

A Brief Historical Overview of the Past Two Decades of Soy and Isoflavone Research^{1,2}

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Abstract

During the past 20 years, a remarkable amount of research into the health effects of soy consumption has been conducted, which in large part can be attributed to the presence of isoflavones in the soybean. Isoflavones first came to the attention of the scientific community in the 1940s because of fertility problems observed in sheep grazing on a type of isoflavone-rich clover. In the 1950s, as a result of their estrogenic effects in rodents, isoflavones were studied as possible growth promoters for use by the animal feed industry, although shortly thereafter, it was shown that isoflavones could also function as antiestrogens. Despite this early work, it was not until the 1990s, largely because of research sponsored by the U.S. National Cancer Institute, that the role of soyfoods in disease prevention began to receive widespread attention. Subsequently, isoflavones and soyfoods were being studied for their ability to alleviate hot flashes and inhibit bone loss in postmenopausal women. In 1995, soy protein attracted worldwide attention for its ability to lower cholesterol. At this same time, isoflavones began to be widely discussed as potential alternatives to conventional hormone therapy. In 2002, it was hypothesized that individuals possessing the intestinal bacteria capable of converting the soybean isoflavone daidzein into the isoflavan equol were more likely to benefit from soy intake. More recently, *in vitro* and animal research has raised questions about the safety of isoflavone exposure for certain subsets of the population, although the human data are largely inconsistent with these concerns. *J. Nutr.* 140: 1350S–1354S, 2010.

Asian populations have consumed foods made from soybeans for centuries, whereas in the West, certain subpopulations, namely Seventh-day Adventists and vegetarians, have used soyfoods for ~100 years, although the quintessential soyfood tofu was first introduced on a large scale to the general U.S. population beginning only in the early 1970s. Health-conscious and ecologically minded consumers were particularly attracted

to soy at this time because it was perceived as being a source of high-quality protein low in saturated fat that was more efficiently produced than animal sources of protein.

A dramatic increase in soyfood consumption during the last decade of the 20th century occurred because of the belief among many consumers that soyfoods might offer health benefits independent of their nutrient content. This increased interest is best viewed in the context of the general recognition underway at this time that plants contain large numbers of potentially beneficial nonnutritive biologically active components commonly referred to as phytochemicals. This knowledge led to the concept of functional foods [initially referred to as designer foods by the National Cancer Institute (NCI)³] and to soy being one of the first foods widely acknowledged to fall into this category. Like all foods, the soybean contains a number of biologically active components, many of which are being actively investigated including, e.g., saponins and lunasin, but unquestionably it is the isoflavones that are responsible for so much of the scientific interest in this legume.

Isoflavones, which have been known to exist in plants for >100 years, have a relatively limited distribution in nature such that among commonly consumed foods by humans, they are found in physiologically relevant amounts only in soybeans and foods derived from this legume (1), although a variety of plants such as red clover (2) are also rich sources. Consequently,

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³ Abbreviations used: ER, estrogen receptor; NCI, National Cancer Institute; ODS, Office of Dietary Supplements.

isoflavone intake among older adults in Japan and Chinese cities such as Shanghai is ~40 mg/d (3), whereas in Europe and the United States it is likely no more than 3 mg/d (4–10). Although soy protein has also been the subject of considerable investigation, especially in regard to its hypocholesterolemic effects (11), recent scientific interest in soy largely parallels the interest in isoflavones. Of the ~2000 soy-related papers currently published annually, more than one-half are related to isoflavones.

Isoflavones, like many phytochemicals of interest to nutritionists, are phytoalexins, substances formed by the host tissue in response to physiological stimuli, infectious agents, or their products, which accumulate to levels that inhibit the growth of microorganisms (12). Isoflavones possess properties (i.e. anti-fungal, antimicrobial, and antioxidant) that enhance the survival of the soybean (12). For this reason, soybean isoflavone concentrations increase greatly in times of stress, such as when moisture is limited, and are influenced by the environmental conditions under which the soybean is grown (13,14).

