

# Adipose Tissue as a Medium for Epidemiologic Exposure Assessment

Lenore Kohlmeier<sup>1</sup> and Martin Kohlmeier<sup>2</sup>

<sup>1</sup>Departments of Nutrition and Epidemiology; <sup>2</sup>Department of Nutrition, University of North Carolina, Chapel Hill, North Carolina; Free University of Berlin, Berlin, Germany

In the United States, adipose tissue is rarely used as a medium for assessment of prior exposures in epidemiologic studies. Adipose tissue aspirations are in general less invasive and carry less risk than phlebotomy. Tissue samples can be analyzed for a wide number of epidemiologically important exposures. Beyond reflecting long-term energy balance, this tissue offers a relatively stable depot of triglyceride and fat-soluble substances, such as fat-soluble vitamins, and pesticides. As a tissue it represents the greatest reservoir of carotenoids in the body. Halogenated hydrocarbons may be measured in concentrations of hundreds-fold greater than those in blood of the same individuals. The composition of adipose tissue also reflects the long-term dietary intakes of a number of essential fatty acids. The turnover times of all of these substances in adipose tissue remain under-researched. Sampling and storage of adipose tissue, homogeneity of sampling sites, turnover times, and the effects of diet, age, gender, race, hormones, and disease on adipose tissue composition are discussed in this review of current knowledge about adipose tissue stability. Experience in the use of adipose tissue sampling in epidemiologic studies in various countries has shown that it is simple to conduct, requires little training, carries little risk, and does not result in excessive participant refusal. — *Environ Health Perspect* 103(Suppl 3):99–106 (1995)

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## Introduction

In the United States, adipose tissue is rarely used as a medium for assessment of prior exposures in epidemiologic studies. Contrary to widely held feelings, adipose tissue actually can be obtained with more ease and less risk than venous blood obtained by phlebotomy. Adipose tissue samples contain many compounds that can be measured reliably and that are related to past exposures of interest for epidemiologic studies. Thus, adipose tissue is not just an amorphous storage site reflecting long-term energy balance; it also constitutes a relatively stable depot of a multitude of different fatty acids, fat-soluble vitamins, pesticides, and other fat-soluble substances. The following review is to draw renewed attention to the considerable potential of adipose tissue analyses as measures of long-term environmental exposures with particular emphasis on dietary intake.

## Adipose Tissue Samples

### The Sampling Procedure

Adipose tissue sampling from living subjects was described in detail by Hirsch in

1960 (1) as a “simple, virtually painless and risk-free method for the removal of such small samples of adipose tissue,” which could be conducted on subjects to collect fat tissue for etiologic research on fatty acid composition of this tissue. Our own experience is based on the collection of more than 1400 adipose tissue biopsies in healthy volunteers (2) and a variety of patients. We found that adipose tissue aspirations were well tolerated and actually perceived as less painful than standard venipuncture. There was not a single instance of ill effects due to the procedure other than transient hematoma formation. Adipose tissue sampling has been used most often in adult populations. Samples also have been obtained from younger individuals, even newborns (3). Perceived invasiveness is the major issue for such sensitive groups, limiting acceptance more than actual risk or the possibility of discomfort. The same is true for other important groups. Thus, we could not identify any published data on the composition of adipose tissue in healthy pregnant women.

The procedure rarely is met with excessive participation refusal, and is simple enough to require only minimal training. Concerns about possible hematoma development after biopsy have not been borne out in practice. This view is corroborated by the experiences from the (EURAMIC) study, a multinational case-control study conducted with a standard protocol in 12 European countries (4). For this investigation on the role of antioxidants in cardiovascular disease,

fat biopsies were obtained from men who had suffered acute myocardial infarction, immediately after they had been admitted to the hospital. In responding to a questionnaire, none of the 12 participating centers reported problems related to the occurrence of hematomas or patients' complaints during the collection of 1300 biopsies.

The method that was employed in the EURAMIC study was an adaptation of procedures described by other authors (1,5). Samples are collected from the upper half of the buttock while the subject is lying face down. In this survey no local anesthesia was used. After disinfection of the skin with alcohol, the subject is asked to tense the buttock to delineate muscle and fat. The upper outer quadrant is grasped between two fingers and the thumb of one hand. A Luer-lock needle (gauge 17, 1.5 mm) is inserted at a 45 degree angle, after which a vacuum tube is connected to the needle. This needle then is moved in and out of the adipose tissue, at which time some fat collects at the top of the Luer adapter between the needle and the tube. The adapter is capped and frozen immediately at  $-80^{\circ}\text{C}$ . Thermally insulated containers with dry ice can be used as a satisfactory alternative for temporary storage in hospitals without adequate ultra-deep freezer facilities.

