Can linoleic acid contribute to coronary artery disease?[1-3]

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ABSTRACT The adipose tissue concentration of linoleic acid was positively associated with the degree of coronary artery disease (CAD) in a cross-sectional study of 226 patients undergoing coronary angiography. Linoleic acid concentration in adipose tissue is known to reflect the intake of this fatty acid. These results are therefore indicative of a positive relationship between linoleic acid intake and CAD. The platelet linoleic acid concentration was also positively associated with CAD. After confounding factors were allowed for, the eicosapentaenoic acid concentration in platelets was inversely associated with CAD for men, and the docosapentaenoic acid concentration in platelets was inversely associated with CAD for women; results consistent with several other studies that suggest that fish, and ω-3 fatty acids derived from fish and fish oils, can beneficially influence macrovascular disease. *Am J Clin Nutr* 1993;58:228–34.

KEY WORDS Linoleic acid, ω-3 fatty acids, polyunsaturated fatty acids, coronary artery disease, atherosclerosis

Introduction

Many studies have examined the relationships between diet and end points of coronary heart disease (CHD) such as angina, myocardial infarction, sudden death, angiographically assessed coronary artery disease (CAD), and coronary mortality. The majority have focused on the lipid components of the diet. A high intake of saturated fatty acids is now considered to be a positive risk factor for CHD and an adequate intake of ω-3 fatty acids is believed to be influential in preventing CHD. The role of linoleic acid (18:2n−6), an ω-6 essential fatty acid, however, is less clear.

The measurement of linoleic acid in adipose tissue provides a good estimate of long-term intake of linoleic acid (1, 2). Several studies have demonstrated an inverse relationship between adipose tissue linoleic acid content and CHD (3–6). Population studies have shown that low concentrations of adipose tissue linoleic acid are associated with increased rates of CHD (3, 4), and that this inverse relationship exists both between and within populations (4). A cross-sectional survey of Scottish men demonstrated that those with previously unidentified CHD, as defined by angina pectoris or myocardial infarction, had a significantly lower concentration of adipose tissue linoleic acid than did men without CHD (5). The inverse relationship between adipose tissue linoleic acid and angina pectoris or myocardial infarction has also been demonstrated in a case-control study (6). These results are supported by the observation that as the vegetable-animal fat ratio increased in Australia and North America, there has been an associated reduction in total mortality. In England and Wales where minimal changes in this ratio occurred, there were also minimal changes in coronary mortality (7). However, not all studies have produced results that would indicate a protective role for linoleic acid. In a study by Blankenhorn et al (8) it was found that increased intake of linoleic acid significantly increased the risk of new atherosclerotic lesions in human coronary arteries.

Evidence for an inverse association between the long-chain ω-3 fatty acids or fish intake and CHD has been accumulating. A reduction in total mortality was demonstrated in a secondary prevention intervention study in which the intervention was fatty fish (9). Prospective studies have found an inverse association between fish intake and CHD incidence (10, 11), although an inverse association has not been demonstrated in all prospective studies (12, 13). Fish intake has also been associated with improved arterial wall characteristics (14). In a study in which platelet fatty acids were measured, eicosapentaenoic acid (20:5n−3) was inversely associated with angina pectoris and docosapentaenoic acid (22:5n−3) was inversely associated with risk of acute myocardial infarction. Also in this study, adipose tissue docosahexaenoic acid (22:6n−3) was inversely associated with acute myocardial infarction (6).

In our study the fatty acids in adipose tissue and platelets were measured. The relationships between each of the fatty acids and the degree of angiographically assessed CAD were examined.

Subjects and methods

Population sample

Some of the subject characteristics are given in **Table 1.** All patients underwent coronary angiography and were on the routine cardiac catheterization list for investigation of chest pain thought to be due to either CAD (97%) or valvular heart disease (3%). Consecutive patients (160 males and 66 females aged 16–80 y) were enrolled over 10 mo. All patients were included. The

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Received June 28, 1992.

Accepted for publication February 26, 1993.

Am J Clin Nutr 1993;58:228–34. Printed in USA.

TABLE 1
Patient characteristics

Characteristic	Men (n = 160)	Women (n = 66)
	%	
Obtained from medical records		
Angina	98	95
Previous myocardial infarction	39	27
Valvular heart disease	2.0	8.2
Hypertension	41	63
Taking medication		
Aspirin	67	49
Nitrates	58	44
Calcium antagonists	48	42
Beta-blockers	41	39
Angiotensin-converting-enzyme inhibitors	16	29
Obtained from questionnaire		
Smokers	14	11
Exsmokers	58	58
Never smoked	28	31
Diabetes mellitus	8.7	9.1
No dietary change	49	44
Dietary change	51	56

project was presented to and approved by the Monash Medical Centre Ethics and Research Committee.

