Human Nutrition: Applied Nutrition (1983) 37A, 365-372.

RELATIONSHIP BETWEEN THE FATTY ACID COMPOSITION OF THE DIET AND THAT OF THE SUBCUTANEOUS ADIPOSE TISSUE IN INDIVIDUAL HUMAN SUBJECTS

Thomas PLAKKÉ*, Johannes BERKEL†, Anton C. BEYNEN, Ruud J. J. HERMUS‡ and Martijn B. KATAN

*Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, The Netherlands. *Present address: Rijkswaterstaat, Koningskade 4, 2500 EX Den Haag; †Present address: Preventicon, Department of Epidemiology, University of Utrecht, Radboudkwartier 261, 3511 CK Utrecht; ‡Present address: Central Institute for Nutrition and Food Research TNO, P.O. Box 360, 3700 AJ Zeist, The Netherlands.*

The fatty acid composition of the diet, measured by means of a two-day record, was compared with the fatty acid composition of subcutaneous body fat in 321 free-living adults. In spite of the known imprecision of dietary survey methods, highly significant correlations ($r = 0.5$) were found between the polyunsaturated fatty acid content or the polyunsaturated-to-saturated fatty acid ratio of the adipose tissue and the corresponding variable in the diet. It was estimated that complete elimination of the noise inherent in the measurement of an individual's fatty acid intake might have resulted in correlation coefficients of the order of 0.8. Analysis of subcutaneous fat biopsies merits consideration as a simple and reproducible measure of the dietary fatty acid composition of free-living individuals.

Introduction

In intervention studies, changes in the mean fatty acid composition of subcutaneous body fat have proved useful for monitoring adherence to an experimental diet (Dayton, Hashimoto & Pearce, 1967; National Diet-Heart Study, 1968; Clarke *et al.*, 1969; Turpeinen, 1973). As we pointed out recently (Beynen, Hermus & Hautvast, 1980), the mean fatty acid composition of the adipose tissue of different, independent groups of human subjects on experimental or constant diets correlated very well with their mean qualitative dietary intake of fatty acids. It has, however, been doubted (National Diet-Heart Study, 1968) whether data on adipose tissue composition would give any useful information on the composition of dietary fatty acids of each separate individual within a group. Unfortunately, few other ways exist to estimate the dietary fatty acid composition of free-living individuals. Dietary survey methods, if carefully performed, can provide data on the mean composition of the diet of groups of subjects, but it is widely acknowledged that they usually do not yield reliable information on individuals (Keys, 1965; Marr, 1971; Liu *et al.*, 1978; Beaton *et al.*, 1979).

Microbiopsies of buttock adipose tissue can be obtained easily and with minimal discomfort for the subjects using the technique of Hirsch *et al.* (1960). We therefore decided to re-examine the question of whether the fatty acid composition of such biopsies could serve as an objective biochemical index of the fatty acid composition of the diet of individuals as opposed to group means. The

study was part of a survey of the nutritional and health status of Seventh-Day Adventists and matched controls in the Netherlands. Seventh-Day Adventists maintain rather strict principles with regard to health, such as abstinence from tobacco and alcohol; some of them adhere to a lacto-ovo-vegetarian diet.

Subjects and methods

The study group consisted of 190 Seventh-Day Adventists, 73 men and 117 women, and 170 control subjects, 69 men and 101 women. All Seventh-Day Adventists had been members of this church for at least ten years. The controls were selected by asking each Seventh-Day Adventist to persuade four friends, neighbours or colleagues, who did not belong to the Seventh-Day Adventist Church, to volunteer for the study. Out of each four potential controls one was randomly selected for participation. Fat biopsies were obtained from 159 Seventh-Day Adventists, 65 men and 94 women, and 162 control subjects, 68 men and 94 women. The age of the subjects ranged from 35 to 55 years and the participants have been described previously (Berkel, 1979).

A two-day record method was used to assess the dietary habits of the subjects. The subjects were individually instructed by an MSc student of the Department of Human Nutrition. Subjects were asked to record everything they consumed on two consecutive days and not to change their habits in any way: for example, if a party had been arranged during the recording days, they were told to go ahead as planned, but to make sure that they did not forget to write anything down. Husbands were asked to fill in their booklets themselves and not leave it to their wives. Subsequently a home visit was made by a dietitian who checked the records, discussed ambiguous items with the subjects and measured the volume of household utensils. Food intake data were converted into nutrients using the 1978 edition of the computerized Dutch food table (Hautvast, 1975).

