

Special article

## Bioactive compounds: Definition and assessment of activity

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Manuscript received March 18, 2009; accepted April 26, 2009

### Abstract

Biomarkers and their role in evaluating efficacy and safety were the topic of the 23rd Hohenheim Consensus Meeting at the University of Hohenheim in Stuttgart. Scientists who had published and reviewed scientific and regulatory papers on the topic were invited, among them basic researchers, toxicologists, clinicians, and nutritionists. The participants were presented with 11 questions (in bold font), which were discussed and answered (in italic font) at the workshop, with the aim of summarizing the current state of knowledge on the subject. The explicatory text accompanying the short answers was produced and agreed on after the conference and was backed up by corresponding references.  
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### Keywords:

Biomarker; Bioactive constituents; Nutraceuticals; Health effect; Xenobiotics; Bioavailability; Intake; Phytochemicals

### Introduction

Current nutritional approaches are beginning to reflect a fundamental change in our understanding of health. Increasing knowledge regarding the impact of diet on regulation at the genetic and molecular levels is changing the way we consider the role of nutrition, resulting in new dietary strategies. Diet not only provides adequate nutrients to meet metabolic requirements, but can also contribute to the improvement of human health. Consequently, extracts of plants or single compounds thereof that are believed to benefit human health need to be identified and developed for the food market to complement a balanced diet. The evaluation of the efficacy and safety of these naturally occurring bioactive compounds presents a challenge to scientists, and the biomarker concept may be

a breakthrough in this respect. Gene expression data hold particular promise for the future as biomarkers.

It was apparent to us that many concepts and terms in the field of nutrition are not understood consistently within the profession. Our objective was to reach a consensus and to provide reliable definitions and descriptions, thus clarifying the situation. The following topics were introduced by the participants at a workshop and were the subject of extensive group discussion.

### What are bioactive compounds?

*Consensus: Bioactive compounds are essential and non-essential compounds (e.g., vitamins or polyphenols) that occur in nature, are part of the food chain, and can be shown to have an effect on human health.*

### Background

Bioactive compounds are also referred to as *nutraceuticals*, a term (coined in 1979 by Stephan DeFelice) that

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reflects their existence in the human diet and their biological activity. Bioactive substances present as natural constituents in food provide health benefits beyond the basic nutritional value of the product.

Nutraceuticals may range from isolated nutrients, dietary supplements, and diets to genetically engineered “designer” foods, herbal products, and processed foods, such as cereals, soups, and beverages. The basis for the increasing interest in nutraceuticals is that knowledge from epidemiologic studies, e.g., that a specific diet or a component of the diet is associated with a lower risk of chronic disease, is transmitted through the media to the consumer.

Until relatively recently, vitamins and other micronutrients have been recommended only to avoid the classic symptoms of deficiency. Modern techniques in biology have given us further insight into the molecular and cellular needs of the organism. For example, clinical symptoms of deficiency can be the result of a long-term low intake of micronutrients. However, well before these symptoms occur, the inadequate delivery of micronutrients to their target tissue causes alterations that may trigger the development of chronic disease. Similar effects on genetic regulation in target tissues may occur if nutrients are overdosed.

The compounds studied most extensively are the antioxidants, an increased intake of which can alter the risk of chronic diseases including cancer and cardiovascular disease.

At the same time, there is evidence that some individuals, despite an optimum intake of micronutrients (as measured based on the recommended daily intake), nonetheless exhibit a high risk for diseases such as cancer or coronary heart disease. These individuals have a mutation in one enzyme or several enzymes involved in micronutrient metabolism or distribution. This “polymorphism” results in low tissue concentration or inadequate function of the micronutrient and obviously has implications for an individual’s health. For instance, the major determinants of plasma homocysteine concentrations are nutritional and genetic factors, and there is evidence from several studies that a mild to moderate increase in plasma homocysteine is strongly associated with the development of atherosclerosis [1]. A common single base-pair exchange of cytosine to thymidine at nucleotide 677 of the methylenetetrahydrofolate reductase gene leads to thermolabile methylenetetrahydrofolate reductase with decreased enzyme activity, resulting in increased plasma homocysteine concentrations [2]. It has been reported that this mutation is associated with the development of coronary artery disease [3]. An intake of folate exceeding the standard recommendation may help to protect against coronary heart disease in cases of high homocysteine in groups with the mutation.

Over the past decade, knowledge of the role of phytochemicals (e.g., polyphenols) in specific pathologies has advanced at an accelerating pace. Different classes of phytochemicals (e.g., phytoestrogens) have been identified as having a preventive effect against specific diseases, mainly at very early stages of disease development, particularly con-

cerning cancer. The fact that these compounds occur mainly in vegetables seems to confirm the epidemiologic evidence that a high intake of vegetables is associated with a lower risk of different kinds of cancer.