Clinical details such as age, diabetic status, smoking history, height, weight, and past dietary change were gathered by using a questionnaire administered at the time of angiography. Self-reported height and weight were used to calculate body mass index (BMI), which was calculated by dividing weight (kg) by the square of height (m). Information about dietary change was obtained with the questionnaire. Each patient was asked whether they had made a conscious decision to make dietary changes at any time in the previous 10 y. For analysis the patients were divided into two groups: no dietary change and dietary change. To estimate the total number of cigarettes smoked, the number of cigarettes smoked per day was multiplied by the duration of time that the patient smoked. The measurement of blood pressure was not included in this study for several reasons. Almost all hypertensive patients were being treated for hypertension. However, the anti-hypertensive drug treatment differed and so too did the duration of treatment and the degree of blood pressure lowering. This would make the interpretation of results from a single blood pressure measurement difficult. Information about history of hypertension and use of particular medication was collected from the medical records. The presence or absence of hypertension and use of particular medications were included in the analysis. Coronary angiography was performed according to the Judkins technique (15) and recorded on 35-mm movie film. Two different scoring systems were used to quantify the degree of CAD: a myocardial score and an extent score.

Angiographic scores

Myocardial score. A myocardial scoring system that takes into account the degree of stenosis of any number of arterial branches and their relative importance in terms of the amount of myocardium supplied, has been developed (16). This scoring system takes into account the severity as well as the location of the coronary lesions. A score from 0 to 15 (best to worst condition) can be given.

Extent score. An angiographic scoring system that has been designed to reflect the proportion of the coronary endothelial surface area affected by atheroma has been developed as an estimate of the extent of coronary atherosclerosis (17). The proportion of the coronary arterial tree with detectable atheroma, identified as luminal irregularity, was scored with a maximum of 10; a score of 0 indicates that no coronary atheroma was detected, and a score of 10 means that 100% of the coronary arteries visualized showed detectable atheroma.

Serum lipid measurements

Fasting blood was drawn from the femoral artery immediately before cardiac catheterization and placed into evacuated glass tubes. The untreated blood was allowed to clot and the serum was separated by using standard procedures. Total cholesterol, triglycerides, and high-density-lipoprotein (HDL) cholesterol were measured in fresh serum.

Total cholesterol and triglycerides were measured enzymatically with commercial kits (13225 and 22203, respectively; Trace Scientific Pty Ltd, Clayton, Victoria, Australia). HDL cholesterol was measured enzymatically as for total cholesterol, after the precipitation of apolipoprotein B–containing lipoproteins by using equal volumes of 20% polyethylene glycol 6000 (Merck-Schuchardt, Munich, Germany) and serum. Low-density-lipoprotein (LDL) cholesterol was derived by using the Friedewald formula adapted to Système International (SI) units (18). Cholesterol and triglyceride measurements were performed on a KONE Progress selective chemistry analyzer (KONE Instruments Corporation, Espoo, Finland).

Fatty acid analysis

The fatty acid compositions of subcutaneous adipose tissue and platelets were measured by using gas chromatography. The methods relating to these measurements are presented below.

For collection and preparation of adipose tissue, $\approx$1–2 mg adipose tissue was taken from the site of catheter insertion immediately after coronary angiography. The sample was frozen at −70 °C for up to 12 mo, until all samples had been collected.

For collection and preparation of platelets, 20 mL EDTA-anticoagulated blood was drawn from the femoral artery immediately before cardiac catheterization and was used for platelet harvesting. The tubes were mixed immediately after blood collection and again before platelet harvesting. Tubes of blood were centrifuged at 110 × g for 15 min and the platelet-rich plasma was removed then recentrifuged at 2000 × g for 10 min at room temperature. The plasma was removed and the platelets were washed twice with 0.9% NaCl containing 1 g EDTA/L. The platelet pellet was then frozen at −70 °C until extraction and methylation.

Extraction and methylation of the adipose tissue and platelet fatty acids were performed by using a modification of the one-step method described by Lepage and Roy (19). Samples were placed into glass tubes with 2 mL of 4:1 methanol:toluene and 200 μL acetyl chloride was added slowly to each tube with continuous mixing, then samples were placed into an oven at 100 °C for 1 h. The tubes were cooled under running water, 2.5 mL potassium carbonate was added, and the tubes were mixed and then centrifuged at 2000 × g for 10 min at room temperature.

