

Adipose tissue biomarkers of fatty acid intake¹⁻³

Ana Baylin, Edmond K Kabagambe, Xinia Siles, and Hannia Campos

ABSTRACT

Background: Biomarkers can provide a more accurate measure of long-term intake than can dietary questionnaires.

Objective: The objective was to identify which adipose tissue fatty acids are suitable biomarkers of intake as assessed with a validated food-frequency questionnaire.

Design: Costa Rican men with a mean (\pm SD) age of 56 ± 11 y ($n = 367$) and women aged 60 ± 10 y ($n = 136$) completed a 135-item food-frequency questionnaire and provided an adipose tissue sample. Fifty fatty acids were identified by capillary gas chromatography. Correlation coefficients were calculated after adjustment for age, sex, body mass index, and smoking status.

Results: The best adipose tissue marker for total intake of saturated fatty acids was 15:0 + 17:0 ($r = 0.18$). Both 15:0 and 17:0 were also the best correlates of dairy product intake ($r = 0.31$ for each). The diet-adipose tissue correlations for n-3 fatty acids were $r = 0.34$ for 18:3, $r = 0.15$ for 20:5, and $r = 0.18$ for 22:6. Fish intake correlated significantly with these adipose tissue n-3 fatty acids. Dietary and adipose tissue n-6 fatty acids were highly correlated: 18:2 ($r = 0.58$) and 18:3 ($r = 0.24$). The best indicators of total *trans* fatty acid intake were *ct*18:2n-6 and *tc*18:2n-6 ($r = 0.58$ for each); total 18:1 *trans* fatty acid ($r = 0.45$) and 16:1 *trans* fatty acid ($r = 0.16$) were the next best indicators.

Conclusions: Adipose tissue is a suitable biomarker of dietary fatty acid intake, particularly for n-3 and n-6 *cis* polyunsaturated fatty acids and *trans* fatty acids. Ideally, adipose tissue and dietary questionnaires should complement, rather than substitute for, each other in epidemiologic studies. *Am J Clin Nutr* 2002;76:750-7.

KEY WORDS Fatty acids, biomarkers, n-3 fatty acids, *trans* fatty acids, adipose tissue, dietary questionnaire, Hispanics

INTRODUCTION

The use of biomarkers to assess dietary intake has increased dramatically in the past few years (1-7). Biomarkers may provide a more accurate and objective measure of long-term intake than dietary questionnaires provide because biomarkers do not rely on memory, self-reported information, or interviewer bias. However, nutrient concentrations in tissue or blood do not always reflect dietary intake because they can be affected by genetic factors, smoking, obesity, physical activity, and metabolism (5).

Adipose tissue is an attractive choice for the study of long-term fatty acid intake because of its slow turnover (8-10) and lack of

responsiveness to acute disease (11, 12). Fatty acids that cannot be synthesized endogenously from carbohydrates are the best candidates for biomarkers of fatty acid intake. These are polyunsaturated fatty acids (PUFAs: n-3 and n-6), *trans* fatty acids, and odd-numbered and branched-chain fatty acids (5). Conversely, saturated fatty acids (SFAs) (except for odd-numbered fatty acids) and monounsaturated fatty acids (MUFAs) are not expected to reflect intake. Their distribution in adipose tissue is still of interest, however, because they may be predictors of disease risk.

In the past, most studies on biomarkers have focused on just a few fatty acids, such as linoleic acid or long-chain n-3 fatty acids (6, 8, 13, 14), even though it is well known that other fatty acids may play an important role in human health (5, 15). Furthermore, the most complete studies on fatty acids were done in developed countries (16-18). The population profile of fatty acids in developing countries deserves attention because the pattern of food intake is changing rapidly and the intake of *trans* fatty acids, in the form of partially hydrogenated vegetable oils, is increasing (19).

In this report, 35 detectable fatty acids out of 50 fatty acids identified by capillary gas chromatography were analyzed to determine which adipose tissue fatty acids most accurately reflect intake as assessed with a validated food-frequency questionnaire (FFQ) (20). We also describe the pattern of fatty acids in adipose tissue and the diet of the Costa Rican population as a reference for future studies in other developing countries.

SUBJECTS AND METHODS

Study population

Participants were the 521 control subjects from a case-control study of diet and heart disease in Costa Rica. Through the use of information available at the Costa Rican National Census and Statistics Bureau, the control subjects were randomly selected to match the cases by age, sex, and area of residence. The catchment area included 18 counties comprising a full range of socioeconomic levels (although middle-income households predominate in Costa Rica) and urban (57%), suburban (28%), and rural (15%)

¹ From the Department of Nutrition, Harvard School of Public Health, Boston (AB, EKK, and HC), and the Proyecto Salud Coronaria, Centro Centroamericano de Población, Universidad de Costa Rica, San José (XS and HC).

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³ No reprints available. Address correspondence to H Campos, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Building 2, Room 353A, Boston, MA 02115. E-mail: hcampos@hsph.harvard.edu. Received August 16, 2001.

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lifestyles (21). The total study area comprised 2225 km² and a population of 1 092 000 persons who are culturally Hispanic American and ethnically Mestizo, as a result of 4 centuries of tripartite (white, Amerindian, and black) racial mixing (22). Subjects were visited at their homes for recruitment and data collection, and 90% of those eligible participated. Subjects were ineligible if they reported a past diagnosis of myocardial infarction or if they were physically or mentally unable to answer the questionnaire because of a stroke or other serious illness. All subjects gave written, informed consent on forms approved by the Ethics Committee of the Harvard School of Public Health and the University of Costa Rica.

