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Biochem. J. (1962) **84**, 124

The Effect of Dietary Changes on the Fatty Acid Composition of Normal Human Depot Fat

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(Received 20 November 1961)

Reports of similar diets but dissimilar depot-fat composition of the herbivora (Hilditch, 1956; Dole, James, Webb, Rizack & Sturman, 1959) and of the different diets but similar composition of human and chimpanzee depot fats (Gunstone, 1955; Kingsbury, Paul, Crossley & Morgan, 1961*b*) emphasize the apparent stability of the depot-fat composition and the overriding importance of phylogenetic rather than dietary factors in determining its fatty acid pattern. However, the fatty acid composition of mammalian depot fat can be varied by large and prolonged dietary alterations (Hilditch, 1956), though such extensive temporary changes in the diets of normal people are unusual so that, if the fatty acid composition of human depot fat does vary markedly in relatively short periods of time, this could provide evidence for the importance of endogenous rather than exogenous determinants of fatty acid composition.

In order to examine the effect of dietary changes on the fatty acid composition of the depot fat, various oils and fats rich in polyunsaturated fatty acids were fed to normal volunteers who were on a controlled basal diet. Serial depot-fat samples were taken and their fatty acid patterns examined. In addition, as the plasma fatty acids are readily influenced by diet (Ahrens *et al.* 1959; Kingsbury, 1961), a simultaneous study of the total plasma fatty acids and of the ester class containing the highest percentage of the polyunsaturated fatty acids (the plasma cholesteryl esters) was carried out both as a check on the absorption of the dietary fatty acids and as an interesting metabolic comparison.

EXPERIMENTAL

Subjects. These were eleven male students, aged 19–23 years, with no history of any lipid abnormality, or of liver, pancreatic, kidney or thyroid disease. Their plasma lipids and proteins were normal and their fasting plasmas clear. All were of normal weight and activity, with no previous idiosyncracies of diet, and their diets over the previous year had been similar. Volunteers with low, medium and high normal plasma cholesterol concentrations were chosen.

Diets. The subjects were given the same basal diet for the experimental period of 6 weeks; the various fats were added to this. The experiment was divided into three phases, each of 2 weeks, consisting of an initial control period and then two consecutive supplemental periods.

The control and basal diets represented the student's normal quantity and type of food, assessed from knowledge of the student's usual diet and by questioning. The menus were planned to avoid dislikes and the concentration of any type of food on particular days. For this reason fried foods were not given. The fat was a mixture of animal and dairy fats; no fish foods were included.

The experiment began with the control diet shown in Table 1 but, as the volunteers considered that the fat content was less than they normally consumed and their plasma cholesterol concentrations fell slightly (Kingsbury, Morgan, Aylott & Emmerson, 1961*a*), the diet was changed after the first 6 days, as shown in Table 1. On this diet the plasma cholesterol concentrations rose to their starting values and the students considered the amount of fat to be similar to that normally eaten.

After the second week on the control diet there followed the two consecutive supplemental periods, each of 2 weeks. In the first fortnight group 1 (2 students) was given 10 g. of ethyl arachidonate/day for 11 days, followed by 10 g. of Ethiv 315 (the 'pentaene' fraction from cod-liver oil) daily

for 12 days. Group 2 (3 students) received 55 g. of cod-liver oil/day for 12 days, followed by 55 g. of corn oil (as a spray-dried powder) daily for 12 days. Group 3 (3 students) was fed as described for group 2 but in the reverse order.

As 120 g. of the corn oil-skimmed-milk powder was fed daily, 55 g. of skimmed-milk powder was added to the other diets. Olive oil (45 g.) was added to the diet of group 1 to bring all the supplements up to a total of 50–60 g. of fat/day. During the supplemental period the fat of the basal diet was reduced by 50–60 g. The supplements were not given on the days that blood was taken so that any temporary fluctuations of the plasma fatty acids would be avoided.

A summary of the diets and periods of supplementation is shown in Table 1.

Sampling for blood and adipose tissue. Samples were taken (1) before the start of the experiment, (2) at the end of the basal period, (3) and (4) after each supplemental period. The samples are numbered thus in the Tables. Blood was obtained by venepuncture, mixed with one-ninth vol. of aq. 3% (w/v) trisodium citrate and cooled to 0°, and the plasma separated.

Depot-fat samples were obtained under local Xylocaine anaesthesia. A small cut was made in the skin with a scalpel point and a short wide-bore (2–3 mm.) needle, attached to a standard rotating lung-biopsy apparatus (apparatus and needles supplied by Downe Bros., Mayer and Phelps, London, W. 1), driven under the skin up to the hilt, the rotation stopped and the needle's end pressed up under the skin against a finger. With the needle *in situ*, an ordinary syringe was substituted for the compressed-air rotator, suction applied and the needle withdrawn containing its plug of fat. Several runs could be made through the same incision if desired. Pressure was applied to the area

for a few seconds and finally a small dressing. The procedure was painless, with only slight and temporary residual discomfort similar to a small bruise, and had the advantage of providing with certainty sufficient fat for a satisfactory analysis.

The depot-fat samples were washed immediately in cold water to remove blood, then several times with acetone to dehydrate the surface, and finally were stored in acetone at –20° under nitrogen.

Extraction of the lipids. Plasma (10 ml.) was added dropwise to 100 ml. of swirling cold ethanol-ether (3:1, v/v), mixed thoroughly and stored at 0° under nitrogen overnight. The samples of depot-fat were homogenized in cold ethanol-ether in an all-glass hand tissue-grinder and stored overnight at 0° under nitrogen.

