The linoleic acid and *trans* fatty acids of margarines[1]

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ABSTRACT Fifty brands of margarine were analysed for *cis*-polyunsaturated acids by lipoxidase, for *trans* fatty acid by infrared spectroscopy, and for fatty acid composition by gas-liquid chromatography. High concentrations of *trans* fatty acids tended to be associated with low concentrations of linoleic acid. Later analyses on eight of the brands, representing various proportions of linoleic to *trans* fatty acids, indicated that two of them contained still higher levels of *trans* fatty acids (>60%) and negligible amounts of linoleic acid. It is proposed that margarine could be a vehicle for the distribution of some dietary linoleic acid and that the level of linoleic acid and the summation of the saturated plus *trans* fatty acids be known to ascertain nutritional characteristics. *Am. J. Clin. Nutr.* 32: 1805–1809, 1979.

Margarine is frequently consumed in a prudent diet because it is a source of polyunsaturated fatty acids, lacks cholesterol, and contains a level of saturated fatty acids lower than that of animal fat. Its composition, however, differs greatly from the oils from which it is derived. That an appreciable portion of vegetable oils is partially hydrogenated before consumption was not taken into account in the studies attempting to relate composition of food supplies to the risk of developing cardiovascular disease (1, 2). This situation was previously discussed (3, 4).

Hardened vegetable fat, processed as for margarine, was found to contain variable amounts of residual linoleic acid (5–9). The inclusion of isomeric forms of this fatty acid would lead to an overestimation of the effective polyunsaturates.

Trans fatty acids in the presence of cholesterol were reported to increase serum cholesterol levels of man only slightly less than a mixture of lauric acid and myristic acid (10). Adverse effects of *trans* fatty acids in the rat were nullified with safflower oil, a rich source of linoleic acid (11). According to Houtsmuller (12), *trans* fatty acids would not be expected to cause undesirable effects, provided sufficient linoleic acid were present in the diet. He proposed that the levels of linoleic acid and *trans* fatty acids should be equal so that appropriate pairs of fatty acids in phospholipids could ensure proper fluidity.

Margarines were therefore examined to determine the relative concentration of the linoleic acid left in the oil and of the *trans* acids formed during hydrogenation.

Materials and methods

Fifty locally available brands of margarine were obtained for fatty acid analyses. A central plug from each product was extracted for lipids with diethyl ether, the extract dried with sodium sulfate, and the ether evaporated. Some samples were also extracted with chloroform-methanol by the procedure of Bligh and Dyer (13), and also by the procedure of Sheppard et al. (14).

The *cis*-polyunsaturated fatty acids (principally linoleate) of the margarines were determined by lipoxidase (linoleate: oxygen reductase, EC 1.13.1.13), using a modification of the method of MacGee (15). The enzyme was obtained from P-L Biochemicals, Milwaukee, Wis., and the stock solution (1 mg/ml) was used after the sample had been saponified overnight. *Trans* fatty acids were determined by infrared spectrophotometry by the methods of Allen (16) and the A.O.A.C. (17). Fatty acids, as their methyl esters, were analyzed on the basis of chain-length and degree of unsaturation with a Hewlett-Packard gas chromatograph, model 7620A, equipped with a six-foot, one-eighth inch column of 10% butanediosuccinate, operated at 190 C, and coupled with a digital integrator.

After sufficient time for possible reformulation of margarines, eight brands were analyzed again. These are designated as the 1977 samples.

Results

The diethyl ether extraction of margarine provided an adequate estimation of the mar-

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garine fat, that is 80% of the product. Lower values, in the order of 74 to 76% fat were obtained with the method of Sheppard et al. (14). The procedure of Bligh and Dyer (13) gave intermediate values.