The amount of sample obtained can be a matter of concern. The goal in the EURAMIC study was to collect 20 to 25 mg of adipose tissue from each participant. With the described sampling procedure

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Address correspondence to Dr. Lenore Kohlmeier, Department of Epidemiology, 2105e McGavran Greenberg Hall, UNC School of Public Health, Chapel Hill, NC 27514-7400. Telephone (919) 966-7450. Fax (919) 966-2089.

there was the possibility that the failure to obtain tissue would not be recognized in time to repeat aspiration. Of the 1300 samples from the EURAMIC study, 27 were found to contain no fat. Close to half of these could be traced to one particular physician who did not master the method. Another 139 samples (10.7% of the samples) weighed 5 mg or less. Many investigators extract and wash the sample before freezing, which would allow optical assessment of the size of the sample.

Adipose tissue samples have been collected by aspiration of other sites including from paraumbilical, femoral, and triceps areas. In studies of fatty acid composition of more than 1600 randomly selected Heidelberg residents (2), adipose tissue was aspirated from paraumbilical sites. This offered the advantage of a lower risk of bleeding. Gluteal sites were preferred in the EURAMIC study, because many of the participating countries considered it advantageous that the subjects could not watch the procedure.

Contamination of the adipose tissue sample with blood may compromise its usefulness depending on the compounds to be measured. Fifty-five percent of the samples obtained for the EURAMIC study were free of blood while 22% contained large amounts of blood. The proportion of bloody samples varied widely between centers, from 90% blood-free samples in one center to more than 80% blood-contaminated samples from other centers. The underlying reasons for these differences are not yet clear, and need to be investigated in more detail. It is important, however, to recognize early in the design phase of any investigation whether blood contamination affects the planned analyses. If contamination has been found to be critical for a particular analyte, it usually will be possible to adapt the sampling protocol appropriately. Changes may include a reduction in the number of cutting or stabbing movements with the needle after the initial puncture; also, the freshly obtained samples may be rinsed in saline.

Contamination of the adipose tissue samples with blood or fluid is also a problem of dilution. Concentrations of substances measured in adipose tissue can be expressed per gram of tissue or per unit of fat. The proportion of fat in the biopsy tissue in the EURAMIC samples ranged from 47 to 83% wet weight as measured through the recovery of standards that had been added to the triglycerides before gas chromatographic measurement of individual fatty acids (5). If the parameter of interest is concentration of a pesticide or of

a fat-soluble vitamin in fat tissue, correction for the amount of fat in the sample should be performed.

### Storage

The proper conditions for short- and long-term storage of the adipose tissue must be met to insure that these valuable samples maintain their integrity until analysis, which may be months or even years later. Storage loss depends upon time, temperature, and the particular analyte of interest. The immediate environment of the samples during storage is also of importance. The high volatility of free short-chain fatty acids can be reduced by maintaining mildly alkaline pH. Highly polyunsaturated fatty acids which are very susceptible to oxidation should be protected by storing them under nitrogen or argon gas (6). An antioxidant may be added to improve stability of very sensitive fatty acids (7). Some compounds decompose rapidly when exposed to light, most notably the fat-soluble vitamins A, E, and K (6,8), while others are stable only at very low temperatures (5,8). For most purposes, immediate storage under nitrogen or argon in tightly closed brown glass vials at  $-80^{\circ}\text{C}$  is adequate to conserve the sample for many months. Since freezing and thawing will result in enzyme release into cells and destruction of membranes the analyses should not require the washing of sample or the separation of cells from the medium after thawing.

