Clinical Nutrition (2007) **26**, 474–482

Available at www.sciencedirect.com

ScienceDirect

http://intl.elsevierhealth.com/journals/clnu

ORIGINAL ARTICLE

Adipose tissue arachidonic acid and the metabolic syndrome in Costa Rican adults

Eric S. Williams[a], Ana Baylin[b], Hannia Campos[a,c,*]

[a]*Department of Nutrition, Room 1-201, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA*
[b]*Department of Community Health, Brown University, USA*
[c]*Centro Centroamericano de Población, University of Costa Rica, Costa Rica*

Received 10 September 2006; accepted 18 March 2007

KEYWORDS
Metabolic syndrome;
Polyunsaturated fatty acids;
Arachidonic acid;
Abdominal obesity;
Insulin resistance

Summary
Background & aims: Arachidonic acid, a precursor to a series of inflammatory mediators, may contribute to the development of insulin resistance. We examined the association between adipose tissue arachidonic acid and the metabolic syndrome in Costa Rica, a country in which the metabolic syndrome is highly prevalent.
Methods: The 484 study participants each provided a fasting blood sample and an adipose tissue biopsy that was analyzed for fatty acid composition. Criteria for the metabolic syndrome were those established in the Third Report of the National Cholesterol Education Program Expert Panel. The data were analyzed by multivariate logistic regression.
Results: Subjects with greater adipose tissue arachidonic acid content had an increasing risk of the metabolic syndrome across quintiles: odds ratio (95% confidence interval), 1.00; 1.51 (0.78–2.91); 2.40 (1.26–4.55); 3.50 (1.84–6.66); and 6.01 (3.11–11.61); test for trend, $P<0.0001$, after adjustment for age, gender and area of residence. Further adjustment for metabolic risk factors, including adipose fatty acids and body mass index, did not significantly modify the result. Adipose tissue arachidonic acid was also independently associated with abdominal obesity, hypertriglyceridemia, elevated fasting glucose, and high blood pressure.
Conclusions: This study identifies arachidonic acid as an important independent marker of metabolic dysregulation. A better understanding of the role of this fatty acid in the pathogenesis of the metabolic syndrome is warranted.

Abbreviations: ATP III, Adult Treatment Panel; BMI, body mass index; FFQ, food frequency questionnaire; HDL, high-density lipoprotein; PPAR-γ, peroxisome proliferator-activated receptor-γ; PUFA, polyunsaturated fatty acid

*Corresponding author. Department of Nutrition, Room 1-201, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA. Tel.: +1 617 432 0100; fax: +1 617 432 2435.
E-mail address: hcampos@hsph.harvard.edu (H. Campos).

0261-5614/$ - see front matter
doi:10.1016/j.clnu.2007.03.004

Introduction

Arachidonic acid, an n-6 polyunsaturated fatty acid (PUFA), is a major component of mammalian cell membranes and may account for up to 25% of all phospholipid fatty acids.[1] Although it is consumed in the diet in meats, eggs, and some fish, it is also synthesized in the liver from linoleic acid, the most abundant dietary PUFA, and transported to other cell types via serum albumin or lipoproteins.[2] A major function of arachidonic acid is to serve as a precursor to the eicosanoid family of autocrine and paracrine hormones that modulates immune and inflammatory responses in the body.[3] Additionally, there is evidence that arachidonic acid may act as a transcriptional regulator by modulating signal transduction at the cell surface, by altering membrane fluidity, or cell–surface interactions by acylating membrane proteins.[4–7]

Arachidonic acid is the precursor of the 2-series of prostaglandins, which have greater biological activity than the 3-series prostaglandins derived from the n-3 PUFA, such as eicosapentaenoic and docosahexaenoic acids.[1] There is a suggestion that excessive production of n-6 eicosanoids from arachidonic acid, such as the 2-series prostaglandins, may give rise to pathophysiological signaling.[8] Indeed elevated tissue levels of arachidonic acid have been associated with a number of disease states, including coronary heart disease,[9,10] breast cancer,[11] obesity,[12,13] diabetes[14] and stress.[15]

Arachidonic acid can act as a strong negative modulator of glucose uptake,[16] and studies have demonstrated higher serum levels of arachidonic acid in diabetic subjects compared to normal matched controls.[17] However, the data on the subject have been conflicting, as other studies have shown a positive correlation between arachidonic acid and insulin sensitivity.[18–20] These inconsistencies are inherent in the large number of *in vitro* studies done on the subject, but epidemiological studies are scarce.

