Public Health Nutrition: 5(6A), 865–871 DOI: 10.1079/PHN2002391

Part C. Recent advances in the evaluation of assessment methods using biomarkers

Biomarkers and the measurement of fatty acids

Lenore Arab[1,2,*] and Jabar Akbar[2]
[1]Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA: [2]Department of Nutrition, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Abstract

Objective: To review the various biomarkers of dietary intakes of fatty acids in human populations, their measurement, limitations and analytical considerations.
Design: Review of the literature.
Results: Although there is no good biomarker of intake of total fat, a number of alternatives exist for assessing the intakes of exogenously produced fatty acids that are consumed. Adipose tissue, erythrocyte membrane concentrations and serum or plasma levels can reflect prior intakes over the past few hours to the past few years. The concentrations of individual fatty acids in these media generally reflect relative levels, and are influenced by a number of factors. Although relatively expensive to analyse, a single analysis by gas chromatography or high-performance liquid chromatography provides information on multiple fatty acids, and is superior to attempting to measure specific fatty acids using traditional dietary assessment methods.
Conclusions: Biomarkers of fatty acids that reflect long-term intake are available for nutritional epidemiology purposes. Analytical methods have become very accurate and able to detect and quantify smaller families, such as *trans*-fatty acids.

Keywords
Fatty acids
Biomarkers
Adipose tissue
Serum lipids
Dietary fat
Nutritional epidemiology

There are many types of biomarker and many potential functions for biomarkers in nutritional epidemiology studies. As presented in Table 1, they can be used as indicators of vulnerability, markers of compliance, markers of disease or pre-clinical disease, for validation purposes and to estimate error matrices[1]. However, the great hope is that they may also replace dietary assessment and therefore provide unbiased, quantitative indicators of prior dietary exposures.

Biomarkers differ from dietary intake in that they reflect both intake and the processes involved in utilisation of the nutrient in question. Thus, individuals with the same dietary intake may differ in absorption, transport, uptake in target tissues, metabolism from storage sites or excretion. This characteristic serves to detract from their value as a quantitative marker of food consumed, and enhances their value as a marker of the available nutrient or food in the study of diet and disease[2]. Despite the limitation of intra-individual variability influencing biomarker levels, they may still be superior for hypothesis testing than biased or non-quantitative self-reported measures of dietary intake.

Another issue is that the time period of exposure reflected by the biomarker differs from that of dietary intake, in that the biomarker reflects both intake and the processes involved in utilisation of the nutrient in question. Biomarkers of diet can in some cases represent an unbiased quantitative dietary intake over a specific period of time, such as the urinary excretion of small, water-soluble nutrients or non-nutrients in food that are catabolised or eliminated within hours of being consumed. Examples are isothiocyanates that are excreted within hours of consumption of cruciferous vegetables; ascorbic acid, which is excreted within a day of consumption; and minerals such as sodium or calcium that are eliminated largely through the urine. Alternatively, they can be markers that accumulate in the body, whose concentrations are associated with intakes over time and present an integrated measure of relative consumption. These longer-term markers are substances that are stored in various organs, of which the sampling without pinpointing a clearly defined exposure time window reflects total ranking of intakes of the individual. To date, few markers exist that can serve this function. One of the best opportunities to use biomarkers of long-term intake is provided by biochemical measures of some of the exogenously produced fatty acids. This review of fatty acid measures as biomarkers of fatty acid intakes addresses issues relevant to the use of biomarkers as replacements for traditional report-based dietary assessment.

*Corresponding author: Email Lenore@unc.edu

Table 1 Types of biomarker in nutritional epidemiology

Biomarkers of dietary exposure:
indicate prior consumption, suitable for ranking or quantification of prior intake
Ranking – markers that discriminate high consumers from low consumers, such as adipose tissue levels of fat-soluble vitamins
Quantification – markers that reflect total intake over a defined period of time, as is the case with 24-hour urinary excretion of calcium, sodium or B vitamin metabolites or doubly labelled water
Biomarkers of compliance:
substances added to the diet to determine amounts consumed
Biomarkers of susceptibility:
enzymes or genetic alleles that reflect functional differences which can affect disease risk or the ability to utilise nutrients
Biomarkers of pre-clinical disease:
biochemicals that indicate metabolic aberrations associated with disease development
Biomarkers for validation:
measures that are used as standards against which other reports of dietary intake are assessed