Data collection

We collected information about sociodemographic characteristics, smoking status, socioeconomic status, dietary intake, and medical history (including a personal history of diabetes and hypertension). Trained fieldworkers collected anthropometric measurements of the subjects while they wore light clothing and no shoes. Measurements were performed in duplicate, and the average was used for analysis. Height was measured with steel anthropometers (GPM Anthropological Instruments, Zurich, Switzerland). To ensure correct readings (to the nearest 0.1 cm) for height, subjects were always positioned against a wall. Weight was measured to the nearest 0.25 kg with the use of mechanical portable scales (Seca, Hamburg, Germany) that were calibrated biweekly. Body mass index (in kg/m²) was calculated.

Biological specimens were collected at each subject's home on the morning after an overnight fast. A subcutaneous adipose tissue sample was collected from the upper buttock with a 16-gauge needle and disposable syringe according to previously described biopsy procedures (23). The adipose tissue samples were stored in a cooler with ice packs at 4 °C and transported to the fieldwork station within 4 h. Frozen adipose tissue was stored at -80 °C, and within 6 mo it was transported over dry ice to the Harvard School of Public Health for analysis.

Dietary assessment

Dietary information was obtained by using a semiquantitative FFQ, which was a modification of the Willett questionnaire (24). The questionnaire included specific items from the Costa Rican diet (25), a typical Central American diet based on rice, beans, plantains, corn tortillas, small salads with chopped vegetables, meat, eggs, and cheese. Although some Western dietary influences are noticed, the study population still prepares and eats most meals at home; 60% reported eating outside of the home < 1 time/wk. The FFQ developed for the Costa Rican population included questions about the intake of 135 food items and 20 vitamin, mineral, and food supplements; the types of fatty acid used for cooking and frying; the consumption of fried foods in the home and elsewhere; and food habits related to meat consumption. Subjects selected the type of fatty acid or oil that was most frequently used for cooking, frying, or baking at home. The questionnaire was administered by trained interviewers.

Nutrient intake was computed by multiplying the consumption frequency of each food by the nutrient content of the specific portion and using composition values from the US Department of Agriculture (26) supplemented with data from food manufacturers and published reports.

In a subset of 120 subjects, we carried out a validation study to compare the FFQ with an assessment of dietary intake

in 7-d, 24-h food recalls (20). For reproducibility, a second FFQ was administered 1 y after the first interview. The Pearson partial correlation coefficients for total fatty acid, SFAs, MUFAs, and PUFAs between the averages of the 2 FFQ assessments and 7-d, 24-h food recall assessments were 0.74, 0.71, 0.64, and 0.75, respectively; they were 0.46, 0.60, 0.47, and 0.59, respectively, between the first and second FFQs ($P < 0.0001$ for all). The ability of the FFQ to assess cholesterol and fatty acid intake was also validated previously by its ability to predict concentrations of plasma lipids and apolipoproteins (25).

Fatty acid analysis

Fatty acids from adipose tissue were extracted from a hexane and isopropanol (3:2 by vol) mixture containing the sample and were esterified with methanol and acetyl chloride as described by Lillington et al (27). After esterification, the methanol and acetyl chloride were evaporated, and the fatty acid methyl esters were redissolved in isooctane. The methyl esters were quantitated by gas-liquid chromatography under the following conditions: fused silica, capillary *cis/trans* column (100 m × 250 mm internal diameter, with a 0.20- μ m film; SP2560; Supelco, Bellefonte, PA); splitless injection port at 240 °C; hydrogen carrier gas at a constant flow of 1.3 mL/min; Hewlett-Packard (now Agilent) Model GC 6890 FID gas chromatograph with 7673 Autosampler injector (Palo Alto, CA); 1 mL injected sample; temperature program of 90–170 °C at 10 °C/min, 170 °C for 5 min, 170–175 °C at 5 °C/min, 175–185 °C at 2 °C/min, 185–190 °C at 1 °C/min, 190–210 °C at 5 °C/min, 210 °C for 5 min, 210–250 °C at 5 °C/min, and 250 °C for 10 min. Peak retention times and area percentages of total fatty acid were identified by injecting known standards (NuCheck Prep, Elysium, MN) and were analyzed with CHEMSTATION A.08.03 software (Agilent Technologies). Fifty fatty acids were analyzed, but only 35 were detected. Fatty acids not detected were 19:0, 22:1, 21:0, *c*24:1n-9, 23:0, *c*22:2n-6, *c*15:1n-5, *c*22:3n-3, *c*17:1n-7, *t*18:3n-3, *c*18:1n-12, *t*20:1n-9, *c*19:1n-9, *t*20:2n-6, and 20:1n-7.

Blinded (indistinguishable from other samples) duplicate samples ($n = 12$) were analyzed throughout the study. The range of CVs for these samples was 3.0–35.6%. In general, peaks near the sensitivity limit (≈ 0.10 of the total area) had larger CVs. The CVs for the most abundant fatty acids were 5.4% for palmitic acid (16:0), 16.0% for stearic acid, 3.0% for oleic acid (18:1n-9), 8.5% for α -linolenic acid (18:3n-3), 5.5% for linoleic acid (18:2n-6), 14.2% for eicosapentaenoic acid (EPA; 20:5n-3), 14.5% for docosahexaenoic acid (DHA; 22:6n-3), 15.7% for 18:1 *trans* fatty acid, and 6.4% for 18:2 *trans* fatty acid.

