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Original Contribution

A Novel Fatty Acid Profile Index—the Lipophilic Index—and Risk of Myocardial Infarction

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The lipophilic index (LI), a mean measure of fatty acid melting points, has been proposed to capture overall fatty acid profile and may play an important role in the etiology of coronary heart disease. We aimed to determine the association between LI in diet and in adipose tissue and metabolic risk factors for myocardial infarction (MI) and risk of MI. We used a population-based, matched case-control study of nonfatal first acute MI conducted in Costa Rica between 1994 and 2004, with 1,627 case-control pairs. The LI is defined as the mean of the melting points of specific fatty acids in diet or adipose tissue. LIs in diet and adipose tissue were significantly associated with higher plasma triglyceride concentrations, low-density lipoprotein cholesterol concentrations, and low-density:high-density lipoprotein cholesterol ratio. Comparing extreme quintiles for the LI in diet or adipose tissue, the odds ratios for MI were 1.57 (95% confidence interval: 1.22, 2.02; P for trend < 0.001) for dietary LI and 1.30 (95% confidence interval: 1.00, 1.69; P for trend = 0.02) for adipose tissue LI in the multivariable models. We hypothesize that a higher LI in diet and in adipose tissue represents decreased fatty acid fluidity and could play an important role in the etiology of coronary heart disease.

biological markers; case-control studies; coronary artery disease; fatty acids; membrane fluidity; myocardial infarction

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LI, lipophilic index; MI, myocardial infarction; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; VCAM-1, vascular cell adhesion molecule 1.

Fatty acids are traditionally classified according to their chain, length, number of double bonds, and configuration—*cis* or *trans* (1). These characteristics underlie the differentiation of fatty acids as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and *trans*-fatty acids. Omega-3 and omega-6 fatty acids share similar characteristics but have different metabolic functions. The current classification is based on structural properties, not on biological properties such as the affinity between fatty acid molecules and their fluidity (1), a biological property which may clarify the metabolic processes underlying coronary heart disease. Health effects of individual fatty acids within these broad categories are heterogeneous. For example, Hu et al. (2) observed that dietary intake of long-chain SFA but not short- to medium-chain SFA was associated with an increased risk of coronary heart disease in the Nurses' Health Study.

One characteristic of fatty acids that refines the current classification is their melting point, which is determined by the length of the fatty acid chain and its degree of unsaturation. The melting point results from van der Waals forces, which agglutinate different fatty acid molecules (3). Thus, the longer and straighter the fatty acid chain, the tighter the packing of the molecules and accordingly the lesser the fluidity.

In tissues, some fatty acids are derived from diet, while others are derived from carbohydrates and from the action of

elongases and desaturases. Dietary fatty acids are incorporated into body tissues such as adipose tissue, plasma phospholipids, and cell membrane phospholipids and determine their viscosity. Fatty acids from diet or those released from adipose tissue serve as metabolic fuel and fulfill structural functions when incorporated into cell membranes. The availability of fatty acids determines fatty acid composition and thus the fluidity of biological specimens such as cholesterol particles and cell membranes. Increased fluidity has been associated directly with the fluidifying effect of high-density lipoproteins (4) and with improvement of insulin binding (5) and insulin action (6) but associated inversely with hypertension (7).

In order to capture the fluidity of the overall fatty acid profile in diet and tissues, we propose the use of the lipophilic index (LI) (8), which is the sum of the product of the quantity of fatty acids consumed by an individual and the respective melting point of each fatty acid divided by the total grams of fat intake. The LI in biological specimens is defined as the mean of the melting points of the specific fatty acids contained in biological specimens weighted by their specific concentrations. We investigated whether the LIs in diet and adipose tissue are associated with a higher risk of myocardial infarction (MI), as well as risk factors for coronary heart disease.

MATERIALS AND METHODS

Study population

All subjects were Hispanic Americans living in the Central Valley of Costa Rica between 1994 and 2004. A detailed description of the study has been published elsewhere (9–11). Briefly, the study catchment area included 18 counties in the Central Valley of Costa Rica that encompassed a wide range of socioeconomic levels and urban, peri-urban, and rural lifestyles. Eligible cases comprised men and women diagnosed with a first acute MI by 2 independent cardiologists at any of the 6 recruitment hospitals. MI was defined according to the World Health Organization criteria (12). Cases were ineligible if they 1) died during hospitalization, 2) were aged 75 years or older on the day of their first MI, or 3) were physically or mentally unable to answer the questionnaire. Cases were enrolled when they were in the step-down unit of the hospital. The mean length of hospitalization was 15 days (9). Each case was individually matched by age (±5 years), sex, and area of residence to a population control. Control subjects were ineligible if they had had a previous MI or if they were physically or mentally unable to complete the questionnaire. The participation rate was 98% for cases and 88% for controls. All participants 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

Cases and controls were visited at their homes by trained personnel, who collected dietary and health information, anthropometric measurements, and biological specimens.

