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The Fatty Acid Composition of Human Depot Fat

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Early studies of human depot fat were limited by available analytical techniques and investigations into the fatty acid composition of the depot fat did not begin until about 1925. Eckstein (1925) reported 1% of myristic acid and traces of lauric acid, 26% of saturated and 63·6% of unsaturated acid, and 0·5, 0·03 and 0·33% of acids with two, three and four double bonds respectively. The dienes and tetraenes, but not the trienes, were confirmed by Wagner (1926), and 60% of unsaturated acids and 25·8–31% of saturated acids were found by Telfer (1928) and McAmis & Anderson (1930).

Cramer & Brown (1943) published the results of analyses made by fractional distillation of the methyl esters of fatty acids from depot fat, together with their new technique of low-temperature crystallization. These authors were the first to report the presence of mono-unsaturated C_{14} and C_{16} acids, together with various isomeric C_{18} di- and mono-unsaturated acids. They found the following acids present: lauric, myristic, tetradecenoic, palmitic, hexadecenoic, stearic, oleic, octadecadienoic, arachidonic and other C_{20} acids. They did not mention the tri-unsaturated acids. Their fat samples were taken from five subjects, of which at least two or three had vascular disease, and the fat taken from various body sites was bulked before analysis.

It seemed possible that the temperature difference between the fat of the body cavities and subcutaneous tissue could influence its composition. However, Cuthbertson & Thompsett (1933) found the iodine values of subcutaneous, omental, perinephric and epicardial depot fat to be 70, 63·5, 63 and 63·5 respectively. This small variation precluded substantial differences in major components but the possibility of differences in the minor components, such as the polyunsaturated acids, still remained, and it seemed interesting to obtain more information on their concentrations in various sites.

There was evidence that disease could influence the composition of human depot fat, for Telfer (1928) found an increase in the percentage of saturated acids, a raised melting point and a decreased iodine value in a patient in a diabetic coma, and Channon & Harrison (1926) found the subcutaneous fat of scleromatous infants to have a higher melting point and lower iodine value than that from normal infants, but no analyses of fatty acid composition were reported in either of these studies.

This paper describes a general study of human depot fat with the techniques of alkaline isomerization and gas–liquid chromatography. Because of the possibility that age, sex, diet, disease and site of sampling might influence the composition of depot fat, it was decided to restrict the samples used for this study to those taken from the abdominal subcutaneous fat of male adults, some 'normal' and some 'atheromatous', and to analyse samples from various sites in one individual.

EXPERIMENTAL

Selection of subjects. Subjects were matched by age into pairs, one of each pair being atheromatous. Four pairs were chosen, with four additional atheromatous patients. The criteria insisted upon for the selection of each group were these:

(*a*) The non-atheromatous patients were defined by a complete absence of any history of angina or ischaemic limb pain, coronary thrombosis or cerebrovascular accident, and by normal pulse rate, blood pressure, fundi and vascular examination. Their electrocardiograms, chest X-ray examinations and fasting plasma cholesterol, phospholipid and glyceride levels were normal and each took a regular amount of exercise per day with some physical recreation at the weekends (golf, gardening, walking etc.).

(*b*) The atheromatous patients all had histories of angina or ischaemic pain or coronary thrombosis or all of these, or they presented as cases of aortic aneurysm and had a negative Wassermann test. Vascular examination usually revealed thickened femoral arteries and reduced peripheral pulses. All had arteriograms which showed large vessel atheroma; the atheromatous arteries were checked macroscopically at operation and the sections of any excised arteries showed the characteristic microscopic appearance of atheroma. This group had also taken daily and weekend exercise until admittance to hospital or until prevented by their lesion.

The following criteria were insisted upon in both the 'normal' and atheromatous subjects:

(*a*) No marked family history of vascular disease; one parent alive and well, over 65; at least one brother or sister of about the same age as the patient, alive and well.

(*b*) No family or personal history of diabetes, thyroid dysfunction, xanthoma or jaundice.

(*c*) No personal history attributable to kidney or pancreatic disease, vascular trauma or clotting abnormalities.

(*d*) Plasma-cholesterol, -phospholipid and -glyceride concentrations were each less than 350 mg./100 ml.

(*e*) No recent marked changes in the patients' weights, no idiosyncracies of diet such as the avoidance of fat or vegetable-oil supplements, and all were non-vegetarians. There had been no change in occupation in the previous 3 years and no unusually prolonged periods of stress were disclosed by questioning.

(*f*) Routine urine examinations were negative and bromosulphthalein liver-function tests were normal.

As a rough provisional guide, the amount of atheroma was assessed as mild, moderate and severe according to the arteriogram and inspection at operation. The term 'mild' was used to mean local areas of irregularity in the arterial intima, 'moderate' as extensive areas with some evidence of ulceration, 'severe' as confluent and ulcerative areas with marked weakening of the arterial wall.

