

Dietary intake and nutritional status

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Summary

Though dietary factors are implicated in chronic disease risk, assessment of dietary intake has limitations, including problems with recall of complex food intake patterns over a long period of time. Diet and nutrient biomarkers may provide objective measures of dietary intake and nutritional status, as well as an integrated measure of intake, absorption and metabolism. Thus, the search for an unbiased biomarker of dietary intake and nutritional status is an important aspect of nutritional epidemiology. This chapter reviews types of biomarkers related to dietary intake and nutritional status, such as exposure biomarkers of diet and nutritional status, intermediate endpoints, and susceptibility. Novel biomarkers, such as biomarkers of physical fitness, oxidative DNA

damage and tissue concentrations are also discussed.

Biomarkers of nutritional exposure and nutritional status: An overview

Food frequency questionnaires (FFQ), multiple food records, and 24-hour recalls are the most common methods to assess dietary intake in nutritional epidemiologic studies (1). The strengths and limitations of dietary assessment methods, as well as nutritional status biomarkers, are summarized in Table 11.1. Generally, the accuracy of the information collected depends on the ability to integrate complex eating patterns concisely and the subject's memory. Current dietary assessment methods may not completely capture nutrient interactions and

metabolism, as food is a complex mixture; thus, the absorption and metabolism of any single nutrient is affected by the presence of another. For example, iron taken with vitamin C is absorbed more efficiently than by itself, but phytate can bind iron and make it unavailable. Cooking is another important factor that can change concentrations of nutrients or can form compounds not normally present in foods. Obtaining this level of detail using dietary assessment instruments is generally not feasible. Furthermore, food composition tables are not available for all nutrients, limiting the assessment of many of them. Finally, there are numerous nutrients, such as selenium and vitamin D, that cannot be measured adequately in the food source.

Table 11.1. Strengths and limitations of intake assessment methods and nutritional status biomarkers

	Assess by	Limitations	Strengths
Estimate of dietary intake	Questionnaire FFQ Food records 24-hour recall Diet history Food composition table	<ul style="list-style-type: none"> - Prone to different types of bias - Dependent on memory - May not capture variability in eating pattern - does not account for absorption or bioavailability when foods are cooked or eaten as complex mixtures - Not comprehensive especially for diaries and recalls - Focused on specific nutrient - Many newer dietary compounds of interest not covered 	<ul style="list-style-type: none"> - Easier to administer in population-based studies - Long-term intake estimate
Nutritional status	Biomarker of: - absolute intake - correlated intake	<ul style="list-style-type: none"> - Collection - Storage - Sensitivity - Specificity - Laboratory variability - Single measure may not be representative of usual 	<ul style="list-style-type: none"> - Objective measure - Error structure different than questionnaire-based information - Integrated measure

Diet and nutrient exposure biomarkers may be independent of the subject's memory or the capacity to describe foods consumed. Biomarkers may provide an integrated measure of intake, absorption and metabolism, which may improve the accuracy of the estimation of the association between the nutrient and disease, but limit the direct interpretation of the connection between intake and disease. However, since biomarkers may be affected by biospecimen collection methods, storage conditions and laboratory variations, these factors must be carefully considered in the study design.

Types of biomarkers

Exposure biomarkers

Biomarkers of absolute intake: Recovery biomarkers

Biomarkers of absolute intake, or recovery biomarkers, reflect a balance between intake and output over a defined period, with relatively high correlation between the absolute dietary intake and the

biomarker (> 0.8) (2). The two well-studied recovery biomarkers are urinary nitrogen and doubly-labelled water. Urinary nitrogen is an example of a recovery biomarker of protein intake. A 24-hour urine collection is required, and subjects should take para-aminobenzoic acid (PABA) tablets with the three main meals of the day to validate the completeness of the collection (3). The amount of nitrogen recovered in a 24-hour urine collection can be converted to protein intake using estimates of the percent of nitrogen excreted in urine (~81%). Doubly-labelled water is another example of a recovery biomarker for energy expenditure, in which the average metabolic rate of a human is measured over a period of time. A dose of doubly-labelled water, in which both the hydrogen and the oxygen have been partly or completely replaced for tracking purposes (i.e. labelled) with an uncommon isotope of these elements, is administered to the individual. The loss of deuterium and O-18 is then measured over time by regular sampling of heavy isotope concentrations in the body water (by sampling saliva, urine or blood);

the methods used to measure the recovered products are technically challenging (4).

