7th International Soy Symposium
Role of Soy in Health and Disease Prevention

March 7-9, 2007, Shangri-La Hotel, Bangkok, Thailand

In the **Opening Address** *Dr Mark Messina, Nutrition Matters, USA* highlighted that this was the first of the International Soy Symposia to be held in Asia. The Permanent Secretary for Thailand opened the conference, commenting that the public is increasingly interested in health and nutrition and that prevention is better than cure. Following a cultural Thai dance *Dr Kraisid Tontisirin, Former Director, Food and Nutrition Division, FAO (Rome), Thailand* gave the **Keynote Address – The Role of Soy in Nutrition & Health Promotion in Asia.**

Dr Tontisirin highlighted the double burden of malnutrition in Asia – i.e. problems of both under and over nutrition in the same population. For example in the Philippines 30% of children are underweight, while 30% of adult women are overweight. In South East Asia and China, 61% of deaths are attributable to non-communicable diseases. The 2002 World Health Report analysed the causes of death globally and concluded that many of the risks have a dietary origin. Since soy is a low cost source of protein, and provides high quality oil, other nutrients and several bioactive compounds, a regular intake can deliver potential health benefits. Scientific research has highlighted the potential benefits of soy in reducing serum total and low density lipoprotein (LDL) cholesterol, and the use of health claims for this effect has been approved in the USA and the UK. While soy is a traditional part of the Asian diet the advance in food science and technology has led to the development of a number of different products containing soy such as soy nuts, energy bars and breakfast cereals. There is a need for further innovative developments to promote the increased consumption of soy products, to meet consumer demands for nutrition and wellbeing.

OPENING SESSION: SOY AND HEALTH – AN OVERVIEW

Dr Mark Messina, Nutrition Matters, USA discussed **Health Effects of Soy – Perspectives on Recent Findings.** There has been a huge amount of research on the health benefits of soy foods over the past two decades, with evidence for benefits against certain cancers, heart disease and menopausal symptoms. However, recent reports in the media have questioned the extent of these effects, leading to consumer confusion. It is difficult to research dietary influences on chronic disease and there is no area of nutrition where the data are fully consistent. The prohibitive cost of conducting dietary trials leads to small numbers of subjects and a shorter duration of trials than is ideal, and endpoints therefore tend to be disease markers rather than disease end-points per se. This all contributes to some inconsistencies in the data. Other inconsistencies arise from variations in subject characteristics such as differences between pre- and postmenopausal women, and from the use of different soy products, the form, and content of bioactive ingredients. The National Institute of Health in the US thus wants to standardise the use of soy in dietary trials, and is organising a conference in September 2007 to progress this issue.

A further source of consumer confusion has been an analysis by the American Heart Association (AHA) of recent trials investigating the effect of soy on serum cholesterol levels, which concluded that the effect is smaller (3-5% reduction in LDL cholesterol) than previously suggested. Nevertheless, the AHA report concluded that many soy products should be beneficial to cardiovascular and overall health because of their

high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat..

Dr Messina then highlighted that the French authorities have set a very low intake limit of 1mg isoflavones per kg body weight per day in adults with soy consumption not advised under the age of 3 years, and Israel has issued recommendations with no basis in the scientific literature. While the precautionary principle is being applied, much of the concern is pure speculation based on rodent research – yet for a number of reasons rodents do not predict effects in humans. Isoflavone metabolism is different between species, and in many animal studies isoflavones are administered directly into the blood stream rather than via the diet.

Despite recent confusing media reports, soy foods remain important additions to a healthy diet. Taking the conclusions of the AHA analysis a 3-5% reduction in LDL cholesterol remains an important and significant effect on public health. The British Medical Journal reported in 1994 that a 1% reduction in LDL cholesterol lowers the risk of coronary heart disease (CHD) by 2-4%, such that a 3% reduction in LDL will result in an approximately 10% reduction in CHD risk – which is impressive for a dietary component. The AHA report did not consider the effects of soy on other risk factors for CHD such as LDL particle size and clot formation, such that the actual benefits may be much greater.

The evidence also suggests benefits of soy for postmenopausal women. A study has shown that soy protein intake is very protective against non-fatal myocardial infarction in postmenopausal women. More than 30 trials have examined the ability of isoflavones to inhibit postmenopausal bone loss and, although the data are mixed, the overall findings are suggestive of a benefit. Evidence suggests that soy intake can also help to alleviate hot flushes. A recent study designed to investigate effects on bone health, found a statistically significant reduction in the frequency of hot flushes after 12 months of treatment, in the group taking 54mg genistein per day compared with placebo.

Is Isoflavone Metabolism the Key to Understanding the Health Effects of Soy? was discussed by *Dr Shaw Watanabe, National Institute of Health and Nutrition, Japan.* Soy has been part of the Asian diet for the past 2000 years. Despite being a traditional food in Japan, soy is being investigated for health properties, and the isoflavone components is a particularly active research topic in Japan as elsewhere in the world. However the mechanism of action of soy is still not clarified.

In addition to being estrogen modifiers, various other functions are associated with the isoflavones. They have a similar structure to estrogen and thus could exert some estrogenic activity.

Estrogenic Compound	Relative Estrogenicity
Estradiol	1000
Daidzein	2
Genistein	10
Estrone	1000
Equol	5

The isoflavones can bind to the two estrogen receptors (ER), ER-_ and ER-_, but with much lower binding affinity than estradiol and they mainly bind to ER-_.