Sampling of depot fat. The size of sample required necessitated its removal during an operation. Samples used in this study were taken from the subcutaneous fat on either side of an abdominal incision at the beginning of the patient's operation. This meant that all the subjects were selected from cases admitted for operations involving an abdominal incision.

To investigate the effect of the site of sampling on fatty acid composition, depot fat was removed *post mortem*, within 8 hr. of death, from a patient with extensive atheroma (cause of death was coronary thrombosis), samples being taken from the subcutaneous fat of the upper part of the right buttock, the left abdominal wall and from the perinephric fat. On removal from the subjects the samples of depot fat were washed immediately with ice–water, followed by several washes with cold acetone, then stored in a vacuum flask in contact with solid CO_2 until required for analysis.

Chemical methods. The wet tissue was cut into small pieces and extracted with redistilled acetone for 5 hr. in a Soxhlet extractor. The residue was repeatedly ground with sand and extracted with redistilled ether. The acetone solution was diluted with water, repeatedly extracted with ether, the total ether extracts were combined and the solvent was removed by distillation under reduced pressure. Since auto-oxidation of the fatty acids would have led to erroneous results, their ultraviolet-absorption spectra were examined after heating in neutral glycol.

The extracted fat was saponified under N_2 and the unsaponifiable material removed and weighed. The iodine, acid and saponification values were determined by standard methods (British Standards Institute, 1950). Polyunsaturated acids were estimated spectroscopically after alkaline conjugation (Hilditch, Morton & Riley, 1945; Hilditch, Patel & Riley, 1951), and the complete ultraviolet spectra of the isomerized acids were examined. The fatty acids were methylated, diazomethane in ether being used, and the methyl esters examined by gas–liquid chromatography on columns (120 cm. long and 4 mm. diam.) of 16% Apiezon L on 100–200 mesh Celite at 200° and 230°. To distinguish between saturated and unsaturated acids the esters were brominated and rechromatographed at 230°.

Plasma cholesterol was determined by the method of MacIntyre & Ralston (1954), phospholipids were determined by the method described by Hawk, Oser & Summerson (1947), total esterified fatty acids according to Morgan & Kingsbury (1959) and the glycerides were calculated by subtracting the fatty acids esterified to cholesterol and phospholipids from the total esterified fatty acids, and multiplying the resultant figure by 1·045 (Kingsbury, 1958).

Interpretation of analytical results. In calculating the amount of arachidonic acid present we used the factors given by Brice, Swain, Herb, Nichols & Riemenschneider (1952). The conditions of isomerization used by these workers were slightly different from those of Hilditch *et al.* (1945, 1951), but the latter did not give conversion factors for this acid. Moreover, analysis by gas–liquid chromatography indicated the presence of a large number of acids in the C_{18}–C_{23} range, and it was impossible therefore to make true corrections since the exact effect of isomerization on these acids is unknown. With these considerations in mind, and from the data given by Brice *et al.* (1952), the amount of arachidonic acid (tetraene) present was calculated and, after correction for this, the amounts of linolenic (triene) acid and linoleic (diene) acid were determined from the figures of Hilditch *et al.* (1945, 1951). In the calculation of the monoene fraction in the isomerization results no account was taken of the palmitoleic acid and myristoleic acid which were known to be present. The monoene figure was obtained from the difference in iodine value of the total fatty acids and that calculated for polyunsaturated fatty acids.

At the time of this study the gas–liquid-chromatography methods available did not separate adequately the C_{18} dienes and trienes, nor the various C_{20} polyunsaturated acids. Consequently, we have grouped them together in the results [Table 1 (*b*)]. Furthermore, very small amounts of many fatty acids of unknown structure were found; these were not included in Table 1 since it was impossible to classify them correctly.

RESULTS

Chemical constants of the fat [Table 1 (*a*)]. The iodine value of the total fat varied from 60 to 67, and that of the separated fatty acids was slightly higher, 62–70. This slight difference was also observed by Telfer (1928), who found an iodine value of 71·0 for the total fat and 78·6 for the fatty acids in the subcutaneous depot fat of a normal adult.

The detailed composition of the non-saponifiable fraction of human depot fat is unknown at present. In these studies its total amount varied between 0·4 and 1·3%. The percentage of fat in the tissue also varied considerably (between 49 and 78%). However, as no associated trends were apparent in any of the other measurements, no conclusions can be drawn from either of these variations.

The acid values obtained were nil, which suggests that the conditions of sampling, storing and extracting were less likely to have caused hydrolysis of the fatty acid esters than those of Telfer (1928) and Channon & Harrison (1926), where values of up to 2·7 were found.

