

Biomarkers of Fat and Fatty Acid Intake¹

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ABSTRACT Unlike other macronutrients such as protein, the amounts and types of fat in the human diet vary tremendously across cultures and over time have changed significantly within Westernized countries. Studies of the effect that fat sources, fat amounts and changes in fat intake have on human disease are extremely difficult to conduct with traditional dietary assessment methods for a number of reasons. These include the hidden nature of many fats, the variation in fatty acids contained in foods and feed and the sensitivity of individuals to questions about fat intake in their diets. For these reasons biomarkers of fat intake are particularly desirable. Fat and fat-soluble substances have the advantages over other nutrients of a long half-life and readily accessible storage depots (in the absence of starvation, undernutrition or eating disorders). Technological advances in quantitative measurements of individual fatty acids, with the help of gas chromatography and mass spectrometry (GCMS)⁴ and high performance liquid chromatography (HPLC), made possible the study of specific isomers of minor fatty acids from small tissue samples. Technological advances also opened the gateways to the study of fats that represent less than 1% of the total fat profiles, such as decosahexanoic acid (DHA), eicosapentanoic acid (EPA) and conjugated linoleic acid (CLA). Biological advances enhanced our appreciation of the differences between fats of differing chain lengths within a family, including the saturated fats. Challenges remain, such as assessing total fat intake, discriminating the contribution of endogenously produced fats, determining how to evaluate the importance of relative versus absolute contributions of fat and accounting for the factors that influence deposition and mobilization of individual fats within and between individuals. Factors that can influence deposition and mobilization include genetic variation, disease status, lifestyle differences (i.e., alcohol consumption and smoking), circulating apolipoprotein levels and the hormonal milieu of the individual and the source tissue. *J. Nutr.* 133: 925S–932S, 2003.

KEY WORDS: • biomarkers • diet assessment • epidemiology • fat intake • fatty acids • nutrition

Biomarkers of total fat intake

By sheer quantity consumed, dietary fat represents one of the greatest dietary exposures in the United States. For most Americans, it represents the greatest single source of energy. Adipose tissue is often viewed as a neutral energy storage medium. However, new research on the bioactivity of fat components in cell structure, function, intercellular communication and genetic transcription is changing this image. As far as dietary recommendations are concerned, both the amount of fat and the type of fat to be consumed remain controversial.

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³ Abbreviations used: CLA, conjugated linoleic acid; DHA, decosahexanoic acid; EPA, eicosapentanoic acid; FIO, flame ionization detectors; GC, gas chromatography; GCMS, gas chromatography and mass spectrometry; HPLC, high performance liquid chromatography; NEFA, nonesterified fatty acid.

The tremendous interest in dietary fat intake and the controversy surrounding the effect of fat intake on chronic diseases and cancer, has heightened the desire for a biomarker that would closely reflect the fat intake of an individual. In addition to being so widely and massively dispersed throughout the diet, fat is also one of the most difficult dietary components to assess through traditional methods for several reasons. Fat is sometimes very difficult for an individual to recognize and quantify. For instance, fat used in food preparation—for frying and cooking or as sauces and dressings—is often added by someone other than the individual under study, making it nearly impossible to identify the source and brand of fat. Even if this were known, it would be particularly tedious to report in detail. In addition, the accuracy of reporting fat is especially prone to bias. Underreporting of fat intake is greater among individuals that are overweight because of social implications (1).

Despite calls for proposals to develop and validate dietary fat biomarkers, a biomarker of the absolute amount of fat consumed remains elusive. However, there are biomarkers that can be used to quantify circulation change in fat intake, as well as biomarkers that reflect the consumption of essential and nonessential exogenously produced fatty acids. Interpreting these biomarkers requires an understanding of fatty acid metabolism, exogenous factors and the contributions of various body pools.

Fatty acid metabolism

Fatty acids are the basic structural components of triglycerides and are also found in phospholipids and cholesterol esters. They are rarely available as free fatty acids *in vivo*. Fats have important functions as storage units for energy, as structural units in membranes and as precursors to eicosanoids. They can therefore be found in serum, membranes and adipocytes. The proportion to be found in each media and molecule appears to depend upon the type of fatty acid. For example, linoleic acid concentrations appear to be greatest in cholesterol esters, followed by phospholipids, and lowest in triglycerides. Oleic acid shows the inverse sequence (2). Arachidonic acid appears to be particularly controlled across tissues (3), and it can represent 27% of the platelet phospholipid content, as little as 2% of plasma and less than 1% of adipose tissue concentrations (2). The fatty acids common in human tissues are characterized in **Table 1**.