Estrogenic Compound	Relative Binding Affinity for ER	
	ER-_	ER-_
17-_-estradiol	100	100
Daidzein	0.1	0.5
Genistein	4	87

A Japanese database of the isoflavone content of food has been developed. The estimated median intake of isoflavones is 40mg/day – which is greater than the intake of flavonoids or carotenoids. Following the ingestion of soy protein powder plasma levels of daidzein and genistein peak at 6 hours and then decline over the next 72 hours.

Daidzein can be metabolized to equol by particular intestinal bacteria. Not all individuals can produce equol, and the bacteria responsible for this phenotype have not yet been identified. To investigate the pharmacokinetics of supplemental equol, three groups of subjects received either 10mg or 30mg equol per day given in one dose, or 30mg per day given as three 10mg doses. Following a single dose plasma equol peaked at 1 hour, while the administration of three doses of equol resulted in 3 peaks of similar plasma equol concentration. Female subjects had twice the peak concentrations of males. The urinary excretion pattern showed two groups, one with higher excretion compared with a group with slow and low levels of excretion, irrespective of dose.

Equol supplementation has been shown to reduce both diastolic and systolic blood pressure in peri-menopausal women with high blood pressure but not in those with normal blood pressure.

Future trials and meta-analyses of the effects of soy should consider whether subjects are equol producers and the source and dose of isoflavones given.

SESSION 1: SOY AND CARDIOVASCULAR DISEASES

Dr Suzanne Ho, The Chinese University of Hong Kong, Hong Kong opened the first session with her talk **Cholesterol Lowering Effects of Soy Protein: A Review of the Clinical and Epidemiological Data.** In view of the apparently contradictory evidence on soy and cholesterol lowering Dr Ho gave a brief overview of the evidence in this important area – cardiovascular disease (CVD) accounted for 36.3% of all deaths in the USA in 2004. Since publication of the Anderson meta-analysis in 1995, which reported a 12.9% reduction in LDL cholesterol, soy protein has gained much attention for its role in improving CVD risk. Many further trials have been conducted. However, varied forms and amount of soy protein have been used, with different, including nil concentrations of isoflavones. Study populations have had lower baseline lipid concentrations than those in some of the earlier trials and the trials have been of small sample size and short duration.

Recently conducted meta-analyses suggest a smaller effect of soy than previously reported by Anderson. The Sacks review reported a 7% reduction in total cholesterol among subjects with initial cholesterol levels between 259 and 333mg/dl, but little

effect in those with mildly elevated or average cholesterol levels. A review by Reynolds included studies testing isolated soy protein supplementation and reported a significant reduction in triglycerides and total and LDL cholesterol with a significant increase in high density lipoprotein (HDL) cholesterol among subjects with or without hypercholesterolaemia. Weggemans reported a 4% reduction in LDL and a 3% increase in HDL cholesterol. An analysis by Zhan and Ho included only trials assessing the effects of soy protein containing isoflavones on lipid profiles. A significant reduction in triglycerides and total and LDL cholesterol with a significant increase in HDL cholesterol was noted, but the changes were related to the level and duration of intake and the gender and baseline serum lipid concentrations.

	LDL	**HDL**
Anderson 1995 [1]	-12.9%	+2.4%
Sacks 2006 (AHA) [2]	-3%	+1.5%
Reynolds 2006 [3]	-4.25mg/dl	
Weggemans 2003 [4]	-4%	+3%
Zhan & Ho 2005 [5]	-5.25%	

Further evidence about the relationship between soy and blood lipids comes from population-based studies. A study in Hong Kong used telephone survey methodology to investigate daily soy protein intake. For men and pre-menopausal women there was a significant association between soy protein intake and the concentration of plasma LDL cholesterol. Nagata has conducted a population-based study in Japan of 1242 men and 3596 women. There was a clear trend for lower cholesterol concentration from the highest to the lowest quartiles of soy protein consumption.

While a hospital based study of postmenopausal Japanese women reported no significant difference in blood cholesterol levels with soy intake, a study in premenopausal normolipidaemic women found a 5.3% reduction in total cholesterol with the consumption of 400ml soy milk supplement per day – which agrees reasonably well with the population studies.

A very recent study has found a positive association with high soy protein intake and change in HDL cholesterol in women aged >55 years (10 years postmenopause), but no effect on LDL cholesterol.

Some of the factors that may explain different effects in the studies include the proportion of the subjects that are responsive to soy intake and that may have a genetic predisposition to respond to soy.

Dr Ho emphasised the benefits of small reductions in cholesterol concentration on reduced risk of CVD, and that these effects can have important implications at the population level. Soy is a food and not a drug so even a small reduction in CVD risk is of considerable importance at the population level. There is likely to be an even bigger effect if soy replaces foods high in saturated fat. Dr Ho concluded that we should enjoy soy foods, but not expect a magic bullet.

In the question and answer session an important issue raised is whether less than 25g soy protein per day reduces blood cholesterol, as lower intakes are a more manageable

amount to eat. Dr Ho concluded from the population studies that lower intakes do have a cholesterol lowering effect.