The saponification value was also fairly constant between 190 and 200, thus indicating an average molecular weight of 262–275, which is very similar

to the figure of 265 reported by Telfer (1928). All these chemical constants were within the ranges reported in the literature. No differences were found between the chemical constants of the depot fat from normal and atheromatous subjects.

Fatty acids

Results from gas–liquid chromatography [Table 1 (*b*)]. The gas–liquid-chromatography results show that oleic acid is by far the largest component of human depot fat, 42–51%; next is palmitic acid (C_{16} saturated), 21–30%, then both palmitoleic acid (C_{16} mono-unsaturated) and stearic acid (C_{18} saturated), both between 5 and 8·5%, then myristic acid (C_{14} saturated), 2–6%. These are almost exactly the figures obtained by Cramer & Brown (1943) by distillation and low-temperature crystallization. The percentages of lauric acid obtained by gas–liquid chromatography were, however, significantly higher than those shown in the results of either Eckstein (1925) or Cramer & Brown (1943), whereas the traces of C_8 and C_{10} acids found by us were not reported in either of these studies.

Further new findings, disclosed by gas–liquid chromatography, are the significant amounts of odd-number carbon atoms straight-chain and also branched-chain fatty acids. The exact sizes of the various branches of the latter acids in human depot fat are not yet known.

No differences were apparent between the concentration of oleic acid and saturated acids from the depot fats of normal and atheromatous subjects.

Results from alkaline isomerization [Table 1 (*c*)]. The high concentration of oleic acid shown by gas–liquid chromatography was confirmed by the results from alkaline isomerization. There were no consistent differences between the amounts of the saturated, total unsaturated or oleic acid fractions from the normal and atheromatous subjects when estimated either by gas–liquid chromatography or alkaline isomerization (Table 2).

Of the acids with more than one unsaturated bond the dienes were present in the highest concentration (5·4–7·4%), and the tetraenes next (0·6–1·3%), whereas Cramer & Brown (1943) found 8–11% of dienes and 0·3–1·0% of tetraenes. These differences may have been due to the particular conditions of isomerization and crystallization employed in the two studies, and perhaps influenced by the fact that we interpreted the dienes and trienes observed after isomerization entirely as C_{18} acids, and the tetraenes as C_{20} acids. This would seem justified, however, as most of the diene fraction must have consisted of linoleic acid and, although small corrections to the conversion formulae might eventually be required, it would be surprising if such corrections completely offset these differences.

No unusual peaks were observed in the ultraviolet-absorption spectra of the isomerized fatty acids. The triene and tetraene peaks were well marked, at least double in both cases (about 270 and 310 mμ respectively), and, as usual, only one of each was used in the conversion formulae. Traces of pentaenes and hexaenes were seen at about 340 and 370 mμ respectively.

Although all samples were examined by the same techniques, any differences in the values for linoleic acid and arachidonic acid found between the normal and atheromatous subjects (Table 2) could not be regarded as definite or sufficient for statistical analysis, in view of the difficulty discussed, in accurately assessing the tetraene concentrations. There did, however, appear to be a higher concentration of trienes in the atheromatous samples. This is discussed below.

The significant amounts of these trienes are a new finding, being more than ten times the amount found by Eckstein (1925). The low values determined by Eckstein probably resulted from the methods used (hexabromide values), which minimize already low triene concentrations (T. P. Hilditch, personal communication). The chain length and structure of these trienes are not known for certain; they have been labelled 'linolenic' for convenience and so calculated as C_{18} acids. It is possible that, as for the dienes and tetraenes, the conversion factors will need correcting when their composition is determined and when suitable standards are available to check the isomerization constants.

Discrepancies between the results of chromatography and alkaline isomerization. The chromatographic values, iodine values and spectroscopic results showed several discrepancies (Table 2): first, there was apparently a 5–6% difference in the amounts of oleic acid; secondly, the observed iodine values and those calculated from the chromatographic results did not agree; thirdly, the combined amounts of di-, tri- and tetra-enes were much lower when determined by chromatography. The differences in the amounts of oleic acid are explicable if it is assumed that the monoene fraction (determined by difference between the observed iodine value and the iodine value of the polyunsaturated acids) contained the amounts of myristoleic acid and palmitoleic acid as shown by chromatography.

The second discrepancy lies in the difference of approximately 6 units between the observed iodine value (average 66·4, range 49·8–62·4) and that calculated from the chromatographic results (average 59·87, lowest possible 47·74, highest possible 66·74); this could be accounted for by the lower concentrations of polyunsaturated acids found by chromatography and is probably caused in part by limitations in the chromatographic

Table 1. *Fatty acid composition of depot fat*

Fatty acid concentrations are expressed as molar percentages of total fatty acids. N, Normal subjects; A, atheromatous subjects.

