprehensive social services provided in Costa Rica, all persons living in the catchment area had access to medical care without regard to income. Therefore, control subjects came from the source population that gave rise to the cases and were not likely to have had cardiovascular disease that was not diagnosed because of poor access to medical care. Participation was 98% for cases and 88% for controls. The methods for this study have been previously published.<sup>14</sup> All subjects gave informed consent on documents approved by the Human Subjects Committee of the Harvard School of Public Health and the University of Costa Rica.

### Data Collection

All study participants ( $n=1819$  case-control pairs) were visited in their homes. At this visit, data on sociodemographic characteristics, smoking, socioeconomic status, medical history, anthropometric measurements, and biological samples were collected. Data and sample collection took place, on average, 3 weeks after hospital discharge. Dietary intake was assessed with a 135-item food frequency questionnaire that was developed and validated specifically to assess fatty acid intake among the Costa Rican population.<sup>16,17</sup> All foods included in the food frequency questionnaire were analyzed for their content of fatty acids, including  $\alpha$ -linolenic acid, and data were incorporated into the nutrient database for analysis. The Costa Rican staples include rice, beans, bread, and plantains, with a small side dish consisting of egg, meat, or cheese and a chopped vegetable like squash. Salads usually consist of cabbage and tomato, and meals are accompanied by sweetened beverages and coffee. Traditional cooking methods use vegetable oils or hard palm shortening for preparing rice and beans and frying the side dishes. Physical activity was determined by asking subjects the average frequency and time spent on several occupational and leisure-time activities during the last year as previously described.<sup>18</sup> These activities were grouped into 6 categories according to their intensity or metabolic equivalent task (MET). One MET is defined as the energy expenditure for sitting quietly or  $\approx 1$  kcal/kg<sup>-1</sup> body weight/h<sup>-1</sup>. A subcutaneous adipose tissue biopsy was collected from the upper buttock with a 16-gauge

needle and disposable syringe following procedures previously described.<sup>17</sup> This simple procedure was carried out by numbing the area with ice and without the use of local anesthesia. Samples were stored at  $-80^{\circ}\text{C}$ , and within 6 months, they were transported over dry ice to the Harvard School of Public Health for analysis.

### Fatty Acid Analysis

Fatty acids were quantified by gas-liquid chromatography as described previously.<sup>17</sup> Peak retention times and area percentages of total fatty acids were identified by the injection of known standards (NuCheck Prep, Elysium, Minn) and analyzed with the Agilent Technologies ChemStation A.08.03 software (Agilent Technologies, Santa Clara, Calif). The between-run coefficient of variation was 8.5% for  $\alpha$ -linolenic acid. Fatty acids in subcutaneous adipose tissue are known to reflect long-term intake<sup>19</sup> and in case-control studies are preferred over other biomarkers of fatty acid intake.<sup>20</sup> In the Costa Rican population, adipose tissue  $\alpha$ -linolenic acid was an excellent biomarker of intake.<sup>16,17</sup>

### Statistical Analysis

All data were analyzed with the Statistical Analysis Systems software (SAS Institute Inc, Cary, NC). The significance of differences in crude means and frequencies for health characteristics and potential confounders were assessed by paired *t* tests and the McNemar test. Odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of MI among deciles of adipose tissue or calorie-adjusted  $\alpha$ -linolenic acid intake were estimated from multiple conditional logistic regression models. In subgroup analyses, ORs and 95% CIs were calculated for tertiles of fish intake (g/d) or EPA+DHA (g/d). Confounders included in the final models were smoking status (never, past, or  $<10$ ,  $10$  to  $20$ , or  $>20$  cigarettes a day), physical activity (quintiles), household income (quintiles), history of diabetes mellitus (yes/no), history of hypertension (yes/no), waist-to-hip ratio (quintiles), calorie-adjusted saturated fat intake (quintiles), linoleic acid (quintiles), and *trans* fatty acids (quintiles) in adipose tissue. Other potential confounders examined but not included in the final models were body mass index, intake of vitamin E, folate, fiber, cholesterol, total energy, alcohol, and dietary or adipose  $\gamma$ -linolenic acid, arachidonic acid, EPA, and DHA.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