Biomarkers of total fat intake

Measurement of the fat intakes of individuals is a long-pursued goal that has met with little success. Fat is also one of the most difficult dietary components to assess through traditional methods. The reasons are many. Invisible fat is very difficult for an individual to recognise and quantify. Fat used in food preparation or in sauces and dressings is often added by others than the individual under study, making it close to impossible for the individual to identify the source and brand of fat. Even if known, it would be tedious to report in detail. Also, reported fat intakes are especially prone to bias. Individuals tend to underreport fat intake because of its social undesirability and the problem is greater among the overweight[3]. Although we still have no good biomarker of the total fat intake of individuals, there are biomarkers sensitive to change in fat intake and biomarkers that reflect the exposure to, or consumption of, essential and non-essential exogenously produced fatty acids.

Biomarkers of fatty acid intake

Unlike total fat, there are biological measurements from readily accessible human tissues that could potentially reflect intakes of specific fatty acids in the diet over varying periods of time. Biomarkers of intake are valid only when the concentrations reflect dietary intakes and are not simply reflecting exogenous processes. Many fatty acids can be synthesised, lengthened or desaturated endogenously. These three processes are relevant to the interpretation of fatty acid measures as biomarkers of consumption. In the Western diet of excess, this is not generally a severe limitation. Although saturated fats can be synthesised endogenously, this rarely occurs in people consuming adequate energy and more than 25% of their energy as fat[4]. Therefore, body fat stores tend to reflect long-term dietary fat consumption, except in individuals who have been fasting or going through cycles of weight loss and weight gain. The profile of adult body fat reflects the profile of polyunsaturated fats after digestion and selective metabolism have expressed their impact upon fat tissue levels. Plasma and membrane levels also relate to dietary intakes at various and different points in time.

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

The media available for assessment of dietary exposures in man most frequently involve blood and urine, but can also include hair, nails, skin, breath, saliva and faeces, depending upon the storage, circulation and stability of the nutrient. Owing to their lipid nature, fatty acids have the strong advantage of being available in long-term compartments such as adipose tissue, medium-term compartments such as erythrocytes and short-term plasma or serum components. Within serum, free fatty acids are present in triglycerides, phospholipids and cholesterol esters.

Chylomicrons in blood are short-lived dietary fat intake markers that peak directly after a meal. Serum triglycerides reflect the dietary intakes of the past hours to days, and when the fatty acid is found in cholesterol esters and phospholipids, it is likely to reflect consumption of a few days earlier[5].

Adipose tissue, because of its stability over time under conditions of energy balance, is an ideal measure of long-term dietary intakes of exogenous fatty acids. It represents an integrated measure over time of intake[6]. As its measure cannot be related directly to a quantitative intake at a clearly defined period of time, it is therefore one of the types of biomarker referred to as ranking or concentration markers.

The safety of adipose tissue aspiration is comparable to that of phlebotomy[7]. Adequate amounts of tissue for specific fatty acid analyses (5–10 mg) can be collected from various sites by aspiration using an 8-gauge needle with or without use of local anaesthesia. The relative distribution of individual fatty acids in adipose tissue is

Table 2 Site-specific concentration of fatty acids*

Fatty acid ($n = 143$)	Perirenal	Abdomen	Buttock
C14:0	2.58 ± 0.82	2.43 ± 0.75	2.16 ± 0.73
C16:0	23.76 ± 2.40	23.13 ± 2.11	21.79 ± 2.51
C16:1	4.90 ± 2.05	5.61 ± 2.16	7.22 ± 2.68
C18:0	6.54 ± 1.54	5.28 ± 1.73	4.00 ± 1.60
C18:1	45.59 ± 2.92	46.97 ± 2.75	48.26 ± 2.83
C18:2	15.36 ± 2.93	15.32 ± 2.85	15.37 ± 2.82
C18:3	0.62 ± 0.20	0.61 ± 0.19	0.61 ± 0.20

* Adipose tissue samples were obtained within 24 hours of death and taken from the retroperitoneal adipose tissue anterior to the left kidney (perirenal) and from the periumbilical region (abdominal)[8].

https://doi.org/10.1079/PHN2002391 Published online by Cambridge University Press

determined from sampling one single site in a uniform way throughout a study. Most commonly, abdominal or gluteal fat is used. Because of ease of access, the upper arm is also used regularly.