(*a*) *Chemical constants*

	Matched pairs								Additional atheromatous subjects			
Subjects	N P.B.	A P.W.	N B.H.	A A.H.	N W.C	A M.G.	N A.F.	A A.P.	M.C.	A.S.	A.J.	C.D.
Age (years)	(38)	(40)	(42)	(43)	(45)	(45)	(68)	(61)	(45)	(48)	(49)	(58)
Fat in tissue (%)	48·7	64·8	67·8	65·3	60·3	59·6	68·4	65·8	72·5	67·4	78·1	64·6
Iodine value of fat	59·6	61·9	63·1	61·0	63·0	61·8	66·7	64·4	65·6	63·7	65·3	61·6
Acid value of fat	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Saponification value of fat	199·6	197·3	198·0	198·2	198·6	197·7	195·1	193·2	190·1	195·0	–	198·5
Unsaponifiable (% of fat)	1·00	0·98	1·18	0·69	0·47	1·34	1·27	0·4	0·4	0·8	0·5	0·4
Iodine value of fatty acids	62·4	64·8	65·9	63·9	65·9	64·6	69·8	67·3	68·5	66·7	68·1	64·2

(*b*) *Gas–liquid-chromatographic results*

Saturated straight-chain fatty acids												
Total	41·0	42·5	39·5	40·5	39·0	43·0	35·5	38·5	36·5	40·5	41·0	41·0
C_8	Trace	–	Trace	–	Trace	Trace	–	Trace	–	Trace	0·5	Trace
C_{10}	Trace	Trace	Trace	Trace	Trace	Trace	Trace	0·5	–	Trace	0·5	Trace
C_{12}	1·5	0·5	1·0	1·0	1·5	1·5	1·0	1·5	0·5	1·0	1·5	1·5
C_{14}	5·5	3·5	4·5	6·0	5·0	6·5	4·0	4·0	2·5	4·0	6·0	6·0
C_{15}	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	1·0
C_{16}	24·5	30·0	25·5	24·5	25·5	25·0	21·0	24·0	25·5	24·0	23·5	24·5
C_{17}	0·5	0·5	0·5	1·0	0·5	0·5	0·5	0·5	0·5	1·0	1·0	1·0
C_{18}	8·0	6·0	5·0	6·5	5·5	6·5	7·5	6·0	6·0	8·5	5·5	6·0
C_{19} / C_{20}	0·5	1·5	2·5	1·0	0·5	2·5	1·0	1·0 0·5	0·5 0·5	1·0 0·5	1·0 1·0	0·5 0·5
C_{22}	–	–	–	–	–	–	–	–	–	–	–	–
Saturated branched-chain fatty acids												
Total	2·0	1·0	2·0	2·5	2·0	1·5	1·5	2·0	1·5	2·0	2·5	2·0
C_{15-16}	0·5	Trace	0·5	0·5	0·5	Trace	Trace	0·5	Trace	0·5	0·5	0·5
C_{17-18}	1·5	1·0	1·5	2·0	1·5	1·5	1·5	1·5	1·5	1·0	1·5	1·5
C_{19-20}	–	–	–	–	–	–	–	Trace	Trace	0·5	0·5	Trace
Unsaturated fatty acids												
Total	56·0	56·5	59·0	56·5	59·5	55·0	63·0	59·5	62·0	57·5	56·5	57·0
C_{14} Myristoleic	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
C_{16} Palmitoleic	5·0	5·0	8·0	6·5	6·0	6·0	5·5	6·5	7·5	6·0	6·0	6·0
C_{18} Oleic	47·0	48·5	47·5	46·5	48·5	45·5	51·0	46·0	47·0	44·0	42·5	42·5
C_{18} Linoleic/linolenic	3·5	2·5	3·0	3·0	4·5	3·0	6·0	5·0	5·5	5·5	5·5	6·5
C_{20} Di-/tri-/tetra-enoic	–	–	Trace	–	–	Trace	–	1·5	1·5	1·5	2·0	1·5

Table 1 (*cont.*)