Information on sociodemographic characteristics, lifestyle history, and medical history was gathered by means of a general questionnaire with closed-ended questions (9). Self-reported diabetes and hypertension were previously validated in this population (9).

Dietary intake was determined by use of a validated 162-item food frequency questionnaire (10, 13). Participants were asked the average frequency and amount of time they had spent in 12 occupational and leisure-time activities during the previous year. Total physical activity was calculated by multiplying the frequency, duration, and intensity (in metabolic equivalents) of each physical activity and the energy expenditure from all activities (9, 14).

Anthropometric measurements were taken with participants wearing light clothing and no shoes. Biological samples were routinely collected in the morning after overnight fasting. Subcutaneous adipose tissue biopsies were collected from the upper buttock with a modified version of the method described by Beynen and Katan (15). During the same visit, blood samples were drawn into tubes coated with 0.1% ethylenediaminetetraacetic acid after a fast of 12–14 hours, immediately stored at 4°C, and protected from light. Blood samples were centrifuged within 6 hours at 2,500 revolutions per minute for 20 minutes to isolate and aliquot plasma and white blood cells. Blood samples were sealed and stored under nitrogen at −80°C until analysis (9). Because biomarkers for coronary heart disease such as serum lipids and inflammation markers might have been altered by the MI or by treatment, biomarker levels were determined only in controls, to control for reverse causation. Triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were analyzed with enzymatic reagents (Boehringer Mannheim (now Roche Diagnostics), Indianapolis, Indiana). Levels of high-sensitivity C-reactive protein (hs-CRP) and vascular cell adhesion molecule 1 (VCAM-1) were ascertained as biomarkers of inflammation. We measured hs-CRP by immunoturbidimetry on a Roche Modular P Chemistry Analyzer (Hoffman-La Roche, Nutley, New Jersey). The average coefficient of variation for 12 blind duplicates was 1.4% (range, 0%–5%). To measure VCAM-1, the Human sVCAM-1 Quantikine assay kit (lot 258820; R&D Systems, Minneapolis, Minnesota) was used. The average coefficient of variation for 12 blind duplicates was 9.6%. All assays were performed with the same batch of reagents.

Fatty acid analysis and calculation of indices of fat lipophilicity

Dietary fatty acid intake was computed by multiplying the consumption frequency of each food by the nutrient content of the specific portion (16). The fatty acid compositions of all foods and oils commonly consumed in Costa Rica were analyzed in the same laboratory, using the same standards and instrumentation for peak identification as were used in analyzing fatty acids in biological tissues (17).

The dietary LI was calculated by multiplying the intake of each fatty acid (in grams) by its specific melting point (°C), summing the products, and then dividing by the sum of fatty

acid intake (in grams) (8):

$$\text{Dietary LI} = \frac{\sum_k \text{Fatty acid (g)}_i \times \text{Melting point (°C)}_i}{\sum_k \text{Fatty acid (g)}_i}.$$

Information about melting points was acquired from the LipidBank database (18). Gas-liquid chromatography was used to quantify fatty acid levels in adipose tissue in the whole population and, in addition, to quantify fatty acid levels in whole blood, plasma, and red blood cells in a subgroup of 200 controls (13, 17). The tissue-specific LI was computed in the same way as the dietary LI, by multiplying the relative density of each fatty acid (percentage) in adipose tissue and by its melting point (in °C), summing the products, and then dividing by the sum of the relative densities of all fatty acids.

Of the 49 quantified individual fatty acids in adipose tissue, data on melting points were available for 37. Of these 37 fatty acids, complete information on all participants in the analyses was available for 30. Since lignoceric acid (24:0) was not quantified in the diet, it was not included in the LI. Thus, the LI was calculated on the basis of 29 fatty acids. Fatty acids that lacked data on melting points or on values for diet, adipose tissue, plasma, or red blood cells represented very small peaks in the chromatogram. Therefore, information on the melting points was available for 98% of the total fatty acids in the diet, 98% in adipose tissue, 96% in plasma, and 86% in red blood cells.

