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VALIDITY OF THE FATTY ACID COMPOSITION OF SUBCUTANEOUS FAT TISSUE MICROBIOPSIES AS AN ESTIMATE OF THE LONG-TERM AVERAGE FATTY ACID COMPOSITION OF THE DIET OF SEPARATE INDIVIDUALS

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The relationship between the fatty acid composition of subcutaneous adipose tissue and diet was estimated in 59 Dutch women aged 32–35 years. Food consumption was estimated by taking the means of nineteen 24-hour recalls administered over a period of two and a half years, August 1981–December 1983. Highly significant correlations were found between linoleic acid content of fat tissue and diet ($r = 0.70$) and also between the linoleic acid-to-saturated fatty acid (linoleic/S) ratio of fat tissue and diet ($r = 0.62$). This confirms the hypothesis that on an individual level the fatty acid composition of the adipose tissue is a valid index for the habitual dietary fatty acid composition of free-living adults. When using one 24-hour recall instead of the average of 19 recalls, the correlation coefficient between the linoleic/S ratio of the diet and that of the adipose tissue was substantially decreased. This demonstrates the weakening effect of the large day-to-day variation in within-person intake on the correlation between a short-term assessment of the nutrient intake of an individual and a biochemical indicator of long-term nutritional status.

adipose tissue; diet; epidemiologic methods; fatty acids

Many problems are encountered in determining food intake by means of survey methods (1, 2). Therefore, there is a great interest in objective biochemical indicators of nutrient intake. It has been known for a long time (3–5) that the mean fatty acid composition of the subcutaneous adipose tissue of groups of subjects reflects the mean fatty acid composition of the diet of the group. The question remained, however, whether data on adipose tissue composition would give any useful information on the composition of dietary fatty acids of free-living individuals. In an earlier study (6), we examined this question by comparing the fatty acid composition from micro-

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Abbreviations: linoleic, linoleic acid; M, monounsaturated fatty acids; P, polyunsaturated fatty acids; S, saturated fatty acids.

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biopsies of buttock adipose tissue with the dietary fatty acid composition as determined by a two-day record method. A two-day record method may provide valid data on the mean composition of the diet of groups of individuals but yields imprecise information on the fatty acid intake of individuals, due to a large day-to-day variation (7–12). In our earlier study (6), the weakening effect of the day-to-day within-person variance on the correlation coefficient between dietary fatty acid composition and adipose tissue fatty acid composition was estimated using the ratio of the day-to-day within-person variance and the between-person variance as published previously (7, 8, 12). The result indicated that the correlation coefficient between the long-term average dietary polyunsaturated over saturated fatty acids (P/S) ratio and the adipose tissue P/S ratio might be as high as 0.85 instead of the observed 0.49. Therefore, we suggested that the fatty acid composition of an individual's fat tissue could be a valid index for the habitual fatty acid composition of the diet of free-living individuals.

In this study, we have tested this hypothesis by taking fat tissue biopsies from 59 young Dutch women after a two-and-a-half-year period in which their food intake was estimated 19 times by a 24-hour recall method.

MATERIALS AND METHODS

Selection of subjects

Women aged 32–35 years taking part in a study on seasonal variation in energy balance were asked by mail to participate in this study. In the letter, the purpose and method of taking a fat biopsy were explained; a reply coupon and a short medical questionnaire were included. The medical questionnaires were screened by a physician. From the 96 subjects contacted, 49 agreed immediately, and another 20 subjects agreed after a telephone call or after a home visit during which additional explanation was given. The remaining 27 refused to cooperate.

From the 69 women who were willing to participate, three subjects were not eligible for the study because they were pregnant or had moved away. The data of seven subjects were discarded, since comparison of their stated nitrogen intake with their urinary nitrogen excretion and of their stated energy intake and physical activity with their changes in body weight suggested that their intake data were not trustworthy. The results presented in this paper refer to the remaining 59 apparently healthy women for whom the 24-hour food recalls were considered valid. Table 1 shows some characteristics of these women by the end of the study.

