Adipose tissue n−6 fatty acids and acute myocardial infarction in a population consuming a diet high in polyunsaturated fatty acids[1–3]

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ABSTRACT

Background: The Jewish population of Israel consumes a diet rich in n−6 polyunsaturated fatty acids (PUFAs), principally linoleic acid. The consequences of this diet for ischemic heart disease (IHD) remain unclear.

Objective: We assessed the association of adipose tissue n−6 fatty acids, which are derived entirely from the diet, with acute myocardial infarction (AMI).

Design: A total of 180 cases and 492 IHD-free controls aged 25–64 were included in a population-based case-control study of Jerusalem residents hospitalized with a first AMI. Diet was assessed by the use of a food-frequency questionnaire and adipose tissue fatty acids by gas chromatography of biopsy samples taken from subcutaneous gluteal tissue. The data were analyzed by multivariate logistic regression.

Results: Dietary PUFAs ($\bar{x}$: 10.1% of energy) correlated ($r = 0.43$, $P < 0.001$) with adipose tissue linoleic acid, which constituted 25.6% of storage fatty acids. High intakes of linoleic acid were not associated with excess risk of AMI (age- and sex-adjusted odds ratio for the third versus the first tertile: 0.96; 95% CI: 0.62, 1.48; NS). In contrast, arachidonic acid, the long chain n−6 derivative of linoleic acid, was positively associated with AMI (age- and sex-adjusted odds ratio: 2.12; 95% CI: 1.33, 3.36; $P = 0.004$). With multivariate adjustment, there was no evidence for an adverse association of linoleic acid with AMI, whereas the risk associated with arachidonic acid persisted, albeit attenuated.

Conclusions: A very high linoleic acid intake does not appear to confer increased risk of nonfatal AMI. Nonetheless, the increased risk associated with arachidonic acid, a finding that requires confirmation, tempers an inference that diets rich in n−6 fatty acids are safe vis-à-vis coronary health. *Am J Clin Nutr* 2003;77:796–802.

KEY WORDS Polyunsaturated fatty acids, adipose tissue fatty acids, linoleic acid, arachidonic acid, myocardial infarction, ischemic heart disease, Jewish population, epidemiology

INTRODUCTION

Dietary recommendations to reduce the risk of ischemic heart disease (IHD) traditionally include limiting total fat consumption to < 30% of total energy and saturated fatty acids (SFAs) to < 10% of energy, replaced in part by monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) (1–4). However, because of uncertainty as to the health effects of a high-PUFA diet, the World Health Organization recommends total PUFA intakes of 3–7% of energy (1) and the British Nutrition Foundation Task Force recommends an average PUFA intake of 7.5%, with linoleic acid (18:2n−6) contributing 6% of energy (safe range: 3–10%) (2). In 1996 the American Heart Association recommended an n−6 fatty acid ceiling of 10% of energy with a preference for MUFA intake up to 15% of energy (3). In 2000 the American Heart Association no longer placed limits on PUFAs, emphasizing the protective effects of unsaturated fatty acids (5), and in 2001 it promoted n−6 and n−3 PUFAs but had become concerned with the potentially adverse effects of MUFAs (6). Such recommendations have been questioned (7), as has a beneficial role of dietary n−6 PUFAs (8). An upper limit for linoleic acid intake as low as 3% of energy has been proposed to avoid suspected "adverse effects of excesses of arachidonic acid [20:4n−6] and its eicosanoid products" (9). An indicator for caution is that high dietary linoleic acid intake may enhance atherogenesis through oxidative modification of LDL (3, 10, 11).

Subcutaneous adipose tissue linoleic acid is derived entirely from dietary sources and, because of its long half-life of ≈680 d (12), reflects long-term intake (13). The Jewish population of Israel has an unusually high intake of PUFAs (14, 15), making up on average 8–10% of energy, confirmed by an extraordinary subcutaneous adipose tissue PUFA content of 25% (16, 17) and a commensurately lower proportion of MUFAs (17). A major determinant of this phenomenon is the use of vegetable oils and margarine in cooking, spreads, and condiments rather than butter and lard, due in part to ritual dietary restrictions. The same holds true for fats used by the food industry. National Food Balance Sheets indicate that, in 1990, vegetable oils made up 71.4% of the fats; margarine, 26.2%; and butter, only 2.4%; by 1999, these figures were 75.8%, 21.4%, and 2.7%, respectively (18, 19). The main

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source of oil is soybeans [82% in 1994 (20)], with a predominant PUFA content.