Statistical analyses

Spearman correlation coefficients adjusted for potential confounders (age, sex, body mass index, and smoking status) were calculated to determine associations between dietary and tissue fatty acids. Spearman correlations and the Wilcoxon rank-sum test were used to identify associations between fatty acid values in adipose tissue or the diet and potential confounders. Dietary and tissue values for fatty acids were normalized when appropriate by the usual transformations (\log_e and square root). Fatty acids in adipose tissue are described as a percentage of the total fatty acids analyzed. Dietary fatty acids are described as a percentage of total fat, a percentage of total energy, and energy-adjusted grams per day.

TABLE 1
General characteristics of the study population¹

| | Men (n = 367) | Women (n = 136) |
|--------------------------|---------------|-----------------|
| Age (y) | 56 ± 11 | 60 ± 10 |
| BMI (kg/m ²) | 25.7 ± 3.9 | 26.3 ± 4.4 |
| Weight (kg) | 71.7 ± 13.1 | 62.1 ± 10.7 |
| Height (cm) | 166.7 ± 6.9 | 153.7 ± 6.6 |
| Total energy (MJ/d) | 9.97 ± 3.02 | 8.99 ± 2.45 |
| Total fat | | |
| (g/d) | 87.0 ± 29.0 | 81.8 ± 25.8 |
| (% energy) | 33.0 ± 6.0 | 34.4 ± 6.1 |

¹ $\bar{x} \pm SD$.

Fatty acid intakes were adjusted for energy by calculating the residuals from a regression of dietary intakes on total energy intake (5). Correlations were conducted with energy-adjusted intakes and with fatty acids expressed as a percentage of total fat. Multiple linear regression models were used to test for trends across diet deciles and to compare the average values for all tissue fatty acids at each decile of the dietary fatty acids and were adjusted for age, sex, BMI, and smoking status. We tested trends by assigning the median value of the corresponding decile to each subject and entering that variable in the model as a continuous variable. Robust estimators of variance were used in the regression models, which eliminated the need to normalize the dependent variable (28). These multiple linear regression models allowed us to describe the relation between dietary and adipose tissue fatty acid without being restricted by assumptions of linearity. Plots from this analysis provide a visual description of that relation. To identify potential food predictors of adipose tissue fatty acids, we performed stepwise linear regression analysis.

All variables were examined for outliers, and erroneous values were corrected if possible and deleted if not. To ensure that results were not sensitive to these values, analyses were repeated with and without outliers that were not obvious errors. Of the 521 subjects recruited, those for whom adipose tissue samples were missing ($n = 16$) or who had outlier values in any dietary fatty acid ($n = 2$) were excluded. The final sample of 503 subjects consisted of 367 men with a mean (\pm SD) age of 56 ± 11 y and 136 women aged 60 ± 10 y. The following minor fatty acids in adipose tissue had missing data because they could not be resolved in the chromatogram (20:0, $n = 97$; 22:0, $n = 163$; 18:3n-6, $n = 98$; and 20:3n-6, $n = 30$). Therefore, the sample size for the specific analysis for these fatty acids was smaller. All data were analyzed with SAS software, version 8 (SAS Institute Inc, Cary, NC).

RESULTS

The general characteristics of this population—anthropometric measurements and total fat and energy intakes—are shown in **Table 1**. The distribution of fatty acids in adipose tissue and in the diet is shown in **Table 2**. Among SFAs, palmitic acid accounted for 22% in adipose tissue and 27% in diet. MUFAs, mostly oleic acid, accounted for the largest proportion of total fatty acid in both adipose tissue (54%) and diet (42%). PUFAs showed a similar proportion in adipose tissue and diet (16% and 18%, respectively). Linoleic acid represented most of the n-6 PUFAs, and α -linolenic acid represented most of the n-3 PUFAs. The n-3 PUFAs of marine origin (EPA and DHA) represented a small percentage of the total n-3 PUFAs in both adipose tissue and diet. Total *trans*