## Results

Table 1 shows the basic characteristics of the population. The nutrient composition (percent energy) was 32% total fat, 55% carbohydrate, and 13% protein. The saturated, monounsaturated, and polyunsaturated contents of the diet were 10%, 12%, and 6%. Rice, beans, sweet beverages, plantains, beef, eggs, milk, bananas, palm shortening, and soybean oil were the major energy contributors.  $\alpha$ -Linolenic acid intake and in adipose tissue was significantly lower in cases than controls ( $P<0.001$ ). Fish intake was similar in cases and controls, and the variation within each group was large. The most widely consumed fish by both cases and controls was marine tropical white fish ( $\approx 70\%$ ), followed by canned tuna. Other fish and seafood contributed  $<0.5\%$ . The distribution of potential confounders by each decile of adipose tissue  $\alpha$ -linolenic acid is shown in Table 2.  $\alpha$ -Linolenic acid in adipose tissue ranged from 0.36% in the lowest decile to 1.04% in the highest decile. Corresponding median levels for  $\alpha$ -linolenic acid intake were 1.11 and 2.35 g/d and 0.42% and 0.86% of calories. A positive trend was found between adipose tissue  $\alpha$ -linolenic acid and physical activity and intake of fiber, *trans* fat, and linoleic acid, as well as with *trans* fat, linoleic acid, and EPA in adipose tissue. An inverse trend was

**Table 1. General Characteristics of the Study Population**

	Controls (n=1819)	Cases (n=1819)
Matching variables		
Age, y	58±11	58±11
Women, %	27	27
Area of residence, % rural	26	26
General characteristics		
Body-mass index, kg/cm <sup>2</sup>	26.4±4.3	25.9±4.0
Waist-to-hip ratio	0.95±0.07	0.97±0.07
Physical activity, MET/d	1.55±0.65	1.50±0.67
Monthly household income, US \$	580±425	506±393
History of diabetes mellitus, %	15	24
History of hypertension, %	30	39
Current smoker $\geq$ 1 cigarette/d, %	21	40
Current drinkers, %	40	37
Total fat, % energy	31.8±5.8	32.4±5.9
Saturated fat, % energy	10.4±2.7	11.1±2.9
Monounsaturated fat, % energy	11.8±3.8	11.9±3.5
Polyunsaturated fat, % energy	6.2±2.0	6.0±2.0
Trans fat, % energy	1.42±0.7	1.45±0.7
Carbohydrates, % energy	55.4±7.3	54.3±7.6
Protein, % energy	13.0±2.1	13.2±2.2
Cholesterol, mg/1000 kcal	118±53	126±58
Fiber, g/1000 kcal	10.0±2.5	9.5±2.4
Fish, g/d*	17.3±15.5	18.3±18.3
$\alpha$ -Linolenic acid		
Intake, g/d*	1.63±0.63	1.57±0.63
Intake, % energy	0.60±0.23	0.58±0.23
Intake, % total fat	1.93±0.77	1.82±0.75
Adipose, % total fat	0.65±0.21	0.62±0.21

Values are mean±SD when appropriate. All variables except *trans* fat and fish are significantly different between cases and controls ( $P<0.05$ ).

\*Adjusted for total energy by use of the residual method.

observed between adipose tissue  $\alpha$ -linolenic acid and intake of saturated and monounsaturated fat and DHA in adipose tissue. Those with a history of hypertension or diabetes were less likely to be in the highest deciles of adipose tissue  $\alpha$ -linolenic acid compared with the lowest. Subjects in the lowest decile of  $\alpha$ -linolenic acid had the lowest intake of fish.

An inverse relationship was observed between adipose tissue  $\alpha$ -linolenic acid and the risk of nonfatal acute MI in a multivariate model that included smoking, physical activity, household income, history of diabetes, history of hypertension, waist-to-hip ratio, saturated fat intake, and linoleic and *trans* fatty acids in adipose tissue (Figure 1A). Compared with the lowest decile of adipose tissue  $\alpha$ -linolenic acid, the ORs for MI for the 2nd through 10th deciles were 0.94 (95% CI, 0.66 to 1.34), 0.85 (95% CI, 0.59 to 1.24), 0.59 (95% CI, 0.40 to 0.87), 0.52 (95% CI, 0.34 to 0.78), 0.51 (95% CI, 0.34 to 0.79), 0.43 (95% CI, 0.30 to 0.67), 0.45 (95% CI, 0.28 to 0.71), 0.37 (95% CI, 0.23 to 0.59), and 0.41 (95% CI, 0.25 to 0.67). Similar results were obtained between dietary  $\alpha$ -linolenic acid and the risk of nonfatal acute MI (Figure 1B).

Compared with the lowest decile of dietary  $\alpha$ -linolenic acid intake, the ORs for MI for the 2nd through 10th deciles were 0.74 (95% CI, 0.53 to 1.04), 0.67 (95% CI, 0.48 to 0.94), 0.69 (95% CI, 0.49 to 0.98), 0.64 (95% CI, 0.45 to 0.91), 0.73 (95% CI, 0.51 to 1.05), 0.71 (95% CI, 0.50 to 1.02), 0.74 (95% CI, 0.51 to 1.06), 0.63 (95% CI, 0.44 to 0.91), and 0.61 (95% CI, 0.42 to 0.88). These associations were very similar to those observed in models that included just the matching variables age, gender, and area of residence. The inverse association between  $\alpha$ -linolenic acid and the risk of nonfatal acute MI was nonlinear regardless of whether the analyses were conducted with  $\alpha$ -linolenic acid assessed in adipose tissue or by food frequency questionnaire. For adipose tissue  $\alpha$ -linolenic acid, no further reductions in risk were obtained beyond the seventh decile. Larger CIs were obtained with dietary questionnaires compared with adipose tissue, indicating higher measurement error when diet is assessed by questionnaire.

