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Fasting Whole Blood as a Biomarker of Essential Fatty Acid Intake in Epidemiologic Studies: Comparison with Adipose Tissue and Plasma

Ana Baylin[1], Mi Kyung Kim[2], Amy Donovan-Palmer[1], Xinia Siles[3], Lauren Dougherty[1], Paula Tocco[1], and Hannia Campos[1,3]

[1] Department of Nutrition, Harvard School of Public Health, Boston, MA.
[2] Hanyang University College of Medicine, Seoul, South Korea.
[3] Central American Center for Population, University of Costa Rica, San Pedro de Montes de Oca, Costa Rica.

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Biomarkers could provide a more accurate measure of long-term intake than questionnaires. Adipose tissue is considered the best indicator of long-term essential fatty acid intake, but other tissues may prove equally valid. The authors evaluated the ability of fasting whole blood, relative to fasting plasma and adipose tissue, to reflect fatty acid intake. Costa Rican men ($n = 99$) and women ($n = 101$) completed a 135-item food frequency questionnaire and provided adipose tissue and blood samples from 1999 to 2001. Fatty acids were identified by using capillary gas chromatography. Correlation coefficients adjusted for age, sex, and body mass index were calculated. Diet-tissue correlation coefficients for α-linolenic acid and linoleic acid, respectively, were 0.38 and 0.43 in whole blood, 0.51 and 0.52 in adipose tissue, and 0.39 and 0.41 in plasma. High correlations were observed between whole-blood α-linolenic and linoleic acid and adipose tissue ($r = 0.59$ and $r = 0.67$) and plasma ($r = 0.96$ and $r = 0.88$), respectively. Results show that fasting whole blood is a suitable biomarker of long-term essential fatty acid intake, and its performance is comparable to that of fasting plasma. Thus, fasting whole blood could be the sample of choice in epidemiologic studies because of its ability to predict intake, its accessibility, and minimum sample processing.

adipose tissue; blood; Costa Rica; fasting; fatty acids; plasma

Abbreviation: FFQ, food frequency questionnaire.

Biomarkers are widely used to assess dietary intake in epidemiologic studies. In general, it is well accepted that these markers may help overcome some methodological problems derived from the use of dietary questionnaires in nutritional epidemiology (1, 2). However, their ability to reflect dietary intake may be affected by nondietary factors such as genetic background, smoking, obesity, physical activity, and metabolism (2).

Biomarkers of individual fatty acids have been widely used because fatty acids play an important role in the development of chronic disease (2). Evaluation is simplified in epidemiologic studies because individual fatty acids can be measured in various readily available tissues (e.g., adipose tissue, plasma, red blood cells). In these tissues, fatty acid measurements in adipose represent mostly triacylglycerol, red blood cells represent phospholipids, and plasma represents a combination of triacylglycerols, cholesterol esters, and phospholipids, found in lipoproteins (2–7). However, a major disadvantage is that the metabolic characteristics of all of these biologic specimens are quite different (8), and some tissues are more likely to reflect dietary intake while others most likely reflect physiologic properties.

Correspondence to Dr. Ana Baylin, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, SPH2, Boston, MA 02115 (e-mail: abaylin@hsph.harvard.edu).

Because not many studies compare the ability of different tissues to predict dietary intake, interpretation of available information is difficult (2).

Adipose tissue is considered the best choice for the study of long-term fatty acid intake mainly because of its slow turnover (6, 9, 10) and its lack of response to acute disease (11, 12). However, adipose tissue is rarely used in epidemiologic studies because of the difficulty in obtaining the samples (6, 13). In general, the use of specimens such as plasma, specific plasma fractions, and red blood cell membranes or phospholipids has been discouraged for studying long-term intake, because they are likely to reflect short-term intake (days and months, respectively) (6), but little data are available comparing these tissues with adipose tissue to reflect long-term intake. The use of fasting whole blood is an attractive, unexplored option for epidemiologic studies because of its accessibility and minimum sample processing. Albert et al. (14) successfully evaluated fatty acids in whole blood in relation to sudden death, but the ability of whole blood to reflect intake relative to individual tissues in population studies is unknown.

We have previously reported that adipose tissue is an excellent biomarker of dietary fatty acid intake assessed by a food frequency questionnaire (FFQ) or seven 24-hour recalls (7, 15). The goal of this study was to evaluate the ability of fasting whole blood to reflect dietary fatty acid intake relative to other tissues, including plasma and adipose tissue.