Insulin resistance is postulated to be responsible for the rapidly increasing incidence of the metabolic syndrome in the world population. Epidemic rates of obesity are a major contributor; however, genetic and dietary factors are also thought to be very important. The metabolic syndrome is associated with a high risk of coronary heart disease, diabetes and premature death, and is especially pervasive in many developing countries. Costa Rica is one such country in which the metabolic syndrome is highly prevalent, despite having *lower* rates of obesity than many industrialized nations. We conducted a population-based study in Costa Rica, a country with low intake of n-6 PUFA,[21] to test the hypothesis that arachidonic acid in adipose tissue is positively associated with the metabolic syndrome.

Materials and methods

Study population

Study participants were the 521 control subjects from a case-control study of heart disease conducted in Costa Rica between 1994 and 1998.[21] The control subjects were chosen via the Costa Rican National Census and Statistics Bureau at random throughout the greater San Jose area, which included 18 counties and comprised a full range of socioeconomic levels (although middle-income households predominate in Costa Rica) and urban (57%), suburban (28%) and rural (15%) lifestyles. The participation rate was 90%. All subjects gave written informed consent on forms approved by the Human Subjects Committee of the Harvard School of Public Health and the Ethics Committee of the University of Costa Rica. The total study area encompassed 2225 km^2 and 1,092,000 people who were culturally Hispanic American and ethnically Mestizo, as a result of four centuries of tripartite (white, Amerindian and black) racial mixing.

Data collection

The interview consisted of a general questionnaire of close-ended questions concerning socio-demographic characteristics, past medical history, family history of diabetes, current medication usage, socioeconomic status and smoking history. Self-reported diabetes and hypertension were validated as previously described.[22] Following the interviews, anthropometrical measurements (height, weight, blood pressure, waist/hip diameter and skinfold thickness) were collected by trained fieldworkers while subjects wore light clothing and no shoes. Additionally, biological specimens (subcutaneous fat aspiration and blood samples) were collected. Subcutaneous adipose biopsies were taken from the top of the buttock via a 16 gauge needle and stored in a 10 cc syringe, where it was immediately immersed in ice for later analysis. Blood samples were drawn in 0.1% EDTA after a 12–14 h fast. Blood tubes were immediately stored at 4 °C and shielded from light.

Dietary assessment

Dietary intake was collected using a food frequency questionnaire (FFQ) that had been developed and validated specifically to assess fatty acid intake among the Costa Rican population.[21,23] Dietary information from the FFQ was used to test whether the adipose tissue associations were independent from dietary intake.

Laboratory analysis

Fatty acids from adipose tissue samples were extracted from a hexane and isopropanol (3:2 by volume) mixture containing the sample and were esterified with methanol and acetyl chloride. After esterification, the methanol and acetyl chloride were evaporated, and the fatty acid methyl esters were quantified by gas–liquid chromatography as previously described.[24] Thirty-five out of 50 fatty acids analyzed were detected by this method. Twelve duplicate samples, indistinguishable from others, were analyzed throughout the study. The between-run coefficients of variation for linoleic and arachidonic acid were 5.5% and 11.2%, respectively.

Blood tubes were centrifuged within 6 h at 2500 rpm for 20 min to separate plasma. Plasma triglyceride, cholesterol and HDL cholesterol levels were assayed with enzymatic reagents (Boehringer–Mannheim). Cholesterol measurements were standardized according to the program specified by the Centers for Disease Control and the National Heart, Lung and Blood Institute.

Metabolic syndrome

As detailed in the third report of the Adult Treatment Panel (ATP III), subjects having three or more of the following criteria were defined as having the metabolic syndrome[25]:

- abdominal girth: waist girth > 102 cm in men or > 88 cm in women;
- hypertriglyceridemia: serum triglycerides ⩾1.69 mmol/L (150 mg/dl);
- low high-density lipoprotein cholesterol: serum HDL cholesterol < 1.03 mmol/L (40 mg/dl) in men or < 1.28 mmol/L (50 mg/dl) in women;
- high blood pressure: blood pressure ⩾130/85 mmHg;
- high fasting glucose: blood glucose levels ⩾5.6 mmol/L (100 mg/dl).

Subjects using antihypertensive or diabetic medication were considered to meet the criteria for high blood pressure or high fasting glucose, respectively. The fasting glucose cutoff was lowered to 100 mg/dl to reflect revised guidelines for impaired fasting glucose.[26]

Statistical analysis

Data analysis was performed using the Statistical Analysis Systems (SAS) software. There were 484 subjects included in the analysis after those with missing data points were excluded. After the data were checked for errors, outliers and distributions, means and frequencies for health characteristics and potential confounders were compared by using the Wilcoxon test. Multiple non-conditional logistic regression analysis was used to examine the effects of adipose tissue fatty acids on the metabolic syndrome; odds ratios and 95% confidence intervals for fatty acid quintiles were determined. Tests for trend were then performed across quintiles using the median value for each of the quintiles modeled as a continuous variable.

