Studies of Adipose Tissue in Man

A Microtechnic for Sampling and Analysis

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ABUNDANT evidence may be found indicating that adipose tissue plays a central role in many phases of lipid and carbohydrate metabolism.[1] Yet, due to analytic limitations as well as to the necessity for surgical excision of tissue, there have been few biochemical studies of this tissue in man. With the advent of gas-liquid chromatography (GLC), precise analyses of fatty acid composition can now be completed with less than a milligram of fat. The present report describes a simple, virtually painless and risk-free method for the removal of such small samples of adipose tissue.[2] Results obtained by the use of this method of adipose sampling will be presented and discussed.

METHODS

Obtaining the Sample

Samples of adipose tissue are aspirated with ease from any subcutaneous area over the trunk or extremities, but the buttock is usually the most convenient site. After preliminary procainization of the skin, a No. 18 thin-walled needle with stylus (T 462 LNR, Becton, Dickinson and Co., Rutherford, New Jersey) is inserted through the procaine wheal 2 to 3 cm. into the subcutaneous adipose layer. The stylus is removed and about 1 ml. of isotonic saline is injected through the needle from a 50 ml. standard venepuncture syringe. Maximum traction on the syringe plunger is applied, and while suction is maintained, the syringe and needle are repeatedly rotated and pushed back and forth in the adipose tissue. These movements are most easily accomplished by grasping the skin and adipose layer between the thumb and forefinger of one hand and the syringe barrel, with retracted plunger, in the other. In this manner a portion of the injected saline is recovered along with numerous minute shiny fat droplets which cling to the inner surface of the syringe. If the first attempt is unproductive, the aspiration can be repeated without relocating the needle or injecting more saline. This simple technic is illustrated in Figure 1.

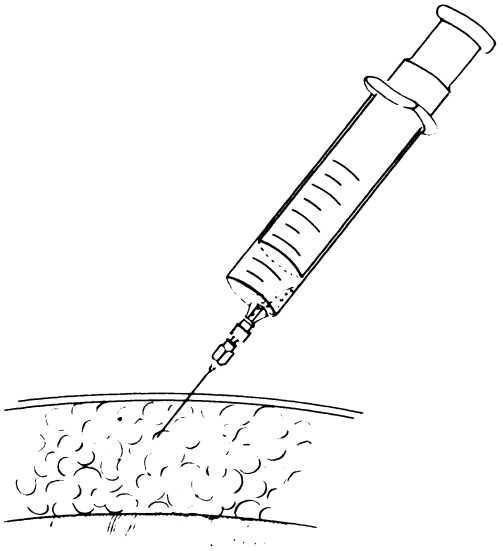

FIG. 1. The technic of adipose aspiration is illustrated by this diagram showing retraction of the syringe plunger and withdrawal of fat droplets from the subcutaneous adipose tissue.

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Preparation and Analysis of the Sample

The entire aspirate is quantitatively transferred from the syringe barrel into a 60 ml. glass-stoppered bottle by washing the syringe interior and plunger with 20 ml. of a 1:1 (v:v) solution of isopropyl alcohol and petroleum ether (30 to 60° c.).

Removal of non-lipid substances such as protein and salt is accomplished as follows: The extract is filtered through ether-treated shark-skin filter paper into a 60 ml. separatory funnel moistened with distilled water. Then the filtrate is washed twice by shaking with 15 ml. of distilled water. At each step the lower phase is discarded, and the final washed upper phase is decanted into a 60 ml. glass-stoppered bottle containing 2 to 4 gm. of anhydrous sodium sulfate. The final volume of the extract is close to 10 ml., and from this a 1 or 2 ml. aliquot is removed for gravimetric determination of total lipid.[3] The weighed solute is discarded. An appropriate portion of the remaining solution (equivalent to 0.2 to 5 mg. of triglyceride) is removed for formation of methyl esters by interesterification with anhydrous methanol.[4] The mixture of methyl esters is then analyzed by a standard technic of GLC. The linearity of detector response of our chromatographic device has been rigorously tested;[5] this is essential to the success of GLC as a quantitative technic.

For identification and accurate measurement of fatty acid composition, elutions are carried out both on polar (ethylene glycol adipate polyester) and non-polar (Apiezon-M) stationary phases at 184.5° and 197° c., respectively. The combined use of polar and non-polar stationary phases enables the most precise resolution of components from eight to twenty-two carbon atoms in length, but for simpler comparative studies of adipose composition, runs are often made on polar columns alone. However, in the latter case the oleic acid isomers are not resolved from the larger oleic acid (18:1) peak. Furthermore, measurements of 16:1, 14:0 and 12:0 are more accurate when obtained by a two-column analysis.

Precautions

All solvents are reagent grade and redistilled in an all glass apparatus. Needles, syringes and glassware are chemically cleaned and extracted in organic solvents to remove all traces of fatty contaminants. Rubber stoppers, plastics, Luer-lock or other devices glued to the syringe should not be used at any stage, since organic solvents may extract interfering substances from such materials. Hence, disposable syringes and needles are unsatisfactory. The aspirate may be stored in the initial extraction solution for many weeks. However, the risk of fatty acid oxidation and other chemical changes increases with time. Therefore, it is wise to complete the further purification and analysis as quickly as possible. In order to avoid oxidative changes, DL-α-tocopherol has recently been added to the isopropyl alcohol-petroleum ether extracting solution (1 μL. per 20 ml.). Whether or not this will prevent all oxidative artifacts is not yet known.