The Jerusalem acute myocardial infarction (AMI) registry showed a higher incidence of AMI (JD Kark, S Goldman, N Goldberger, R Fink, and B Adler, unpublished observations, 2002) than in most World Health Organization MONICA program centers (21). The question arises whether the frequency of AMI relates to dietary practices, in particular the unusual fatty acid intake of the Israeli population. In the present study, we assessed the association of subcutaneous adipose tissue fatty acid composition with AMI in a population-based case-control study in Jerusalem.

SUBJECTS AND METHODS

Subjects

The methods of recruitment of the study sample for this population-based case-control study have been described (22). We reviewed consecutive patients from the Jewish population of Jerusalem aged 25 to 64 y with a first AMI who were admitted to the 4 city hospitals, all of which are university affiliated. Patients were excluded if they were institutionalized or within 6 mo of termination of pregnancy. Participants were interviewed, had blood drawn, and underwent a subcutaneous adipose tissue needle biopsy during their hospitalization. Patients were included in the analysis if they met the criteria of the World Health Organization MONICA program for definite AMI (23) (87% of the cases) or if an explicit clinical diagnosis of AMI appeared in the discharge summary. In all, 326 patients (271 men and 55 women) participated. A review of all discharge diagnoses that recorded acute MI (in 3 of the 4 hospitals) indicated that 85% of all patients were identified by the study.

The control group constituted Jerusalem residents who were sampled from the National Population Registry after stratification by age and sex. For each 10-y age and sex group (between 25 and 64 y), persons were randomly sampled with a predetermined weighted sampling ratio that took into account the expected age distribution of the cases to ensure adequate representation, with a male-to-female ratio of 2:1 among the controls. Potential participants were contacted by mail and subsequently by telephone. After verification of eligibility criteria, identical to those of the cases, controls were invited to the study center. A total of 617 persons (410 men, 207 women) attended (83% response rate). Nine women aged 25 to 34 y were excluded from the analysis because no cases occurred in this category.

Preexisting IHD may lead to alterations in the diet and, consequently, in the adipose tissue fatty acid composition, which could bias the comparisons. Therefore, we excluded both cases and controls with a prior diagnosis of IHD, defined as any of the following: a previous reported AMI, self-report of physician-diagnosed IHD, self-report of chest pain (a positive result on the Rose questionnaire) confirmed by a physician, or use of medication for chest pain. As a result, 26.4% of cases and 10.9% of controls were excluded from the analysis, leaving 240 eligible cases and 542 controls. Of these, 12.9% of cases and 2.8% of controls refused the biopsy and 12.1% of cases and 6.5% of controls did not have an adipose tissue determination for logistic or technical reasons. Therefore, 180 cases (149 men, 31 women) without previously diagnosed IHD and 492 IHD-free controls (334 men, 158 women) remained for analysis.

The study was authorized by the Hadassah University Hospital ethics review board. All subjects gave signed informed consent.

Variable definition and data collection

Data collected by trained interviewers included sociodemographic variables, self-reported medical history, family history of IHD, medication use, weight and height, smoking status, alcohol and exercise behaviors, and a detailed food-frequency dietary assessment. For the purpose of the case-control comparison, hypercholesterolemia, hypertension, and diabetes were defined as a positive response to the question "Has a doctor ever told you that you have…?" Weight and height used to compute body mass index were self-reported. Alcohol intake in volume of ethanol per week was estimated from the reported usual weekly intake of wine, beer, spirits, mixed drinks, and liqueur. The weekly energy expenditure attributable to exercise was derived from a detailed questionnaire that assessed the frequency, intensity, and duration of the 3 main exercise activities of each individual in addition to walking, stair climbing, gardening, housework, and home maintenance, and was calculated according to Taylor et al (24).