Within a person, fatty acid profiles may differ across sites. Data on the influence of site on fat profiles are presented in Table 2, indicating similarity across sites but also a tendency for medium-chain saturated fatty acids to be less concentrated and monounsaturated fats to be more highly concentrated in the buttock[8]. The proportion of saturated fatty acids appears to be highest in deep-seated sites (perirenal) vs. subcutaneous sites (abdomen and buttocks), as evidenced from autopsies of a racially mixed group of adults. Abdominal tissue in this study was more heavily saturated than the gluteal fat. Although differences were generally low, for some minor fatty acids they were as great as 40%. Polyunsaturated fatty acid profiles, however, were not significantly different in this sample, suggesting that the exogenously produced fatty acids are less site-specific.

Gas chromatography (GC) or high-performance liquid chromatography (HPLC) is applied to quantify the peaks[9]. Although the half-lives of individual fatty acids in adipose tissue may differ, from the few studies of turnover of linoleic acid in adipose tissue, the average half-life of fatty acids reflects an integrated measure over one to two years of intake[6].

Under conditions of adequate or overnutrition, without weight swings and in persons of good health, fatty acid stores – both polyunsaturated and saturated – are likely to reflect dietary intakes. However, pregnancy can affect fatty acid profiles of the mother, as deposition of fat is enhanced.

Measurement of fatty acids by GC or HPLC

Measuring the relative proportions of individual fatty acids involves careful sample collection, sample preparation and chromatographic analysis requiring hours per column run, resulting in throughput of dozens of samples per day, at best, under automated injection procedures.

Usually, fatty acids are chromatographed in the form of methyl esters. Analyses of short-chain (C2–C6) and medium-chain (C8–C12) fatty acids pose particular problems. Special precautions in the analysis of such volatile methyl esters are required to prevent excessive losses during solvent evaporation. The less-volatile short-chain free acids can be separated on a variety of liquid phases, fortified with a non-volatile acid such as phosphoric acid[9], which reduces dimerisation of the free acids.

The three methods used commonly to analyse fatty acids are GC, liquid chromatography (LC) and gas chromatography–mass spectrometry (GC–MS). GC is the most popular method. Analysis of medium- and long-chain fatty acids as their methyl esters is usually carried out on a polar column. These columns separate fatty acid methyl esters based on carbon number (chain length) and position of the double bonds. Most fatty acid analysis by GC uses a flame ionisation detector (FID). This non-specific detector ionises carbon-containing compounds (e.g. fatty acids) in a flame and quantifies the ions as they elute from the column. Unfortunately, analysis occurs via a destructive method and further analysis is impossible. When measuring fatty acids, the sample itself is composed of many individual fatty acids that have to be accounted for and separated (i.e. profile). A typical profile is composed of 20–25 peaks that correspond to individual fatty acids ranging in length from 14 to 22 carbon atoms. Individual fatty acids are identified by comparing their retention times with the retention times of known standards.

GC–MS is a powerful tool to ensure the correct identification of specific fatty acids or to elucidate the chemical structure of fatty acid metabolites. Mass spectrometry (MS) can detect and characterise compounds, giving definitive structural information. The combination of GC and MS (GC–MS) is particularly useful for lipid/fatty acid studies because of the ease with which complex mixtures may be separated and identified. However, the high cost and specialised personnel required to maintain the GC–MS preclude its use in many laboratories. Argentation thin layer chromatography (TLC) is another technique that can separate fatty acids based on the total number of ethylenic bonds. However, it does not separate fatty acids with the same number of carbon atoms.