Dr Paul Nestel, Baker's Heart Research Institute, Australia then discussed the **Impact of Soyfoods and Isoflavones on Arterial Health.** He began by explaining that the arteries contain a large density of ER-_ in men as well as women, and this stimulates cellular signalling proteins. Estrogens are epidemiologically and prospectively associated with less CHD risk, and this is probably very similar for isoflavones. Soy isoflavones inhibit *in vivo* coronary artery obstruction by platelets and improve *in vitro* coronary dilatation.

Professor Nestel has undertaken studies investigating arterial compliance. Loss of arterial compliance leads to stiffness of the arteries and is a risk factor for CHD. The methodology has improved recently leading to more reliable results. Newly diagnosed CHD patients usually have less arterial compliance than normal individuals, and in prospective studies increasing stiffness of arteries predicts adverse CV events. Can arterial compliance be improved? In a crossover trial with two 6-week intervention periods in 21 peri- and postmenopausal women 25% of the subjects showed significantly greater arterial compliance following the period with isoflavone supplementation (45mg genistein and 38mg daidzein) than with placebo (P=0.011), demonstrating that isoflavones significantly reduce arterial stiffness. Since women lose the capacity to dilate arteries in forearm vessels after the menopause, this suggests an estrogen/receptor interaction, and hence these findings are of interest.

Further studies have investigated whether metabolites of isoflavones are equally bioactive to the isoflavones. Dehydroequol, a metabolite of daidzein has been shown to stimulate vasodilatation in a dose-dependent manner and this effect is mediated via nitric oxide. Another metabolite of daidzein, trans tetrahydrodaidzein, significantly reduced arterial stiffness and blood pressure when given at doses of 1g per day for 5 weeks to a group of 25 overweight, dyslipidaemic subjects. Another study has shown that genistein taken daily for 6 months improved flow mediated vasodilatation (FMVD) of the brachial artery, an index of endothelial function.

Professor Nestel concluded that vascular function is a most consistently observed target of isoflavones, that isoflavones reduce arterial stiffness, and that metabolites of daidzein are equally biologically active in inducing these effects.

Professor Ken Setchell, Cincinnati Children's Hospital Medical Center, Ohio,USA discussed Improvement of Markers of Cardiovascular Risks Using Pasta Naturally Enriched with Isoflavones. There is a debate whether soy protein, soy isoflavones or some interaction between these two components are responsible for the cholesterol-lowering effect. Isoflavone supplements alone have been shown to have no or little effect on blood lipids. Working in Italy, Professor Setchell has developed pasta enriched with naturally sourced isoflavones from soy germ. The pasta is indistinguishable from conventional pasta, and delivers 33mg isoflavones per serving with only 0.6g soy protein – giving the opportunity to investigate the effects of isoflavones.

In a study in 62 newly diagnosed patients with hypercholesterolaemia a number of end points were measured following consumption of isoflavone-enriched or

conventional pasta. The study was a single crossover design, with subjects following a step II diet at baseline. The test group incorporated isoflavone-enriched pasta into their diet for 4 weeks compared with conventional pasta in the control group. The test group then switched to a further 4 weeks on conventional pasta. The LDL cholesterol level at baseline was 185 mg/dl ±4 and this was reduced to 169mg/dl±4 after 4 weeks on the isoflavone-enriched pasta (8.6% reduction), and returned to 181mg/dl after the further 4 weeks on conventional pasta. In contrast LDL cholesterol in the placebo group was unchanged throughout the study.

Other endpoints in the study investigated inflammation, blood vessels and antioxidants. C-reactive protein (CRP) was measured as a marker of inflammation. Hypercholesterolaemia results in low-grade inflammation and the study group were approaching the upper limit of the normal range for CRP. After 4 weeks on the conventional pasta CRP levels worsened in the control group. In the group receiving isoflavone-enriched pasta, CRP was reduced by 42% and after a further 4 weeks on conventional pasta CRP had returned to baseline levels. Brachial artery FMVD was improved after 4 weeks on the isoflavone-enriched pasta compared with the baseline values, while there was no beneficial change in the controls.

In this study 69% of the group were equol producers, which is unusually high for a Western population. In the isoflavone-enriched pasta group, the equol producers showed a considerably greater reduction in CRP and increase in FMVD compared with the non-producers.

To explain these profound effects Professor Setchell raised the possibility that there may be a unique interaction between the wheat and the isoflavones. In soy germ glycosidic forms of the isoflavones predominate over the aglycone forms, while in the isoflavone-enriched pasta the glycosides are dramatically reduced and the aglycones predominate. This may be due to the presence of beta-glucosidase in the wheat, representing an important food matrix effect. Professor Setchell also pointed out that in Western soy foods (soy flour, protein isolate, soy milk) the glycosidic forms of the isoflavones predominate, whereas in Asian fermented soy foods (tempeh, miso) the aglycone forms predominate. He hypothesised that this difference may possibly explain why the proportion of equol producers is higher in Asian than in Western populations, and why there was an unusually high proportion of equol producers in the Italian study.

In the question and answer session Professor Setchells highlighted that the real benefit of soy is in prevention. It is not realistic to believe that trials can demonstrate the reversal of a major disease of the vasculature, but incorporating soy earlier in life may prevent the changes that lead to cardiovascular events.