Urinary nitrogen and doubly-labelled water are the only validated recovery biomarkers, but it must still be assumed that the testing period is representative of the subjects' usual habits. The relative complexity and high cost of these methods prevents these biomarkers from being applied to large cohort studies. Thus, these biomarkers are often used as gold standards for validating dietary questionnaires or developing correction factors to estimate measurement attenuation.

Biomarkers of correlated intake: Concentration biomarkers

Biomarkers of correlated intake are based on concentrations in the body (i.e. in blood, urine, saliva, hair, nails or tissue), reflecting current intake status. Concentration biomarkers are correlated with intake, such that higher concentrations of these biomarkers result from higher intake. The measured concentration is a consideration of intake, uptake, and metabolism. Concentration

biomarkers can enhance dietary assessment, or in some cases be the primary method of assessment of nutrient exposure.

Nutrients

This type of biomarker may be used to enhance assessment and measurement of dietary components that are currently captured by dietary questionnaires. Vitamin C is thought to protect against oxidative stress, but assessment of intake is complicated by the varying concentration in foods and the widespread and episodic use of vitamin C supplements. Vitamin C is water soluble and responsive to short-term changes in intake; any single measure of vitamin C may not accurately rank subjects' typical exposure. Because the serum or plasma must be stored using metaphosphoric acid or other preservatives, few epidemiologic studies use vitamin C biomarkers (5).

Vitamin E, especially α -tocopherol, has been the focus of a great deal of scrutiny because of its potential benefits in reducing the risk of cancers and cardiovascular diseases (6,7). The correlation of estimates of vitamin E intake from questionnaires with serum concentrations is highly variable, since most dietary vitamin E is obtained from vegetable oils used in cooking (8) and intake of such oils is not estimated well by food frequency questionnaires (FFQs)(9). For example, the correlation between the FFQ-estimated vitamin E intake and serum α -tocopherol ranged from 0.47 in Dutch men to -0.08 in Italian men in the European Prospective Investigation into Cancer and Nutrition (EPIC) (10). Many studies have found an association between serum α -tocopherol levels and chronic disease risk, but not with dietary estimates of vitamin E.

For example, in the EPIC study, high serum concentrations of α -tocopherol were associated with significantly lower risks of gastric cancer, but estimated dietary intake of vitamin E was not (11).

Biomarkers as the primary method of assessment. This type of biomarker may be used to measure intake for dietary components that are not currently captured by dietary questionnaires. The selenium content of foods is highly dependent on local soil concentrations, which range over several orders of magnitude. Wheat is an important selenium source in many populations, but the selenium content of wheat can vary considerably; therefore, wheat used to produce flour, bread, pasta and other noodles from different geographic areas can result in variable levels of selenium. Several well-established biomarkers of selenium have been developed, including serum and toenail selenium, which provide a valid estimate of selenium status. More than 20 studies have examined the association between serum, plasma or nail selenium and risk of prostate cancer; a meta-analysis concluded that serum and plasma selenium were consistently lower in cases compared with controls (12). Serum and toenail selenium are common validated biomarkers of selenium status (9).

Iron is another example of a concentration biomarker than may better reflect exposure and provide a more informative assessment of the association between iron and disease than intake estimates. Dietary iron is acquired from plant and animal sources, as well as fortified grain in some countries. There are large differences in the bioavailability and absorption pathways of heme and non-heme iron, suggesting that estimating total iron intake will not give a

useful estimate of true exposure. In addition, because menstruation can lead to very different amounts of iron loss in women, the estimation of intake may not be biologically relevant. There are several biomarkers for iron, including serum iron and serum ferritin; both are subject to homeostatic control and influenced by inflammation, respectively.