Statistical analysis

Participants who had missing values for LI in adipose tissue were excluded from the analysis. Also excluded were participants in whom major confounders were observed or whose caloric intakes were extreme (<500 kcal or >3,500 kcal in women or <800 kcal or >4,000 kcal in men). Missing values for income were entered as an additional category in multivariable models. Thus, 1,627 matched case-control pairs remained for the analysis of the LI in adipose tissue. The data available for the controls were as follows: plasma HDL cholesterol for 1,600 controls; LDL cholesterol (19) for 1,561 controls; triglycerides for 1,615 controls; hs-CRP for 1,322 controls; and VCAM-1 for 1,359 controls. The fatty acid composition of whole blood, red blood cells, and plasma was available for 200 additional controls.

Variables not normally distributed were log-transformed. Baseline characteristics of cases and controls were compared with Wilcoxon's and chi-square tests. We used Spearman's correlation coefficients to examine the correlations, first between specific fatty acids and the LI in diet and in the different tissues and second between the LI in diet and in the different biological specimens. Using linear regression models, we calculated least-square means of the major biomarkers of coronary heart disease (specifically, HDL and LDL cholesterol, LDL:HDL cholesterol ratio, triglycerides, hs-CRP, and VCAM-1) across quintiles of the LI in diet and in adipose tissue. In order to estimate the association between the LI in diet or in adipose tissue and MI, we fitted conditional logistic regression models dividing the participants according to quintile of the LI. For linear trend tests, each quintile of the LI was assigned its median value, and the resulting variable was considered as continuous in the analyses. Models were adjusted for matching factors, waist:hip ratio, physical activity, total caloric intake, history of hypertension, smoking status, and alcohol intake. There was an association between α-linolenic acid and arachidonic acid and MI in our study population, and these fatty acids were also correlated with the LI (20, 21). Therefore, we included these fatty acids in the multivariable models. We adjusted for the PUFA:SFA ratio in sensitivity analyses to examine the association of the LI independently of the PUFA:SFA ratio.

All significance levels were 2-sided. All statistical analyses were performed with SAS software, version 9.1.3 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Compared with controls, cases revealed higher diet and adipose tissue LIs, higher consumption of SFA, and lower consumption of PUFA (Table 1). Baseline characteristics of participants according to quintiles of diet and adipose tissue LI are displayed in Appendix Table 1.

Table 2 shows the melting points of specific fatty acids and Spearman's correlation coefficients for correlations between specific fatty acids and the LI in diet, adipose tissue, plasma, and red blood cells. We also calculated fatty acid levels in whole blood. Since whole blood consists mainly of plasma, results for whole blood were similar to those observed for plasma (data not shown). In adipose tissue, Spearman's correlation coefficient was −0.34 for the LI and α-linolenic acid and −0.44 for the LI and arachidonic acid.

Overall, LDL cholesterol, LDL:HDL ratio, and the concentrations of triglycerides in plasma were positively associated with the LIs in adipose tissue and in diet among controls (Table 3). The LI of diet was inversely associated with HDL cholesterol, while the LI of adipose tissue was inversely associated with hs-CRP and VCAM-1. In adipose tissue, Spearman's correlation coefficient was −0.04 ($P = 0.20$) for correlation between hs-CRP and α-linolenic acid and 0.26 ($P < 0.001$) for correlation between hs-CRP and arachidonic acid. The adjustment for α-linolenic acid did not substantially change the results for the associations between LI in adipose tissue and hs-CRP. On the other hand, the association between LI in adipose tissue and hs-CRP was attenuated after adjustment for arachidonic acid (for the first quintile, adjusted mean = 2.6 mg/L, 95% confidence interval (CI): 2.3, 3.1; for the fifth quintile, adjusted mean = 2.0 mg/L, 95% CI: 1.7, 2.2 (P for trend = 0.001)) but was not attenuated for VCAM-1 (for the first quintile, adjusted mean = 721 ng/mL, 95% CI: 688, 756; for the fifth quintile, adjusted mean = 652 ng/mL, 95% CI: 623, 683 (P for trend < 0.001)).

The LI of fatty acids in adipose tissue was associated with an increased risk of MI in the crude model and after adjustment for potential confounders. However, the association was attenuated upon adjustment for α-linolenic acid, a fatty acid already identified as associated with MI in our study (Table 4). Participants in the highest quintile of LI had a

Table 1. Characteristics of Cases and Controls in a Matched Case-Control Study of Nonfatal First Acute Myocardial Infarction, Costa Rica, 1994–2004

