Establishing Health Benefits of Bioactive Food Components: a basic research scientist's perspective

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Abstract

Bioactive food components or functional foods have recently received significant attention because of their widely touted positive effects on health beyond basic nutrition. However, a question continues to lurk: are these claims for ‘super foods’ backed by sound science or simply an exaggerated portrayal of very small ‘benefits’? Efforts to establish health benefits by scientific means pose a real challenge in regards to defining what those benefits are, as well as how effective the foods are in justifying any health claim. This review discusses the pitfalls associated with the execution, interpretation, extrapolation of the results to humans and the challenges encountered in the dietary research arena from a basic scientist’s perspective.

Introduction

Consuming certain foods for their health-promoting properties have been in existence for over a millennium across different cultures. Yogurt and honey, for example, are recognized for their health benefits and their combination was considered “the food of the gods” in ancient India [1]. For several thousand years, use of herbs, as in traditional Chinese medicine, took into account possible food interactions as well as food combinations that enhanced the healing effects when treating specific diseases [2,3,4]. To date, a substantial number of studies have shown that dietary food components can impart profound beneficial effects on human health ranging from the reduction of potential risk factors to the prevention of or delay in the onset of chronic diseases [5,6,7]. However, despite the perceived safety, low cost and health claims attributed to diet- or food-derived components/compounds, the precise food components and the underlying mechanisms responsible for the biological effects remain elusive.

Recently, greater public health interest in bioactive food components led to attention from the regulatory and scientific community as well as the popular press. For example, a recent ad hoc Federal working group proposed a working definition: "Bioactive food components are constituents in foods or dietary supplements, other than those needed to meet basic human nutritional needs, that are responsible for changes in health status." [8]. A 2013 workshop (International Union of Nutritional Sciences (IUNS) 20th International Congress of Nutrition (ICN) held in Granada, Spain, September 15–20, 2013 and a 2013 symposium “Are Dietary Bioactives Ready for Recommended Intakes?” held in April 21, 2013, at the ASN Scientific Sessions and Annual Meeting (Experimental Biology 2013, Boston, MA,) examined how to establish putative health effects of bioactive food components and suggested using the Dietary Reference Intake (DRI) approach that establishes nutrients requirements [9]. Recommendations for future research were also proposed [10•,11•]. However, despite a number of efforts to identify and establish the beneficial health effects of bioactive food components, the available data are often conflicting and the field of dietary research is challenged by competing interests (academic, scientific, political, consumer, industry).

The goal of this review seeks to outline, from a basic scientist’s perspective, the experimental challenges encountered by scientists interested in establishing the health effects of bioactive food components. Potential experimental approaches available will be discussed to promote and facilitate the design and execution of studies that focus on food bioactives and health. Throughout the review, some of our previous work will be cited as examples to exemplify the key points we have identified as challenges.

Challenge 1. Experimental considerations for assuring quality *in vitro* data.

The isoflavone genistein, a well-documented health-promoting bioactive component from soy, activates multiple cellular pathways in a concentration-dependent manner in the human prostate cancer cells LNCaP and breast cancer cells MCF-7 [12,13]. The highest reported concentration for genistein in tissue/tissue fluid is at 10 μ M [13]. However, the majority of studies reporting anticarcinogenic and antiproliferative properties of genistein utilize much higher concentrations than what is physiologically relevant *in vivo*. In our laboratory, we observed using the microarray approach that low concentrations of genistein inhibits hormone-(androgen/estrogen)-induced gene expression and proliferation activities. However, at high concentrations, (> 10 μ M) it may be detrimental through the induction of DNA damage [14,15](Figure 1). Another example relates to resveratrol, a phytochemical considered to possess anticarcinogenic properties. We found that resveratrol inhibits cell growth through induction of DNA-damage-related cyclin inhibitor p21 expression in LNCaP at higher concentrations. However, at lower concentrations, resveratrol decreases prostate-specific antigen (PSA), a classic androgen-responsive prostate cancer marker [16] (Figure.2). The circulating concentration of resveratrol is less than 5 μ M [16]. Hence, considering physiological achievable concentration, we concluded that the anti-androgenic/estrogenic effects may prevail over apoptosis [13]. These findings make us ponder how to ascertain the true health benefits of bioactives and caution us about the importance of how we might interpret the data. Oftentimes, scientists try to push the *in vitro* system by using higher dosages in their experimental design which may result in targeting multiple molecular and signaling pathways. This approach may render the underlying mechanisms of action uncertain and confusing. Thus, consideration of physiologically attainable concentrations would be important as cell death/apoptosis may not be the specific end point associated with a health benefit and may be more reflective of cellular stress (DNA damage).