(*c*) *Alkaline isomerization*

	Patient	Age (years)	Total sat. (balance) N	Total sat. (balance) A	Total unsat. N	Total unsat. A	'Monoenes' 'Oleic' (calc.) N	'Monoenes' 'Oleic' (calc.) A	Dienes 'Linoleic' N	Dienes 'Linoleic' A	Trienes 'Linolenic' N	Trienes 'Linolenic' A	Tetraenes 'Arachidonic' N	Tetraenes 'Arachidonic' A	Ratios: Trienes/Tetraenes N	Ratios: Trienes/Tetraenes A	Ratios: Trienes/Dienes N	Ratios: Trienes/Dienes A
Matched pairs																		
(1)	P.B.	38	41·0	.	59·0	.	50·9	.	6·6	.	0·6	.	0·9	.	0·7	.	0·09	.
	P.W.	40	.	37·2	.	62·8	.	55·5	.	5·8	.	0·7	.	0·8	.	0·9	.	0·12
(2)	B.H.	42	36·9	.	63·1	.	55·6	.	5·8	.	0·4	.	1·3	.	0·3	.	0·07	.
	A.H.	43	.	37·6	.	62·4	.	55·4	.	5·8	.	0·6	.	0·6	.	1·0	.	0·10
(3)	W.C.	45	37·4	.	62·6	.	53·9	.	7·5	.	0·5	.	0·7	.	0·7	.	0·07	.
	M.G.	45	.	38·5	.	61·5	.	53·1	.	7·0	.	0·7	.	0·7	.	1·0	.	0·10
(4)	A.F.	68	34·1	.	65·9	.	56·8	.	7·6	.	0·3	.	1·2	.	0·2	.	0·04	.
	A.P.	61	.	36·1	.	63·9	.	55·1	.	7·1	.	1·1	.	0·6	.	2·0	.	0·16
														Average	0·5	1·2	0·07	0·12
														Range	0·2–0·7	0·9–2·0	0·04–0·09	0·1–0·16
Additional atheromatous subjects	M.C.	45	.	35·1	.	64·9	.	57·0	.	5·4	.	1·5	.	1·0	.	1·5	.	0·28
	A.S.	48	.	36·6	.	63·4	.	55·5	.	5·8	.	1·1	.	1·0	.	1·1	.	0·19
	A.J.	49	.	35·5	.	64·5	.	55·8	.	7·0	.	0·9	.	0·8	.	1·1	.	0·11
	C.D.	58	.	38·7	.	61·3	.	53·4	.	6·2	.	1·1	.	0·6	.	1·8	.	0·18
															Average	1·4	.	0·19
															Range	1·1–1·8	.	0·11–0·28

Table 2. *Comparison of results obtained by gas–liquid chromatography and alkaline isomerization*

		Percentage of total fatty acids	
		Normal	Atheroma
(1) Gas–liquid chromatography			
Saturated acids	Av.	37·5	42·3
	Range	37·0 –43·0	38·0 –44·5
Unsaturated acids	Av.	59·4	57·6
	Range	56·0 –63·0	55·0 –62·0
Oleic acid	Av.	48·6	45·3
	Range	47·5 –51·5	42·5 –48·5
C_{18} Linoleic/Linolenic	Av.	4·25	4·5
	Range	3·0 – 6·0	2·5 – 6·5
C_{20} Di-/tri-/tetra-enoic	Range	Trace	Trace–2·0
(2) Alkaline isomerization			
Saturated acids	Av.	37·3	36·9
	Range	34·1 –41·0	35·1 –38·9
Unsaturated acids	Av.	59·7	63·1
	Range	42·6 –65·9	61·3 –64·9
'Oleic'	Av.	54·3	55·1
	Range	50·9 –56·8	53·1 –57·0
'Linoleic'	Av.	7·0	6·3
	Range	5·8 – 7·6	5·4 – 7·1
'Linolenic'	Av.	0·45	0·96
	Range	0·3 – 0·6	0·6 – 1·5
'Arachidonic'	Av.	1·0	0·76
	Range	0·7 – 1·2	0·6 – 1·0
Triene/Tetraene	Av.	0·5	1·3
	Range	0·2 – 0·7	0·9 – 2·0
Triene/diene	Av.	0·07	0·16
	Range	0·04– 0·09	0·10– 0·28

Table 3. *Effect of site of sample on fatty acid composition of depot fat*

Fat from one atheromatous male subject (54 years) was analysed.

Site...	Abdomen (left side)	Buttock (right side)	Perirenal
Yield of fat from tissue (%)	73·3	63·4	58·7
Iodine value of total fat	66·5	65·9	63·6
Iodine value of fatty acids	69·0	69·3	65·2
Percentage unsaponifiable	1·9	1·5	1·3
	Percentage of total fatty acids		
Chromatographic results			
Saturated acids			
C_{12}	1·0	1·0	1·0
C_{14}	5·5	5·5	5·5
C_{16}	23·5	22·5	23·5
C_{18}	4·5	4·5	5·5
Unsaturated acids			
C_{16} (Palmitoleic)	6·0	6·5	5·5
C_{18} (Oleic)	50·5	49·0	49·0
C_{18} (Di- and tri-enes)	4·5	4·5	5·5
C_{20} (Di-, tri- and tetra-enes)	1·0	1·5	1·0
Spectroscopic results			
Dienes ('Linoleic')	6·6	6·6	6·3
Trienes ('Linolenic')	0·7	0·7	0·7
Tetraenes ('Arachidonic')	0·5	0·5	0·5
Pentaenes	0·1	0·1	0·1
Ratio, trienes:tetraenes	1·4	1·4	1·4

methods available at the time of the study (1956–57).