TABLE 2
Distribution of fatty acids in adipose tissue and in the diet¹

| | Adipose tissue % of total fatty acids | Diet | |
|-----------------------------|--|----------------|------------------------|
| | | Total fat % | Energy-adjusted g/d |
| Saturated fatty acids | 26.21 ± 3.42 | 36.43 ± 5.95 | 25.93 ± 6.13 |
| 12:0 | 0.02 ± 0.03 | 0.66 ± 0.50 | 0.47 ± 0.38 |
| 14:0 | 1.02 ± 0.41 | 3.12 ± 1.45 | 2.24 ± 1.16 |
| 15:0 | 0.19 ± 0.07 | 0.02 ± 0.03 | 0.01 ± 0.02 |
| 16:0 | 21.60 ± 2.67 | 27.01 ± 4.66 | 19.52 ± 4.17 |
| 17:0 | 0.21 ± 0.06 | 0.04 ± 0.02 | 0.03 ± 0.01 |
| 18:0 | 2.98 ± 0.96 | 5.53 ± 1.53 | 3.98 ± 1.41 |
| 20:0 | 0.19 ± 0.10 | 0.01 ± 0.01 | 0.01 ± 0.00 |
| 22:0 | 0.03 ± 0.05 | 0.03 ± 0.03 | 0.02 ± 0.02 |
| 24:0 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 |
| Monounsaturated fatty acids | 54.08 ± 4.38 | 42.20 ± 4.51 | 30.54 ± 8.78 |
| 14:1n-5 | 0.14 ± 0.09 | 0.01 ± 0.02 | 0.00 ± 0.02 |
| 16:1n-7 | 6.22 ± 2.05 | 1.83 ± 0.51 | 1.31 ± 0.45 |
| 18:1n-9 | 44.30 ± 2.75 | 39.61 ± 4.63 | 28.70 ± 8.52 |
| 18:1n-7 | 2.45 ± 0.57 | 0.51 ± 0.23 | 0.36 ± 0.15 |
| 20:1n-12 ² | 0.11 ± 0.09 | 0.24 ± 0.13 | 0.17 ± 0.10 |
| 20:1n-9 ² | 0.52 ± 0.12 | | |
| Polyunsaturated fatty acids | | | |
| n-6 | 15.29 ± 3.33 | 17.44 ± 4.57 | 12.37 ± 3.70 |
| 18:2n-6 | 13.37 ± 3.24 | 17.06 ± 4.55 | 12.10 ± 3.68 |
| 18:3n-6 | 0.14 ± 0.17 | 0.19 ± 0.08 | 0.14 ± 0.06 |
| 20:2n-6 | 0.22 ± 0.06 | NA | NA |
| 20:3n-6 | 0.29 ± 0.10 | 0.02 ± 0.01 | 0.01 ± 0.01 |
| 20:4n-6 | 0.44 ± 0.13 | 0.19 ± 0.09 | 0.13 ± 0.06 |
| 22:4n-6 | 0.22 ± 0.07 | NA | NA |
| n-3 | 0.99 ± 0.20 | 1.53 ± 0.43 | 1.27 ± 0.36 |
| 18:3n-3 | 0.55 ± 0.16 | 1.30 ± 0.36 | 1.08 ± 0.29 |
| 20:3n-3 | 0.02 ± 0.01 | NA | NA |
| 20:5n-3 | 0.04 ± 0.02 | 0.06 ± 0.08 | 0.04 ± 0.06 |
| 22:5n-3 | 0.21 ± 0.06 | 0.02 ± 0.02 | 0.02 ± 0.01 |
| 22:6n-3 | 0.17 ± 0.06 | 0.18 ± 0.16 | 0.13 ± 0.12 |
| n-7 | | | |
| 18:2n-7 ³ | 0.67 ± 0.18 | 0.03 ± 0.03 | 0.02 ± 0.02 |
| <i>trans</i> Fat | 3.12 ± 0.99 | 4.76 ± 2.24 | 3.98 ± 1.85 |
| <i>r</i> 14:1n-5 | 0.02 ± 0.02 | 0.01 ± 0.02 | 0.00 ± 0.02 |
| <i>r</i> 16:1n-7 | 0.08 ± 0.03 | 0.16 ± 0.05 | 0.14 ± 0.06 |
| <i>r</i> 18:1n-12 | 0.54 ± 0.25 | 0.50 ± 0.29 | 0.35 ± 0.20 |
| <i>r</i> 18:1n-9 | 0.63 ± 0.23 | 0.57 ± 0.40 | 0.40 ± 0.27 |
| <i>r</i> 18:1n-7 | 0.54 ± 0.22 | 0.35 ± 0.25 | 0.24 ± 0.17 |
| All <i>trans</i> 18:1 | 2.24 ± 0.80 | 2.94 ± 1.43 | 2.47 ± 1.23 |
| <i>tt</i> 18:2n-6 | 0.34 ± 0.13 | 0.32 ± 0.27 | 0.23 ± 0.20 |
| <i>ct</i> 18:2n-6 | 0.61 ± 0.25 | 0.71 ± 0.53 | 0.52 ± 0.40 |
| <i>tc</i> 18:2n-6 | 0.36 ± 0.15 | 0.66 ± 0.49 | 0.48 ± 0.37 |

¹ $\bar{x} \pm SD$; of the 50 fatty acids analyzed, data are presented for the 35 that were detected; $n = 503$; NA, not available.²The corresponding dietary intake includes all isomers of 20:1.³Consisted of 9*c*,11*c*-octadecadienoic acid.

fatty acids represented 3% of the fatty acids in adipose tissue. The percentage of *trans* fatty acids in diet was similar. Most of the *trans* fatty acids were represented by 18:1, and 18:2 provided the next largest representation. Just a small percentage corresponded to the shorter-chain species (14:1 and 16:1).

There were no material differences by age either in adipose tissue or dietary fatty acids. However, we found a lower proportion

TABLE 3

Partial Spearman correlation coefficients between adipose tissue and dietary saturated, monounsaturated, and polyunsaturated fatty acids¹