Both plasma and depot-fat extracts were then treated alike. The cold ethanol-ether suspensions were brought to boiling under nitrogen, cooled, filtered, evaporated to low volume *in vacuo*, diluted with 100 ml. of light petroleum (b.p. 40–60°) and then one-third vol. of water was added. The supernatant light-petroleum layer was separated, washed three times with an equal volume of water, dried over anhydrous sodium sulphate and evaporated to a moist residue in weighed flasks *in vacuo* in a boiling-water bath, and the remainder of the solvent removed at temperatures below 60°. The last traces of solvent were removed by a stream of oxygen-free nitrogen at 37° and the flasks allowed to come to room temperature in the stream of nitrogen before weighing. The weighed lipids were then dissolved in *n*-hexane to give solutions containing 5–10 mg./ml., and stored at –20° under nitrogen.

Separation of the plasma cholesteryl esters. These were separated from the total-lipid extract of plasma by the method of Barron & Hanahan (1958). Fresh non-activated

Table 1. *Diets of the basal and supplemental periods of the experiment*

	Supplements	Total kcal. (±100)	Dry wt. (g. ± 10 g.)	Carbo- hydrate (g. ± 10 g.)	Protein (g. ± 10 g.)	Fat (g. ± 10 g.)	Fat (%) of total kcal.)	Fat (%) of dry wt.)
Control diet								
First week	—	3155	707	390	112	127	41	18
Second week	—	3649	782	405	127	169	41	21
Experimental diet								
Basal diet	—	2919	669	391	98	107	33	16
First period	Ethyl arachidonate (10 g.) + olive oil (45 g.) + skimmed- milk powder (55 g.)	660	110	25	17	55	76	50
Second period	Ethiv 315* (10 g.) + olive oil (45 g.) + skimmed-milk powder (55 g.)	660	110	25	17	55	76	50
First period	Cod-liver oil (55 g.) + skimmed-milk powder (55 g.)	660	110	25	17	55	76	50
Second period	Corolac† (120 g.) (=55 g. of corn oil)	660	120	25	17	55	76	50
First period	Corolac† (120 g.) (=55 g. of corn oil)	660	120	25	17	55	76	50
Second period	Cod-liver oil (55 g.) + skimmed-milk powder (55 g.)	660	110	25	17	55	76	50
Total diet	—	3582	767	416	115	162	40	21

* Ethiv 315: a cod-liver-oil fraction rich in C₂₀ pentaenes and C₂₂ hexaenes (British Cod Liver Oils Ltd.).

† Corolac: a powder obtained by spray-drying a 50% (v/v) emulsion of corn oil in skimmed milk (L. E. Pritchett and Co. Ltd.).

silicic acid (3 g.) was washed with diethyl ether, 15% (v/v) benzene in *n*-hexane and then pure *n*-hexane. A slurry of the silicic acid in *n*-hexane was run into columns adapted from 10 ml. graduated pipettes and compressed with nitrogen at a pressure of 3 lb./in.² to a steady level (approximately half the original volume). Then 3 ml. of the plasma lipid extract (containing 15–20 mg. of lipid) was layered on the column and the cholesteryl esters were eluted with 18–20 ml. of 15% benzene in *n*-hexane and under a nitrogen pressure of 3 lb./in.² (flow rate 1–1.5 ml./min.). The eluates were evaporated to dryness and the lipids weighed as before.

Replicate 'standard' plasma extracts run several times through this procedure showed no significant changes in their polyunsaturated fatty acid concentrations or iodine values. The eluates contained no phospholipid or free cholesterol and only traces of glyceride.

Depot-fat samples. These were accepted as being composed solely of glycerides, for previous studies had shown that over 97% of adipose tissue was saponifiable (Kingsbury *et al.* 1961b), and column purification did not alter the analysis. The depot-fat extracts were therefore not purified further.

Alkaline isomerization. The polyunsaturated fatty acids of the supplements, plasma cholesteryl esters and depot fat were estimated by a modification of the method of Holman & Hayes (1958) in which the time of conjugation was reduced to 15 min. as this gave greater extinction values in the pentaene and hexaene region. The conjugated solutions were colourless. The lipids were not saponified before alkaline isomerization as there was no significant change in the extinction of the conjugated products even when the non-saponifiable material was removed.

Iodine values. These were measured on the depot-fat samples by a micro-adaptation of the Wijs procedure. The following average iodine values were used for calculating the monoene values from the measured polyunsaturated fatty acid and iodine values: monoenes 90, dienes 180, trienes 270, tetraenes 330, pentaenes 390 and hexaenes 460.

Gas-liquid chromatography. The fatty acid compositions of the total plasma and depot-fatty acids from two of the volunteers (II and VIII) were determined by gas-liquid chromatography of their methyl esters. Insufficient cholesteryl esters remained for examination by this technique.

(a) **Preparation of the methyl esters.** The fats were heated under reflux in an oxygen-free nitrogen atmosphere with an excess of methanolic potassium hydroxide solution (0.5N) in the presence of a small amount of quinol as antioxidant. The unsaponifiable matter was extracted with redistilled light petroleum (b.p. 40–60°), and the soaps were converted into the fatty acids by acidification with aqueous sulphuric acid (10%, v/v). The fatty acids were extracted with light petroleum (b.p. 40–60°), the solvent was removed in a stream of nitrogen, and the residual fatty acids were converted into the methyl esters by treatment with ethereal diazomethane solution.

(b) **Gas-liquid chromatography conditions.** A Pye Argon Chromatograph with a radium D detector was used with 5% (w/v) polyvinyl acetate on acid- and alkali-washed Celite as the stationary phase, at a temperature of 175°, with an argon flow rate of 40 ml./min. The heating block was in thermal equilibrium with the surroundings; hence

the temperature varied slightly with the ambient temperature during the analyses.

Three or four different amounts (0.025–0.1 μl.) of each sample were run under different sensitivities to obtain complete analyses for both major and minor components.