Results

Table 1 shows the characteristics of the study population, separated into those that did not meet the ATP III definition of the metabolic syndrome (no) and those that satisfied three or more of the clinical criteria for the syndrome (yes). Overall, the prevalence of the metabolic syndrome in this

Table 1 General characteristics of study participants with and without metabolic syndrome.

	Metabolic syndrome*				*P*
	No $n = 273$		Yes $n = 211$		
	Mean	SD	Mean	SD	
General characteristics					
Age, y	55	11	60	10	<0.0001
Gender, % female	20		35		0.0002
Residence, % rural	13		19		0.064
Current smoker, % ⩾1 cig/day	30		23		0.060
Income, USD/mo	600	493	528	486	0.08
Physical activity, METS†	1.49	0.80	1.45	0.73	0.91
Body mass index, kg/m2	24.5	3.3	27.7	4.2	<0.0001
Triceps skinfold, cm	16.0	8.7	21.0	10.9	<0.0001
Subscapular skinfold, cm	19.8	8.6	27.8	11.3	<0.0001
Suprailiac skinfold, cm	14.9	8.7	20.3	11.2	<0.0001
Fasting plasma lipids and blood glucose, mg/dL					
Total cholesterol	200	40	203	37	0.24
Total triglyceride	175	102	254	115	<0.0001
HDL cholesterol	45	12	38	9	<0.0001
Glucose	71	22	87	40	<0.0001
Individual components of the metabolic syndrome, %					
Abdominal obesity	5		37		<0.0001
Hypertriglyceridemia	48		92		<0.0001
Low HDL cholesterol	39		88		<0.0001
High blood pressure	45		93		<0.0001
High fasting glucose	4		34		<0.0001
Self-reported hypertension, % yes	13		45		<0.0001
Self-reported diabetes, % yes	4		22		<0.0001

*According to the Adult Treatment Panel III criteria.
†METS, metabolic equivalent task.

cross-sectional study was 43%, but higher in women than men. Compared with those without the metabolic syndrome, those with the syndrome were older and more likely to live in rural areas. Among the individual components of the metabolic syndrome, hypertriglyceridemia and high blood pressure were the most common, followed by low HDL and abdominal obesity. Table 2 summarizes dietary intake and fatty acid distribution in adipose tissue for the two groups. No significant differences in dietary intake were observed between subjects with and without the metabolic syndrome. In contrast, the adipose fatty acid distribution differed significantly between the two groups. Overall, subjects with the metabolic syndrome were characterized by lower saturated and higher monounsaturated fatty acid content in adipose tissue. Lower 18-carbon PUFA and higher long-chain PUFA were also characteristic of the metabolic syndrome regardless of whether the double bonds were at the n-3 or n-6 position.

The distribution of potential confounders by quintiles of adipose tissue arachidonic acid among subjects without the metabolic syndrome is shown in Table 3. Increased arachidonic acid in adipose was correlated with female gender and

Table 2 Dietary intake and adipose tissue fatty acids of study participants.

	Metabolic syndrome				*P*
	No $n = 273$		Yes $n = 211$		
	Mean	SD	Mean	SD	
Total calories, kcal	2368	748	2271	646	0.29
Carbohydrates, %energy	54.1	8.3	54.2	7.9	0.94
Protein, %energy	13.4	2.4	13.7	2.9	0.24
Total fat, %energy	33.2	6.1	33.4	5.9	0.69
Saturated fat, %energy	11.6	2.7	11.8	2.8	0.49
14:0	0.85	0.38	0.82	0.39	0.29
16:0	7.9	2.1	7.8	2.2	0.47
18:0	2.5	0.6	2.5	0.7	0.76
Monounsaturated fat, %energy	12.4	3.5	12.4	3.3	0.95
16:1n-9	0.45	0.15	0.44	0.16	0.35
18:1n-9	10.1	3.5	10.0	3.2	0.70
Polyunsaturated fat, %energy	5.6	1.5	5.6	1.6	0.51
18:2 n-6	5.0	1.5	5.0	1.8	0.53
18:3 n-6	0.03	0.03	0.03	0.03	0.89
20:4 n-6	0.09	0.04	0.09	0.05	0.23
18:3 n-3	0.48	0.13	0.47	0.13	0.71
20:5 n-3	0.04	0.03	0.04	0.02	0.18
22:6 n-3	0.07	0.06	0.07	0.06	0.19
Trans unsaturated fat, %energy	1.24	0.80	1.24	0.86	0.88
Cholesterol in mg/1000 cal	122	51	130	80	0.70
Fiber, g/1000 kcal	10.6	3.1	11.0	2.8	0.04
Sodium intake, mg/d	2118	756	1974	644	0.05
Alcohol intake, g/d	8.5	18.7	5.7	12.9	0.06
%alcohol users	57		54		0.46
Fatty acids in adipose tissue[a]					
14:0	1.03	0.49	1.01	0.46	0.77
16:0	21.7	2.67	21.6	2.76	0.59
18:0	3.14	1.04	2.77	0.93	<0.0001
16:1	5.92	2.07	6.68	2.24	0.0002
18:1	44.1	2.8	44.5	3.0	0.10
18:2 n-6	13.6	3.2	13.0	3.4	0.02
18:3 n-6	0.16	0.21	0.11	0.12	0.17
20:4 n-6	0.41	0.12	0.48	0.13	<0.0001
18:3 n-3	0.56	0.16	0.53	0.16	0.004
20:5 n-3	0.04	0.02	0.04	0.02	0.0005
22:6 n-3	0.16	0.05	0.18	0.06	0.002
Total *trans*	3.14	1.01	2.84	0.94	0.001