In deteriorated samples, new unidentified peaks appear on the chromatograms as unsaturated components progressively diminish. On adipate columns at 184.5° c., these new peaks have adjusted retention times of 7.40 and 7.00 relative to methyl stearate, and less frequently there are peaks at 0.945, 2.82 and 3.92. On Apiezon columns, an artifactual component precedes 12:0 with a retention time of 0.050 relative to methyl stearate. It is hoped that the addition of tocopherol to the extraction medium and the prompt processing of all sam-

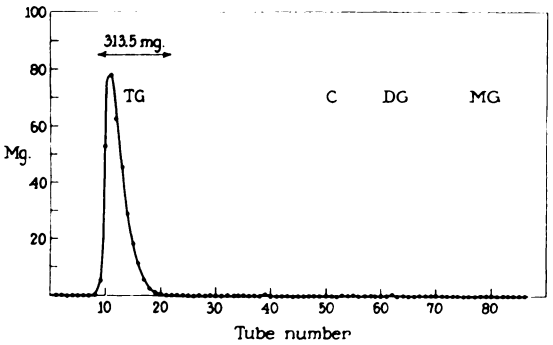

FIG. 2. Silicic acid chromatography of 313.6 mg. of adipose lipid (gradient elution with ethyl ether in petroleum ether). The elution of triglycerides (TG) from an 18-gram column of silicic acid by gradient elution.[5] The absence of components other than triglycerides is evident. (C = cholesterol, DG = diglyceride, MG = monoglyceride.)

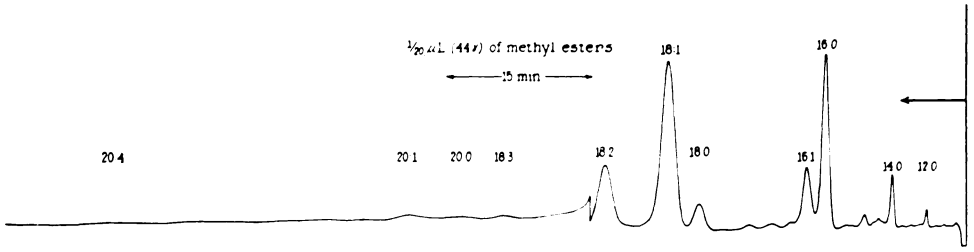

FIG. 3. Gas-liquid chromatogram (EGA polyester) of the methyl esters of normal adipose tissue at 180° c. with origin at right margin. Major peaks are labeled by chain length (number before colon) and by double bonds (number after colon).

ples will prevent the occurrence of these artifacts. When more than a trace of these components is noted, the samples must be discarded.

Subjects

In the studies to be described 145 subjects have been examined. These include healthy medical students and laboratory staff, as well as hospital in- and outpatients with a variety of clinical disorders. In many instances hospital patients on special diets were studied. These diets were administered as liquid formulas prepared from egg white, dextrose and a single fat. This technic has been described in detail elsewhere.[6] A few special analyses of fat removed at autopsy or during surgery were obtained through the courtesy of the Departments of Pathology and Surgery of the New York Hospital-Cornell Medical Center. The Departments of Pathology, Pediatrics and Obstetrics at the New York University-Bellevue Medical Center and Columbia-Presbyterian Medical Center have also made certain subjects available for study.

In many subjects blood was drawn at the time of biopsy of adipose tissue. Serum lipids were extracted by the method of Folch,[7] and the three major lipid ester classes were then separated chromatographically on columns of silicic acid.[8] Finally, fatty acid analysis of each ester class was performed by GLC. The small fraction of free or non-esterified fatty acids was separated by titration from another aliquot of serum by the method of Dole.[9] A sufficient quantity of these acids could be titrated from 10 to 15 ml. of serum to permit satisfactory GLC analysis. Since this extract is contaminated with traces of phospholipid (phosphatidyl serine and possibly other polar lipids are titratable under these conditions), the fatty acids were freed from these contaminants by passage over several grams of silicic acid in ethyl ether. Such batch treatment is highly effective in the differential removal of phospholipids from complex lipid mixtures.

RESULTS

To date, 391 adipose aspirations have been performed on 145 subjects. The procedure has been found completely free of complications. It is to be expected, however, that cases of procaine sensitivity or adverse psychic reaction may be encountered. A brief preliminary questioning reduces the likelihood of such occurrences.

Lipid Constituents of Adipose Tissue

The yield of lipid has averaged 3.68 mg. per aspiration with a range of zero to 34.3 mg. Since aspirations may be repeated with ease, it is feasible to remove as much as 25 mg. from a single subject within ten to twenty-five minutes. The lipid removed from adult adipose tissue is more than 99 per cent triglyceride. Determinations carried out on large pooled samples of aspirate revealed only 0.3 per cent total cholesterol and less than 0.1 per cent phospholipid. When a single, large sample of adipose tissue from the anterior abdominal wall was obtained surgically and promptly extracted and analyzed by silicic acid chromatography, it was found that the large non-phospholipid, non-sterol moiety was exclusively triglyceride. Figure 2 shows that 313.5 mg. of the 313.6 mg. applied was found in the triglyceride peak. If unesterified cholesterol (C), diglyceride (DG) or monoglyceride (MG)

TABLE I

Micro- vs. Macro-Sampling of Subcutaneous Adipose Tissue

(Comparison of Data Obtained at the Same Anatomic Site (Abdominal Panniculus) by Needle Aspiration and by Surgical Excision of Superficial and Deep Adipose Layers)

Fatty acid	Needle aspiration	Surgical excision	
		Superficial	Deep
14:0	2.6	2.1	2.3
16:0	23.3	22.4	23.0
16:1	5.4	4.3	4.3
18:0	4.6	5.7	4.8
18:1*	47.7	50.4	51.7
18:2	9.3	10.7	9.0

* This includes the isomers of 18:1.

had been present, they would have appeared in the designated locations. In contrast to adult adipose tissue, subcutaneous fat removed at the autopsy of a premature, stillborn infant contained 2.2 per cent phospholipid and 1.2 per cent total cholesterol. By histologic examination this specimen was seen to be far more cellular, with less of the signet-ring cell appearance characteristic of adult adipose tissue.