Vitamin D is a third example of a nutrient that is not well measured by intake estimates. Liver, fatty fish, ergocalciferol in mushrooms, and fortified milk are major dietary sources of vitamin D; however, for most people, the primary source of vitamin D is produced internally upon exposure of the skin to ultraviolet B (UVB). This production depends on the melanin content of the skin and the amount of UVB exposure. Estimating sun exposure is complex, because of differences in time spent outside, amount of exposed skin, weather conditions and sunscreen use. Thus, circulating 25-hydroxy vitamin D is considered a more reliable indicator of vitamin D status, capturing both dietary intake and endogenous production. 25-hydroxy vitamin D has been used in several prospective epidemiologic studies to assess the role of vitamin D in chronic disease prevention (13).

Non-nutritional components

An important aspect of the connection between diet and chronic disease is the assessment of potentially hazardous dietary components. The human diet may contain inadvertent contaminants that are formed during food processing or cooking. Examples of contaminants formed during cooking are heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs). Some developing countries lack an integrated food delivery

system that affords the chance to regulate some undesirable food contaminants, such as mycotoxins or by-products of processing (e.g. silica from grinding grain or nitrosamines in salted fish).

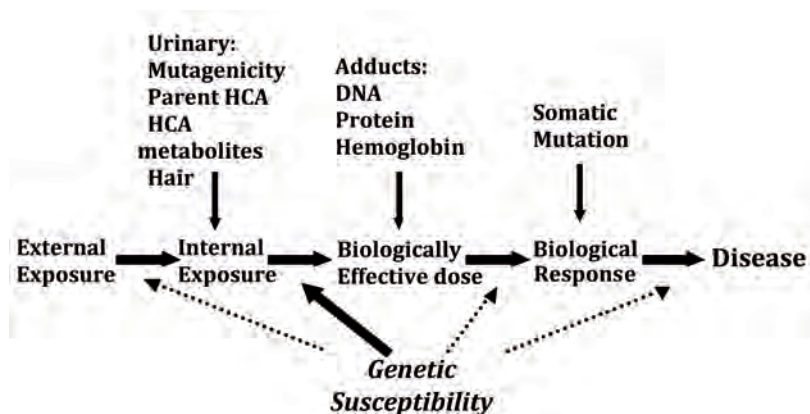
Food-cooking by-products.

HCA and PAHs, both known carcinogens in animal models, are formed in the highest concentrations in meat cooked well-done using high-temperature cooking methods, such as pan-frying or grilling. The assessment of exposure to these compounds can be estimated using questionnaires, but may benefit from the use of biomarkers of exposure. Moreover, there is no national database available for food by cooking methods. A limited database, CHARRED, has been created that is based on the type of meat, cooking method and the degree of doneness (<http://charred.cancer.gov/>).

HCA are formed from the reaction at high temperatures between creatine or creatinine (found in muscle meats), amino acids, and sugars (14–17). HCA undergo extensive metabolism by phase I and II enzymes. Various biomarkers of HCA have been investigated in urine, blood and hair, with each having advantages and limitations.

Urine is a useful biological fluid for the measurement of exposure to various classes of carcinogens, since large quantities may be obtained non-invasively. HCA are rapidly absorbed from the gastrointestinal tract and eliminated in urine as multiple metabolites, with several percent of the dose present as the unmetabolized parent compound within 24 hours of consuming grilled meats. HCA in urine have short half-lives, however, and may not be ideal measures of “usual” intake in etiologic studies, especially if there is substantial day-to-day variability.

Figure 11.1. Potential heterocyclic amines (HCAs) biomarkers (71)



With a large sample size, though, urinary HCAs could still be used to validate intake of HCAs as estimated by questionnaires.

HCA-DNA adducts can be measured in lymphocytes and HCA metabolites bound to circulating blood proteins, such as haemoglobin (Hb) or serum albumin (SA). The measurement of these biomarkers can provide an estimate of exposure and the biologically effective dose, but they do not provide a measure of genetic damage directly in the target tissue. DNA and protein adducts of HCA have been detected in experimental animal models by ³²P-postlabelling. There is a paucity of data on HCA biomarkers in humans, however, as their detection and quantification remains a challenging analytical problem: the concentration of HCAs in the diet is at the parts-per-billion level, and the quantity of HCA biomarkers formed in humans occurs at very low levels. Accumulation of HCAs in human hair, which may serve as a potential long-term biomarker to assess chronic exposure of HCAs, has been suggested but not yet validated (18,19). Similar, but larger, issues exist for PAHs, as these compounds are even more ubiquitous in the food source and environment.