Subcutaneous adipose tissue samples were collected from the buttock according to the method of Hirsch *et al.* (1960), with the exception that saline was not injected and anaesthetics were not used. The samples were stored at −20 °C and analysed after all samples had been collected. Methylesters of the component fatty acids (Metcalfe, Schmitz & Pekka, 1966) were analysed by gas-liquid chromatography using a 1.5 meter glass column filled with 10 per cent SP 2330 on 100/120 mesh Chromosorb packing (Supelco, Inc., Bellefonte, PA 16823), with nitrogen as a carrier gas and an oven temperature of 200 °C. Data are presented in terms of mass percentage of the methylesters of the component fatty acids. Sampling site as a source of error was investigated by taking biopsies from both the left and the right buttock in 15 subjects. The difference in percentage linoleic acid was 0.15 ± 0.09 mass per cent (mean ± s.d.). As a quality control check, two samples of a commercial frying fat were analysed in each run. The combined within- and between-run variation for this material over a two-month period, expressed as relative s.d., was 1 per cent for major and 2 per cent for minor peaks.

Results

Table 1 presents the composition of the diet as calculated from food records.

Table 1. *Composition of the diet of Dutch Seventh-Day Adventists and control subjects determined by two-day food records.* We have assumed that 100 g of fat contained 95 g of fatty acids. 1 kcal = 4.186 kJ, P = polyunsaturated, M = monounsaturated and S = saturated fatty acids.

	Seventh-Day Adventists				*Controls**	
	Lacto-ovo vegetarians		*Omnivores*			
Component†	*Males* ($n = 18$)	*Females* ($n = 31$)	*Males* ($n = 55$)	*Females* ($n = 86$)	*Males* ($n = 69$)	*Females* ($n = 101$)
Total energy (kcal/day)	2390 ± 575	2080 ± 590	2465 ± 480	1865 ± 435	2585 ± 535	2100 ± 401
Animal protein (energy %)	5 ± 3	6 ± 2	7 ± 2	8 ± 3	8 ± 2	9 ± 3
Vegetable protein (energy %)	7 ± 3	6 ± 2	5 ± 1	5 ± 1	4 ± 1	5 ± 1
Total fat (energy %)	33 ± 6	35 ± 7	36 ± 6	35 ± 7	37 ± 6	37 ± 7
P (energy %)	7 ± 3	7 ± 3	6 ± 2	7 ± 3	6 ± 3	6 ± 2
M (energy %)	11 ± 2	11 ± 3	12 ± 2	12 ± 2	13 ± 3	13 ± 3
S (energy %)	13 ± 4	15 ± 4	16 ± 3	15 ± 4	16 ± 3	16 ± 3
P/S ratio	0.70 ± 0.49	0.56 ± 0.28	0.43 ± 0.20	0.51 ± 0.32	0.43 ± 0.24	0.39 ± 0.19
Carbohydrates (energy %)	55 ± 5	52 ± 6	51 ± 6	50 ± 7	43 ± 8	45 ± 8
Alcohol‡ (energy %)	0	0	0	0	8	3

* None of the controls were vegetarian.

† Mean ± s.d.

‡ As the distribution of alcohol intake was highly skewed s.d. are not given.

Separate data are given for males and females, and for vegetarians and omnivores. The omnivorous subjects had a dietary pattern similar to that in other Western populations (Friend, Page & Marston, 1979). Although the Seventh-Day Adventists on the whole had a lower fat and higher carbohydrate intake than the control subjects, the differences were slight.

The relative percentage of dietary polyunsaturated fatty acids was highest for lacto-ovo-vegetarians and lowest for the control subjects (Table 2). The omnivorous Seventh-Day Adventists formed an intermediate group. The percentage of polyunsaturated fatty acids in the adipose tissue is compatible with this classification, although the differences were smaller (Table 2).

The intake by the control subjects of polyunsaturated fatty acids (Table 2), which essentially represent linoleic acid, appears to be markedly higher than that by British subjects (Thomson *et al.*, 1982). This is reflected by a lower relative percentage of linoleic acid in the adipose tissue of British subjects (Katan & Beynen, 1981), when compared to the control subjects in the present study (Table 2). However, the content of linoleic acid in the adipose tissue of our Dutch subjects agrees well with that in Americans (Katan & Beynen, 1981).