Figure 2 shows the correlation between adipose tissue and dietary  $\alpha$ -linolenic acid. The median intake of  $\alpha$ -linolenic acid in the seventh decile of  $\alpha$ -linolenic acid in adipose tissue was 1.79 g/d or 0.65% energy. The correlations between dietary  $\alpha$ -linolenic acid and adipose EPA, docosapentaenoic acid, and DHA were  $r=0.05$ ,  $r=-0.06$  and  $r=-0.11$ , respectively. We also examined the correlation between dietary  $\alpha$ -linolenic acid and EPA, docosapentaenoic acid, and DHA in plasma or erythrocytes in a subset of 200 study participants. The correlations were  $r=0.16$ ,  $r=0.20$ , and  $r=-0.03$ , respectively, for plasma and  $r=-0.08$ ,  $r=0.03$ , and  $r=-0.09$ , respectively, for erythrocytes.

It has been shown that the association between  $\alpha$ -linolenic acid and risk of MI is most evident among those consuming low levels of fish (EPA+DHA <100 mg/d).<sup>21</sup> We examined this hypothesis by evaluating the association between adipose tissue  $\alpha$ -linolenic acid by tertiles of fish intake or dietary EPA+DHA. Table 3 (top) shows the association between  $\alpha$ -linolenic acid in adipose tissue and MI by tertiles of fish intake. The highest tertile of  $\alpha$ -linolenic acid in adipose tissue was associated with a 50% lower risk among those with the lowest fish intake (median, 3.3 g/d), a 52% lower risk among those in the middle tertile of fish intake (median, 13.4 g/d), and a 55% lower risk among those with the highest fish intake (32.4 g/d). The association between  $\alpha$ -linolenic acid in adipose tissue and risk of MI by tertiles of dietary EPA+DHA is shown in the bottom of Table 3. The highest tertile of  $\alpha$ -linolenic acid in adipose tissue was associated with a 49% lower risk among those with the lowest intake of EPA+DHA (0.13 g/d), a 44% lower risk among those in the middle tertile of EPA+DHA intake (0.24 g/d), and a 58% lower risk among those with the highest intake of EPA+DHA (0.52 g/d).

## Discussion

We conducted a case-control study to assess the relationship between adipose tissue  $\alpha$ -linolenic and the risk of nonfatal acute MI in Costa Rica. We found that both dietary and adipose levels of  $\alpha$ -linolenic acid were associated with a large and significant reduction in the risk of nonfatal acute MI. The relationship between  $\alpha$ -linolenic acid and MI was nonlinear;

**Table 2. Population Characteristics by Deciles of Adipose Tissue  $\alpha$ -Linolenic Acid Among Controls**

	Deciles of Adipose Tissue $\alpha$ -Linolenic Acid										<i>P</i>
	1	2	3	4	5	6	7	8	9	10	
Median, % total fatty acids	0.36	0.45	0.5	0.55	0.6	0.64	0.7	0.77	0.88	1.04	for trend
$\alpha$ -Linolenic acid intake, g/d*	1.11	1.25	1.38	1.38	1.52	1.62	1.79	1.83	2.08	2.35	<0.0001
$\alpha$ -Linolenic acid intake, % energy	0.42	0.46	0.51	0.51	0.57	0.6	0.65	0.67	0.76	0.86	<0.0001
Waist-to-hip ratio	0.95	0.94	0.96	0.96	0.95	0.94	0.95	0.95	0.95	0.95	0.36
Physical activity, MET/d	1.49	1.54	1.52	1.49	1.46	1.45	1.58	1.65	1.61	1.71	<0.0001
Monthly household income, US \$	428	510	581	619	676	640	625	599	526	517	0.26
Current smokers $\geq$ 1 cigarettes/d, %	29	25	17	18	23	16	20	21	25	15	0.21
Current drinkers, %	39	42	43	40	45	41	46	41	38	32	0.56
History of diabetes mellitus, %	22	13	17	12	17	14	15	12	12	14	0.003
History of hypertension, %	38	32	31	28	31	27	27	30	33	25	0.01
Daily dietary intake											
Energy, kcal/d	2430	2425	2428	2431	2490	2450	2497	2437	2438	2492	0.62
Saturated fat, % energy	12	11	11	11	11	10	10	9	10	9	<0.0001
Monounsaturated fat, % energy	12	12	12	12	12	12	12	11	11	11	0.002
Linoleic acid, % energy	5	5	6	6	7	7	7	7	7	7	<0.0001
<i>Trans</i> fat, % energy	1.20	1.39	1.44	1.34	1.41	1.46	1.49	1.45	1.55	1.49	0.0003
EPA, % energy	0.04	0.04	0.04	0.05	0.04	0.05	0.05	0.04	0.04	0.05	0.11
DHA, % energy	0.06	0.07	0.07	0.08	0.07	0.08	0.08	0.07	0.08	0.07	0.16
Cholesterol, mg/1000 kcal	127	124	122	120	111	116	116	109	123	118	0.04
Fiber, g/1000 kcal	9	10	10	10	10	10	10	10	10	11	<0.0001
Fish, g*	14	16	16	19	17	18	19	17	18	18	0.03
Adipose tissue, % total fatty acids											
EPA	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	<0.0001
DHA	0.15	0.15	0.14	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.005
Linoleic	10	12	14	14	15	16	17	18	18	21	<0.0001
<i>Trans</i>	2.15	2.42	2.6	2.55	2.67	2.69	2.7	2.72	2.72	2.75	<0.0001