### Analyses of Adipose Tissue

Adipose tissue is a biomarker of significance for epidemiologic research of dietary exposures in relation to cancer. In contrast to more traditional methods of assessment of long term dietary intakes (9,10), it holds the promise of being able to sample past dietary habits without bias. It can also reduce the random error inherent in dietary assessment based upon calculations of intakes from subjective reports and the estimated content in the food as reflected by the averages found in tables of food composition. Assessment of diet-related exposures in epidemiologic research is generally based upon subjective reporting of the past by the subject or a surrogate. This may be the immediate past, as reflected by one or more 24-hr recalls, which, because of the expense are seldom carried out for multiple days; complete diet histories, which address usual food consumption behavior at each meal or eating period; or food frequencies, which ask about the rate or frequency of consumption of specific food items or food groups over a day, week, or month (11,12).

The latter is, for logistic reasons, applied most often. However, it takes a questionable approach to the difficult cognitive task of acquiring accurate and valid information on food intake (13,14). These methods are also of questionable use in case-control studies, because of the potential for response bias. This can occur when the subjects under study who have been recently diagnosed with a disease, such as breast cancer, report their dietary fat intakes differently than controls. The reason for this might be an increased awareness of diet, linked to the concern about this diagnosis. It may reflect an inaccurate overreporting of foods believed to cause the disease, as the subject searches to come to terms with the diagnosis. It may also reflect changes in eating behavior of recent origin due to the onset of disease; particularly with diseases of the gastrointestinal tract.

More objective measures can be derived from serum samples. These however, are much more likely to reflect acute needs, altered dietary intakes, or disturbed transport mechanisms than is adipose tissue (15,16). In so far as adipose tissue represents a stable, long-term reservoir that integrates exposure levels over time, it provides an ideal medium for the assessment of diet-related exposures that are lipophilic and measurable in this storage depot.

### Pesticides in Adipose Tissue

The idea of using adipose tissue as a medium of assessing exposure is not new. For 20 years the U.S. Public Health Service has been collecting and storing adipose tissue samples in the National Human Adipose Tissue Survey (NHATS) (17). This survey targeted polychlorinated biphenyls and organochlorine pesticides for study. Since 1967 it has collected approximately 12,000 samples of adipose tissue. These specimens, however, are largely from deceased individuals (85–90%) and the remainder from patients undergoing surgery. They were sampled from 47 urban or metropolitan statistical areas, and they were severely under quota; both characteristics have been cause for criticism of the survey (17). The ratios of adipose tissue to blood concentrations of many pesticides are more than one to a hundred, as seen in Table 1. The conclusions of a National Academy of Science report on monitoring human tissues were that since adipose tissue contains the highest concentrations of some of the most persistent chemicals in humans, this is the most sensitive way to assess exposure to such chemicals and should be continued where feasible (17).

**Table 1.** Adipose tissue to blood ratios for halogenated hydrocarbons.<sup>a</sup>

Halogenated hydrocarbons	Ratios of concentrations in adipose tissue vs blood
Halogenated aromatic compounds	
DDT	184–792
DDE	52–412
TCDD	89–135
4-Chlorobiphenyl	130
4,4-Dichlorobiphenyl	70
2,4,5,2',4',5'-Hexachlorobiphenyl	400
Halogenated aliphatic compounds	
	15–16
Aromatic amine compounds	
<i>p</i> -Nitroaniline	7
<i>p</i> -Phenylenediamine	0.1

<sup>a</sup>Committee on National Monitoring of Human Tissues (16).

### Fatty Acids in Adipose Tissue

Adipose tissue is made up largely of fatty acids which are stored there mostly in the form of neutral fat (triglycerides). Almost all of the fatty acids in adult humans, and therefore also in adipose tissue, are derived from the diet since *de novo* synthesis contributes less than 1 g of fatty acids per day (18). Most of the fatty acids found in adipose tissue are straight-chain, even-numbered carbonic acids with chain lengths between 14 and 20 carbons, and with or without double bonds between carbons. In most people oleic, palmitic, linoleic, and palmitoleic acid (given here in the order of their predominance) make up about 80% of the fat mass. Until recently, many publications gave the proportions of only these five fatty acids and very few others. Also, because of insufficient separation, some fatty acids were lumped with others. Because of this, the original figures for the oleic acid content of adipose tissue, which were reported to be close to 50% of the total fat mass, had to be corrected downwards by almost one-fifth, to about 40% (19). Currently, more than 40 additional fatty acids (Tables 2 and 3) can be regularly detected if state-of-the-art analytical methods are used (19–22). Several of those fatty acids that are present at very low concentrations in adipose tissue have increasingly attracted interest. Some of these (highly polyunsaturated fatty acids) are precursors of prostaglandins and other very active compounds. Others can indicate the use of particular types of fat or foods (transmonounsaturated fatty acids, branched-chain fatty acids). The accurate and precise measurement of these minor components can provide a new and, as yet, little used tool to relate adipose composition to dietary habits.