To round up the first session *Dr John Erdman, University of Illinois,* discussed **Putting the Coronary Benefits of Soy into Perspective.** He explained that of the 38 dietary trials included in the Anderson 1995 meta-analysis the range of soy protein intakes was 17-124g per day (average 47g/day), and in some of these studies soy protein represented the entire protein intake. Some were hospital-based studies, and some subjects had very high baseline cholesterol levels. Consequently, large reductions in the cholesterol concentrations were observed in these populations. More recent studies suggest that the reduction in cholesterol with soy may not be as large as

originally proposed, especially in free-living populations. Clinical trials with larger numbers of subjects are needed to ascertain whether smaller intakes of soy products are relevant for cholesterol lowering.

SESSION 2: SOY AND CANCER PREVENTION

Dr Mark Messina, Nutrition Matters, USA opened the second session with his talk **Making Sense of the Soy and Breast Cancer Relationship.** There is a huge amount of interest in the potential role of soy foods in reducing the risk of breast cancer. However, despite an impressive amount of research over the past 15 years, there is no clear consensus that adult soy intake reduces breast cancer risk. A recent meta-analysis of the epidemiological data including 18 studies concluded that soy intake may be associated with a small reduction in breast cancer risk: odds ratio, OR=0.86 for all women. There was no association with Asian countries.

While there are several putative chemopreventive agents in soy, research has mainly focused on the isoflavones. However, the clinical data also provide little persuasive evidence for a protective effect of soy although there is some evidence that estrogen metabolism is favourable altered.

However, a more positive emerging hypothesis is that intake of soy in early life may reduce breast cancer risk. Animal data suggest that exposure to genistein during the first 3 weeks of life reduces chemically-induced tumour development during adulthood, and these findings are supported by the results of epidemiological studies, One study has shown a 50% reduction in breast cancer risk in women who were the highest soy consumers at 13-15 years of age. A further study by the National Cancer Institute interviewed women and mothers to assess soy intake at age 5-11, 11-19 and 20+ years. Those in the highest tertile of soy consumption at age 5-11 were 58% less likely to have breast cancer at a later age (OR 0.42) leading some commentators to suggest that where there is a history of breast cancer in a family, girls should be given soy foods at an early age.

Dr Messina also considered the data on safety of soy consumption for breast cancer patients. Whereas hormone replacement therapy (HRT) increases breast tissue density, which is linked to breast cancer risk, isoflavones do not have this effect.

Dr Messina concluded that we are unlikely to ever conclude that adult intake of soy reduces breast cancer risk, but early intake may reduce risk. Clinical studies are needed to further investigate this hypothesis. Finally, from his analysis of the evidence he concluded that soy is not contraindicated for any group of women.

Dr Gertarud Maskarinec Cancer Research Center of Hawaii, USA further explored the early intake hypothesis in her talk **Exploring the Possible Influence of Soy Consumption during Early Life on Adult Breast Cancer Risk.** She began by agreeing with Mark Messina that the adult intake hypothesis does not look exciting, but that the timing of soy consumption may be crucial for a preventive effect against breast cancer. There are various potential mechanisms for such an effect. For example studies suggest that the isoflavone genistein may help breast nodules to mature to a type that is more resistant to proliferation. A possible direct effect is that due to their weak estrogenicity isoflavones accelerate differentiation of breast tissue structures, similar to the effect of an early pregnancy. Alternatively soy foods may act indirectly

through body composition or body weight, determinants of hormonal development and age at menarche.

Evidence for the early intake hypothesis comes from epidemiological studies, including the observation that breast cancer rates doubled among first generation Japanese migrants to Hawaii, and tripled during the second generation. Third and Fourth generation immigrants now have similar breast cancer rates to Caucasians. This is not due to inter-marriage and suggests that some etiologic factor may act during childhood or adolescence. Dr Maskarinec then described preliminary research to enable a study in children to be undertaken, to further investigate the hypothesis.

Soy and Prostate Cancer – An update, was the title of the talk by *Professor Mindy Kurzer, University of Minnesota, USA*. Rates of prostate cancer have historically been low in Asian countries, and though there has recently been a huge increase in rates (for example between 1978 and 1997 prostate cancer rates increased by 100% in Japan and Singapore), they remain much lower than in Western countries. Is it possible that the change in prostate cancer rates is linked to soy consumption?

This hypothesis is supported by *in vitro*, animal and epidemiological studies. The epidemiological data show that prostate cancer incidence increases after native Asians migrate to the USA. Although a meta-analysis of 8 epidemiological studies found that only 3 of the studies showed significant differences, the pooled estimate of risk from the 8 studies overall showed a 30% reduction in prostate cancer risk with soy consumption (OR 0.70, 95% CI 0.59,0.83; P<0.001).

There are a number of possible mechanisms for a protective effect of soy. These include down regulation of androgen receptors, inhibition of androgen synthesis, increased apoptosis, reduced cell proliferation and reduced metastasis, among others. Studies investigating the effects of genotype have found that polymorphisms influence the effect of phytoestrogen-rich foods on prostate cancer risk.

However clinical studies show mixed results, with no consistent effects for the consumption of soy on intermediary biomarkers of the disease. One review has shown virtually no effect of soy isoflavones on circulating levels of reproductive hormones. However a study has shown that in men with prostate cancer soy supplementation beneficially increases prostate specific antigen (PSA) doubling time (i.e. reduces the increase in PSA over time). A further study has reported a trend to reducing PSA with 3-months soy isoflavone supplementation.