Values are means when appropriate.

\*Adjusted for total energy by use of the residual method. n=1819.

ie, risk did not decrease with intakes  $\geq$ 0.65% energy (1.79 g/d). These results suggest that increasing intake of vegetable oils rich in  $\alpha$ -linolenic acid could reduce the risk of MI.

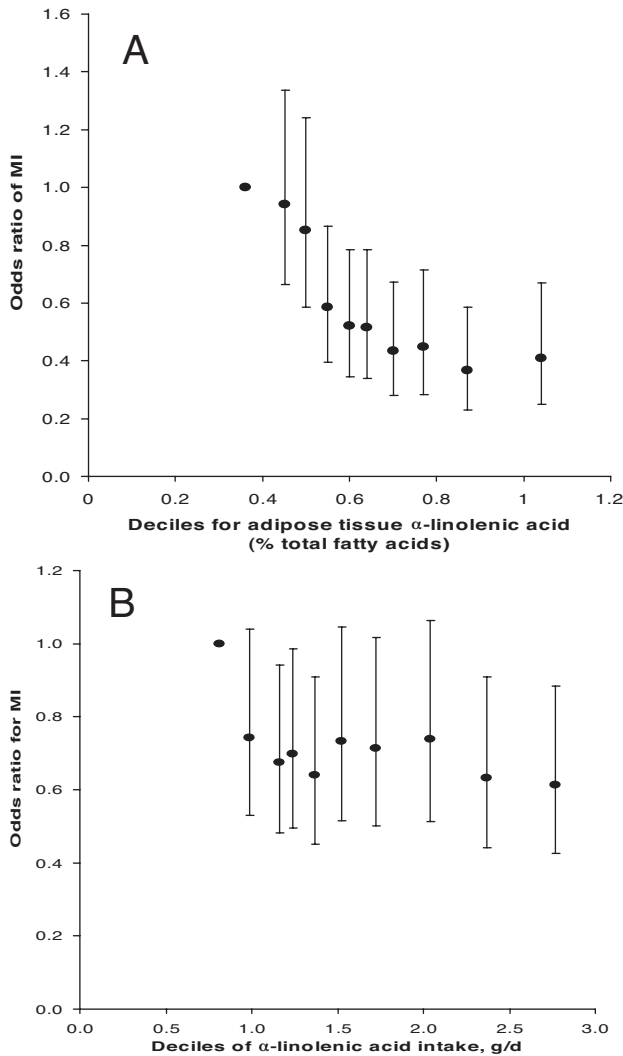
Our data are consistent with a clinical trial<sup>9</sup> reporting a protective effect of a Mediterranean diet rich in  $\alpha$ -linolenic acid against the risk of MI. Although this trial was not specifically designed to test the effects of  $\alpha$ -linolenic acid, investigators found that when measuring plasma fatty acids 2 months after randomization, plasma  $\alpha$ -linolenic acid was significantly and independently associated with improved prognosis.<sup>9</sup> A study in India found that the use of mustard oil, which is rich in  $\alpha$ -linolenic acid, was associated with lower MI risk compared with sunflower oil.<sup>8</sup> The ORs for MI were 0.49 when mustard oil was used for cooking and 0.29 when it was used for frying. This apparent protective effect is consistent with the present study. The ORs for MI comparing the seventh decile to the first decile were 0.43 for adipose tissue and 0.71 for dietary intake. However, other studies have found that although increased  $\alpha$ -linolenic acid intake was associated with lower risk of fatal ischemic heart disease and sudden cardiac death, it was not associated with MI.<sup>4,6</sup>

Our data indicate that  $\alpha$ -linolenic acid is associated with decreased MI risk at low intake levels and that the amount of

$\alpha$ -linolenic acid associated with maximum benefit is small. The difference in intake of  $\alpha$ -linolenic acid between the lowest and seventh quintiles was 0.68 g/d. Soybean oil and canola oil, which are good plant sources of  $\alpha$ -linolenic acid, contain  $\approx$ 6% to 8%  $\alpha$ -linolenic acid. This suggests that the amount of  $\alpha$ -linolenic acid contained in  $\approx$ 2 teaspoons of these oils, taken daily, could be enough to reduce the risk of nonfatal acute MI when the baseline level of intake is low. This level of intake also can be easily achieved with intake of flaxseed oil ( $\approx$ 1 to 2 mL) or walnuts ( $\approx$ 6 to 10 halves).