The dietary food-frequency instrument, a 145-item questionnaire tailored to Israeli food preferences that used photographed food models based on measured portion sizes, enabled us to assess energy intake, total fat intake, and type of fat. The nutrient content of these items was derived from Israeli and US food tables, supplemented by special laboratory analysis of many of the items. The validity of the tables was assessed by laboratory analyses of composite daily food intakes, which showed good agreement between calculated and measured results. The validity of the questionnaire in terms of dietary fat intake, as assessed with biomarkers in the full control group without exclusion of those subjects with preexisting IHD ($n = 542$), appeared to be satisfactory. The Spearman correlation of dietary PUFAs (expressed as a percentage of total fat intake) with subcutaneous adipose tissue linoleic acid was 0.42 ($P < 0.001$); that of dietary SFAs with adipose tissue myristic acid (14:0) was 0.36 ($P < 0.001$) and that with adipose tissue palmitic acid (16:0) was 0.27 ($P < 0.001$). The Spearman correlation of dietary MUFAs with adipose tissue oleic acid was weak, 0.13 ($P = 0.01$). The Spearman correlations of the frequency of fish intake with erythrocyte membrane eicosapentaenoic acid (20:5n−3) and docosahexaenoic acid (22:6n−3) in the full control group ($n = 562$) were 0.39 and 0.38, respectively.

Adipose tissue

Subcutaneous adipose tissue was aspirated from the upper outer quadrant of the buttocks as previously described (25). In the cases, 47% of fat aspirations were performed within 3 d of admission, 79% within 6 d, and 94% within 10 d. After extraction and methylation of the samples, fatty acid percentage composition was determined in coded samples by gas-liquid chromatography (25). Peaks from 14:0 to 20:4, including labeled unidentified peaks, were taken as 100%. The 18:3 peaks [α-linolenic (18:3n−3) and γ-linolenic (18:3n−6) acids] were not always separable. *trans* Fatty acid peaks were not determined. CVs calculated from 35 blindly labeled split samples of adipose tissue were as follows: 14:0, 18.2%; 16:0, 4.2%; 16:1, 12.6%; 18:0, 12.5%; 18:1, 2.2%; 18:2, 3.0%; and 20:4, 34.0%. We subsequently use the term *oleic acid*, the predominant 18:1 fatty acid, to represent the 18:1 MUFAs.

Statistical methods

Associations between nutrients and storage fatty acids were assessed by partial correlation, adjusted for age and sex. Tests for a sex interaction in the association of the fatty acids and risk factors with AMI were not statistically significant, except for palmitic acid in the analysis of variance but not in the logistic models.

TABLE 1
Characteristics of the cases (patients with a first acute myocardial infarction) and of a population-based control group in Jerusalem

	Cases[1] (n = 180)	Controls[1] (n = 492)	Odds ratio[2] (95% CI)	P value[2]
Age (y)	54.1 ± 8.2[3]	52.1 ± 9.4	—	0.003
Sex (% men)	82.8	67.9	—	0.0001
Hypercholesterolemia (%)	27.8	17.7	1.82 (1.21, 2.75)	0.004
Hypertension (%)	37.2	29.7	1.34 (0.92, 1.95)	0.12
Diabetes (%)	17.2	10.0	1.70 (1.03, 2.82)	0.039
BMI (kg/m^2)	26.1 ± 4.0	25.5 ± 4.0	1.04 (0.99, 1.09)	0.10
Current smoking (%)	47.8	31.0	2.00 (1.39, 2.89)	0.0002
Alcohol intake (mL/wk)	22 ± 45	29 ± 60	0.9946 (0.9905, 0.9986)	0.009
No alcohol intake (%)	37.2	28.3	2.03 (1.38, 3.00)	0.0003
Exercise (kcal/wk)	1277 ± 1341	1708 ± 1748	0.9998 (0.9997, 1.000)	0.024
Family history of CHD (%)	45.6	35.8	1.66 (1.16, 2.37)	0.0057

[1] Unadjusted data.
[2] Adjusted for age and sex by logistic regression; odds ratios were calculated per unit increase in BMI, alcohol intake, and energy expenditure and for the presence or absence of the categorical trait.
[3] $\bar{x}$ ± SD.