HPLC is another method of choice for fatty acid analysis. This analytical procedure yields the versatility of analysing either free or derivatised (methyl esters) fatty acids. The column eluates are monitored continuously by means of a flow-through detector, which should be insensitive to solvent flow rate, temperature or solvent composition. The eluates may be collected, by means of a fraction collector, and used for further analyses. This method of analysis uses an ultraviolet detector, thus resulting in samples that are not destroyed (unlike the cases of FID and MS). Unfortunately, HPLC does not always cleanly separate every isomer[9]. It is not uncommon for a laboratory to use a combination approach: after running each sample through HPLC, the same sample is then run through an MS detector or submitted for GC analysis.

The separation time for each sample determines the spectrum of fatty acids clearly separated. Scientists typically use a 30 m column to carry out such fatty acid separations. The length of such a column is standard, along with an optimal separation time of about 35 minutes. There is also a 60 m long column that allows additional separation, but also roughly doubles the separation time of the 30 m long column. The issue of cost efficiency also has to be considered. However, determination of positioning of the double bonds, *cis* vs. *trans*, requires longer columns[10].

https://doi.org/10.1079/PHN2002391 Published online by Cambridge University Press

Table 3 International and ethnic comparisons of fatty acid profiles

Fatty acid	US-born Americans*	Greece-born*	Greece (Crete) urban*	Greece (Crete) rural*	Israeli Ashkenazim†	Israeli non-Ashkenazim†	New Zealand Maoris‡	New Zealand Europeans‡	Pukapkans in Pukapuka‡	Pukapukans in Rarotonga‡	Manifold
C10:0	n/a	n/a	n/a	n/a	n/a	n/a	0.10 ± 0.02	0.06 ± 0.02	0.27 ± 0.04	0.20 ± 0.03	4.50
C12:0	0.63 ± 1.59	0.91 ± 0.75	0.24 ± 0.44	0.62 ± 0.75	0.39 ± 0.17	0.27 ± 0.08	0.34 ± 0.07	0.33 ± 0.04	11.5 ± 1.39	10.8 ± 1.30	47.9
C13:0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.34 ± 0.06	0.58 ± 0.16	1.71
C14:0	4.37 ± 4.16	2.59 ± 0.52	1.37 ± 0.34	1.96 ± 1.85	2.29 ± 0.52	1.72 ± 0.54	3.44 ± 0.20	4.19 ± 0.25	16.1 ± 1.34	14.7 ± 1.64	11.7
C15:0	n/a	n/a	n/a	n/a	n/a	n/a	0.64 ± 0.04	0.55 ± 0.03	0.49 ± 0.07	0.50 ± 0.12	1.31
C16:0	19.6 ± 2.14	18.6 ± 1.74	15.2 ± 1.45	15.1 ± 1.56	20.6 ± 2.63	18.9 ± 2.63	23.9 ± 0.75	23.3 ± 0.71	20.4 ± 0.92	20.3 ± 1.03	1.58
C17:0	n/a	n/a	n/a	n/a	n/a	n/a	0.67 ± 0.07	0.57 ± 0.04	0.45 ± 0.22	0.39 ± 0.10	1.72
C18:0	2.92 ± 2.11	4.03 ± 0.97	2.76 ± 1.73	2.57 ± 1.37	4.96 ± 1.22	4.60 ± 1.11	4.85 ± 0.37	4.98 ± 0.72	2.50 ± 0.31	2.70 ± 0.44	1.99
C20:0	n/a	n/a	n/a	n/a	n/a	n/a	0.53 ± 0.04	0.52 ± 0.06	0.15 ± 0.08	0.32 ± 0.12	3.53
Total saturated	27.5 ± 5.55	26.1 ± 2.22	19.6 ± 2.93	20.3 ± 2.21	28.5 ± 2.84	25.5 ± 3.10	34.6 ± 1.09	34.6 ± 1.34	52.6 ± 2.24	50.7 ± 2.60	2.69
C12:1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1.05 ± 0.12	0.81 ± 0.13	1.30
C14:1	0.78 ± 0.95	0.65 ± 0.52	1.25 ± 1.58	1.29 ± 1.92	n/a	n/a	n/a	n/a	2.96 ± 0.37	2.62 ± 0.42	4.55
C16:1	6.92 ± 2.03	7.34 ± 1.18	6.58 ± 1.53	5.68 ± 1.81	3.70 ± 1.00	3.39 ± 0.94	7.23 ± 0.46	8.96 ± 0.40	9.64 ± 0.44	9.45 ± 1.32	2.84
C17:1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.60 ± 0.10	0.70 ± 0.14	1.17
C18:1	51.1 ± 4.30	52.7 ± 2.82	62.8 ± 4.02	62.6 ± 4.03	39.6 ± 2.64	39.1 ± 3.01	52.1 ± 0.75	49.4 ± 1.09	28.95 ± 1.62	31.4 ± 2.80	2.17
C19:1	n/a	n/a	n/a	n/a	n/a	n/a	0.28 ± 0.03	0.30 ± 0.03	0.10 ± 0.06	0.22 ± 0.10	3.00
C20:1	n/a	n/a	n/a	n/a	n/a	n/a	0.82 ± 0.07	0.95 ± 0.10	0.61 ± 0.15	0.91 ± 0.19	1.56
Total monounsaturated	58.9 ± 4.99	60.6 ± 2.85	70.7 ± 3.71	69.5 ± 4.03	43.3 ± 3.35	42.5 ± 3.36	60.4 ± 0.65	59.65 ± 0.74	44.1 ± 2.25	46.5 ± 2.88	1.66
C18:2	13.7 ± 3.30	13.3 ± 2.20	9.78 ± 1.67	10.2 ± 3.23	22.2 ± 3.63	25.6 ± 4.52	2.60 ± 0.13	3.02 ± 0.11	3.22 ± 0.38	2.60 ± 0.26	9.86
C18:3	n/a	n/a	n/a	n/a	2.44 ± 0.57	2.66 ± 0.47	0.93 ± 0.08	0.82 ± 0.08	0.06 ± 0.05	0.22 ± 0.14	44.3
Total polyunsaturated	13.7 ± 3.30	13.3 ± 2.20	9.78 ± 1.67	10.2 ± 3.23	24.9 ± 4.60	28.3 ± 4.85	3.53 ± 0.09	3.84 ± 0.08	3.28 ± 0.19	2.82 ± 0.20	8.82