Fatty acids are simple in structure, with a carboxyl group at one end and a methyl group at the other end of a carbon backbone. This backbone usually ranges between 6 and 24 carbons in length and is generally even-numbered. Their nomenclature is derived from the number of carbon atoms, the number of double bonds and the position of the first double bond on the carbon chain opposite the carboxyl group. **Figure 1** provides an example of the naming conventions used. Many but not all fatty acids can be synthesized, lengthened or desaturated endogenously. These three processes affect the use of fatty acid measures as biomarkers of consumption. Saturated fatty acids have no double bonds in the carbon backbone. Saturated fatty acids can be synthesized endogenously from acetyl CoA units by carbohydrate feeding. Synthesis of fat however is reported to be relatively rare in people who consume more than 25% of their energy as fat (4). Thus their adipose tissue stores tend to reflect dietary fat consumption except in the case of fasting or dietary cycling during which the long-term stores may be depleted.

Endogenous alteration of dietary saturated fatty acids can also occur through elongation. Lengthening the existing saturated fatty acids, two carbons at a time, creates new fatty acids. This generally occurs in the endoplasmic reticulum (5).

The third major alteration of fatty acids within the body is through desaturation, resulting in the formulation of a double bond. The mammalian lack of a Δ^{12} desaturase prevents conversion of oleic acid into linoleic acid [(n-9) to (n-6)

conversion]. Lack of the Δ^{15} desaturase prevents the conversion of linoleic into α -linolenic acid or the interconversion of (n-6) and (n-3) fatty acids in man. Plants can perform both of the conversions. Therefore dietary sources of these families of (n-6) and (n-3) fatty acids are the sole contributors to stores in man.

Exogenously produced fatty acids

Polyunsaturated fats are essential for life and cannot be produced endogenously. They are the precursors for eicosanoid synthesis, and their role in human nutrition was established in 1929 (6). Transformation of these polyunsaturates through the cyclo-oxygenase pathways results in prostaglandin, prostacyclin and thromboxane production. The lipooxygenase pathways lead to leukotrienes, hepxylins and lipoxins that are 20-carbon cyclized metabolites of dihomogamma-linolenate, arachidonate or eicosapentanoate. The precursor fatty acid determines the product as seen in **Figure 2**.

Because essential polyunsaturated fats cannot be synthesized *de novo* by humans and play an important role in health, markers of these biologically active fatty acids are of particular interest. Polyunsaturated fats contain more than one double bond and are generally liquid (i.e., oils) at room temperature rather than solid or semisolid (i.e., margarine or butter). Linoleate, arachidonate, dihomogamma-linolenate and eicosapentanoate are the most prevalent in human tissues. After digestion, metabolism and selective storage affect fat tissue levels, the profile of adult body fat reflects the profile of these dietary fats. The factors influencing fatty acid profiles in adipose tissue are presented in **Table 2** and discussed below.

The majority of fatty acids in human tissue are nonessential and are both dietarily supplied and endogenously produced. For those interested in exogenous sources of fatty acids and their relationships to chronic disease, one of the strongest biomarkers of long-term intake available is the relative distribution of individual fatty acids in adipose tissue. Transport is presumed to be nonselective. Adipose samples may be aspirated from one of various sites (most commonly gluteal or abdominal) and measured by gas chromatography (GC) or HPLC. Quantities as small as 10 mg are adequate for these analyses. The long-established procedure for sampling adipose tissue (7) carries a low risk of infection (8).

Half-lives of individual fatty acids in adipose tissue may differ. At best, one can judge from the few studies of turnover of

TABLE 1

Names of common fatty acids in human tissues and types of carbon bonds

Number of carbons	Saturated	Monounsaturated	Polyunsaturated (n-3)		Polyunsaturated (n-6)	
12	Laurate 12:0	Lauroleate 12:1				
14	Myristate 14:0	Myristoleate 14:1 (n-5)				
16	Palmitate 16:0	Palmitoleate 16:1 (n-7)				
18	Stearate 18:0	Oleate 18:1 (n-9) cis	Elaidate 18:1(n-9) trans	α -Linolenate 18:3 (n-3)	Linoleate 18:2 (n-6)	γ -Linolenate 18:3 (n-6)
20	Arachidate 20:0	Gadoleate 20:1 (n-11)	Gondoate	Eicosapentanoate 20:5 (n-3)	Dihomo- γ -linoleate 20:3 (n-6)	Arachidonate 20:4 (n-6)
22	Behenate 22:0	Euricate 22:1 (n-9)		n-3 Docosahexanoate 22:5 (n-3)	n-3 Docosapentanoate 22:6 (n-3)	n-6 Docosapentanoate 22:5 (n-6)
24	Lignocerate 24:0					n-6 Docosahexanoate 22:6 (n-6)