The concentration of total *cis*-methylene-interrupted polyunsaturated fatty acids (largely 18:2ω6) in the fat of 50 brands of margarine is shown in Figure 1. The most frequent occurrence was in the range of 5 to 10% linoleic acid. More than half of the margarine fats contained less than 10% linoleic acid whereas two exceeded 40%. The corresponding values for the *trans* fatty acids (Fig. 2), showed that the most frequent occurrence was in the range of 30 to 35% and the least frequent under 5%. The inverse relationship in Figure 3 demonstrates the high level of *trans* acids associated with low levels of linoleic acid. This was particularly evident in the print or block margarines, but three tub products contained more than 25% *trans* acid and less than 5% linoleic acid in the total fatty acids. Margarine fat having more than 20% linoleic acid was derived solely from vegetable fat, and had the lowest levels of *trans* fatty acids.

In Table 1 are given the P/S ratios and the total octadecadienoic acids (18:2), as determined by gas-liquid chromatography, the concentration of linoleic acid from the lipoxidase assay, and the summation of saturated plus *trans* fatty acids. It is noteworthy that the two highest P/S ratios, above 3, occurred with inferior levels of linoleic acid and high concentrations of saturated plus *trans* fatty acids. The group of margarines with a P/S ratio from 1.5 to 1.9 appeared to be the superior print margarines. Generally, the tub margarines with similar or higher levels of linoleic acid contained lower levels of saturated plus *trans* fatty acids than the print type, but there were outstanding exceptions. A wide range in the linoleic concentration occurred in both the print margarines (2 to 32%) and the tub margarines (2 to 45%). The products containing marine oil, with or without animal fat, had the lowest concentrations of linoleic acid and from 54 to 59% saturated plus *trans* fatty acids.

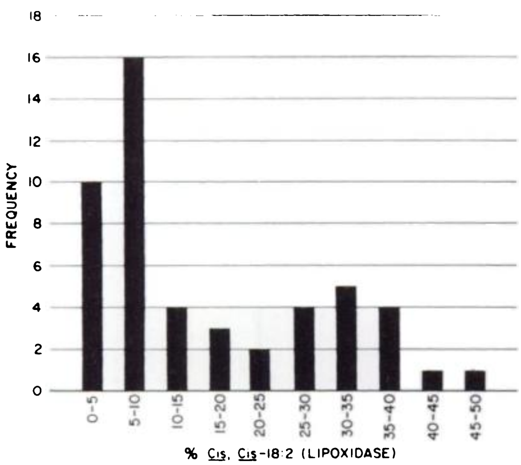

FIG. 1. Distribution of linoleic acid (*cis, cis*-18:2, as determined by lipoxidase) in margarine.

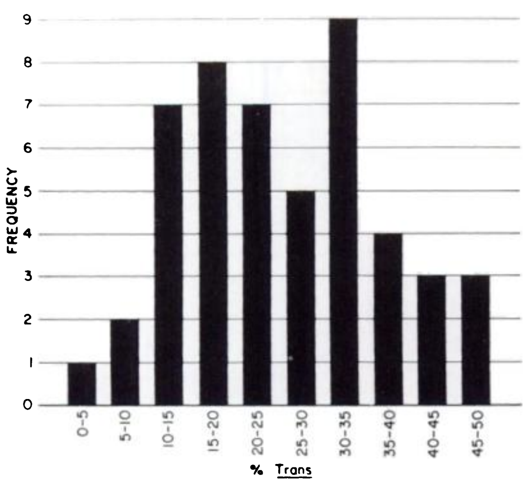

FIG. 2. Distribution of *trans* fatty acids in margarine.

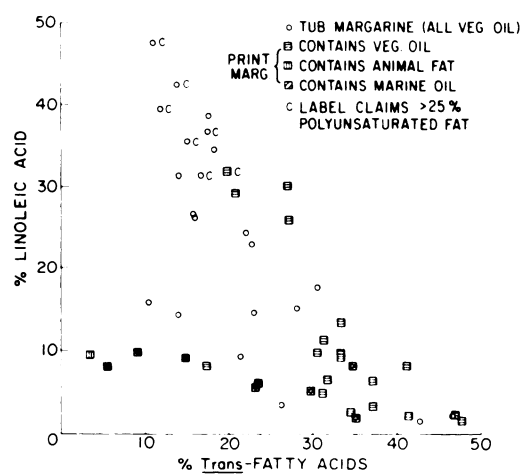

FIG. 3. Relationship between linoleic acid and *trans* fatty acids in margarine.