n/a – not available.
* Supragluteal adipose tissue aspirations[12].
† Subcutaneous abdominal fat tissue samples[13].
‡ Gluteal adipose tissue samples[14].

Silver-ion high-performance liquid chromatography (Ag^+-HPLC) is commonly used to analyse samples for conjugated linoleic acid and other *trans*-fatty acids[11]. This method differs from HPLC/MS in that the column is composed of stable silver ions that are linked via ionic bonds to phenylsulfonic acid moieties instead of a constant bombardment of hydrogen ions. It is possible to control many of the chromatographic parameters, especially mobile phase composition, flow rate and column temperature, with a high degree of accuracy. In addition, the column can be reused, the analysis time is short, and sample separation is simple and rapid. Samples may be analysed by GC before and after the separation, thus allowing determination of the quantity of *trans*-fatty acids.

General issues relative to interpreting dietary intake biomarkers

Measurement error is inherent in any biomarker. It derives from sampling, transport and handling, and, with fatty acids, is also related to the amount of the individual fat in the sample available for analysis[1]. Assessment is related to the size of the peak derived in the chromatogram and proximity to other peaks in the elution sequence. Minor fatty acids measured with GC may show coefficients of variation greater than 25%.

An understanding of the denominator of any concentration measure is essential to any biomarker-based measure. Serum can be diluted or concentrated depending upon physiological factors. Toenails have individual growth rates and therefore reflect different exposure times. Urine requires a time denominator for collection or standardisation against a constant such as creatinine in healthy individuals. Fatty acid levels in any biomarker medium have the special characteristic of being a proportion, not an absolute measure. The interpretation of fatty acid measures requires recognition that the measurement is the percentage that a fatty acid contributes to the profile of all fatty acids, not the absolute amount. Thus, greater intake of a specific fatty acid can drive down the relative percentage of another, even though its intake is unaltered. For this reason, quantifiable standards need to be run during the analyses.

