Biomarkers as Endpoints in Intervention Studies

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20.1. INTRODUCTION: WHY ARE BIOMARKERS NEEDED IN INTERVENTION STUDIES?

The methods of molecular epidemiology can be applied in proving the efficacy of preventing hazard exposure, or intervening in the development or progression of a disease. A public health intervention would involve reducing exposure to a potentially hazardous material, often through identifying points of unintentional exposure through occupational monitoring of the environment or personnel. Where such exposures have occurred, chemoprevention approaches may provide an intervention with chemical agents, including minor dietary constituents and/or pharmaceuticals, to halt or slow the disease process. It is essential to prove the efficacy of either type of intervention.

The most desirable intervention study design is a double-blinded, randomized, placebo-controlled clinical trial that directly measures protection against the disease of interest. The definitive proof that any dietary or pharmaceutical intervention prevents disease and/or enhances some physiological process is provided by feeding or administering the test substance at a given level for sufficient time to enable the disease process to occur in the absence of the substance. In practice, however, such a model is almost impossible to attain in most intervention studies, in part because of the length of time that most diseases take to develop. For example, cancer is known to show a 20–30 year lag between disease initiation and development, and the possibility of attaining compliance in a dietary or pharmaceutical trial over such a long period is completely unrealistic. Phase III trials, with cancer reduction as the endpoint, require tens of thousands of subjects, followed-up for times of 5 years or more (Kelloff et al. 1992). For this reason, the Chemoprevention Branch of the National Cancer Institute (NCI) have focused a significant part of their efforts on finding surrogate endpoints (biomarkers) with high reliability and predictive value for cancer. The NCI and other US agencies convened a workshop on ‘Biomarkers as indicators of cancer risk reduction following dietary manipulation’ (Kavanaugh et al. 2006; Prentice 2006; Schatzkin 2006; Ferguson et al. 2006) that summarizes current views on many of the issues associated with using biomarkers as endpoints of intervention studies.

A biomarker has been defined as ‘a measurable, biological parameter that predicts the risk of human disease, disorders or conditions, but is not a measure of the disease, disorder or condition itself’ (Rothman et al. 1995), i.e. biomarkers serve as early indicators of disease in asymptomatic people. They have been used to assess exposure to potential environmental hazards, to gain insight into disease mechanisms and to understand acquired or inherited susceptibility. Thus, biomarkers cover the whole spectrum from hazard exposure to disease pathogenesis. Their successful application to intervention studies requires an understanding of disease natural molecular Epidemiology of Chronic Diseases, Edited by C. P. Wild, P. Vineis, and S. Garte
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history, the mechanism of the intervention and the characteristics and limitations of the biomarker. Schatzkin et al. (1993) have distinguished biomarkers from surrogate disease endpoints as follows:

Whereas any biologic phenomenon can be considered a biomarker, an intermediate end-point is defined as being on the causal pathway between exposure and disease. An intermediate end-point is a valid surrogate for a disease in relation to a given exposure if, and only if, that exposure causes a similar change in the occurrence of both the intermediate end-point and the disease.

The acceptable endpoint of an intervention study might be a single biomarker, or batteries of different markers. In the case of cancer, the NCI’s stated criteria (Kelloff et al. 1992) include:

- Differential expression in normal and high-risk tissue.
- Appearance early in carcinogenesis (the earlier a reliable biomarker appears, the greater is the chance for successful intervention with a chemopreventive agent).
- High sensitivity, specificity, and accuracy relative to cancer.
- Ease of measurement (use of non-invasive techniques and small tissue samples is preferable).
- Demonstration of modulation by chemopreventive agents.
- Correlation of modulation with decreased cancer incidence.

The same principles also apply to the use of biomarkers in proving efficacy of protection against other diseases.