TABLE 1
Criteria used to judge fatty acid composition

Type of margarine	P/S[a]	Percentage weight of 18:2		Percentage weight of *trans* plus saturated fatty acids
		Total by GLC	Total *cis*-PUFA by lipoxidase[b]	
V[c] Print	3.66	22.0	2.6	52.9
	3.06	18.3	1.6	53.8
	1.89	36.7	29.1	40.3
	1.82	32.9	30.2	45.2
	1.80	32.9	26.0	45.1
	1.52	34.2	31.8	42.9
	1.38	20.5	8.1	56.9
	1.35	21.8	2.6	51.1
	1.28	28.6	15.8	60.8
	1.19	21.6	11.4	51.5
	1.00	19.2	6.5	54.1
	0.87	20.3	15.8	36.8
	0.85	17.7	11.4	53.7
	0.76	18.4	6.5	57.7
	0.75	18.8	9.7	60.5
	0.75	16.4	9.7	56.7
	0.69	16.5	4.9	56.8
	0.63	13.4	3.2	59.3
	0.48	14.0	8.2	46.5
	0.28	6.0	2.1	62.7
	0.28	9.4	8.0	45.4
V Tub	2.53	42.8	26.4	32.9
	2.53	44.9	42.4	31.7
	2.44	45.1	45.0	29.6
	2.41	37.7	37.0	35.1
	2.33	41.5	31.5	32.6
	2.30	19.9	14.5	25.4
	2.29	37.7	31.5	35.5
	2.05	34.7	34.0	38.6
	1.99	16.3	1.6	51.5
	1.97	36.2	36.0	33.9
	1.94	30.4	23.0	39.4
	1.94	34.8	34.0	39.4
	1.78	32.2	32.0	38.9
	1.78	29.4	24.4	40.2
	1.59	30.0	26.6	36.4
	1.52	16.8	2.2	60.9
	1.51	26.4	3.5	44.8
	1.38	26.0	15.2	48.2
	1.30	30.5	14.6	48.2
	1.14	21.5	17.8	49.9
	1.81	17.3	9.4	46.0
V and A[d]	0.91	18.1	8.2	55.2
	0.56	18.5	9.2	48.1
	0.38	12.8	9.8	45.8
V and A and M[e]	0.34	9.8	5.9	55.2
	0.29	9.6	5.9	56.5
V and M	0.73	17.5	5.2	54.1
	0.35	6.7	1.7	59.1
A	0.30	10.3	9.6	42.5

[a] P/S, polyunsaturated: saturated fatty acids, determined by gas-liquid chromatography (GLC). Total 18:2 = P. [b] Total *cis*-methylene-interrupted fatty acids which would include linolenate. The results represent the maximum linoleate; PUFA, polyunsaturated fatty acids. [c] Vegetable oil. [d] Animal fat in print margarine. [e] Marine fat in print margarine.

In the later series of analyses (Table 2), two of the eight margarines contained more than 60% *trans* fatty acids and negligible amounts of linoleic acid.

Discussion

If margarine is consumed as a source of polyunsaturated fatty acids, that is, linoleic acid, the P/S ratio is inappropriate and sometimes misleading. The indiscriminate P includes biologically inactive isomers of linoleic acid and the S would not include the *trans* fatty acids which tend to resemble saturated fatty acids in biological systems. A ratio of two variables in a complex mixture provides no quantitative information. It is possible to have a high P/S ratio in an oil that has little of either P or S, and is predominantly monounsaturated. The concentration of linoleic acid in a margarine is the essential information on polyunsaturates.