Professor Kurzer described a new study in which subjects received 40g soy protein given in two doses per day and providing 107 mg isoflavones (expressed as aglycone) for 6 months compared to milk protein. The 58 high-risk subjects (average age 68 years) had undergone a prostate biopsy because they were at risk of prostate cancer. There was no effect of soy consumption on sex hormone binding globulin (SHBG), testosterone, total or free PSA or PSA%. Soy had no effect on ER_ expression but improved estrogen metabolism. There was a significant reduction in androgen receptor expression in the prostate, which indicates a reduced risk of prostate cancer. There was no effect on circulating estrogens, but there was an increase in urinary estradiol and estrone, both associated with reduced risk of prostate cancer. Moreover at the end of the study the results showed that progression to prostate cancer in the 6-

month period was significantly reduced in men consuming soy, supporting the suggestion that soy consumption lowers risk of prostate cancer.

Professor Kurzer concluded that research into soy and prostate cancer is at a much earlier stage than for breast cancer. More proteomic and DNA work is needed to determine which genes are influenced by soy. Additional intervention studies investigating tissue biomarkers and more prospective cohort studies are also needed.

The next three presentations were on the topic of **Soy and Cancer – Studies in Asia**. *Dr Yu-Tang Gao, Shanghai Cancer Institute, China* described a population based case-control study conducted in China to evaluate soy food intake and breast cancer risk in 1459 breast cancer cases and 1556 age-matched controls. Data collected by food frequency questionnaire (FFQ) showed that the average level of soy intake is relatively high compared with Western populations and that there is a wide range of intake (grams soy per week: median of 655g, with 25th percentile of 350g and 75 th percentile of 1250g). The OR for breast cancer showed a 34% reduction in risk in the highest compared with the lowest decile for soy intake (OR 0.66, 95% CI 0.46-0.95). A more pronounced effect was observed among those women who reported no recent change in soy food intake (OR 0.46, 95% CI 0.28-0.75). The urinary excretion of phytoestrogens was significantly lower in breast cancer cases. The effect of soy intake during adolescence (13-15 years) was also investigated and was found to be inversely associated with the risk of breast cancer in adult life. After adjustment for confounding factors, the OR for adult breast cancer risk in the highest compared with the lowest quintile of adolescent soy intake was 0.51 (95% CI 0.40, 0.65). The results support the hypothesis that high soy intake during adolescence may reduce the risk of breast cancer in later life.

A further case-control study investigated soy intake and risk of endometrial cancer in 832 cases and 846 age-matched controls. The OR for endometrial cancer was 0.77 in the highest quartile of isoflavone intake compared with the lowest quartile. Finally, a new large-scale population-based prospective cohort study involving 73,294 women aged 40 to 70 years and living in urban Shanghai has shown that the relative risk for colorectal cancer is 0.65 after 7 years of follow-up for the highest compared with the lowest tertile of soy intake.

Dr Chisato Nagata, Gifu University, Japan explained that the rate of breast cancer in Japanese women has increased since 1970 but remains lower than in Caucasians, or in Hawaii and Japanese Immigrants in Hawaii. Since soy intake has been suggested to lower breast cancer incidence, average isoflavone intake levels were assessed for the period 1975 to 2000 from the Japanese National Nutrition Survey. Mean intakes were slightly higher in men (25.2 mg/day) than in women (24.4 mg/day). Three case control and 3 cohort studies have been conducted in Japan to investigate the relationship between soy and breast cancer. Overall the data are inconsistent, with only 1 cohort and 1 case control study showing a significant inverse association.

Dr Nagata also referred to a meta-analysis of 18 epidemiological studies of soy intake and breast cancer risk and showed an overall relative risk of 0.93 for the case-control studies..

Dr Adeline Seow, National University of Singapore, Singapore discussed the significant increase in rates of various cancers that has been observed in Singapore over the last few years. Singapore is a dense urban population and while many of the Chinese population maintain the traditional Chinese diet, there is a gradual transition to a Western diet. The Singapore-Chinese Health Study is investigating cancer and dietary associations at a number of specific sites. A collaborative project has shown that women in the lowest quartile of soy intake are more likely to have mammography showing a higher risk of breast cancer. The increase in breast cancer rates over the last 40 years or so shows a birth cohort effect. The lines of each cohort delineate early in life, which is consistent with the early intake hypothesis.

Data from the Singapore-Chinese Health Study show that there is no association for lung cancer in smokers with soy. However, in non-smokers the upper tertile of soy intake had only half the risk compared with the lower tertile. Dr Seow concluded that there may be value in investigating other sites of cancer in this study.

SESSION 3: SOY AND BONE HEALTH

Dr William Wong, Baylor College of Medicine, USA began this session with a discussion of **Soyfoods, Isoflavone Supplements and Bone Health: A Review of the Existing Data and Results from the Osteoporosis Prevention Using Soy Study (OPUS).** Dr Wong referred to an analysis of 17 *in vitro* studies, 24 *in vivo* studies in animal models, 15 epidemiological studies and 17 dietary intervention studies which concluded that phytoestrogens have bone-sparing effects in the long term. However the size of the effect and the mechanism of action are not well defined. More recent studies also show potential benefits of soy consumption or isoflavone intake on bone, except for one study.