At the same time, state-of-the-art technologies, including biotechnology, have led to nutritional discoveries, product innovations, and mass production on an unprecedented scale. These developments have spawned an important and dynamic new area of research, resulting in increasing numbers of nutritional products with potential medical and health benefits.

Scientific and technologic developments are increasing the possibilities of modifying traditional foods and developing new food sources to meet these newly discovered requirements. Using modern genetics, chemistry and molecular biology, the scientific community is now able to design and manufacture foods having specific characteristics.

These new products represent a major departure from traditional foods, in part because they are based on a new approach to nutrition, i.e., as having the potential to lower the risk of chronic disease. Current nutritional approaches are beginning to reflect a fundamental change in our understanding of health. Increasing knowledge of the impact of diet on regulation at the genetic and molecular levels will lead to a rethinking of health goals, resulting in dietary strategies based on aggregation rather than individual factors.

Herbal remedies claimed for treatment of different diseases have entered the market in many cases without any scientific basis with respect to efficacy or safety [4]. They are composed of several compounds, each with a potential effect on a cellular level. It is not always clear which compound is the effective one, or whether a specific combination may be necessary. In addition, it cannot be excluded that harmful components may exist in herbal products. Consequently, the health benefits of phytochemicals should be evaluated using isolated compounds or well-characterized mixtures or extracts in selected and validated *in vitro* and *in vivo* systems. These include bioavailability, dose–effect levels in target tissues, and the definition of biological markers (biomarkers) that allow the examination or investigation of an effect of a phytochemical at a very early stage of disease development.

### What is a biomarker?

*Consensus: A biomarker is a chemical or biological test result in an analyzed biological material related to a certain exposure, susceptibility, or biological effect.*

### Background

The term *biological marker*, or *biomarker*, is widely used in medicine, biology, ecology, and environmental chemistry as a generic term for test results on biological material that give some indication of the status of the organism, environment, or ecosystem from which the sample was derived. This status may relate to exposure, susceptibility, effect, or risk.

Biomarkers that may help to define the potential of a phytochemical in the chemoprevention of certain diseases such as cancer, coronary heart disease, and advanced macular degeneration can be taken to determine dose–effect levels, bioavailability and, most importantly, safety of specific nutraceuticals in specific cells and tissues.

At a minimum, a biomarker should be correlated to the specific pathology and to the nutraceutical when tested under *in vitro* and *in vivo* conditions. The following examples demonstrate an approach designed to elucidate the effectiveness of a nutraceutical using different biomarkers.

In the literature, there are many definitions. The differences are related to the specific subject of the journal where the definition is published, e.g.:

- “Biomarker could be defined as a measurement made on body tissue, body fluid or excretion to give a quantitative indication of exposure to a chemical and which may give an estimate of the risks consequent on that exposure” [5], related to the subject of food chemistry
- “A biomarker is any substance, structure or process that can be measured in the human body or its products and may influence or predict the incidence or outcome of disease” [6], related to cancer epidemiology
- “The biomarker would specifically and sensitively reflect a disease state and could be used for diagnosis as well as for disease monitoring during and following therapy” [7], related to therapy

Functionality also suggests that “a biomarker is a molecule that indicates an alteration in physiology from normal having clinical utility” [7], a definition that is limited only by its relation to physiology and the symptoms of disease. Examples also exist of other broad definitions, e.g., “biomarkers have been defined as indicators of actual or possible changes of systemic organ tissue, cellular and sub-cellular structural and functional integrity which can be used, either singly or in batteries, to monitor health and exposure to compounds in populations and individuals” [8].

None of these definitions, including the consensus definition given to the question above, is sufficient to encompass all the qualities of a biomarker. Two further definitions often mentioned in texts are related to accuracy or specificity, e.g., “one of the basic requirements of a (dietary exposure) biomarker is that it accurately reflects intake of the dietary constituents of interest” [9], and “the essential qualities of biomarkers—responsiveness, specificity, ease of use, applicability and relevance—apply particularly in (diet and health)” [10].

Thus, apart from meeting the general requirements of a biomarker, any such results must come from tests that have been validated according to methodologic practices and the ability of the biomarker to actually reflect the exposure, susceptibility, effect, or risk as determined. The expectations of the validation processes differ among the various analytical methods and clinical endpoints. In the current text we assume that, for a test result to be used as a biomarker, such validation has

Table 1  
Definitions of biomarker, clinical endpoint, and surrogate endpoint

Biomarker	A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention
Clinical endpoint	A characteristic or variable that reflects how a patient feels, functions, or survives
Surrogate endpoint	A biomarker that is intended to substitute for a clinical endpoint

taken place and has been published. In the case of diseases such as cancer and cardiovascular disease, there are considerable problems in the validation of early markers of risk.