Short-, medium-, and long-chain fatty acids

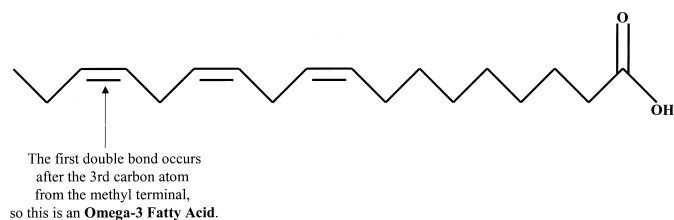
Fatty acids with less than eight carbons are considered short-chained. These are generally water soluble, except for those in milk lipids, which are the primary dietary source of short-chain fatty acids. The fatty acids 8–15 carbons in length are medium-chained and are normally intermediaries in the biosynthesis of long-chain fatty acids. Milk and coconut fat are rich sources of medium-chain fatty acids. Neither of these classes of fatty acids is generally stored in significant amounts in body lipids. The long-chain fatty acids are those found in the biomarker media discussed here. They come in various families, characterized by the number and placement of double bonds. Animal fat, butter, palm oil and peanuts are rich sources of long-chain saturated fatty acids. Particularly high levels of long-chain polyunsaturated fatty acids are present in membranes of the retina, brain synapses and sperm.

Trans fatty acids

Unsaturated fats containing carbon-to-carbon double bonds can be oriented such that the remaining hydrogens are adjacent (*cis*) or opposite (*trans*) to one another on the molecule. This isomerization influences structure, melting point and the ability of the fatty acid to fit tightly in a membrane structure or to bond to a receptor. *Trans* fats may occur naturally through the rumination process, but they largely occur through the industrial hydrogenation of polyunsaturated fats to enhance their stability and save them from oxidation. Margarine can contain 15–25% of their fat in the *trans* formation (10). In the U.S. diet, the amount of *trans* fats consumed increased over the past decades and averages ~5% of total fat stores. Mean adipose tissue levels are lower in many European countries than in the U.S. (11). The most commonly found *trans* fat is the 18:1(n-9): elaidic acid. *Trans* fats are roughly similar to saturated fats in their three-dimensional spatial configuration but are not as precisely aligned. Their biological activity may stem from their effective competition with other fatty acids for desaturase enzymes.

Monounsaturated fats

Although they are not precursors to eicosanoid production, monounsaturated fats receive much attention for their neutral or protective role in serum lipid levels and for their potential involvement in breast carcinogenesis. Monounsaturated fats



Linolenic Acid
or 9,12,15-Gammalinolenic Acid
or 18:3Δ^{9,12,15}
or 18:3ω³

FIGURE 1 Nomenclature schematic for polyunsaturated fatty acids.

linoleic acid in adipose tissue that the average half-life of fatty acids reflects an integrated measure over 1–2 y of intake (9). The degree to which adipose tissue levels of fatty acids correlate with reported dietary intakes can be seen in Table 3. With the strongest dietary assessment tools after deattenuation, the correlation coefficients are as low as 0.26 for mono-unsaturated fats and as high as 0.80 for DHA, a metabolite of EPA. Direct comparability, as expressed by correlation coefficients close to one, cannot be expected for a number of reasons. One reason is that the biomarker is subject to absorption, metabolism and all of the factors that have an impact on metabolic efficiency. The medium being sampled is not necessarily a storage site for the nutrient, and the nutrient may have been utilized before reaching storage (as is the case of the circulating β-carotene levels of smokers). Sampling, handling, storage and lab measurement contribute to some extent. Other discrepancies may be caused in part by measurement errors in reported dietary intakes of the daily and seasonal variations of habitual intake and sources of the adipose tissue and differences in utilization. The measurement error associated with dietary intake reports often includes difficulty in identifying fat sources, particularly for foods not prepared at home. Also included is error in the inference of fat composition of reported foods based on database values of food composition that generally exceed the measurement error of biomarkers. As seen in Table 3, the exogenously produced fatty acid biomarkers are more closely associated with dietary reports than are the groups of fats (e.g., saturated fatty acids), which can be synthesized endogenously.

