

# Soy and other legumes: ‘Bean’ around a long time but are they the ‘superfoods’ of the millennium and what are the safety issues for their constituent phytoestrogens?

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The recognition that legumes and, in particular, soybeans provide not only an excellent source of vegetable protein but also contain appreciable amounts of a number of phytoprotectants has increased general awareness of their potential nutritional and health properties. Since the discovery that soybeans are one of the richest dietary sources of bioavailable phytoestrogens, this legume has been elevated to the forefront of clinical nutritional research. These natural ‘selective oestrogen receptor modulators’ have been shown to be bioactive. The recent approval by the Food and Drug Administration in the United States for a health claim for soy protein reducing risk for heart disease by its effects on lowering cholesterol levels has led to the increased awareness of the health benefits of soy protein. However, the presence of high levels of phytoestrogens in soybeans has also led to concerns over the potential safety of soy foods. This review will focus on the cardioprotective benefits of legumes and discuss the hypothetical concerns regarding the constituent phytoestrogens.

**Key words:** legumes, soy, phytoestrogen, isoflavone, cholesterol, thyroid, cancer, infant.

## Introduction

The importance of consuming pulses, or beans as they are more commonly known, for good health was recognized by Hippocrates as long ago as 400 BC. Their value has long been appreciated in many Asian and Far Eastern countries, in Mexico, South America and the Middle East, where a great variety of legumes, ranging from soybeans, chick-peas, green peas, lentils, pinto beans and all manner of coloured varieties of beans, have featured prominently in traditional diets. Despite our knowledge of the nutritional attributes of legumes, the consumption of legumes by Western populations remains relatively low. For the United States legume consumption is estimated to average less than one-quarter a serving per day, and less than one-third of all adults consume beans over any three-day period.<sup>1</sup> Figures from the 1995 National Nutrition Survey show that Australians consume an average of only 9.8 g of legume and pulse products and dishes each day.<sup>2</sup> Some of the difficulties in encouraging increased dietary intake of legumes are to overcome the perception of a negative taste barrier and the lack of cooking skills where beans are concerned. Many consumers also appear to believe that beans are either not a source of protein or, at best, a ‘poor-man’s protein’ that is good enough only for animal feed. Based on the Protein Digestibility Corrected Amino Acid Score (PDCAAS) most legumes rank high, and some soy proteins are equivalent to egg albumin protein in quality.<sup>3</sup>

It has been known for some time that legumes are nutrient-dense. There is some variation in the nutrient composition among different legumes but, overall, beans bring to the table an excellent source of protein, complex carbohydrates,

dietary fibre, oligosaccharides, minerals and phytochemicals, all of which have a bearing on different disease states.<sup>1,4</sup> What they don’t bring may be as nutritionally important, and in this respect legumes are deficient in cholesterol and lactose, and have a relatively low proportion of saturated fats. In addition, legumes are notable for the uniformly low blood glucose response they produce<sup>5,6</sup> and are recognized as a source of prebiotics, which have been described as ‘colonic foods’ that selectively stimulate the growth of beneficial intestinal microflora.<sup>7</sup> Furthermore, their inclusion in the diet is likely to be indirectly beneficial by contributing to an overall decrease in the amount of animal protein that would otherwise be consumed.

The reader is directed to several excellent reviews and references therein which have been published recently covering the nutritional attributes of legumes,<sup>1,4,8</sup> and for this reason these are not discussed in detail here. Instead we have focussed on the potential cardioprotective role of legumes and particularly the phytochemicals contained within legumes, with specific reference to the bioactive isoflavones.

As Fig. 1 illustrates, all legumes contain isoflavones. However, most clinical and nutritional studies of legumes have been carried out on soybeans. Driven largely by the recognition that soybeans are one of our richest dietary

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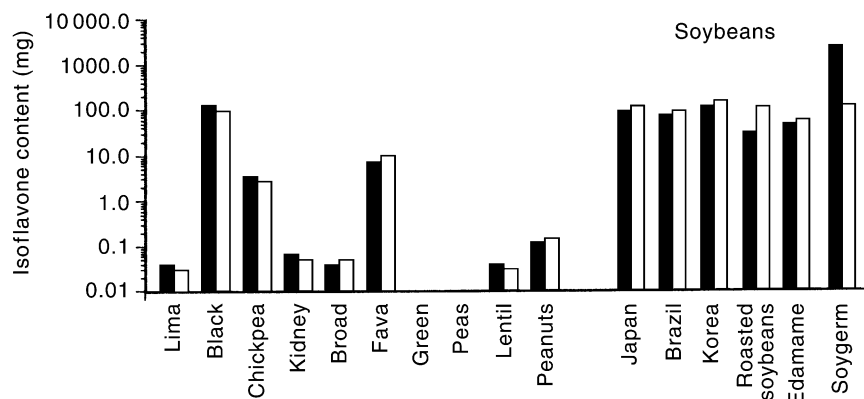


Figure 1. Isoflavone content of selected legumes expressed per 100 g (■) and per serving-size (□).