Mycotoxins. Fungal carcinogens are another example of a food contaminant whose study may benefit from the use of an exposure biomarker. Aflatoxin (AFB1) is produced by *Aspergillus flavus* and other related species, and plays an important role in the high rates of hepatocellular carcinoma seen in southern China and parts of Africa (20). Assessment of AFB1 exposure by questionnaire is very limited, as the amount of infection in grain and the amount of toxin produced varies by locality, crop and storage conditions. In communities where most food is grown and stored at home, the ability to develop general exposure metrics applicable to questionnaire data is minimal. Therefore, the development of biomarkers of exposure to these dietary contaminants is critical.

Extensive work on the metabolism of AFB1 led to the identification of AFB1 adducts with DNA and albumin, including AFB1-DNA adducts in urine (21), as correlates of the effective dose of AFB1. Using urinary AFB1-DNA adducts as a biomarker, a nested case-control study demonstrated a 5-fold increased risk of liver cancer in subjects who had measurable levels of these adducts (22). In the same study, dietary aflatoxin intake

was estimated by crossing the concentration of directly measured aflatoxin in food samples and typical intake by questionnaire for each of the contaminated food items. The intake estimates showed no association with risk of liver cancer (22).

Biomarkers of intermediate endpoints

Biomarkers of intermediate endpoints are defined as "...an exogenous substance or first metabolite or the product of an interaction between nutritional exposure and some target molecule or cell that is measured in a compartment within an organism." (23).

Biomarkers of intermediate endpoints have been used extensively to address the association between energy balance and chronic disease. There are multiple serologic indices able to reflect a state of obesity and/or physical activity, including circulating sex steroid and metabolic hormones, as well as inflammatory markers. Serum estradiol levels are higher in obese, compared to lean, post-menopausal women (24). Obesity is also associated with increased levels of adipokines (e.g. leptin and adiponectin), which can be related to insulin resistance (25), characterized by elevated insulin and glucose levels. Inflammatory markers, such as C-reactive protein and adiponectin, are suspected to be on the causal pathway between obesity and chronic disease. Obesity results in excessive production of storage lipids and high circulating levels of glucose, both of which create a proinflammatory oxidative environment (26,27). In addition, individuals who are physically active, after adjustment for body mass index (BMI), have decreased serum estradiol, estrone

and androgens (28,29), and male athletes have low testosterone levels (30). Physical activity can improve insulin sensitivity, and thus decrease insulin levels (31). Proinsulin is enzymatically cleaved into insulin and C-peptide in the pancreas (32), and as C-peptide has a longer half-life than insulin (32), it is a better measure of insulin secretion (33–35). Increased physical activity has been associated with a reduction in inflammatory markers in many studies (36–39).

Other examples of biomarkers of intermediate endpoints include blood cholesterol, which are related to risk of cardiovascular disease and also related to saturated fat intake, and blood pressure, which is related to hypertension and also related to sodium intake.

Biomarkers of susceptibility

Humans have a myriad of enzymes that have evolved to maintain cellular homeostasis, including enzymes that metabolize exogenous environmental compounds and nutrients ingested in food. This metabolism allows the utilization of nutrients and the subsequent detoxification and excretion of potentially harmful compounds and metabolites. The genes encoding metabolic enzymes are polymorphically expressed in humans; molecular biology and enzymology studies have shown that there are many polymorphisms that have a functional consequence for the expressed protein. Therefore, the interaction of genetic polymorphisms with consumed nutrients or with foodborne promutagens could serve to modulate diet-influenced disease etiology. Including genetic heterogeneity may provide a better characterization of nutrient exposure and disease risk relationship. Several areas in which genetics may

influence relationships between diet and disease risk are described below.