[a]Age-adjusted adipose tissue fatty acids presented as a percent of total fatty acids.

Table 3 Characteristics of study participants without the metabolic syndrome[a] by quintiles of adipose tissue arachidonic acid.

	Quintiles of arachidonic acid in adipose tissue					*P* for trend
	1	2	3	4	5	
General characteristics						
Median adipose arachidonic acid*	0.27	0.34	0.39	0.46	0.56	
Age, y	55	54	55	54	55	0.58
Gender, % female	13	13	13	28	33	0.0009
Area, % rural	17	13	11	4	18	0.80
Current smoker, % $\geqslant$ 1 cig/day	23	32	43	24	28	0.85
Income, USD/mo	655	700	470	512	629	0.45
Physical activity, METS	1.55	1.56	1.49	1.36	1.54	0.59
Body mass index, kg/m^2	23.1	24.0	24.2	25.7	25.7	$<$0.0001
Triceps skinfold, cm	13.1	13.4	15.2	19.2	19.1	$<$0.0001
Subscapular skinfold, cm	15.5	18.3	18.7	22.8	24.8	$<$0.0001
Suprascapular skinfold, cm	11.6	12.6	13.6	18.3	19.6	$<$0.0001
Fasting plasma lipids and blood glucose, mg/dL						
Total cholesterol	192	205	198	207	197	0.48
Total triglyceride	157	170	179	195	176	0.18
HDL cholesterol	43.6	43.2	44.6	45.6	46.5	0.04
Glucose	68.9	74.6	65.5	71.2	70.6	0.92
Dietary variables, %energy						
Carbohydrate	55.4	53.6	54.0	53.5	53.1	0.18
Protein	13.1	13.7	13.1	13.5	13.6	0.54
Total fat	32.6	34.2	34.0	33.5	32.6	0.92
Saturated fat	11.2	12.0	11.6	11.6	11.6	0.66
14:0	0.81	0.96	0.84	0.79	0.89	0.98
16:0	7.56	8.48	7.92	7.78	7.93	0.91
18:0	2.43	2.69	2.50	2.48	2.54	0.84
Monounsaturated fat	12.0	12.8	13.0	12.3	12.3	0.85
16:1n-9	0.43	0.49	0.45	0.47	0.47	0.53
18:1n-9	9.49	10.50	10.75	9.77	10.34	0.49
Polyunsaturated fat	5.8	5.7	5.8	5.9	5.2	0.13
18:2 n-6	5.3	5.0	5.1	5.4	4.6	0.12
18:3 n-6	0.04	0.03	0.03	0.04	0.03	0.52
20:4 n-6	0.09	0.09	0.09	0.10	0.10	0.21
18:3 n-3	0.51	0.47	0.47	0.49	0.46	0.17
20:5 n-3	0.05	0.04	0.04	0.04	0.04	0.59
22:6 n-3	0.08	0.06	0.07	0.07	0.09	0.62
Trans unsaturated fat	1.35	1.22	1.21	1.36	1.19	0.39
Fatty acids in adipose tissue						
14:0	1.18	0.94	1.06	0.98	0.93	0.02
16:0	22.61	22.12	21.74	21.33	20.43	$<$0.0001
17:0	0.23	0.24	0.22	0.19	0.19	$<$0.0001
18:0	3.59	3.53	3.15	2.72	2.65	$<$0.0001
16:1	5.05	5.20	5.74	6.54	7.06	$<$0.0001
18:1	42.92	44.44	44.07	44.09	45.10	0.001
18:2 n-6	14.36	13.64	13.67	13.72	12.88	0.03
18:3 n-6	0.16	0.18	0.16	0.14	0.16	0.99
18:3 n-3	0.63	0.57	0.56	0.56	0.50	$<$0.0001
20:5 n-3	0.03	0.03	0.04	0.04	0.05	$<$0.0001
22:6 n-3	0.13	0.15	0.15	0.16	0.19	$<$0.0001
Total *trans*	3.60	3.11	3.17	3.11	2.78	0.0002

$N = 273$.

[a]According to the Adult Treatment Panel III criteria.