When adipose triglyceride is interesterified with methanol, the resultant fatty acid methyl esters are separated and can be identified as shown in the gas-liquid chromatogram of Figure 3. The major peaks are labeled as to chain length and content of double bonds. The predominance of 16:0 (palmitic) and 18:1 (oleic) acids is evident, but a large number of lesser components can also be seen. In all, there may be as many as thirty-five or forty peaks, but six major acids always comprise more than 90 per cent of the total.

Validation of Aspiration Technic

A comparison of the concentrations of the six major fatty acids in tissue removed by various technics and from different sites has served to validate this method of adipose sampling. In Table I the concentrations of these six acids (myristic, palmitic, palmitoleic, stearic, oleic and linoleic) found by percutaneous aspiration from the anterior abdominal wall are compared with analyses made on tissues removed surgically from the superficial and deep portions of the subcutaneous fat layer at the same site as the needle aspiration. The similarity of results indicates that the aspiration method of adipose removal provides a representative sample of tissue. Table II compares the fatty acid compositions of aspirates from different superficial sites in the same subject. With the possible exception of the 16:1 and 18:0 acids, the differences seem negligible. In Table III a sample aspirated at autopsy from the subcutaneous fat of the buttock is compared with fatty tissue removed from between the fasciculi of the psoas muscle and also from the omentum, perinephric area and pericardium. Although the fatty acid composition of omental fat is practically indistinguishable from that of the needle aspirate, the other deep sites show slight changes which are most pronounced in the pericardial sample. In this location there is a definite, although small, decrease in C_{18} unsaturated acids.

It is clear that a needle aspirate of subcutaneous fat provides a highly representative sample of the adipose fatty acids in all regions of the body, as well as a representative sample of the fatty acids present locally. In many animal species, superficial adipose fat is more unsaturated than the fat of deeper, internal depots.[10] However, as Cuthbertson and Tompsett noted earlier,[11] these differences are negligible in man. This uniformity in composition of human depot fat is presumably the result of

TABLE II

Similarity of Fatty Acid Structure at Various Subcutaneous Sites

(Comparison of the Fatty Acid Structure of Adipose Tissue Removed by Needle Aspiration from Different Subcutaneous Sites in the Same Subject)

Fatty Acid	Site				
	Buttock	Abdomen	Thigh	Arm	Interscapular Area
14:0	2.0	2.6	2.0	2.1	1.9
16:0	22.7	23.3	21.1	22.3	22.6
16:1	6.2	5.4	5.8	4.4	3.9
18:0	3.7	4.6	4.3	5.7	6.7
18:1*	49.3	47.7	50.0	49.0	49.3
18:2	9.9	9.3	10.3	10.3	10.7

* This includes the isomers of 18:1.

TABLE III
Superficial vs. Deep Adipose Tissues
(Comparison of Adipose Fatty Acid Composition in Fatty Tissue Removed from a Single Superficial and Four Deep Sites in the Same Subject)

Fatty Acid	Needle Aspiration	Excision			
	Buttock	Psoas Muscle	Omentum	Perinephric Area	Pericardium
14:0	2.0	2.8	2.6	1.9	3.1
16:0	22.7	25.4	20.5	20.1	27.2
16:1	6.2	4.8	7.0	4.3	6.8
18:0	3.7	4.7	4.8	6.5	4.4
18:1*	49.3	48.5	49.0	51.4	45.4
18:2	9.9	8.9	10.8	10.3	8.5

* This includes the isomers of 18:1.

wearing clothes and taking other measures to maintain a warm skin, since animals grown in warm environments show similar uniformity in composition.[12]

Normal Fatty Acid Composition of Subcutaneous Adipose Tissue

Having examined the validity of this technic, it was thought essential to establish a set of values which might be considered as the "normal" fatty acid composition. The selection of a normal composition is highly arbitrary for, as will be shown, dietary alterations leave an imprint on adipose composition without in any way rendering it abnormal. Furthermore, newly born premature and full term infants have different compositions of adipose tissue, neither of which can be said to be abnormal. To designate these patterns as normal serves only to provide a standard or reference for relating adipose and other compartments of lipids and for the study of changes in adipose tissue as a function of age, metabolic state, disease and diet.

To establish a reference composition, twelve healthy subjects between the ages of twenty and thirty-five were selected. All were on random diets and gave no history suggesting dietary peculiarities, metabolic disease or recent change of weight. The means and standard deviations of fatty acid concentrations in these subjects are shown in Table IV. The twenty-two acids listed comprise 98 per cent of the total; however, it is notable that seven acids account for 92 per cent. An additional group of acids of uncertain identity which occur occasionally and in only trace amounts have

TABLE IV
Fatty Acid Analyses of Subcutaneous Adipose Tissues Obtained by Needle Aspiration from Normal Subjects Between the Ages of Twenty and Thirty-Five Years (Five Women and Seven Men)

Fatty Acid	Mean ± Standard Deviation
12:0	0.7 ± 0.1
14:0	3.3 ± 0.1
14:1	0.6 ± 0.05
14:0 branched*	0.1 ± 0.1
15:0	0.6 ± 0.1
15:0 branched*	0.3 ± 0.3
16:0	19.5 ± 2.1
16:1	6.9 ± 0.1
17:0	0.2 ± 0.2
17:0 branched*	1.0 ± 0.3
18:0	4.2 ± 1.1
18:1	41.2 ± 4.4
18:1 isomers*	5.1 ± 1.0
18:2	11.4 ± 1.4
18:3	0.4 ± 0.1
19:0 branched*	0.5 ± 0.2
20:0	0.6 ± 0.1
20:1	0.6 ± 0.3
20:2	0.1 ± 0.1
20:2*	0.1 ± 0.1
20:3	0.2 ± 0.1
20:4	0.2 ± 0.1

NOTE: Underlined components comprise 92 per cent of the total mixture.
* Identity not established.