MATERIALS AND METHODS

Study population

Participants were 101 women and 99 men recruited as controls in an ongoing case-control study of diet and heart disease in Costa Rica between 1999 and 2001. The study design and population have been described previously (16). All subjects gave 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, dietary intake, and medical history. Anthropometric measurements were gathered by trained field-workers from subjects wearing light clothing and no shoes. Measurements were performed in duplicate, and the mean was used for analysis. A subcutaneous adipose tissue biopsy was collected from the upper buttock with a 16-gauge needle and disposable syringe following procedures described previously (13). Approximately 2 mg of adipose tissue was stored in Wheaton borosilicate glass vials (Wheaton Science Products, Millville, New Jersey) with solid Teflon caps (DuPont, Wilmington, Delaware) containing approximately 1 ml of hexane:isopropanol.

At the subjects' homes, fasting blood samples were collected into 10-mm blood collection tubes containing either 0.1 percent ethylenediaminetetraacetic acid or 10 percent dimethyl sulfoxide and were immediately placed in ice at 4°C. Within 4 hours, blood from tubes containing 0.1 percent ethylenediaminetetraacetic acid was centrifuged at 1,430 *g* for 20 minutes at 4°C to separate plasma from red blood cells. Samples collected in 10 percent dimethyl sulfoxide were stored unspun. Within 6 months, samples were transported over dry ice to the Harvard School of Public Health for long-term storage in tanks containing compressed nitrogen.

Dietary assessment

Dietary information was obtained by using a 135-item FFQ, modified from the Willett questionnaire (17), that was developed and validated specifically for use in the Costa Rican population (15). This FFQ inquired about intake of 135 food items as well as 20 vitamin, mineral, and food supplements; types of fat used for cooking and frying; consumption of fried foods in and away from home; and food habits related to meat consumption. Each item had a specified portion size and nine possible coding responses, which ranged from never or less than one per month to six or more per day. Portion sizes for the food items in the FFQ were calculated on the basis of standardized serving sizes specified by the School of Nutrition at the University of Costa Rica (15).

All subjects were visited at their homes and were interviewed about their dietary intake during the past year. Subjects selected the type of fat or oil used most frequently for cooking, frying, or baking at home. This selection was confirmed by visual inspection of the oil containers at the time of the home visit. Energy and nutrient intakes were estimated from the US Department of Agriculture food composition tables at the Channing Laboratory (17). To estimate the amount of each oil used for cooking and frying at home, we complemented the FFQ data by asking each study subject about his or her recipes for staple dishes (rice and beans) and fried foods and incorporated them into the FFQ. An important aspect of the study is that the fatty acid composition of all foods and oils commonly consumed in Costa Rica was assessed in the same laboratory and by using the same standards and instrumentation for peak identification that were used to assess fatty acid in biologic tissues.

The Costa Rican diet is a typical Central American diet consisting of rice and beans as the main staple; plantain; corn tortillas; a small salad and chopped vegetables, with meat, eggs, or cheese; and tropical fruits. Intake of fish is very low in this population. Fifty-two percent of the population use soybean oil for cooking, 23 percent use palm oil, and the remaining 25 percent use other oils (mainly corn and sunflower). The study population still prepares and eats most meals at home; 60 percent reported eating outside of the home only once or less than once per week.

Fatty acid analysis

All fatty acid analyses were carried out with fasting samples. Lipids from adipose tissue were extracted from a mixture of 200 μl of hexane:isopropanol (3:2) containing the sample and were esterified by using methanol and acetyl chloride, as described by Lillington et al. (18). Fatty acids

in plasma and whole blood were extracted into isopropanol and hexane containing 50 mg of 2.6-di-tert-butyl-*p*-cresol as an antioxidant. Fatty acids were transmethylated with methanol and sulfuric acid, as described by Zock et al. (19, 20). After esterification, adipose and blood samples were evaporated, and the fatty acid methyl esters were redissolved in iso-octane and were assessed by gas-liquid chromatography, as follows: fused silica capillary *cis*/*trans* column SP2560, 100 m × 250 μm internal diameters × 0.20 μm film (Supelco, Belefonte, Pennsylvania); splitless injection port at 240°C; hydrogen carrier gas at 1.3 ml/minute, constant flow; Hewlett-Packard (now Agilent Technologies) model GC 6890 flame ionization detector gas chromatograph with 7673 Autosampler injector (Palo Alto, California); 1 μl of sample injected; temperature program of 90–170°C at 10°C/minute, 170°C for 5 minutes, 170–175°C at 5°C/minute, 175–185°C at 2°C/minute, 185–190°C at 1°C/minute, 190–210°C at 5°C/minute, 210°C for 5 minutes, 210–250°C at 5°C/minute, and 250°C for 10 minutes.