Convenience is not the only reason for using biomarkers as the endpoint of a clinical trial. Taking a study to a disease endpoint may be unethical, since no hypothesis is foolproof, and there is a small but finite possibility that it could be wrong. This is illustrated vividly by the β-carotene trials (Box 20.1). The rationale for suggesting β-carotene was mainly based on related high-risk populations with low β-carotene levels in the USA (O’Keefe et al. 1996). However, the studies based on the USA populations often showed benefits against other diseases. In this trial, the study endpoint was to measure the incidence of a disease linking the population being studied with that disease. The reduction in incidence of, or mortality from, chronic disease without knowing the mechanism of action. Indeed, it was suggested that trials would help elucidate possible mechanisms. The strongest argument for early intervention trials was that if such studies were delayed until the major research questions were answered, it would be unethical to deny the control group the intervention or difficult to find a control group because of widespread antioxidant consumption.

In the event, these trials generally had negative endpoints. While a large number of possible explanations have been attempted, the observations generally provide some lessons. Although the use of high-risk groups appeared justified, it may have been part of the problem. The population groups chosen were either heavy smokers or asbestos-exposed workers, i.e. they were groups that had already been exposed to high risk and likely to have already been experiencing early disease processes. It is plausible that β-carotene or other antioxidants could have been beneficial in other populations in which disease had not already been initiated, i.e. at a very early stage of the disease process (the normal population). If biomarkers had been used, the studies could have been aborted at an earlier stage.

Kavanaugh et al. (2006) detail a research agenda for optimizing the use of biomarkers in subsequent cancer prevention trials. Similar considerations also apply to other disease endpoints.

Box 20.1 Lessons from the β-carotene trials

It is instructive to consider what has been learned from largely negative trials with disease endpoints, such as those with β-carotene (Altmann et al. 1996; Omenn et al. 1996). In these examples, the investigators chose high-risk populations to increase the probability of disease occurrence or recurrence in the study group during a short intervention. There appeared to be several advantages to this. Not only did such an approach reduce study numbers and time of study (and therefore costs), it was thought to enhance the quality of the study by making it more likely that there would be continuity of personnel involved in the trial. However, because there was little understanding of the mechanism by which β-carotene was likely to act, biomarkers could not be rationally selected as intermediate endpoints. It was considered sufficient to show the efficacy of the intervention by proving a reduction in incidence of, or mortality from, chronic disease without knowing the mechanism of action. Indeed, it was suggested that trials would help elucidate possible mechanisms. The strongest argument for early intervention trials was that if such studies were delayed until the major research questions were answered, it would be unethical to deny the control group the intervention or difficult to find a control group because of widespread antioxidant consumption.

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for suggesting that dietary supplementation with β-carotene would benefit population health was mainly based upon epidemiological studies that related high plasma β-carotene levels in individuals with low disease risk in the same individuals. However, the endpoint of two major intervention studies based in Europe (Albanes et al. 1996) or the USA (Omenn et al. 1996) was that the interventions enhanced, not reduced, the risk of the diseases that they had been designed to protect against. Box 20.1 discusses the issues uncovered in this trial, and its aftermath.

In practice, experimental studies with disease endpoints are less common than experimental studies linking the test intervention to surrogate disease outcomes.

20.2. IDENTIFICATION AND VALIDATION OF BIOMARKERS

If biomarkers are to provide an effective index of efficacy, they must provide a quantifiable index of reduction in hazard exposure, disease initiation or progression. This implies their being able to measure responses across the range of intakes being studied, being able to be measured with precision and high sensitivity, and being applicable to the population group being studied. For example, Alberts et al. (1994) considered rectal mucosal proliferation indices as biomarkers for interventions, but the protocols they developed are applicable to other biomarkers. They emphasized the importance of ongoing quality control/quality assurance programmes in order to achieve high accuracy and reproducibility with minimal variability, and outlined a series of steps that can be followed in the validation process. Validation of a given biomarker is essential if it is to provide meaningful information in intervention studies. A valid biomarker in this context is one that allows the correct inference to be drawn regarding the effect of an intervention on the true clinical endpoint of interest.