| Adipose tissue fatty acids ² | Corresponding dietary fatty acid | Animal fat | Vegetable fat | SFAs | MUFAs | PUFAs | | <i>trans</i> Fatty acids |
|---|----------------------------------|------------|---------------|-------|-------|-------|-------|--------------------------|
| | | | | | | n-6 | n-3 | |
| Saturated fatty acids | | 0.18 | -0.13 | 0.17 | -0.01 | -0.17 | -0.05 | -0.12 |
| 12:0 | 0.03 | 0.02 | 0.05 | 0.01 | 0.04 | 0.04 | 0.08 | 0.05 |
| 14:0 | 0.15 | 0.07 | -0.03 | 0.05 | 0.01 | 0.00 | 0.05 | -0.08 |
| 15:0 | 0.04 | 0.11 | 0.01 | 0.15 | 0.07 | 0.03 | 0.05 | -0.09 |
| 16:0 | 0.13 | 0.15 | -0.19 | 0.15 | -0.06 | -0.26 | -0.13 | -0.18 |
| 17:0 | -0.02 | 0.17 | 0.08 | 0.17 | 0.16 | 0.15 | 0.15 | 0.06 |
| 18:0 | 0.17 | 0.15 | 0.07 | 0.13 | 0.15 | 0.13 | 0.16 | 0.09 |
| 20:0 | -0.12 | -0.02 | 0.17 | -0.06 | 0.07 | 0.27 | 0.26 | 0.26 |
| 22:0 | -0.12 | -0.04 | 0.00 | 0.00 | -0.01 | -0.10 | -0.09 | -0.11 |
| 24:0 | 0.01 | 0.02 | 0.05 | -0.01 | -0.01 | 0.08 | 0.09 | 0.06 |
| Monounsaturated fatty acids | | 0.11 | -0.29 | 0.14 | -0.08 | -0.44 | -0.31 | -0.32 |
| 14:1n-5 | 0.04 | 0.00 | -0.09 | 0.00 | -0.05 | -0.10 | -0.04 | -0.10 |
| 16:1n-7 | -0.06 | -0.03 | -0.24 | -0.02 | -0.19 | -0.33 | -0.23 | -0.22 |
| 18:1n-9 | 0.05 | 0.20 | -0.22 | 0.25 | 0.05 | -0.39 | -0.27 | -0.28 |
| 18:1n-7 | -0.02 | -0.05 | -0.19 | -0.08 | -0.17 | -0.22 | -0.17 | -0.09 |
| 20:1n-12 ³ | 0.05 | 0.10 | 0.03 | 0.10 | 0.07 | 0.08 | 0.07 | 0.04 |
| 20:1n-9 ³ | 0.04 | 0.15 | -0.15 | 0.12 | 0.04 | -0.17 | -0.10 | -0.08 |
| Polyunsaturated fatty acids | | -0.24 | 0.36 | -0.29 | 0.08 | 0.58 | 0.36 | 0.35 |
| n-6 | | | | | | | | |
| 18:2n-6 | 0.58 | -0.24 | 0.36 | -0.29 | 0.08 | 0.58 | 0.36 | 0.34 |
| 18:3n-6 | 0.24 | -0.09 | 0.05 | -0.10 | -0.05 | 0.13 | 0.13 | 0.26 |
| 20:2n-6 | NA | -0.11 | 0.16 | -0.19 | 0.04 | 0.33 | 0.20 | 0.21 |
| 20:3n-6 | -0.16 | -0.07 | 0.06 | -0.15 | -0.03 | 0.19 | 0.07 | 0.10 |
| 20:4n-6 | 0.04 | -0.03 | -0.01 | -0.09 | -0.03 | 0.05 | -0.04 | 0.00 |
| 22:4n-6 | NA | -0.02 | -0.01 | -0.06 | -0.03 | 0.03 | -0.07 | 0.05 |
| n-3 | | -0.13 | 0.24 | -0.16 | 0.07 | 0.38 | 0.31 | 0.22 |
| 18:3n-3 | 0.34 | -0.19 | 0.30 | -0.19 | 0.08 | 0.44 | 0.34 | 0.28 |
| 20:3n-3 | NA | -0.01 | 0.04 | -0.04 | 0.03 | 0.13 | 0.15 | 0.05 |
| 20:5n-3 | 0.15 | 0.03 | -0.06 | -0.05 | -0.04 | 0.02 | 0.13 | -0.02 |
| 22:5n-3 | 0.03 | -0.01 | -0.01 | -0.02 | 0.00 | 0.02 | 0.02 | 0.00 |
| 22:6n-3 | 0.18 | 0.00 | -0.02 | -0.04 | -0.03 | 0.02 | 0.05 | 0.01 |
| n-7 | | | | | | | | |
| 18:2n-7 | 0.10 | -0.02 | 0.10 | 0.05 | 0.06 | 0.13 | 0.12 | 0.13 |

¹For all *r* values >0.15, *P* < 0.001; *n* = 503; of the 50 fatty acids analyzed, data are presented for the 35 detected; NA, not available; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

²Missing values for adipose tissue fatty acids: 12:0, *n* = 7; 20:0, *n* = 77; 22:0, *n* = 163; 24:0, *n* = 5; 18:3n-6, *n* = 98; and 20:3n-6, *n* = 30.

³The corresponding dietary intake includes all isomers of 20:1.

of SFAs and a higher proportion of MUFAs in adipose tissue in the women than in the men, regardless of intake (data not shown).

Spearman correlation coefficients between dietary and adipose tissue fatty acids adjusted for age, sex, BMI, and smoking status

are shown in **Tables 3** and **4**. SFAs showed low correlations overall. The best correlation was found when 15:0 and 17:0 fatty acids in adipose tissue were added together (*r* = 0.18). Adipose tissue oleic acid was correlated inversely with vegetable fatty acid and

TABLE 4

Partial Spearman correlation coefficients between adipose tissue and dietary *trans* fatty acids¹

| Adipose tissue fatty acids ² | Corresponding dietary fatty acid | Animal fat | Vegetable fat | SFAs | MUFAs | PUFAs | | <i>trans</i> Fatty acids |
|---|----------------------------------|------------|---------------|-------|-------|-------|------|--------------------------|
| | | | | | | n-6 | n-3 | |
| <i>trans</i> Fatty acids | | -0.17 | 0.34 | -0.18 | 0.06 | 0.42 | 0.34 | 0.54 |
| <i>t</i> 14:1n-5 | 0.09 | 0.07 | 0.07 | 0.04 | 0.07 | 0.13 | 0.11 | 0.09 |
| <i>t</i> 16:1n-7 | 0.12 | 0.04 | 0.17 | 0.05 | 0.13 | 0.24 | 0.23 | 0.16 |
| <i>t</i> 18:1n-12 | 0.30 | -0.14 | 0.28 | -0.11 | 0.07 | 0.27 | 0.17 | 0.32 |
| <i>t</i> 18:1n-9 | 0.43 | -0.11 | 0.28 | -0.10 | 0.08 | 0.31 | 0.26 | 0.40 |
| <i>t</i> 18:1n-7 | 0.36 | -0.04 | 0.30 | 0.00 | 0.14 | 0.35 | 0.27 | 0.36 |
| All <i>trans</i> 18:1 | 0.43 | -0.15 | 0.33 | -0.13 | 0.08 | 0.37 | 0.28 | 0.45 |
| <i>tt</i> 18:2n-6 | 0.53 | -0.23 | 0.31 | -0.25 | 0.01 | 0.38 | 0.31 | 0.52 |
| <i>ct</i> 18:2n-6 | 0.58 | -0.24 | 0.28 | -0.30 | -0.04 | 0.42 | 0.36 | 0.58 |
| <i>tc</i> 18:2n-6 | 0.61 | -0.22 | 0.21 | -0.31 | -0.08 | 0.37 | 0.34 | 0.58 |