Peak retention times were identified by injecting known standards, and purity ranges were all above 99 percent (NuCheck Prep, Elysium, Minnesota); Agilent Technologies ChemStation A.08.03 software was used for analysis. We established that the sample processing and freezing did not affect the fatty acid measurements by comparing two pools of frozen and fresh samples, and short- versus long-term freezing. No consistent differences were evident. Coefficients of variation for all of the fatty acids studied were monitored continuously by analyzing pooled samples (indistinguishable from other study samples) throughout the analyses of the study samples. In general, peaks near the sensitivity limit (close to 0.10 of the total area) were associated with larger coefficients of variation. The major determinant of measurement error in different tissues is the relative abundance of each fatty acid. For example, the area percentage for arachidonic acid is 0.48 in adipose tissue, and the coefficient of variation is 5.9 percent. However, arachidonic acid is most abundant in plasma (6.02 percent), and the coefficient of variation is 1.8 percent. Thus, the coefficient of variation of the most abundant fatty acids is very low, 5 percent on average, regardless of the tissue. In particular, coefficients of variation for linoleic acid were 2.6 percent in adipose tissue, 0.9 percent in plasma, and 2.1 percent in whole blood; coefficients of variation for α-linolenic acid were 3.9 percent in adipose tissue, 3.2 percent in plasma, and 3.3 percent in whole blood. We identified a total of 50 fatty acids in the three tissues. However, fatty acids that were undetectable, or those with a very low concentration, are not reported here. Fatty acids analyzed in tissues include fatty acids in all fractions. In particular, fatty acids in plasma and whole blood include those from triglycerides, cholesterol esters, phospholipids, and free fatty acids.

Statistical analyses

In this paper, fatty acids are described in all biologic specimens as a percentage of total fatty acids measured, expressed as mean (standard deviation). In diet, they were analyzed as a percentage of total fat and in terms of energy-adjusted grams per day by calculating the residuals from regressing dietary fatty acid levels on total energy intake (2). Data are presented as a percentage of total fat, but the correlations with energy-adjusted intake yielded indistinguishable results. Monounsaturated, polyunsaturated, saturated, and *trans*-fatty acid totals were recalculated from the sum of those fatty acids detected or available.

Partial Spearman correlation coefficients adjusted for age, sex, and body mass index were calculated to determine associations between dietary and tissue fatty acids. Correlation coefficients were considered significant at a level of $p < 0.05$. Spearman correlation coefficients were also used to calculate associations among fatty acids in the three tissues. For α-linolenic acid, multiple linear regression models were used to test for trends across diet deciles and to compare the mean values for all tissue fatty acids at each decile of diet fatty acids, adjusting for age, sex, and body mass index. Test of trends were computed by assigning the median value of the corresponding decile to each subject and entering that variable as a continuous one in the model. Robust estimators of the variance were used in regression models, thus eliminating the need for normalizing the dependent variable in the models described above (21). Correlation coefficients were compared by using a two-sided *t* test for dependent samples with $n - 3$ degrees of freedom (22).

All data were analyzed with the Statistical Analysis Systems software package, version 8 (SAS Institute, Inc., Cary, North Carolina).

TABLE 1. General characteristics* of Costa Rican adults evaluated to determine the ability of fasting whole blood to accurately reflect fatty acid intake, 1999–2001

	Men ($n = 99$)	Women ($n = 101$)
Age (years)	57 (11)	62 (12)
Body mass index (kg/cm^2)	26 (3.7)	27.1 (5.0)
Height (cm)	168 (7)	153 (6)
Weight (kg)	73 (12)	64 (13)
Total energy (MJ/day)	11.6 (3.9)	9.4 (3.1)
Total cholesterol (mmol/liter)	4.39 (0.85)	4.69 (0.8)
Low density lipoprotein cholesterol (mmol/liter)	2.50 (0.74)	2.71 (0.72)
High density lipoprotein cholesterol (mmol/liter)	1.33 (0.25)	1.45 (0.22)
Triglycerides (mmol/liter)	1.21 (0.65)	1.21 (0.62)
Total fat (% energy)	35.4 (6.9)	35.1 (5.5)
Saturated fat (% energy)	11.8 (2.7)	12.1 (2.5)
Monounsaturated fat (% energy)	13.7 (5.2)	13.3 (4.2)
Polyunsaturated fat (% energy)	6.6 (2.0)	6.4 (2.0)
Carbohydrates (% energy)	51.6 (7.2)	54.8 (6.4)
Protein (% energy)	12.3 (1.9)	12.1 (2.2)

* Values are expressed as mean (standard deviation).