Characteristic	Cases ($n = 1,627$)		Controls ($n = 1,627$)		*P* Value
	Mean (SD)	%	Mean (SD)	%	
Age, years	59.1 (10.8)		58.7 (11.0)		N/A
Female sex		27		27	N/A
Lipophilic index					
Adipose tissue	23.82 (2.43)		23.62 (2.37)		0.01
Diet	29.56 (4.61)		28.63 (4.74)		<0.001
History of diabetes		25		16	<0.001
History of hypertension		39		31	<0.001
Current smoker		39		20	<0.001
Current alcohol drinker		46		53	<0.001
Waist:hip ratio	0.97 (0.07)		0.95 (0.07)		<0.001
Physical activity, METs/day	34.1 (15.4)		34.8 (14.5)		0.001
Total energy intake, kcal/day	2,484 (652)		2,359 (622)		<0.001
Total fat intake (% of total energy intake)	32.1 (5.7)		31.8 (5.8)		0.06
SFA	10.9 (2.8)		10.3 (2.7)		<0.0001
MUFA	11.8 (3.5)		11.8 (3.9)		0.14
PUFA	6.0 (2.0)		6.3 (2.1)		<0.001
Trans-fatty acid	1.3 (0.6)		1.3 (0.6)		0.10
Dietary PUFA:SFA ratio	0.63 (0.31)		0.68 (0.33)		<0.001
Adipose tissue PUFA:SFA ratio	0.71 (0.22)		0.73 (0.22)		0.005

Abbreviations: MET, metabolic equivalent of task; MUFA, monounsaturated fatty acids; N/A, not applicable; PUFA, polyunsaturated fatty acids; SD, standard deviation; SFA, saturated fatty acids.

30% greater risk of MI, and the linear trend was statistically significant. The dietary LI was associated with an increased risk of MI. Participants in the fifth quintile of dietary LI showed a 57% higher risk of MI compared with participants in the first quintile, and the linear trend test was statistically significant. Adjustment for the PUFA:SFA ratio strengthened the associations. Spearman's correlation coefficients for correlation between the LI in diet and different biological specimens among controls are shown in Table 5. The strongest correlation was observed between the LI in adipose tissue and the LI in plasma.

DISCUSSION

Results of this large case-control study showed positive associations between diet and adipose tissue LI and the risk of MI. The LI in adipose tissue was also consistently and positively associated with plasma LDL cholesterol concentration, LDL:HDL cholesterol ratio, and plasma triglyceride concentration among controls. However, an inverse association was found between LI in adipose tissue and plasma VCAM-1 concentration.

The estimated LI was developed to summarize the concentrations of individual fatty acids in an index that could be used to estimate the mean fluidity of fatty acids consumed in the diet or present in biological samples. The LI achieves this estimation by measuring the quality (melting point) and quantity of fatty acid. Higher melting points—which quantify the lipophilic attraction of fatty acids—correlate with a higher number of carbon atoms and a greater degree of saturation. For example, the fatty acids 12:0 and 24:0 have melting points of 44.2°C and 87.75°C, respectively, and the fatty acids 20:4 n6 and 20:0 have melting points of −49.5°C and 76.75°C, respectively. In addition, melting points are increased by a *trans* configuration rather than a *cis* configuration. For example, 18:2 n-6 fatty acid has a melting point of −5°C, whereas 18:2 n-6tt fatty acid has a melting point of 28.5°C.

We found a positive association between the LI in adipose tissue and plasma triglyceride concentrations, on the one hand, and between the LI in diet and plasma triglyceride concentrations on the other. We hypothesize that a higher LI, indicating less fluidity, may be associated with an increased risk of MI due to an excessive accumulation of plasma triglycerides, which can lead to insulin resistance and lipotoxicity. Conversely, a lower LI, indicating more fluidity and a higher proportion of unsaturated fatty acids, can diminish liver triglyceride production and increase fatty acid oxidation by suppressing the expression of lipogenic genes (22). We also found a positive association between diet and adipose tissue LI, on the one hand, and plasma LDL cholesterol concentration on the other. Since the LDL receptor is a cell-surface receptor of nucleated cells and since fatty acids can alter the properties of membranes (23), greater fluidity may also decrease the concentration of circulating cholesterol by increasing its clearance. In addition, the diet and adipose tissue LIs were correlated with plasma LI. Since increased plasma phosphatidylcholine fluidity has been associated with enhanced lecithin-cholesterol acyltransferase—a key enzyme in lipoprotein metabolism—amelioration of lipid metabolism by raised fatty acid fluidity may be explained by improved lecithin-cholesterol acyltransferase activity. The incorporation of fatty acids that decrease the membrane fluidity into phospholipids of cell membranes is another mechanism that may contribute to the association between LI and MI, because this fatty acid incorporation may impair oxygen permeability in cell membranes by decreasing membrane fluidity (24, 25), the handling of cell calcium, or the regulation of intracellular pH (26, 27). Thus, increased membrane rigidity associated with a higher LI may impair endothelial cell function by decreasing oxygen diffusion.