The association between  $\alpha$ -linolenic acid and MI observed in this study could be due to the potential antiinflammatory properties of  $\alpha$ -linolenic acid found in some<sup>22,23</sup> but not all studies.<sup>24,25</sup> In epidemiological studies,<sup>22,23</sup> increased  $\alpha$ -linolenic acid intake in the range observed in this study ( $\approx$ 0.6 g/d) was associated with decreased plasma concentration of markers of inflammation (C-reactive protein,<sup>22,23</sup> vascular cell adhesion molecule-1,<sup>23</sup> and E-selectin).<sup>23</sup> Consistent with these findings, intervention studies found that  $\alpha$ -linolenic acid lowers C-reactive protein plasma concentration,<sup>26–28</sup> vascular cell adhesion molecule-1,<sup>26,29,30</sup> and E-selectin.<sup>26,30</sup> However, results from intervention studies were obtained

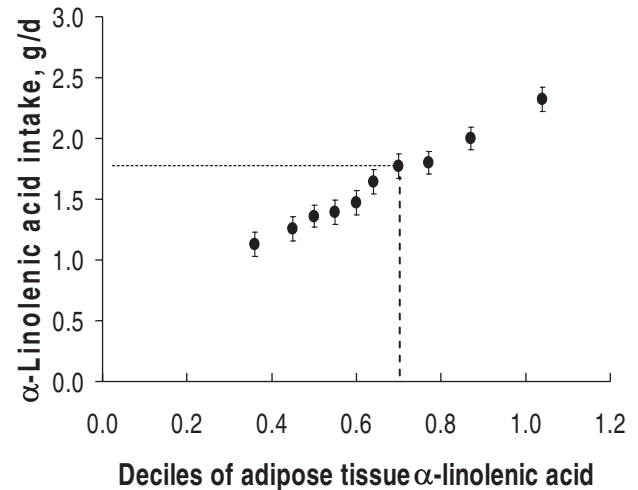




**Figure 1.** ORs and 95% CIs for MI by deciles of  $\alpha$ -linolenic acid in adipose tissue (A) or intake (B) adjusted for smoking status, physical activity, household income, history of diabetes mellitus, history of hypertension, waist-to-hip ratio, saturated fat intake, and linoleic and *trans* fatty acids in adipose tissue.

with very high intakes of  $\alpha$ -linolenic acid (range, 5 to 15 g/d), and their relevance to the present study is uncertain. The potential antiinflammatory effects of  $\alpha$ -linolenic acid could be mediated in part through its conversion to EPA and DHA by the action of desaturase and elongase enzymes.<sup>31</sup> However, the finding that this conversion is generally low (<8%)<sup>25,32</sup> and the observation of poor correlations between dietary  $\alpha$ -linolenic acid and long-chain fatty acids in adipose tissue, plasma, or erythrocytes in this study suggest that  $\alpha$ -linolenic acid could exert direct protective antiinflammatory effects. It has been shown that  $\alpha$ -linolenic acid decreases the nuclear transcription factor  $\kappa$ B, a major transcription factor involved in the regulation of inflammatory genes.<sup>33,34</sup> Furthermore,  $\alpha$ -linolenic acid inhibits the production of nitric oxide and downregulates inducible nitric oxide synthase, cyclooxygenase-2, and tumor necrosis factor-R gene expression in murine macrophages.<sup>34</sup>

Other metabolic effects of  $\alpha$ -linolenic acid could potentially explain our findings. In the National Heart, Lung, and



**Figure 2.** Median levels and 95% CIs for dietary  $\alpha$ -linolenic acid by deciles of  $\alpha$ -linolenic acid in adipose tissue. Line denotes the level at which no further association between  $\alpha$ -linolenic and risk of MI was observed in Figure 1.