Data on food consumption and nutrient intake

Food consumption was assessed 19 times by means of 24-hour recalls. The interviews were conducted by trained dietitians during home visits in the period August 1981–December 1983. The first 14 interviews were carried out at monthly intervals and the last five interviews at intervals of 2–3 months. Per subject, at least two interviews were obtained for each day of the week.

TABLE 1

Age, body weight, body height and body mass index of the 59 young adult Dutch women, by end of study of the relationship of fatty acid composition of subcutaneous adipose tissue and diet, August 1981–December 1983

Variable	Mean	SD*	Minimum	Maximum
Initial age (years)	32.9	1.7	30.0	35.0
Body weight (kg)	60.4	6.1	46.0	75.0
Body height (cm)	167.3	5.8	155.7	190.0
Body mass index (kg/m^2)	21.6	1.7	17.7	26.8

* SD, standard deviation.

The portion sizes of the foods most frequently used were checked by the dietitian by weighing on a Soehnle 8600 balance; other foods were estimated in household measurements. The food consumption data were converted into nutrient intakes using the 1981 edition of the computerized Dutch nutrient data bank (13). This data base made it possible to calculate the saturated, monounsaturated, and polyunsaturated fatty acid content and the linoleic acid content of the diet, linoleic acid referring almost exclusively to the *cis, cis* isomer. On the average, five per cent of the total amount of fatty acids fell under the category "degree of saturation unknown". The set-up of the data base is such that the sum of the fatty acid categories equals the total (crude) fat; the non-fatty acid part of food lipids has not been taken into account. For statistical calculations, fatty acids were expressed in grams per 100 g fat.

Physical activity

Physical activity was also assessed 19 times by 24-hour recalls. The activities were classified into seven categories by level of energy expenditure (14).

Anthropometric measurements

Body weight without clothes was measured to the nearest 0.5 kg by the participants themselves the day after every dietary interview before breakfast and after the bladder had been emptied, using high quality bathroom scales provided and calibrated by us. The dietitians measured the subjects' heights to the nearest 0.5 cm with a microtoise.

Adipose tissue collection and fatty acid analysis

Three months after the food consumption study had been finished, subcutaneous adipose tissue samples were collected during a home visit. The samples were taken from the buttock, as described by Beynen and Katan (15), using an evacuated blood sampling tube and a 1.5 mm needle. It has been shown (15) that this method is rapid and safe and that subjects judge it to be no more painful or unpleasant than a routine blood sampling from an arm vein.

Methyl esters of the component fatty acids (16) were analyzed by gas-liquid chromatography using a 1.8 m glass column filled with 10 per cent Silar 5CP on 100–120 mesh Chromosorb WHP packing (Chrompack, Cat. No. 00910, Middelburg, The Netherlands) with helium as a carrier gas. The oven temperature was 180 C and was programmed to rise to 215 C in 28 minutes. This yielded a good separation of fatty acids from C8:0 to C24:1. In addition, *cis*- and *trans*-isomers of unsaturated C16 and C18 fatty acids were quantitated separately on a 6 m column containing 15 per cent OV-275. Data are presented and statistical calculations were made in terms of g/100 g fatty acid methyl esters. Monounsaturated fatty acids were defined as the sum of *cis*- and *trans*-isomers of C14:1, C16:1, C18:1, and C20:1; polyunsaturated fatty acids as the sum of all di- and polyenoic fatty acids; and linoleic acid as *cis, cis* C18:2 ($n - 6$). With a view to quality control, two samples of a commercial frying fat were analyzed in each run. The coefficient of variation for this material over a two-months' period was 0.5 per cent for peaks containing more and 1.5 per cent for peaks containing less than 2 g/100 g fatty acid methyl esters; thus, for a peak containing 1 g/100 g the standard deviation was 0.015 g/100 g.

Urinary nitrogen analysis

The subjects collected their urine for a 24-hour period in the week following each of the first 14 interviews, and total urinary nitrogen was determined by the Kjeldahl method.

Statistical methods

Pearson correlation coefficients were calculated between the fatty acid composition of the diet and the corresponding variables of the adipose tissue.