We found an unexpected inverse association between LI from adipose tissue, hs-CRP, and VCAM-1. The association between the LI and hs-CRP was attenuated when arachidonic acid in adipose tissue was adjusted for, which suggests that the association between the LI and hs-CRP might have stemmed from the potentially deleterious effects of arachidonic acid, which counteract its characteristic advantage—that is,

Table 2. Melting Points for Different Fatty Acids and Spearman's Correlation Coefficients (*r*) for Correlations Between Individual Fatty Acids and the Lipophilic Index Among Controls, Costa Rica, 1994–2004

FA	Melting Point, °C	Sample[a]							
		Diet (*n* = 1,627)		Adipose Tissue (*n* = 1,627)		Plasma (*n* = 200)		Red Blood Cells (*n* = 200)	
		Individual FA Intake, % (% of Total Fat Intake)	*r*	Individual FA Level, %	*r*	Individual FA Level, %	*r*	Individual FA Level, %	*r*
Saturated FAs									
12:0	44.2	0.82	0.50**	0.05	0.10**	0.02	0.35**	0.002	0.06
14:0	53.9	2.74	0.51**	1.24	0.29**	0.74	0.66**	0.20	0.41**
15:0	52.3	0.31	0.44**	0.21	0.24**	0.17	0.24[a]	0.11	0.50**
16:0	63.1	24.03	0.57**	21.32	0.90**	22.78	0.89**	24.15	0.88**
17:0	61.3	0.34	0.37**	0.20	0.57**	0.28	0.04	0.36	0.60**
18:0	69.6	7.90	0.29**	2.74	0.64**	6.76	−0.14	14.99	0.30**
19:0	68.6	0.08	0.29**	0.07	0.06*	0.07	−0.12	0.10	0.25**
20:0	76.75	0.21	0.00	0.10	0.39**	0.19	−0.33**	0.40	0.72**
22:0	81.5	0.10	−0.44**	0.01	0.16**	0.48	−0.50**	1.87	0.68**
Monounsaturated FAs									
14:1 n-5c	−4	0.21	0.41**	0.19	−0.18**	0.04	0.56**	0.002	0.11
16:1 n-7c	0	1.47	0.09**	6.62	−0.34**	2.64	0.57**	0.35	0.13
18:1 n-9c	16	30.96	0.10**	42.10	0.02	20.74	0.64**	13.47	0.61**
18:1 n-7c	15	1.39	−0.16**	2.24	−0.33**	1.71	0.21*	1.13	0.43**
20:1 n-9c	23.25	0.17	−0.17**	0.43	0.16**	0.14	0.34**	0.19	0.20*
Polyunsaturated FAs									
n-6									
18:2 n-6c	−5	20.13	−0.59**	15.58	−0.51**	28.09	−0.67**	9.68	−0.50**
18:3 n-6	−11.15	0.05	0.03	0.07	−0.27**	0.39	−0.19*	0.04	−0.54**
20:4 n-6	−49.5	0.29	0.10**	0.47	−0.44**	6.02	−0.68**	11.56	−0.85**
n-3									
18:3 n-3c	−11.15	1.96	−0.42**	0.65	−0.34**	0.49	−0.11	0.08	−0.65**
20:5 n-3c	−54.1	0.14	0.01	0.04	−0.15**	0.41	−0.48**	1.26	0.36**
22:5 n-3c	−54.1	0.09	0.15**	0.18	−0.17**	0.41	−0.46**	1.48	−0.81**
22:6 n-3c	−44.15	0.24	−0.09**	0.14	−0.11**	1.49	−0.37**	3.31	−0.83**
n-7									
18:2 n-7ct	−4.5	0.32	0.38**	0.56	−0.27**	0.19	0.17*	0.09	0.05
Trans-FAs									
16:1 n-7t	31.0	0.19	0.39**	0.15	−0.07*	0.13	−0.13	0.12	0.29**
18:1 n-12t	56.5	0.50	0.26**	0.60	0.07*	0.28	0.11	0.23	0.45**
18:1 n-9t	45.5	0.75	0.10**	0.49	0.19**	0.24	−0.06	0.19	0.11
18:1 n-7t	44.0	1.15	0.32**	0.36	0.35**	0.18	−0.12	0.29	0.03
18:2 n-6tt	28.5	0.23	−0.15**	0.26	−0.34**	0.15	0.03	0.04	0.07
18:2 n-6ct	1.0	0.53	−0.30**	0.55	−0.38**	0.26	−0.14	0.11	−0.05
18:2 n-6tc	1.0	0.59	−0.18**	0.31	−0.29**	0.22	−0.08	0.10	−0.34**

Abbreviation: FA, fatty acid.