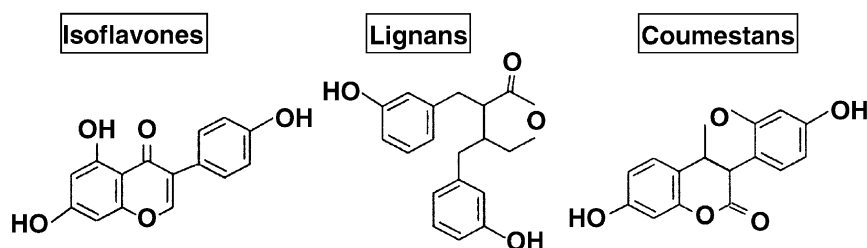


Figure 2. The three principal classes of phytoestrogens

sources of phytoestrogens,<sup>9–13</sup> this legume has undergone a renaissance in recent years.

### Phytoestrogens

Interest in the health-related effects of phytoestrogens has surged in recent years. Numerous dietary intervention studies have been performed in areas related to lowering cholesterol and cardiovascular disease, their effects on bone and as an alternative to conventional hormone replacement for postmenopausal women; these have been extensively reviewed elsewhere.<sup>14,15</sup> The results, although variable among studies, have largely been of a positive nature with regard to their health-related effects. The three main classes of phytoestrogens (Fig. 2) that have been most studied are the lignans,<sup>16</sup> for which flaxseed is a very rich source,<sup>17</sup> the isoflavones,<sup>18</sup> which are found in high concentrations in soybeans,<sup>9–13</sup> and the coumestans, which are much less frequently consumed in the human diet.

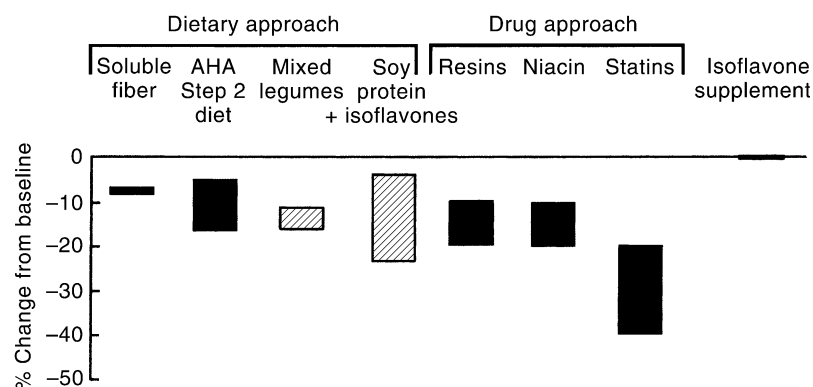
These oestrogen mimics have a wide range of hormonal and non-hormonal properties that may be the key to explaining why it is that countries where legumes are a nutritional staple have a low incidence of many of the common diseases that cause morbidity and mortality in the Western world.<sup>15,19</sup> It is our contention that increasing the dietary intake of phytoestrogens via a greater intake of legumes would have global implications for improving human health.

Can then soybeans and other legumes become the 'super foods' of the new millennium? The recent approval by the United States Food and Drug Administration (FDA) to allow the food industry to make health claims for soy protein lowering the risk of heart disease by its beneficial effects on blood lipids will unquestionably increase the credibility and awareness of legumes beyond just cardiovascular disease protection.<sup>20</sup> Approval of this health claim was based on a review of compelling data from more than 50 clinical studies, some of which were included in a meta-analysis,<sup>21</sup> which

showed that soy protein has hypocholesterolemic effects. It has been known for more than 40 years that soy protein can lower plasma cholesterol in subjects with hypercholesterolemia,<sup>22,23</sup> and a recent study of adults living in Japan showed that the plasma cholesterol concentration was inversely proportional to the daily intake of soy protein.<sup>24</sup>

The FDA approval for soy protein reducing the risk for heart disease has been controversial for several reasons. It has been argued that soy protein has little or no effect in lowering plasma cholesterol in healthy subjects or patients with mildly elevated plasma cholesterol, and that the amount of soy protein required for cholesterol reduction is high. In considering the former issue, we have summarized in Fig. 3 the effectiveness of soy protein and other mixed legumes in reducing plasma low-density lipoprotein (LDL) cholesterol compared to other cholesterol-lowering strategies.