In many respects, the biological effects of isoflavones first came to the attention of the scientific community in the 1940s because of breeding problems experienced by female sheep in Western Australia grazing on a type of clover rich in isoflavones (15–17). Three decades later, Setchell et al. (18) established that isoflavone-rich soy, which was part of the standard diet of cheetahs in North American zoos, was a factor in the decline of fertility in these animals. It is easy therefore to understand why nutritionists, if they thought of isoflavones at all at this time, viewed them largely as antinutrients. Interestingly, in the 1950s, isoflavones were being studied by the animal feed industry as possible growth promoters because of their reported estrogenic effects in rodents (19–22). By the 1960s, the determination of the relative binding affinities of isoflavones for estrogen receptor (ER) alpha helped firmly establish these soybean constituents as phytoestrogens (23,24).

For the most part, there was little interest in isoflavones within the nutrition community throughout the 1980s. One notable exception is the now-classic work by the pioneering isoflavone researcher, Kenneth D. R. Setchell, who showed that in response to soy consumption, isoflavone excretion increased dramatically and that only a minority (~25% of Westerners) of participants possessed the intestinal bacteria capable of converting the soybean isoflavone daidzein into the isoflavan equol (25,26). Sixteen years later, Setchell et al. (27) proposed that equol was an especially beneficial compound and that those individuals who possessed equol-producing intestinal bacteria were more likely to benefit from soyfood consumption than those who did not. This hypothesis is currently a very active area of research.

The view toward isoflavones as only being phytoestrogens required modification as a result of Akiyama et al. (28) serendipitously discovering in 1987 that genistein, the primary soybean isoflavone, was an inhibitor of protein tyrosine kinase, an enzyme frequently overexpressed in cancer cells (29). Since then, genistein has been extensively studied for its ability to affect a diverse array of intracellular signaling cascades (30,31) that control cell growth (30). As a result of the protein tyrosine kinase finding, it became clear that isoflavones were complex molecules that could no longer be viewed simply as phytoestrogens and that soyfoods might account for the low incidence of breast cancer in Japan, a notion supported by animal (32) and epidemiologic (33) research published in the early 1990s. As already noted, although largely overlooked, the ability of isoflavonoids to function as antiestrogens and thus possibly

reduce risk of hormone-sensitive cancers as a result was already part of the scientific literature in the 1960s (23–26).

In 1990, there was sufficient preliminary evidence for the NCI to sponsor a workshop on the role of soy in reducing cancer risk (34). The findings from this meeting led the NCI to initiate a multi-million dollar research program evaluating the anticancer effects of soyfoods. This declaration of interest, which was largely based on the proposed chemopreventive effects of isoflavones, greatly increased interest in both soy and isoflavones in a wide range of areas. One of these areas was the alleviation of menopausal symptoms.

In 1992, Adlercreutz et al. (35) were the first to suggest that soyfoods, because they contain isoflavones, might at least partially account for why Asians and Japanese women in particular were less likely to report experiencing menopause-related hot flashes. The first trial to examine this hypothesis was published in 1995 (36); since then, >50 trials evaluating the efficacy of isoflavone-containing products have been conducted (see references for reviews) (37,38).

Upon reflection, it is now apparent that in the relatively short recent history of isoflavone and soy research, 1995 was a seminal year. In that year, Anderson et al. (11) published a meta-analysis that attracted widespread attention to the hypocholesterolemic effects of soy protein, although Italian investigators had demonstrated dramatic reductions in cholesterol in hypercholesterolemic participants in response to soy protein as early as the late 1970s (39,40). In many respects, the meta-analysis indirectly led to the approval by the U.S. FDA of a health claim for soy protein and coronary heart disease 4 years later (41). Interestingly, Anderson et al. (11) suggested that isoflavones might account for 60–70% of the cholesterol-lowering effects of soy protein; as a result of this suggestion, considerable investigation of the hypocholesterolemic properties of isoflavones was undertaken (42). Although there is only weak support for the cholesterol-lowering effects of isoflavones, and the cholesterol-lowering potency of soy protein is less than initially thought (43,44), the effects of isoflavones on a variety of coronary heart disease risk factors have been extensively evaluated (45). For example, in 2001, Walker et al. (46) were the first to show that the isoflavone genistein markedly increased nitric oxide-dependent dilation in forearm vasculature.