However, we have no complete explanation for these differences in the polyunsaturated acids at present, nor for the opposite nature of the slight changes in the total unsaturated and oleic acid fractions between normal and atheromatous samples observed by gas–liquid chromatography and spectroscopy. Until these discrepancies are resolved it seems most desirable to use both methods of analysis.

Effect of sample site on fatty acid composition (Table 3). Analysis of the three samples of depot fat from various sites in one atheromatous individual, reported in Table 3, showed only small variations in the fatty acid composition. The only observed differences were the slightly lower iodine value and increased stearic acid content of the sample of perinephric fat, together with the overall variation in the percentage of unsaponifiable material. There were no differences in the concentrations of the polyunsaturated acids and the triene:tetraene ratio was greater than 0·8, in agreement with the other atheromatous samples. This similarity in composition found in depot fat from various body sites confirms the similarity of iodine values obtained by Cathcart & Cuthbertson (1931) and G. A. Rose (personal communication) in subcutaneous, omental and perinephric fat.

General features. The serum-lipid levels, weights or the social status of these patients (Table 4) did not appear to show any association with the severity of the disease process or the concentration of any fatty acid in the depot fat, although it is possible that a much larger series might have shown a statistically significant correlation not suggested by these studies.

Differences between normal and atheromatous samples. One interesting feature of the results of this study was that the triene values for the atheromatous patients were higher than for the normals, a difference which could be emphasized by comparing the triene:tetraene or triene:diene ratio in the two groups of subjects (Table 2). It can be seen that no overlapping occurred between the ranges of these two ratios in the two groups. These were the only consistent differences shown between the two groups of subjects, and in fact the triene:tetraene ratio seemed roughly proportional to the severity of the atheroma (but not to the plasma-lipid levels), as can be seen by comparing Tables 1 (*c*) and 4.

Table 4. *Plasma-lipid concentrations of atheromatous subjects*

In the first column numbers in parentheses indicate matched pairs shown in Table 1 (*c*). G, Neutral glycerides; P, phospholipids; C, total cholesterol.

Patient	Age (years)	Lesion	Severity of atheroma	Occupation	Weight (lb.)	Plasma lipids (mg./100 ml.) G	P	C
Normal								
P.B. (1)	38	Laparotomy (intestinal obstruction)	—	Policeman	178	16	182	195
B.H. (2)	42	Duodenal ulcer	—	Carpenter	174	30	193	186
W.C. (3)	45	Post-traumatic arterial occlusion	—	Postman	146	53	216	195
A.F. (4)	68	Hernia	—	Retired clerk	157	28	178	183
Atheromatous								
P.W. (1)	40	Claudication (right iliac obstruction)	Mild–moderate	Station foreman	170	74	196	173
A.H. (2)	43	Abdominal mass (aortic aneurysm)	Moderate–severe	Medical	190	120	236	216
M.G. (3)	45	Claudication angina (aortic aneurysm)	Moderate–severe	Foreman painter	182	42	190	196
A.P. (4)	61	Claudication (iliac and aortic obstruction)	Severe	Purifying-plant attendant	162	58	249	238
M.C.	45	Abdominal mass (aortic aneurysm)	Severe	Chartered accountant	147	85	256	257
A.S.	48	Claudication (iliac obstruction)	Moderate–severe	Engineer	182	131	231	230
A.J.	49	Claudication (iliac obstruction)	Moderate	Costing clerk	158	62	235	212
C.D.	58	Claudication (aortic aneurysm)	Severe	Postman	146	109	212	224

However, it should be emphasized that this study was intended as a general investigation and the methods were not primarily directed to an accurate determination of the trienes and tetraenes, so that in some respects any conclusions drawn about these differences are tentative.

DISCUSSION

The physical characteristics of the depot-fat samples of this study were very similar to those from different body sites reported previously in the literature, and similar to the reported composition of human depot-fat samples bulked from various sites (Cramer & Brown, 1943). Moreover, as the small differences found in some animals between the fatty acid content of subcutaneous and perinephric fat (Hilditch, 1956) were not found in the subjects of this study, it seems that the site has very little influence on the composition of human adipose tissue and for metabolic studies samples may be taken from the most convenient site. Thus at present there is no evidence to suggest that the various sites of adipose tissue (excepting the special case of the mammary gland) cannot be regarded as part of a homogeneous compartment.