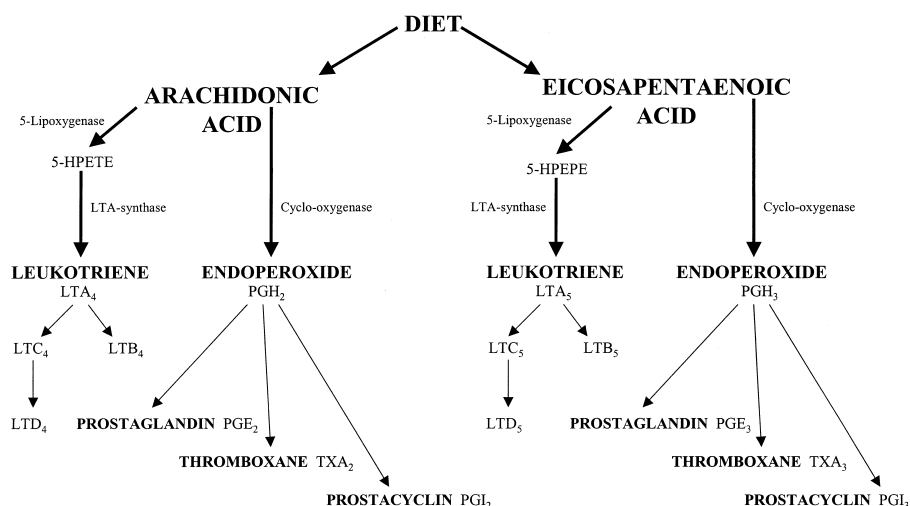


FIGURE 2 Prostaglandin pathway.

TABLE 2

Factors influencing measured fatty acid biomarker levels

- Intake through the diet
- Relative amounts of other fatty acids in the media (because fatty acids are expressed as percent of total, not absolute amounts of blood or fat)
- Supplement use (such as fish oil capsules)
- Genetic polymorphisms of elongase and desaturase enzymes
- Tissue-sampling site
- Tissue-sampling procedure
- Amount sampled (detection limits)
- Sample handling and storage
- Analytic method
- Lipolysis/Fasting
- Nutritional status (Fe, Zn, Cu, Mg sufficiency)
- Lipogenesis
- Diseases:
 - Cystic Fibrosis
 - Malabsorption
 - Liver Cirrhosis
 - Diabetes
 - Zellweger Syndrome
- Oxidation

can influence lipid metabolism and eicosanoid production through enzymatic inhibition and saturation.

Most of the studies of monounsaturated fats are based upon intake of olive oil—a rich source of oleic acid and the principal monounsaturated fatty acid in the Western diet. Canola oil is also a rich dietary source of this fat. When biomarkers of oleic acid are studied, the results are not consistent with an oleic acid effect independent of olive oil as a dietary source (12).

Polyunsaturated fats

There are three families of polyunsaturated fats common in human tissues. They are the (n-3), (n-6) and (n-9) fatty acids. As mentioned earlier the former two are not endogenously synthesized and, for lack of an enzyme, cannot be interconverted.

Omega-6 (n-6) fatty acids/arachidonic acid

Eicosanoids are oxygen-containing metabolites of fatty acid precursors in the (n-3) and (n-6) families. The dominant precursor is arachidonic acid, the availability of which is tightly regulated in vivo by esterification and by mobilization from cellular glycerolipids. Arachidonic acid can be formed from linoleic acid desaturation and elongation, which is thought to

TABLE 3

Correlations between adipose tissue fatty acids and dietary records

Adipose tissue fatty acids	FFQ ¹	14 days of records	14 days deattenuated
Polyunsaturated fat	0.44	0.57	0.63
Linoleic acid	0.44	0.51	0.57
Eicosapentanoic acid	0.47	0.44	0.63
Decosahexanoic acid	0.41	0.55	0.80
Saturated fatty acid	0.24	0.46	0.49
Monounsaturated fat	0.05	0.19	0.26

¹ FFQ refers to food frequency questionnaire (39,54)

occur primarily within the liver. Dietary sources rich in preformed arachidonic acid include eggs and lean meats, such as poultry, organ meats and fish. Evening primrose oil is a rich source of γ -linolenic acid [(18:3(n-6))].

Arachidonic acid is stored in cell membranes and can be mobilized by phospholipids. It can be released in response to injury. Arachidonic acid is regulated well within the body and is the principle precursor in eicosanoid synthesis (3). Eicosanoids are fast-acting, high-potency hormones that are produced locally from free fatty acids and serve as second messengers. Seminal fluid is particularly rich in eicosanoids, 20-carbon metabolites of arachidonic acid, dihomo- γ -linolenic acid or eicosapentanoic acid, which have been synthesized via cyclooxygenase or lipoxygenase pathways in microsomes. They are very complex in their activity because they can demonstrate biphasic responses depending upon concentrations. In addition, different eicosanoids arising from the same precursor fatty acid can have opposing actions. Prostacyclin and thromboxane A2 are examples of this because the former inhibits platelet aggregations and the latter stimulates platelet aggregation. Eicosanoids from different precursor fatty acids also tend to express contradictory effects, such as in the case of prostaglandin E1 and E2 effects described below. An overabundance of one precursor can drive down production of the products of another.

