



6th International Symposium on

The Role of Soy in Preventing & Treating Chronic Disease

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een samenvatting van:

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alleen bestemd voor (para)medici

This was the 6th in this series of international symposia focusing on recent advances in the key areas of soy and health research. The symposium highlighted a number of randomized-controlled trials recently undertaken in humans and provided information about some on-going trials, the results of which are keenly awaited. Over 50 research scientists presented details of their research on soybeans, soy foods and soybean components, and more than 80 posters were presented.

COGNITIVE FUNCTION

L. Dye (University of Leeds, UK) began the symposium with an overview of clinical studies investigating the effect of soy isoflavones on cognition. It is known that estrogen can modulate specific neuronal processes important to cognitive function. Gender differences in cognitive performance and differences across the menstrual cycle have also been observed. Hence it is hypothesised that bioactive soy isoflavones, which mimic the effects of estrogens, may also influence cognitive function and may offer potential benefit to post-menopausal women for whom a decline in cognitive function is a common complaint.

Specific tests have been designed to assess different types of cognitive function, including memory, non-verbal learning, long- and short-term verbal learning, verbal fluency, spatial ability, executive functions such as planning ability, vigilance and attention, and psychomotor functioning. To date only a small number of intervention studies have investigated the effects of soy isoflavones on cognitive endpoints in comparison with studies done on other health effects of soy. One 10-week placebo-controlled study (File et al 2001) found that a high-soy diet (100 mg soy/day) was associated with improved verbal and non-verbal episodic memory and mental flexibility. Verbal fluency and planning ability were also improved in women but not in men.

A well-conducted double-blind, randomised, placebo-controlled trial in young adult females has recently tested the effects of 68 mg soy isoflavones (aglycone weight)/day, administered in a soy food matrix across two consecutive menstrual cycles (Hill et al 2005). Long-term verbal memory was improved in the first and last week of intervention.

A handful of randomized controlled trials have been conducted in

postmenopausal women aged 45 years to 75 years, for time periods of 6 weeks to 12 months, with doses of soy isoflavones ranging from 60 to 110 mg/day. These 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.

Improving cognition can potentially help to delay the age at which Alzheimer's disease starts to affect brain function. **C. Gleason (University of Wisconsin, USA)** discussed a double-blind, randomized, placebo-controlled pilot study in 30 healthy, well-educated older men and postmenopausal women (60+ years, women not on HRT) that aimed to identify target groups for soy isoflavone intervention.

A dose of 100 mg soy isoflavones/day for 6 months was associated with improvements in a language test (category fluency) in women but not in men. However a test of executive function showed a benefit of isoflavones that was dependent on genetic predisposition to Alzheimer's disease via the ApoE 4 allele. Having one or two copies of this allele increases risk for Alzheimer's disease by 3- and 9-fold respectively, and the benefit of soy isoflavones on executive function was only observed in men without the ApoE 4 allele. This pilot study has thus focused attention on subject characteristics that may influence response to treatment, such as gender and ApoE genotype.

H. Kim (University of Alabama at Birmingham, USA) discussed a study in transgenic mice that used a proteomics approach to investigate the potential basis of soy isoflavone protection against cognitive dysfunction. Oxidative stress is a risk factor for Alzheimer's disease and different polyphenols appear to be neuroprotective. The aim of the study was to investigate whether grape-seed extract or soy protein isoflavones would attenuate the oxidation of brain proteins. The results are the first proteomic identification in mammalian tissue of proteins affected by complex dietary supplements showing a beneficial effect.

INFLAMMATORY DISEASES

H. Aukema (University of Manitoba, Canada) discussed a study undertaken in a rat model of genetically determined kidney disease. In a 2X2 design, maternal rats were fed soy protein or a casein control 2-weeks prior to conception and up to weaning, while the offspring were additionally fed soy protein or casein in the post-weaning period. In the other two groups weanling rats were fed soy protein or casein in the post-weaning period alone.

Feeding soy-protein in the post-weaning diet confirmed earlier findings of reduced disease progression. However, the new and exciting finding from this study was that exposure to soy via the maternal diet was also associated with reduced renal inflammatory cell infiltration in the offspring and this was even more reduced when combined with soy-protein feeding in the post-weaning period.