Houtsmuller (12) argued that since essential fatty acids, if available, occupied the 2-position and straighter chain fatty acids the 1-position of glycerophospholipids, there should be equal amounts of linoleic acid and *trans* fatty acids in the diet. Elaidic acid did not appear to interfere with the synthesis of prostaglandins so long as the diet contained adequate linoleic acid. For the structural components of membranes, it would appear that the essential fatty acids for the 2-position of the glycerophospholipids should approximate the concentration of the straighter chain fatty acids, that is, the saturated and *trans* acids.

Trans fatty acids have been shown to be readily oxidized in the rat (18, 19). In cardiac tissue, however, triglyceride levels increased when the diet contained partially hydrogenated soybean oil, and elaidic acid showed an affinity for the cardiolipin molecule (20, 21). Also *trans* fatty acids differed from *cis* isomers and more closely resembled saturated fatty acids in acyl transferase reaction and incorporation into glycerophospholipids (22–24). Vergroesen (10) found that they exhibited a hypercholesterolemic effect in man but Mattson et al. did not (25). Addition of elaidic acid lowered the growth response to linoleic acid in an essential fatty acid deficient rat (11). Overall, there appears to be little justification for taking into account the saturated but not the *trans* fatty acids of a partially hydrogenated fat.

High levels of *trans* fatty acids without detectable linoleic acid in a margarine is an unfortunate situation. If the *trans* fatty acids, such as elaidic acid, are to be increased, so should the linoleic acid. It would be desirable to reduce high levels of *trans* fatty acids and to retain some of the original linoleic acid in the final product, thereby furthering the supply and distribution of an essential nutrient.

The technical assistance of L. Thompson was appreciated.

TABLE 2
Percentage by weight of fatty acids in 1977 margarines

Fatty acid	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
14:0	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1
15:0	0.2	0.1						
16:0	5.9	12.5	4.4	11.9	12.4	12.4	11.2	4.1
16:1	0.3	0.2	0.3	0.1	0.1	0.1	0.1	0.3
17:0	0.1	0.1					0.1	0.1
18:0	6.4	6.3	9.4	6.4	6.0	6.6	7.1	6.3
18:1	64.2	36.5	72.2	36.5	49.6	36.9	39.8	73.0
18:2	16.0	42.9	8.2	44.0	31.2	43.1	35.9	10.4
18:3	4.9	1.0		0.6	0.4	0.7	5.0	1.1
20:0	0.5	0.3	0.6	0.3	0.3	0.2	0.3	0.7
20:1	1.4	0.2	2.8	0.2			0.3	3.6
20:0	0.1		2.0					1.3
Total saturated	13.3	19.4	16.6	18.7	18.8	19.3	18.9	12.6
Total PUFA[a]								
18:2 + 18:3	20.9	43.9	8.2	44.6	31.6	43.8	40.9	11.5
cis PUFA	14.4	36.1	ND[b]	37.8	25.3	33.5	30.0	ND[b]
trans fatty acid	25.7	12.0	64.1	12.3	27.9	13.6	21.8	64.8

[a] Polyunsaturated fatty acids. [b] Not detectable.