Blood Institute Family Heart Study, individuals who reported  $\approx 0.6$ -g/d-higher linolenic acid intake had lower blood pressure,<sup>35</sup> lower plasma triglycerides concentration,<sup>36</sup> fewer calcified atherosclerosis plaques,<sup>37</sup> and less carotid atherosclerosis.<sup>38</sup> In one intervention study, increasing  $\alpha$ -linolenic acid by 3 g/d resulted in lower plasma low-density lipoprotein cholesterol and apolipoprotein B concentrations.<sup>39</sup> Other studies that used higher doses (7 to 15 g/d) found similar results.<sup>25,26</sup> These findings, however, have not been consistent.<sup>40</sup>

It is possible that  $\alpha$ -linolenic acid would have a more prominent role among populations with low fish intake because EPA can inhibit the action of delta-5 and delta-6 desaturase activity, which is required for  $\alpha$ -linolenic acid conversion.<sup>41</sup> Consistent with this hypothesis, 1 study showed that 1-g/d intake of  $\alpha$ -linolenic acid was associated with a 50% lower risk of nonfatal MI among men consuming very

**Table 3. Multivariate ORs and 95% CIs for Risk of MI by Tertiles of Adipose Tissue  $\alpha$ -Linolenic Acid, Fish Intake, and Dietary EPA+DHA**

	Tertiles of Adipose Tissue $\alpha$ -Linolenic Acid, median (% total fatty acids)		
	1 (0.44)	2 (0.61)	3 (0.83)
Fish intake median, g/d			
3.3	1.00 Ref	0.68 (0.48–0.97)	0.50 (0.34–0.74)
13.4	1.00 Ref	0.62 (0.44–0.88)	0.52 (0.35–0.77)
32.4	1.00 Ref	0.65 (0.46–0.93)	0.55 (0.37–0.80)
EPA+DHA intake median, g/d			
0.13	1.00 Ref	0.66 (0.46–0.94)	0.49 (0.34–0.73)
0.24	1.00 Ref	0.51 (0.36–0.72)	0.44 (0.29–0.65)
0.52	1.00 Ref	0.73 (0.51–1.03)	0.58 (0.39–0.85)

Model included smoking, waist-to-hip ratio, income, history of diabetes, history of hypertension, intake of saturated fat, physical activity, and adipose tissue *trans* and linoleic acid. n=3668.

low (<100 mg/d) long-chain n-3 fatty acids from fish, but no association was found among those with higher intake.<sup>21</sup> However, consistent with results from the Family Heart Study,<sup>42</sup> we found that an association with a reduced risk of nonfatal MI was evident regardless of fish intake or long-chain fatty acids EPA+DHA, which were on average >100 mg/d even in the lowest tertile (median, 130 mg/d). Nevertheless, fish intake overall is low in the studied population, and it is still possible that no association would be found in populations with very high intakes.

The data presented here suggest that despite the increase in soybean oil consumption in the last 20 years, a large portion of the Costa Rican population does not meet the recommended  $\alpha$ -linolenic acid intake. In the United States, an adequate intake for  $\alpha$ -linolenic acid has been set at 1.6 g/d for men and 1.1 g/d for women.<sup>43</sup> Furthermore, a range of  $\alpha$ -linolenic acid intake between 0.6% and 1.2% of energy (1.3 to 2.6 g/d for a 2000-kcal diet) has been proposed in the United States as an acceptable macronutrient distribution range. The acceptable macronutrient distribution range represents the range of an energy source that is associated with reduced risk of chronic disease while providing adequate intake of essential nutrients. Our data indicate that 50% of the Costa Rican population was below the minimum acceptable macronutrient distribution range. It is possible that this group, in which the median  $\alpha$ -linolenic acid intake ranged between 0.42% and 0.57% energy, may benefit the most from increased intake of  $\alpha$ -linolenic acid. Further increases to intakes as high as 0.86% energy were not associated with extra protection.

The main limitation of this study is the use of a retrospective case-control design in which causal effects cannot be studied. Case-control studies that use dietary questionnaires also are more prone to reporting bias than prospective studies because responses from the cases could be modified by the MI. This study used both dietary questionnaires and biomarkers as an indicator of dietary information. The most reliable conclusions may be drawn when both methods yield similar results, as in the present study. Given the potential benefits of  $\alpha$ -linolenic acid, large clinical trials in populations with low  $\alpha$ -linolenic acid intakes are warranted.

### Conclusions

Increased  $\alpha$ -linolenic acid intake is associated with reduced risk of nonfatal acute MI. This relationship is nonlinear; the risk did not decrease with intakes between 1.79 g/d (0.65% energy) and 2.35 g/d (0.86% energy). Furthermore, fish or EPA+DHA intake at the levels found in this population did not modify the observed association. Thus, it is possible that consumption of vegetable oils rich in  $\alpha$ -linolenic acid could confer important cardiovascular protection in many countries where intake is low.

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### Disclosures

None.