* $P < 0.05$; ** $P < 0.001$.

[a] Mean lipophilic index: diet, 28.63; adipose tissue, 23.61; plasma, 19.62; red blood cells, 25.11.

its low melting point (20, 21). VCAM-1 is released by endothelial cells in response to cytokines. Moreover, the fatty acids in adipose tissue are not affected by acute stimuli (28, 29). Because of these 2 factors, adipose tissue may not be the optimal substance for studying the association between the LI and VCAM-1.

Table 3. Mean Levels of Biomarkers for Cardiovascular Disease Among Controls, by Quintile of Lipophilic Index in Adipose Tissue and in Diet, Costa Rica, 1994–2004

Biomarker	No. of Controls	Quintile of Lipophilic Index										*P* for Trend
		1		2		3		4		5		
		Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Adipose tissue												
LDL cholesterol, mg/dL[a]	1,561	102	98, 107	106	102, 110	108	104, 113	108	103, 112	112	107, 116	0.001
HDL cholesterol, mg/dL[b]	1,600	52	51, 54	52	50, 53	52	50, 53	51	49, 52	52	50, 53	0.28
LDL:HDL cholesterol ratio[b]	1,561	2.0	1.9, 2.1	2.2	2.0, 2.3	2.2	2.1, 2.3	2.3	2.2, 2.4	2.3	2.2, 2.4	<0.001
Triglycerides, mg/dL[b]	1,615	133	125, 142	132	124, 141	136	127, 145	139	130, 148	146	136, 156	0.01
hs-CRP, mg/L[a]	1,322	3.0	2.6, 3.5	2.5	2.2, 2.9	2.5	2.2, 2.9	2.2	1.9, 2.5	1.8	1.6, 2.1	<0.001
VCAM-1, ng/mL[a]	1,359	717	685, 749	694	664, 725	688	658, 720	669	640, 700	655	626, 685	<0.001
Diet												
LDL cholesterol, mg/dL[a]	1,561	101	97, 106	108	104, 112	108	103, 112	109	105, 113	108	104, 112	0.03
HDL cholesterol, mg/dL[b]	1,600	53	52, 54	52	50, 53	52	50, 53	52	50, 53	50	48, 51	0.001
LDL:HDL cholesterol ratio[b]	1,561	2.0	1.9, 2.1	2.1	2.0, 2.2	2.2	2.1, 2.3	2.2	2.1, 2.4	2.3	2.2, 2.4	<0.001
Triglycerides, mg/dL[b]	1,615	134	125, 143	130	121, 139	137	128, 147	140	131, 150	142	133, 152	0.04
hs-CRP, mg/L[a]	1,322	2.4	2.1, 2.7	2.5	2.2, 2.9	2.5	2.1, 2.9	2.5	2.2, 2.9	2.2	1.9, 2.5	0.27
VCAM-1, ng/mL[a]	1,359	682	653, 714	671	641, 703	678	648, 709	678	648, 709	710	680, 742	0.10

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; VCAM-1, vascular cell adhesion molecule 1.

[a] Adjusted for age, sex, living area, physical activity, total energy intake, history of diabetes mellitus, history of hypertension, and smoking status.

[b] Adjusted for age, sex, living area, physical activity, total energy intake, history of diabetes mellitus, history of hypertension, smoking status, and current alcohol drinking status.

The LI offers an alternative way of examining fatty acids. Nevertheless, it has a few limitations. First, some *trans*-fatty acids make a negligible contribution to calculation of the LI. *Trans*-fatty acids have melting points close to 0°C (18), and the relative proportion of these fatty acids in biological specimens is small (30). However, it is known that dietary and adipose tissue *trans*-fatty acids are strong markers of an increased risk of coronary heart disease (31–33). A second limitation is that the estimated LI depends on the source of fatty acids; therefore, the pathophysiological meaning of the LI by itself is complex. For example, the level of 18:0 (stearic acid), a fatty acid with one of the highest melting points, is 2.2 times higher in red blood cells than in plasma. Moreover, it is positively associated with LI in red blood cells and inversely associated with LI in plasma. Furthermore, 20:4 n-6 (arachidonic acid), one of the fatty acids with the lowest melting points, is a major contributor to the LI in plasma (6%) and red blood cells (12%), but its contribution in diet and adipose tissue is negligible (0.29% in diet and 0.47% in adipose tissue). Thus, the correlations between disease and LIs derived from different sources should be interpreted cautiously. The LI calculated for dietary and adipose tissue fatty acids comprises mainly triglycerides and may not reflect membrane fluidity at all, even if an association between the LI and MI might be expected. The fatty acids most likely to represent membrane fluidity would be those measured in red blood cells. The final limitation lies in the nature of the associations themselves and in their relationship to disease. Positive associations between diet and adipose tissue, on the one hand, and diet and plasma, on the other, and inverse associations between diet and red blood cells all suggest that the LIs for adipose tissue and plasma