TABLE V
Fatty Acid Composition of Human Adipose Tissue (Results by Present Technic and by Classic Methods)

Fatty Acid	Needle Aspiration and GLC Analysis (12 samples), 1960	Autopsy Specimens—Classic Methods (5 samples), 1943
12:0	0.7	0.5
14:0	3.3	3.5
14:1	0.6	0.4
16:0	19.5	25.0
16:1	6.9	6.4
18:0	4.2	7.0
18:1*	46.3	45.9
18:2	11.4	9.6
20:4	0.2	0.7
Other 20 carbon acids	1.7	1.2
Total	94.8	100.2

NOTE: The means of twelve "normal" samples obtained by the present technic are compared with the means of five autopsy samples analyzed by classic methods.[13] Note that acids with an odd number of carbon atoms and other small components are not listed among the results obtained by the new method. This accounts for the difference in the two totals.

* This includes the isomers of 18:1.

not been listed; these account for the remaining 2 per cent. Only negligible amounts of acids with chain length greater than twenty carbon atoms are seen. The high standard deviations of some of these acids most likely express interindividual differences and are not attributable to the analytic methods employed, for it has been shown[5] that replicate analyses of a given mixture by GLC are accomplished with standard deviations less than 1.5 per cent of the mean.

The "normal" adipose composition shown in Table IV corresponds well with the analyses made over fifteen years ago by Cramer and Brown.[13] Using fractional distillation and low temperature crystallization, they analyzed five samples of human adipose tissue; Table V presents the mean of these data. It is not clear whether the small differences in these two sets of data are of analytical origin or truly represent differences in the adipose tissues analyzed.

Comparison with the Fatty Acids of Serum

It is not possible to provide an exact statement of the metabolic relation of adipose tissue to any serum lipid compartment by simple comparisons of fatty acid compositions of these entities. Nevertheless, such comparisons may suggest certain important interrelations. In Figures 4 and 5 the adipose composition (as indicated by a bar graph of seven acids: 14:0, 16:0, 16:1, 18:0, 18:1, 18:2 and 20:4) of a healthy twenty-two year old man on a random diet is compared with the composition of four serum lipid classes. Both adipose and serum samples were obtained at 8:00 A.M. following a twelve-hour fast. Although the adipose composition is seen to be practically indistinguishable from the reference values of Table IV, it is noteworthy that no serum lipid group is precisely equivalent to adipose tissue in its array of fatty acids. The triglyceride and non-esterified fatty acids show the closest resemblance

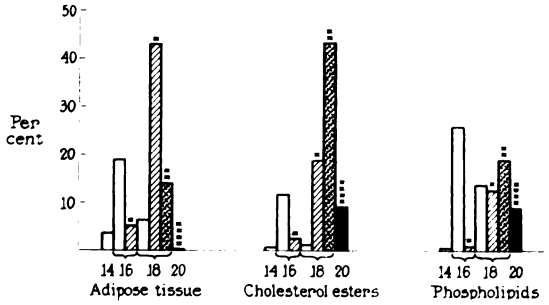

FIG. 4. Fatty acid composition of adipose tissue and serum lipids (random diet) compared with serum cholesterol ester and phospholipid fatty acids. Chain length and number of double bonds of each acid are indicated. The individual studied had been on a random diet with no history of metabolic or dietary abnormalities.

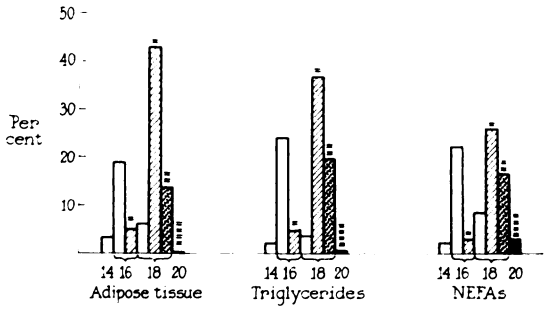

FIG. 5. Fatty acid composition of adipose tissue and serum lipids (random diet) with serum triglyceride and non-esterified fatty acids (NEFA's) in the same subject as in Figure 4.

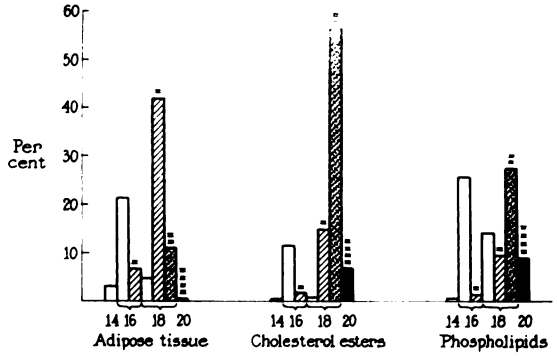

FIG. 6. Fatty acid composition of adipose tissue and serum lipids (corn oil diet) compared with serum cholesterol ester and phospholipid fatty acids. This subject had been on a diet containing 40 per cent of calories as corn oil (linoleic acid = 54 per cent) for ten weeks. Note the large amount of linoleic acid ($18^{=}$) in the serum fractions.

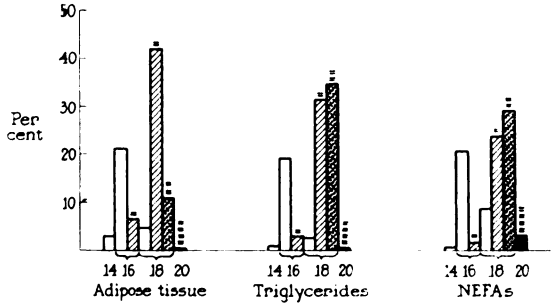

FIG. 7. Fatty acid composition of adipose tissue and serum lipids (corn oil diet). The fatty acids of serum triglycerides and non-esterified fatty acids (NEFA's) compared with adipose tissue in the same subject shown in Figure 6.