### References

1. Wang C, Harris WS, Chung M, Lichtenstein AH, Balk EM, Kupelnick B, Jordan HS, Lau J. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr*. 2006;84:5-17.
2. Lang T. Food control or food democracy? Re-engaging nutrition with society and the environment. *Public Health Nutr*. 2005;8:730-737.
3. Harris WS. Alpha-linolenic acid: a gift from the land? *Circulation*. 2005;111:2872-2874.
4. Hu FB, Stampfer MJ, Manson JE, Rimm EB, Wolk A, Colditz GA, Hennekens CH, Willett WC. Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. *Am J Clin Nutr*. 1999;69:890-897.
5. Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men: the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Epidemiol*. 1997;145:876-887.
6. Albert CM, Oh K, Whang W, Manson JE, Chae CU, Stampfer MJ, Willett WC, Hu FB. Dietary alpha-linolenic acid intake and risk of sudden cardiac death and coronary heart disease. *Circulation*. 2005;112:3232-3238.
7. Baylin A, Kabagambe EK, Ascherio A, Spiegelman D, Campos H. Adipose tissue alpha-linolenic acid and nonfatal acute myocardial infarction in Costa Rica. *Circulation*. 2003;107:1586-1591.
8. Rastogi T, Reddy KS, Vaz M, Spiegelman D, Prabhakaran D, Willett WC, Stampfer MJ, Ascherio A. Diet and risk of ischemic heart disease in India. *Am J Clin Nutr*. 2004;79:582-592.
9. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation*. 1999;99:779-785.
10. Hibbeln JR, Nieminen LR, Blasbalg TL, Riggs JA, Lands WE. Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. *Am J Clin Nutr*. 2006;83:1483S-1493S.
11. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases, part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. *Circulation*. 2001;104:2746-2753.
12. Zatonski W, Campos H, Willett W. Rapid declines in coronary heart disease mortality in Eastern Europe are associated with increased consumption of oils rich in alpha-linolenic acid. *Eur J Epidemiol*. 2008;23:3-10.
13. Reddy KS. Cardiovascular disease in non-Western countries. *N Engl J Med*. 2004;350:2438-2440.
14. Kabagambe EK, Baylin A, Ascherio A, Campos H. The type of oil used for cooking is associated with the risk of nonfatal acute myocardial infarction in Costa Rica. *J Nutr*. 2005;135:2674-2679.
15. Colon-Ramos U, Kabagambe EK, Baylin A, Ascherio A, Campos H, Peterson KE. Socio-economic status and health awareness are associated with choice of cooking oil in Costa Rica. *Public Health Nutr*. 2007;10:1214-1222.
16. Kabagambe EK, Baylin A, Allan DA, Siles X, Spiegelman D, Campos H. Application of the method of triads to evaluate the performance of food frequency questionnaires and biomarkers as indicators of long-term dietary intake. *Am J Epidemiol*. 2001;154:1126-1135.
17. Baylin A, Kabagambe EK, Siles X, Campos H. Adipose tissue biomarkers of fatty acid intake. *Am J Clin Nutr*. 2002;76:750-757.
18. Campos H, Siles X. Siesta and the risk of coronary heart disease: results from a population-based, case-control study in Costa Rica. *Int J Epidemiol*. 2000;29:429-437.
19. Dayton S, Hashimoto S, Dixon W, Pearce ML. Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. *J Lipid Res*. 1966;7:103-111.
20. Kark JD, Manor O, Goldman S, Berry EM. Stability of red blood cell membrane fatty acid composition after acute myocardial infarction. *J Clin Epidemiol*. 1995;48:889-895.
21. Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS, Rimm EB. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation*. 2005;111:157-164.
22. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, Martin A, Andres-Lacueva C, Senin U, Guralnik JM. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab*. 2006;91:439-446.