*Adipose tissue fatty acids are presented as % of total fatty acids.

all measurements of increased adiposity. Arachidonic acid was inversely correlated with saturated fatty acids and 18-carbon PUFAs, and positively correlated with monounsaturated fatty acids and long-chain PUFAs.

Adipose tissue arachidonic acid was significantly associated with increased risk of the metabolic syndrome as shown in Table 4. The odds ratios became progressively higher across quintiles of arachidonic acid, a finding largely unaltered by adjustment for several dietary and non-dietary potential confounders. Adjustment for linoleic acid, a precursor to the n-6 family of PUFA, did not modify the association between arachidonic acid and the metabolic syndrome. Alpha-linolenic acid, which may have a positive impact on cardiovascular health, did not modify this association either. The results remained basically unchanged after the adjustment of other fatty acids including *trans* fatty acids and long-chain n-3 and n-6 PUFAs. Despite the observed association between several fatty acids and metabolic risk (Table 2), none of them except one (palmitoleic acid) remained significant in the multivariate model. Because of the strong association between body mass index (BMI) and the metabolic syndrome in Table 1, models were further adjusted for BMI. This attenuated the association between adipose tissue arachidonic acid and the metabolic syndrome somewhat; however, the association remained statistically significant (Table 4, model 6).

As the ATP III definition of the metabolic syndrome includes five distinct metabolic derangements, it is possible that the fatty acids studied affect each component to varying degrees, and even in varying directions. Table 5 repeats the logistic regression analysis to model each of the five defining components of the metabolic syndrome separately (abdominal girth, high triglycerides, low HDL cholesterol, high blood pressure, high fasting glucose). Odds ratios are shown for each quintile of fatty acid content in adipose tissue. The highest quintile of arachidonic acid corresponded with unfavorable odds ratios for every component of the syndrome, except low HDL cholesterol. Odds ratios were particularly exaggerated for abdominal girth with a 3-fold increase between the first and second quintile and an 18-fold increase overall.

Discussion

We conducted a population-based study of the metabolic syndrome in Costa Rica, applying the criteria established in ATP III. We found a strong positive association between adipose tissue arachidonic acid and the metabolic syndrome. The persistence of this association in multivariate analysis after adjustment for various potentially confounding variables attested that it was an independent correlate of the syndrome. Together with results from a prior study in this population that showed an association between adipose tissue arachidonic acid and non-fatal myocardial infraction, these data raise the concern that arachidonic acid may be detrimental to metabolic and cardiovascular health.[27–29]

Recent studies have highlighted the association between adipose tissue fatty acid composition and obesity.[12,13] In a study of obese Mediterraneans, central obesity was positively associated with n-6 PUFAs and inversely associated with monounsaturated fatty acids and n-3 PUFAs in adipose tissue.[13] Consistent with results from our study, a cross-sectional study of 88 children from Cyprus and Crete found that BMI was more strongly associated with arachidonic acid than with any other adipose tissue PUFA.[30] Of note is the striking association between arachidonic acid and abdominal obesity in our study, where those in the highest level of arachidonic acid in adipose tissue were almost 20 times more likely to have abdominal obesity. Supporting these

Table 4 Odds ratios for the metabolic syndrome by quintile of arachidonic acid in adipose tissue.

	Quintiles of adipose tissue arachidonic acid					*P* for trend
	1	2	3	4	5	
Median adipose arachidonic acid	0.280	0.360	0.428	0.489	0.625	
Ratio, cases/controls	22/74	31/66	41/56	52/45	65/32	
Age-, sex-, and residence-adjusted	1.00	1.51 (0.78, 2.91)	2.40 (1.26, 4.55)	3.49 (1.83, 6.66)	6.01 (3.11, 11.61)	<0.0001
Model 1	1.00	1.50 (0.78, 2.90)	2.44 (1.28, 4.65)	3.58 (1.87, 6.87)	6.16 (3.16,11.98)	<0.0001
Model 2	1.00	1.56 (0.80, 3.03)	2.47 (1.29, 4.73)	3.74 (1.94, 7.21)	6.12 (3.12,12.00)	<0.0001
Model 3	1.00	1.51 (0.78, 2.93)	2.36 (1.23, 4.52)	3.43 (1.78, 6.61)	5.55 (2.82, 10.92)	<0.0001
Model 4	1.00	1.45 (0.74, 2.83)	2.33 (1.22, 4.47)	3.38 (1.75, 6.54)	5.67 (2.88, 11.13)	<0.0001
Model 5	1.00	1.49 (0.76, 2.93)	2.36 (1.22, 4.57)	3.49 (1.78, 6.84)	5.68 (2.84, 11.34)	<0.0001
Model 6	1.00	1.17 (0.58, 2.39)	1.59 (0.79, 3.21)	1.93 (0.93, 3.99)	2.72 (1.28, 5.76)	0.004

Model 1: adjusted for smoking, physical activity, income. Further adjustment for intake of saturated fat, carbohydrate, alcohol, fiber, folate, and use of vitamin E or multivitamins did not modify the results.
Model 2 : Model 1 plus linoleic in adipose.
Model 3: Model 1 plus linolenic in adipose.
Model 4: Model 1 plus *trans* in adipose.
Model 5: Model 1 plus significant adipose tissue fatty acid confounders.
Model 6: Model 5 plus BMI.