The attenuation of correlation coefficients due to day-to-day within-person variation was calculated according to the formula (7, 8, 12, 17):

$$r = r_u(1 + R^2/k)^{-1/2}$$

where r_u = "true" correlation coefficient; r = observed correlation coefficient; k = number of independent recalls per subject; and R = ratio of intra-individual coefficient of variation over inter-individual coefficient of variation in dietary fatty acids.

Analyses of variance were performed with both logarithmically transformed and untransformed data. The results of the transformed data did not differ basically from those of the untransformed data. In this report, we present coefficients of variation resulting from the analysis with the transformed data as this is the appropriate way of modeling proportionality between intra-individual variation and individual mean level (see Appendix).

A regression analysis was conducted to examine whether the fatty acid composition of the diet can be predicted from the fatty acid composition of the adipose tissue.

RESULTS

The composition of the diet determined by taking the average of the nineteen 24-hour recalls is presented in table 2. The dietary pattern is typical for a Western population (12) and is similar to that found in earlier studies on the food consumption of young Dutch adults (18).

The ratio of the intra-individual coefficient of variation over the inter-individual coefficient of variation given in table 2 makes it possible to estimate the attenuation of the correlation coefficients between the dietary fatty acid composition and the fatty acid composition of the adipose tissue. A high ratio for the fatty acid intake would cause underestimation of the correlation coefficients if only one 24-hour recall per subject was used. As can be seen in table 2, the ratio was highest for the energy percentage from protein; this was caused by the very low between-subject coefficient of variation. In general, the ratios found in this study are comparable to the findings of Beaton et al. (12), who used six 24-hour recalls. Exceptions are the ratios for the

TABLE 2

Composition of the diet as determined by the 24-hour recall method repeated 19 times in 2.5 years in 59 young adult Dutch women in a study of the relationship of fatty acid composition of subcutaneous adipose tissue and diet, August 1981–December 1983, and the ratio of the within-subject variation over the between-subject variation in nutrient intake. Comparison with data by Beaton et al. (12)

Variable	Mean ± SD*	Coefficient of variation			
		Within subjects	Between subjects	Ratio within/between	
				Current study	Beaton study
Energy (kcal/day)	2,132 ± 617	0.25	0.16	1.6	1.2
Protein (% of energy)	13 ± 5	0.27	0.09	3.0	2.0
Fat, total (% of energy)	38 ± 8	0.20	0.11	1.8	1.6
Saturated fatty acids (% of energy)	16 ± 4	0.23	0.13	1.8	1.4
Monounsaturated fatty acids (% of energy)	15 ± 4	0.24	0.13	1.9	1.8
Polyunsaturated fatty acids (% of energy)	6 ± 2	0.36	0.19	1.9	3.3
Linoleic acid (% of energy)	5 ± 2	0.42	0.23	1.8	
P/S ratio†	0.40 ± 0.20	0.42	0.22	1.9	2.8
M/P ratio†	2.78 ± 1.16	0.34	0.19	1.8	
Carbohydrate, total (% of energy)	44 ± 9	0.16	0.13	1.2	1.3
Mono- and disaccharides (% of energy)	23 ± 7	0.26	0.21	1.2	
Alcohol (% of energy)	5 ± 2	‡	‡	‡	‡

* The 19 recalls were averaged per subject; the mean and standard deviation (SD) of these 59 averages are given.

† S, M, and P: saturated-, mono- and polyunsaturated fatty acids.

‡ No analysis of variance was made, because the distribution was skewed.

energy percentages from protein and polyunsaturated fatty acids and for the P/S ratio. The higher values for the latter two variables in the study by Beaton et al. are due to a higher within-subject variation.

The average fatty acid composition of the adipose tissue agreed well with the findings in our earlier study (6) and with data from the United States (19), as is shown in table 3. Dietary polyunsaturated fatty acids consist mainly of linoleic acid, which cannot be synthesized de novo by man. The results from our study confirm earlier findings (5) that long-term (relative) linoleic acid intake is well reflected in the proportion of this acid in the adipose tissue (table 3). This was less so for the percentages of monounsaturated fatty acids (M) and saturated fatty acids (S) in the adipose tissue. This is not surprising, as the body synthesizes these fatty acids from various precursors and can also convert them into each other.