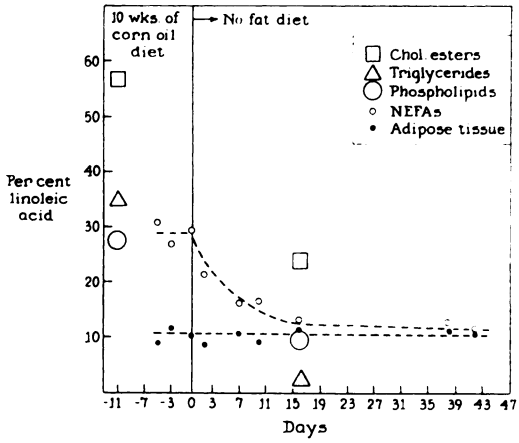

FIG. 8. Dietary effects on the linoleic acid (18:2) content of adipose tissue and four serum lipid fractions. A diet rich in corn oil (40 per cent of calories) was changed isocalorically to a fat-free diet without altering protein or total calories. Note the rapid changes in all serum compartments, but not in adipose tissue.

to adipose tissue, but even these contain far larger amounts of 18:2 and 20:4.

It would appear that no serum lipid group derives its fatty acids from the adipose tissue in a simple, unselected way. More likely, the serum lipids represent mixtures of acids of two origins, dietary and adipose fat. As dietary and metabolic situations vary, either the diet or adipose tissue may become the primary source of serum fatty acids. Although these changing circumstances are most quickly reflected in the triglyceride and non-esterified fatty acids, the composition of cholesterol esters and phospholipids is also involved. Thus, Figures 6 and 7 compare the adipose and serum lipid constituents after ten weeks of corn oil feeding in a thirty-four year old man with hypercholesterolemia, in whom one-fifth of total calories was derived from linoleic acid. Even though each of the serum lipids reflected this high intake of 18:2, the composition of the adipose tissue remained unchanged. When the regimen was changed to a fat-free and hence linoleic acid-free diet by substituting carbohydrate for fat calories, the linoleic acid content of the serum non-esterified fatty acids quickly fell to the level present in adipose tissue (Fig. 8). All other serum lipid compartments also diminished in linoleic acid content. It would appear that adipose tissue becomes a major source of circulating fatty acids when the diet is fat free. Although other possible sources of plasma lipid (such as hepatic lipogenesis) must be considered as well, it appears that adipose fat stands in readiness to provide a sustained fundamental pattern for serum lipid components, whereas dietary fat contributes inconstantly. Thus, knowledge of adipose fatty acid composition would seem to be of paramount significance in the study of fatty acid metabolism in man. Subsequent sections will examine certain changes in adipose composition as a function of age, metabolic state, disease and diet.

Changes With Age

Most of the samples have been from subjects ranging in age from twenty to sixty years.

TABLE VI
Change in Adipose Composition with Age*

Fatty Acid	Subject				
	Four Premature Infants (1,500, 1,610, 1,733, 1,760 gm.)	Three Full Term Infants (4,545, 3,778, 4,100 gm.)	Two Children (8 and 11 yr.)—Mild Rheumatic Fever	Twelve Normal Adults (20 to 35 yr.)	Three Normal Adults (46, 56 and 60 yr.)
14:0	3.7 ± 0.4	3.0	3.7 ± 0	3.3 ± 0.1	2.3 ± 0.2
16:0	26.7 ± 4.4	40.2 ± 0.9	17.1	19.5 ± 2.1	21.2
16:1	10.0 ± 1.4	14.6 ± 0.3	9.8 ± 1.5	6.9 ± 0.1	6.7
18:0	8.4 ± 2.3	5.1	4.7	4.2 ± 1.1	4.0
18:1	26.4 ± 2.7	25.2 ± 1.3	41.4	41.2 ± 4.4	46.2
18:2	12.0 ± 1.0	1.3 ± 0.1	12.8	11.4 ± 1.4	11.5
18:3	0.2 ± 0.2	1.8 ± 0.2	0.6	0.4 ± 0.1	0
20:3	1.2 ± 0.8	3.9 ± 2.5	0.2	0.2 ± 0.1	0.3
20:4	0.9 ± 0.6	0.3	0.2	0.2 ± 0.1	0.3

* All figures are means. Standard deviations are included when means show interesting variations.

In this group we have seen no variations in adipose fatty acid which can be correlated with age. However, newly born premature and full term infants show startling differences from the normal adult pattern. These are illustrated in Table VI. The first column (adipose composition of four premature infants) shows higher concentrations of 16:0, 16:1, 18:0, 20:3 and 20:4 than seen in normal young adults (column 4), but there is a much lower concentration of 18:1. Even more striking changes are seen in three full term newly born infants (column 2) in whose adipose tissue linoleic acid (18:2) has nearly disappeared, while contents of 16:0, 16:1 and 20:3 are even larger than in the premature infants.

It might be expected that progressive development of the fetus in the last trimester of pregnancy would be associated with adipose changes more closely approximating the average adult pattern. Yet, the full term infant shows greater adipose differences from normal than does the premature. It is interesting to speculate on possible explanations for this finding. The accumulation of fat in fetal adipose depots is known to occur primarily toward the end of pregnancy.[14] The premature and full term infants described in Table VI weighed an average of 1,651 gm. and 4,141 gm., respectively; but it is estimated[14] that this threefold change in total body weight parallels an almost twelvefold change in fat content from roughly 75 to 875 gm. Although there is little doubt that some fatty acids can pass from the maternal circulation to the fetus,[15] there is also good evidence that most fetal fat is synthesized *in situ*.[16] The present findings support this view. The similarity of data from the premature infants and young adults, especially the 18:2 contents of the two groups, suggests that early in pregnancy fetal adipose fatty acids originate from a maternal source. But late in pregnancy, lipogenesis from carbohydrate may predominate, in which case a decrease in 18:2 and increase in 16:0 and 16:1 would be expected. These same findings (increasing 16:0 and 16:1 and decreasing 18:2) occur in the rat fed a high carbohydrate, nearly fat-free diet.[17] Evidently, calories in excess of those required for fetal growth are available for lipogenesis late in gestation. Perhaps the enzymatic apparatus responsible for the conversion of carbohydrate to fat in adipose tissue matures late in pregnancy, giving rise to this sudden burst of fat production. In any event, the fat present at term shows a pattern characteristic of those fatty acids which are synthesized from carbohydrate.