Table 5 Odds ratios for components of the metabolic syndrome by quintile of arachidonic acid in adipose tissue.

	Quintiles of adipose tissue arachidonic acid				
	1	2	3	4	5
Median adipose arachidonic acid	0.280	0.360	0.428	0.489	0.625
Basic model					
Adjusted for age, sex and area of residence					
Abdominal obesity	1.00	4.37 (0.89,21.46)	7.59 (1.65,34.96)	16.49 (3.75,72.58)	22.86 (5.22,100.2)
High triglycerides	1.00	2.37 (1.31, 4.29)	1.91 (1.07, 3.42)	3.56 (1.89, 6.68)	3.40 (1.81, 6.38)
Low HDL cholesterol	1.00	1.37 (0.77, 2.42)	2.13 (1.18, 3.83)	1.51 (0.84, 2.70)	1.74 (0.96, 3.13)
High blood pressure	1.00	1.00 (0.53, 1.88)	1.39 (0.74, 2.62)	2.42 (1.23, 4.76)	2.63 (1.31, 5.27)
High fasting glucose	1.00	1.51 (0.78, 2.915)	2.40 (1.26, 4.55)	3.50 (1.84, 6.66)	6.01 (3.11, 11.61)
Multivariate model					
Adjusted for significant confounders*					
Abdominal obesity	1.00	4.41 (0.87,22.50)	6.52 (1.37,30.94)	14.07 (3.09,63.99)	19.18 (4.20,87.56)
High triglycerides	1.00	2.28 (1.23, 4.22)	1.86 (1.02, 3.42)	3.74 (1.91, 7.31)	3.47 (1.77, 6.80)
Low HDL cholesterol	1.00	1.19 (0.66, 2.16)	2.09 (1.14, 3.85)	1.51 (0.81, 2.81)	1.80 (0.96, 3.39)
High blood pressure	1.00	1.00 (0.52, 1.95)	1.27 (0.65, 2.48)	2.10 (1.01, 4.34)	2.25 (1.06, 4.78)
High fasting glucose	1.00	1.49 (0.76, 2.93)	2.36 (1.22, 4.57)	3.49 (1.78, 6.84)	5.68 (2.84, 11.34)

*This refers to Model 5 shown in Table 4 that includes age, sex and residence plus smoking, physical activity, income and significant adipose tissue fatty acid confounders.

observations in humans, studies in mice have shown that arachidonic acid can promote the differentiation of clonal preadipocytes, and it is hypothesized that the prostaglandin I_2 (prostacyclin) receptor with subsequent cyclic AMP production and upregulation of peroxisome proliferator-activated receptor-γ (PPAR-γ) plays a role in this adipogenesis pathway.[27]

A group of investigators that studied age-related changes in the fatty acid composition of adipose tissue reported a decrease in Δ-6 desaturase activity with age, which was more marked in women than in men. Along with this change, the relative content of arachidonic acid in adipose tissue progressively increased with age in women but not in men in the study.[31] This is consistent with our finding that women have significantly higher levels of adipose tissue arachidonic acid than men. Women in our study were also more likely to meet criteria for the metabolic syndrome than men, even after adjustment for age and other potential confounders in multivariate analysis (data not shown).

The adverse health outcomes associated with arachidonic acid in our study may be specific to adipose tissue, as phospholipid arachidonic acid in muscle has been positively associated with insulin sensitivity.[18] Other studies have shown that arachidonic acid in platelets or plasma is lower in patients with heart disease than in controls.[28,29] Therefore, it is possible that individual tissues (adipose, plasma, erythrocytes) may represent different metabolic pools of arachidonic acid and findings in one tissue may not be generalizable.

In the present study, there were no large differences in arachidonic acid intake between those with and without the metabolic syndrome, as roughly assessed by the food frequency questionnaire (FFQ). In spite of this, significant differences were observed in arachidonic acid in adipose tissue. This finding is consistent with a recent study showing no correlation between dietary arachidonic acid and that in plasma, adipose or whole blood.[32] Thus, tissue differences in arachidonic acid between subjects are most likely attributable to individual differences in metabolism.