(c) **Presentation of results.** 'Carbon numbers' for the components in each run were derived graphically by plotting the chain length of the saturated straight-chain components against the logarithms of retention distance. The apparent chain length or carbon number of the other components was then found by interpolation. The carbon numbers for each run were averaged to give the mean carbon number for each component.

The carbon numbers of the fatty acids causing the majority of the 'peaks' shown in the analyses of human plasma, depot fats and supplements were established on both polar and non-polar phases by the use of markers, progressive hydrogenation, fractionation, isomerization and by comparison with retention volumes quoted in the literature.

Peak identification was carried out by running authentic samples of acids with an even number of carbon atoms/molecule: C₁₂–C₂₂ saturated acids, and C₁₆, C₁₈, C₂₀ and C₂₂ monoenoic acids, C₁₈, C₂₀ and C₂₂ dienoic acids, C₁₈ and C₂₀ trienoic acids, C₂₀ tetraenoic acid, C₂₀ pentaenoic acid and C₂₂ hexaenoic acid.

RESULTS

Fatty acid composition of the dietary supplements. The total polyunsaturated fatty acids in the various supplements estimated by isomerization and by gas-liquid chromatography are shown in Table 2 (a). Reasonable correlation was obtained between the two types of analysis, except for the monoenes of the 'pentaene' fraction. We are uncertain at present which value is the more accurate.

The fatty acid composition of the three supplements as found by gas-liquid chromatography is shown in Table 2 (b). The fish oil and its 'pentaene' extract contained much higher concentrations of pentaenes and hexaenes than human depot fat and also a greater percentage of C₂₀ monoene (12% as opposed to less than 2% in human fat and less than 1% in corn oil). Hence it could be expected that the most noticeable alteration in human depot fatty acids after feeding the fish oil would be in the C_{20:1}, C_{20:5} and C_{20:6} fatty acids (with the standard notation for unsaturated fatty acids). Thus the 55 g. supplement of corn oil gave approximately 25 g. of linoleic acid/day; an equal amount of cod-liver oil provided 3 g. of dienes, 2 g. of trienes, 2.5 g. of tetraenes, 5 g. of pentaenes and 3.5 g. of hexaenes, whereas the 'pentaene' fraction supplied approximately 3 g. of pentaenes and 2 g. of hexaenes/day. The ethyl arachidonate was over 95% pure C₂₀ tetraene by gas-liquid chromatography and isomerization; hence approx. 9 g. of this acid was supplied daily.

Polyunsaturated acids of the cholesteryl esters. The isomerization analyses revealed that, without ex-

Table 2 (a). *Polyunsaturated fatty acid composition of the dietary supplements*

Fatty acids were determined by (a) gas-liquid chromatography and (b) by alkaline conjugation.

Components	Composition (%)					
	Corn oil*		Cod-liver oil*		'Pentaene' fraction*	
	(a)	(b)	(a)	(b)	(a)	(b)
Total saturated	16	17	18	24	1.9	0
Monoenes	34	(calc.) 35	50	(calc.) 46	15.7	(calc.) 28
Dienes	49	(calc.) 47	5.4	(calc.) 6.3	8.1	(calc.) 10.4
Trienes	0.5	1.0	1.4	2.8	4.5	6.1
Tetraenes	0	0	4.6	4.7	10.4	13.6
Pentaenes	0	0	9.5	9.0	31.0	28
Hexaenes	0	0	7.4	6.7	22.2	19

* Iodine values for corn oil, cod-liver oil and 'pentaene' fraction were 121, 152 and 312 respectively.

Table 2 (b). *Fatty acid composition of the dietary supplements*

Fatty acids were determined by gas-liquid chromatography.

Fatty acid	Composition (%)		
	Corn oil	Cod-liver oil	'Pentaene' fraction
C ₁₀	—	0.8	—
C ₁₂	—	0.4	—
C ₁₃	—	—	1.8
C ₁₄	—	2.3	1.9
C _{14:1}	—	0.1	1.9
C ₁₅	—	0.6	1.2
C _{15:br.}	—	0.6	1.2
C ₁₆	12.0	11.5	0.6
C _{16:1}	—	7.8	6.7
C _{16:2}	—	1.2	2.7
C ₁₇	—	0.9	2.2
C _{17:br.}	—	0.9	2.2
C ₁₈	2.5	3.6	0.7
C _{18:1}	24.0	25.6	6.9
C _{18:2}	49.0	2.7	3.1
C _{19:br.}	—	—	0.8
C _{19:3}	0.5	1.4	2.1
C _{19:4}	—	2.7	6.1
C ₂₀	0.5	—	—
C _{20:1}	—	11.7	1.4
C _{20:3}	—	0.5	0.7
C _{20:3}	—	—	1.0
C _{20:4}	—	1.4	3.6
C ₂₂	0.5	—	—
C _{20:5}	—	8.2	25.9
C _{22:1}	—	4.9	—
C _{22:2}	—	1.0	1.7
C _{22:3}	—	—	1.4
C _{22:4}	—	0.5	0.6
C _{22:5}	—	1.3	4.7
C _{22:6}	—	7.4	22.2

ception, the plasma cholesteryl esters of all the volunteers showed marked increases in those polyunsaturated fatty acids dominant in the supplements with which they were being fed [Table 3 (a)]. Thus arachidonic acid increased the tetraenes from 4 to 24 %; the pentaenes rose from 1 to 7 % when

cod-liver oil was fed, whether before or after corn oil; and the dienes rose from 28 to 55 % when corn oil was fed before, but only from 36 to 45 % when it was fed after, cod-liver oil. The increase of dienes may appear less than actually occurred, for all volunteers showed a decrease in the cholesteryl ester dienes when no supplemental dienes were given, as in the control period, and while the ethyl arachidonate was being fed. Dienes were present in the remaining supplements [Tables 2 (a) and (b)]. The mean diene decrease of all the volunteers during the initial fortnight of control diet without supplements was from 46 to 36 %.