TABLE 2. Distribution of fatty acids in the diet, adipose tissue, plasma, and whole blood in Costa Rican adults, 1999–2001*

	Diet (% total fat)	Adipose tissue (% total fatty acids)	Plasma (% total fatty acids)	Whole blood (% total fatty acids)
Saturated fatty acids				
12:0	0.81 (0.42)	0.04 (0.05)	0.02 (0.03)	0.01 (0.02)
14:0	2.72 (1.15)	1.13 (0.48)	0.74 (0.31)	0.52 (0.23)
15:0	0.29 (0.12)	0.22 (0.09)	0.17 (0.05)	0.14 (0.05)
16:0	25.73 (4.91)	20.53 (2.83)	22.78 (2.24)	21.92 (1.88)
17:0	0.31 (0.10)	0.20 (0.06)	0.28 (0.06)	0.28 (0.06)
18:0	7.45 (1.38)	2.65 (0.92)	6.76 (0.74)	9.42 (0.92)
20:0	0.22 (0.04)	0.09 (0.05)	0.19 (0.04)	0.26 (0.05)
22:0	0.09 (0.05)	0.004 (0.007)	0.48 (0.14)	0.83 (0.19)
Total saturated fatty acids	37.90 (6.62)	25.00 (3.52)	31.85 (2.24)	34.07 (1.86)
Monounsaturated fatty acids				
14:1(n-5)	0.19 (0.08)	0.18 (0.11)	0.04 (0.03)	0.03 (0.02)
16:1(n-7)	1.29 (0.39)	6.73 (2.15)	2.64 (0.96)	1.86 (0.78)
18:1(n-9)	32.35 (7.02)	42.82 (2.97)	20.74 (2.72)	18.23 (2.31)
18:1(n-7)	1.30 (0.32)	2.28 (0.46)	1.71 (0.31)	1.52 (0.26)
20:1(n-9)	0.17 (0.04)	0.43 (0.10)	0.14 (0.04)	0.17 (0.04)
Total monounsaturated fatty acids	35.57 (7.11)	52.49 (4.02)	25.91 (3.50)	23.32 (2.89)
n-3 polyunsaturated fatty acids				
18:3(n-3)	1.71 (0.62)	0.62 (0.18)	0.49 (0.18)	0.37 (0.14)
20:3(n-3)	0.02 (0.01)	0.02 (0.01)	0.01 (0.01)	0.01 (0.01)
20:5(n-3)†	0.14 (0.17)	0.02 (0.02)	0.34 (0.23)	0.81 (0.47)
22:5(n-3)	0.07 (0.04)	0.19 (0.05)	0.41 (0.12)	0.90 (0.19)
22:6(n-3)	0.21 (0.21)	0.14 (0.05)	1.49 (0.38)	2.39 (0.55)
Total n-3 polyunsaturated fatty acids†	2.13 (0.74)	0.95 (0.25)	2.62 (0.61)	4.54 (0.87)

Table continues

RESULTS

General characteristics of the participants are described in table 1. The distribution of fatty acids in diet, adipose tissue, plasma, and whole blood, expressed as a percentage of total fatty acids, is described in table 2. The distribution of fatty acids varied substantially among tissues. Monounsaturated fatty acids were more abundant in adipose tissue, representing 52 percent of total fatty acids, while they accounted for 26 percent in plasma and 23 percent in whole blood. On the other hand, plasma and whole blood were richer in *cis*-polyunsaturated fatty acids, 38 percent and 40 percent, respectively, than was adipose tissue (17 percent). The individual fatty acids distribution also varied considerably. For example, whole blood had a high proportion of docosahexaenoic acid, 22:6(n-3), and arachidonic acid, 20:4(n-6) (2.4 percent and 9.1 percent, respectively) compared with adipose tissue, where they represented 0.14 percent and 0.48 percent. Although whole blood is a combination of plasma and red blood cells, it resembled more the fatty acid composition of plasma than of red blood cells (data not shown).