Maternal exposure to soy protein was equally effective as post-weaning exposure in reducing renal cell hyperproliferation in diseased kidneys in the offspring. Maternal soy protein compared with casein was also associated with less proteinuria in the offspring, an indicator of improved renal function. These findings indicate that in this animal model of kidney disease maternal soy-protein feeding has long-term beneficial effects on renal inflammation and disease progression in the offspring.

M. Kauppila (University of Turku, Finland) discussed a rat study in which dietary soy was concluded to reduce perivascular, stromal and glandular inflammatory changes in the prostate, while body weight gain, prostate size and hormone concentrations in serum remained unaltered. When fed isoflavonoid-free soy an equivalent decrease in the number of inflammatory foci was observed and the urine content of isoflavonoids was markedly decreased. He concluded that the anti-inflammatory activity of soy is not related to the isoflavone content.

EQUOL

M. Kurzer (University of Minnesota, USA) gave an overview of the equol hypothesis and future directions for research. Equol is a metabolite of the soy isoflavone daidzein produced by bacterial fermentation in the intestinal tract. Equol has higher bio-activity than all the other isoflavones and shows greater affinity for the estrogen receptor (ER) and greater antioxidant activity.

However only 20-30% of Western adults produce equol in comparison to 50% of the Japanese population. Since most research studies are conducted in mixed samples of both equol producers and non-producers, the magnitude of any effects that only occur in equol producers could be diluted, while conversely being exaggerated in non-equol producers.

Recent studies have suggested benefits of equol production against breast cancer risk, against risk factors for cardiovascular disease, prostate and cervical cancer risk, bone health and menopausal symptoms. However, other studies have not shown these effects, and there is potential for publication bias in this area where studies with positive findings are more likely to be published. Hence the equol hypothesis remains controversial.

To confirm or refute the hypothesis larger studies are needed with recruitment of subjects by equol status. In addition, further data can be gathered from existing studies – samples from previous clinical trials could be re-analysed by equol status of the subjects, while unpublished data on equol could be systematically collected and analysed.

If it is confirmed that equol production improves response to soy, an important aim of future research will be to determine whether dietary or other factors can initiate equol production in non-equol producers.

I. Rowland (University of Ulster, UK) explained that due to the presence of a chiral centre, equol exists in two optically active isomeric forms (R- and S-equol). It has been shown that the intestinal flora exclusively produces S-equol. S-equol has a relatively high affinity for ER- β (only) and thus acts as a selective

estrogen receptor modulator (SERM). In contrast R-equol is thought to have different binding affinity for the estrogen receptor, with greater affinity for ER- α .

In an interesting *in vitro* study both racemic and S-equol were found to inhibit the growth of breast and prostate cancer cell lines. However, whilst S-equol was unable to prevent chemically-induced DNA damage in breast cells, racemic equol did show preventive effects. This implicates the R- rather than the S-enantiomer as being responsible for the antioxidant effects of equol *in vitro*, which may have implications for the chemopreventive properties of equol *in vivo*.

T. Larkin (University of Wollongong, Australia) discussed a study investigating whether concurrent daily intake of soy with a probiotic yogurt (LGG, *L. acidophilus* and *bifidobacteria*) or with a prebiotic (resistant starch baked into a bread, providing 16-20g resistant starch/day) would affect isoflavone metabolism and plasma lipids in comparison with consumption of soy alone. The study consisted of a run-in period, and two 5-week dietary periods separated by a 4-week wash-out period. The 31 hyperlipidemic subjects (19 males and 12 postmenopausal females) all had total blood cholesterol above 5.5 mmol/L.

At the end of the 5-week period, soy consumption was found to significantly increase plasma daidzein and genistein, but following consumption of a test soy meal there was no effect on plasma and urinary isoflavones. Consumption of either the probiotic or prebiotic did not significantly affect plasma isoflavone levels and had no effect on equol production.

Regarding equol status, 12 of the subjects consistently produced equol, 10 produced equol sporadically and 9 did not produce equol at all. However, when analysed by equol status there were no significant effects of the dietary treatments on plasma equol or urinary equol excretion.