In addition to concentration, selection of the types of target cells for *in vitro* experiments can also generate varying results. If the target organ/tissues are internal, such as liver, breast or prostate, direct exposure to hundreds of μ M concentrations may not be realistic physiologically. On the other hand, if the epithelial lining such as the intestine or lung where the cells interact directly with the compounds is taken as the *in vitro* model, exposure to much higher concentrations is more likely to induce biological effects compared to internal organs or tissues [17]. Therefore, consideration of exposure concentrations, selection of target cells and understanding the precise mechanisms at play would be critical in establishing health benefits for any food bioactives. Moreover, where one is able to introduce an overwhelming concentration into the system, it may beget a situation reminiscent of a double-edged sword.

Challenge 2. Experimental considerations for *in vivo* animal models.

To date, a number of studies, including our own, have reported dissimilar effects while trying to recreate *in vitro* findings in an animal model [16,18,19,20]. We observed that, despite potent antiproliferative/anticarcinogenic effects seen in cell culture systems, prolonged exposure to resveratrol in a xenograft model unexpectedly led to increased tumor angiogenesis and inhibition of tumor apoptosis [16]. Nonetheless, before ruling out any health effects of bioactives in such cases, we need to consider the variables that may affect what appeared to be contradictory experimental outcomes.

Most compounds entering the circulation have short half-lives (hours) and exist at physiologic levels of μ M or less [21,22,23*]. However, higher pharmacological dosages are often used to study food bioactives in animal models, resulting in an effect that is more of a drug/supplement effect rather than a food effect. By pushing the system, one deviates from the goal of unraveling

the true health effects of bioactives, as it would occur in a whole body system at physiological levels.

When using animal models, routes of administration (incorporation into the diet vs parenterally or orally administered) may be an additional factor to be considered. Foods are mostly consumed orally and the bioactive molecules would have to pass through the digestive tract to be absorbed. In such instances, knowledge about the bioactive's bioavailability and bioaccessibility will help to adjust the dosage and facilitate interpretation of the biological effects [24]. Moreover, results of studies of bioactives administered through injections (intraperitoneal, intravenous, etc.) or gavage will need careful interpretation because the kinetics of exposure may differ significantly from those obtained with oral administration [23,25].

Humans typically consume food in three meals a day. Therefore, when designing an animal study, we must consider information about human consumption over a period of time. Calculations using several available algorithms in the literature to determine specific doses for treatment should be carried out to ensure the validity of human to animal conversions in relation to time and manner of food consumption [26,27]. Additionally, the growing conditions and site of production may result in different food composition [28,29]. An accurate analysis of specific food components would be critical to safeguard the accuracy of the observed health efficacies. Moreover, we consume food on a regular basis during our life time. Over time, the food consumption pattern or the food choices we make can impart a profound impact on our overall health. The ability to recapitulate such changes may not be feasible using only *in vitro* models. However, animal models that examine longer exposure time to a dietary component may be more relevant, as the manifestation of health benefits may need to have continuous and longer-term exposure.