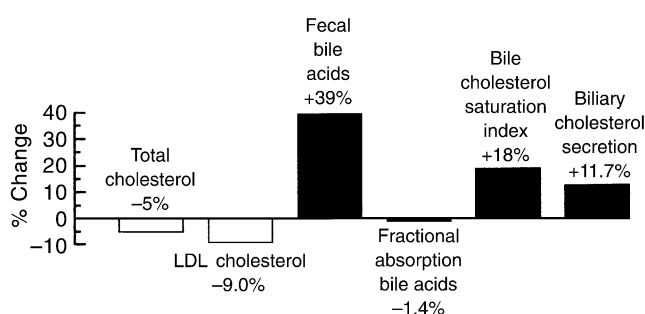
As is evident in some people with very high serum cholesterol concentrations, the hypocholesterolemic effect of soy protein is similar to that of several drug therapies and certainly comparable to, if not better than, that achieved by the American Heart Association (AHA) Step 2 (Fig. 3) diet to which it appears to have an additive effect.<sup>21</sup> The failure of modest amounts of soy protein to reduce serum cholesterol in normocholesterolemic subjects reflects the complexity of cholesterol homeostasis, although a study of healthy normocholesterolemic premenopausal women did show a mean reduction in serum total cholesterol of 9.6% when 60 g of textured vegetable protein was consumed each day for a one-month period.<sup>25</sup> In our opinion, one problem with the FDA health claim for soy protein reducing the risk for heart disease is that it implies a medicinal use for soy protein rather than a nutritional preventative role. It is doubtful that many Asians consume as much as 25 g of soy protein each day. A recent dietary survey of 4838 Japanese adults revealed that the average daily intake of soy protein by men was 8.00 g and by women 6.88 g, yet even at these relatively modest



**Figure 3.** The effectiveness of mixed legumes and soy protein in reducing LDL cholesterol.

levels of intake plasma lipids were low.<sup>24</sup> When the relationship between serum cholesterol and soy protein intake was examined, the effect of soy protein was similar to that concluded from the meta-analysis.<sup>21</sup> We believe that the real potential of soy protein lies in the ability to prevent increases in blood cholesterol, and the maximum benefits of soy protein and other legumes may be more apparent with earlier introduction into the diet. The AHA figures for 1998 indicate that at least 98 million adults have sufficiently elevated serum cholesterol levels to require some form of cholesterol-reducing strategy. More disturbing is the relatively large number of children with significantly high serum cholesterol levels. This is estimated in the United States to be about 26 million. The latest national figures for Australia from the Heart Foundation indicate that in 1989 over 4.5 million adults had elevated cholesterol levels.<sup>26</sup>

The main mechanism by which cholesterol is eliminated from the body is by its conversion into bile acids, which are then excreted in the faeces. Interruption of the enterohepatic circulation of bile acids leads to up-regulation of cholesterol 7 $\alpha$ -hydroxylase and HMG CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, which are the rate-limiting enzymes for bile acid and cholesterol synthesis. Legumes, in general, have a propensity to lower LDL cholesterol by this mechanism.<sup>27,28</sup> A detailed study of the changes in cholesterol and biliary lipids in healthy adults fed a diet of mixed legumes consisting of 60% beans (mainly red, navy and lima beans), 27% peas and 13% lentils revealed a 9% reduction in LDL cholesterol concomitant with a 39% increase in faecal bile acid excretion, although faecal neutral sterols were unchanged (Fig. 4). One consistent effect of diets high in legumes is the increase in cholesterol saturation that occurs, which seems to be the result of a decrease in the molar percentage of phospholipid in bile. It has been suggested that



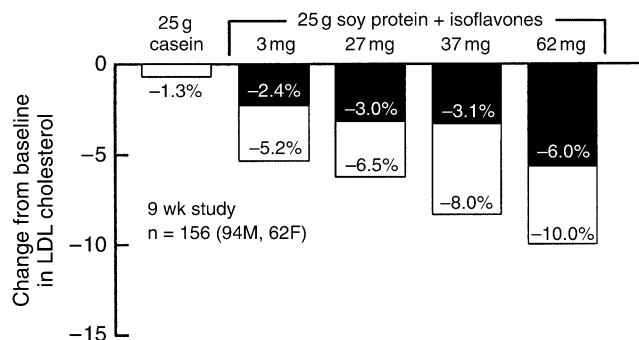
**Figure 4.** Changes in lipids in healthy adults consuming a diet of mixed legumes (data taken from study by Duane<sup>27</sup>).

such an increase in the cholesterol saturation index, which may be due to the presence of saponins in legumes, could lead to a predisposition of cholesterol gallstones in patients at risk. However, to our knowledge there are no data to suggest that people regularly consuming legumes have an increased risk for gallstone disease. Interestingly, vegetarians tend to have a lower prevalence of gallstones than non-vegetarians.<sup>29,30</sup> Clearly, the cholesterol-lowering effect of legumes is not just confined to soybeans.