Total cholesterol was significantly decreased with both the soy+probiotic and soy+prebiotic treatments (-4.7%, $P=0.038$ and -5.5%, $P=0.003$, respectively). Soy+prebiotic produced the most significant reduction in LDL-cholesterol (-7.3%, $P=0.005$). There were no significant effects on HDL-cholesterol or triglycerides. This suggests a synergistic action between soy and probiotic and soy and prebiotic treatments on total and LDL-cholesterol, which may be useful in the management of hypercholesterolaemia.

C. Nagata (Gifu University Graduate School of Medicine, Japan) undertook a case-control study ($n=455$) to investigate any association between urinary excretion of equol and the risk of breast cancer in Japanese women. The cases were 160 women newly diagnosed with breast cancer and controls were individually matched by age and menopausal status. Dietary soy intake, assessed by validated semi-quantitative food frequency questionnaire, was not significantly different between cases and controls. However urinary levels (from spot urine samples) of daidzein and genistein were significantly higher in cases than controls while the proportion of women with detectable levels of urinary equol was significantly greater in controls than cases (19 vs 8%). The conclusion was that equol or other factors related to equol production might be associated with decreased risk of breast cancer.

T. Clarkson (Wake Forest University School of Medicine, North Carolina, USA) described a study in postmenopausal female monkeys designed to investigate whether equol production *per se* accounts for the significant benefits of soy consumption on plasma lipid profile seen in monkeys. The control group consumed a casein-lactalbumin-based diet containing 0.2 mg cholesterol/kcal, while the intervention group consumed the same diet with the addition of 52.4 mg racemic equol/1800 kcal. After 8 months there were no benefits of equol consumption compared with the control group. Thus the beneficial effect of soy consumption on plasma lipids is not due to equol production, but is related to other factors.

ABSORPTION OF SOY BIOACTIVES

T. Hedlund (Health Sciences Center, Aurora, USA) discussed a study investigating the effects of long-term dietary habits on daidzein metabolism in 45 healthy men (aged 19-65 years). The baseline plasma levels of various isoflavonoids were measured and following daily consumption of a soy beverage for 1 week isoflavonoid levels were measured in plasma and prostatic fluid.

Men who had consumed 30 mg or more soy isoflavones/day for at least 2 years were 5.3 times more likely to be equol producers than men who were low consumers of soy isoflavones ($P=0.014$). In addition, men who had consumed animal meat regularly were 4.7 times more likely to be equol producers than men who did not consume meat ($P=0.023$). Ability to produce equol was not linked to BMI, age, or to consumption of yoghurt, dairy products, fruit or American-style fast food.

The study also found that daidzein and its metabolites, but not genistein, were present at higher levels in prostate fluid than in plasma (median = 4-13 times that in plasma), increasing the potential for direct effects in the prostate.

B.O. de Lumen (University of California, USA) described an animal study showing that the soy peptide lunasin, shown to have cancer preventive properties, is bioavailable following oral administration to male and female mice. Synthetic lunasin labelled with tritium was present in many tissues that are targets for the most common cancers, such as prostate, colon, mammary gland and lungs. It was also found in the brain demonstrating that it crosses the blood-brain barrier. In a 4-week rat study, intact lunasin was detected in rat liver.

It was concluded that soy protease inhibitors protect the cancer preventive peptide lunasin from digestion *in vivo* and that lunasin is bioavailable. Further studies are needed to investigate the efficacy of lunasin.

CANCER

A higher risk of prostate cancer among male migrants from Asia to the United States has led to the hypothesis that soy foods may be protective, possibly through an effect on circulating sex steroids. **G. Maskarinec (Cancer Research Center of Hawaii, USA)** discussed a pilot study to determine the feasibility of recruiting healthy men for a soy-based intervention study and to test dietary compliance with a protocol of high-soy intake.

In a cross-over design half of the subjects (total n=23) undertook a high-soy diet or maintained their usual diet for 3 months. This was followed by a 1-month wash-out period, and the subjects then swapped to the other diet.