The cholesteryl ester pentaenes did not increase during the 'pentaene' supplement, however (group 1, last period); we have attributed this provisionally to the effects of the arachidonic acid previously fed to these volunteers for, even at the end of the experiment, their tetraenes were still raised, i.e. 9 as compared with 3 %. It seemed unlikely that this small pentaene response was caused by deficient absorption when marked pentaene increases occurred during the cod-liver-oil supplements. With this exception, the changes in the cholesteryl esters reflected, very quickly, the dominant polyunsaturated fatty acid content of supplements.

Total plasma fatty acids. These include the acids esterified with cholesterol. A summary of the results obtained by gas-liquid chromatography for the saturated and monoenoic fatty acids, C_{20:4}, C_{20:5} and C_{22:6} acids is shown in Table 4.

The results show that the total polyunsaturated fatty acids of the plasma varied in a similar way to those in the cholesteryl ester fraction of the plasma [determined by alkaline isomerization, Table 3 (a)], as instanced by the results for C_{20:4} and C_{20:5} fatty acids for subject II, and for C_{20:5} and C_{22:6} fatty acids for subject VIII. Unfortunately the third sample from subject VIII was lost, so no compari-

son in this case can be made between the plasma lipids and the plasma cholesteryl esters which showed, by alkaline conjugation analysis, an increase in dienoic acid after feeding corn-oil supplements. The C_{22} hexaene concentrations appeared to be too low in subject II for reliable analysis by gas-liquid chromatography. Further, this method of analysis has demonstrated a considerable variation in saturated and C_{14} and C_{18} monoenoic acid concentrations which could not be detected by alkaline conjugation. There was, however, a remarkable constancy in the percentage of $C_{16:1}$ acid in both subjects, even though changes occurred in the concentrations of all the other fatty acids.

Fatty acids in depot fats. Alkaline isomerization gave [Table 3 (b)] overall mean values of 6.8 % of dienes (range 6.4–7.8), 0.69 % of trienes (0.45–1.1), 0.45 % of tetraenes (0.21–0.47), 0.12 % of pentaenes (0.07–0.16) and 0.14 % of hexaenes (0.12–0.23) in the depot-fat fatty acids of all the volunteers at the beginning of the experiment, which are within the normal range (Kingsbury *et al.* 1961*b*). These mean

values changed slightly during the initial control fortnight to 7.0 % of dienes, 0.90 % of trienes, 0.39 % of tetraenes, 0.18 % of pentaenes and 0.22 % of hexaenes, in contrast with a mean diene decrease from 46 to 36 % in the cholesteryl esters during the same period.

There were, however, small individual variations in the initial 2 weeks. Thus increases occurred in the dienes, trienes and tetraenes of both subjects IV and VII, in the pentaenes of subject VIII and in the hexaenes of subject VII, but these changes were neither consistent nor as marked as the diene decrease in the cholesteryl esters.

When the depot fats were examined by alkaline conjugation, after each supplemental period, very few changes were found. Corn oil produced no marked change in total dienes in either group 2 or 3. In group 1 the ethyl arachidonate produced a small but consistent increase in tetraenes (0.33–0.57 %) and the 'pentaene' fraction (Ethiv 315) resulted in increases in the pentaenes and hexaenes of 0.18–0.43 % and 0.19–0.35 % respectively.

Table 3 (a). *Polyunsaturated fatty acid composition of plasma cholesteryl esters of the subjects*

Fatty acids were determined by alkaline conjugation. Group numbers are as indicated in Table 1. For definition of 'Group average', see text.

Group	Subjects	Sample no.	Dienes (%)	Trienes (%)	Tetraenes (%)	Pentaenes (%)	Hexaenes (%)
			Group average	Group average	Group average	Group average	Group average
1	I (J.T.)	1	50.7	2.0	4.2	0.9	0.2
		2	45.4	2.6	3.9	1.3	0.4
		3	30.3	2.8	28.9	2.1	1.0
		4	29.1	2.3	10.4	1.7	1.8
	II (P.C.)	1	54.0	2.4	4.3	1.2	1.6
		2	38.0	2.1	3.4	0.9	1.4
		3	22.0	0.9	18.8	1.2	0.6
		4	32.0	0.7	9.2	1.2	1.6
	III (P.D.)	1	39.6	1.9	2.5	0	0
		2	33.2	4.4	2.9	0	0
		3	31.9	3.1	9.2	8.0	0
		4	35.6	Neg.	1.3	2.4	0
	IV (S.E.)	1	45.0	2.2	5.3	1.9	1.0
		2	40.0	3.0	5.5	1.7	1.1
		3	31.0	4.8	5.8	9.7	9.6
		4	46.0	2.4	5.0	2.1	2.2
	V (B.R.)	1	48.5	2.7	4.8	1.1	0.3
		2	45.0	3.1	7.2	1.6	1.2
		3	45.0	2.7	11.2	11.3	1.9
		4	54.0	1.9	6.3	2.1	1.3
3	VI (B.W.)	1	39.0	3.3	6.7	1.3	0.4
		2	32.0	3.2	5.6	1.4	1.3
		3	68.7	4.7	8.9	1.4	0.5
		4	33.5	1.5	6.5	8.2	1.5
	VII (P.K.)	1	44.8	3.9	4.6	1.3	1.3
		2	30.6	3.3	3.2	5.4	0.8
		3	52.5	4.4	4.7	6.7	0.9
		4	37.5	5.8	6.3	6.4	7.1
	VIII (D.E.)	1	38.0	3.4	5.2	1.3	0.9
		2	24.0	4.5	7.3	2.2	1.3
		3	47.0	3.4	6.7	1.3	1.4
		4	30.0	0.6	6.3	5.3	3.3
		1	52.0	2.2	4.2	1.0	0.4
		2	42.0	2.3	3.6	1.1	0.4
		3	26.0	1.9	24.0	1.6	0.6
		4	30.0	0.4	9.8	1.4	0.6
		1	44.0	2.8	5.3	1.9	0.8
		2	39.0	3.0	5.5	1.7	0.6
		3	36.0	4.8	5.8	9.7	1.4
		4	45.0	2.4	5.0	2.1	0.8

Table 3 (b). *Polyunsaturated fatty acid composition of the depot fats of the subjects*

Fatty acids were determined by alkaline conjugation. Group numbers are as indicated in Table 1. For definition of 'Group average', see text.