Gas–liquid chromatography showed the depot fat to be a complex mixture, especially in the C_{16}–C_{22} region, and there were many acids not shown in Table 1 (*b*) as their structure was unknown and complete resolution was not possible on Apiezon columns. There were 1–2% of both branched-chain and odd-numbered fatty acids. Whether these fatty acids were formed *in vivo* or were derived from the diet is unknown.

The existence of significant amounts of the short-chain C_8 and C_{10} acids is also a new finding, and the percentage of lauric acid was much higher than reported by Eckstein (1925) and Cramer & Brown (1943).

Alkaline isomerization showed the presence of both pentaenes and hexaenes, but, as suitable standards were not available at the time of analysis and as the method was not designed to measure accurately small amounts of these acids, no attempt has been made to estimate the quantities present. The significant amount of trienes is also a new finding: the chain lengths of these trienes were not established, but gas–liquid chromatography showed certainly the presence of linolenic acid.

Thus although the analysis showed the presence of many unusual acids and also significant amounts of many polyunsaturated acids, the lack of standards, the empirical nature of the alkaline isomerization for the pentaene and hexaene fractions and the limited separation on Apiezon columns in gas–liquid chromatography undoubtedly contributed to the discrepancies observed between the results of the two analytical methods. For this and other reasons a repeat study is being undertaken in this laboratory at present.

Probably more than 97% of the fat in the adipose tissue consisted of glycerides, and as such their component fatty acids can be compared with those from other acyl esters and with glycerides from other sites. Published work on this subject in man is almost confined to the plasma lipids and fat deposited in the vessel walls of atheromatous patients. Unfortunately, of those who have published studies by gas–liquid chromatography, Ahrens *et al.* (1959) did not report the fatty acid compositions of their two patients before a very large and unusual alteration of their dietary fats. Bottcher, Keppler, ter Haar Romeny, Boelsma van Houte & van Gent (1958) did not report their results or the clinical definition of their subjects in detail. Dole, James, Webb, Rizack & Sturman (1959) grouped the triglycerides and cholesteryl esters together, as did James, Lovelock, Webb & Trotter (1957). Finally, Tuna, Reckers & Frantz (1958), although using gas–liquid chromatography to separate the fatty acids, estimated the polyunsaturated acids by isomerization.

Therefore the comparison shown in Table 5 for normal subjects has been restricted to analyses with iodine values, alkaline isomerization and the older chemical methods of estimation. It can be seen that the fatty acid composition of depot fat is similar to the glycerides of plasma and milk from fasting subjects but differs markedly from the total plasma fatty acids, and especially from the cholesteryl esters, by its much lower content of polyunsaturated fatty acids. The phospholipid esters have a diene and triene content similar to the glycerides but a higher concentration of tetraenes. The fatty acid pattern of each class of ester therefore appears to be different, but within each class the pattern seems to have broad similarities, whether in plasma, milk or depot fat. Thus the total plasma-fatty acid composition is the average of that of the various esters, whereas that of depot fat is dominated by the glyceride fraction, as one would expect from its low content of non-saponifiable matter.

In the comparison of the normal and atheromatous subjects it was realized that a person's age is not necessarily identical with the biological age of his arterial tissues and that the term 'non-atheromatous' only excluded atheroma severe enough to be recognizable either clinically or at operation. However, failing more absolute measurements, the definitions described earlier were accepted as excluding those cases in which atheroma is found secondarily, or where there was a strong familial predisposition, or metabolic factors such as a marked idiosyncrasy of diet. Certainly there was a

very large difference in the degree of atheroma between the two types of patients. In these two groups, the observed difference between the triene values and apparently altered triene: tetraene and triene: diene ratios is reminiscent of the increased triene concentrations, in relation to the dienes and tetraenes, in the depot fat of animals deficient of certain fatty acids in their diets and which Jorgensen & Holman (1958) believed to be the best indicator of the 'essential fatty acid state' of the animal.

Thus it seemed possible that these patients might be suffering from a deficiency of those polyunsaturated fatty acids said to prevent this alteration of fatty acid composition in animals (Nunn & Smedley-MacLean, 1938; Fulco & Mead, 1959; Dam, Kristensen, Nielson & Sandegard, 1959; Rieckehoff, Holman & Burr, 1949; Privett, Jorgensen & Holman, 1959). However, in view of the difficulties in the estimation of polyunsaturated acids, due partly to the preliminary state of gas–liquid-chromatographic separation at the time of experiments and to the discrepancies between the results of the two methods of analysis, we do not wish to discuss this point further at present. Experiments designed to compare small quantities of polyunsaturated acids are in progress.