TABLE 2
Descriptive statistics for the study population*

Characteristic	Men	Women
Extent score (10)†	4.3 ± 1.9 [159]‡	3.4 ± 2.3 [66]
Myocardial score (15)§	7.7 ± 3.6 [159]‡	6.4 ± 4.25 [66]
Age (y)	59 ± 10.7 [159]	61 ± 10.3 [66]
Body mass index‖	25.7 ± 2.59 [132]	25.2 ± 4.30 [57]
Total cholesterol (mmol/L)	5.8 ± 0.93 [159]	6.4 ± 1.1 [66]
LDL cholesterol (mmol/L)	4.0 ± 0.86 [155]	4.4 ± 1.0 [66]
HDL cholesterol (mmol/L)	0.98 ± 0.28 [155]	1.25 ± 0.42 [66]
Triglycerides (mmol/L)	1.8 ± 0.96 [159]	1.7 ± 0.77 [66]
Cigarettes smoked ¶	741 ± 760 [129]	344 ± 606 [57]

* $\bar{x}$ ± SD; *n* in brackets.
† Scoring system for extent of coronary atherosclerosis, from 0 to 10.
‡ Significantly greater than women $P < 0.0001$,
§ Scoring system for degree of arterial stenosis, from 0 to 15.
‖ In kg/m^2.
¶ Estimate of the total number of cigarettes smoked over a lifetime.

The upper toluene phase was removed and placed into small glass tubes, then dried under nitrogen.

Immediately after extraction and methylation of the fatty acids the methylated fatty acids were dissolved in 50 μL chloroform for injection. A Shimadzu GC-9A gas chromatograph was used with a flame ionization detector and a Shimadzu Chromatopac C-R3A integrator (Shimadzu Corporation, Kyoto, Japan). A 50-m glass capillary column with an 80% cyanopropyl silicone polar phase was used. The methyl ester peaks were identified with standards obtained from Nu Chek Prep, Inc (Elysian, MN). Temperature programming was used for determining fatty acids. The starting temperature of 130 °C was raised at 5 °C/min until 190 °C was reached, then increased more slowly at 1.2 °C/min until 200 °C was reached. The temperature was then held constant at 200 °C for 15 min. The CV in determining the percentages of the individual fatty acids varied depending on the concentration of the fatty acid in the sample. For major components (> 5%) the CV ranged from 1.2% to 4.7%. For trace fatty acids (< 1%) the CV ranged from 7.4% to 14%.

Statistics

The data-analysis package used for all the statistical analyses performed was *SAS* (20,21). At a univariate level, Spearman's rank correlation coefficient (*r*) was used to determine the degree and direction of association between two variables. To control for covariates, the *PARTIAL* option was used. The Wilcoxon rank-sum test was performed to test whether there were differences between two population means.

Results

Descriptive statistics

Some descriptive statistics for the study population are presented in **Table 2.** The extent score ranged from 0 to 8.5 and was significantly higher for men than for women. The myocardial score ranged from 0 to 15 and was also significantly higher for men than for women. Nineteen patients (8.4%) had no detectable CAD, with both extent and myocardial scores of 0. The degree of correlation between the two scores was high ($r = 0.72$, $P < 0.0001$). The mean concentration, as a percentage of the total fatty acids in adipose tissue and platelets are presented in **Table 3.**