Group	Subjects	Sample no.	Iodine values	Dienes (%)	Trienes (%)	Tetraenes (%)	Pentaenes (%)	Hexaenes (%)	Monoenes (calc.)
			Group average	Group average	Group average	Group average	Group average	Group average	
1	I (J. T.)	1	62	6.8	0.54	0.46	0.07	0.22	50
		2	68	6.8	0.81	0.36	0.17	0.30	56
		3	61	6.3	1.04	0.66	0.13	0.10	42
		4	62	6.4	1.07	0.33	0.37	0.18	68
	II (P. C.)	1	64	6.3	0.83	0.28	0.13	0.18	54
		2	60	6.4	0.69	0.33	0.18	0.43	49
		3	65	7.5	1.04	0.50	0.23	0.13	51
		4	58	6.9	0.88	0.35	0.49	0.28	43
	III (P. D.)	1	79	7.8	1.14	0.47	0.25	0.12	65
		2	80	7.3	1.10	0.39	0.39	0.09	67
		3	70	6.5	1.00	0.46	0.38	0.21	56
		4	73	6.9	1.30	0.61	0.32	0.27	58
	IV (S. E.)	1	57	6.6	0.45	0.25	0.31	0.07	47
		2	73	7.6	1.10	0.41	0.38	0.11	53
		3	78	7.6	0.71	0.97	0.48	0.24	56
		4	74	7.4	0.90	0.97	0.49	0.22	58
	V (B. P.)	1	68	6.5	0.60	0.21	0.11	0.17	60
		2	75	6.4	0.76	0.33	0.15	0.21	67
		3	72	6.2	0.90	0.49	0.21	0.32	61
		4	75	6.8	0.70	0.31	0.17	0.29	65
3	VI (B. W.)	1	52	6.5	0.55	0.28	0.16	0.22	48
		2	59	7.0	0.86	0.34	0.12	0.17	47
		3	65	7.7	0.91	0.38	0.16	0.16	51
		4	58	6.9	0.58	0.27	0.21	0.32	45
	VII (P. K.)	1	72	6.8	0.81	0.39	0.36	0.14	60
		2	60	7.6	1.00	0.97	0.63	0.18	43
		3	70	6.8	0.83	0.85	0.41	0.10	57
		4	72	7.8	0.81	0.83	0.42	0.15	55
	VIII (D. E.)	1	58	7.1	0.60	0.41	0.08	0.16	52
		2	62	8.9	1.04	0.49	0.17	0.17	46
		3	68	8.3	0.82	0.44	0.18	0.10	55
		4	72	7.1	1.11	0.76	0.80	0.90	57

Table 4. *Fatty acid composition of total plasma fatty acids of two subjects*

Fatty acids were determined by gas-liquid chromatography.

Subject ...	Sample no. ...	Composition (%)							
		VIII (D. E.)				II (P. C.)			
		1	2	3	4	1	2	3	4
	Fatty acid								
	Myristic acid	2.9	1.5	—	3.0	5.6	5.1	2.0	3.4
	Myristoleic acid	4.7	2.9	—	4.6	2.6	1.1	0.8	1.9
	Palmitic acid	16.0	18.0	—	15.0	14.0	30.0	28.0	20.0
	Palmitoleic acid	6.0	6.7	—	6.7	7.8	7.8	7.2	8.6
	Stearic acid	6.9	5.3	—	6.6	7.3	12.6	11.2	7.8
	Oleic acid	22.0	27.0	—	18.0	19.0	24.0	30.0	28.0
	Arachidonic acid	3.8	2.5	—	2.1	0.3	0.5	1.9	0.7
	C _{20:5}	—	0.5	—	2.0	0.2	0.1	0.2	0.5
	C _{22:6}	—	0.5	—	1.6	—	—	—	—

Similar small increases occurred in the hexaenes in group 2 (0.18–0.31%) and in the pentaenes and hexaenes of group 3 (0.15–0.38% and 0.16–0.53% respectively) after the cod-liver-oil supplements. Gas-liquid chromatography of the depot-fat

samples from subjects II and VIII (Table 5) confirmed these results, with the exception of hexaene changes. Again the alterations were less marked than those in the corresponding cholesteryl esters.

Alkaline conjugation therefore showed that the polyunsaturated fatty acid composition of the depot fat from all the volunteers was similar, that small individual variations occurred, but that the supplements had little effect except where these contained appreciable amounts of those fatty acids present only in low concentrations in human depot fat, e.g. arachidonic acid, pentaenoic acids and hexaenoic acids, when small increases occurred in the corresponding tissue acids.

The iodine values of the depot-fat samples shown in Table 3 (b) gave calculated total monoene values of 43–65% in the basal period; individual values varied only slightly except in subject VII (60–43%). The total monoene changes did not appear to follow any regular pattern.