Omega-3 (n-3) fatty acids

Omega-3 (n-3) fatty acids derive primarily from cold-water fish, but some plants are also rich in (n-3) fatty acids. Although they represent a much smaller proportion of the diet, the (n-3) fatty acids are extremely important because of their impact on eicosanoid production. Their impact is direct, with eicosapentanoic acid serving as the precursor to the odd-numbered products of the three and five classes in contrast to the two and four families produced from the arachidonic acid pathway. The impact of (n-3) fatty acids is also indirect in that they compete successfully with (n-6) fatty acids for the cyclooxygenase enzymes, and their products often have the inverse physiologic effect of the (n-6) fatty acid eicosanoid products (13). For example, the prostaglandin E2 products can be pro-inflammatory and can induce vasodilatation, pain, fever and edema, whereas consumption of fish oil (at 2 g EPA plus DHA/d) is anti-inflammatory and decreases responsiveness to bacterial infection. The purported preventive associations between (n-3) fatty acids, cardiovascular disease and cancer have driven much of the interest in this family of fatty acids.

Conjugated linoleic acid

Conjugated linoleic acid (CLA) refers to positional isomers of linoleic acid in which the two double bonds are at 9 and 11 (the predominant form in foods) or at 10 and 12, instead of 9 and 12. This mild difference in structure may result in a drastic difference in their impact on tumor development. In animal experiments, linoleic acid stimulates carcinogenesis, whereas CLA feeding at 1% of the diet was shown to suppress mammary carcinogenesis (14). CLA is introduced into the diet by rumen bacteria that contain the isomerases capable of converting linoleic acid to CLA. For this reason and because of the amounts consumed, the primary dietary sources are milk and fat-rich dairy products. Recently, 0.5 and 1% of CLA in the diet were shown to reduce terminal end bud density and tumor load in chemically induced mammary carcinomas in rodents (15). In the same model, CLA was shown to suppress linoleic acid

metabolites including arachidonic acid, which may explain part of the effect through an influence on eicosanoid biosynthesis.

Measurement of fatty acid

Measurement of the relative proportions of individual fatty acids in human tissue is one of the more demanding nutritional biomarker analytic procedures for several reasons. Before analysis, accuracy of the methods requires careful sample collection and sample preparation. The need for separation of dozens of individual fats from a single sample requires chromatography and individual runs in a column that limits the throughput to dozens of samples per day under automated injection procedures and depends on the desired set of fatty acids to be precisely quantified. The specifics of the methods have been the subject of major works and multiple articles and can only be summarized here (16,17,18,19). Each procedure involves separation, identification and quantitation stages. Hydrolysis of fatty acids to unesterified forms is often required.

Thin layer chromatography or silica cartridges are used to separate the lipid fractions. Then either GC, HPLC or gas liquid chromatography (GLC) are used to separate the individual fatty acids. This choice depends on the need for precise quantitative analyses of small proportions of the total fat, the volatility of the fatty acid, the comparison in mass between fatty acids of interest and the polarity of the compound availability of equipment and cost. Due to equipment and running costs of HPLC, GLC—when performed in open-tubular columns of fused silica—is less expensive (15). However, for isolation and separation of particular fatty acids, HPLC is preferred (15). HPLC is also carried out at ambient temperatures, which prevents the heat-related rearrangement of fatty acids with labile moieties (15). Most importantly these analyses must be carried out with the intent to minimize autooxidation of the samples (15). For this, blanketing with nitrogen is often chosen (15).

The identification of peaks through elution order is commonly based on relative retention times and equivalent chain lengths. This does not however allow for identification of unknown peaks. Flame ionization detectors (FIO) are used to ionize carbon-containing compounds and quantify the ions as they pass through the collector. Mass spectrometry (GC/MS) with a mass ion detector is more precise and can identify compounds based on their total mass; however it is still prohibitively expensive for use in large studies. GC works well for simple, shorter and volatile fatty acid mixtures. In addition, GC can afford the epidemiologist a total fatty acid profile in a single run by separating based on the fatty acid chain length, the number of double bonds and the positioning of these bonds. Polyunsaturated fatty acids are more effectively separated with HPLC (19).

The most challenging separations are those involving identification of individual *cis*- and *trans*-isomers of fatty acids and small peaks such as those from conjugated linoleic acid. These families of fatty acids are either of equivalent molecular weight or are present in very small fractions that call for longer columns and run times. Therefore, the choice of an analytic method depends on the need for precise quantitative analyses of small proportions of the total fat, the volatility of the fatty acid, the comparison in mass between fatty acids of interest and the polarity of the compound availability of equipment and cost.

Quantitation of the weight of specific fatty acids depends upon the addition of internal standards in known weights early in the analysis. These standards need to be similar in composition to the experimental sample.

Factors influencing fatty acid measurements

Interpretation of the levels of fatty acids measured in a human population requires an understanding of the factors that can influence either the measurement or the actual level in the tissue of interest. From an analytic point of view, measurement error is inherent in any biomarker. For fatty acids the degree of error using traditional methods of assessment is related to the size of the peak derived in the chromatogram for that fat and its proximity to other peaks in the elution sequence. Coefficients of variance for minor fatty acids, such as CLA, may be greater than 25%.