In the same year in which Anderson et al. (11) published their meta-analysis, a Wake Forest University research group, extremely active in the soy field, helped popularize the notion that isoflavones possessed mixed ER agonist/antagonist properties and were a possible alternative to conventional hormone therapy (47). Soon thereafter, investigators began referring to isoflavones as natural selective ER modulators (48), a classification that gained support from the identification of the second ER, ER β (49), and the finding that isoflavones preferentially bind to ER β compared with ER α (50,51). Later work demonstrated that isoflavones also preferentially transactivate ER β [for a review, see Reiter et al. (52)].

A final landmark development in 1995 was the publication of animal research by Lamartiniere et al. (53–55) showing that isoflavone exposure early in life reduces breast cancer risk during adulthood. In addition to the animal studies, this hypothesis has considerable epidemiologic support (56–59) and is consistent with an emerging school of thought that emphasizes the important role early life events have in the etiology of breast cancer (60–62).

Not surprisingly, given the recognized role of estrogen, there has been considerable interest in the potential skeletal effects of isoflavones, although in 1996, the investigators responsible for

publishing the first animal study to demonstrate skeletal benefits suggested it was the ability of genistein to inhibit tyrosine protein kinase activity that was responsible for this effect (63). That year, the first rodent study was published showing isoflavone-rich soy protein improved bone mineral density (64) and 2 years later the first clinical study showing this was the case in postmenopausal women appeared in the literature (65). Since then, >30 trials have examined the effects of isoflavone-containing products on bone mineral density in postmenopausal women (see references for reviews of the literature) (66,67). The ability to conduct clinical trials, especially those longer in duration, was greatly aided by the development of isoflavone supplements, which first became available in 1996.

Along with research of the possible skeletal benefits of isoflavones for postmenopausal women, there has been interest in understanding the impact of soy on cognitive function. The first clinical trial in this area that reported a benefit was published in 2001 (68). One year before this publication an prospective epidemiologic study, whose primary endpoint was heart disease in men, found an association between tofu intake and the development of cognitive impairment in older age (69). However, other epidemiologic data do not concur with this observation (70). For a review of the clinical trials, the reader is referred to the reference (71).

Not surprisingly, the federal government has funded much of the isoflavone research in the United States, but their involvement in this field is not limited to funding. For example, in 1999, the USDA in conjunction with Iowa State University created an online database of the isoflavone content of foods (72). Also in 1999, The Office of Dietary Supplements (ODS), Office of Research on Women's Health, and National Institute on Aging sponsored a workshop to evaluate the effects of phytoestrogens on diseases affecting older men and women (73). In 2005, the ODS sponsored a comprehensive review of the soy-related clinical literature, which was conducted by the Agency for Healthcare Research and Quality at Tufts University (74). And in July of 2009, the ODS convened a workshop aimed at providing guidance for future clinical research involving soy (75).

It would be remiss not to mention that although there continues to be considerable enthusiasm for the potential health benefits of soyfoods and isoflavones, concerns about the safety of isoflavones, based largely on their estrogen-like properties, have occurred in parallel. In fact, isoflavones are not just classified as phytoestrogens and mixed estrogen agonists/antagonists but also as endocrine disruptors (76–78). Evaluations of isoflavone safety have been undertaken by governmental and quasi-governmental agencies in several European countries as well as in Japan and Israel and at the time of this writing, the European Food Safety Authority is currently conducting an evaluation. Most notable among the concerns, which in all cases are based almost exclusively on in vitro and animal research (the human research, including both clinical and epidemiologic data, are supportive of safety) is that isoflavone-containing products pose a risk to estrogen-sensitive breast cancer patients and women at high-risk of developing this disease (79) and that isoflavone exposure via the consumption of soy infant formula may harm the long-term development of infants (80). The latter issue was reviewed by the National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction in 2006 (81,82), and 2009 (83). At the most recent meeting the expert panel concluded that there was minimal concern about the safety of soy infant formula (83). For a discussion of the breast cancer (79,84) and infant formula issues (80,85,86), the reader is referred to the references.

Finally, there is recognition of the need to more precisely identify those factors contributing to the inconsistent clinical data such as interindividual differences in isoflavone metabolism, the health status (at-risk compared with normal risk, healthy compared with unhealthy) and metabolic profile (i.e. receptor polymorphisms) of study participants and especially, differences in the chemical composition of intervention products. Establishing those variables that play such a role may go a long way toward achieving a more precise understanding of the health effects of soyfood and isoflavone consumption.

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