It is almost certain that the cholesterol-lowering effect of soy protein and other legumes is accounted for by multiple factors. Some of these are discussed elsewhere.<sup>31</sup> Soy protein appears to up-regulate the expression of LDL receptors.<sup>32</sup> This has been confirmed from the finding that dietary isoflavones had no cholesterol-reducing effect in a LDLr-null mouse model deficient in LDL receptors and did not protect against atherosclerosis.<sup>33</sup> Effects of soy protein and isoflavones on gene expression have also been demonstrated in other animals.<sup>34</sup> The lack of cholesterol in soy protein and the relatively high proportion of polyunsaturated to saturated fatty acids are also factors in the overall hypocholesterolemic effects of soy protein, as these will contribute to a positive effect on blood lipids. Reducing significantly cholesterol intake will lower LDL cholesterol and it has been shown that soy reduces the absorption of cholesterol and bile acids.<sup>35</sup>

The role that phytoestrogens play in the hypocholesterolemic effect of soy protein is controversial, and the potential importance of isoflavones in reducing risk for cardiovascular disease may not have been recognized in the health claim as a result of the limited data that was presented. It was speculated in 1982 that soy isoflavones contribute to the cholesterol-lowering effects of soy protein,<sup>36</sup> but several studies have recently failed to demonstrate a significant cholesterol-lowering effect when isoflavone supplements were administered.<sup>37,38</sup> Similarly, it appears from animal and human studies that the cholesterol-lowering effect of soy protein is also lost or significantly reduced when the isoflavones are removed.<sup>39-42</sup> In an attempt to tease out the contribution played by isoflavones in the lowering of lipids, Crouse *et al.* fed 25 g of soy protein from which the isoflavones were first extracted by alcohol washing and then added back at different levels to recreate at the highest level the amounts naturally found in soy protein (Fig. 5).<sup>42</sup>

Their study showed a dose-dependent effect of isoflavones in the presence of soy protein on reducing LDL cholesterol in 156 adults. When the study subjects were subdivided according to their baseline serum LDL cholesterol



**Figure 5.** Teasing out the hypocholesterolemic effect of soy protein and isoflavones. (□) All (LDLc 3.62–5.17 mmol/L). (■) Subjects > 4.29 mmol/L LDLc. Constructed from data published by Crouse *et al.*<sup>42</sup>

concentrations, the lipid-lowering effect was more evident in those subjects with LDL cholesterol levels greater than 4.29 mmol/L, where mean 10% reduction was observed at a dietary intake of 62 mg isoflavones per day.<sup>42</sup> All of these studies pose intriguing questions regarding mechanism of action of soy protein and its constituent isoflavones on lipids. The reason for a need for both the soy protein matrix and isoflavones to attain lipid reduction remains unclear. While it is recognized that high serum cholesterol is a risk factor for cardiovascular disease, there are other properties of soy protein and isoflavones that also contribute to lowering risk.

The antioxidant effects of isoflavones<sup>43–45</sup> are important in reducing the susceptibility of LDL cholesterol to oxidation. Oxidized LDL cholesterol is not recognized by the LDL receptor but, moreover, is taken up by scavenger receptors that ultimately deposit cholesterol in the arterial wall to form the fatty streaks that lead to atheroma.<sup>46,47</sup> Several recent studies have shown that soy protein containing isoflavones reduces the susceptibility of LDL cholesterol to oxidation.<sup>48–52</sup> In addition, isoflavones appear to exert favourable effects on the arterial wall.<sup>40,53–55</sup> Improvement in arterial compliance in the absence of significant effects on serum cholesterol has been demonstrated for isoflavone supplements,<sup>37,38</sup> and even small reductions in blood pressure have been noted.<sup>56</sup>

### Functional foods based on soybean isoflavones

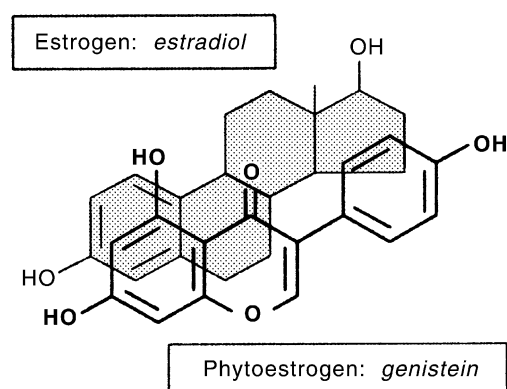
The low incidence of hormone-related diseases in countries where soy foods are regularly consumed is what originally stimulated interest in isoflavones.<sup>18,36,57,58</sup> The concept that there may be advantages to having functional foods that are enriched in phytoestrogens is gaining momentum within the food industry. The speed at which all this is happening is of some concern, and, if trends continue, the food chain may soon become saturated with phytoestrogens. All this is fuelled by the recent decision by the United States FDA to allow a health claim for soy protein in reducing risk for heart disease<sup>20</sup> because of the compelling evidence that this phytoestrogen-rich food is hypocholesterolemic.<sup>21,42</sup> Not unexpectedly, a plethora of soy isoflavone extracts and supplements are now commercially available<sup>59</sup> for which little is known about their metabolism and effects, but a great deal is known about the potency of isoflavones when ingested at very high doses.<sup>60–64</sup> It is this potency that has led to con-