Table 2. *Mean values ± s.d. of the proportion of polyunsaturated fatty acids in the diet and in the adipose tissue of Dutch Seventh-Day Adventist lacto-ovo vegetarians (SDA-vegetarians), in Seventh-Day Adventist omnivores (SDA-omnivores) and in the control subjects.*

		Polyunsaturated fatty acids (g/100 g of total fatty acids)	
Subgroup		*Dietary fat*	*Adipose tissue*
SDA-vegetarians	($n = 43$)	23.0 ± 8.5*	18.6 ± 5.2
SDA-omnivores	($n = 116$)	19.7 ± 7.6*	17.8 ± 4.7
Controls	($n = 162$)	17.4 ± 6.4*	15.3 ± 3.2*

*Significantly different from both other subgroups ($P < 0.05$, two-tailed Student's *t*-test).

Linear correlation coefficients were calculated for all combinations of dietary and adipose tissue relative percentages of polyunsaturated, monounsaturated and saturated fatty acids as well as their ratios. Table 3 documents these correlation coefficients as calculated for all subjects studied. The highest correlation coeffi-

Table 3. *Linear correlation coefficients between the fatty acid composition of the diet and of the adipose tissue for all subjects combined (n = 321).* P = polyunsaturated, M = monounsaturated and S = saturated fatty acids, expressed as g/100 g total fatty acids. All values higher than 0.11 differ significantly from zero at the 95 per cent level. With this number of subjects, a correlation coefficient of 0.50 has 95 per cent confidence limits of 0.41 and 0.58.

Adipose tissue variable	*Dietary fat variable*					
	P	*M*	*S*	*P/S*	*M/P*	*S/M*
P	0.54	−0.22	−0.47	0.51	−0.52	−0.26
M	−0.39	0.22	0.30	−0.36	0.34	0.13
S	−0.24	0.03	0.24	−0.22	0.27	0.17
P/S	0.51	−0.19	−0.46	0.49	−0.49	−0.27
M/P	−0.48	0.16	0.43	−0.43	0.50	0.27
S/M	0.05	−0.10	−0.00	0.04	−0.00	0.05

cients were obtained when polyunsaturated fatty acids were present in both the dietary and adipose tissue parameter. Sex and age were not taken into consideration in this analysis, since their influence on adipose tissue fatty acid composition of adults is negligible (Heffernan, 1963; Heffernan, 1964; Insull & Bartsch, 1967; Baker, 1969).

It could be argued that the observed relationship between dietary and adipose tissue fatty acids for the total sample (Table 3) was caused by subgroups with differing dietary habits. In general, if a sample such as ours is not distributed normally but instead contains clusters of subjects at the extreme ends of the scale, then correlation coefficients may be artificially elevated. However, Table 4 reveals that statistically significant correlation coefficients between the fatty acid composition of the diet and that of the adipose tissue were also obtained within each of the three dietary subgroups separately. This suggests that the relationship between dietary and adipose tissue fatty acids holds for individuals.

Table 4. *Linear correlation coefficients between the fatty acid composition of the diet and the adipose tissue within the subgroups of Seventh-Day Adventist lacto-ovo vegetarians (SDA-vegetarians, n = 43), Seventh-Day Adventist omnivores (SDA-omnivores, n = 116) and controls (n = 162).* P = polyunsaturated, M = monounsaturated and S = saturated fatty acids, expressed as g per 100 g of total fatty acids.

Aipose tissue variable	*Subgroup*	*Dietary variable*		
		P	*P/S*	*M/P*
P	SDA-vegetarians	0.60	0.59	−0.50
	SDA-omnivores	0.57	0.49	−0.60
	Controls	0.40	0.39	−0.38
P/S	SDA-vegetarians	0.61	0.63	−0.50
	SDA-omnivores	0.51	0.46	−0.52
	Controls	0.40	0.38	−0.39
M/P	SDA-vegetarians	−0.45	−0.40	0.44
	SDA-omnivores	−0.52	−0.44	0.62
	Controls	−0.39	−0.38	0.38

All correlation coefficients were significantly different from zero at the $P = 0.01$ level.