Table 4 shows the observed Pearson correlation coefficients and the calculated "true" or unattenuated correlation between the fatty acid composition of the adipose tissue and the diet in this study and in the normal healthy controls in our previous study (6). The attenuation factor of $(1 + R^2/k)^{-1/2}$ for the P/S ratio was about 0.91 for the present study, as opposed to 0.62 for the previous study, in which only two days were recorded per person. The unattenuated correlation coefficients predicted from the present data are similar to those calculated in our previous study.

A separate analysis was conducted for

TABLE 3

Proportion of fatty acids (g/100 g fatty acids) in the adipose tissue and in the diet of 59 young adult Dutch women (mean ± SD), August 1981–December 1983. Data on the adipose tissue of 51 young adult US Caucasian men (19) are given for comparison

Fatty acid variable	Adipose tissue (g/100 g fatty acids methyl esters)			Dietary fat (g/100 g fat)	
	Dutch women		US men	Dutch women	
	Mean	SD*	Mean	Mean	SD*
Saturated	25.31	2.05	29.1	41.17	2.6
C10:0	0.03	0.01			
C12:0	0.46	0.15			
C14:0	2.80	0.50	2.5		
C15:0	0.30	0.07			
C16:0	17.88	1.18	22.4		
C17:0	0.31	0.05			
C18:0	3.53	0.90	4.2		
Monounsaturated	54.35	2.52	57.9	37.90	2.0
C14:1	0.56	0.14			
C16:1 *trans*	0.88	0.21	77.0		
C16:1 *cis*	6.80	1.23			
C18:1 *trans*	3.07	0.70	50.9		
C18:1 *cis*	41.64	2.16			
C20:1	1.41	0.27			
Polyunsaturated	18.00	2.16		15.37	2.7
C18:2 *trans, trans*	0.42	0.08			
C18:2 *cis, trans*	0.50	0.10	12.8		
C18:2 *cis, cis*	14.21	2.10			
C18:3 ($n - 3$)	0.87	0.16			
C18:3 ($n - 6$)	0.27	0.05			
C20:2	0.39	0.06			
C20:3	1.34	0.26			
Unknown	2.3			5.5	

* SD, standard deviation.

subjects with large fluctuations in body weight, because Dayton et al. (20) have shown that these fluctuations disturb the relationship between the fatty acid profile of adipose tissue and the average fatty acid composition of the diet. The data of women with a change in body weight by more than 3 kg from the first to the last three measurements (mean absolute weight change 5.4 ± 1.5 kg in two and a half years) were compared with those of women with a more stable body weight. Table 5 and figure 1 show that this change in weight indeed weakens the correlation of the dietary fatty acid composition and the fatty acid composition of the adipose tissue, although the correlation coefficients are still rather high even for the group with large changes in body weight.

DISCUSSION

Dietary surveys are usually conducted to characterize the food and nutrient intake of populations with emphasis on group means rather than on the results of the individuals in the group. As long ago as

TABLE 4

Observed and unattenuated correlation coefficients between the fatty acid composition of the diet and of the adipose tissue. Data are given for the control group in the authors' previous study (6) (men and women, n = 162) and for the present group of young adult Dutch women (n = 59) studied August 1981–December 1983*

Fatty acid variable	Correlation coefficient			
	Previous study (average of a 2-day record)		Present study (average of nineteen 24-hour recalls)	
	Observed	Unattenuated*	Observed	Unattenuated*
P/S ratio†	0.38	0.65	0.57	0.63
M/P ratio†	0.38	0.61	0.63	0.69
P†	0.40	0.64	0.68	0.75
Linoleic/S ratio†			0.62	0.68
M/Linoleic ratio†			0.63	0.69
Linoleic acid			0.70	0.77

* The value expected if the mean intake during an infinite number of survey days is assumed. It was calculated by the formula presented in the Statistical Methods section.

† P = polyunsaturated fatty acids; Linoleic = linoleic acid; M = monounsaturated fatty acids; S = saturated fatty acids.