International variation in adipose tissue fatty acid concentrations

Evidence of the influence of dietary differences on human fat deposits is provided in international comparisons and comparisons of ethnic groups residing in different parts of the same country. Table 3 presents the mean levels of individual fatty acids in the USA, Greece, New Zealand and Israel for natives and emigrants from three countries[12–14]. This table illustrates the fact that almost as much difference can be found in different geographical areas within a country as between countries. An example of this is the profound difference in total saturated fatty acids of the Pukapkans relative to the US-born Americans. The proportions of monounsaturated fatty acids did not differ as much. However, a nearly fourfold difference in the total polyunsaturated fatty acids was noted. These international comparisons within a genetically similar group emphasise the importance of dietary influences upon fatty acid profiles.

Seasonal differences in fatty acid levels in the trunk and extremities

Few studies have systematically examined seasonal variation in fatty acid composition of adipose tissue. One early study, conducted in 1969 with the current technology at that time, showed higher levels in summer of medium- and long-chain saturated fatty acids and lower levels of

Table 4 Seasonal measures of individual fatty acids[15]

	Leg and forearm		Chest and abdomen	
Fatty acid	Winter	Summer	Winter	Summer
C12:0	n/a	0.3 ± 0.05	0.3 ± 0.06	0.4 ± 0.13
C14:0	2.0 ± 0.12	3.1 ± 0.43	3.4 ± 0.17	3.2 ± 0.28
C14:1	1.4 ± 0.13	1.2 ± 0.17	0.7 ± 0.10	0.9 ± 0.08
C16:0	16.5 ± 0.40	21.0 ± 1.21	24.1 ± 0.77	24.4 ± 1.57
C16:1	14.6 ± 1.20	10.1 ± 0.88	7.9 ± 0.24	8.4 ± 0.44
C18:0	1.5 ± 0.40	2.3 ± 0.38	4.0 ± 0.28	4.4 ± 0.65
C18:1	44.7 ± 0.72	41.9 ± 0.78	39.2 ± 0.78	38.5 ± 0.88
C18:2	16.4 ± 0.68	16.5 ± 0.60	16.8 ± 0.76	15.9 ± 1.69
C18:3	3.0 ± 0.18	3.6 ± 0.12	3.7 ± 0.32	3.8 ± 0.35
Total				
Saturated	20.0 ± 0.66	26.7 ± 1.29	31.7 ± 0.84	32.4 ± 1.21
Monounsaturated	60.7 ± 1.12	53.2 ± 0.64	47.8 ± 0.68	47.8 ± 0.90
Polyunsaturated	19.4 ± 0.85	20.1 ± 0.70	20.5 ± 0.87	19.7 ± 1.69

n/a – not available.

https://doi.org/10.1079/PHN2002391 Published online by Cambridge University Press

monounsaturated fats in the extremities. However, this was not the case in the samples of the chest and abdomen. The sum of all polyunsaturated fat levels was approximately 20% in this population and did not differ across sites or season[15] (Table 4).

Fatty acids of special interest

Five classes of fatty acids are of particular interest in relation to dietary intakes. The polyunsaturated fats are of special interest because they are produced exogenously and stem from specific food sources, depending upon the availability of specific enzymes that can help in the formation of the polyunsaturated bonds. Among the polyunsaturated fats, three families are distinguished: the $n-3$, $n-6$ and $n-9$ fatty acids. Essential fatty acids of the $n-3$ and $n-6$ families are needed for eicosanoid production. Eicosanoids are fast acting, high-potency hormones that are produced from free fatty acids locally. These are oxygen-containing metabolites of fatty acid precursors of the $n-3$ and $n-6$ families. The most ubiquitous precursor is arachidonic acid. Dietary sources rich in arachidonic acid include eggs, lean meats such as poultry, organ meats and fish. Evening primrose oil is a rich source of γ-linolenic acid (C18:3$n-6$). The $n-3$ fatty acids stem largely from cold-water fish. The primary contributor from this family to eicosanoid production is eicosapentanoic acid. It produces odd-numbered prostaglandins of the 3- and 5- classes as compared with the 2- and 4- families produced from the arachidonic acid pathway. Their products often have the inverse physiological effect of the $n-6$ fatty acid eicosanoid products[16]. For example, prostaglandin E_2 products can be pro-inflammatory whereas fish oil is anti-inflammatory.