cerns regarding the possibility that phytoestrogens may be a double-edged sword:<sup>65</sup> on the one hand offering benefits to some groups, while perhaps also creating risk to others. So what are the issues relating to soy and its isoflavones, and are they hypothetical or real? Below we briefly overview the background to phytoestrogens and then focus specifically on the evidence, or lack thereof, that has led to questions regarding the safety of soy foods for human consumption.

### Bioavailability and behaviour of isoflavones

Soy proteins and most soy foods contain the isoflavones, daidzein and genistein, mainly in the form of their respective glycosidic conjugates.<sup>10,11,13,66,67</sup> The basic chemical structure of isoflavones is almost identical to that of the steroidal oestrogens.<sup>68</sup> Figure 6 shows how closely the structures of genistein and estradiol superimpose on each other and, for this reason, it is perhaps not surprising that isoflavones have an affinity for oestrogen receptors because many compounds having a phenolic ring show an affinity for the oestrogen receptor.<sup>69</sup> However, recent X-ray crystallographic analysis of the oestrogen receptor<sup>70</sup> reveals that isoflavones behave much more like selective oestrogen receptor modulators (SERM) compared to oestrogens with regard to the conformational changes that take place on receptor binding.<sup>71</sup>

When ingested, phytoestrogens are metabolized in the intestine by the action of the microflora, absorbed, transported to the liver and eliminated primarily by renal excretion in urine.<sup>72</sup> Hydrolysis of the  $\beta$ -glycoside moiety is essential for bioavailability because there is no evidence that intact isoflavone–glycosides are absorbed, and there is ample  $\beta$ -glucosidase activity<sup>73</sup> along the length of the intestine to effect hydrolysis of the sugars from a very early age of life.<sup>74</sup> On first-pass, conjugation occurs with glucuronic acid, a reaction catalysed by one of the uridine diphosphate (UDP)-glucuronosyl transferases.<sup>75</sup> This reaction appears to take place both in the intestine and the liver.<sup>76–78</sup> In common with endogenous oestrogens,<sup>79</sup> isoflavones circulate in plasma, undergo enterohepatic recycling, and are excreted in urine mainly in the form of glucuronide and sulfate conjugates.<sup>58,80</sup> Concentrations of isoflavones range 100–600 ng/mL (or 0.4–2.5  $\mu$ mol/L) following the ingestion of modest amounts of soy protein foods, which far exceed plasma estradiol concentrations that are typically 30–60 pg/mL (120–240 pmol/L).<sup>19</sup> It is perhaps not surprising that at such



**Figure 6.** Comparison of the chemical structures of the soybean isoflavone, genistein, and estradiol.

high circulating plasma isoflavone concentrations significant hormonal effects can be demonstrated, especially given their low binding affinity for serum proteins.<sup>81,82</sup> Genistein and equol, for example, show an almost 10-fold lower affinity for sex hormone binding globulin (SHBG) than estradiol, so that a much greater proportion of these isoflavones are available for receptor occupancy.

#### **Phytoestrogens: Nature's selective oestrogen receptor modulator (SERM)**

There is no question that isoflavones are biologically active. They have a myriad of hormonal and non-hormonal properties,<sup>19,72,83,84</sup> and behave physiologically in a similar manner to endogenous oestrogens in binding to oestrogen receptors. However, with regard to the classical oestrogen receptor ER $\alpha$ , the binding affinity of isoflavones is highly variable, but relatively low compared with estradiol.<sup>85</sup> Genistein and equol, the intestinal bacterial metabolite of daidzein,<sup>58</sup> show similar affinities for ER $\alpha$  and considerably less for daidzein, while the methoxylated isoflavones, formononetin and biochanin A, which are the main isoflavones in commercial clover supplements being targeted at postmenopausal women, show extremely poor affinity toward ER $\alpha$ . The substitution of a methyl group in the B-ring of the isoflavone sterically hinders binding to the receptor. Isoflavones however, should be considered as reasonable oestrogens based on their almost eight-fold greater affinity toward ER $\beta$ .<sup>86,87</sup> This oestrogen receptor has been localized to a number of tissues that are specific sites of oestrogen action, including but not limited to bone, brain, vascular endothelia and bladder.<sup>86,88</sup> It is probable therefore that isoflavones exert selective oestrogen actions in these tissues in a manner not dissimilar from the actions of the anti-oestrogen tamoxifen.<sup>89</sup> These actions may be useful in managing acute and chronic conditions associated with oestrogen deficiency. So the time has arrived to no longer view phytoestrogens as oestrogens but rather as natural SERM, and in this there may be long-term benefit from early and chronic dietary intakes of these bioactive compounds. In this regard it has already been demonstrated in an animal model that prepubertal and neonatal exposures to the isoflavone genistein, either by the intraperitoneal<sup>90</sup> or dietary<sup>91</sup> route, leads to the animal becoming refractory to breast cancer when exposed to carcinogen later in life. These observations in a well-established model for human breast cancer suggest that it is the early and long-term dietary intake of phytoestrogens that may better explain the low incidence of this disease in Asian women. This contention is reinforced by the changing trends in disease patterns as the Asian diet in countries such as Japan is becoming more Westernised.