23. Lopez-Garcia E, Schulze MB, Manson JE, Meigs JB, Albert CM, Rifai N, Willett WC, Hu FB. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. *J Nutr.* 2004;134:1806–1811.
24. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation.* 2003;108:155–160.
25. Goyens PL, Spilker ME, Zock PL, Katan MB, Mensink RP. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. *Am J Clin Nutr.* 2006;84:44–53.
26. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *J Nutr.* 2004;134:2991–2997.
27. Bemelmans WJ, Lefrandt JD, Feskens EJ, van Haelst PL, Broer J, Meyboom-de Jong B, May JF, Tervaert JW, Smit AJ. Increased alpha-linolenic acid intake lowers C-reactive protein, but has no effect on markers of atherosclerosis. *Eur J Clin Nutr.* 2004;58:1083–1089.
28. Rallidis LS, Paschos G, Liakos GK, Velissaridou AH, Anastasiadis G, Zampelas A. Dietary alpha-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. *Atherosclerosis.* 2003;167:237–242.
29. Rallidis LS, Paschos G, Papaioannou ML, Liakos GK, Panagiotakos DB, Anastasiadis G, Zampelas A. The effect of diet enriched with alpha-linolenic acid on soluble cellular adhesion molecules in dyslipidaemic patients. *Atherosclerosis.* 2004;174:127–132.
30. Thies F, Miles EA, Nebe-von-Caron G, Powell JR, Hurst TL, Newsholme EA, Calder PC. Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. *Lipids.* 2001;36:1183–1193.
31. Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu Rev Nutr.* 2004;24:345–376.
32. Burdge G. Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. *Curr Opin Clin Nutr Metab Care.* 2004;7:137–144.
33. Perez-Martinez P, Lopez-Miranda J, Blanco-Colio L, Bellido C, Jimenez Y, Moreno JA, Delgado-Lista J, Egido J, Perez-Jimenez F. The chronic intake of a Mediterranean diet enriched in virgin olive oil, decreases nuclear transcription factor kappaB activation in peripheral blood mononuclear cells from healthy men. *Atherosclerosis.* 2007;194:e141–e146.
34. Ren J, Chung SH. Anti-inflammatory effect of alpha-linolenic acid and its mode of action through the inhibition of nitric oxide production and inducible nitric oxide synthase gene expression via NF-kappaB and nitrogen-activated protein kinase pathways. *J Agric Food Chem.* 2007;55:5073–5080.
35. Djousse L, Arnett DK, Pankow JS, Hopkins PN, Province MA, Ellison RC. Dietary linolenic acid is associated with a lower prevalence of hypertension in the NHLBI Family Heart Study. *Hypertension.* 2005;45:368–373.
36. Djousse L, Hunt SC, Arnett DK, Province MA, Eckfeldt JH, Ellison RC. Dietary linolenic acid is inversely associated with plasma triacylglycerol: the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Clin Nutr.* 2003;78:1098–1102.
37. Djousse L, Arnett DK, Carr JJ, Eckfeldt JH, Hopkins PN, Province MA, Ellison RC. Dietary linolenic acid is inversely associated with calcified atherosclerotic plaque in the coronary arteries: the National Heart, Lung, and Blood Institute Family Heart Study. *Circulation.* 2005;111:2921–2926.
38. Djousse L, Folsom AR, Province MA, Hunt SC, Ellison RC. Dietary linolenic acid and carotid atherosclerosis: the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Clin Nutr.* 2003;77:819–825.
39. Goyens PL, Mensink RP. The dietary alpha-linolenic acid to linoleic acid ratio does not affect the serum lipoprotein profile in humans. *J Nutr.* 2005;135:2799–2804.
40. Wendland E, Farmer A, Glasziou P, Neil A. Effect of alpha linolenic acid on cardiovascular risk markers: a systematic review. *Heart.* 2006;92:166–169.
41. Chen Q, Nilsson A. Desaturation and chain elongation of n-3 and n-6 polyunsaturated fatty acids in the human CaCo-2 cell line. *Biochim Biophys Acta.* 1993;1166:193–201.
42. Djousse L, Pankow JS, Eckfeldt JH, Folsom AR, Hopkins PN, Province MA, Hong Y, Ellison RC. Relation between dietary linolenic acid and coronary artery disease in the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Clin Nutr.* 2001;74:612–619.
43. Institute of Medicine. *Dietary Reference Intakes: Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.* Washington, DC: National Academies Press; 2002.

### CLINICAL PERSPECTIVE

Long-chain n-3 fatty acids from fish, eicosapentaenoic acid and docosahexaenoic acid, reduce cardiovascular mortality, but availability of fish is limited and probably insufficient to meet worldwide needs.  $\alpha$ -Linolenic acid, an essential n-3 fatty acid found in vegetable cooking oils such as soybean and canola oils and other products of plant origin could be a viable alternative to fish oils. We determined whether  $\alpha$ -linolenic acid was associated with risk of nonfatal acute myocardial infarction in 1819 case-control pairs from a population-based study in Costa Rica. Increased dietary  $\alpha$ -linolenic acid assessed by questionnaire and in adipose tissue was associated with 39% and 59% lower risk of myocardial infarction, respectively. The relationship between  $\alpha$ -linolenic acid and myocardial infarction was nonlinear, and it was evident only at low intake levels. Risk of myocardial infarction decreased by 57% when median intakes of 1.79 g/d (0.65% energy) were compared with 1.11 g/d (0.42% energy), but it did not decrease further with intakes >1.79 g/d. The amount of  $\alpha$ -linolenic acid associated with risk reduction was small, and it could be obtained with 2 teaspoons of soybean or canola oils, which are common plant sources of  $\alpha$ -linolenic acid. This level of intake also could be easily achieved with intake of flaxseed oil ( $\approx$  1 to 2 mL) or walnuts ( $\approx$  6 to 10 halves). Fish or eicosapentaenoic acid and docosahexaenoic acid intake at the levels found in this population did not modify the observed association. In summary, consumption of vegetable oils rich in  $\alpha$ -linolenic acid could confer important cardiovascular protection.