Gas-liquid-chromatographic analysis of depot fats of subjects II and VIII. The results are shown in Table 5. The carbon numbers of the fatty acids as

established on polar and non-polar columns are also given. The initial samples from both subjects were very similar in composition, except for the lower percentages of myristoleic acid, palmitic acid and palmitoleic acid and the higher percentages of stearic acid and those fatty acids with carbon numbers above 20 in subject II. At the end of the control period linoleic acid had increased from 5.8 to 6.7% in subject II and from 5.9 to 9.7% in subject VIII. These changes were also shown by alkaline conjugation and were the converse of the changes in the cholesteryl-ester fatty acids. The myristoleic acid concentrations in both the depot and plasma fatty acids were very similar during this period.

At the end of the first supplemental period, subject VIII, who had received corn oil, showed marked decreases in the concentrations of myristoleic acid (3.0–0.5%) and palmitic acid (28.6–

Table 5. *Fatty acid composition of depot fat of two subjects*

Fatty acids were determined by gas-liquid chromatography.

Carbon number		Subject ...	...	Composition (%)								Fatty acid groups
Non-polar column	Polar column			Sample no.	Fatty acid	VIII (D. E.)				II (P. C.)		
		1	2			3	4	1	2	3	4	
10.00	10.00		Decanoic acid	—	—	—	0.1	—	—	—	0.5	A
12.00	12.00		Lauric acid	1.0	0.2	1.1	0.8	0.7	1.5	—	1.3	
14.00	14.00		Myristic acid	4.5	1.4	3.1	2.1	4.2	3.6	—	3.4	
13.70	14.50		Myristoleic acid	4.7	3.0	0.5	2.1	1.1	1.0	—	5.4	
			Total	10.2	4.6	4.7	5.1	6.0	6.1	—	10.6	
13.00	13.00		C ₁₃	—	—	—	—	—	—	—	—	B
15.00	15.00		C ₁₅	0.4	0.6	0.5	1.1	0.7	0.7	—	2.8	
17.00	17.00		C ₁₇	0.2	0.5	0.2	0.4	0.5	0.4	—	3.4	
13.70	13.25 } 13.35 }		C _{14:br.}	—	—	—	1.2	0.2	—	—	3.6	
15.70	15.20 } 15.50 }		C _{16:br.}	0.3	0.2	0.2	1.4	0.3	0.2	—	4.8	
17.35	17.35 } 17.60 }		C _{18:br.}	0.4	0.8	0.9	1.0	0.5	0.5	—	3.8	
			Total	1.3	2.1	1.8	5.1	2.2	1.8	—	18.4	
16.00	16.00		Palmitic acid	31.4	28.6	17.9	17.9	20.3	19.5	—	12.9	C
15.05	16.45		Palmitoleic acid	11.0	13.8	11.8	9.2	8.1	6.7	—	8.0	
18.00	18.00		Stearic acid	1.6	3.7	2.7	4.9	4.9	4.7	—	6.5	
17.60	18.35		Oleic acid	41.3	34.9	47.5	41.1	42.8	50.9	—	26.7	
17.45	18.90		Linoleic acid	5.9	9.7	8.0	6.4	5.8	6.7	—	5.7	D
17.45	19.75		Linolenic acid	0.1	0.4	0.2	0.3	0.1	0.2	—	0.3	
19.60	20.35		C _{20:1}	0.3	1.0	0.7	1.2	0.9	0.8	—	1.0	E
19.30	20.90		C _{20:2}	0.2	0.2	0.1	1.7	0.3	0.1	—	—	
19.20	21.10		C _{20:3}	—	0.2	0.1	—	0.5	0.1	—	1.0	
18.90	21.60		Arachidonic acid	0.1	0.2	0.4	0.1	0.3	0.2	—	0.4	
18.90	22.10		C _{20:5}	0.1	—	0.1	0.6	0.2	—	—	0.5	
21.50	22.25		C _{22:1}	—	—	—	—	0.3	0.1	—	0.8	
21.20	22.90		C _{22:2}	0.1	0.1	0.9	0.4	—	—	—	0.3	
21.00	23.60		C _{22:4}	0.03	Trace	0.2	0.1	0.1	0.03	—	0.1	
20.75	24.05		C _{22:5}	0.1	0.1	0.1	0.8	—	—	—	—	
20.55	24.40		C _{22:6}	0.1	1.0	2.9	1.1	0.3	0.1	—	0.1	
Total of acids with a polar carbon number ≥ 20				1.0	2.8	5.5	6.0	2.9	1.4	—	4.2	

Table 6. *Comparison of total unsaturated fatty acids of depot fat of two subjects*

Unsaturated fatty acids were determined (a) by gas-liquid chromatography and (b) by alkaline conjugation and determination of iodine values.

Subject	Sample no. ... Fatty acids	Composition (%)							
		1		2		3		4	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
VIII (D. E.)	Monoenes	54.0	52.0	50.0	46.0	60.0	55.0	53.0	57.0
	Dienes	6.0	7.1	10.0	8.9	8.4	8.3	8.7	7.1
	Trienes	0.3	0.6	1.9	1.0	1.2	0.8	1.4	1.1
	Tetraenes	0.1	0.4	0.2	0.5	0.5	0.4	0.06	0.8
	Pentaenes	0.1	0.1	0.05	0.2	0.2	0.2	1.3	0.8
	Hexaenes	0.13	0.2	1.02	0.2	2.9	0.1	1.10	0.9
II (P. C.)	Monoenes	53.0	54.0	59.0	49.0	—	51.0	42.0	43.0
	Dienes	6.6	6.4	6.9	6.4	—	7.5	6.4	6.9
	Trienes	1.1	0.8	1.2	0.7	—	1.0	2.0	0.9
	Tetraenes	0.7	0.3	0.3	0.3	—	0.5	0.5	0.4
	Pentaenes	0.2	0.1	—	0.2	—	0.2	0.5	0.5
	Hexaenes	0.3	0.2	0.1	0.2	—	0.2	0.1	0.3