Dr Wong then discussed the OPUS study (osteoporosis prevention using soy) that is investigating isoflavone intake in postmenopausal women. It is funded by the United States Department of Agriculture (USDA) and the Cooperative State Research Education and Extension Service (CSREES). It is a multi-centre trial in three study sites in the USA, set up to investigate the safety of isoflavone supplements, whether they reduce bone loss in postmenopausal women, if any benefit is maintained over 2-years of continuous treatment, and if there is any change in bone metabolism.

The 400 subjects were randomised into the placebo group, a group receiving 80 mg isoflavones per day, and a group receiving 120 mg isoflavones per day. The subjects also received 1000 mg calcium carbonate and 400 IU vitamin D per day. Preliminary results show that isoflavone supplementation at these levels is safe, and that there is a favourable effect on whole body bone mineral density, but no effect on whole body bone mineral content or on bone mineral content and bone mineral density of the lumbar spine and pelvic regions. There is also no difference in bone metabolism between the groups.

In the question and answer session, it was highlighted that other long-term studies are currently underway and there should be a lot of new data coming out over the next few years.

In his talk **Japanese Population Based Osteoporosis Study (JPOS) – Intake of Fermented Soybean (Natto) and Reduced Bone Loss in Postmenopausal Women**

Dr Yukihiro Ikeda, Kinki University School of Medicine, Japan described a 3-year Japanese population-based osteoporosis study in 4550 women aged 20-79 years. Dr Ikeda's study investigated habitual intake of the traditional fermented soy food 'natto' in 944 women who participated in this study, and in whom bone mineral density and change in bone mineral density were measured. Natto contains isoflavones and a high content of menaquinone-7 (vitamin K2).

In premenopausal women (aged 20-44 years), there was no association between natto intake and bone mineral density at the spine, total hip or femoral neck assessed by dual energy x-ray absorptiometry (DEXA). In postmenopausal women (aged 44 years and over) higher natto intakes (>4 times per week) were associated with greater bone mineral density at the total hip and there was a positive association between natto intake and the rate of change in bone mineral density at the femoral neck and the distal third of the radius. After adjusting for potential confounders an association for higher intakes of natto and a beneficial effect on rate of change of bone mineral density in the femoral neck in postmenopausal women remained significant. There was no relationship between intake of tofu or other soybean products and the rate of bone mineral density change in postmenopausal women. Dr Ikeda concluded that the potential mechanism for the effect of natto should be addressed.

Dr Yu-Tang Gao, Shanghai Cancer Institute, China then discussed **Soyfood Consumption and Risk of Bone Fracture among Postmenopausal Women.** The Shanghai Women's Health Study is a large cohort study in which the population has a high and wide range of soy intake. It is thus ideal to investigate associations between soy intake and the risk of bone fracture. The study was launched in March 1997 and 75,221 women were recruited at baseline. The analysis included 24,403 postmenopausal women who had never used hormone replacement therapy and who reported no history of bone fracture. A first follow-up was conducted between 2000 and 2002, and a second follow-up between 2002 and 2004, with a very high response rate for each. The OR for bone fracture adjusted for age and energy intake was 0.63 in the highest compared with the lowest quintile of soy protein intake. For soy isoflavone intake the OR was 0.65. If the group was then stratified into time since the menopause, the OR for women within 10 years of the menopause was 0.52, compared with 0.71 for women who had undergone the menopause longer than 10 years ago.

Dr Gao concluded that this shows evidence of a significant inverse association between soy and risk of fracture in postmenopausal women, particular those in the early years since the menopause. The size of the cohort is so large that we can be confident of this data.

Dr Suzanne Ho, The Chinese University of Hong Kong, Hong Kong then discussed **Bone Health in Adolescent Girls and Young Women in Hong Kong and Implications on Acquisition of Peak Bone Mass.** While a cross sectional study in Hong Kong in postmenopausal women has shown a positive linear association for both increased femoral neck bone mineral density and bone mineral content with quartiles of soy intake, there have been few studies in young women. A prospective study in Hong Kong investigated 187 healthy women aged 30-40 years over 38 months. Diet was assessed by FFQ and bone endpoints were determined by DEXA. There was a significant dose response relationship for bone loss across quartiles of

soy intake. After adjusting the data for confounders, 9% of the variance in bone change was explained by soy intake.

An intervention study has investigated bone health effects in 210 adolescents (14-16 years). The effects of 375 ml calcium fortified soymilk, providing 600 mg calcium and 54 mg isoflavones per day were compared with a non-placebo control group over a 1-year period. The end points were change in bone mineral density and bone mineral content at the spine and hip, assessed by DEXA. Both groups showed similar background dietary intakes, age, BMI and physical activity levels. At the 1-year follow-up there was a greater percentage change in bone mineral density and bone mineral content at all regions of the hip in the supplemented group, compared with controls. There was no difference in spine bone mineral content or density between the two groups.

Dr Ho concluded that 375 ml calcium fortified soy milk is an effective strategy to optimise peak bone mass and to increase hip bone mineral density and content in this study population with a low background intake of calcium. While it is not possible to determine if the effects are due to the additional calcium or to the soy, a potentiating effect of soy cannot be excluded.

SESSION 4: SOY AND THE MENOPAUSE

Dr Aedin Cassidy, University of East Anglia, Norwich, UK discussed **Soyfoods, Isoflavone Supplements and Alleviation of Menopausal Symptoms**. Hot flushes are the most common symptom of the menopause. They occur in 50% of women and can last for many years. While estrogen can alleviate the symptoms of hot flushes, following safety concerns the use of HRT is now in decline and the search for complementary therapies is on. The low levels of hot flushes experienced by women in Japan and China have led to a significant amount of research investigating the potential role of soy and soy isoflavones in reducing menopausal symptoms.