References

1. ANTAR, M. A., M. A. OHLSON AND R. E. HODGES. Changes in retail market food supplies in the United States in the last seventy years in relation to the incidence of coronary heart disease, with special reference to dietary carbohydrates and essential fatty acids. Am. J. Clin. Nutr. 14: 169, 1964.
2. KAHN, H. A. Change in serum cholesterol associated with changes in the United States civilian diet, 1909–1965. Am. J. Clin. Nutr. 23: 879, 1970.
3. SINCLAIR, H. M. Deficiency of vitamin E and myocardial infarction. Lancet 2: 500, 1973.
4. Report of the Committee on Diet and Cardiovascular Disease. Health and Welfare Canada, December 1976.
5. BEARE, J. L., D. TOVELL AND T. K. MURRAY. The total *cis*-methylene-interrupted fatty acids in Canadian margarines. Can. Med. Assoc. J. 93: 1209, 1965.
6. BEARE, J. L., C. HEROUX AND T. K. MURRAY. Variations in polyunsaturates of special margarines. Can. Med. Assoc. J. 96: 1175, 1967.
7. MULJANICH, P., AND R. OSTWALD. Fatty acids in newer brands of margarine. J. Am. Dietet. Assoc. 56: 29, 1970.
8. CARPENTER, D. L., AND H. T. SLOVER. Lipid composition of selected margarines. J. Am. Chem. Soc. 50: 372, 1973.
9. NAZIR, J., J. MOORECROFT AND M. A. MISHKEL. Fatty acid composition of margarines. Am. J. Clin. Nutr. 29: 331, 1976.
10. VERGROESEN, A. J. Dietary fat and cardiovascular disease: possible modes of action of linoleic acid. Proc. Nutr. Soc. 31: 323, 1972.
11. PRIVETT, O. S., F. PHILLIPS, H. SHIMASAKI, T. NOZAWA AND E. C. NICKELL. Studies of effects of *trans* fatty acids in the diet on lipid metabolism in essential fatty acid difficient rats. Am. J. Clin. Nutr. 30: 1009, 1977.
12. HOUTSMULLER, U. T. M. Proceedings of the International Fat Congress. Marseille, France, 1976.
13. BLIGH, E., AND W. DYER. Rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911, 1959.
14. SHEPPARD, A. J., W. D. HUBBARD AND A. R. PROSSER. Evaluation of eight extraction methods and their effects upon total fat and gas liquid chromatographic fatty acid composition (analyses) of food products. J. Am. Oil Chem. Soc. 51: 416, 1974.
15. MACGEE, J. Enzymatic determination of polyunsaturated fatty acids. Anal. Chem. 31: 298, 1959.
16. ALLEN, R. R. A rapid method for the determination of *trans* unsaturated fatty acids in fats and derivatives. J. Am. Oil Chem. Soc. 46: 552, 1969.
17. Official Methods of Analysis of the AOAC (12th ed.). Washington, D.C.: Association of Official Analytical Chemists, 1975, p. 497, method 28.052.
18. COOTS, R. H. A comparison of the metabolism of *cis, cis*-linoleic, *trans, trans*-linoleic and a mixture of *cis, trans*- and *trans, cis*-linoleic acids in the rat. J. Lipid Res. 5: 473, 1964.
19. ANDERSON, R. L., AND R. H. COOTS. The catabolism of the geometric isomers of uniformly ^{14}C-labelled Δ9-octadecenoic acid and uniformly ^{14}C-labelled Δ9,12-octadecadienoic acid by the fasting rat. Biochim. Biophys. Acta 144: 525, 1967.
20. EDWIN, P. W., AND F. A. KUMMEROW. Incorporation and distribution of dietary elaidate in the major lipid classes of rat heart and plasma lipoprotein. J. Nutr. 102: 783, 1972.
21. HSU, C. M. L., AND F. A. KUMMEROW. Influence of elaidate and erucate on heart mitochondria. Lipids 12: 486, 1977.
22. LANDS, W. E. M., M. L. BLANK, L. J. NUTTER AND O. S. PRIVETT. A comparison of acyltransferase activities in vitro with the distribution of fatty acids in lecithins and triglycerides in vivo. Lipids 1: 224, 1966.
23. PRIVETT, O. S., L. J. NUTTER AND F. S. LIGHTLY. Metabolism of *trans* acids in the rat: Influence of the geometric isomers of linoleic acid on the structure of liver triglycerides and lecithins. J. Nutr. 89: 257, 1966.
24. TAKATORI, T., F. C. PHILLIPS, H. SHIMASAKI AND O. S. PRIVETT. Effects of dietary saturated and *trans* fatty acids on tissue lipid composition and serum LCAT activity in the rat. Lipids 11: 272, 1976.
25. MATTSON, F. H., E. J. HOLLENBACH AND A. M. KLIGMAN. Effect of hydrogenated fat on the plasma cholesterol and triglyceride levels of man. Am. J. Clin. Nutr. 28: 726, 1975.