TABLE 3
Fatty acid composition of adipose tissue and platelets*

Fatty acid	Men	Women
	% of total fatty acids	
Adipose tissue fatty acids		
Lauric (12:0)	0.43 ± 0.28 [106]	0.38 ± 0.17 [39]
Myristic (14:0)	2.95 ± 0.80 [106]	2.67 ± 0.67 [39]
Palmitic (16:0)	23.1 ± 1.96 [106]	22.4 ± 2.47 [39]
Stearic (18:0)	4.47 ± 1.29 [104]	4.27 ± 1.39 [39]
Palmitoleic (16:1n−7)	5.87 ± 2.37 [106]	5.72 ± 2.25 [39]
Oleic (18:1n−9)	42.6 ± 3.11 [104]	42.5 ± 2.62 [39]
Linoleic (18:2n−6)	12.6 ± 3.82 [106]	13.7 ± 3.83 [39]
Homo-γ-linolenic (20:3n−6)	0.17 ± 0.05 [105]	0.25 ± 0.11 [39]
Arachidonic (20:4n−6)	0.32 ± 0.11 [105]	0.41 ± 0.12 [39]
Alpha-linolenic (18:3n−3)	0.56 ± 0.17 [106]	0.61 ± 0.23 [39]
Eicosapentaenoic (20:5n−3)	0.10 ± 0.05 [68]	0.11 ± 0.04 [26]
Docosapentaenoic (22:5n−3)	0.15 ± 0.06 [104]	0.20 ± 0.08 [39]
Docosahexaenoic (22:6n−3)	0.11 ± 0.06 [101]	0.18 ± 0.10 [39]
Platelet fatty acids		
Palmitic (16:0)	17.9 ± 1.23 [135]	18.2 ± 1.52 [55]
Stearic (18:0)	17.9 ± 1.11 [135]	17.7 ± 1.18 [55]
Palmitoleic (16:1n−7)	1.58 ± 0.37 [134]	1.61 ± 0.36 [55]
Oleic (18:1n−9)	15.7 ± 1.41 [135]	15.8 ± 1.22 [55]
Linoleic (18:2n−6)	5.46 ± 1.56 [135]	5.42 ± 1.07 [55]
Homo-γ-linolenic (20:3n−6)	0.98 ± 0.23 [134]	1.01 ± 0.21 [55]
Arachidonic (20:4n−6)	16.9 ± 1.50 [135]	16.7 ± 1.14 [55]
Docosatetraenoic (22:4n−6)	1.49 ± 0.36 [135]	1.51 ± 0.31 [55]
Alpha-linolenic (18:3n−3)	0.39 ± 0.16 [129]	0.37 ± 0.13 [49]
Eicosapentaenoic (20:5n−3)	2.14 ± 0.31 [135]	1.04 ± 0.35 [55]
Docosapentaenoic (22:5n−3)	0.86 ± 0.20 [134]	0.80 ± 0.25 [55]
Docosahexaenoic (22:6n−3)	2.13 ± 0.41 [135]	2.08 ± 0.33 [55]

* $\bar{x}$ ± SD; *n* in brackets.

Risk factors, medication, and fatty acids

The correlations between several CAD risk factors and the two CAD scores are shown in **Table 4.** Age showed the strongest association with the two CAD scores. Cigarette smoking was also positively associated with CAD. Associations between the established serum lipid risk factors and the CAD scores were

TABLE 4
Spearman's rank correlation coefficients describing the associations between coronary artery disease (CAD) risk factors and CAD scores

	Extent score		Myocardial score	
Risk factors	Men	Women	Men	Women
Age	0.29*	0.39*	0.42†	0.41*
Cigarette smoking	0.09	0.29‡	0.21‡	0.18
Body mass index	−0.16	0.09	−0.01	0.08
Total cholesterol	0.03	0.13	0.05	0.10
LDL cholesterol	0.10	0.15	0.11	0.13
HDL cholesterol	−0.05	−0.14	−0.05	−0.24‡
Triglycerides	−0.03	0.19	0.03	0.27‡

* $P < 0.001$.
† $P < 0.0001$.
‡ $P < 0.05$.

generally weak and rarely significant, particularly for men. For men, none of the associations was significant. For women, HDL cholesterol was inversely associated with the myocardial score and triglycerides were positively associated with the myocardial score. For the total population, HDL cholesterol was significantly inversely associated with both the extent score ($r = -0.14$, $P < 0.05$) and the myocardial score ($r = -0.16$, $P < 0.05$). Both men and women classed as hypertensive had a significantly higher extent score ($P < 0.05$) and myocardial score ($P < 0.05$) than those without hypertension as a risk factor for CAD.

The correlations between several of the CAD risk factors and fatty acids in adipose tissue and platelets are presented in **Table 5.** The other risk factor that was related to several of the fatty acid measurements was hypertensive status. Men with hypertension had significantly higher adipose tissue linoleic acid ($P < 0.05$) concentrations than men without hypertension. Women with hypertension had significantly lower platelet linoleic acid than women without hypertension ($P < 0.05$). Dietary change by men was associated with a significantly higher concentration of adipose tissue linoleic acid ($P < 0.05$). Diabetic status was not associated with any of the fatty acids.

Treatment of the patients with aspirin, nitrates, beta-blockers, calcium antagonists, or angiotensin-converting-enzyme (ACE) inhibitors were also related to concentrations of several of the fatty acids. Aspirin was associated with significantly higher platelet eicosapentaenoic acid for men ($P < 0.05$) and higher platelet linoleic acid ($P < 0.01$) and α-linoleic acid ($P < 0.05$) for women. Nitrates were associated with lower adipose tissue lauric acid ($P < 0.05$) for men and lower platelet stearic acid ($P < 0.05$) for women. Beta-blockers were associated with higher adipose tissue oleic acid for women ($P < 0.05$). Calcium antagonists were associated with higher concentrations of adipose tissue eicosapentaenioc acid ($P < 0.05$) and platelet docosapentaenoic acid ($P < 0.05$) for women. ACE inhibitors were associated with higher adipose tissue docosapentaenoic acid for men ($P < 0.01$).