Our study has some limitations. First, to the authors' best knowledge, the ATP III definition of the metabolic syndrome has not been previously validated in the Costa Rican population, and there has been a concern that the criteria used may not be applicable to all populations, such as Asians, for example.[33,34] However, the ATP III definition has been used prospectively in several diverse American cohorts, which have included Latin American subjects.[35,36] Additionally, although the association of arachidonic acid with the metabolic syndrome is suggestive, the cross-sectional nature of this study precludes any inference of causality. Also, though the odds ratios calculated in the study were adjusted for potential confounders, we cannot rule out the potential for residual confounding from other variables either measured or unmeasured in our study. Of note, adipose tissue arachidonic acid in our study correlates considerably with BMI ($r = 0.40$),[9] which confounds to some degree its association with the metabolic syndrome. The metabolic syndrome is inexorably intertwined with obesity, and in light of this, our statistical models were further adjusted for BMI as a marker of obesity. Despite this, however, the association between adipose tissue arachidonic acid and metabolic syndrome in our study remained significant.

The implications of our finding associating adipose tissue arachidonic acid with the metabolic syndrome are 2-fold. First, a deeper understanding of the genetic and metabolic factors that influence arachidonic acid levels in adipose is warranted. Second, further studies on the role of increased adipose tissue fatty acids in the pathogenesis of the metabolic syndrome can clarify whether the increased

content of arachidonic acid we measured in subjects with the syndrome is a cause or a consequence of metabolic dysregulation in the body.

Conclusion

These findings associate increased adipose tissue arachidonic acid with a high prevalence of the metabolic syndrome. This association is probably not related to dietary arachidonic acid consumption and is likely the result of a complex interaction of presently unknown genetic and metabolic factors. Further studies in this regard to understand the high prevalence of the metabolic syndrome in Costa Rica will have a significant impact on public health in this aging population.

Acknowledgments

This work was supported by Grants HL 60692 and HL 071888 from the National Institutes of Health. ESW was supported by the Foreign Language and Area Studies grant from the U.S. Department of Education and a traveling fellowship from Harvard Medical School. We are grateful to Xinia Siles for data collection and study management, to the study participants, to the fieldworkers of Proyecto Salud Coronaria in San Jose, Costa Rica, and to the Costa Rican National Census and Statistics Bureau for making the recruitment of our study subjects possible.

Contributors: Hannia Campos (principal investigator, study design, grant application, project supervision, manuscript editing), Ana Baylin (data management and analysis, manuscript editing), Eric Sean Williams (data analysis, interpretation and preparation of the manuscript).

Disclosures: The authors of this paper have no financial disclosures or conflicts of interest.