¹For all *r* values >0.15, *P* < 0.001; *n* = 503; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

²Missing values for adipose tissue fatty acids: 12:0, *n* = 7; 20:0, *n* = 97; 22:0, *n* = 163; 24:0, *n* = 5; 18:3n-6, *n* = 98; and 20:3n-6, *n* = 30.

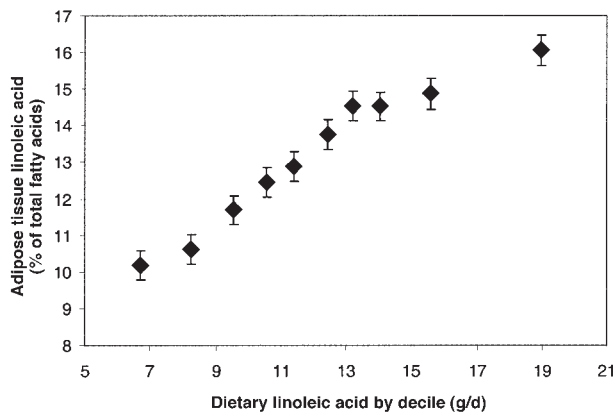


FIGURE 1. Mean (\pm SEM) adipose tissue linoleic acid plotted against median daily linoleic acid intake by decile after adjustment for age, sex, BMI, and smoking status. $r = 0.58$, $P < 0.01$; $n = 503$.

PUFAs (Table 3) but correlated directly with animal fatty acid and SFAs; it showed no correlation with its corresponding dietary fatty acid. PUFAs exhibited stronger correlations; the best corresponded to linoleic acid (0.58) and α -linolenic acid (0.34). The correlation coefficient for total PUFAs between adipose tissue and diet was 0.57. Arachidonic acid (20:4n-6) did not correlate with its corresponding dietary fatty acid or with dietary PUFAs. With 2 exceptions (*t14:1n-5* and *t16:1n-7*), *trans* fatty acids (Table 4) showed the strongest correlations with their corresponding dietary fatty acids. In particular, the highest correlations were for *ct*-linoleic acid and *tc*-linoleic acid (0.58 and 0.61, respectively). Similar results were obtained after stratification of the samples by sex or when dietary intake was expressed as a percentage of total fat.

The average concentrations and SEMs for adipose tissue linoleic acid within each decile of intake are shown in **Figure 1**. This plot shows that a wide range of intake can be discerned by adipose tissue measurements. A similar dose-response curve for α -linolenic acid is shown in **Figure 2**. The total intake of *trans* fatty acids plotted against adipose tissue *tc*-linoleic acid, which indicated that *tc*-linoleic acid in adipose tissue can be a good marker of *trans* fatty acid intake, is shown in **Figure 3**. Almost identical results were found for *ct*-linoleic acid. These

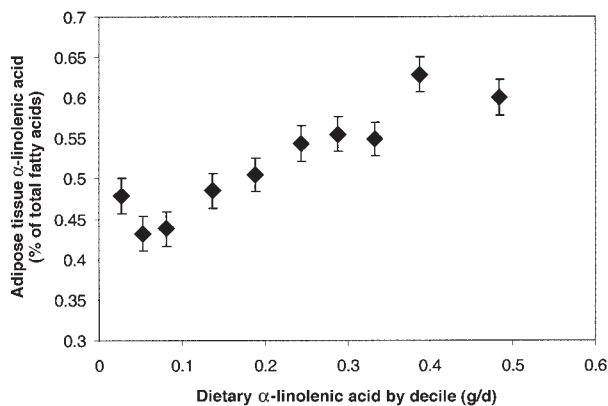


FIGURE 2. Mean (\pm SEM) adipose tissue α -linolenic acid plotted against median daily α -linolenic acid intake by decile after adjustment for age, sex, BMI, and smoking status. $r = 0.34$, $P < 0.01$; $n = 503$.

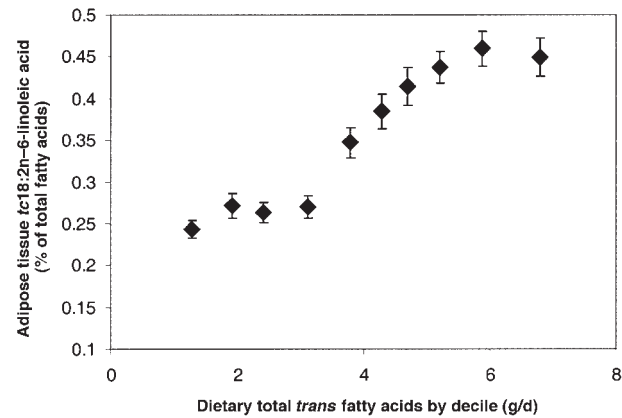


FIGURE 3. Mean (\pm SEM) adipose tissue *tc*-linoleic acid plotted against median daily *trans* fatty acid intake by decile after adjustment for age, sex, BMI, and smoking status. $r = 0.58$, $P < 0.01$; $n = 503$.

dose-response relations were virtually unchanged when dietary intake was expressed as a percentage of total fat.