TABLE 5

Effect of changes in body weight on the correlation coefficient between the fatty acid composition of the diet and the fatty acid composition of the adipose tissue in 59 young adult Dutch women, August 1981–December 1983. "Changed" denotes subjects (n = 27) who had lost or gained more than 3 kg in 2.5 years. "Stable" denotes the others (n = 32)*

Fatty acid variable	Weight category status	Correlation coefficient r	95% confidence limits for r	One-tailed p value‡
Linoleic/S ratio†	"Changed"	0.54	0.23–0.76	
	"Stable"	0.75	0.54–0.87	0.09
M/Linoleic ratio†	"Changed"	0.54	0.21–0.64	
	"Stable"	0.77	0.57–0.88	0.07
Linoleic†	"Changed"	0.62	0.32–0.81	
	"Stable"	0.82	0.66–0.91	0.06

* Calculated as the difference between the means of the first three and the last three measurements.

† Linoleic = linoleic acid; M = monounsaturated fatty acids; S = saturated fatty acids.

‡ For difference between values of "Stable" and "Changed" groups.

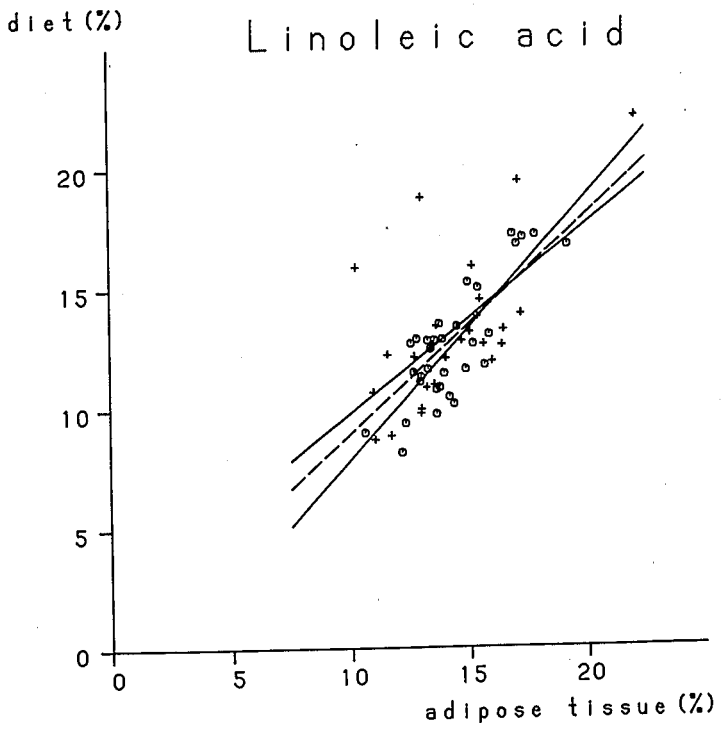

FIGURE 1. Linear regression ($y = -0.14 + 0.91x$) of linoleic acid (g/100 g fat) in the diet of 59 young adult Dutch women assessed with a 24-hour recall method repeated 19 times during two and a half years, August 1981–December 1983, on the corresponding value in adipose tissue. + denotes women ($n = 27$) who lost or gained more than 3 kg during the survey period ($y = 1.96 + 0.79x$). O denotes women ($n = 32$) with a more stable body weight ($y = -3.12 + 1.10x$).

1952, Chalmers et al. (21) stated that unless repeated surveys are made of the same individuals, inter-individual variation will be overestimated and intra-individual variation will be underestimated. As a consequence, the dietary intakes of individuals found in surveys covering one or only a few days fail to correlate with other characteristics of these individuals even if there is ample evidence that those characteristics are affected by the diet (7–9, 12, 17).