Schatzkin et al. (1990) developed protocols for establishing whether a given biomarker is a valid intermediate endpoint between exposure to a hazard and disease incidence. They recommend using prospective cohort or, if these are not available, case-control studies, in order to quantify the strength of the biomarker–disease association. The biomarker provides a valid disease surrogate if the attributable proportion is close to 1.0, but not if it is close to 0. In most examples where the attributable proportion is in between 1.0 and 0, the intermediate endpoint must reflect an established exposure–disease relationship.

The importance of biomarker validation is illustrated by the failure of a putative colorectal cancer-predictive endpoint, the aberrant crypt (Hardman et al. 1991). This measure had been used as an endpoint of a number of animal studies, especially in the 1980s, and it appeared to be true that dietary components that increased aberrant crypts also enhanced colon cancer incidence in longer-term studies. However, it did not provide an accurate measure of intervention. Hardman and co-workers compared seven different dietary regimes for their ability to modulate colon carcinogenesis in Sprague–Dawley rats by the known colon carcinogen 1,2-dimethylhydrazine. If the studies had only progressed as far as aberrant crypts, they would have falsely predicted the ability of the different diets to protect against colon carcinogenesis.

20.3. USE OF BIOMARKERS IN MAKING HEALTH CLAIMS

An appropriately designed intervention study can be used as the basis for a health claim, and this is often the main reason that such studies are done. While the exact basis for such claims differs between geographical regions, there are some general principles that govern the weight attached to individual studies. For example, the Scientific Advisory group of the Australia/New Zealand Food Safety Authority (FSANZ 2006) considers health claims for foods or dietary regimes, and has stated:

Assessment of the quality of primary evidence includes (but may not be limited to) assessment of the following elements:

- The completeness and appropriateness of the described methodology.
- Appropriate and accurate description and quantification of exposure to the diet, food or food component.
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- Appropriate and accurate quantification of the health-related outcome.
- Sample size.
- Sample and measurement bias.
- Potential confounding variables.
- Inclusion of appropriate controls.
- Study duration.
- Appropriate statistical methods.

The second and third of these may involve surrogate outcomes or biomarkers. Biomarkers used in studies relating diet, food or food components to a health outcome include serum lipid levels, blood pressure, bone mineral density and adenomatous polyps. While either a disease endpoint or a surrogate outcome is adequate as a study endpoint, it is essential that the outcome measured is one relevant to the study hypothesis. Studies using biomarkers are only useful where there is a well-accepted and validated predictive relationship between the biomarker and the health or disease outcome, and the biomarker is biologically plausible.

20.4. BIOMARKERS OF STUDY COMPLIANCE

In considering the quality of evidence provided by a given trial, one of the most important questions to be asked is whether the study participants adhered to the intervention throughout. There is good evidence that simply asking the participants this question will not necessarily lead to the correct answer (Eliassen et al. 2006), stressing the need for biomarker validation of compliance to interventions, especially when comparing populations of different ethnicity. In pharmaceutical trials, compliance may be assessed by checking medicine cabinets, etc., to see whether there is remaining medication. In dietary trials, compliance may be more difficult to assess, and biomarker approaches become important. Compliance may need to be measured at several different stages in the study, especially in longer-term studies where dietary patterns may change during the duration of the trial.

Biomarker-mediated indices of exposure are commonly performed in industrial monitoring to provide indices of risk. For example, Waidyanatha et al. (2001), used headspace solid-phase microextraction of 0.5 ml urine specimens, followed by gas chromatography–mass spectrometry, to provide an accurate measure of urinary benzene to serve as a biomarker of exposure among benzene-exposed workers and unexposed subjects in Shanghai, China. However, studies of this nature imply a large amount of prior information on the chemistry of the material to which the group is exposed, dose–response relationships, and the level of risk provided by the material in question, i.e. benzene.

In the context of an intervention study, it is not risk per se but reduction of risk that is important, and the quality of the data is dependent not only upon study compliance but also upon the material reaching its biological target.