Table 4. Odds Ratios for Myocardial Infarction According to Quintile of Lipophilic Index in Adipose Tissue and in Diet, Costa Rica, 1994–2004

Model[a]	Quintile of Lipophilic Index										*P* for Trend
	1 (*n* = 650)		2 (*n* = 651)		3 (*n* = 651)		4 (*n* = 651)		5 (*n* = 651)		
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Adipose tissue[b]											
Model 1	1	Reference	0.97	0.77, 1.21	1.03	0.82, 1.30	1.13	0.90, 1.41	1.39	1.10, 1.76	0.002
Model 2	1	Reference	0.92	0.72, 1.18	1.07	0.83, 1.38	1.11	0.87, 1.43	1.30	1.00, 1.69	0.02
Model 3	1	Reference	0.91	0.71, 1.17	1.05	0.81, 1.35	1.06	0.82, 1.38	1.20	0.91, 1.59	0.12
Model 4	1	Reference	0.93	0.72, 1.19	1.12	0.87, 1.44	1.21	0.93, 1.57	1.47	1.12, 1.94	0.001
Model 5	1	Reference	0.93	0.72, 1.19	1.10	0.85, 1.42	1.16	0.88, 1.53	1.38	1.02, 1.86	0.01
Model 6	1	Reference	1.04	0.79, 1.38	1.25	0.91, 1.73	1.32	0.92, 1.89	1.60	1.07, 2.39	0.02
Diet[c]											
Model 1	1	Reference	1.38	1.11, 1.71	1.41	0.13, 1.77	1.69	1.35, 2.11	1.83	1.45, 2.30	<0.001
Model 2	1	Reference	1.32	1.04, 1.69	1.38	1.07, 1.77	1.46	1.14, 1.87	1.57	1.22, 2.02	<0.001
Model 7	1	Reference	1.59	1.11, 2.28	2.03	1.32, 3.13	2.37	1.44, 3.89	2.68	1.39, 5.16	0.002

Abbreviations: CI, confidence interval; MET, metabolic equivalent of task; OR, odds ratio; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

[a] Model 1: adjusted for matching factors. Model 2: additional adjustment for abdominal obesity (measured as waist:hip ratio), physical activity (METs/day, in quintiles), total caloric intake, history of hypertension, smoking (current smoking status, in tertiles), and alcohol intake (current alcohol intake, in tertiles). Model 3: model 2 + α-linolenic acid in adipose tissue (in quintiles). Model 4: model 2 + arachidonic acid in adipose tissue (in quintiles). Model 5: model 2 + α-linolenic acid in adipose tissue (in quintiles) + arachidonic acid in adipose tissue (in quintiles). Model 6: model 2 + PUFA:SFA ratio in adipose tissue (in quintiles). Model 7: model 2 + PUFA:SFA ratio in diet (in quintiles).

[b] Median values for each quintile: quintile 1—20.75; quintile 2—22.44; quintile 3—23.62; quintile 4—24.85; quintile 5—26.77.

[c] Median values for each quintile: quintile 1—23.31; quintile 2—26.30; quintile 3—28.53; quintile 4—31.81; quintile 5—35.69.

probably represent dietary fatty acid composition, whereas the LI in red blood cells most likely represents fatty acid metabolism. Therefore, studies on the association between LIs and disease using different biological specimens in the same population would provide more valuable insight into the markers that most likely reflect disease.

Besides these limitations of the LI, our study itself had 4 limitations. First, the prevalent case-control design impaired the ability to establish an objective temporal relationship between exposure and outcome. On the other hand, our study measured fatty acid levels in adipose tissue, which are unlikely to change with acute disease because of the slow turnover of adipose tissue (34, 35). Therefore, it is unlikely that our results for adipose tissue can be explained as reverse causality. Yet, as indicated above, this tissue may not be the most desirable for evaluation of LI, because it does not measure fatty acid levels in cell membranes. A second limitation of this study is that because of the potential for laboratory error, biomarkers are susceptible to measurement error. Third, melting points for 12 fatty acids were not available in the LipidBank database (18). Despite this limitation, fatty acids with available melting points accounted for an average of 98% of adipose tissue fatty acids and 98% of dietary fat. Thus, if the missing data were included, the results would probably remain unchanged. Finally, we cannot exclude the possibility of residual confounding as an explanation for our results. For example, hs-CRP was measured only among controls; therefore, for this inflammatory marker, we were unable to adjust the association between MI and the LI of dietary or adipose tissue. Nevertheless, the comprehensive information that was collected regarding potential confounders allowed adjustment for the main ones. Furthermore, point estimates and linear trends were consistent among the different multivariable models in suggesting a positive association. Therefore, residual confounding probably does not explain the observed results.