Therefore, we grouped the sexes in the analysis and included an interaction term for palmitic acid in the relevant multivariate analysis. Analysis of variance applying the general linear models procedure was used to compare mean adipose tissue fatty acid composition in cases and controls. Covariate-adjusted odds ratio estimates of the relative risk of AMI for fatty acids categorized in sex-specific tertiles (determined in the control group) were obtained by unconditional logistic regression. The Hosmer and Lemeshow chi-square statistic for goodness-of-fit was not statistically significant in the logistic models. We tested for differences between tertiles of adipose tissue fatty acids with 2 degrees of freedom, and tested for trend with the fatty acid defined as a continuous variable. Two-tailed nominal P values are presented. SPSS version 9 for PC (SPSS, Chicago) was used for the analyses.

RESULTS

Characteristics of the study sample

The cases were on average 2–3 y older than the controls [men ($\bar{x}$ ± SD): 53.5 ± 8.5 and 51.2 ± 9.9 y for the cases and controls, respectively; women: 56.9 ± 5.7 and 54.0 ± 8.0 y for the cases and controls, respectively]. The ethnic distribution according to country of origin (Israel or in Europe, Asia, or North Africa) did not differ significantly between cases and controls. The cases had a higher prevalence of hypercholesterolemia, diabetes, smoking, and family history of IHD; had a lower alcohol intake; and exercised less than did the controls. Trends for hypertension and overweight were in the expected direction (**Table 1**). Men were ≈5 times as likely as women to be hospitalized with a first AMI between the ages of 25 and 64 y.

Dietary intake in the control group

The salient features in this population were an unusually high intake of PUFAs ($\bar{x}$ ± SD: 10.1 ± 3.2% of energy) and an extraordinary ratio of PUFAs to SFAs (P:S; median: 0.91). More than 90% of the study population reported PUFA consumption in excess of 6% of energy and almost 25% in excess of 12% of energy. Less than 10% of the population had a P:S < 0.5, whereas in almost 25% the ratio was > 1.2. Mean SFA intake represented 11.1%; MFAs, 12.9%; total fat, 36.6%; carbohydrate, 47.1%; and protein, 16.2% of energy. The PUFA intake was derived largely from vegetable oils, margarine, and seeds (55%) and bread and baked products (17%). Use of butter was modest, and lard was not used in this population. Intake of fresh fruit, fresh and cooked vegetables, and natural fruit juice was relatively high, as in Mediterranean countries, with a weekly median of 27 servings.

Subcutaneous adipose tissue in the control group

The PUFA-rich diet was reflected in the unadjusted adipose tissue composition, with a high linoleic acid content ($\bar{x}$ ± SD: 25.6 ± 3.7%) and a commensurately low proportion of 18:1 MUFAs (41.1 ± 2.5%), predominantly oleic acid (18:1n−9). The 10th to 90th percentile range for linoleic acid was 21–31% and that for oleic acid was 38–44%. The mean proportion of arachidonic acid was 0.57 ± 0.26%. The substantial correlation (r = 0.56) between linoleic and α-linolenic acids suggests a common dietary source, probably soybean oil products.

Association of dietary macronutrients with adipose tissue fatty acids in the control group

Age- and sex-adjusted findings included a reasonably strong correlation of dietary PUFAs expressed as a percentage of total fat with adipose tissue linoleic acid (r = 0.43, P < 0.001); a weaker association with α-linolenic acid (r = 0.16, P = 0.001); no relation with arachidonic acid, the n−6 desaturation and elongation product; and inverse associations with adipose tissue oleic acid (r = –0.18, P < 0.001) and palmitoleic acid (16:1; r = –0.16, P < 0.001).

Dietary SFAs were inversely associated with adipose tissue linoleic acid (r = –0.38, P < 0.001) and α-linoleic acid (r = –0.14, P = 0.005) but not with arachidonic acid (r = 0.05). Dietary MUFAs were not associated with adipose tissue n−6 fatty acids.

Adipose tissue fatty acid composition and acute myocardial infarction

In age- and sex-adjusted ANOVA (**Table 2**) and logistic (**Table 3**) models, there was no significant association of linoleic acid, γ-linolenic acid, or α-linolenic acid with AMI. Arachidonic acid had a significant positive relation with AMI (Table 2) that was nonlinear and similarly elevated in the upper 2 tertiles (Table 3). Associations were positive with palmitoleic acid (P = 0.002) and unidentified chromatographic peaks (P = 0.011) and tended to be inverse with stearic acid (18:0; P = 0.054) and oleic acid (P = 0.097) (Table 2).