The relationships between particular fatty acids in adipose tissue with the same fatty acid measured in platelets are presented in **Table 6.** In general, the associations between the same fatty acids in the different tissues were not strong. The fatty acid with the highest concordance between adipose tissue and platelets was linoleic acid.

Adipose tissue fatty acids and CAD

The relationships between adipose tissue fatty acids and the two CAD scores are presented in **Table 7.**

Men. Myristic acid was inversely associated with the myocardial score. This association is not significant after age was adjusted for ($r = -0.16$, $P > 0.05$). Palmitoleic acid was inversely associated with the extent score. After age, triglycerides, and cigarette smoking were adjusted for, the association remained significant ($r = -0.24$, $P < 0.05$).

Linoleic acid was positively associated with both the extent score and the myocardial score. After age was adjusted for, the

TABLE 5
Spearman's rank correlation coefficients describing the associations between fatty acids in adipose tissue and platelets and risk factors for coronary artery disease

	Men						Women					
Fatty acid	Age	Smoking	BMI	LDL-C	HDL-C	TG	Age	Smoking	BMI	LDL-C	HDL-C	TG
Adipose tissue fatty acids												
Lauric (12:0)	−0.15	−0.04	−0.17	0.03	0.09	−0.04	−0.17	−0.03	−0.30	−0.16	0.15	−0.18
Myristic (14:0)	−0.13	−0.02	−0.17	0.02	−0.02	0.11	−0.21	0.00	−0.38*	−0.22	0.05	0.11
Palmitic (16:0)	−0.08	0.01	−0.04	0.02	−0.10	0.31†	−0.11	0.18	0.04	−0.12	0.07	0.25
Stearic (18:0)	0.07	−0.21*	−0.23*	−0.20	0.05	−0.13	0.11	0.09	−0.50†	−0.14	0.27	−0.17
Palmitoleic (16:1n−7)	−0.21*	0.24*	0.06	0.00	−0.01	0.19*	−0.14	−0.09	0.15	0.08	−0.05	0.08
Oleic (18:1n−9)	−0.05	0.06	0.07	0.05	−0.01	0.01	−0.10	0.03	0.06	−0.05	−0.25	0.02
Linoleic (18:2n−6)	0.18	−0.09	0.02	0.07	0.04	−0.25†	0.29	−0.01	0.12	0.18	0.02	−0.09
Homo-γ-linolenic (20:3n−6)	0.13	0.09	0.32†	0.07	−0.07	−0.02	0.32*	0.06	0.45*	0.03	0.03	0.28
Arachidonic (20:4n−6)	−0.06	0.11	0.19	−0.12	0.02	0.08	0.07	−0.06	0.52†	0.07	0.06	0.17
Alpha-linolenic (18:3n−3)	−0.03	−0.16	0.05	0.05	0.07	−0.08	0.10	−0.04	−0.12	−0.01	0.27	−0.21
Eicosapentaenoic (20:5n−3)	−0.11	0.15	−0.02	−0.14	0.25*	−0.19	−0.21	−0.19	−0.34	−0.04	0.19	−0.54†
Docosapentaenoic (22:5n−3)	0.16	−0.02	0.04	−0.20*	−0.00	−0.00	0.42†	−0.19	0.22	−0.04	−0.00	0.28
Docosahexaenoic (22:6n−3)	−0.03	−0.02	0.12	−0.15	0.09	−0.07	0.34*	−0.05	0.17	−0.11	0.26	−0.01
Platelet fatty acids												
Palmitic (16:0)	−0.01	−0.10	−0.00	−0.14	0.07	0.11	0.44†	0.04	0.01	0.23	0.09	0.23
Stearic (18:0)	−0.07	−0.13	−0.18	0.08	0.13	−0.19*	0.01	−0.06	−0.44†	−0.01	−0.10	−0.02
Palmitoleic (16:1n−7)	0.22*	0.07	−0.03	−0.09	0.20*	−0.09	0.11	0.14	−0.20	−0.05	−0.05	0.06
Oleic (18:1n−9)	−0.11	0.08	0.03	−0.14	−0.15	0.23†	−0.04	−0.12	0.27	−0.06	0.10	0.13
Linoleic (18:2n−6)	0.11	−0.07	0.01	−0.09	−0.14	0.11	0.09	−0.11	−0.06	−0.21	0.09	−0.14
Homo-γ-linolenic (20:3n−6)	−0.06	0.07	0.12	0.04	−0.19*	0.18*	0.30*	0.04	0.02	0.14	0.05	0.20
Arachidonic (20:4n−6)	−0.20*	−0.04	0.14	0.16	0.03	−0.03	−0.25	−0.04	0.10	0.12	0.05	0.12
Docosatetraenoic (22:4n−6)	0.01	0.04	0.09	0.04	0.05	−0.06	−0.36†	−0.06	0.14	−0.24	−0.09	−0.12
Alpha-linolenic (18:3n−3)	0.07	−0.07	−0.08	0.06	−0.06	−0.01	−0.04	−0.12	0.20	−0.06	−0.10	0.07
Eicosapentaenoic (20:5n−3)	−0.14	−0.02	0.14	0.19*	−0.07	0.07	−0.15	0.19	0.01	−0.12	0.04	−0.10
Docosapentaenoic (22:5n−3)	−0.06	0.04	−0.04	0.17	0.02	−0.09	−0.17	0.00	−0.14	−0.15	0.26	−0.30*
Docosahexaenoic (22:6n−3)	−0.03	0.11	−0.05	0.08	0.04	−0.08	−0.18	0.00	0.13	0.09	−0.12	0.07