Designing a package of biomarkers may be especially important when considering a dietary regime, where more than one component may be mechanistically involved in the desired biological effects. For example, Fowke et al. (2006), were looking to show protection against cancer by Brassica vegetables. As they pointed out that, not only do these vegetables contain micronutrients that may scavenge free radicals, they also contain isothiocyanates (ITC) and indoles (e.g. indole-3-carbinol) that induce Phase I and Phase II enzymes, leading to the oxidation, reduction and metabolism of endogenous and exogenous carcinogens. The authors performed a randomized cross-over trial of 20 subjects, comparing the effects of a Brassica vegetable intervention with those of a micronutrient and dietary fibre supplementation. They considered the effects of the interventions on urinary excretion of F2-isoprostanes (F2-iP), as a stable biomarker of systemic oxidative stress. They not only estimated Brassica intake by repeated 24 h dietary recalls and food frequency questionnaire methodology, but also checked the accuracy of the information they were given by comparing the values thus obtained with dietary intakes estimated through urinary ITC levels. In this example, they were able to show that the group on the Brassica intervention arm were excreting more ITC in the urine than the other experimental group, from which they implied (as required) a higher Brassica intake.

Martini et al. (1995) did controlled feeding studies that validated plasma α-carotene, β-carotene, and lutein as foods in gene ker of Brassica family to moni tor and distinguish general approaches in monitoring higher concentrations of plasma α-tocopherol and β-carotene, as well as other tissue carotenoids. Accumulation of these lipids in tissues is generally assessed by a combination of biochemical and metabolic assessment of plasma levels (Handelman et al. 2006). Other biomarkers of interest include in diet and in blood, such as vitamin A and carotenoids, fatty acids, and others. The most appropriate biomarker for monitoring compliance is often the measure of exposure or biomarker activity that is the most accessible and may be easily determined.

Other biomarkers considered include DNA, RNA, proteins, microRNAs, and epithelial cells. For example, DNA methylation and miRNA expression have been used to assess diet and lifestyle exposures. DNA methylation is a reversible chemical modification of DNA that can be used as a biomarker of exposure to environmental chemicals, such as benzene. miRNA expression has been used to assess exposure to environmental chemicals, such as benzene, and to assess the health effects of exposure to these chemicals.

In conclusion, biomarkers are important tools for assessing and monitoring exposure to environmental chemicals, but they must be carefully selected and validated for each specific study and chemical. The choice of biomarkers should be guided by the study hypothesis, the study design, and the available knowledge about the chemical and its metabolism. Biomarkers should be validated for both accuracy and precision, and the results should be interpreted with caution, taking into account the limitations of the biomarkers and the study design.
and lutein as biomarkers of intake of carotenoid-rich foods in general, and lutein as an intake biomarker of commonly consumed vegetables in the *Brassica* family. Their methods allowed them not only to monitor intake of some plant foods, but also to distinguish among plant food groups. This general approach has been optimized to distinguish multivitamin intake, as reflected by significantly higher concentrations of plasma retinol, β-carotene and α-tocopherol, from high fruit and vegetable intake, resulting in higher lutein and zeaxanthin and β-cryptoxanthin concentrations (Eliassen et al. 2006). It should be noted, however, that high concentrations of carotenoids in plasma do not necessarily provide an indication of tissue levels, since some tissues will selectively accumulate certain carotenoids. For example, lutein and zeaxanthin accumulate at high levels in the retina of the eye (Handelman et al. 1988).

Other biomarkers that enable an objective assessment of nutrient consumption include energy expenditure, urinary nitrogen, selected fatty acid measurements and various blood micronutrient concentrations (Prentice et al. 2002). A measure of levels of the material in either urine or plasma may not be as useful as an index of a biological effect. For example, Baylin and Campos (2006) compared the various types of fatty acid biomarkers that have been used to assess compliance in dietary lipid intervention trials. They concluded that fatty acids in adipose tissue provide a biomarker of choice for long-term intake studies, but there are practical limitations to the tissue’s accessibility and measurement, and the measure does not reflect a short term outcome. They were unable to distinguish the desirability of plasma vs. erythrocytes on the basis of the data available to date. The type of study (short- or long-term), the metabolic characteristics of the population group being studied and the probable variability in the fatty acids of interest will help in the selection of the most appropriate tissue to reflect the true intake. If the study design is comparing groups with differing isocaloric intakes, certain fatty acids may not be appropriate for use, especially when trying to assess small differences. Serum cholesterol ester may be the most appropriate serum fraction for measuring short-term but not long-term dietary compliance.