We next performed a stepwise linear regression to identify adipose tissue fatty acids that marked intake of specific food items. Soybean oil accounted for 30% of the variance in total adipose tissue *trans* fatty acids, and margarine accounted for 6%. Note that in Costa Rica, all commercial soybean oil is partially hydrogenated. The intake of dairy products was the best correlate of 15:0 and 17:0 ($r = 0.31$ for each). The correlation between 15:0 in adipose tissue and the intake of dairy products (predominantly 2% milk, cheese, and sour cream) is shown in **Figure 4**. Almost identical results were obtained when 17:0 was used instead of 15:0. This relation was detected even when 15:0 and 17:0 did not correlate with their respective fatty acids in the diet, probably because of incomplete information on these 2 fatty acids in our database. The relation between fish intake (including dark-meat fish, light-meat fish, and shellfish) and adipose tissue DHA is shown in **Figure 5**. DHA and EPA correlated with dietary fish intake, but docosapentaenoic acid (DPA; 22:5n-3) did not. DHA was the best correlate of fish intake. The lowest deciles of fish

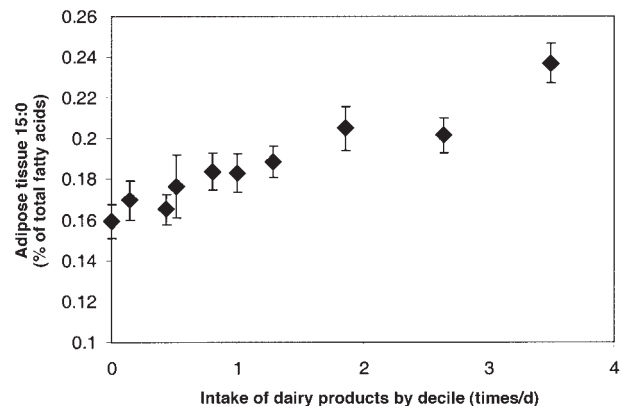


FIGURE 4. Mean (\pm SEM) adipose tissue 15:0 plotted against median daily intake of dairy products by decile after adjustment for age, sex, BMI, and smoking status. $r = 0.31$, $P < 0.01$; $n = 503$.

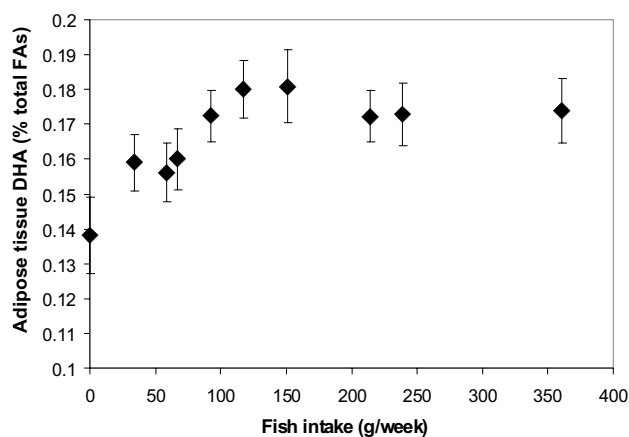


FIGURE 5. Mean (\pm SEM) adipose DHA (docosahexaenoic acid) plotted against median weekly fish intake by decile after adjustment for age, sex, BMI, and smoking status. $r = 0.15$, $P < 0.01$; $n = 503$.

intake corresponded to the lowest amounts of DHA in adipose tissue. DHA in adipose tissue appeared to reach a plateau at an intake of ≈ 100 g fish/wk.

DISCUSSION

We found that adipose tissue PUFAs (linoleic and α -linolenic acids) and *trans* fatty acids (18:2 and 18:1) are good biomarkers of their respective dietary intakes. DHA and 15:0 were good discriminators for the intake of fish and dairy products, respectively. Our study explored biomarkers of fatty acid intake in a very complete dataset for both adipose tissue and diet. In view of the high participation (90%) and the way in which the subjects (population controls) were chosen, the findings presented here may be representative of the entire population from which the subjects were selected within the strata of the matching factors (sex, age, and geographic area).

The distribution of fatty acids in adipose tissue in our study was in the range expected on the basis of other studies (6, 11, 13, 16–18, 29–32). Because SFAs and MUFAs can be synthesized endogenously, proportions of both types of fatty acid are not expected to be similar in adipose tissue and diet. Total adipose tissue *trans* fatty acids are in the lower range in comparison with the totals in the US population and in some Northern European populations (13, 16–18, 31–34). However, the intake of *trans* fatty acids in the Costa Rican population is higher than the intakes in other European populations, in particular those of France (35) and Spain (36). In Costa Rica, the intake of 18:1 *trans* fatty acids is comparable to or lower than that in Western countries, whereas the intake of 18:2 *trans* fatty acid is among the highest reported to date (17, 33, 37). This difference is probably due to the high intake in Costa Rica of partially hydrogenated soybean oil, which is high in 18:2 *trans* fatty acid. In fact, 30% of the variability in total adipose tissue *trans* fatty acids is due to soybean oil, which is used in the everyday cooking of rice, beans, and fried food. Margarine and baked products were the next major source of *trans* fatty acids. In contrast, the partially hydrogenated soybean oil used for cooking does not contribute to *trans* fatty acid intake in other populations (17, 34, 35). The distribution of marine $n-3$ fatty acids in adipose tissue in this study with low to moderate intake of fish (\bar{x} , 14.5 g/d;

median, 13.1 g/d) is also similar to other studies in which subjects had similar fish consumption (38).