While there is limited evidence that fruit and vegetable intake is associated with risk of breast cancer (40), it is possible that this association may differ according to an individual's genetic profile. This is because fruits and vegetables contain compounds that serve to decrease oxidative load; reactive oxygen species are also endogenously generated or neutralized by numerous enzymes. Studies reported that a reduced breast cancer risk was particularly evident in women who had greater fruit and vegetable consumption and were among a subgroup with genetic variants in catalase (rs1001179) (41) and myeloperoxidase (rs2333227) (42), which is related to higher antioxidant capabilities.

A polymorphism of manganese superoxide dismutase (MnSOD) (rs1799725, Ex2+24T > C) in the mitochondrial targeting sequence results in a change of amino acids that is thought to alter antioxidant capacity. In the Physicians Health Study, investigators found that there was a significant interaction between prostate cancer risk, the MnSOD CC genotype, and low baseline plasma antioxidant levels; those with the CC genotype and low antioxidants had almost a four-fold increased risk of prostate cancer (43). These findings are also replicated in the Prostate, Lung, Colorectal and Ovarian (PLCO) study, where the MnSOD variant genotype was associated with increased risk of prostate cancer, particularly among men with lower intakes of dietary and supplemental vitamin E (44).

The Human Genome Project has opened unprecedented opportunities to comprehensively investigate inherited genetic

variations. Ongoing work is exploiting these opportunities through the National Cancer Institute's Cancer Genetic Markers of Susceptibility (CGEMS) to characterize vitamin D/calcium-related pathway genetics and prostate cancer risk in the PLCO trial. Several single nucleotide polymorphisms that predicted serum 25(OH)D concentrations were identified (45). Studies of genetic variants that determine serum micronutrient concentrations, as well as adiposity and height, are ongoing with genome-wide scan data (46–52). Exploiting this information would provide a more coherent measure of chronic disease risk associated with dietary and nutrition exposures. Also, individualising dietary recommendations necessitates a detailed understanding of all genetic and physiological variables that influence the interaction of gene-diet and their relation to disease process. Thus, the potential benefits of understanding the interrelationships between genetic variation and nutrition are enormous.

Novel biomarkers

Physical activity as measured by markers of physical fitness

There has been a great deal of effort to expand studies of energy balance and its role in health and disease using both estimates of caloric intake and BMI. Recently, more attention has been paid to the other side of the energy balance equation, namely physical activity. Assessment of physical activity has primarily used questionnaire assessments of physical activity at work, during leisure time, and increasingly, activities of daily living (housework, etc.). Although physical fitness can be measured using factors such as resting pulse

or aerobic capacity, this captures neither the amount of energy expended by a subject nor the amount of low-intensity activities, which may have health benefits (53). Epidemiologists are starting to use accelerometers to accurately capture activity over a test period. However, further work will be required to deploy these devices on a large scale; measurements will be restricted to a small number of days, and for etiologic studies this could only be meaningful in prospective studies.

Alternatively, biomarkers of effect have been examined to explore physical activity hypotheses. For example, one hypothesis for the protective effect of physical activity on breast cancer is the alteration of circulating hormone levels (54). Post-menopausal women with lower serum levels of sex steroid hormones have lower risk of breast cancer. Physical activity may lower these serum concentrations, but whether this is dependent on greater physical activity leading to lower BMI is unclear. Because adipose tissue is an important source of sex steroid hormones in post-menopausal women, determining whether the effect of physical activity on breast cancer risk is independent of BMI will require careful evaluation of serum sex steroid levels to assess the mechanism of action (54). Assessing the other potential mechanisms of action for the association of physical activity and cancer will require the use of molecular markers for inflammation and immunity (53).