### 20.6. BIOMARKERS RELEVANT TO MORE THAN ONE DISEASE

**Oxidative stress**

The notion of disease prevention through antioxidant intervention partly stems from the fact that fruits and vegetables contain antioxidants, and are linked to lower disease rates in those who consume high levels of them. Protection against DNA damage by plant food products can be demonstrated *in vitro*. Dalle-Donne *et al.* (2006) claim...
Box 20.2 Steps in designing appropriate biomarkers for intervention studies

The examples are taken from a series of studies on the possibility of Oltipraz protection against liver cancer in Qidong, People's Republic of China (Kensler et al. 2005; El-Nezami et al. 2006; see also Chapter 25 for further details).

1. Establish what type of disease an intervention might protect against, and what population would be most responsive. In the examples provided here, liver cancer is known to be a significant risk factor for the population, and there is good evidence linking it to aflatoxin B exposure (Kenzler et al. 1998).

2. If the causal factor in high disease risk is known, establish how this is working and whether there is a good animal model on which preventive hypotheses can be tested. Aflatoxin B1 is known to act as a tumour initiator through the formation of DNA adducts in both animals and humans. Cancer risk in male F344 rats has been shown to relate to the formation and disappearance of aflatoxin–albumin adducts of rats chronically exposed to aflatoxin B1 (Egner et al. 1995).

3. Check what tissue is easily collected and reflective of the disease process in the animal model. The urinary excretion of aflatoxin B(1)-N(7)-guanine (AFB-N(7)-guanine) reflects tissue levels of this important DNA adduct in the rodent (Egner et al. 1995). Additionally, serum aflatoxin adduct biomarkers complement this urinary information.

4. Ensure that this same biomarker will be also appropriate in humans. Both serum aflatoxin–albumin adducts and urinary aflatoxin metabolites have been associated with increased liver cancer risk in prospective studies (Wild and Turner 2002).

5. Consider what type of intervention might reduce the formation of this lesion. In the Oltipraz trials, there was good preliminary data that this chemical modulated enzyme induction. In animal models, it reduced the risk of cancer through aflatoxin B1 exposure, and this chemoprevention effect was mimicked by enhanced excretion of urinary aflatoxin metabolites or by serum aflatoxin–albumin adduct biomarkers (Egner et al. 1995).

6. Once the scientific basis for an intervention is established and a biomarker validated, human studies can proceed. Kenzler and co-workers (2005) have extensively described the results of their Oltipraz chemoprevention trials in this high-risk human population in China.

that, because oxidative stress markers can be measured accurately and objectively, they provide valuable indicators of responses to therapeutic interventions. Sies (1997) defined oxidative stress as a ‘disturbance in prooxidant-antioxidant balance in favor of the former, leading to potential damage’. High levels of oxidative stress contribute significantly to the age-related development of some cancers through DNA damage, while lipid peroxidation plays a role in the development of cardiovascular disease. In diabetes, hyperglycaemia leads to the autoxidation of glucose, glycation of proteins and the activation of polyol metabolism. These changes accelerate oxidative stress, which may play an important role in the development of complications in diabetes, such as lens cataracts, nephropathy and neuropathy (Osawa and Kato, 2005). Protein carbonyl content has been used as a general biomarker of severe oxidative protein damage. High levels of protein carbonylation are associated with various human diseases, including Alzheimer's disease, chronic lung disease, chronic renal failure, diabetes and sepsis (Dalle-Donne et al. 2006).

More generally, biomarkers of oxidative stress have been used as an index of susceptibility to degenerative disease, as a means of studying mechanisms of action for certain chemopreventive agents and of defining the optimal intake of those antioxidants (Ferguson et al. 2006). (Halliwell 2002) points to mass spectrometric measurements of various families of isoprostanes (F2-, F3- at DNA base o promising bi intervention (2006) used stable biom studies com nutrient/diet urine sample each interve transformed repeated m F2-1P lev intervention and other di There ha validity of of action bi and tissue n have been u disease. MI the oxidati acids with i been conc Bowen and problems w dency to bi acids, there tissues and MDA is nc react with and it for as ribose, technical to modify design of t standardizt endpoint.