**Table 2.** Fatty acids usually constituting between 1 and 50% of total fat mass in adipose tissue of men and women on Westernized diets.<sup>a</sup>

Systematic designation	Trivial name	Typical abundance, %
18:1 <i>cis</i> (n-9)	Oleic acid	42
16:0	Palmitic acid	20
18:2 <i>cis,cis</i> (n-6)	Linoleic acid	14
16:1	Palmitoleic acid	6
18:0	Stearic acid	3.7
14:0	Myristic acid	2.7
18:1 <i>cis</i> (n-7)	Elaidic acid	1.8

<sup>a</sup>The information on the fatty acids in the respective groups was derived from several sources (19–22).

### Antioxidants in Adipose Tissue

The interest in potential preventive effects of antioxidants on cancerogenesis has been fueled by the multitude of epidemiologic studies showing a protective effect both within and across cultures with the increased consumption of fruits and vegetables. Particularly because of the consistent findings on carotene intake and lung cancer, interest has focused on the carotenes. In most of these studies, carotene intake has been measured indirectly through questions about dietary habits. This is difficult to assess for a number of reasons. Carotene absorption is dependent upon the presence of fat in the meal; food concentrations of carotenes are a function of cultivar and species; the available carotene is affected by food preparation (23) and the functional status of the intestine. Therefore, exposure estimates based on objective internal indicators should, if available and stable, be more accurate expressions of the true carotene availability to the subject of study.

The kinetics of  $\beta$ -carotene absorption and tissue accumulation are less well understood than those of dietary fatty acids. It is known that dietary  $\beta$ -carotene is absorbed with concurrent dietary fat

intakes (< 5%), and none is absorbed without fat (23). Serum  $\beta$ -carotene concentration increases 6 to 50 hr after ingestion (24,25). At low doses, a serum steady-state condition is reached within 5 to 20 days. Half-life of  $\beta$ -carotene in serum is approximately 10 days; the half-life in adipose tissue is still unknown. Fat storage depots in adipose tissue represent a major reservoir for  $\beta$ -carotene.

Although the isolation and extraction of carotenoids has been mastered since the 1930s, the advent of high performance liquid chromatography (HPLC) has enabled detection and accurate measurement of minute concentrations of the 600 known carotenoids (26). The importance of carotenoids other than  $\beta$ -carotene, and the knowledge that they make up 80% of the carotenoids in serum came much later, with the first publication on this topic in 1983 (27). Research on the formation, distribution, and biological relevance of various *cis*-isomers is just beginning (27).

If one is interested in carotenoids, although the concentrations in the liver are greatest, the absolute amount of reserves of carotenoids in adipose tissue, due to the total mass, is unsurpassed (28). First measurements of individual carotenes in adipose tissue revealed 40-fold concentration differences in  $\beta$ -carotene between individuals (29). Lycopene was found to be present in concentrations equal to those of  $\beta$ -carotene in adipose tissue. In the EURAMIC study, tocopherols and carotenes were measured in adipose tissue (Table 4). The concentrations ranged from 0.18 to 0.55  $\mu\text{g/g}$  fats across the countries. The detection limit then was 0.2  $\mu\text{g/g}$  for  $\beta$ -carotene. Mean  $\alpha$ -tocopherol contents of adipose tissue per country were expectedly greater than that of  $\beta$ -carotene, and ranged from 90 to 230  $\mu\text{g/g}$  fat. The coefficient of variation was 7%.