#### **Issues related to safety and toxicity of soy and isoflavones**

Several concerns have been expressed recently in connection with soy and phytoestrogens.<sup>92,93</sup> These are relevant as far as hypothetical grounds are concerned, but are not at all supported by clinical experiences.<sup>94</sup> It seems absurd to be questioning the safety of soy, or other legumes for that matter, when these foods have formed the dietary backbone of many cultures and when we are aware that the major diseases com-

mon to Westerners are of relatively low incidence in countries where soy foods are a staple.

In our opinion, the two main areas of legitimate relevance relate to the issue of early infant exposure to isoflavones from soy infant formula,<sup>95,96</sup> and the question of whether women who are at high risk for breast cancer or with pre-existing malignancy should avoid the use of phytoestrogen-rich soy foods or supplements. It has been suggested that soy isoflavones may be a cause for dementia and brain abnormalities.<sup>97</sup> Many myths have also surfaced, all having been the food for much media hype and alarm. It has been claimed that phytoestrogens are the cause of endocrine disruption in humans, and that these compounds interfere with pubertal development in boys and girls. Most of the concerns regarding safety are based on hypothetical considerations and are warranted, but there is little scientific evidence to support any of them. So what is the evidence?

#### **Soy foods, isoflavones and breast cancer**

Perhaps the most important issue to address is the role of phytoestrogens in breast cancer,<sup>65,83,98</sup> and this is particularly relevant to women who have either been diagnosed with breast cancer or who fall into the category of being at high risk for the disease. Some 80% of all breast cancers are ER-positive<sup>99</sup> (note that there are no clinical tests as yet to distinguish if tumours are ER $\alpha$  or ER $\beta$  positive), meaning they are stimulated to grow in the presence of oestrogen. The standard therapeutic approach that has been employed for more than 30 years for this type of breast cancer is to administer the 'oestrogen-like' drug, tamoxifen, which binds to these receptors antagonizing the action of endogenous oestrogen.<sup>89</sup> More recently, drugs that inhibit aromatase have been developed to inhibit the local synthesis of oestrogen in breast tissue.<sup>100,101</sup> The close similarity in the chemical structure of isoflavones to estradiol (Fig. 6) has unfortunately led phytoestrogens to be viewed as 'oestrogens'. As a result, and despite a lack of supporting clinical evidence for concern, some health professionals have recommended that women with breast cancer should avoid eating soy foods.

X-ray crystallographic analysis of the binding of genistein to the oestrogen receptor reveals it is similar to the manner in which the recently approved SERM, Raloxifene binds.<sup>89</sup> This drug was developed with the goal of finding an ideal oestrogen, one that would offer postmenopausal women the benefits of oestrogen, particularly on preventing osteoporosis and heart disease, without the negatives of increasing risk for breast and uterine cancers. Data from the Multiple Outcomes Raloxifene Evaluation (MORE) study of 7705 postmenopausal women followed over a 4-year period showed a highly protective effect of Raloxifene compared to placebo on the occurrence of breast cancer.<sup>102</sup> As soy isoflavones behave as natural SERM by virtue of their ER-binding characteristics,<sup>70</sup> it is logical to expect a protective effect of soy foods on breast cancer rather than the opposite. In 1984, soon after we discovered high levels of isoflavones in the urine and blood of people consuming soy foods, we proposed that these non-steroidal oestrogens would be beneficial in protecting against or treating breast cancer.<sup>18</sup> While this hypothesis is yet to be definitively proven, there is no evidence to indicate the opposite is true. On the contrary, there is a wealth of data from *in vitro* studies of breast cancer

cells grown in culture and from *in vivo* animal studies, where soy, or its isoflavones, have been administered, confirming the anticancer actions of phytoestrogens.<sup>15,83,98,103</sup> However, prospective clinical studies are needed to definitively establish if soy and isoflavones can protect against breast cancer, and 'secondary prevention' trials in breast cancer patients may help to clarify the safety of consuming soy foods for such patients. With regard to the latter, a small animal study did show that the chemotherapeutic effects of tamoxifen in animals with chemically induced mammary cancer was enhanced significantly when the fermented soy food miso was fed to the animals.<sup>104</sup> If this finding can be extrapolated to humans, then it should put at rest fears of consuming soy foods by breast cancer patients being treated with tamoxifen. Based on the evidence presented above, and in the absence of data to the contrary, there seems no reason to deter women with breast cancer from consuming soy foods.