Challenge 3. Transition from animal models to humans: the ultimate challenge

The ultimate goal of dietary research is to translate scientific findings to humans. Animal models have proven to be instrumental for pre-clinical testing. However, the genetic and physiological makeup of the animals oftentimes fall short in mimicking the complexity of human biological processes, resulting in inconclusive/contradictory findings in clinical trials [30,31,32]. Furthermore, accumulating data indicates that genetic differences or predisposition of an individual may contribute to differential responsiveness in an individual [33]. For example, we previously reported differential changes in circulating β -carotene or lycopene amongst individuals following consumption of a watermelon or tomato juice supplement [34]. The beneficial health outcome of carotenoids will depend on the responsiveness of individuals to certain foods. Hence, understanding of interindividual variability would be important to ascribe a recommendation for intake and define its health benefit. Additionally, gender differences in dietary intake patterns and metabolic profiles may also be important factors to consider when interpreting data extracted from clinical trials [35]. Moreover, long term exposure to food components may result in its accumulation in vital organs in toxic amounts and thus warrants caution. A possible approach to mitigate toxic accumulation may be the use of compartmental modeling to predict whole body distribution pattern [36,37]. The results can be used to facilitate targeted analysis of specific tissues for the accumulation and metabolism of a food component. Additionally, in the quest of a more realistic translational alternative, one could consider use of *in vitro* human cell-based assays, and/or humanized mice [38,39,40]. Careful experimental consideration and use of the emerging translational alternatives will render more reliable data and reduce the chances of placing patients at risk.

Challenge 4. New paradigm in dietary research and issues with dietary supplements and functional foods

Recent advances in microbiome research suggest that the health effects of bioactive food components may be indirect and occur through modulation of the microbiome or through processing of a food component by the microbiome [41,42]. Given the complex nature of interaction between the food matrix and gut microbiome, a systematic approach [43] may be beneficial in providing a better view of the overall landscape of the physiological system.

Regarding bioactives that are in use as dietary supplements, there are clear distinctions in what one is exposed to through consumption as food versus taking it in a pill form. Supplements in the form of a pill provides a one-time bolus and not considered part of a meal and hence not comparable. Food is also composed of a complex mixture where interaction between components may occur. Such interaction may subsequently change the bioavailability and bioefficacies of a health-promoting agent. The lesson of the grapefruit-drug interactions serves as the best example to illustrate potentially serious health hazards caused by such an interaction [44,45]. The ideal study would be one having a food with all the matrix without the food components of interest. This could be achieved through genetic engineering of food or the development of specific processing methods. Efforts to enhance food to create functional food for health is no longer a novel concept [46,47]. However, given the lack of precise understanding of how a compound works, interaction between bioactives and other macro-, micro-nutrients as well as the emerging role of the microbiome in health and disease, careful and thorough evaluation of the efficacy of functional foods is imperative before the “super food” reaches the consumer.

Conclusions

Despite the existence of numerous studies concerning bioactive components and health, many of the findings remain inconclusive, leading to questioning of the credibility of health claims attributed to such bioactives. The complexities associated with dietary research call for a framework upon which recommendations can be made in order to achieve a beneficial health effect. This review was not meant to be a comprehensive overview of how to establish health benefits of a food bioactive but delineate the challenges we currently face in conducting experiments that will ultimately facilitate realistic interpretation of laboratory-based dietary data into potential health benefits. A sound science-based dietary recommendation would be the holy grail in establishing the health-promoting effects of food bioactives.

Conflict of Interest

There are no conflicts of interest.

Acknowledgements

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Figure1

Working Model

Figure2

Figure3

Figure 1. Schematic representation of biphasic action of phytochemicals. Hormones, cytokines induce proliferation of cancerous cells. At physiological concentrations, phytochemicals induce xenobiotic metabolism and reduce cell proliferation through inhibition of hormones and cytokines. However, at higher concentrations, phytochemicals exhibit a pharmacological effect, inducing apoptosis and DNA damage through modulation of genes involved in the p53 mediated pathways. p53; tumor suppressor p53; p21; a cyclin inhibitor; BAX; a pro-apoptotic member of the bcl-2 protein family.

Figure 2. Concentration-dependent effects of genistein on cellular pathways. At higher concentrations, genistein modulates DNA damage, cellular stress, while at physiologic concentrations (5-10 μ M), genistein inhibits cell growth and hormone (androgen/estrogen) [13].

Figure 3. Concentration-dependent effects of resveratrol on cancer markers. Resveratrol, at higher concentrations, induces p21 expression, a cyclin inhibitor regulating cell cycle progression, however, at the same time, reduces prostate specific antigen PSA, a classic prostate cancer marker [16]. P21; cyclin -dependent kinase inhibitor 1.

Working Model