Adipose tissue showed the highest correlations with dietary intake (table 3). The highest diet-adipose correlations were found for 18:3(n-3), 18:2(n-6), and 18:2 *trans*-fatty acids. Diet-plasma and diet–whole blood correlations were generally lower than those for diet-adipose, particularly for 18:3(n-3) and 18:2(n-6) ($p = 0.024$ and $p = 0.039$ for the diet-adipose vs. the diet-plasma comparison), although, for 18:2(n-6), the diet-adipose versus the diet–whole blood comparison was not significant ($p = 0.07$). In this population, the correlations between diet and biomarkers of long-chain (n-3) fatty acids were relatively similar among all tissues, albeit low. It should be noted that there were no good biomarkers of arachidonic acid, $r \leq 0.12$, for all tissues. The correlation between linoleic acid intake and tissue arachidonic acid was also negligible: $r = -0.08$, $r = -0.02$, and $r = -0.06$ for adipose tissue, plasma, and whole blood, respectively. Saturated fatty acids and monounsaturated fatty acids showed low correlations overall. The highest

TABLE 2. Continued

	Diet (% total fat)	Adipose tissue (% total fatty acids)	Plasma (% total fatty acids)	Whole blood (% total fatty acids)
n-6 polyunsaturated fatty acids				
18:2(n-6)	18.87 (6.17)	15.71 (3.53)	28.09 (4.22)	22.38 (3.22)
18:3(n-6)	0.03 (0.06)	0.05 (0.02)	0.39 (0.16)	0.28 (0.12)
20:3(n-6)	0.04 (0.02)	0.33 (0.12)	1.68 (0.38)	1.75 (0.37)
20:4(n-6)	0.24 (0.11)	0.48 (0.13)	6.02 (1.58)	9.08 (1.61)
22:4(n-6)	0.03 (0.02)	0.21 (0.07)	0.26 (0.10)	1.28 (0.31)
Total n-6 polyunsaturated fatty acids	19.25 (6.18)	17.04 (3.56)	36.74 (4.66)	35.10 (3.45)
n-7 polyunsaturated fatty acids				
18:2(n-7)*ct*‡,§	0.30 (0.14)	0.54 (0.17)	0.19 (0.10)	0.16 (0.07)
Total polyunsaturated fatty acids†	21.38 (6.56)	17.33 (3.70)	38.39 (4.98)	39.87 (3.30)
Trans-fatty acids				
16:1(n-7)*t*	0.17 (0.08)	0.16 (0.04)	0.13 (0.05)	0.12 (0.04)
18:1(n-12)*t*	0.46 (0.23)	0.62 (0.22)	0.28 (0.13)	0.26 (0.11)
18:1(n-9)*t*	0.67 (0.29)	0.48 (0.17)	0.24 (0.12)	0.24 (0.11)
18:1(n-7)*t*	1.05 (0.43)	0.31 (0.13)	0.18 (0.08)	0.25 (0.12)
Total 18:1 *trans*-fatty acids	2.18 (0.82)	1.41 (0.46)	0.70 (0.28)	0.75 (0.29)
18:2(n-6)*tt*	0.19 (0.16)	0.26 (0.08)	0.15 (0.05)	0.12 (0.05)
18:2(n-6)*ct*	0.47 (0.30)	0.55 (0.14)	0.26 (0.12)	0.21 (0.11)
18:2(n-6)*tc*	0.52 (0.28)	0.29 (0.08)	0.22 (0.05)	0.20 (0.06)
Total 18:2 *trans*-fatty acids	1.18 (0.73)	1.10 (0.26)	0.63 (0.19)	0.53 (0.18)
Total *trans*-fatty acids	3.57 (1.50)	2.69 (0.62)	1.47 (0.44)	1.40 (0.43)

* Values are expressed as mean (standard deviation). Fatty acids are expressed as a percentage of total fatty acids. $n = 200$ unless otherwise indicated.

† $n = 91$ for adipose tissue, $n = 63$ for plasma, and $n = 62$ for whole blood.

‡ 9c,11*t*-octadecadienoic acid (conjugated linoleic acid).

§ *c*, *cis*; *t*, *trans*.

correlation corresponded to the odd-chain carbon fatty acid 15:0. Similar results were obtained after stratification of the samples by sex (data not shown).

Whole blood and plasma were highly correlated for most fatty acids (figure 1). In general, correlations were high between adipose tissue and plasma/whole blood.

Figure 2 describes the mean proportion and 95 percent confidence intervals for tissue α-linolenic acid within each decile of intake. These three plots illustrate a clear dose-response curve for adipose tissue, plasma, and whole blood. Adipose tissue performed better than plasma or whole blood in representing dietary intake.