Dr Cassidy discussed two recent systematic reviews that have investigated the overall effects of isoflavone supplement studies. One of these reviews concluded that isoflavone supplementation may produce slight to moderate reductions in the number of hot flushes and the benefit may be more apparent in women who experience a higher number of hot flushes. The other review used different inclusion criteria and concluded that the data are sometimes inconsistent. Trials examining the effects of soy foods have generally been of different designs, with many poor quality studies, such that a meta-analysis is currently not possible.

So far most studies have been short term. More rigorous trials are needed to investigate the optimally effective dose of soy, and to compare soy head to head with other therapies. It also needs to be established how critical the timing of the intervention is – should it be peri- or postmenopausal? Other questions are whether there are stronger effects in equol producers, phenotype effects, whether combination therapies work, and the duration of the intervention that is needed. It is possible that lifetime exposure may explain the inter-country differences, and taking soy only at the menopausal period may not mimic this effect.

Soy Protein as an Alternate to HRT was discussed by *Dr Gita Radhakrishnan, University College of Medical Sciences and GTB Hospital, Delhi, India*. As the role

of women in professional and social arenas is increasing, there is a fall in traditional family support for this life stage. Soybeans are for practical purposes the only nutritionally relevant dietary sources of isoflavones, and supplementation has been shown to ameliorate the vasomotor symptoms of the menopause. Soy protein has also been shown to have positive effects on cardiovascular and skeletal health in postmenopausal women. Hence there is vast potential for soy as a nutritional supplement for all groups in India.

Dr Louise Dye, University of Leeds, UK discussed **The Impact of Soy Isoflavones on Cognitive Function in Younger and Older Women.** To date there have been very few studies of soy isoflavones with cognitive endpoints, especially with objective measures of performance. Of three studies investigating these effects in premenopausal women the results overall show a small and subtle effect of supplementation on verbal memory, and that 8-10 weeks is needed to see any effect. The studies in postmenopausal women have used different forms of soy and different doses, different intervention periods, and the age-range of subjects has been large. The results generally show stronger cognitive effects of soy isoflavones than in premenopausal women, though the effects are mainly confined to improvements in memory and certain aspects of frontal lobe function. Some of the beneficial effects are confined to women within 10 years of the menopause suggesting that there may be a critical time point for isoflavone benefits. In the light of these positive preliminary findings, further investigation is warranted to determine whether these effects are sustainable in the longer-term.

Possible mechanisms include a direct impact on the central nervous system, changes in estrogenic activity, genetic vulnerability, diet-gene interactions, antioxidant effects of isoflavones, and potential metabolic effects. Future research should address the need for longer term studies, to investigate effects in equol producers, to consider the effects of ingesting aglycone vs glycosidic forms of the isoflavones, to look at effects in men as well as women, and to investigate genetic vulnerability.

Equol Supplementation and Postmenopausal Symptoms in Japanese women was discussed by *Dr. Shaw Watanabe, National Institute of Health and Nutrition, Japan*. It has been shown that premenopausal women who are equol producers show a benefit on scores for 'profile of mood states' (POMS), which includes subjective measures such as confusion, fatigue, depression and anger. However this benefit is lost in postmenopausal women. In this new study subjects received placebo, or a high (30 mg) or low daily dose of equol (10 mg), and the effects on the severity of 23 menopausal symptoms was investigated. The doses of equol were chosen to mimic levels in equol producers.

No difference was observed in serum levels of steroid hormones by treatment group or by pre- or postmenopausal status. The POMS score was significantly reduced in both equol groups compared with placebo. Nervousness was also significantly reduced in both equol groups. The overall score of menopausal symptoms was significantly reduced in the non-equol producers. There were no adverse effects reported for either dose of equol.

The final talk in this session was given by *Dr Yoshihisa Niwata,Otsuka Pharmaceutical Co Ltd, Japan* who discussed **Individual Differences in Equol-**

Producing Status and the Use of Equol-Producing Bacteria. Some data show that about 50% of Japanese produce equol, compared with only 30% of Western populations. Factors that influence equol production include low fat and high carbohydrate meals, and green tea but how to improve equol-producing status has not yet been identified.

There are two potential routes to delivering the benefits of equol in the form of functional foods: equol containing foods or probiotics that colonize the gut flora. Four equol producing bacteria have been identified. Safety issues include ingestion history, their existence in the human intestine, and virulence factors. A further issue is to show resistance to gastric juice with the bacteria remaining viable in the colon. One such bacterium, *Lactococcus garvieae* exists in the human intestine and has been found in 35.3% of study populations. A study has shown that it remains viable in the colon and maintains production of equol, determined by the presence of equol in feces. However the presence of equol in blood was not tested in this study.

Dr Niwata concluded that the use of equol producing bacteria for the development of functional foods is a great opportunity to spread the health benefits of equol, particularly those who do not produce equol naturally. However, in the question and answer session it was highlighted that further research on the benefits of equol is needed before claims can be made for benefits, and before equol containing products can be marketed.