Other factors, summarized in Table 2, can play a role in the inaccuracy of fatty acid measurement. Some functions occur before arrival of the sample for laboratory analysis. These include the sampling techniques and the handling and storage of the sample (20). For example, larger samples of adipose fat derived from surgery or punch biopsy are not as susceptible to the problems of detection limits. Inappropriate handling of the sample can result in oxidation of the polyunsaturated fatty acids through air exposure or contact with iron from erythrocytes. Samples stored for long periods of time before analysis, especially in a large study in which the samples are being run in a single laboratory, may suffer degeneration or the consequences of refrigeration loss (21). Long storage periods, resulting from a cohort or nested case-control design or simply from a backlog of samples to be analyzed, can result in changes in the profiles of the polyunsaturated fats, because these are temperature and oxygen sensitive. Careful storage under nitrogen gas and at -80°C will minimize this loss. The throughput for these analyses can be a limiting factor in sample turnover causing analyses in many cases to take months or even years to be completed. The tissue-sampling site can also have an impact on the values derived. For example, a comparison of fatty acid profiles from a deep-seated site (perirenal) and two subcutaneous sites (abdominal and buttocks) from autopsies of a racially mixed group of adults showed the proportion of saturated fatty acids to be highest in the perirenal adipose tissue and lowest in the buttock. Monounsaturated fatty acids were highest in the buttock (22). Abdominal tissue was more heavily saturated than the gluteal fat. The extremes of difference were as great as 40%. Polyunsaturated fatty acid profiles however were not significantly different across these three sites, which fortunately allows a choice of the most convenient sampling site. Another study comparing abdominal fat with fat from the inner and outer thigh showed higher levels of saturated fatty acid in the abdominal fat (23). In this study the polyunsaturated fat levels differed; the highest levels were in the outer thigh, the lowest levels were in the abdomen and in-between levels were in the inner thigh. These differences were as great as 30% for saturated fatty acids and 17% for the polyunsaturates.

Even if there were consistent and comparable mean levels of individual fatty acids across sites, it would be unwise to mix sites within a study. Nutritional status can also adversely influence the fatty acid profiles of the dietary fat intakes of individuals. Because the desaturases are metalloenzymes, adequate amounts of iron, zinc, copper and magnesium may be required for fatty acid metabolism to function normally.

Interpretation of fatty acid concentrations

One aspect of major importance in the interpretation of fatty acid measures is that the basis of measurement is the percentage an individual fatty acid contributes to the profile of all fatty acids, not the absolute amount of the fatty acid

consumed. Thus greater intakes of a specific fatty acid can drive down the relative percentage of another fatty acid even though its intake is unaltered. For this reason quantifiable standards of defined amounts of specific fatty acid must be run during the analyses.

When adipose tissue concentrations are used, an extrapolation of total body stores (based upon measurement or estimation of total body fat) might be appropriate if the biomarker is to reflect total stores as a measure of prior intakes. Height and weight can be used to extrapolate the body fat pool using the formulas derived by Womersley (24). Multiplication of the concentration in adipose tissue by the estimated total body fat should result in an estimate of the total body burden of that fatty acid. This type of extrapolation is not possible with serum fatty acid measurement. Serum reflects recent intake, not long-term stores, and is poorly correlated with adipose tissue levels in most individuals (19,25).

Fasting and weight gain

During weight maintenance, the turnover of triglyceride fatty acids in adipose tissue is estimated to be very low with half-lives of 1–2 y for linoleic acid (7,26). Adipose tissue requires 3–4 y to equilibrate to the diet in normal weight individuals when a change toward increased (n-6) fatty acids has been initiated (9). Studies of weight loss and gain in obese subjects shows that the fatty acid composition of adipose tissue, both gluteal and abdominal, are relatively stable. However, a 20% decrease in weight in obese subjects over short periods of time (2–10 wk) results in decreases of as much as 15% in (n-3) fatty acids (18:3-3) in both abdominal and gluteal tissue (27). Polyunsaturated fatty acids are released more rapidly than saturated fatty acids during long-term energy deficits. Thus homeostatic conditions are needed for the biomarker to reflect accurately long-term intake and not to be biased by lipolysis.

Choice of a medium for biomarker-based measures of fatty acids

Fatty acids can be measured as free fatty acids in serum, components of circulating triglycerides, components of erythrocyte membranes, phospholipids or cholesterol esters, or adipose tissue from various sites.