The presence of larger than normal amounts of 20:3 in full term infants should not be overlooked. In rats with essential fatty acid deficiency 5,8,11-eicosatrienoic acid is formed in

TABLE VII
Adipose Composition in Various Clinical States

Fatty Acid	State and Subject					
	Normal: 5 Women; 7 Men (20–35 yr.)	Acute Myocardial Infarction: 5 Men (32–65 yr.)	Postpartum State: 3 Women (20–35 yr.)	Obesity: Woman, 58 yr.; 114 kg.	Diabetes: 32 year old Woman; Insulin 40 units/day for 1½ yr.	Wilson's Disease: 39 year old Woman
14:0	3.3 ± 0.1	3.3	1.8 ± 0*	2.4	2.9	2.0
16:0	19.5 ± 2.1	25.0 ± 0.9*	20.6	25.0	18.9	18.0
16:1	6.9 ± 0.1	4.9 ± 0.8*	6.7	6.9	9.8	12.7
18:0	4.2 ± 1.1	4.9	5.5	3.4	4.7	1.9
18:1	41.2 ± 4.4	44.8	45.4	43.6	47.9	42.4
18:2	11.4 ± 1.4	9.0 ± 0.4*	12.1	9.1	11.0	11.8
18:3	0.4 ± 0.1	0.2	0.3	0.3	0.2	0.2
20:3	0.2 ± 0.1	0.1	0.2	0.3	0.1	0.4
20:4	0.2 ± 0.1	0.3	0.5 ± 0.5*	0.5	0.0	0.4

* Standard deviations included when means appear appreciably different from normal values.

excess from oleic acid;[18] indeed a rise in concentration of this C_{20}-triene is one of the earliest biochemical manifestations of this deficiency state.[19] It remains for future studies to determine whether or not the chemical structure of this 20:3 acid in full term infant adipose tissue is the same as that in the weanling rat fed a fat-free diet.

Effects of Various Clinical Situations

There is reason to believe that some disorder of adipose tissue may play an important role in several clinical states. Thus, in obesity the size of the adipose tissue is the chief abnormality, even if the etiologic mechanisms are found elsewhere. Since adipose tissue is a primary locus of insulin action,[1] diabetes also may be looked on as a disorder with an important biochemical expression in adipose tissue. Yet, in several cases of obesity and of diabetes, no significant alterations in the fatty acid composition of adipose tissue were found. An example of each is given in Table VII.

Table VII lists data obtained from five men dying within several days after suffering acute myocardial infarctions. The small changes seen in these acutely ill patients, as well as in the chronically bed-ridden patient with Wilson's disease, are not as yet meaningful. It may be that, when more information on adipose composition has been gathered, such small changes will be interpretable and helpful in the further understanding of these disorders.

The unexpected finding of low linoleic acid in full term infants poses the question of what differences in adipose tissue composition there may be in parturient women. However, Table VII shows no significant abnormalities of 18:2, 16:0 or 16:1 content in postpartum women.

A small group of patients with specific disorders of lipid metabolism (hyperlipemia, hypercholesterolemic xanthomatosis, and Von Gierke's disease with hyperlipemia) have been studied, but no systematic changes typical of these disease entities have been found. In these situations, the antecedent dietary therapy has usually left specific marks on adipose composition (Table VIII). The hypercholesterolemic subject consuming linseed oil as a major source of fat calories showed changes in adipose fatty acids which would be expected with a slow exchange between a typical mixture of adipose fatty acids and that found in linseed oil itself. A similar exchange occurred in the hyperlipemic patient fed a diet rich in corn oil (18:2 = 54 per cent). In the child with glycogenosis who had required high carbohydrate feedings for over five years, a mixture low in 18:2 and high in 16:0 and 16:1 was encountered,

TABLE VIII
Change in Adipose Composition with Diet

Fatty Acid	Diet and Subject: Random Diet: 12 Normal Adults, 20–35 yr. (mean ± S.D.)	Linseed Oil, 3 oz./day for 1 yr.: Hypercholesteremic Man, 44 yr. with Arteriosclerotic Heart Disease		Corn Oil, 2 oz./day for 2 yr.: Hyperlipemic Man, 57 yr.		High Carbohydrate, Low Fat Diet for 5 yr.: Glycogenosis (Von Gierke's Disease): 6 yr. old Male Child
14:0	3.3 ± 0.1	1.2		0.7	(0.3)†	3.1
16:0	19.5 ± 2.1	14.7	(5.4)*	15.9	(12.7)†	22.6
16:1	6.9 ± 0.1	5.8		5.8		22.1
18:0	4.2 ± 1.1	5.4	(4.0)*	2.2	(2.7)†	1.0
18:1	41.2 ± 4.4	35.5	(22.6)*	44.0	(30.7)†	43.1
18:2	11.4 ± 1.4	20.5	(16.4)*	25.9	(53.5)†	3.3
18:3	0.4 ± 0.1	13.7	(51.1)*	0.8		0.3

* Fatty acid composition of linseed oil.
† Fatty acid composition of corn oil.

as in the case of full term infants. However, in this instance, the 20:3 acid was not significantly increased.

These three cases emphasize the importance of long standing dietary effects in shaping the composition of adipose tissue. The subsequent section considers these effects in further detail.