A third family actively under study is the *trans*-fatty acids group[17]. The dietary sources of *trans*-fats have increased dramatically in the last decades. Although these fatty acids can occur naturally through the rumination process, the primary source in the American diet is from foods in which polyunsaturated fats have been hydrogenated to enhance their stability and prevent oxidation, such as margarines, which contain 15–25% of their fat in the *trans* formation[17]. The most common *trans*-fatty acid is C18:1$n-9$ or elaidic acid. Their biological activity may stem from their effective competition with other fatty acids for desaturase enzymes.

Monounsaturated fats have recently received much attention for their neutral or protective role in serum lipid levels and for their potential involvement in breast carcinogenesis. Canola oil is a rich dietary source of this fat.

The fifth class of interest is the only fatty acid type shown to be clearly and strongly preventive against cancer (in animal models) *in vivo*: the conjugated linoleic acids (CLA). These positional isomers of linoleic acid have two double bonds at C9 and C11 (the predominant form in foods) or C10 and C12, instead of at C9 and C12 as found in linoleic acid. This mild difference in structure results in a drastic difference in their impact on tumour development. In animal experiments, linoleic acid stimulates carcinogenesis whereas CLA feeding at 1% of the diet has been shown to suppress mammary carcinogenesis and to suppress linoleic acid metabolites including arachidonic acid[18,19]. CLA is introduced into the diet by rumen bacteria that contain the isomerases capable of converting linoleic acid to CLA. Consequently, the primary dietary sources are milk and fat-rich dairy products.

Diet–biomarker relationships

Comparisons of dietary intake reports with biomarker measurements conducted within the same population are often expected to yield high correlations. In fact, correlation coefficients are often lower than 0.3, unless adjustments for deattenuation are made. However, these may over-inflate the true relationship. Studies that use repeated days of dietary records or multiple 24-hour recalls tend to have greater correlations with adipose tissue markers than those that are food-frequency based[20]. The reasons for the discrepancies are many, and are summarised in Table 5. Measurement error is certainly responsible for reductions in correlation. Sampling, handling, storage, laboratory measurement; the problems of self-reported dietary intakes; the difficulty individuals have in identifying fat sources, particularly for foods they did not prepare; and the error in inference of fat

Table 5 Sources of discrepancy between dietary assessment and biomarkers of fatty acid in the same individuals (sources of variance)

Biomarkers	Subjective dietary assessment
Sampling day	Memory of consumption
Sampling site	Portion size estimation
Laboratory measurement error	Frequency estimation
Absorption	Mixed dishes and their decomposition
Medium not reflective of uptake (stores)	Unknown recipes
Carrier molecules	Bioavailability
Genetic variance in uptake, transport, metabolism	Fortification
	Supplement use
	Instrument-based biases

https://doi.org/10.1079/PHN2002391 Published online by Cambridge University Press

composition of reported foods based on food composition database values – all contribute. In addition, factors such as selective uptake may also influence biomarker-based measures. But beyond this, direct comparability resulting in high correlations cannot be expected for several reasons. First, the biomarker is subject to absorption, metabolism and all of the factors that impact on metabolic efficiency. Second, the site sampled will not necessarily reflect the same time period of consumption as the dietary intake records. Third, despite high intakes, the nutrient may have been utilised prior to reaching storage, as is the case with β-carotene biomarker levels in smokers.

Conclusion

Dietary assessment can be enhanced, replaced and assessed through the use of biomarkers such as those derived from measuring adipose tissue polyunsaturated fatty acids. The investment in effort and cost may be greater than for dietary assessment with traditional tools. However, this cost is more than balanced by the value of an objective measure of exposure. Biomarkers in general cannot replace traditional dietary assessment methods. There are too few biomarkers of exposure available for the time periods of interest. However, biomarkers of dietary exposure do belong in every nutritional epidemiology study in one or more of the functions outlined in Table 1. Nutritional epidemiologists need to be proactive in insisting on the best possible dietary assessment in studies contributing to our knowledge on the relationships between health and diet. Getting the wrong answer is simply too costly to choose the alternative.