* $P < 0.05$.
† $P < 0.01$.

TABLE 6
Spearman's rank correlation coefficients describing the associations between the adipose tissue fatty acids and the same fatty acids measured in platelets

Fatty acid	Men	Women
Palmitic (16:0)	0.07	−0.08
Stearic (18:0)	0.31*	0.24
Palmitoleic (16:1n−7)	0.22†	−0.01
Oleic (18:1n−9)	0.16	0.27
Linoleic (18:2n−6)	0.46‡	0.49§
Homo-γ-linolenic (20:3n−6)	0.18	0.45*
Arachidonic (20:4n−6)	−0.05	−0.22
Alpha-linolenic (18:3n−3)	0.06	0.23
Eicosapentaenoic (20:5n−3)	−0.33†	0.21
Docosapentaenoic (22:5n−3)	−0.11	0.15
Docosahexaenoic (22:6n−3)	−0.03	0.33†

* $P < 0.01$.
† $P < 0.05$.
‡ $P < 0.0001$.
§ $P < 0.001$.

TABLE 7
Spearman's rank correlation coefficients describing the associations between adipose tissue fatty acids and coronary artery disease scores

	Extent score		Myocardial score	
Fatty acid	Men	Women	Men	Women
Lauric (12:0)	−0.09	0.01	−0.18	−0.01
Myristic (14:0)	−0.12	−0.15	−0.20*	−0.25
Palmitic (16:0)	−0.04	−0.12	−0.09	−0.16
Stearic (18:0)	0.00	0.08	−0.09	−0.01
Palmitoleic (16:1n−7)	−0.21*	−0.18	−0.10	−0.08
Oleic (18:1n−9)	−0.15	−0.03	−0.18	0.05
Linoleic (18:2n−6)	0.20*	0.24	0.25†	0.21
Homo-γ-linolenic (20:3n−6)	0.24*	0.04	0.21*	0.08
Arachidonic (20:4n−6)	−0.03	−0.11	0.12	−0.03
Alpha-linolenic (18:3n−3)	0.08	0.14	0.05	0.04
Eicosapentaenoic (20:5n−3)	0.22	−0.15	0.10	−0.24
Docosapentaenoic (22:5n−3)	0.10	0.11	−0.00	0.19
Docosahexaenoic (22:6n−3)	0.02	0.33*	−0.01	0.33*

* $P < 0.05$.
† $P < 0.01$.

association between linoleic acid and the extent score was not significant ($r = 0.14$, $P > 0.05$). The association with the myocardial score remained ($r = 0.19$, $P < 0.05$). After all covariates that were related to adipose tissue linoleic acid were adjusted for, namely age, triglycerides, hypertensive status, and dietary change, linoleic acid was not significantly associated with the extent score ($r = 0.13$, $P > 0.05$), but the association with the myocardial score remained ($r = 0.20$, $P < 0.05$). Adipose tissue homo-γ-linolenic acid was also positively associated with both CAD scores. After age and BMI were adjusted for, homo-γ-linolenic acid was significantly associated with the extent score ($r = 0.27$, $P < 0.05$) but not with the myocardial score ($r = 0.09$, $P > 0.05$).

Women. Docosahexaenoic acid was positively associated with both CAD scores. However, after age and BMI were adjusted for, the associations between both the extent score ($r = 0.13$, $P > 0.05$) and the myocardial score ($r = 0.14$, $P > 0.05$) were not significant.