It is remarkable that adipose tissue oleic acid shows the best correlation with saturated fat intake, even better than that of any adipose tissue SFA. Positive correlations between serum MUFAs and dietary SFAs have been reported by others (39, 40). The positive correlation may hold because the sources of MUFAs and SFAs are the same or because dietary SFAs are rapidly desaturated in the body (40).

Previous studies (6, 12, 13, 16–18, 41) showed high correlations (0.40–0.75) between diet and adipose tissue total PUFAs ($n-6$ and $n-3$). We have found high diet-adipose tissue correlations for linoleic acid and α -linolenic acid. We did not find a correlation between dietary PUFAs and adipose tissue arachidonic acid, which suggests that there is little endogenous conversion of linoleic acid to arachidonic acid. Consistent with this finding, the proportions of arachidonic acid in adipose tissue remained constant when an increase or decrease in adipose tissue linoleic acid was induced by changes in PUFAs fed to marmoset monkeys (42). In humans, no arachidonic acid was found in adipose tissue after supplementation with a diet high in arachidonic acid (43). It has been suggested that the physiologic mechanisms controlling fatty acid homeostasis in humans shunt dietary arachidonic acid into specific metabolic pathways, which prevents its accumulation in adipose tissue (44).

Our data show high correlations between adipose tissue and dietary *trans* fatty acids. Higher correlations were found for 18:2 *trans* fatty acid. Adipose tissue *trans* fatty acids also showed moderate correlations with dietary $n-6$ and $n-3$ PUFAs because the main source common to them all is soybean oil. Some studies have also shown high correlations for total *trans* fatty acids, in the range of 0.5 to 0.7 (32, 33, 37), although others have shown lower correlations (17, 18).

In the present study, the intake of dairy products correlated with adipose tissue 15:0 and 17:0 fatty acids. These fatty acids did not correlate with their corresponding dietary fatty acids, most likely because current nutrient databases (26) do not include complete data on these 2 fatty acids. Wolk et al (45) arrived at similar results by assuming a fixed percentage of 15:0 and 17:0 from dairy products to correlate their dietary content with their corresponding presence in adipose tissue. Adipose tissue 15:0 and 17:0 in our study were lower than those previously reported by Wolk et al (45) (0.19% compared with 0.35% for 15:0 and 0.21% compared with 0.25% for 17:0), which probably reflects a much lower intake of dairy products in Costa Rica than in Sweden. Because the intake of ruminant fat, another source of 15:0 and 17:0 fatty acids, is not high in the Costa Rican population (1–2 times/wk), these 2 fatty acids in adipose tissue can be considered markers for fatty acids from dairy products. Other studies have shown similar results using serum as a marker for the intake of milk fat (46, 47).


We found a modest relation between fish intake and adipose tissue DHA. This is not surprising, because the most widely consumed fish in Costa Rica, sea bass and other local whitefish, contain a higher proportion of DHA (19%) than of EPA (5%) or DPA (4%) (unpublished data from our laboratory). This relation appeared to reach a plateau at an intake of ≈ 100 g/wk. We cannot determine whether this association reflects a real biological phenomenon or a combination of measurement error and a narrow range of intake, particularly in a population whose intake of fish is low. It has been suggested that people with a higher intake of fish may overestimate the amount of fish consumed, in an effort to suggest a healthier diet (48). Nevertheless, most studies suggest a nonlinear association between fish intake and the amount of EPA, DPA, or DHA in adipose

tissue (49, 50), plasma (10, 48), and erythrocytes (10). A plateau is not evident in some studies in adipose tissue (10, 38), but, particularly in the study by Marckmann et al (38), there were only 3 subjects with the higher intake. More data are needed to explore this association, although direct comparisons and interpretation of this research are difficult across populations, given the large variation in n-3 fatty acids from fish and different intakes of fish.

Because some authors reported better correlations when working with data calculated as a percentage of total fat rather than with energy-adjusted data (32), we carried out the analysis by both methods. We did not find any important differences between the 2 methods. The fatty acids whose correlations were slightly higher by the percentage method were palmitic and oleic, mostly because of their high correlations with total fat intake.

It should be noted that, in the context of nutritional epidemiology, all sources of error from questionnaires, together with technical errors in the measurement of the biomarker and other sources of physiologic variation in the biochemical indicator, tend to weaken correlations (5). Therefore, it is not surprising that correlation coefficients in the range of 0.4 to 0.6 are considered relatively high. A correction for measurement error can increase correlation coefficients and thus provide a more precise result (5, 51). In the current study, we were unable to correct for measurement error because multiple adipose tissue samples and FFQs were not available. Thus, the correlations presented here may be underestimated. Nevertheless, we do show that a correlation of 0.34 for adipose tissue α -linolenic acid is good enough to discriminate between small differences in intake.

A possible disadvantage of the correlation coefficient is that it is a function of the accuracy of the questionnaire and the true between-person variation in the population being studied. This limits the generalizability of the correlation coefficient to those populations in which between-person variation is similar to that in the test population (5), and it emphasizes the importance of conducting these studies in various populations.

In summary, PUFAs and *trans* fatty acids in adipose tissue are good biomarkers of their corresponding dietary fatty acids. DHA and odd-numbered SFAs (15:0 and 17:0) may be suitable biomarkers of their respective intake in fish and dairy products. 

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