Oxidative capacity of diet as measured by markers of oxidative DNA damage

The impact of dietary antioxidants on the incidence of cancer has been widely studied by assessing

intake using FFQs or food records, as well as by status biomarkers, such as serum vitamin measures. An alternative biomarker strategy is to measure the amount of oxidative stress in an individual with a biomarker that integrates antioxidant intake, oxidative stress from exogenous and endogenous sources, and individual response to this stress (genetic and epigenetic factors). Oxidative stress can lead to modification of DNA nucleotides. The DNA adduct 7,8-dihydro-8-2'-deoxyguanosine (8-oxodG) has been widely used as a marker of oxidative stress (55). Direct measurement of this DNA adduct in peripheral blood mononuclear cells or urine reflects the sum of oxidative damage and the repair of this damage. Oxidative stress can also lead to the oxidation of thymine and the formation of 5-hydroxymethyl-2'deoxyuridine (HMdU). This adduct is immunogenic, and autoantibodies against it can be monitored as a marker of oxidative stress (56). Several studies have investigated the responsiveness of these markers to dietary intake and modification (57,58). Observational and intervention studies suggest that diet can significantly modify the amount of oxidative stress as measured by the DNA modification markers. Moreover, these markers can be employed directly, or alternatively used in conjunction with a diet/behaviour index. For example, the concentration of 8-oxodG could be measured in a subset of a cohort, and the dietary and other questionnaire data examined to build a predictive model for this oxidative stress marker. If a sufficiently powerful model can be built, it can then be used to examine the association between the index and the disease in the full cohort. This may be a more powerful technique for the integration of antioxidant and

prooxidant exposures than is an index that arbitrarily assigns points based on median splits of intake for purportedly antioxidative foods or nutrients.

Exposure status as measured by tissue concentrations

Most previous biomarker studies of nutrient status or carcinogen exposure have used easily accessible biological compartments (e.g. serum, urine, hair or nails) to assess the association between exposure and cancer risk. Recently, the developing interest in molecular epidemiology has provided the impetus to use tissue banks to provide measures of exposure directly in the target tissue.

Large numbers of studies have examined the association between nutrient intake and the risk of disease. Some nutrients such as trace elements or minerals, however, are not amenable to intake estimation. For example, meaningful estimates of zinc intake are difficult because the bioavailability varies strongly with the other dietary constituents in the same meal; phytate from whole grain can almost completely block zinc absorption. Also, serum zinc may not be a sensitive indicator of status, because serum zinc concentration is under tight homeostatic control. Therefore, an alternative method has been devised whereby the concentration of zinc in the target tissue of interest is measured directly (59). This uses a sensitive technique to measure the zinc concentrations in the biopsy tissue directly, thus giving a clearer assessment of the importance of the element in the studied tissue.

An alternative use of tissue biomarkers is to directly assess the exposure to carcinogens that is derived from the diet. Antibodies against the adduct created when

activated benzo[a]pyrene interacts with DNA have been used to assess the association between PAH exposure and breast cancer using breast biopsies. Using an immunohistochemical assay, an association was found between PAH-DNA adducts in breast tissue and the risk of breast cancer (60), but this work requires careful interpretation (61).

Studies using target tissue may be restricted to easily or routinely biopsied organs, such as those often biopsied during screening exams or positive exam work-ups (e.g. colon, prostate or breast). The direct assessment of nutrient status or carcinogen exposure in the target tissue may lead to a clearer understanding of the nutrient or exposure in the disease process.

Urinary mutagenicity

A urinary mutagenicity test using *Salmonella typhimurium* indicator strains (Ames test) has been used to monitor populations occupationally or environmentally exposed to genotoxic compounds (62). Genotoxic compounds in the diet may originate from contaminants in the food chain or from by-products of food preparation; for example, the urine of individuals who consumed well-done meat can be highly mutagenic (63). Mutagenic activity of the urine is substantially increased when the urine is acid-hydrolysed. Mutagenicity of unhydrolysed urine likely reflects excretion of unmetabolized mutagens, whereas the mutagenicity of hydrolysed urine reflects the excretion of both metabolized and unmetabolized mutagens. Other dietary components, such as cruciferous vegetables or parsley, may decrease urinary mutagenicity by enhancing the level of conjugation.