DISCUSSION

This study evaluated biomarkers of fatty acid intake in a very complete data set that included three types of biologic specimens and diet in the same population. A major strength of the study is that all foods common to the diet of the studied population were assessed in the same laboratory and by using the same standards and instrumentation for peak identification that were used to assess fatty acids in biologic tissues. Overall, the distribution of fatty acids varied substantially between the different tissues, reflecting metabolic changes and different physiologic roles. Linoleic acid, α-linolenic acid, and 18:2 *trans*-fatty acids showed the highest correlations with diet in all tissues. Adipose tissue fatty acids had the highest correlations with fatty acid intake. The ability to reflect fatty acid intake was comparable between whole blood and plasma, albeit lower than for adipose tissue.

Even though several studies in different populations have evaluated biomarkers of fatty acids in various tissues, data on the ability of diverse tissues to predict intake in the same population are scarce (6, 23–25). Comparisons between previous studies are complex because of the use of different dietary assessment methods in addition to population differences in age, sex, lifestyles, and other factors that may affect the tissue-diet associations. As a result, for example, the correlation coefficient between polyunsaturated fatty acids in plasma and intake varied widely (from 0.16 to 0.73) across studies (24, 26–29). In addition, it becomes impossible to identify whether differing results obtained between

TABLE 3. Partial Spearman correlation coefficients for associations between each tissue fatty acid and the corresponding dietary fatty acid in Costa Rican adults, 1999–2001*

	Adipose tissue	Plasma	Whole blood
Saturated fatty acids			
15:0	0.20	0.26	0.23
16:0	0.10	0.14	0.14
17:0	0.07	0.09	0.09
18:0	0.05	0.01	0.03
Total saturated fatty acids	0.04	0.11	0.14
Monounsaturated fatty acids			
18:1(n-9)	0.15	0.21	0.19
Total monounsaturated fatty acids	0.06	0.14	0.12
n-3 polyunsaturated fatty acids			
18:3(n-3)	0.51	0.39	0.38
20:5(n-3)†	−0.08	0.28	0.22
22:6(n-3)	0.26	0.31	0.23
Total n-3 polyunsaturated fatty acids†	0.39	0.23	0.23
n-6 polyunsaturated fatty acids			
18:2(n-6)	0.52	0.41	0.43
18:3(n-6)	−0.08	0.05	0.00
20:3(n-6)	0.02	−0.14	−0.16
20:4(n-6)	0.11	0.12	0.05
Total n-6 polyunsaturated fatty acids	0.51	0.38	0.40
n-7 polyunsaturated fatty acids			
18:2(n-7)*ct*‡,§	0.25	0.26	0.28
Total polyunsaturated fatty acids	0.51	0.39	0.40
Trans-fatty acids			
16:1(n-7)*t*	0.27	0.20	0.23
Total 18:1 *trans*-fatty acids	0.24	0.08	0.10
18:2(n-6)*tt*	0.27	0.10	0.07
18:2(n-6)*ct*	0.41	0.29	0.31
18:2(n-6)*tc*	0.42	0.28	0.27
Total 18:2 *trans*-fatty acids	0.40	0.24	0.26
Total *trans*-fatty acids	0.31	0.11	0.14

* Spearman correlation coefficients were adjusted for age, sex, and body mass index. $p < 0.05$ for $r > 0.14$. $n = 196$ unless otherwise indicated.

† $n = 91$ for adipose tissue, $n = 63$ for plasma, and $n = 62$ for whole blood.

‡ 9c,11*t*-octadecadienoic acid (conjugated linoleic acid).

§ *c*, *cis*; *t*, *trans*.