### Soy foods and dementia

A recent report has suggested that isoflavones in tofu may be the cause of dementia and brain abnormalities in Japanese Americans living on the island of Oahu.<sup>97</sup> A study of 8005 adults followed since 1991 showed a dose-dependent increased risk of up to 2.8-fold for the development of vascular dementia when two to three or more servings of tofu were consumed weekly. Cognitive Abilities Screening Instrument (CASI) scores and brain weights were lowest in those subjects consuming the most tofu. The investigators first concluded that this study 'provides evidence that soy (tofu) phytoestrogens cause vascular dementia'.<sup>97</sup> Their study, while interesting, merely showed an association and not a cause/effect. The study had limited data on the diet and other factors could have explained the increased risk amongst the tofu eaters. Interestingly, miso was one of 26 foods that were examined, yet similar relationships were not reported for this phytoestrogen-rich fermented soy food. The investigators later concluded that age, education and history of a prior stroke explained 27.8% of the variance in CASI scores, while tofu intake accounted for only 0.8%.<sup>105</sup> Unless you are a Japanese American living on Oahu and consuming tofu, there seems little reason for concern and this report provides absolutely no evidence that phytoestrogens are the incriminating agents in brain ageing and cognitive decline. It should be noted that the incidence of dementia in mainland Japan is no higher than it is in the United States where tofu is not commonly consumed<sup>106</sup> and, interestingly, preliminary results from the Adventist Health Study showed that the risk for dementia was highest in meat eaters, not vegetarians consuming soy foods.<sup>107</sup> If phytoestrogens affect brain function, it is more likely to be in a positive manner, as we are aware that previous use of hormone replacement therapy by postmenopausal women is associated with decreased risk for development of dementia and improved cognitive function.<sup>108–110</sup> The brain is rich in oestrogen receptors, particularly ER $\beta$ , and it has been shown in rats that isoflavones rapidly appear in brain tissue after intraperitoneal injection.<sup>19</sup>

### Soy isoflavones and the infant

Perhaps the most controversial safety concerns about phytoestrogens have focused attention on soy infant formula. We first drew attention to the isoflavone content of soy formula

in 1987,<sup>12</sup> and reconfirmed this observation a decade later<sup>95</sup> while establishing that commercially available soy formulas made from soy protein isolates deliver to the infant between 22 and 45 mg of total isoflavones (mostly  $\beta$ -glycosides) each day.<sup>95,96</sup> This is in a similar range to the estimated isoflavone intake of Japanese adults adhering to a traditional diet containing soy foods.<sup>24</sup> All of the soy infant formulas produced for Western countries have been formulated with isolated soy protein and fortified with micronutrients since 1960, but in South Korea formula based on 'whole soybeans' has also been in commercial use for 30 years. Whole soybean-based formulas contain on average about five-fold higher levels of isoflavones than the formulas made from isolated soy protein products (data not shown but available on request). The plasma concentrations of isoflavones in infants fed soy formulas that were made from isolated soy protein are, not surprisingly, very high, exceeding endogenous oestrogen levels by 13 000–22 000 times.<sup>95</sup> This finding has led to concerns over the safety of soy formulas, although these are not supported by any clinical data. The difficulty in evaluating the safety of soy, or its constituent isoflavones, is that there is no satisfactory animal model to study infant development, and human studies are unlikely to yield definitive data unless they are powered high and done prospectively. This seems unlikely given the prohibitive costs of such an approach, so we are left with the experience from more than 40 years of soy formula use to draw conclusions. To this end, there are no clinical data pointing to adverse short or long-term effects from soy formula use in infancy. While clearly we would recommend that all infants be breast-fed, for those infants that cannot be or for women not wishing to breast-feed, it is our view that there is little reason to be concerned over the use of these formulas, while there may even be some long-term benefits to be gained from early exposure to phytoestrogens.<sup>90</sup>