**Table 3.** Fatty acids (abbreviated systematic designation) constituting usually less than 1% of total fat mass in adipose tissue of men and women on Westernized diets.<sup>a</sup>

10:0									
12:0									
15:0	<i>iso</i> 14:0	<i>tr</i> 14:1	<i>cis</i> 14:1						
	<i>iso</i> 15:0	15:1							
		<i>tr</i> 16:1n-7							
17:0	<i>iso</i> 17:0	17:1							
	<i>cis</i> 18:1n-5	<i>tr</i> 18:1n-10	<i>tr</i> 18:1n-9	<i>tr</i> 18:1n-8	<i>tr</i> 18:1n-7	<i>tr</i> 18:1n-6	<i>tr</i> 18:n-5		
	<i>cis, tr</i> -18:2n-6	<i>tr, cis</i> -18:2n-6	<i>tr, tr</i> 18:2n-6	18:3n-3	18:3n-6	18:4n-3			
20:0	20:1n-9	20:1n-7	20:2n-6	20:3n-3	20:3n-6	20:3n-9	20:4n-6	20:5n-3	
21:0									
22:0	22:1n-9	22:2n-6	22:4n-3	22:4n-6	22:5n-6	22:6n-3			
23:0									
24:0	24:1								
26:0									

*tr*, trans fatty acid. <sup>a</sup>The information on the fatty acids in the respective groups was derived from several sources (19–22).

**Table 4.** Carotenoid and tocopherol composition of human adipose tissue.

Component	Mean <sup>a</sup>	Standard deviation	Range
β-Carotene	20.2	9.3	9.0–42.1
α-Carotene	5.7	3.2	2.7–14.7
Lycopene	18.5	10.7	0.8–41.0
α-Tocopherol	80.6	8.1	70.1–95.3
γ+α-Tocopherol	18.1	7.9	3.7–29.1
δ-Tocopherol	1.3	1.0	0.6–5.2

<sup>a</sup>Mean percentage of total carotenoids or tocopherols (n=19); adapted from Parker (29).

As might be expected, serum and tissue concentrations are not in a fixed relationship to one another. In some individuals, β-carotene in serum was two to four times that in adipose tissue; in others the ratios were in the opposite direction, with carotene and carotenoid values in adipose tissue two to five times as high as in serum (29).

Retinoids, although they do not have antioxidant capabilities, have also been of interest in relation to cancer due to their potent effect on cell differentiation. It has recently been reported that adipocytes store retinoid and synthesize and secrete retinol-binding protein. This extra hepatic synthesis of retinol-binding protein may play an important role in the conservation, recycling, and reutilization of retinol (30).

Other naturally occurring fat-soluble antioxidants of interest include tocopherols, tocotrienols, and quinones including ubiquinone, phyloquinone, and bilirubin. α-Tocopherol, the other parameter of primary interest in the EURAMIC study, can be measured in serum, plasma, platelets, red blood cells, feces and adipose tissue. We maintain that, for epidemiologic studies, adipose tissue concentrations are the measurement of choice.

Measurements of tocopherol in serum offer a precision of 3 to 5% (31). The sensitivity of the measure is at least 0.6 μg/μl. α-Tocopherol levels have been well researched in adipose tissue, where the concentrations are greater per gram tissue than in any other human tissue (Table 5). Tissue uptake is a linear function of the log of the dose (32), and shows correlation coefficients of 0.7 with dietary history intake values (6).

The utility of concentrations of antioxidants in adipose tissue as an unbiased measure of prior exposure in case-control studies depends upon its independence from the presence of disease. Concern has been expressed that antioxidants in adipose tissue may be influenced by the presence of a tumor

growth. Studies on the concentrations of antioxidants in various quadrants of breast tissue, both adjacent and distal to tumors, have shown that the proximity of a tumor does not alter concentration levels of retinol or carotenoids expressed as μg/g of tissue or fat. Tocopherol levels were actually greater in the tumor-adjacent tissue, but not significantly so (34). The time frame of dietary exposure and adipose tissue deposition remains even more poorly researched than that of the fatty acids in adipose tissue.

### Sources of Intraindividual Variation of Adipose Tissue Composition

The measured composition of adipose tissue is subject to considerable analytical as well as biological variation. Most of the available information comes from measurements of the fatty acid composition. Much less is known about variation affecting other analytes.

#### The Role of Diet

Fatty acids in the form of neutral fat contribute more than one-third of all energy to the diets of most Americans. Some of those fatty acids are completely broken down and utilized by muscles and the liver for energy production (35,36). Another, much smaller, proportion consisting only of polyunsaturated fatty acids is converted into prostaglandins and other related compounds with high biological activity (37). Whatever is consumed and afterwards spared from utilization will be conserved, most of it as triglycerides in adipose tissue. Some of these fatty acids are structurally altered before storage. The most frequent structural alterations involve either chain elongation whereby two carbons are added at a time, or the partial desaturation of the

fatty acid through the introduction of a double bond (38). Endogenous chain shortening and saturation of double bonds also contribute to structural changes of the fatty acids after absorption. All of these metabolic events determine which fatty acids can potentially reflect previous dietary patterns and how other dietary components exert their influences.