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Against liver injury; see also Kenzler et al. (2006). Cancer aflatoxin-adducted liver. The altered modulation effect on the own to act as in rat praz trials, animal models of aflatoxin-bis, human lipoxygenase of severe neuropathy of the proteinous human diabetes and obesity. The estimates of background levels of DNA oxidation in human cells range over three orders of magnitude, depending on the method used. In part, this is because oxidation occurs readily during sample preparation, creating a serious potential artefact (Collins 2005). Nevertheless, using validated, reliable biomarker assays for DNA oxidation, it is possible to demonstrate a decrease in oxidative damage after supplementation with isolated antioxidants or whole plant foods in humans. A number of bioactive compounds that function in disease prevention commonly act as antioxidants or show ability to quench singlet-oxygen. It should be recognized, however, that this does not necessarily mean that they protect against disease through their antioxidant properties, since many such compounds may enhance immune response, promote gap junction communications, modify nuclear DNA signalling and modify production of eicosanoids (Ferguson et al. 2006).

Inflammation

Inflammation is an important contributor to atherothrombosis. The C-reactive protein (CRP) is not only a biomarker of inflammation, but it is also a direct participant in atherogenesis. CRP consistently predicts new coronary events, including myocardial infarction and death, in patients with ischaemic heart disease. CRP and other markers of inflammation may also be useful in the diagnosis and management of childhood asthma (Li et al. 2005).

Cancer

Various biomarkers have been suggested to relate to the risk of certain cancers. As with other diseases, the closer to the formation of a frank tumour, the more likely is the marker to be informative. For example, the likely number of new cases of colorectal cancer has been estimated through measurement of numbers of adenomatous polyps as a biomarker. However, even estimating such polyps is invasive, labour-intensive and costly. Measurements of recurrence of adenomatous polyps over short time periods can be inaccurate because of a large potential rate of missing small adenomas. More importantly, they may not provide an accurate biomarker, since not all polyps progress to form cancers (Wargovich 2006). It should also be recognized that a chemopreventive intervention may be affecting adenoma development, rather than adenoma formation, and a negative answer in a chemoprevention trial that estimates numbers of new polyps could be a false-negative result.
In 1992, Rozen considered measurement of rectal epithelial proliferation as a biomarker of risk for colorectal neoplasia, and response in intervention studies. The justification for using this marker is that a phase of epithelial hyperproliferation typically precedes frank colorectal carcinogenesis. Although the marker seemed of some value in experimental studies, it has been less conclusive in humans. Part of the reason is technical, including difficulties in obtaining tissue samples and sampling error, the type of labelling technique used, lack of an objective method for quantifying proliferation and confounding factors influencing the degree of proliferation. Schatzkin (2006) also pointed out that rectal epithelial proliferation is not necessary for colorectal cancer development, and there may be alternative pathways that bypass this, such as an apoptotic pathway. An agent that reduces hyperproliferation will only be likely to reduce cancer, provided that it does not also reduce apoptosis.

Environmentally induced lung cancer has been identified as a serious problem in industrialized nations, and polycyclic aromatic hydrocarbons (PAHs) and related compounds recognized as lung carcinogens. Because the disease is invariably fatal, lung cancer prevention has been seen as an important target for intervention. Talaska et al. (1996) describe optimization of methods for collecting cells from bronchial-alveolar lavage or sputum, and validation of measures of carcinogen–DNA adduct levels in the lung-derived cells thus available. In practice, however, it has been more common and less invasive to determine levels of the marker in surrogate molecules, such as protein, or markers from surrogate tissues, such as lymphocyte DNA, to assess the risk to the target organ, the lung.