From a comparison of the experimental results presented here and the data given by Hilditch (1956), it is obvious that, although human fat is similar to that of other wholly land animals, it differs from them in one aspect or another, except for the depot fat of the chimpanzee (Gunstone, 1955). This fat appears very similar in composition to the corresponding human fat: thus the chimpanzee has 2% of myristic acid, 29% of palmitic acid, 7% of stearic acid, 5% of palmitoleic acid, 44% of oleic acid, 8% of C_{18} dienes and 1·7% of unsaturated C_{20} and C_{22} acids.

As phylogenetic influences appear to be the most important determinant of depot-fat composition, as shown by the similar diets but dissimilar depot-fat

Table 5. *Percentage fatty acid composition and iodine value of fatty acid esters in plasma, milk and adipose tissue of normal human adults*

Figures are arranged in order of increasing iodine values and the reference numbers (last column) refer to the list of references given below.

Sample	Iodine value	Fatty acids: Mono-unsat.	Total sat.	Dienes	Trienes	Tetraenes	Pentaenes	Hexaenes	Ref. no.
Glycerides									
Milk	52*	34*	48*	7·8	1·0	0·70	0·30	0·30	1
Depot fats	68·9	51·9	36·0*	10·2	.	1·0	.	.	2
	63	54·3	37·3	7·0	0·45	1·0	Trace	Trace	3
Plasma	78	44·7	37·4	14·27	1·34	1·26	0·47	0·54	4
Phospholipids									
Plasma	101·8	35·5	37·6	14·53	0·97	8·16	1·76	1·42	4
Total fatty acids									
Red blood cells	100·7	22*	50*	7·84	0·0	13·1	2·87	4·11	5
Plasma	105·0	39·2	31·0	19·4	2·0	5·1	1·3	1·3	6
	107·3	34·7	32·1	22·3	2·1	5·5	0·91	1·25	5
	113·0	54·4	20·46	15·9	1·07	5·7	1·29	1·09	4
	.	.	.	10·0	1·6	2·0	0·9	1·8	7
	.	.	.	15·0	1·97	2·89	0·83	0·7	8
	.	.	.	22·0	1·59	6·03	0·76	1·13	9
Cholesteryl esters									
Plasma	137	28·6	16·2	41	0·7	6·2	0·7	1·2	10
	142·7	23·5	18·7	47·4	0·7	8·0	1·0	0·7	11 and 4
	146	32·7	11·9	43·8	4·0	6·1	0·8	0·17	12
	.	20·2	5·0	62	.	12·8	.	.	13
	160	Approx. 5*	Approx. 23*	53·2	5·1	9·5	1·4	2·5	14

* Calculated from the respective authors' data.

1. Insull, Hirsh, James & Ahrens (1959).
2. Cramer & Brown (1943).
3. Present study.
4. Luddy, Barford, Riemenschneider & Evans (1958).
5. Evans, Waldron, Oleksyshyn & Riemenschneider (1956).
6. Pikaar & Nijhof (1958).
7. Herdenstam (1960).
8. Holman & Hayes (1958).
9. Hammond & Lundberg (1955).
10. Tuna, Reckers & Frantz (1958).
11. Riemenschneider, Luddy & Morris (1958).
12. Wright, Pitt & Morton (1959).
13. Klein & Jansen (1959).
14. Lewis (1958).

compositions of the Herbivora (Hilditch, 1956), and now also by the converse example of a similar fat composition but dissimilar diet of Western man and the chimpanzee, it would seem that the composition of human and chimpanzee fat indicates the existence of a special primate group. However, this cannot be known for certain until the long-term effects of various diets on the composition of human fat are known and until more comprehensive analyses of animal fats are available.

SUMMARY

1. The fatty acid composition of the depot fat from 12 subjects, both normal and atheromatous, has been studied by gas–liquid chromatography and alkaline isomerization.

2. The site from which the fat sample is taken has very little effect on its fatty acid pattern.

3. Human depot fat was found to contain 42–51% of oleic acid, 21–30% of palmitic acid, 5–8·5% of palmitoleic acid and stearic acid, 5–8% of 'linoleic' acid and under 3% of acids with more than two double bonds.

4. Significant amounts of odd-numbered and branched-chain fatty acids, C_8 and C_{10} saturated acids and of tri-unsaturated acids (trienes), pentaenes and hexaenes occur in human depot fat.

5. The fatty acid composition of the depot fat is similar to that of the glycerides of plasma and milk as determined by alkaline isomerization and iodine values; it also shows some similarities with that of the phospholipids of these sites but not with the cholesteryl esters or total plasma fatty acids.

6. No significant differences were found between the depot-fat composition of normal and atheromatous subjects, excepting possibly an increased concentration of tri-unsaturated acids, particularly in relation to the dienes and tetraenes, in the atheromatous samples.

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