Effects of Diet

In adults in caloric balance, dietary effects on adipose tissue are produced only slowly; in short term studies no effects are seen. This is illustrated in Figure 9, in which concentrations of saturated, mono- and diunsaturated fatty acid groups are indicated by bars. After ten weeks on a formula containing forty per cent of calories as corn oil, the adipose tissue of this subject was normal in fatty acid composition. Then, after thirty-eight days on a fat-free high carbohydrate formula, the adipose pattern was still unaffected. During ten weeks on high intakes of corn oil, this patient ingested more than 3.5 kg. of linoleic acid, an amount roughly three times that present in his entire adipose tissue. Yet, the adipose linoleic concentration did not rise. During thirty-eight fat-free days the patient's caloric expenditure was of the same order of magnitude as the total caloric value of his entire adipose depot, yet body weight did not change nor was adipose composition altered. Clearly, there must have been intense lipogenesis from carbohydrate; but, the lack of accumulation of 16:0 and 16:1 and lack of decrease in 18:2 strongly argues against the participation of the entire adipose depot in the new synthesis of fatty acids.

When formulas rich in corn oil are fed over extremely long periods, slow changes in adipose composition are seen eventually, and they continue until an adipose pattern is evolved which is very similar to that of the fed corn oil acids. Figure 10 is a composite of the adipose changes in eight different subjects receiving diets rich in corn oil (usually 40 per cent of calories) from eight to 160 weeks. Changes are almost imperceptible up to twenty weeks. However, at 160 weeks the adipose tissue has become rich in diunsaturated acid (essentially all 18:2) and it closely resembles a mixture of corn oil and normal adipose triglyceride in proportions of 7 to 3. There is no reason to believe that even further adipose changes might not have occurred if the corn oil feedings had been continued for longer periods of time.

COMMENTS

This technic, whereby numerous samples of human adipose tissue can be analyzed rapidly and accurately, opens many investigative pathways. Only a few of these have been explored

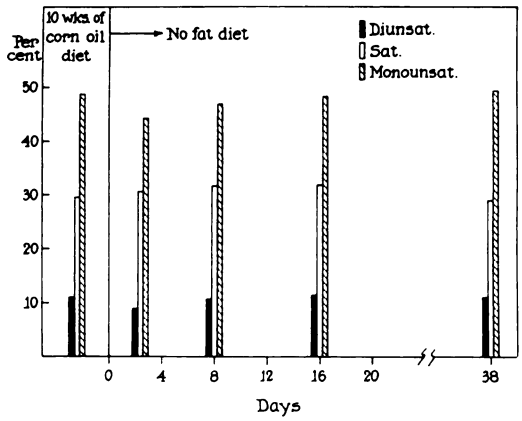

FIG. 9. The effects of fat-free diet on the composition of adipose tissue in terms of diunsaturated (18:2), monounsaturated (16:1 and 18:1) and saturated (14:0, 16:0 and 18:0) acids. These findings supplement those of Figure 8.

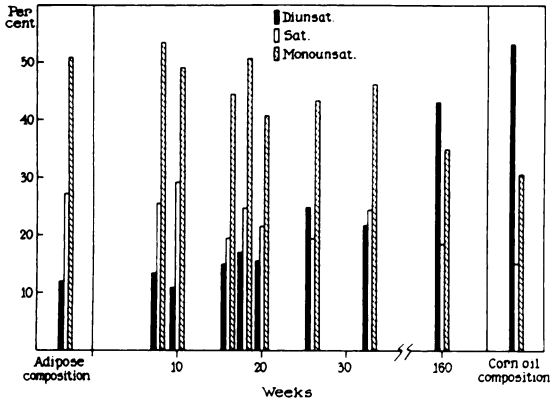

FIG. 10. Long term dietary effects on adipose tissue. The adipose compositions of eight patients on diets rich in corn oil (usually 40 per cent of calories) are compared with the normal adipose composition as well as with the fatty acid structure of corn oil. For abbreviations, see legend to Figure 9.

in a preliminary way in this paper, and thus it is too soon to assess with any finality the many forces which create specific fatty acid compositions in adipose tissue. Obviously, there are broad similarities to the well known early investigations of "hard" and "soft" animal fats. The classic work of Mendel and Anderson in this area, reviewed nearly thirty years ago,[20] might have predicted many of the present findings. But, one aspect of the present study and of the older animal work as well remains particularly puzzling.

If one considers the rate at which dietary fat

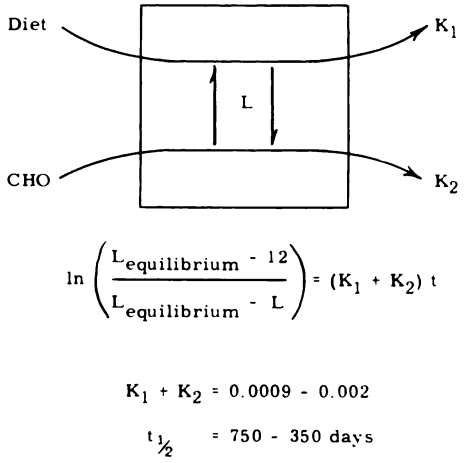

FIG. 11. Kinetics of adipose fatty acids. Adipose tissue is represented as a single pool of completely miscible fatty acids of constant size with linoleic acid concentration = L. L is being turned over under two influences, at least: (1), mixing with dietary fat (K_1); and (2) new synthesis of fatty acids from carbohydrate coupled with removal of acids for energy (K_2). When a diet rich in linoleic acid is fed, there is a slow increase in L from 12 (normal content at start) to $L_{equilibrium}$. In estimating the $t_{1/2}$ from the data of Figure 10, $L_{equilibrium}$ has been considered to lie somewhere between 44 per cent (the observed L in one patient after 160 weeks of high corn oil intake) and 53.5 per cent (a theoretical maximum equal to the 18:2 content of corn oil). Using several points from Figure 10 for L and t, $(K_1 + K_2)$ is found to range between 0.0009 and 0.002, hence $t_{1/2}$ is between 750 and 350 days.