Platelet fatty acids and CAD

The relationships between the platelet fatty acids and the two CAD scores are presented in **Table 8.**

Men. A positive association between linoleic acid and both the CAD scores was found. These associations remained after age was adjusted for [$r = 0.25$, $P < 0.01$ (extent score), $r = 0.24$, $P < 0.01$ (myocardial score)]. Arachidonic acid was inversely associated with both CAD scores. After age was adjusted for, the associations between arachidonic acid and the CAD scores were not significant [$r = -0.14$, $P > 0.05$ (extent score), $r = -0.12$, $P > 0.05$ (myocardial score)]. Platelet arachidonic acid was also inversely associated with platelet linoleic acid concentration ($r = -0.34$, $P < 0.0001$). To determine whether the inverse association between linoleic acid and arachidonic acid for men could be explained by age, cigarette smoking, diabetic status, dietary change, plasma cholesterol concentration, or any other serum lipid measurement, the association for each of these factors was adjusted for. None of these variables were able to explain (even partially) this inverse association.

The long-chain ω-3 fatty acids were not significantly inversely associated with either of the CAD scores. However, after LDL cholesterol concentration and aspirin use were adjusted for, both of which were significantly related to platelet eicosapentaenoic acid as well as age, eicosapentaenoic acid was inversely associated with the myocardial score ($r = -0.18$, $P < 0.05$).

Women. Arachidonic acid was inversely associated with the myocardial score. After age was adjusted for, this association was not significant ($r = -0.22$, $P > 0.05$). The associations between the long-chain fatty acids and the CAD scores were not significant. After calcium antagonist use was adjusted for, which was related to the concentration of docosapentaenoic acid, docosapentaenoic acid was significantly inversely associated with both the extent score ($r = -0.28$, $P < 0.05$) and the myocardial score ($r = -0.37$, $P < 0.01$). After age was further adjusted for, the association with the extent score was not significant ($r = -0.24$, $P > 0.05$) but remained significant for the myocardial score ($r = -0.33$, $P < 0.05$).

TABLE 8
Spearman's rank correlation coefficients describing the associations between platelet fatty acids and coronary artery disease scores

	Extent score		Myocardial score	
Fatty acid	Men	Women	Men	Women
Palmitic (16:0)	−0.06	0.23	−0.04	0.19
Stearic (18:0)	−0.03	0.05	−0.16	−0.11
Palmitoleic (16:1n−7)	−0.02	0.06	0.04	0.11
Oleic (18:1n−9)	−0.03	0.01	−0.05	0.14
Linoleic (18:2n−6)	0.27*	0.00	0.26*	−0.08
Homo-γ-linolenic (20:3n−6)	0.09	0.02	−0.01	0.10
Arachidonic (20:4n−6)	−0.19†	−0.24	−0.19†	−0.28†
Docosatetraenoic (22:4n−6)	−0.08	−0.25	0.04	−0.20
Alpha-linolenic (18:3n−3)	0.17	0.01	0.12	−0.05
Eicosapentaenoic (20:5n−3)	−0.07	−0.01	−0.12	−0.10
Docosapentaenoic (22:5n−3)	−0.16	−0.19	−0.09	−0.24
Docosahexaenoic (22:6n−3)	−0.06	−0.12	−0.05	−0.05

* $P < 0.01$.
† $P < 0.05$.

Relationship outcomes

A summary of the major relationship outcomes observed for adipose tissue and platelet fatty acids after confounding factors were adjusted for, is presented in **Table 9.**

TABLE 9
Major relationship outcomes after confounding factors were adjusted for*

Men
1) Adipose tissue palmitoleic acid (16:1n−7) was inversely associated with the extent score ($r = -0.24$, $P < 0.05$).
2) Adipose tissue linoleic acid (18:2n−6) was positively associated with the myocardial score ($r = 0.20$, $P < 0.05$).
3) Platelet linoleic acid (18:2n−6) was positively associated with both the extent score ($r = 0.25$, $P < 0.01$) and the myocardial score ($r = 0.24$, $P < 0.01$).
4) Adipose tissue homo-γ-linolenic acid (20:3n−6) was positively associated with the extent score ($r = 0.27$, $P < 0.05$).
5) Platelet eicosapentaenoic acid (20:5n−3) was inversely associated with the myocardial score ($r = -0.18$, $P < 0.05$).

Women
1) Platelet docosapentaenoic acid (22:5n−3) was inversely associated with the myocardial score ($r = -0.33$, $P < 0.05$).

* Possible confounding factors were age and other variables associated with the particular fatty acids. *r* is Spearman's rank correlation coefficient.

Discussion

The myocardial score has been used routinely as a means for quantitatively presenting the information obtained in a coronary angiogram. This score takes into account both severity and location of coronary lesions. Because severity is measured, the score may relate to factors that complicate atherosclerotic lesions in addition to the atherosclerosis itself. It has one major disadvantage if the relationships with risk factors for CAD are being examined. The score is biased by lesion location. The extent score is an estimate of the aggregate degree of atherosclerosis in the coronary arteries. This score is not biased by lesion location, it is quantitative, and it has been shown to be correlated more strongly with CAD risk factors than with a vessel score or a stenosis score (17). The extent score and the myocardial score were significantly associated, but the degree of association suggests that different aspects of CAD are being assessed. However, in general, those patients with more of the myocardium threatened by CAD also had a higher percentage of their coronary arteries affected by atherosclerosis.