Discussion

Dietary surveys, if properly conducted can give reliable estimations of the mean fatty acid composition of the diet of groups. Estimates for individuals, however, show a large standard error (Keys, 1965; Marr, 1971; Liu *et al.*, 1978; Beaton *et al.*, 1979), and little or no progress has been made in reducing this error. Our present observations suggest that the composition of body fat, which has already proved its usefulness in assessing the mean fatty acid composition of groups (Beynen *et al.*, 1980), can also be used to assess the qualitative dietary fatty acid intake of separate individuals.

The data show rather high correlation coefficients between the fatty acid composition of the diet and that of the subcutaneous fat stores in individuals. The highest correlation coefficients were observed when polyunsaturated fatty acids were present in both the dietary and the adipose tissue parameter. This

may be related to the fact that the main polyunsaturated fatty acid in the body, linoleic acid, is entirely of exogenous origin. The relative percentage of saturated and monounsaturated fatty acids in adipose tissue, on the other hand, may be influenced by factors other than their content in the dietary fat, eg carbohydrate intake, alcohol consumption and interconversion of saturated and monounsaturated fatty acids.

The half-life of adipose tissue fatty acids in man is of the order of 600 days (Hirsch *et al.*, 1960; Dayton *et al.*, 1966) and thus the fatty acid profile of body fat should reflect the average fatty acid composition of the diet over a period of about three years (Clarke *et al.*, 1969; Turpeinen, 1973; Beynen *et al.*, 1980). For obtaining information on dietary habits over such a long period, a cross-check dietary history method might appear more appropriate than the two-day record method used in the present study. However, we felt that this method was too demanding for our subjects and might have jeopardized their cooperation. We assumed, moreover, that in this age group dietary habits would not have changed much over the past few years. This assumption seems to be justified by the observed, relatively high correlation coefficients between dietary and adipose tissue fatty acids.

If the fatty acid composition of body fat is to be accepted as a valid measure of the fatty acid composition of the diet, then the observed correlation coefficient between the two ought to be close to unity, provided that both parameters can be accurately measured. We observed a correlation coefficient between eg the ratio of polyunsaturated to saturated fatty acids (P/S ratio) in the diet and in the adipose tissue in the order of 0.50, which implies that 25 per cent of the variance of adipose tissue fatty acids between individuals is accounted for by differences in dietary fat composition. However, it is well known that such correlations are degraded by the shortcomings of the dietary survey method for individuals. The reasons for this have been amply reviewed (Keys, 1965; Liu *et al.*, 1978; Beaton *et al.*, 1979). An important problem with the dietary record method is the large day-to-day variation in the amount and composition of consumed by one person. The composition of adipose tissue, on the other hand, is quite insensitive to short-term fluctuations (Hirsch *et al.*, 1960; Dayton *et al.*, 1966, 1967).

The weakening effect of within-person variability in food intake on the correlation coefficient between diet and physiological variables can be calculated. For this calculation one needs to know the quotient of two variances related to dietary intake, namely day-to-day variance within persons, and the variance between persons. For the dietary P/S ratio, Beaton *et al.* (1979) found relative standard deviations within and between persons of 52 and 26 per cent, respectively, for 24-hour recalls in North American adults. With two-day records and a variance ratio of $(52/26)^2=4$, any correlation between the dietary P/S ratio and physiological parameters such as body fat composition would be degraded by a factor of $\sqrt{(1+4/2)} = 1.73$ (Beaton *et al.*, 1979). This suggests that the correlation between the long-term average dietary P/S ratio and the adipose tissue P/S ratio might be as high as 0.85 (1.73 x 0.49) instead of the value of 0.49 observed here. In that case 72 per cent of the variance between individuals in fat tissue would be accounted for by variance in dietary fatty acid intake. The value of 0.85

agrees well with the correlation coefficient of 0.82 earlier calculated (Beynen *et al.*, 1980) between the mean P/S ratios of the diet and of the adipose tissue of various groups of people. In this case the effect of within-person day-to-day fluctuations in food intake was eliminated by using group means.

In conclusion, we suggest that the fatty acid composition of an individual's fat tissue is a valid index for the habitual fatty acid composition of the diet of individuals. Absolute proof of this is difficult to obtain because the true long-term food intake of a free-living individual is almost impossible to measure (Keys, 1965; Marr, 1971; Liu *et al.*, 1978; Beaton *et al.*, 1979). However, our data, combined with statistical considerations regarding dietary survey methods suggest a very high correlation. An additional advantage of the biopsy method is that, unlike dietary survey methods, the day-to-day variation for an individual is negligible (Hirsch *et al.*, 1960; Dayton *et al.*, 1966, 1967).