### Soy isoflavones and thyroid function

Recent attention has been given to the possible goitrogenic effects of soy isoflavones because of their ability to interfere with the enzyme thyroid peroxidase (TPO) and to compete for iodine as a substrate.<sup>111,112</sup> The IC<sub>50</sub> for inhibition of TPO by the isoflavone, genistein, is considerably higher than the circulating concentration of free genistein in the plasma of infants fed soy formulas.<sup>95,113</sup> What is not mentioned is the finding that many flavonoids found in fruits and vegetables are also inhibitory on TPO with similar IC<sub>50</sub> values.<sup>112</sup> The average dietary intake of flavonoids<sup>114</sup> is similar to that of isoflavones when soy foods are consumed. If soy foods are a concern for the development of subclinical thyroid disease, then recommendations to avoid fruits and vegetables should equally be given; this is absurd given current nutritional guidelines. The susceptibility to thyroid disease is strongly linked to iodine deficiency and for most of the Western world there is adequate iodine in the diet, while soy formulas have been fortified with iodine since 1960. If thyroid problems are on the increase, it is more likely to be due to reductions in salt intake, as iodized salt is one of our main sources of iodine. The most important evidence for lack of effect of soy isoflavones on thyroid hormone status comes from our failure to find more than 12 case reports of thyroid disease precipitated by soy,<sup>21,115–121</sup> when in the past 40 years an esti-

mated 18 million infants have been raised on soy formulas in the United States alone. In a frequently cited report of a cohort of 59 children with autoimmune thyroid diseases, it was suggested that the frequency of soy infant formula use was greater in the patient group compared with a group of healthy siblings and controls.<sup>122</sup> This might be expected as autoimmune thyroid disease is gender biased occurring mainly in females,<sup>123</sup> as seen by the 4 : 1 female : male ratio in the patient group in their study. Yet the comparison groups of infants comprised mainly males. Interestingly, of the 59 children with autoimmune thyroid disease reported in the study, 35% were raised on cow milk formula, 34% on breast milk and 31% were fed soy formula.<sup>122</sup> The clinical experience with soy infant formula far outweighs the usual experience required for the approval of new pharmaceutical agents. Indeed, side-effects from common drugs that are prescribed every day far outnumber the known reported cases of adverse effects from soy infant formula, and if drug criteria for safety are applied to soy infant formula then it is, without question, safe. Thyroid hormones are crucial to normal growth and development in early life and many studies have shown that the growth and development of infants fed soy formula is no different from that of infants fed cow milk formula or breast milk.<sup>124,125</sup>

### Conclusion

Now that a health claim for soy protein is being allowed in the United States, soybeans and soy foods may well become the 'superfood of the millennium'. With regard to safety of these legumes, we are left to draw upon experiences from the long history of use of soy foods and other legumes in people living in countries where these are a dietary staple or in vegetarian groups. We know that both groups have high and sustained plasma isoflavone levels. In these groups, epidemiology speaks for the safety of soy foods, and this is reinforced by the recent observation that Westernization of the Japanese diet is now being linked to the increasing incidence of many hormone-dependent diseases for which we believe that phytoestrogen-rich diets are protective. Finally, there is a paucity of information regarding the pharmacokinetics and bioavailability of isoflavones<sup>59,126–128</sup> from foods and supplements, which makes it difficult to rationalize the most appropriate recommended dietary intake for optimal health benefits without endangering humans to potentially deleterious effects that could occur with mega-doses. Phytoestrogens have been found to cause reproductive abnormalities in several animal species.<sup>60–62,64</sup> These effects can be explained by species differences in the metabolism of phytoestrogens or by chronic ingestion of extremely high doses. In our opinion, there is no logical reason for the development of functional foods with high levels of isoflavones or other phytoestrogens, and it is of some concern that some food manufacturers are now developing or marketing foods that are fortified with isoflavone levels in the range 90–160 mg per serving. People living in countries where soy is a staple rarely consume these levels of intake. Recent dietary surveys of Japanese adults indicate the daily soy protein intake to be in the range of 6–9 g, which we estimate would be expected to contain a total of 18–30 mg of isoflavones, at most.<sup>24</sup> A more appropriate and safe approach to functional food development should be to have modest levels (10–20

mg/serving) of intake of phytoestrogens throughout the day and to maximize the potential long-term beneficial effects of these bioactive phytoprotectants based upon knowledge of their pharmacokinetics.<sup>59</sup> This, after all, is more typical of what happens in cultures where legumes are a staple. We therefore concur with dietary recommendations promoting an increased intake of pulses. The World Health Organization's view is that for chronic disease prevention the consumption of a daily minimum of 30 g of pulses, together with nuts and seeds, is helpful for the prevention of some types of cancer and coronary heart disease.<sup>129</sup> The nutritional benefits of legumes are well established. In closing, we quote the recent statement, 'Forget electricity, the microchip and the car. Without legumes, we wouldn't have made it out of the Dark Ages'.<sup>130</sup>

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