References

1 Kohlmeier L. What you should know about your marker. In: Kok FJ, van't Veer P, eds. *Biomarkers of Dietary Exposure. Proceedings of the 3rd Meeting on Nutritional Epidemiology*. London: Smith-Gordon, 1991; 15–25.
2 Pearce N, de Sanjose S, Boffetta P, Kogevinas M, Saracci R, Savitz D. Limitations of biomarkers of exposure in cancer epidemiology. *Epidemiology* 1995; **6**: 190–4.
3 Lichtman SW, Pisarska K, Berman ER, Pestone M, Dowling H, Offenbacher E, *et al*. Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. *N. Engl. J. Med.* 1992; **327**: 1893–8.
4 Hellerstein MK. De novo lipogenesis in humans: metabolic and regulatory aspects. *Eur. J. Clin. Nutr.* 1999; **53**(Suppl. 1): S53–65.
5 Kohlmeier L. Future of dietary exposure assessment. *Am. J. Clin. Nutr.* 1995; **61**: 702S–9S.
6 Beynen AC, Katan MB. Rapid sampling and long-term storage of subcutaneous adipose-tissue biopsies for determination of fatty acid composition. *Am. J. Clin. Nutr.* 1985; **42**: 317–22.
7 Handelman GJ, Epstein WL, Machlin LJ, van Kuijk FJGM, Dratz EA. Biopsy method for human adipose with vitamin E and lipid measurements. *Lipids* 1988; **23**: 598–604.
8 Malcom GT, Bhattacharyya AK, Velez-Duran M, Guzman MA, Oalmann MC, Strong JP. Fatty acid composition of adipose tissue in humans: differences between subcutaneous sites. *Am. J. Clin. Nutr.* 1989; **50**: 288–91.
9 Hendrikse PW, Harwood JL, Kates M. Analytical methods. In: Gunstone FD, Harwood JL, Padley FB, eds. *The Lipid Handbook*, 2nd ed. London: Chapman & Hall, 1994; 319–58.
10 Christie WW. Detectors for high-performance liquid chromatography of lipids with special reference to evaporative light-scattering detection. In: Christie WW, ed. *Advances in Lipid Methodology – One*. Glasgow: The Oily Press, 1992; 239–71.
11 Sehat N, Rickert R, Mossoba MM, Kramer JKG, Yurawecz MP, Roach JAG, *et al*. Improved separation of conjugated fatty acid methyl esters by silver ion–high performance liquid chromatography. *Lipids* 1999; **34**: 407–13.
12 Fordyce MK, Christakis G, Kafatos A, Duncan R, Cassady J. Adipose tissue fatty acid composition of adolescents in a US–Greece cross-cultural study of coronary heart disease risk factors. *J. Chronic Dis.* 1983; **36**: 481–6.
13 Blondheim SH, Horne T, Davidovich R, Kapitulnik J, Segal S, Kaufmann NA. Unsaturated fatty acids in adipose tissue of Israeli Jews. *Isr. J. Med. Sci.* 1976; **12**: 658–61.
14 Shorland FB, Czochanska Z, Prior IAM. Studies on fatty acid composition of adipose tissue and blood lipids of Polynesians. *Am. J. Clin. Nutr.* 1969; **22**: 594–605.
15 Moriya K, Itoh S. Regional and seasonal differences in the fatty acid composition of human subcutaneous fat. *Int. J. Biometeorol.* 1969; **13**: 141–6.
16 Horrobin DF. Nutritional and medical importance of gamma-linolenic acid. *Prog. Lipid Res.* 1992; **31**: 163–94.
17 Panel on Macronutrients, Food and Nutrition Board, National Academy of Science Letter. *Report on dietary reference intakes for trans fatty acids* [online]. Available at http://www.nas.org, 2002.
18 Ip C, Singh M, Thompson HJ, Scimeca JA. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Res.* 1994; **54**: 1212–5.
19 Banni S, Angioni E, Casu V, Melis MP, Carta G, Corongiu FP, *et al*. Decrease in linoleic acid metabolites as a potential mechanism in cancer risk reduction by conjugated linoleic acid. *Carcinogenesis* 1999; **20**: 1019–24.
20 Kohlmeier L, Bellach B. Exposure assessment error and its handling in nutritional epidemiology. *Annu. Rev. Public Health* 1995; **16**: 43–59.

https://doi.org/10.1079/PHN2002391 Published online by Cambridge University Press