SESSION 5: SOY AND OTHER HEALTH OUTCOMES

In the final session, the first topic to be addressed was **Soy in Infant Nutrition.** *Dr Russell Merritt, Ross Laboratory,USA* explained that soy protein has been used in infant feeds in the West for nearly 100 years and soy protein infant formulas have evolved to become safe and effective alternatives for infants who cannot tolerate human or cows' milk. Dr Merritt reviewed the safety data on feeding soy formula to infants. There are no data from adult or infant populations indicating that dietary isoflavones adversely affect human health development or reproduction.

Dr John Erdman, University of Illinois,USA then discussed the emerging area of **Dietary Protein, Soy Protein and Weight Management.** In theory high protein diets may preserve lean body mass, may increases the thermic effect and may contribute to satiety. The source as well as the quantity of protein may be important. *In vitro* and animal data suggest there may be a benefit of soy protein. While the Shanghai Women's health study found no benefit of consuming high levels of soy on body weight, other studies do suggest that there may be an effect. One intervention study in which soy was used as the protein source for 12 weeks showed a greater weight loss in the treatment group, but all the food was provided making it easy for the subjects to keep to the diet. There was no difference in percentage body fat, but total and LDL cholesterol were reduced suggesting an effect of the treatment. In a meal replacement study, subjects received either 5 soy protein replacements or 2 milk-based replacements for 12 weeks. Compliance was less with the soy group, and the study showed slightly greater weight loss with the casein diet. A further study in 43 women with BMI 30-40 for 16 weeks showed a 13% weight loss in both groups with no difference between the soy or casein meal replacement groups.

Studies investigating satiety suggest a short-term effect of soy compared with whey for a single meal, while other studies have shown no effects on satiety.

A number of questions remain. Does soy in a normal *ad libitum* diet have any beneficial metabolic outcomes? Will lifelong soy be more beneficial than short-term use of soy during weight loss diets? Are certain populations more metabolically receptive to soy? Will soy minimise loss of bone mass during weight loss? If it is shown that soy is beneficial to weight loss and that portion controlled meal replacements can work, then the products must taste good. A variety of soy products will be needed with appropriate advertising regarding dietary guidance.

Jung Um Kim, Inje University, Korea discussed **Soy and Diabetes.** Since soy has a low glycemic index and a low glycemic load its consumption could improve glycemic control in diabetic and insulin-resistant individuals. Several studies have also shown that soy protein, phytoestrogens and soy foods offer benefits in the clinical management of diabetes. For example in one randomised, double-blind, placebo controlled crossover trial 20 patients with type 2 diabetes ingested 50 g soy protein containing >165 mg isoflavones per day and 20 g soy fibre for 6 weeks. Compared with the control group LDL cholesterol was reduced by 10%, triglycerides by 22% and the LDL/HDL ratio by 14%, but there was no change in glycaemic control. In a study in 11 patients with type 1 diabetes consuming an enteral nutrition formula containing 16% soy protein improved postprandial hyperglycaemia compared with a standard diet. Studies have also shown a renal-protective effect due to the amino acid composition or isoflavone effects of soy.

Dr Kim concluded that human trials have shown that soy is beneficial for diabetics for blood glucose and lipid control, and for alleviating the symptoms of diabetic nephropathy. However to date there have been very few studies and further clinical and epidemiological research is needed to determine recommended soy intakes and the mechanisms of action. It is also necessary to develop acceptable products so subjects are willing to stay in the intervention trials.

The final talk in this session, **Upper Limits for Isoflavone Recommendation – Perspectives of the Japanese Expert Panel** was given by *Dr Yoshiko Ishimi, National Institute of Health and Nutrition, Japan.* Under the Japanese 'FOSHU' regulations permission has been granted to use a health claim for isoflavones and bone health in soymilk and in a soft drink containing 40 mg isoflavone conjugate. The claim states "keep calcium in the bone", and is based on data from a study showing a reduced level of bone resorption markers in the urine in postmenopausal women given 45 mg isoflavones for 2 weeks.

Submission of a dossier for a health claim on an isoflavone supplement triggered a safety review, as there is no dietary habit for such products in Japan and there were concerns about the potential for excessive intakes. The review considered daily Japanese intakes of soyfoods and data from experimental studies. Guidelines from the review are not to exceed 30 mg soy isoflavone (in the aglycone form) per day from supplements (not soyfoods), in addition to the daily diet, which provides approximately 70-75 mg isoflavones per day. Pregnant and nursing women, infants and children are advised not to take isoflavone supplements.

These recommendations for both the health claim and the maximum level were based on data from one study each and this was commented on in the question and answer session, as well as the unconventional way in which the upper level had been determined from the data.

CONCLUDING SESSION

*Dr Emorn Wasantwisut, Institute of Nutrition, Mahidol University, Thailand***,** in her talk **Role of Soy in Disease and Health Prevention – Challenges For Future Research and Communication,** drew together some overall conclusions of the symposium. A great deal of effort has been focused on the potential health benefits of soy beyond its nutritional value. In addition to the cholesterol lowering effects of soy, soy isoflavones appear to provide several positive health effects including the reduction in risk of some cancers, reduction of menopausal symptoms and promotion of bone health. Soybean can thus be seen as an emerging functional food in addition to its well-known nutritional properties. Dr Wasantwisut commented that we need more systematic reviews and gap analysis, with more priority questions and more studies of good design..

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