The shortest-term markers of fat intake are proportions of fatty acids in chylomicrons. These reflect the dietary fat intakes that enter the enterohepatic circulation directly after a meal. Other serum or plasma measures reflect the dietary intakes of the past few hours (triglyceride) or the past few days (cholesterol ester and phospholipid fatty acids) (28). Free fatty acids are bound to albumin and released from adipose when insulin and glucose are low. The plasma nonesterified fatty acid (NEFA) levels are largely determined by their rate of liberation from adipose tissue. Serum triglycerides are bound to apolipoproteins, and the rate of very low density lipoprotein secretion from the liver also influences circulating NEFA levels. Postprandially, these fatty acids are cleared to the adipose tissue under the action of lipoprotein lipase. Mobilization of adipose-tissue fatty acids is affected by hormone-sensitive lipase, lipoprotein lipase and fatty acid esterification (29).

The next most immediate biomarker medium is the serum or plasma levels of individual fatty acids, which can reflect intake over the last few days or meals. Serum fatty acids levels were shown to be sensitive indicators of changes in the polyunsaturated fat intakes of the diet (30,31). However serum triglyceride levels fluctuate greatly, with coefficients of variation of 12–30% and with laboratory error accounting for

less than 5% of the total variance (32,33). A single triglyceride measure, based on the experience of lipid research clinics, is likely to be only within 40% of the true average value for triglycerides, compared with 13% for cholesterol. Serum triglycerides may need an average of 10 baseline values to approach a coefficient of variance of 13% (28).

Red cell membranes and platelets are of interest as the biomarker media for fatty acid analyses because they reflect longer-term intake than circulating triglycerides but are still accessible through phlebotomy. Red cell membranes, for example, reflect intake aggregated over the lifespan of erythrocytes, or ~120 d, the half-life of erythrocytes. Membrane lipids differ from storage lipids in that they contain a high proportion of long-chain polyunsaturated fatty acids and rarely include triglycerides. Red blood cells provide a marker reflecting the last month and offer a more aggregated time period than does serum. The method requires pretreatment (lysis), protection against oxidation and centrifuging before storage until the final gas chromatographic analyses can be undertaken. For the use of this medium, whole blood specimens that contain red blood cells are collected, suspended in phosphate buffer and centrifuged. After removal of plasma and buffer coat, the packed red cells are resuspended in buffer, and hematocrit is measured and recorded. The red blood cells may then be stored in a –80°C freezer where they can remain stable for upwards of 5 y (34,35). Red blood cell membrane phospholipids reflect fatty acid profiles of serum for saturated and monounsaturated fats but may contain lower levels of (n-3) and (n-6) fatty acids (19). The need for immediate and appropriate handling of this medium (membrane lysis and isolation) at the time of phlebotomy is a limitation (19,21,24,36–39).

Adipose tissue is a preferred medium for the measurement of fatty acids as a reflection of long-term dietary intakes when no severe weight loss has occurred. Adipose tissue, be it gluteal, abdominal, subscapular, pectoral or from another site, reflects long-term storage of fats under homeostatic conditions. This is because of the oxidative, low fuel requirements of adipocytes and the large energy content. White adipose tissue is metabolically active. It controls the release of nonesterified fatty acids into the circulation and the uptake of dietary fatty acids into the adipocyte via lipoprotein lipase. However, because most populations studied are adequately to overly nourished, these influences do not have a strong impact on the stored fat profile.

The collection of adipose tissue, although still relatively unusual in the U.S., represents a safe and simple method of sample collection that is comparable to phlebotomy (8). Because there is no need for intact adipocytes, the tissue can be collected by aspiration using a 15-gauge needle with or without the use of local anesthesia (40). Training videos and complete methodologies on this are available from the author.

Fatty acid stores, both polyunsaturated and saturated, are likely to reflect more accurate dietary intakes under conditions of adequate nutrition or over-nutrition, lack of weight swings and good health. To some extent, age of the subject is relevant to the use of adipose tissue as a biomarker of exposure. Fat storage and release at birth and in early life (or adolescence) differ from later stages of development and adulthood. Pregnancy can also affect the fatty acid profiles of the mother, because deposition of fat is initially enhanced (40,41).

Effects of disease on fatty acid profiles

Healthy tissue levels can also be influenced by the fatty acid demands of diseases. For example, tumors selectively take up high amounts of polyunsaturated fatty acids (PUFAs). Because

tumors appear to show greater affinities for polyunsaturated fats (42), there is concern that tissue adjacent to a tumor may no longer reflect long-term consumption. One publication suggests that there could be no differences in mean averages between tumor adjacent breast tissue and other quadrants of the breast. Although the distribution of individual differences is not presented, the statistical analysis is not well detailed, and there is an issue of low study power in this sample of 10 women (43).

More obvious are the effects of diseases, such as cystic fibrosis involving pancreatic insufficiency, malabsorption of fats and cirrhosis of the liver, which will affect lipoprotein production (44). Similarly, patients with other lipid metabolism disorders such as Zellweger Syndrome, where synthesis of DHA is impaired, would also have biomarker profiles biased by these diseases (43).