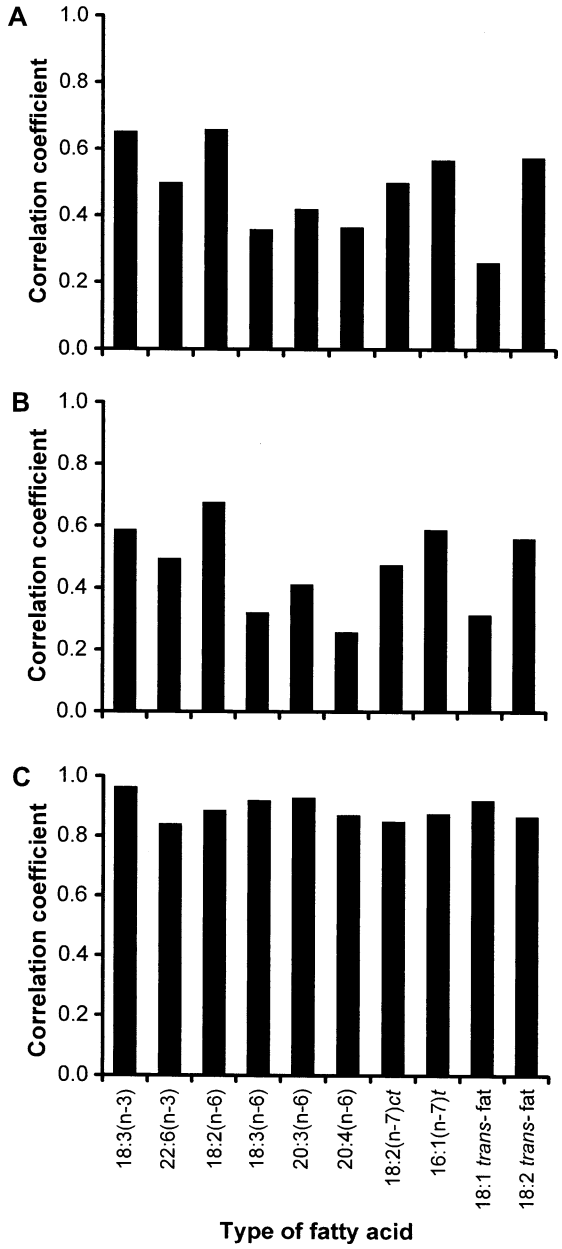

FIGURE 1. Spearman correlation coefficients for pairwise comparisons among tissues from Costa Rican adults, 1999–2001. $p < 0.05$ for $r > 0.14$. A: adipose tissue vs. plasma; B: adipose tissue vs. whole blood; C: plasma vs. whole blood. *c*, *cis*, *t*, *trans*.

two tissues (e.g., plasma vs. red blood cells) are due to these other factors or to differences in the tissue per se (24, 25). This study evaluated the ability of different tissues to reflect intake within the same population and therefore offers internal consistency with regard to tissue comparisons. However, the strength of the association is a function of the true between-person variation in the population being studied; therefore, the ability to generalize the results beyond those populations in which between-person variation is similar to that in the test population is limited (2). For example, in Japan, where intake of long-chain (n-3) fatty acids is high, correlations between long-chain (n-3) fatty acids in diet and

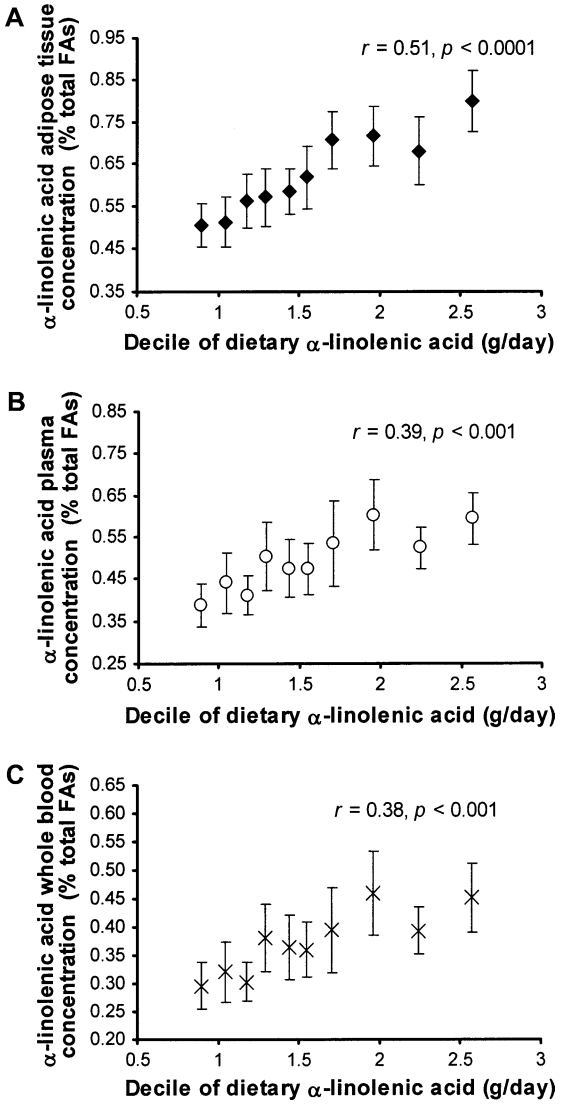

FIGURE 2. Relation between dietary α-linolenic acid and tissue α-linolenic acid in Costa Rican adults, 1999–2001. A: α-linolenic acid in diet vs. adipose tissue; B: α-linolenic acid in diet vs. plasma; C: α-linolenic acid in diet vs. whole blood. Symbols are least-squares means of tissue α-linolenic acid plotted against median daily α-linolenic acid intake by decile, adjusted for age, sex, and body mass index. Bars, 95% confidence intervals. FAs, fatty acids.

plasma were higher than for linoleic or α-linolenic acid (30). On the other hand, in Costa Rica, where fish intake is low (median, 20 g/day), lower correlations between long-chain (n-3) fatty acids in adipose tissue and diet were obtained.