TABLE 2
Subcutaneous adipose tissue fatty acid composition in cases (patients with acute myocardial infarction) and controls, adjusted for age and sex[1]

Fatty acid	Cases (n = 180)	Controls (n = 492)	Difference	P value (ANOVA)
14:0	1.57 ± 0.05	1.65 ± 0.03	−0.08 ± 0.05	0.12
16:0	19.51 ± 0.18	19.26 ± 0.11	0.25 ± 0.20	0.20
16:1	5.89 ± 0.12	5.48 ± 0.07	0.41 ± 0.13	0.002
18:0	2.99 ± 0.08	3.16 ± 0.05	−0.17 ± 0.09	0.054
18:1	40.94 ± 0.32	41.30 ± 0.12	−0.36 ± 0.22	0.097
18:2n−6	25.26 ± 0.30	25.49 ± 0.18	−0.24 ± 0.33	0.47
18:3n−3[2]	1.28 ± 0.03	1.23 ± 0.02	0.05 ± 0.04	0.17
18:3n−6[2]	0.69 ± 0.04	0.74 ± 0.02	−0.05 ± 0.04	0.27
20:4n−6	0.62 ± 0.02	0.57 ± 0.01	0.05 ± 0.02	0.025
n−3:n−6[3]	0.048 ± 0.001	0.046 ± 0.001	0.002 ± 0.001	0.074
Unidentified peaks	1.24 ± 0.05	1.10 ± 0.03	0.14 ± 0.05	0.011

[1] $\bar{x}$ ± SE.
[2] n = 149 cases, 432 controls.
[3] (18:3n−3)/(18:3n−6 + 18:2n−6 + 20:4n−6).

We examined the ratio of n−3 to n−6 fatty acids because of the recognized substrate competition between the 2 pathways for desaturation and elongation enzymes, n−3 fatty acids being represented by α-linolenic acid and n−6 fatty acids by the combination of linoleic, γ-linolenic, and arachidonic acids. Associations with AMI were not significant (Tables 2 and 3).

With multivariable adjustment using logistic models that included a single fatty acid (Table 3), the association with arachidonic acid and the absence of an association with linoleic acid persisted. Further adjustment for body mass index, ethnic origin, and degree of religious observance had no measurable effect (data not shown). In a sex-specific analysis for linoleic acid, there was no association in men and a nonsignificant inverse association in women (data not shown).

Subsequent logistic models simultaneously incorporated several fatty acids (**Table 4**). Findings were similar in age- and sex-adjusted and in multivariate-adjusted analyses. There was an inverse association of adipose tissue linoleic acid with AMI when adjusted for myristic, palmitoleic, oleic, and arachidonic acids (all independent predictors of AMI; Table 4, model a) that was significant only when linoleic acid was introduced as a continuous variable (age- and sex-adjusted P = 0.033 and multivariate-adjusted P = 0.026) but not when tertiles were compared. In sex-specific analyses, the association was inverse in both sexes, more so in women (multivariate-adjusted P = 0.13 in men and P = 0.07 in women; data not shown). There was no association in models that adjusted for MUFAs alone (palmitoleic and oleic acids; Table 4, model b), for SFAs alone (myristic, palmitic, and stearic acids; Table 4, model c), or for various other combinations of fatty acids (data not shown). The association of arachidonic acid with AMI was moderately attenuated with fatty acid adjustment, but remained statistically significant (Table 4, models a and d). Neither of the 18:3 variables (Table 4, model e) was significantly associated with AMI.

The analysis was repeated including only patients for whom biopsies were performed within 3 d of admission. The absence of a positive relation of linoleic acid with AMI persisted. Although the association with arachidonic acid diminished, a comparison of the strength of the association for samples drawn within 3 d compared with >3 d after admission did not show significant differences.