Acknowledgements—The authors thank W.A. van Staveren and A.M. Kool-Hulshof, for advice and for carrying out the dietary survey; P. van de Bovenkamp for analytical support; B.A. Scholte for data processing; and J.G.A.J. Hautvast for his interest in these studies. The study was supported by the Netherlands Heart Foundation, grants No. 76.057 and No. 78.093.

References

Baker, G.L. (1969): Human adipose tissue composition and age. *Am. J. Clin. Nutr.* 22, 829.

Beaton, G.H., Milner, J., Corey, P., McGuire, V., Cousins, M., Stewart, E., de Ramos, M., Hewitt, D., Grambsch, P.V., Kassim, N. & Little, J.A. (1979): Sources of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *Am. J. Clin. Nutr.* 32, 2546.

Berkel, J. (1979): The clean life. Some aspects of nutritional and health status of Seventh-Day Adventists in the Netherlands. PhD Thesis, State University of Utrecht, The Netherlands.

Beynen, A.C., Hermus, R.J.J. & Hautvast, J.G.A.J. (1980): A mathematical relationship between the fatty acid composition of the diet and that of the adipose tissue in man. *Am. J. Clin. Nutr.* 33, 81.

Clarke, J.A., Hedley, E., Marr, J.W. & Wood, J.M.H. (1969): Dietary aspects of a controlled trial of soya-bean oil in myocardial infarction. *Nutr.* (London) 23, 136.

Dayton, S., Hashimoto, S., Dixon, W. & Pearce, M.L. (1966): Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. *J. Lipid Res.* 7, 103.

Dayton, S., Hashimoto, S. & Pearce, M.L. (1967): Adipose tissue linoleic acid as a criterion of adherence to a modified diet. *J. Lipid Res.* 8, 508.

Friend, B., Page, L. & Marston, R. (1979): Food consumption patterns in the United States: 1909-13 to 1976. In *Nutrition, lipids and coronary heart disease*, ed R.I. Levy, pp. 489–522, New York: Raven Press.

Hautvast, J.G.A.J. (1975): Ontwikkeling van een systeem om gegevens van voedingsenquêtes met behulp van de computer te verwerken. *Voeding* 36, 356.

Heffernan, A.G.A. (1963): The fatty acid composition of human adipose tissue. *Clin. Sci.* 25, 423.

Heffernan, A.G.A. (1964): Fatty acid composition of adipose tissue in normal and abnormal subjects. *Am. J. Clin. Nutr.* 15, 5.

Hirsch, J., Farquhar, J.W., Ahrens, E.H. Jr., Peterson, M.L. & Stoffel, W. (1960): Studies of adipose tissue in man. A microtechnic for sampling and analysis. *Am. J. Clin. Nutr.* 8, 499.

Insull, W. Jr. & Bartsch, G.E. (1967): Fatty acid composition of adipose tissue related to age, sex and race. *Am. J. Clin. Nutr.* 20, 13.

Katan, M.B. & Beynen, A.C. (1981): Linoleic acid consumption and coronary heart disease in U.S.A. and U.K. *Lancet* 2, 371.

Keys, A. (1965): Dietary survey methods in studies on cardiovascular epidemiology. *Voeding* 26, 464.
Liu, K., Stamler, J., Dyer, A., McKeever, J. & McKeever, P. (1978): Statistical methods to assess and minimize the role of intra-individual variability in obscuring the relationship between dietary lipids and serum cholesterol. *J. Chron. Dis.* 31, 399.
Marr, J.W. (1971): Individual dietary surveys: purposes and methods. *Wld Rev. Nutr. Diet.* 13, 105.
Metcalfe, L.D., Schmitz, A.A. & Pekka, J.R. (1966): Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.* 18, 514.
National Diet-Heart Study. Final report. American Heart Association Monograph 48. (1968): *Circulation* 37, Suppl. 1.
Thomson, M., Logan, R.L., Sharman, M., Lockerbie, L., Riemersma, R.A. & Oliver, M.F. (1982): Dietary survey in 40-year-old Edinburgh men. *Hum. Nutr.: Appl. Nutr.* 36A, 272.
Turpeinen, O. (1973): Primary prevention of heart disease by diet. In *Lipid metabolism and atherosclerosis*, ed D.W.K. Cotton, pp. 49–53. Amsterdam: Excerpta Medica.