References

1. Zhou L, Nilsson A. Sources of eicosanoid precursor fatty acid pools in tissues. *J Lipid Res* 2001;**42**:1521–42.
2. Simopoulos AP. Essential fatty acids in health and chronic disease. *Am J Clin Nutr* 1999;**70**:560S–9S.
3. Habenicht AJ, Salbach P, Goerig M, et al. The LDL receptor pathway delivers arachidonic acid for eicosanoid formation in cells stimulated by platelet-derived growth factor. *Nature* 1990;**345**:634–6.
4. Gearing KL, Gottlicher M, Widmark E, et al. Fatty acid activation of the peroxisome proliferator activated receptor, a member of the nuclear receptor gene superfamily. *J Nutr* 1994;**124**:1284S–8S.
5. Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. *J Nutr* 2001;**131**:1129–32.
6. Ginsberg BH, Jabour J, Spector AA. Effect of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. *Biochim Biophys Acta* 1982;**690**:157–64.
7. Hallak H, Muszbek L, Laposata M, Belmonte E, Brass LF, Manning DR. Covalent binding of arachidonate to G protein alpha subunits of human platelets. *J Biol Chem* 1994;**269**:4713–6.
8. Rustan AC, Nenseter MS, Drevon CA. Omega-3 and omega-6 fatty acids in the insulin resistance syndrome. Lipid and lipoprotein metabolism and atherosclerosis. *Ann N Y Acad Sci* 1997;**827**:310–26.
9. Baylin A, Campos H. Arachidonic acid in adipose tissue is associated with nonfatal acute myocardial infraction in the central valley of Costa Rica. *J Nutr* 2004;**134**:3095–9.
10. Kark JD, Kaufmann NA, Binka F, Goldberger N, Berry EM. Adipose tissue n-6 fatty acids and acute myocardial infraction in a population consuming a diet high in polyunsaturated fatty acids. *Am J Clin Nutr* 2003;**77**:796–802.
11. Bagga D, Anders KH, Wang HJ, Glaspy JA. Long-chain n-3-to-n-6 polyunsaturated fatty acid ratios in breast adipose tissue from women with and without breast cancer. *Nutr Cancer* 2002;**42**: 180–5.
12. Decsi T, Molnar D, Koletzko B. Long-chain polyunsaturated fatty acids in plasma lipids of obese children. *Lipids* 1996;**31**: 305–11.
13. Garaulet M, Perez-Llamas F, Perez-Ayala M, et al. Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. *Am J Clin Nutr* 2001;**74**:585–91.
14. Pelikanova T, Kazdova L, Chvojkova S, Base J. Serum phospholipid fatty acid composition and insulin action in type 2 diabetic patients. *Metabolism* 2001;**50**:1472–8.
15. Deby-Dupont G, Ducarne H, de Landsheere C, et al. Intense rises of unesterified arachidonate plasma levels in stressed humans. *Biomed Pharmacother* 1983;**37**:386–91.
16. Tebbey PW, McGowan KM, Stephens JM, Buttke TM, Pekala PH. Arachidonic acid down-regulates the insulin-dependent glucose transporter gene (GLUT4) in 3T3-L1 adipocytes by inhibiting transcription and enhancing mRNA turnover. *J Biol Chem* 1994;**269**:639–44.
17. Simopoulos AP. Omega-6/omega-3 fatty acid ratio and trans fatty acids in non-insulin-dependent diabetes mellitus. *Ann N Y Acad Sci* 1997;**827**:327–38.
18. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N Engl J Med* 1993;**328**:238–44.
19. Storlien LH, Kriketos AD, Calvert GD, Baur LA, Jenkins AB. Fatty acids, triglycerides and syndromes of insulin resistance. *Prostaglandins Leukot Essent Fatty Acids* 1997;**57**:379–85.
20. Clifton PM, Nestel PJ. Relationship between plasma insulin and erythrocyte fatty acid composition. *Prostaglandins Leukot Essent Fatty Acids* 1998;**59**:191–4.
21. Baylin A, Kabagambe EK, Siles X, Campos H. Adipose tissue biomarkers of fatty acid intake. *Am J Clin Nutr* 2002;**76**:750–7.
22. Campos H, Siles X. Siesta and the risk of coronary heart disease: results from a population-based, case-control study in Costa Rica. *Int J Epidemiol* 2000;**29**:429–37.
23. Kabagambe EK, Baylin A, Allan DA, Siles X, Spiegelman D, Campos H. Application of the method of triads to evaluate the performance of food frequency questionnaires and biomarkers as indicators of long-term dietary intake. *Am J Epidemiol* 2001;**154**:1126–35.
24. Lillington JM, Trafford DJ, Makin HL. A rapid and simple method for the esterification of fatty acids and steroid carboxylic acids prior to gas–liquid chromatography. *Clin Chim Acta* 1981;**111**: 91–8.
25. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *J Am Med Assoc* 2001;**285**: 2486–97.
26. Hanley AJ, Karter AJ, Williams K, et al. Prediction of type 2 diabetes mellitus with alternative definitions of the metabolic syndrome: the Insulin Resistance Atherosclerosis Study. *Circulation* 2005;**112**:3713–21.

27. Massiera F, Saint-Marc P, Seydoux J, et al. Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern? *J Lipid Res* 2003;**44**:271–9.
28. Wood DA, Riemersma RA, Butler S, et al. Linoleic and eicosapentaenoic acids in adipose tissue and platelets and risk of coronary heart disease. *Lancet* 1987;**1**:177–83.
29. Wang L, Folsom AR, Eckfeldt JH. Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Nutr Metab Cardiovasc Dis* 2003;**13**:256–66.
30. Savva SC, Chadjigeorgiou C, Hatzis C, Kyriakakis M, Tsimbinos G, Tornaritis M, Kafatos A. Association of adipose tissue arachidonic acid content with BMI and overweight status in children from Cyprus and Crete. *Br J Nutr* 2004;**91**:643–9.
31. Bolton-Smith C, Woodward M, Tavendale R. Evidence for age-related differences in the fatty acid composition of human adipose tissue, independent of diet. *Eur J Clin Nutr* 1997;**51**: 619–24.
32. Baylin A, Kim MK, Donovan-Palmer A, et al. Fasting whole blood as a biomarker of essential fatty acid intake in epidemiologic studies: comparison with adipose tissue and plasma. *Am J Epidemiol* 2005;**162**:373–81.
33. Rosenbaum P, Gimeno SG, Sanudo A, Franco LJ, Ferreira SR. Analysis of criteria for metabolic syndrome in a population-based study of Japanese-Brazilians. *Diabetes Obes Metab* 2005;**7**:352–9.
34. Kanauchi M, Kawano T, Kanauchi K, Saito Y. New ''pre-diabetes'' category and the metabolic syndrome in Japanese. *Horm Metab Res* 2005;**37**:622–6.
35. Lorenzo C, Okoloise M, Williams K, Stern MP, Haffner SM. The metabolic syndrome as predictor of type 2 diabetes: the San Antonio heart study. *Diabetes Care* 2003;**26**:3153–9.
36. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *J AM Med Assoc* 2002;**287**:356–9.