DISCUSSION

Foremost among the strengths of our study are the use of a validated biomarker of long-term dietary intake of essential fatty acids instead of reliance solely on a questionnaire-based instrument (13, 26) and the application of a population-based design with a good response rate. The inclusion of first AMI events only and the exclusion of both cases and controls with previous clinically manifest IHD enhanced the validity of our comparisons. A potential limitation of a hospital-based study, however, is that IHD

TABLE 3
Association of n−3 and n−6 subcutaneous adipose tissue fatty acids, introduced separately into logistic models, with acute myocardial infarction[1]

	Age and sex adjustment				Multivariate adjustment[2]			
Fatty acid	Tertile 1,[3] OR	Tertile 2,[3] OR (95% CI)	Tertile 3,[3] OR (95% CI)	P[4]	Tertile 1, OR	Tertile 2, OR (95% CI)	Tertile 3, OR (95% CI)	P[4]
18:2n−6	1.00	1.04 (0.68, 1.59)	0.96 (0.62, 1.48)	NS	1.00	1.00 (0.65, 1.57)	0.92 (0.59, 1.46)	NS
18:3n−3	1.00	0.82 (0.51, 1.33)	1.20 (0.76, 1.88)	NS	1.00	0.79 (0.48, 1.31)	1.30 (0.81, 2.08)	NS
18:3n−6	1.00	0.68 (0.43, 1.09)	0.81 (0.51, 1.27)	NS	1.00	0.64 (0.39, 1.05)	0.86 (0.53, 1.38)	NS
20:4n−6	1.00	1.90 (1.19, 3.03)	2.12 (1.33, 3.36)	0.004	1.00	2.04 (1.24, 3.34)	1.97 (1.20, 3.21)	0.01
n−3:n−6[5]	1.00	0.68 (0.42, 1.09)	0.96 (0.62, 1.51)	NS	1.00	0.75 (0.45, 1.23)	1.05 (0.66, 1.68)	NS

[1] n = 180 cases, 491 controls (data were missing for 1 participant). OR, odds ratio.
[2] Adjusted for age (y); sex; self-reported hypertension, hypercholesterolemia, and diabetes [indicator (yes or no) variables]; alcohol intake (mL ethanol/wk); and cigarette smoking (nonsmoker or 1–10, 11–20, or ≥21 cigarettes/d as dummy variables).
[3] Tertile medians: 18:2n−6, 21.8%, 25.7%, 29.1%; 18:3n−6, 0.34%, 0.79%, 1.10%; 18:3n−3, 0.93%, 1.19%, 1.57%; 20:4n−6, 0.38%, 0.53%, 0.75%; n−3:n−6, 0.037, 0.046, 0.056.
[4] Tested with 2 df.
[5] 18:3n−3/(18:2n−6 + 18:3n−6 + 20:4n−6).

TABLE 4
Association of n−3 and n−6 subcutaneous adipose tissue fatty acids, introduced simultaneously into logistic models, with acute myocardial infarction[1]

	Age and sex adjustment[2]				Multivariate adjustment[3]			
Fatty acid	Tertile 1, OR	Tertile 2, OR (95% CI)	Tertile 3, OR (95% CI)	P^4	Tertile 1, OR	Tertile 2, OR (95% CI)	Tertile 3, OR (95% CI)	P^4
18:2n−6 models[5]								
Model a	1.00	0.92 (0.57, 1.49)	0.70 (0.37, 1.30)	NS[6]	1.00	0.86 (0.52, 1.44)	0.64 (0.33, 1.23)	NS[7]
Model b	1.00	1.08 (0.69, 1.69)	1.00 (0.59, 1.69)	NS	1.00	1.08 (0.67, 1.74)	1.01 (0.58, 1.76)	NS
Model c	1.00	1.05 (0.65, 1.67)	0.89 (0.51, 1.53)	NS	1.00	0.98 (0.59, 1.61)	0.81 (0.45, 1.44)	NS
20:4n−6 models[5]								
Model a	1.00	1.89 (1.14, 3.14)	1.70 (1.04, 2.79)	0.030	1.00	2.02 (1.21, 3.37)	1.47 (0.87, 2.50)	0.026
Model d	1.00	1.87 (1.15, 3.03)	1.74 (1.06, 2.84)	0.030	1.00	1.99 (1.19, 3.32)	1.52 (0.90, 2.56)	0.031
18:3, model e[5]								
18:3n−3	1.00	0.71 (0.43, 1.19)	0.87 (0.51, 1.48)	NS	1.00	0.69 (0.40, 1.19)	0.93 (0.53, 1.63)	NS
18:3n−6	1.00	0.80 (0.48, 1.32)	1.02 (0.61, 1.71)	NS	1.00	0.78 (0.46, 1.32)	1.10 (0.64, 1.90)	NS