Change in fat intake

In the early 1970s Keys (45) and Hegsted (46) reported almost simultaneously on the precision with which changes in the absolute intakes of families of fatty acids (polyunsaturated and saturated fats) can predict changes in serum cholesterol level. Both introduced formulas that predicted change in serum cholesterol as a function of dietary fatty acid changes.

Keys found that $\Delta y = 1.35(2\Delta S - \Delta P) + 1.5\Delta Z$ where ΔS and P are the changes in saturated and polyunsaturated fatty acids as percentages of calories and Δy is the change in plasma cholesterol in mg/dl (45). This can be rearranged to predict the change in saturated fat intake or polyunsaturated fat in an individual's diet based on knowledge of change in serum cholesterol assuming either that the other fats did not change or their amount of change is known. These formulas are then $\Delta S = \Delta y - 1.5\Delta z + 1.35\Delta p/2.7$ and $\Delta P = (2.7\Delta S - \Delta y + 1.5\Delta z)/1.35$. Similarly, Hegsted reported the change in serum cholesterol to be equal to $2.16\Delta S - 1.65\Delta P + 0.176\Delta C$ where $C = \text{mg change in cholesterol per } 1000 \text{ kcal}$ (46). This rearranges to $\Delta S = (\Delta y + 1.65\Delta P - 0.176\Delta C)/2.16$ and $\Delta P = (2.16\Delta S + 0.176\Delta C - \Delta Y)/1.65$. It can therefore predict either the change in ΔS and ΔP as a function of other dietary change or the effect of change in individual fats on serum cholesterol as a marker of change. More recently Mensink (47) and Kris-Etherton (48) published formulas that present more precision by adding changes in monounsaturated fat intakes and by taking into account the chain length of the polyunsaturated fat source. Because these formulas are so robust, changes in serum cholesterol levels—under otherwise homeostatic conditions with regard to weight status—and intakes of other fats and dietary cholesterol could be used as a biomarker of change in fat intake. These formulas were recently refined to be more specific with regard to individual fatty acids. The estimates from these authors are presented in Table 4.

Biomarkers of food intake

Some fatty acids are considered to be indirect biomarkers of the consumption of individual foods or food groups. This is the case when the food source is the primary source of the fatty acid and the fatty acid is stable in the sampling medium. Examples of this are the levels of short-chain fatty acids present in milk (49) (i.e., vacinic acid) and the (n-3) polyunsaturated fats found primarily in marine animals (EPA and DHA) (50,51). The correlations between dietary reports of fish consumption and serum phospholipid EPA were 0.58–0.75 (50). In erythrocyte membranes, levels of EPA were 0.44 (52). Subcutaneous adipose tissue levels of C15:0 and C17:0 in adult Swedish women were correlated with dairy-fat intake in this high dairy-

TABLE 4

Magnitude of effect of change in fatty acid intakes on serum cholesterol^{1,2}

Citation	Sat FAT change	PUFA change	MUFA change	Cholesterol change
Hegsted (1965)	2.16	-1.65	—	0.67
Hegsted (1993)	2.10	-1.16	—	0.67
Keys	2.7	-1.35	—	$1.52 \times \sqrt{\text{chol diff}}$
Mensink	1.51	-0.60	-0.12	
Yu	2.02	-0.96 C12-16 -0.3 C18:0	—	

¹ ~2-mg/dl change in cholesterol per 1% change in saturated fatty acid intake, and a 1.3-mg reduction per 1% increase in PUFA

² Sat FAT, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid

fat consuming population (49). For C15:0, the correlation with the prior 4 wk of consumption was 0.63 before deattenuation, and the correlation was 0.42 for C17:0 (49).

Stearic acid levels are high in chocolate (~30%) (51, 53). However, the utility of this fatty acid as a biomarker of chocolate consumption is limited by its conversion in the liver to oleic acid after desaturation (51). In some populations, the levels of oleic acid are taken as an indicator of the consumption of olive oil. All of this presumes that the individual is not using the fat for energy generation and that the intakes of other fats are relatively homogeneous.

CONCLUSIONS

Biomarkers of exogenously produced fatty acids are among the best available biomarkers of previous dietary intakes. Under homeostatic weight conditions and if properly sampled, collected and analyzed, the biomarkers can represent long-term intakes of individual fats. However, caution must be exercised in assuring that the appropriate analytic method is used for the fatty acids of interest. Standards should be run with the samples. Statistical analyses must adjust properly for the inherent colinearity in these measures. Other potential modifying factors, such as disease or metabolic differences, must be ruled out as potential sources of bias or modification. Unfortunately, no biomarker of total fat intake is available. Changes in saturated or polyunsaturated fat intakes may be identifiable through changes in serum cholesterol levels when intakes of other fatty acids remain constant. The detection of change is a new use for nutritional biomarkers.

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