17.9 %) in his depot fat, with increases of oleic acid (34.9–47.5 %), arachidonic acid (0.2–0.4 %) and C_{22} dienoic acid (0.1–0.9 %). There was, however, no increase in linoleic acid, the dominant fatty acid of the supplement. In the second supplemental period, when cod-liver oil was fed, the concentrations of linoleic acid (8.0–6.4 %) and arachidonic acid (0.4–0.11 %) fell, whereas the acids with an odd number of carbon atoms/molecule or with branched chains increased from 1.8 to 5.1 %, and the C_{20} monoenes, dienes and pentaenes and C_{22} pentaenes increased from 0.7 to 1.2 %, 0.1 to 1.7 %, 0.1 to 0.6 % and 0.1 to 0.8 % respectively. The percentage of palmitic acid remained low at 18 %. Therefore in this period increases occurred of the fatty acids present in cod-liver oil, except for the C_{22} hexaenes. Alkaline conjugation, however, showed an increase of total hexanes (Table 6) and, in this instance, the authors accept the conjugation results as more reliable.

The value of the analyses of subject II, who received ethyl arachidonate followed by Ethiv 315, was reduced by the loss of sample 3. However, changes occurred in the overall supplemental period; the concentrations of palmitic acid and oleic acid fell markedly (19.5–12.9 % and 50.9–26.7 % respectively), whereas the short-chain acids increased from 6.1 to 10.6 %, the acids with an odd number of carbon atoms or with branched chains from 1.8 to 18.4 %, and those acids with carbon numbers greater than 20 increased from 1.4 to 4.2 % (including C_{20} pentaenoic acid from 0.2 to 0.5 %). It is not possible, in the absence of sample 3, to know whether the arachidonate increase of 0.2–0.4 % represents the residue of an increment present after the arachidonate feeding; by alkaline conjugation, however, the highest concentration of total tetraene was in sample 3, so this suggestion

seems possible. The C_{22} hexaene increase again appeared only in the alkaline-conjugation results.

The sharp decrease in oleic acid concentration of subject II to 27 % in sample 4 was not so evident in the total monoene results estimated by alkaline conjugation and iodine value because of the compensatory increases in the C_{14} , C_{16} , C_{20} and C_{22} monoenes as shown by gas-liquid chromatography. Thus the total percentage of monoenes of sample 4 as estimated by either alkaline conjugation and iodine value or by gas-liquid chromatography was very similar [42 and 43 % respectively (Table 6)].

The gas-liquid-chromatographic and alkaline-conjugation analyses of the total polyunsaturated acids (for both subjects) were in reasonable agreement except for the hexaene values. More reliance has been placed on the alkaline-conjugation results as the hexaene concentration appeared to be too low for reliable comparison by gas-liquid chromatography.

It appeared, therefore, that marked changes occurred in the depot-fat saturated and monoenoic acids of both volunteers during the supplemental periods, and some in the basal period. The concentration of palmitoleic acid, however, was relatively constant, varying between 11 and 14 % in subject VIII and 7 and 8 % in subject II, despite the concurrent variations in the other acids. An interesting change common to both was the decrease in palmitic acid during the experiment. The polyunsaturated acids of both volunteers changed slightly but only the supplemental fatty acids of cod-liver oil and its 'pentaene' fraction, and possibly arachidonic acid, appeared in the depot fats. The dienes of the corn-oil supplements did not affect the depot-fat dienes which, however, seemed to be affected in some way by the experi-

ment, for in both volunteers these increased during the control period and decreased during the subsequent supplemental periods.

DISCUSSION

One unexpected result of changing the volunteers from their customary diet to the controlled diet was the consistent decrease in the diene content of their plasma cholesteryl esters. The percentages of dienes in the fats of both customary and experimental diets were very similar, as was the amount of fat eaten daily (judged by the volunteers); hence the decrease did not appear to be due to a large decrease of dienes in the diet. Also there was no consistent decrease in the total trienes, tetraenes, pentaenes and hexaenes or increase in the plasma cholesterol concentrations (Kingsbury *et al.* 1961*a*), and therefore presumably no possible dilution of the 'available' dienes by their esterification with a greater number of cholesterol equivalents. It is possible that an increased synthesis of the saturated and monoene fatty acids from carbohydrate sources could have been responsible; however, there were no significant changes of body weight. We cannot explain these diene changes at present.

The changes in the concentration of plasma-cholesteryl-ester dienes, which was lowered during the basal period and which increased when corn oil was fed, were not accompanied by corresponding changes in the total dienes of the depot fat (subjects II and VIII, Table 5). This was not due to malabsorption of the supplemental polyunsaturated acids, or to their oxidation or isomerization during the processes of absorption and metabolism, since the plasma cholesteryl-ester- and the depot-fat pentaenes and hexaenes both increased after feeding the 'pentaene' fraction. Nor would it seem to be due to the large mass of the subcutaneous fat for, if the newly received fatty acids were shared evenly throughout the various plasma and depot-fat esters, this mass would dilute the responsiveness of the various esters equally. Hence the fatty acids within the body do not form an homogeneous evenly-reacting pool, but are subjected to selective esterification. This indicates that it is the properties of the relevant esterases which determine into which ester particular fatty acids are received. The mass and turnover of the tissue glycerides would then additionally reduce the effect of dietary fatty acids.

Gas-liquid chromatography revealed extensive changes in the total plasma fatty acids between the various experimental periods, and these variations were not restricted to the supplemental fatty acids. This did not appear to be the passive consequence of changes in the plasma concentrations of differently composed esters, for the concentrations of

plasma cholesterol, phospholipid and glyceride hardly changed during the control period (unpublished observations), and also the concentration of palmitoleic acid remained approximately constant.