[1] n = 180 cases, 491 control (data were missing for 1 participant). OR, odds ratio.
[2] Also adjusted for unidentified peaks.
[3] Also adjusted for self-reported hypertension, hypercholesterolemia, and diabetes [indicator (yes or no) variables]; alcohol intake (mL ethanol/wk); and cigarette smoking (nonsmoker or 1–10, 11–20, or ≥21 cigarettes/d as dummy variables).
[4] Tested with 2 df.
[5] Models a to e included the following fatty acids; model a, 18:2, 14:0, 16:1, 18:1, and 20:4; model b, 18:2, 16:1, and 18:1; model c, 18:2, 14:0, 16:0, and 18:0; model d, 20:4, 14:0, 16:1, and 18:1; and model e, 18:3n−3, 18:3n−6, 14:0, 16:1, 18:1, and 20:4.
[6,7] Test for trend with linoleic acid introduced as a continuous variable: [6] P = 0.033, [7] P = 0.026.

deaths occurring in the community cannot be incorporated in a design in which biopsy samples are taken from live consenting patients.

The high PUFA intake in our study population was reflected in the extraordinary linoleic acid adipose tissue content and is in line with reports in Israel over the past 50 y (14–17), including a recent national nutrition survey (D Nitzan-Kalusky, unpublished observations, 2002). The principal finding of our study was the absence of a risk association of AMI with linoleic acid in this context. There was an unexpected positive association of AMI with arachidonic acid, which is in the n−6 fatty acid pathway.

Is dietary linoleic acid safe in regard to IHD?

The absence of increased risk associated with stored linoleic acid is not conclusive evidence for its safety. First, the upper confidence bounds for the odds ratio in several models did not exclude a modest harmful effect.

Second, it may be that both too little and too much linoleic acid are harmful. In populations with a low intake, linoleic acid may be protective [as in earlier adipose tissue studies in Scotland (27) and Norway (28)], whereas in Israel, linoleic acid or its metabolites may be neutral or deleterious (29). In the United States, where linoleic acid intake is intermediate, an inverse association of dietary linoleic acid with IHD death but not with nonfatal AMI was reported in men (30), whereas in women dietary PUFAs were protective overall (31). These findings are not inconsistent with our study of hospital-admitted AMI patients, heavily weighted to men. In the EURAMIC study, in which the mean adipose tissue concentration of 8 European countries was one-half that of Israel (12.9% compared with 25.6%), there was no overall relation of adipose tissue linoleic acid with AMI (17). Could the high incidence of IHD in the Jerusalem population relate to this unusual dietary feature [in addition to other reasonable explanations such as the extremely low alcohol consumption (Table 1), low HDL-cholesterol concentrations (32), and possibly factors in the psychosocial domain relating to the stressors and tensions prevailing in the region]? Our findings do not preclude a second PUFA threshold above which risk may be uniformly increased. A few reports are compatible with this hypothesis of a deleterious effect of high intake of linoleic acid (33–35).

Third, attenuation of the relation between dietary and adipose tissue PUFAs at high intakes could reduce the usefulness of adipose tissue as a dietary marker and obscure an association with AMI. However, regression analysis did not show evidence for a meaningful curvilinear relation (data not shown). Furthermore, the association of dietary PUFAs with adipose tissue linoleic acid (r = 0.43) was consistent, with correlations of 0.34–0.58 reported elsewhere for differing PUFA intakes (27, 36, 37). These are probably underestimates of the true association. A correlation of 0.77, derived from multiple 24-h dietary recalls and corrected for measurement error (13), is likely to be more realistic.

Fourth, the PUFA content may need to be considered in relation to antioxidant protection. The EURAMIC study (17) suggested that a high linoleic acid intake in the presence of a low antioxidant intake is associated with an increased risk of AMI, consistent with the LDL oxidative modification hypothesis. Although the Israeli population appears to have a relatively high average intake of fruit and vegetables (38), which was confirmed in our study, a low intake of vegetables was an independent predictor of all-cause mortality in a Jerusalem cohort (39). Fifth, the association of adipose tissue arachidonic acid with AMI suggests the need for some caution in inferring that high linoleic acid intakes as in Israel are safe.