To our knowledge, this was the first study of the association between diet and adipose tissue LI and MI. Because of the comprehensive social services provided in Costa Rica, all persons living in the catchment area had access to medical care. Therefore, controls came from the source population that gave rise to the cases and were not likely to have had

Table 5. Spearman's Correlation Coefficients for Correlations Between Lipophilic Index in Diet and Different Biological Specimens Among 200 Controls With Data for Plasma and Red Blood Cells, Costa Rica, 1994–2004

Lipophilic Index	Lipophilic Index			
	Diet	Adipose Tissue	Plasma	RBC
Diet	1	0.21**	0.18**	−0.10
Adipose tissue	0.21**	1	0.31***	−0.002
Plasma	0.18**	0.31***	1	0.14*
RBC	−0.10	−0.002	0.14*	1

Abbreviation: RBC, red blood cells.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

undiagnosed cardiovascular disease attributable to poor access to medical care. Furthermore, participation was high among cases (98%) and controls (88%); thus, our findings are likely to represent the entire population from which the cases came.

In conclusion, the LI, an innovative tool for evaluating fatty acids by their ability to affect membrane fluidity in adipose tissue and in diet, was associated with MI. The association between MI and the LI could be mediated by plasma LDL cholesterol and triglyceride concentrations, which were positively associated with the LI in both adipose tissue and diet. Accordingly, the LI, if evaluated in addition to individual fatty acids, may aid in interpreting the results of studies that examine the role of fatty acids in health and disease. Additional research on the specific weight of certain fatty acids in the overall measure of lipophilicity and on the potential effects of the LI in different biological specimens is warranted.

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Appendix Table 1. Baseline Characteristics of Participants in a Case-Control Study of Nonfatal First Acute Myocardial Infarction According to Quintile of Lipophilic Index in Diet and Adipose Tissue, Costa Rica, 1994–2004

Characteristic	Quintile of Lipophilic Index											
	Diet						Adipose Tissue					
	1		3		5		1		3		5	
	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
Age, years	59.2 (10.3)		58.7 (11.3)		59.6 (10.8)		59.7 (11.2)		58.4 (10.9)		59.3 (10.6)	
Female sex		27		28		27		49		22		16
History of diabetes		20		21		18		19		20		26
History of hypertension		38		35		33		44		31		33
Current smoker		26		25		37		23		30		35
Current alcohol drinker		49		54		44		42		54		52
Waist:hip ratio	0.96 (0.07)		0.96 (0.08)		0.96 (0.07)		0.95 (0.08)		0.96 (0.07)		0.96 (0.07)	
Physical activity, METs/day	34.7 (13.5)		32.3 (13.6)		36.0 (17.6)		33.6 (12.9)		34.0 (14.9)		36.3 (18.3)	
Total energy intake, kcal/day	2,233 (585)		2,501 (660)		2,410 (641)		2,284 (639)		2,436 (615)		2,479 (637)	
Total fat intake (% of total energy intake)												
SFA	7.7 (1.6)		10.7 (2.0)		13.2 (2.3)		9.7 (2.6)		10.7 (2.8)		11.6 (2.8)	
MUFA	11.1 (4.9)		12.2 (3.6)		11.5 (2.2)		11.2 (3.4)		11.9 (3.9)		11.9 (3.6)	
PUFA	8.1 (2.0)		6.5 (1.3)		3.7 (0.7)		6.7 (2.0)		6.2 (2.0)		5.4 (2.0)	
Trans-fatty acids	1.0 (0.5)		1.5 (0.6)		1.0 (0.4)		1.3 (0.6)		1.3 (0.6)		1.2 (0.6)	
Dietary PUFA:SFA ratio	1.12 (0.28)		0.63 (0.09)		0.28 (0.04)		0.78 (0.32)		0.66 (0.32)		0.51 (0.28)	
Adipose tissue PUFA:SFA ratio	0.84 (0.21)		0.77 (0.20)		0.54 (0.14)		1.00 (0.18)		0.71 (0.12)		0.48 (0.10)	

Abbreviations: MET, metabolic equivalent of task; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SD, standard deviation; SFA, saturated fatty acids.