The results for men from both adipose tissue and platelets show a positive association between linoleic acid and CAD. Adipose tissue is a good indicator of long-term intake of linoleic acid (1, 2), whereas platelet fatty acids reflect shorter-term intake. A correlation of 0.46 (Table 6) between adipose tissue linoleic acid and platelet linoleic acid for men is consistent with the different time frame of the measurements. However, factors other than diet can also influence the concentration of linoleic acid in both adipose tissue and platelets. Those factors that affect platelet linoleic acid might not affect adipose tissue linoleic acid. Although the degree of association between linoleic acid in adipose tissue or platelets and the CAD scores for men was not high, it was stronger than many of the other recognized risk factors for CAD. Together the results from platelets, and adipose tissue in particular, are indicative of a positive association between linoleic acid intake and CAD.

Much of the evidence now available suggests an inverse relationship between linoleic acid and CHD (3–6). However, one study found that an increased intake of linoleic acid significantly increased the risk of new atherosclerotic lesions in human coronary arteries (8). Interestingly, the end point in this study was also angiographic. Studies finding an inverse relationship for adipose tissue linoleic acid have used either rates of mortality from CHD (3, 4), angina pectoris, or acute myocardial infarction (5, 6) as an end point.

If the positive associations observed between linoleic acid and CAD in this study are due to a positive association between linoleic acid intake and CAD, as suggested by our results, then one possible explanation for the inconsistent findings may be that high linoleic acid intake is a risk factor for coronary atherosclerosis, just as low linoleic acid intake would appear to be a risk factor for coronary events (3–6). The mean concentration of linoleic acid in the adipose tissue of the study population was 12.6% for men and 13.7% for women, which is considerably higher than the mean concentration of linoleic acid in the adipose tissue of European populations studied previously (3–6).

Several mechanisms that might account for the positive associations between linoleic acid and the CAD scores can be proposed. Higher concentrations of linoleic acid may increase the risk of oxidative modification of lipoproteins. The oxidative modification of LDL in particular is understood to be a pathway to atherosclerosis (22). Another possibility is that linoleic acid was a marker for the intake of other factors in food that increase the risk of CAD. A further alternative is that the observed associations for linoleic acid do not relate to intake, but to factors that influence the metabolism of fatty acids in vivo. The results from this study, however, provide insufficient evidence to support any proposed mechanisms.

Adipose tissue palmitoleic acid was inversely associated with the extent score. This is indicative of an inverse association with intake. However, the relationship between intake of saturated or monounsaturated fatty acids and adipose tissue concentration is not as strong as that for linoleic acid. Associations between saturated or monounsaturated fatty acids measured in adipose tissue and CAD therefore do not provide strong evidence for a relationship with intake. Adipose tissue homo-γ-linolenic acid was positively associated with the extent score. This association may be related to intake or to the metabolism of linoleic acid in vivo. Several other significant associations between adipose tissue fatty acids and the CAD scores were observed. All of these associations could be explained by age and other covariates.

At a univariate level, not one of the long-chain ω-3 fatty acids was significantly inversely associated with the CAD scores. After confounding factors were adjusted for, eicosapentaenoic acid was inversely associated with the myocardial score for men and docosapentaenoic acid was inversely associated with the myocardial score for women. These results are supported by findings from Wood et al (6), who found that platelet docosapentaenoic acid was inversely associated with acute myocardial infarction, and eicosapentaenoic acid was inversely associated with angina pectoris (6). The results are also consistent with several other studies that suggest that fish intake may be beneficial for CHD risk (9–11). One of the most important mechanisms for the inverse relationship between the fish oils, or the ω-3 fatty acids, and CHD is likely to be the effects of ω-3 fatty acids on eicosanoid metabolism such that a more vasodilatory state with reduced

platelet aggregation results (23, 24). However, ω-3 fatty acids have been shown to reduce plasma triglyceride concentration (25–27) and blood pressure (28, 29), both of which may reduce the rate of progression of coronary atherosclerosis. The inverse associations between platelet eicosapentaenoic acid for men and docosapentaenoic acid for women, and the myocardial score provide further evidence for a relationship between the intake of this fatty acid and reduced risk of CAD. This is consistent with the proposition that an adequate intake of these fatty acids is beneficial to CAD.

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