Arachidonic acid

This association, which requires confirmation, may have been underestimated as a result of the large laboratory error for small chromatographic peaks (the CV for arachidonic acid was 34%), and is consistent with a hypothesis that high n−6 fatty acid intake may be deleterious (7, 9). In studies conducted elsewhere, in which the populations were characterized by much lower linoleic acid intakes, there was no association of adipose tissue arachidonic acid with AMI (17, 27), although erythrocyte membrane

arachidonic acid was higher in sudden cardiac death cases (40). Eicosanoids produced from arachidonic acid regulate physiologic functions that may increase coronary risk (2). However, arachidonic acid is present only in small amounts in storage triacylglycerol (≈0.5%); its main biological role is in membrane phospholipids, where it is a major component (≈14%). Adipose tissue and erythrocyte membrane arachidonic acid were weakly associated in our study ($r = 0.11$, $P = 0.014$). Therefore, it is unclear whether adipose tissue arachidonic acid reflects physiologically relevant levels.

Severe limitation of linoleic acid intake to 3% of energy, about one-third the mean intake in our population, has been recommended to reduce substrate competition and untoward interactions between the n−3 and n−6 fatty acid pathways (7, 9). Arachidonic acid can be derived directly from the diet, where it is found mainly in meat, poultry, eggs, and fish. Dietary enrichment with arachidonic acid in the short term did not affect its concentration in adipose tissue, but reduced its endogenous accumulation from linoleic acid and increased its incorporation into different tissues and lipid classes (41, 42). We found little association between adipose tissue linoleic acid and arachidonic acid, or between dietary PUFA intake and adipose tissue arachidonic acid.

The relation between dietary intake and tissue concentrations is complex and may be affected by interactions with the n−3 fatty acid pathway (43, 44). Might an apparent protective effect associated with low adipose tissue arachidonic acid as evident in our study reflect these metabolic pathways? Analyses of the ratio of n−3 to n−6 fatty acids and of α- and γ-linolenic acids separately did not show statistically significant associations with AMI. The EURAMIC study (45) reported no significant association of adipose tissue α-linolenic acid (consistent with our findings) or docosahexaenoic acid with AMI. Further adjustment for toenail mercury concentration showed an inverse association of docosahexaenoic acid with AMI (46). A modestly sized case-control study in Norway (47), however, showed an unusually strong protective association of the marine adipose tissue n−3 fatty acids with AMI but an increased risk associated with α-linolenic acid that was attributed to confounding by other fatty acids in a common food source. The weight of the current evidence points to a protective association of n−3 fatty acids with IHD mortality, in particular, with reduced susceptibility to dysrhythmia and sudden cardiac death, but an inconsistent relation with nonfatal AMI (48, 49).

Linoleic acid or oleic acid?

The intake of MUFAs, ≈13% of energy, which was lower than in other Mediterranean countries (16–29% of energy) (50), was mirrored by the relatively low adipose tissue oleic acid content [41% compared with 46% in the EURAMIC study (17)]. Recent American Heart Association Nutrition Committee dietary guidelines recommend replacement of SFAs by unsaturated fat, whether PUFAs or MUFAs (5). Some observers view the overall evidence for a protective effect of dietary PUFAs, and its principal n−6 component linoleic acid, as unconvincing (8, 30, 51); at the same time, there is iconoclastic evidence for a possible atherogenic role of MUFAs (6, 52). Inconsistencies also exist at the mechanistic level. Whereas a dietary increase in the content of oleic acid in LDL at the expense of linoleic acid protected against oxidative modification (10, 11) and reduced the uptake of LDL by macrophages (50, 53), a high linoleic acid content of phospholipids in HDL diminished the concentration of adhesion molecules when compared with oleic and palmitic acids (54), consistent with a protective effect.

In view of the high incidence of AMI in Israel, we cannot dismiss the possibility that very high intakes of n−6 fatty acids may not be innocuous. However, the absence of excess risk associated with linoleic acid, at the extreme of its international distribution, is reassuring.

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JDK designed the study, supervised the data collection and analysis, and drafted the manuscript. NAK contributed to the study design, developed the dietary instrument, and contributed to the manuscript. NG constructed the data files and undertook the data analysis. FB took part in quality-control aspects of the study. EMB was responsible for the adipose tissue fatty acid determinations and contributed to the manuscript. All authors were free of any conflicts of interest.

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