We are unaware of any experimental evidence which explains the surprisingly steady palmitoleic acid concentration found in the plasma (particularly in subject II, samples 1, 2 and 3; and also in subject VIII, samples 2 and 4) even in the presence of marked changes among the other acids. A similar constancy was found for the palmitoleic acid concentrations of the depot-fat samples, so that it appears to be a total-body-fat phenomenon. Whether this will be confirmed by future studies and, if so, will be useful in delineating 'normal' values remains to be seen.

From the depot-fat results it appears that the fatty acids present in the body can be divided into several distinct groups (Table 5). Group A contains the short-chain (C_{10} - C_{14}) acids; group B, the acids with an odd number of carbon atoms/molecule or branched chains; group C, palmitic acid, palmitoleic acid, stearic acid and oleic acid; group D, linoleic acid and linolenic acid; group E, those fatty acids with carbon numbers (on polar columns) above 20. The changes in concentration of the acids of groups A, B and E were usually in the same direction as the other acids in the group. The changes in linoleic acid and linolenic acid (group D) were not related to those of any other acid or group. Group C consists of acids in which large changes could occur irrespective of other members of the group or of the supplements being fed, and these changes were often associated with marked changes in groups A, B and E. Similar groupings appeared in the total plasma fatty acid results (unpublished observations), so that again the changes in those fatty acids (saturated and monoenes) not predominant in the supplements appeared to be a general body phenomenon.

Gas-liquid chromatography of the plasma fatty acids showed the cholesteryl tetraene, cholesteryl pentaene and cholesteryl hexaene increases to be arachidonic acid, C_{20} pentaene and C_{22} hexaene respectively. The supplemental acids were therefore absorbed unchanged and available *in vivo* to depot-fat metabolism. Unless stored under nitrogen and at low temperatures, these polyunsaturated fatty acids become oxidized very easily; hence the metabolism of small quantities of these acids in the body fluids without significant isomerization could indicate the presence of an efficient antioxidant system *in vivo*.

The overall composition of the depot fat, as determined by gas-liquid chromatography, alkaline conjugation and iodine values of these male volunteers aged 19-25 years is similar to that reported for older men aged 40-70 years (Kingsbury

et al. 1961*b*). Thus there appears to be no significant difference in the depot-fat fatty acid composition of men between 19 and 70 years old.

Nevertheless the present studies have shown that, with the possible exception of palmitoleic acid, the tissue fatty acids are subject to considerable flux. When marked changes occurred in the total percentage of those fatty acids classified as groups A, B or E, the opposite change occurred in palmitic acid or oleic acid, or both; this is seen between samples 2 and 4 of subject II and samples 1 and 4 of subject VIII (Table 5). The concentration of these group C acids could change, however, without an associated marked change in the acids of any other particular group. Hence it seems that changes in group C acids can be produced endogenously without equivalent fatty acid changes in either diets or supplements, whereas the minor amounts of groups A, B and E acids appear more sensitive to the effects of the diet. The metabolism of linoleic acid and linolenic acid (group D) seems separate from that of the acids of groups A, B, E or C, for the changes in group D were completely independent of other fatty acid changes or of alterations in diets or supplements.

The increases in tetraenes, pentaenes and hexaenes, but not of dienes, in the depot fat suggests that the oxidation of the former acids is in some way protected or that the weight of the supplement was sufficient to increase recognizably the relatively small mass of depot pentaenes and hexaenes, or both. Any of these possibilities may be correct for the plasma increments persisted for at least 14 days after the end of the supplemental period; and, as the pentaene and hexaene concentrations of 0.2% in the tissues were one-thirtieth of the linoleic concentration, the 5 g. daily pentaene supplements corresponded to an enormous supplement of 150 g. of linoleic acid/day, i.e. five times the daily amount of linoleic acid fed in the corn oil. The total supplemental linoleic acid in the corn oil therefore could have been insufficient to raise the total body-depot-fat linoleic acid concentration by a significant 2% in the presence of an appreciable rate of oxidation. These possible explanations of the different diene and pentaene-hexaene changes need exclusion before more complicated mechanisms are considered.

If the pentaene increases were due solely to more polyunsaturated acid being fed than the amount oxidized, assuming little synthesis *in vivo*, then, if the daily supplements of linoleic acid were continued or increased, there should be a reasonably constant point in the normal individual at which the concentration of the depot linoleic acid begins to increase.

The changes in the saturated and monoene acids of the depot fats were often different from those in

the plasma in the same period, so that it appears impossible to make assumptions about the one from the analysis of the other.

SUMMARY

1. Supplements of ethyl arachidonate, a cod-liver-oil fraction rich in C₂₀ pentaenes and C₂₂ hexaenes, corn and cod-liver oils were substituted for the equivalent amount of fat in a controlled diet and given separately over periods of 14 days to normal volunteers.

2. The fatty acids in serial samples of their total plasma, plasma cholesteryl ester, and depot fat were examined by alkaline isomerization and gas-liquid chromatography.

3. The dietary polyunsaturated acids appeared rapidly in the plasma lipids but only sparsely in the depot fats.

4. Marked individual changes occurred in the monoene and saturated fatty acids of the plasma and depot fat, apparently unrelated to the type of dietary fat. The fatty acid changes of the plasma and depot fat were often dissimilar.

5. It is concluded that the fatty acid composition of the body is in a continuous state of flux, often unrelated to dietary fat, and that changes in the lipids of one body compartment are not reliable indications of changes in another.

We are very grateful to Unilever Ltd. for their support and for a grant to K. J. K., and to Roche Products Ltd. for a grant to D. M. M. We acknowledge the help of Mr de Witt, Marfleet Refining Co., with the gas-liquid chromatography identifications, especially of the long-chain unsaturated fatty acids. We should also like to emphasize our complete dependence on the generous co-operation of the student volunteers, and the hospital dietician, Miss Spriggs, for advice and the planning and maintenance of the dietary programme, and to thank her staff for the care taken to make the diet extremely palatable.

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