exchanges with adipose fat, using Figure 10 as a guide, the exceedingly slow nature of this process is evident. One reasonable model of adipose fatty acid turnover is that of a large pool of acids being turned over by (1) the entrance of dietary fatty acids; (2) the synthesis of fatty acids *de novo* from carbohydrate; and (3) the exit of acids, most likely in the form of serum non-esterified fatty acids.[1] When dietary fat is suddenly enriched in linoleic acid, the rate at which a new equilibrium of linoleic acid content in adipose tissue is reached could indicate an over-all rate of fatty acid turnover in the depots, providing that all adipose fatty acids are readily miscible and can be treated as a single pool. Using the data of Figure 10 for this calculation, one arrives at the extraordinarily slow half-life of 350 to 750 days. Figure 11 shows such a calculation. When different points from Figure 10 are used,

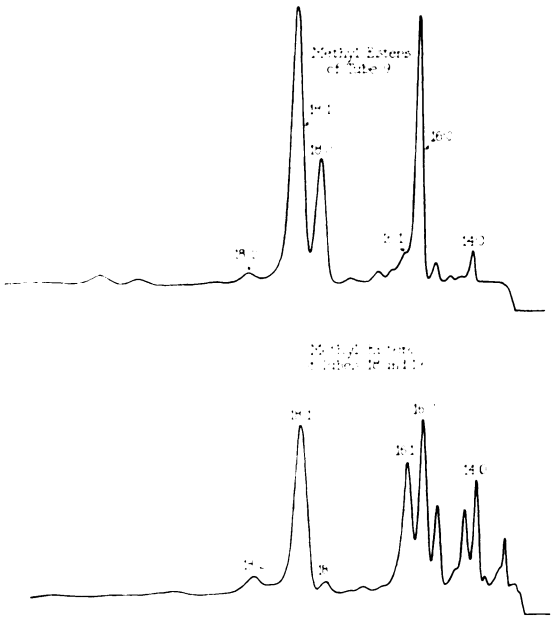

FIG. 12. GLC analyses of triglyceride fatty acids removed from different portions of the triglyceride elution curve shown in Figure 2. The presence of at least two completely different "species" of triglycerides is evident. Stationary phase = ethylene glycol adipate polyester, temperature = 185° C.

$(K_1 + K_2)$ ranges between 0.0009 and 0.0002, and the $t_{1/2}$ is therefore between 750 and 350 days.

However, numerous isotope studies have demonstrated that the turnover of depot fatty acids is far more rapid than these calculations indicate. Rittenberg and Schoenheimer[21] injected and fed mice with deuterated water and showed that depot fatty acids gain deuterium from body water in a curve reaching the midpoint at only five to nine days. More recently, Pihl, Bloch and Anker[22] demonstrated that labeled acetate was quickly incorporated into carcass fat of the adult rat with a half-life of sixteen to seventeen days and into the unsaturated acids of depot fat with a half-life of nineteen to twenty days. Furthermore, many recent studies of different types provide abundant evidence for brisk metabolic activity in adipose tissue.

One reconciliation of these contradictory approaches to the problem of depot fatty acid turnover is to postulate the existence of at least two separate metabolic compartments in adipose fat. If ready exchange of fatty acids between these pools does not occur, one basic assumption for the calculations of Figure 11 is not valid. The much larger compartment might serve as an inert storage for fat calories, exchanging only slowly with dietary fat. The much smaller and rapidly turning over compartment may be in close metabolic relation to dietary, serum and liver lipids. This smaller pool may also be the site of fatty acid synthesis from carbohydrate. Such compartmentalization could occur on a cellular, subcellular or even molecular level. The small cytoplasmic portion of adipose cells, or even the organelles within the cytoplasm, might contain special fatty acids both in the chemical and metabolic sense. Indeed, the different fatty acid positions (α and β) on the triglyceride molecule are not equivalent: lipases hydrolyze the α-ester linkages preferentially,[23] and furthermore, there are specific arrangements of saturated and unsaturated fatty acids at the α- and β-positions in most natural fats.[24] Thus, a possibility of intramolecular compartmentalization also exists.

It is certainly true that adipose fatty acids are organized into different species of triglyceride within adipose tissue. These variations between triglycerides become evident in preliminary separations by silicic acid chromatography. Figure 12 shows analyses of methyl esters prepared from the early and late portions of the triglyceride curve of Figure 2. In tube 9 the triglyceride is rich in 16:0, 18:0 and 18:1, but in tubes 18 and 19 a completely different array of shorter and more polar fatty acids occurs. Are these different species of triglycerides representative of one or another functional compartment of adipose tissue? Newer technics of triglyceride separation now under study in this laboratory may be informative in application to this problem.[25]

The possibility that the bulk of adipose fat is an inert pool of stored calories representing a sample of dietary fat integrated over many years makes the technic of adipose aspiration particularly attractive from the standpoint of nutritional epidemiology. If definitive proof of this idea is obtained, the technic may supplement and lend greater accuracy to nutritional surveys.

SUMMARY

A method is described for simple, virtually painless sampling of adipose tissue in man. Such samples (0 to 34.3 mg. in size) have been obtained from 145 subjects and analyzed for fatty acid composition by gas-liquid chromatography. In this way, a typical or "normal" pattern of adipose fatty acid composition in healthy, young adults has been established. Small groups of older, healthy persons as well as those with coronary artery disease, obesity and diabetes have shown no marked differences from the average pattern of healthy younger subjects. But, newly born premature and term infants have distinctive alterations. Furthermore, controlled dietary manipulations in adult life can give rise to slow changes in adipose composition. The possible significance of these findings is discussed.

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