Prostate-specific antigen (PSA) is the most commonly used biomarker in prostate cancer, although other measures have been considered for use. Collette et al. (2005) questioned whether PSA could be considered a valid surrogate endpoint for survival in hormonally treated patients with metastatic prostate cancer. Their meta-analysis used data from 2161 patients with advanced prostate cancer, treated either with bicalutamide or with castration. Endpoints studied were PSA response, PSA normalization, time to PSA progression and longitudinal PSA measurements. They were able to show that PSA was useful at the individual patient level, but that the correlation between the effect of intervention on any PSA endpoint and overall survival was generally low. They thus concluded that the effect of hormonal treatment on survival cannot be accurately predicted from observed treatment effects on PSA endpoints. This emphasizes the fact that, although a marker is commonly used as a study endpoint, this does not necessarily imply that it has been validated.

DNA adducts are well recognized as a biomarker of exposure to various environmental, life style or occupational chemical carcinogens that have been shown to be modified through various interventions (Santella 1997). For example, administration of chlorophyllin three times a day led to a 50% reduction in the median level of urinary excretion of aflatoxin-N(7)-guanine compared to placebo (Egner et al. 2003). This excreted DNA adduct biomarker is derived from the ultimate carcinogenic metabolite of aflatoxin B(1), aflatoxin-8,9-epoxide, and is associated with increased risk of developing liver cancer in prospective epidemiological studies.

While a decrease in urinary DNA adducts would seem to be a desirable endpoint of intervention studies, implying reduction of exposure, it is also important to realize that urinary DNA adducts are a product of a DNA repair process. While a reduction in these levels may indeed imply an effective reduction in carcinogen exposure, the same result could be achieved by the undesirable endpoint of similar exposure levels, coupled with reduced DNA repair activity. It may be more appropriate to measure adducts levels in cells. For example, DNA adducts of various industrial carcinogens and oxidative DNA damage can be measured in blood mononuclear cells and exfoliated oral and bladder cells from human subjects (Egner et al. 2003). Such DNA damage may also be implied by a measure of DNA breakage, such as the single cell gel electrophoresis or COMET assay (Karunasinghe et al. 2004).

Other biomarkers suggested for cancer include genomic markers, such as micronuclei and specific chromosomal alterations (Bonassai et al. 2007).
Specific genetic markers include oncogenes, growth factors and their receptors, and tumour suppressor genes, such as the ras gene family, the myc family, erb B1, int-2/hst-l and the p53 tumor suppressor gene. Squamous cell differentiation markers include keratins, involucrin and transglutaminase 1, while rectal epithelial proliferation has been implied by proliferating cell nuclei antigen (PCNA).

Cardiovascular disease
A clinician’s manual of clinical tests would accept total, LDL- and HDL-cholesterol and blood pressure as biomarkers for cardiovascular disease (CVD) risk. However, an increasing number of other biomarkers are being used, including plasma triglycerides (TG), heart rate (HR), heart rate variability (HRV), atrial fibrillation (AF), arterial compliance, endothelial vasodilator function, intima-media thickness (IMT) and plaque stability.

A meta-analysis of 17 population-based prospective studies, including a total of 46 413 men and 10 864 women, confirmed a strong relationship between plasma TG and CVD, with every 1 mmol/l increase in plasma TG associated with a 30% increase in relative risk of CVD in men and 75% in women (Hokanson and Austin 1996). The Prospective Cardiovascular Münster (PROCAM) study enrolled 19 698 people aged 16–65 years, examined their lipid profile and known or suggested CVD risk factors at study entry, and then followed them for 8 years in order to relate CVD risk factors to the occurrence of fatal or non-fatal myocardial infarct and sudden cardiac death (Assman et al. 1998). Again, elevated TG appeared to be the most predictive risk factor for CVD, after controlling for all other important factors. Similarly with the Copenhagen Male study, which followed 3000 middle-aged and elderly Danish men, free of CVD at enrolment, for 8 years (Jeppesen et al. 1998). Several other studies (Dyer et al. 1980; Kannel et al. 1987; Shaper et al. 1993; Wannamethee et al. 1995; Palatini et al. 1999, 2002; Jouven et al. 2001), suggest that increased HR is an independent risk factor for SVD and SCD. Hartikainen et al. (1996) found that decreased HR variability (HRV) predicted SCD and arrhythmic events in patients who had survived MI. Vulnerability of the atherosclerotic plaque to rupture (atherosclerotic plaque stability), arterial dysfunction (as decreased compliance or elasticity) or altered vasomotor reactivity were all suggested to be important early markers of CVD risk (Plutzky 1999; Blacher et al. 1999; Simons et al. 1999).