Current challenges and future directions

Dietary assessment

Food frequency questionnaires (FFQs) are the main dietary instrument used by nutritional epidemiologists, but in recent years this method has become controversial. Whether or not it is time to abandon the use of FFQs has been discussed (64,65). The inconsistencies in diet-disease associations observed in epidemiologic studies have been highlighted. Further emphasized were results from a methodologic study that used doubly labelled water as a gold standard for energy intake and urinary nitrogen for protein intake (4). Both energy and protein estimated by the FFQ were measured very poorly. The authors also argue that the associations observed using dietary biomarkers and food diaries are not detectable when FFQs are used (66,67). These assertions have been questioned, and it has been stated that some inconsistencies are to be expected in an area as complex as diet both due to chance and real biological interactions (68). The authors further assert that when large numbers of studies have been pooled the data are consistent, and the ability of the doubly-labelled water study to measure within-person variability has been questioned (68). The association between fat and breast cancer using food records has been seen in two cohort studies, one in the United Kingdom and the other in the Women's Health Initiative (69), in contrast to the null results using a FFQ (70). Further discussion must be undertaken to decide how to estimate dietary intake. For example, are all foods and nutrients substantially misclassified as observed for energy and protein

intake? Should we use other forms of dietary instruments or possibly a combination of instruments? It is, however, crucial that dietary intake be estimated with less error if we are to correlate intake to a biomarker. An automated, web-based FFQ or 24-hour recall is currently being developed, and these new tools may help to improve dietary assessment.

Dietary biomarkers

There are several important issues that need consideration when deciding whether to use dietary biomarkers in an epidemiologic study. The application of dietary biomarkers is most likely to be useful in prospective cohort studies, as the biological samples will be collected and stored before the clinical manifestation of the disease.

In general, there are limited types of biospecimens that are easily available and can be used for measuring nutrients such as blood, urine, hair, nail, faeces, saliva, and tissue biopsies. These may not be specimens from the organ or site of interest; for example, fat soluble vitamins are stored in the liver, adipose tissue, cell membrane, and with smaller amounts in blood components.

Sample collection and storage of biospecimens in an appropriate manner is crucial for nutritional biomarkers. Certain nutrients must be collected under specific conditions (e.g. trace mineral-free tubes for zinc). Zinc contamination can be in dust, thus stringent laboratory conditions must be used to measure this mineral in biological

samples. Other nutrients need to be stored with preservatives to maintain their integrity, such as vitamin C, which needs to be preserved with metaphosphoric acid. Such stringent control may not be a problem in smaller studies with targeted hypotheses, but it can become a constraint in large, prospective studies with competing interests and limited amounts of biological material.

Measurement of nutritional biomarkers in repeat samples is important for many dietary components that have short half-lives, such as water-soluble vitamins and meat-cooking carcinogens. Therefore, it is preferable to have biological specimens from multiple days to derive an estimate of usual nutritional status. A related issue to a short half-life is the need to collect fasting samples as certain nutrients respond with a postprandial spike for several hours. It is important to take into consideration the metabolism of the nutrient with the study aims and design.

The data generated from the Human Genome Project offer great opportunities to utilize genetic information. This rapidly expanding technology will provide valuable information to help understand disease etiology in a comprehensive way, but it will also provide a formidable challenge in design, analysis and implementation of molecular epidemiology studies. The application of molecular epidemiology to nutrition and disease prevention is in its infancy. Further investigations of diet, genetic variability, and disease risk will better

elucidate the complex relationships between diet and disease risk, and support recommendations for healthful eating.

Conclusions

This chapter reviews types of biomarkers related to dietary intake and nutritional status. Exposure biomarkers include biomarkers of absolute intake and correlates of intake. Absolute intake biomarkers are often thought of as a gold standard to validate dietary questionnaires, with relatively high correlation with dietary intake. Concentration biomarkers reflect intake status, with moderate correlations in relation to nutritional (e.g. serum vitamin D, serum vitamin E, and toenail selenium) or non-nutritional components (e.g. food-cooking by-products, and mycotoxins). Intermediate biomarkers of biologic effect have been used extensively to address the association of surrogate endpoints of nutritional exposures. Biomarkers of susceptibility often include genetic heterogeneity, which may help better characterize nutrient exposure and disease–risk relationship. Novel biomarkers, such as biomarkers of physical fitness, oxidative DNA damage, and urinary mutagenicity, have been developed in this rapidly growing field. As analytic methods improve and more biochemical indicators are validated as measures of dietary intake, their use in nutritional epidemiology is likely to expand.

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