There are many fatty acids in human adipose tissue which cannot be produced by human enzymes. Their proportion in adipose tissue is governed by intake and metabolic breakdown. Thus, the relative amounts of polyunsaturated fatty acids in adipose tissue are closely related to the amounts that are consumed habitually (1,21,39,40). Such direct relationships also link consumption and adipose tissue concentrations of branched chain fatty acids (40). The proportions of several trans-unsaturated fatty acids in the body fat also have been found to reflect long-term intakes of these potentially harmful compounds (21). Similarly, the proportions of saturated and monounsaturated fatty acids depend on dietary intakes (20,21,41,42), but these relationships usually are much weaker. Low or very high intake of one fatty acid also may affect adipose tissue concentrations of another fatty acid. Thus, individuals with low linoleic acid intake had reduced proportions of both linoleic and dihomogamma-linolenic acid in adipose tissue (43). Dihomogamma-linolenic acid is indigenously produced from linoleic acid by a process of chain elongation and desaturation. With a decreased supply of precursor its concentration is consequently reduced.

Another example of a more complex relationship between fatty acid composition of diet and adipose tissue is overt essential fatty acid deficiency in children where the low linoleic acid proportions are accompanied by an increased concentrations of eicosatrienoic acid due to compensatory synthesis of this fatty acid from oleic acid (44).

The correlations between the proportion of a particular fatty acid in the diet and in the sampled adipose tissue is often much weaker than expected. It is important to realize that only a small part of this attenuation of the relationship can be explained by measurement error. Other dietary and nondietary factors also exert a strong influence on the fatty acid composition of adipose tissue through the metabolic relationships such as those outlined above. A higher proportion of fat in the diet may increase the sum of palmitic and

**Table 5.** α-Tocopherol content of human tissues.<sup>a</sup>

Tissue	μg/g tissue	mg/g lipid
Adipose tissue	150.0	0.2
Adrenal	132.0	0.7
Testis	40.0	1.0
Pituitary	40.0	1.2
Platelets	30.0	1.3
Heart	20.0	0.7
Muscle	19.0	0.4
Liver	13.0	0.3
Ovary	11.0	0.6
Plasma	9.5	1.4
Uterus	9.0	0.7
Kidney	7.0	0.3
Erythrocytes	2.3	0.5

<sup>a</sup>Adapted from Machlin (33).

stearic fatty acids in adipose tissue (40), increase oleic acid content of adipose tissue (45), and decrease the proportions of arachidonic acid (40), and of several monounsaturated (16:1n-6, 18:1n-7, and 18:1n-5) fatty acids (19). A high intake of monounsaturated fat has similarly been associated with a higher proportion of saturated fatty acids (40) and a lower proportion of several polyunsaturated fatty acids (19,21,40). The total amount of carbohydrate in the diet in one study was associated with a smaller proportion of palmitoleic and a higher proportion of oleic acid in adipose tissue (40). In cross-sectional surveys alcohol consumption was related to higher palmitoleic acid and lower linoleic acid proportions (46,47).

Often, relationships between habitual consumption of a fatty acid and its relative predominance in adipose tissue are seen most clearly in ecologic comparisons or population surveys. Intervention studies can fail to demonstrate an effect of dietary alterations. A case in point has been the experience with eicosapentanoic acid, a fatty acid that has been shown to reduce platelet aggregation (48,49). The proportion of eicosapentanoic acid in adipose tissue has been found to be related to long-term intakes in a population survey (29). Consumption of a diet with 0.5% of the fat as eicosapentanoic acid (EPA) for 100 days, however, did not induce a measurable increase in the EPA content of adipose tissue (49). The important issue here is the rate of change in adipose tissue composition following changes in the diet. The half-life of fatty acids has been estimated at 1 to 2 years (1,50). There may be more than one reason for this wide range of estimates. For one, it has been suggested that in addition to the fatty acid pool with slow turnover, another kinetically distinct pool of fatty acids may exist in adipose tissue which may have a half-life as short as 70 days (39). Adipose tissue turnover also appears to differ between femoral, gluteal, and abdominal sites (51). Finally, half-lives may differ between fatty acids (52). Definitive data on overall as well as site-or fatty acid-specific turnover times are sorely lacking. A review of the available literature reveals many inconsistencies and leaves more questions open than are answered. Particularly puzzling has been a study that detected a marked increase in the linoleic acid content of adipose tissue only 2 weeks after healthy women had switched to a diet with increased polyunsaturated fat content (53). In earlier studies comparable changes

were reported only after many months of increased linoleic acid intakes (50,54).