The endothelium is important in maintaining arterial vasomotor tone and modulating vasoconstrictor, inflammatory, chemotactic and proliferative processes in the artery wall. Abnormalities of endothelial function have been associated with other CVD risk factors. Flow-mediated dilation (FMD) in the brachial artery is strongly correlated with coronary artery FMD, and has been suggested as a non-invasive method for assessing the extent of coronary artery disease (Takase et al. 1998; Celermajer et al. 1994; Enderle et al. 1998).

It is important to recognize that many of these suggestive relationships have only been established through correlations in observational studies. While RCTs would provide useful proof, it is difficult to affect one of these above measures without having a concomitant effect on the others. Nevertheless, questions are being raised about their increasing use. For example, the primary biological mechanism suggesting that omega-3 polyunsaturated fatty acids (PUFA) would protect against CVD is their anti-arrhythmic effects, but this endpoint has not translated to reduced CVD events when investigated further. One randomized controlled trial (Raitt et al. 2005) observed a negative effect of omega-3 PUFA supplementation and arrhythmia, leading to questions about the relevance and reproducibility of this biomarker.

Diabetes
Biomarkers are important for both the diagnosis and management of type II diabetes mellitus (DM). Serum glucose levels, before and after a glucose challenge, is the most common marker of impaired glucose tolerance and pre-diabetic state. Management focuses primarily on such peripheral blood biomarkers as daily serum glucose levels, in association with predictors of long-term morbidity.
20.7. BIOMARKERS THAT PREDICT THE OPTIMIZATION OF HEALTH OR PERFORMANCE

Traditional nutritional science has focused on providing nutrients to nourish populations, and prevent deficiency diseases. However, a more modern approach focuses on maintaining or improving health and optimized performance of individuals through tailored dietary regimes. A considerable challenge is in how to define a 'healthy' phenotype. Rational personalization of diet utilizes the approaches of 'nutrigenetics' and 'nutrigenomics' (Ferguson 2006). Nutrigenetics considers the implications of human variation, including single nucleotide polymorphisms, copy-number polymorphisms and epigenetic phenomena, on dietary requirements and disease susceptibility, while nutrigenomics considers how diet influences gene transcription, protein expression and metabolism. Both sciences are defined by systems biology approaches that integrate several of the '-omics' technologies (transcriptomics, proteomics, metabolomics and metabonomics) with more classic pathological and nutritional science. These approaches have the potential to enable discovery of early indicators for disease disposition, differentiate dietary responders from non-responders, and to lead to the discovery of beneficial bioactive food components.

and what levels or concentrations should be tested. In selection of a biomarker, there is an inevitable conflict between the degree of invasiveness of tissue sampling and the necessity to get close to disease and affected tissue for optimal predictivity. Tissues such as peripheral blood lymphocytes are commonly used because of the ease of harvesting. However, there are questions as to whether a systemic effect can predict changes in disease risk in a different tissue. It is necessary to understand the potential mechanism if a biomarker is to provide the maximum amount of information. Biomarkers of oxidative stress or of inflammatory response may have utility in predicting interventions against a range of different events. There may be value in more specific biomarkers, especially for chronic diseases such as cancer, cardiovascular disease or diabetes. Population subgroups at high risk of disease have been used in intervention studies, but answers provided by these groups may not be representative of the general population. The optimal design of an intervention trial using biomarkers might stratify volunteers according to genotype.

Preliminary intervention studies utilizing rationally designed and well-validated biomarkers may well provide many of the answers that are needed to reduce disease risk in human populations.

References


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