### What are established biomarkers of efficacy with respect to health effects?

*Consensus: An established biomarker of efficacy is an indicator of an improvement of a physiologic function or a decrease in risk factors for a disease.*

#### Background

We are witnessing significant progress in nutritional knowledge that will change the way we consider the role of nutrition in the future. The diet may not only provide adequate nutrients to meet the metabolic requirements, but could also contribute to the improvement of human health. As a consequence, extracts of plants or single compounds thereof that are believed to benefit human health need to be identified and developed for the food market to complement a balanced diet. The evaluation of the efficacy of naturally occurring bioactive compounds is considered a challenge to scientists, and the biomarker concept may be a breakthrough in this respect.

Most naturally occurring bioactive substances are non-essential to the human body. This means that there are no known typical signs or symptoms if these substances are not regularly consumed. For example, although inadequate vitamin C intake eventually leads to the classic deficiency syndrome of scurvy, such syndromes do not exist for most naturally occurring substances with a postulated health-promoting potential, such as flavonoids, polyphenols, phytoestrogens, and others. This means that one precondition

Table 2  
Examples of biomarkers and clinical endpoints

Cardiovascular disease	
Biomarker	Serum cholesterol, blood pressure, etc.
Clinical endpoint	Myocardial infarction, stroke
Osteoporosis	
Biomarker	Markers of bone formation, bone resorption and bone density
Clinical endpoint	Bone fractures
Cancer, e.g., prostate	
Biomarker	Prostate-specific antigen
Clinical endpoint	Prostatic neoplasm or metastases

for adding these non-essential bioactive substances to foods is proof of their efficacy in terms of a defined health marker.

In proving that a substance occurring naturally in the food chain has a defined health effect, scientists are confronted with a problem. The health-promoting effect of these naturally occurring bioactive substances must be preventive, fundamentally distinguishing them from curative drugs. This means that the effect of these compounds on the human body may be very small over relatively short periods—months or a few years—but can contribute significantly to health when they are consumed throughout life as part of the daily diet.

It is also known that many chronic human illnesses begin to develop early in life, but are only manifested, i.e., impair individual health, very much later. This time lag between the start of an illness and individually observable symptoms applies to cardiovascular disease, osteoporosis, and many forms of cancer and may last as long as several decades. Atherosclerotic changes can be found in situ in the blood vessels of adolescents and young adults, or even earlier [11]. However, clinical manifestations such as myocardial infarction, stroke, or intermittent claudication will generally appear only two or three decades later. The same is true of osteoporosis, which results in bone weakness and an increased risk of fractures. Bone is strongest from 20 to 30 y of age. Afterwards, in women earlier than in men, there is a continuous decline in bone strength, with many people developing fractures at 50 to 80 y of age. Similarly, in malignant diseases of epithelial tissues, up to 20 y may elapse between the initiation, i.e., the appearance of the first malignant cell, and clinical manifestation of the cancer.

Investigating preventive activity in naturally occurring bioactive substances when their effect may only be moderate thus poses a serious dilemma in nutritional research. To facilitate discussion of this issue, we must first briefly examine what scientific possibilities exist for demonstrating the efficacy of these naturally occurring bioactive substances.

In simplified terms, the various categories of scientific tests comprise *in vitro* tests (molecular and cellular assays), animal experiments, and human studies, the last of these being subdivided into epidemiologic and intervention studies. Often epidemiologic studies, e.g., to investigate the relation between diet and the occurrence of a disease in a relatively large population, lead to a hypothesis, which is investigated further *in vitro* and in animal experiments. Intervention studies may then be performed to demonstrate efficacy in humans taking a specified amount of a bioactive substance each day under precisely defined and controlled conditions [12].

The measurements used may be intermediate endpoints or clinical endpoints. Clinical endpoints, such as myocardial infarction, fractures, and the occurrence or course of cancers, are typically used to investigate the efficacy of drugs, i.e., for therapeutic purposes. However, for the reasons discussed above, clinical endpoints appear less suitable for studying the efficacy of bioactive substances, i.e., for prevention. We therefore need to take a closer look at the concept of biomarkers and their role in demonstrating the efficacy of bioactive substances.

Table 1 lists the definitions of “biomarker,” “surrogate marker,” and “clinical endpoint” proposed by Zeger [13] at a National Institutes of Health workshop on biomarkers and surrogate endpoints. A surrogate endpoint, i.e., a biomarker suitable for replacing a clinical endpoint, thus appears to be the appropriate parameter for demonstrating the efficacy of naturally occurring bioactive substances in human intervention studies provided it meets certain criteria [14]: 1) it must display good specificity and sensitivity; 2) it must be standardized and validated; 3) it must be non-invasive or, at most, minimally invasive; and 4) it must be inexpensive, if it is to be widely used.

With these provisos, the biomarker concept could be an essential element not only in the efficacy testing of bioactive substances but also in nutritional science in general. Using biomarkers, the moderate effects of natural bioactive ingredients on the human body could be studied without excessive strain on resources. Some examples of biomarkers are presented in Table 2.

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