Further unresolved issues relate to the importance of total fat mass, the rate of fat accretion and impact of weight loss. Any of these may affect fat composition (51,55), although the current level of knowledge does not allow accurate predictions of their quantitative effects. Attempts at correlating dietary intakes of various groups of fatty acids with adipose tissue levels have shown that the strength of association varies greatly, depending upon the method of dietary assessment used to determine intakes, and the groups of fatty acids being examined. According to the findings of Tjønneland (22), food frequency questionnaires seem to produce much lower associations than 14 days of dietary records, as seen in Table 6. She also found that the associations were frequently stronger in men than in women. The only fatty acid showing relatively consistent results across methods and countries is eicosapentanoic acid (20:5n-3).

#### Homogeneity of Fat from Various Body Sites

The composition of human adipose tissue may vary between body sites, but the differences are not directly related to the depth of the tissue. Subcutaneous (from buttock and abdomen) and deep body sites (interscapular, perirenal) were shown by Brook CGD (56) to be similar in their composition. Peripheral areas were found to contain a greater proportion of monounsaturated and less saturated fatty acids, but did not differ in their linoleic acid content (51,56). Brown fat of rats was found to contain much higher proportions of stearic and lauric acid than white adipose tissue at the expense of lower propor-

tions of oleic and palmitoleic acid (57). Such differences may be of importance in very young children. In adults exclusively white fat will be collected from the typical sampling sites, which obviates the issue.

### Other Factors Influencing Adipose Tissue Composition

#### Gender and Age

Gender-specific differences in the fatty acid composition of adipose tissue appear to be small in comparison to diet-related variation but have been found in several surveys (45,58-60). Women tended to deposit more unsaturated fat, and men proportionally more saturated fat in their adipose. Table 7 lists the proportions of the predominant fatty acids in adipose tissue from randomly selected, healthy young men and women in Germany (2).

The most significant age differences are usually found between very young children and adults (1,3,61). The ratio of 16- to 18-carbon fatty acids, regardless of the degree of saturation, was found to be highest in very young children. This ratio approximates adult proportions by the age of five.

#### Race

Racial differences in adipose tissue metabolism were found between 20-year-old black African and Caucasian men (62). No significant differences between the total amount of subcutaneous fat exists, and the fat distribution across the body appeared to be similar, as was the suprailiac mean fat cell size. Black Africans have higher epinephrine-stimulated lipolytic values and LPL activity. It is not yet clear how these differences translate into variant composition.

**Table 6.** Correlations between dietary intakes and adipose tissue fatty acids.

Adipose tissue fatty acids	Dietary intake estimated by						
	FF <sup>a</sup>	14 days <sup>b</sup>	14 days, adj <sup>c</sup>	FF <sup>a</sup>	Adj FF	19-24 hr <sup>d</sup>	Deatten <sup>e</sup>
	Denmark <sup>f</sup>			United States <sup>g</sup>		Netherlands <sup>h</sup>	
Saturated	0.24	0.46	0.49	0.02	0.05		
Monounsaturated	0.05	0.19	0.26	0.08	0.09		
Polyunsaturated	0.44	0.57	0.63	0.15	0.25	0.68	0.75
18:2n-6	0.44	0.51	0.57	0.13	0.23	0.70	0.77
18:3n-3	0.12	0.36	0.40	0.07	0.01		
20:5n-3	0.47	0.44	0.63	0.43	0.43		
22:6n-3	0.41	0.55	0.80				

<sup>a</sup>Food frequency questionnaire. <sup>b</sup>Two 7-day, weighed diet records. <sup>c</sup>Corrected for within-to-between-person variance ratios. <sup>d</sup>19 to 24 hr recalls, conducted at roughly monthly intervals. <sup>e</sup>Deattenuated 24-hr recall data. <sup>f</sup>Pearsons correlation coefficients,  $n=86$ . Data from Tjønneland et al. (22). <sup>g</sup>Spearman's correlation coefficients,  $n=115$ . Data from London et al. (21). <sup>h</sup>Pearsons correlation coefficients,  $n=59$ . Data from Van Staveren et al. (20).