Also of interest is that results from the Costa Rican study, in which data collected between 1995 and 1999 were used, showed that the correlation between dietary intake and α-linolenic acid in adipose tissue was 0.31 (7). This correlation was attributed mostly to the low content of α-linolenic acid in the main dietary source of α-linolenic acid, partially hydrogenated soybean oil (7). The amount of α-linolenic acid in soybean oil has increased from 1.8 percent in 1995 to 4.5 percent in 2001 (unpublished results from our laboratory). As a result, the range of intake increased, and the correlation between dietary intake and α-linolenic acid in adipose tissue in this study, where subjects were recruited between 1999 and 2001, increased to 0.51.

Our data generally are consistent with those from previous studies showing that adipose tissue is an excellent biomarker of fatty acid intake. High correlations (from 0.40 to 0.75) have been reported for polyunsaturated fatty acids (7, 12, 31–37) and *trans*-fatty acids (from 0.34 to 0.76) (7, 33, 34, 36, 38). Our results are also in line with those comparing the ability of two biomarkers to reflect intake. For example, a study in Norway found that dietary α-linolenic acid was better reflected in adipose tissue than in plasma (24). Another study showed that the correlation between diet and adipose linoleic acid was higher than the correlation between diet and red blood cells (25).

The finding that fasting whole blood is a suitable biomarker of fatty acid intake is of particular interest; given the ease of collection, processing, and storage, it could be used as a cost-effective alternative to adipose tissue or plasma in large-scale epidemiologic studies. The distribution of fatty acids in whole blood was comparable to that in plasma, and the correlations between fatty acids in plasma and whole blood were very high (~0.95). Furthermore, the reproducibility for whole blood was similar to that for other tissues. Still, the use of fasting whole blood as a biomarker of fatty acid intake is uncommon. To our knowledge, only one epidemiologic study has used whole-blood fatty acids as biomarkers of intake (14).

In our study, we did not separate the different fractions of plasma:cholesterol ester, phospholipids, and triacylglycerols. It has been suggested that the triacylglycerol fraction is less regulated and therefore more responsive to short-term dietary intake (2), but experimental studies show that all three fractions respond similarly to short-term increases in dietary intake (9, 39, 40). Analysis of each fraction is complex and time consuming; thus, these fractions are more prone to increased measurement error. We have shown that plasma fatty acids reflect dietary intake in a way similar to adipose tissue, although correlation coefficients were generally lower. In large-scale epidemiologic studies, there is always a trade-off between precision in classifying people and budget constraints. We believe that a more detailed subfraction analysis would not improve extensively the ability of plasma to classify people according to dietary intake when fasting blood is used. At the same time, it would make analysis of these studies less affordable.

Note that, although linoleic acid in all tissues is a suitable marker of intake, other (n-6) fatty acids including arachidonic acid were not correlated with intake. Dietary linoleic acid did not correlate with arachidonic acid in any of the tissues studied. Furthermore, correlations for arachidonic acid between tissues were relatively low. These data confirm those from previous studies suggesting that arachidonic acid in tissues is metabolically controlled and levels do not depend on dietary intake, and that different pools of arachidonic acid in the body do not interchange easily (41–43). Saturated and monounsaturated fatty acids in tissues are not

expected to accurately reflect intake in observational studies. However, odd-chain saturated fatty acids (15:0 and 17:0) from dairy products that are not endogenously synthesized may reflect to some extent dietary intake, as supported by our results on 15:0 fatty acids and previous studies in this and other populations (7, 44–46).

In conclusion, in this population, fasting whole blood was a suitable biomarker for long-term essential fatty acid intake, and its performance was comparable to that of plasma. Adipose tissue, if available, is the most reliable choice. Thus, fasting whole blood could be the sample of choice in epidemiologic studies because of its ability to predict intake, its accessibility, and minimum sample processing.

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