In the present study, we have used the mean of nineteen 24-hour recalls obtained over a two-and-a-half-year period as a reference method to test the hypothesis that the fatty acid composition of an individual's subcutaneous buttock fat tissue is a valid index of the long-term fatty acid composition of the diet. The half-life time of adipose tissue in humans in energy balance is approximately 600 days (4, 22), and thus its composition should reflect the dietary fatty acid intake over the preceding two and a half years. The results shown in table 4 confirm that, at least for apparently healthy young adult women, the fatty acid composition of an individual's subcutaneous (buttock) fat tissue is a valid index of the long-term fatty acid composition of the diet. In considering these results, we should realize that the between-person variation coefficient in fatty-acid intake in our study-group was rather small (table 2) and that in populations with a wider spread the correlation between the fatty acid composition of the diet and of the adipose tissue would be even higher. The lowest observed Pearson correlation coefficient was 0.57 for the P/S ratios and the highest was 0.70 for the *cis-cis* linoleic acid values. The correlation was 0.62 for the linoleic/S ratio, which means that almost 40 per cent of the variation between individuals in this ratio in fat tissue is accounted for by variation

in apparent dietary intake. If the number of recalls per person would be further increased, the correlation coefficient would finally approximate a value as high as 0.68 (table 4). On the other hand, with only one 24-hour recall per person the degrading factor would be 0.48 and, theoretically, the correlation coefficient for the linoleic/S ratio in adipose tissue and diet would approximate a value of 0.32. We found values ranging from 0.14 to 0.50 with a median of 0.28 on considering the nineteen 24-hour recalls separately. We could not find any systematic time or seasonal effect on the size of the correlation coefficient.

Thus, this study demonstrates the weakening effect of a large day-to-day variation in intake on the correlation coefficient between a short-term assessment of the nutrient intake of an individual, and a biochemical indicator of the long-term nutritional status.

Yet, even with nineteen 24-hour recalls, about half of the variance in the fatty acid composition of the adipose tissue could not be explained by the intake data. This may be due partly to differences in fatty acid metabolism between subjects and, for a greater part, to errors made in recalling the types and amounts of foods eaten, identification of ingredients in ready-to-eat foods, and shortcomings of the food composition table used to calculate the fatty acid contents of the foods (23, 24).

The linear regression of the percentage of polyunsaturated fatty acid in the diet on the corresponding variable in the adipose tissue was compared with data from an ecologic study based on 14 population means, published by Beynen et al. (5). The slope of the regression line in the ecologic study was $b = 1.2$, but if population groups with sizes smaller than $n = 10$ were discarded the slope equaled 1.1. Thus, between populations a difference of 1 g/100 g fatty acids in adipose tissue polyunsaturated fatty acid content predicts a difference in intake of 1.1 g/100 g of fat. In the present study, the slope of the regression line for the 59 individuals was $b = 0.9$. Excluding subjects with great changes in weight, the slope equaled 1.1. The slope of the regression line for group means in the population-based study was thus similar to that for individuals in the present study. This indicates that the expected differences of the percentage of polyunsaturated fatty acid in the diet for given differences of the corresponding variable in the adipose tissue are the same for individuals with a stable body weight as they are on an aggregate level. This suggests that we are dealing with a fairly general physiologic mechanism.

The question arises whether individuals in other categories of the population would demonstrate the same linear regression. In adults, the effects of age and sex on the fatty acid composition of the adipose tissue are negligible (25, 26). However, in addition to large fluctuations in body weight, some pathologic factors might affect the linear regression of the fatty acid composition of the diet on the corresponding variable in the adipose tissue (27).

We have shown that the fatty acid composition of body fat tissue biopsies is a valid indicator of the long-term dietary fat composition of healthy individuals, especially for subjects who did not experience large gains or losses in body weight during the preceding two to three years. This biochemical indicator may be of value for investigations into the relationship between nutrition and cancer, studies on dietary compliance, and studies evaluating the long-term effects of nutrition education programs.

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APPENDIX

When intra-individual variation is known to be related to mean individual level, it is often considered appropriate to express variability as a coefficient of variation (CV) instead of in terms of the standard deviation (SD) itself.

In this case, it is implicitly understood that the assumption of homoscedasticity in the analysis of variance model, i.e., equal intra-individual variances for each subject, is violated.

A logarithmic transformation allows for a proportional increase in the SD with mean level of measurement, i.e., it accomplishes the desirable property of homogeneity of variances of the transformed variables.

When employing the natural logarithm, the coefficient of variation of the untransformed variable is approximately equal to the square root of the residual variance (standard error of estimate) of the transformed variable.