**Table 7.** Average proportions of the five predominant fatty acids in adipose tissue from a representative survey of 20- to 40-year-old healthy women and men in Germany.<sup>a</sup>

Fatty acid <sup>b</sup>	Women (SD) <sup>b</sup>	Men (SD) <sup>c</sup>
Palmitic acid	25.3 (2.2)	26.2 (2.5)
Palmitoleic acid	7.6 (1.7)	6.7 (1.6)
Stearic acid	4.2 (1.2)	5.5 (1.7)
Oleic acid	47.6 (2.4)	47.9 (2.9)
Linoleic acid	15.3 (3.2)	13.7 (2.8)

<sup>a</sup>These data from Arab et al. (2) have not been corrected for differences in dietary habits or other life-style factors. <sup>b</sup>Percent of combined fatty acid methyl ester (FAME) mass. <sup>c</sup>n=630. <sup>d</sup>n=323.

### Smoking

The effects of other lifestyle factors that may influence adipose tissue composition, such as physical activity, medication use, use of dietary supplements, or smoking have been hardly researched. There have been reports that smoking lowered linoleic acid in smokers compared to nonsmokers (2,43).

### Climate

Other than the influences described above, little is known about potential environmental influences of climate on adipose tissue composition. Cooler temperatures have been thought to lead to increased storage of unsaturated fats in subcutaneous tissues (63). It is not clear whether the high degree of unsaturation that has been observed in the adipose tissue of traditionally living Greenland Inuit (48) attests to the existence of such a relationship or is a chance finding related

only to the high polyunsaturated fatty acid consumption in these people.

### Hormones

Nuclear estrogen receptors can be down-regulated in isolated adipocytes. Estrogen treatment has been shown to reduce adipocyte size significantly, probably through drastic reductions in lipoprotein lipase activity in the adipose tissue (64). In rats, stress increased the size of adipocytes significantly, particularly in the mesenteric region. This effect may have been partly due to glucocorticoids (65). Thus there are clearly documented effects of hormones on the integrity of the adipose tissue. What the repercussions of this are on concentrations of substances of interest stored within the adipocyte remains poorly understood.

### Disease

The little work on the associations between adipose tissue and disease suggests that adipose tissue is remarkably resistant to alterations caused by nonwasting diseases. Earlier, the composition of breast tissue proximal to a tumor was reported to be unaltered. Studies of atopic dermatitis, to test the assumption of an abnormal fatty acid desaturation associated with this condition have also been negative (66). In patients with Crohn's disease, resection length was associated with the composition of fatty acids in the adipose tissue, and increased proportions of unsaturated fatty acids were found; but the authors attribute this to an increased demand for precursors or local prostaglandin synthesis, rather than to disease activity (67). In cystic fibrosis, on the other hand, where

there is a malabsorption of fat, an expected reduction in polyunsaturated and monounsaturated fatty acids is seen, as compared to normal volunteers (68). All told, there appears to be little cause for concern that disease states are directly affecting the adipose tissue composition. If this holds true, it will add another argument for using adipose tissue as a medium for assessment of long-term exposures in case-control studies.

### Conclusions

Dietary assessment in epidemiologic studies is still sorely limited by the ability to acquire unbiased, accurate, quantitative information on long-term, diet-related intakes. A possible advance, particularly as applicable to case-control studies, can be achieved by enhanced use of adipose tissue samples. Adipose tissue, as a medium for assessment of long-term exposures in epidemiologic studies, presents fewer problems in collection, analyses, and handling than expected. Aspiration of adipose tissue, to collect at least 5 mg of sample is simple to learn, and can be conducted on a number of sites on the body simply and without significant risk to the subject.

This medium holds promise for the study of antioxidants and their interrelationships with polyunsaturated fatty acids, as well as for the study of the long-term effects of pesticide exposures. HPLC has enhanced tremendously the ability to use minute samples for measurement of a broad spectrum of carotenoids present at umole levels in the adipose.

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