The high-soy diet consisted of replacing dairy products, meat and snacks with 2 servings of soymilk, tofu or soy nuts per day. The aim was to achieve an intake of 75 mg soy isoflavones, and the results showed that an intake of 60 mg/day was achieved. Urinary analysis confirmed differences in soy intake between the two diet periods. There was no difference in serum testosterone between the groups, or on a profile of mood states but there was a modest, statistically significant reduction in serum prostate specific antigen with the soy intervention.

How soybeans are grown may affect their content of bioactive compounds. For example glyceollins, a type of isoflavonoid, are present in stressed soybeans and have potential estrogen-antagonistic effects. **J.M. Cline (Wake Forest University School of Medicine, North Carolina, USA)** described a 3-week study in postmenopausal female monkeys (n=30) to examine the anti-estrogenic effects of glyceollin-enriched soy protein on biomarkers for cancer risk. The findings showed that glyceollins are absorbed and rapidly cleared following consumption, and that they may enhance the antiestrogenic properties of standard soy protein isolate in the breast and uterus.

The rate of breast cancer in Asians is 25-30% of that in US whites, though is increasing, while that in Asian migrants to the US is much higher than in non-migrants. Similarly the rate in US-born Japanese is about 80% of that in US whites. This raises the question why are the rates so much higher in the US and why are they increasing? **A. Wu (University of Southern California, USA)** described a large population-based case-control study of breast cancer among Asian-American women in Los Angeles County that aimed to test the hypothesis that a high intake of soy foods reduces the risk of breast cancer.

Details were obtained on use of exogenous hormones, family history, dietary history, body size, menstrual and reproductive information, and other lifestyle factors. Data for approximately 2500 cases and controls show that a high intake of soy during both adolescence and adult life is associated with a 40% risk reduction for breast cancer compared with a low intake of soy at both of these life stages. A low intake in adolescence and a high intake in adulthood is associated with 12% risk reduction, while a high intake in adolescence and a low intake in adulthood is associated with 30% risk reduction.

There was not much difference when the results were analysed by estrogen receptor status, or ER/PR status, and very little difference of adult soy intake for tumour stage. However significant reductions in risk were apparent for both pre- and postmenopausal women after adjusting for relevant dietary and non-dietary confounders. A more detailed analysis has suggested that the benefits of soy in postmenopausal women are most pronounced in women with low body weight and in women not using HRT.

I. Rowland (University of Ulster, UK) described the EU-funded multi-disciplinary research programme 'Phytoprevent'

which ran from 2001 to 2004. The aim was to investigate the role of foods rich in phytoestrogens (isoflavones and lignans) on risk reduction of prostate and breast cancers. The project involved analytical studies, in vitro studies, studies in animal models and a human dietary intervention trial.

In vitro experiments, to investigate the effects of isoflavones on various stages of cancer, showed that low concentrations of isoflavones reduced chemically induced DNA damage in breast and prostate cancer cell lines, and suggested a beneficial effect of isoflavones on breast tumour promotion. In a mouse model of mammary cancer perinatal exposure to isoflavones had no effect on tumour development. However post-weaning and life-long exposure to isoflavones was shown to delay tumour development and to reduce tumour incidence.

In the human intervention trial, 39 male and 24 female subjects, all in good health, were given either soy foods (providing 42 mg isoflavones/day) or rye bread (providing 170 mg lignans/day) for 21 days. The lignan group showed no significant changes in urinary estrogen profile. However in the female subjects consuming the isoflavone-containing soy foods, significant changes were seen in the urinary estrogen profile that were consistent with a lower risk of breast cancer (i.e. an increase in the ratio of 2-methoxyestradiol to 2-hydroxyestradiol, 4-hydroxyestradiol to estradiol, and 2-hydroxyestradiol to 16-hydroxyestradiol, and a decrease in the ratio of 2-hydroxyestradiol to 4-hydroxyestradiol).

L. Thompson (University of Toronto, Canada) described a study in mice investigating whether the combination of soy and flaxseed and their respective phyto-estrogens-genistein, and the lignans enterolactone or enterodiol could reduce the growth of established estrogen-receptor-positive breast cancer more effectively compared with soy or genistein alone. It was found that combining phytoestrogen or phytoestrogen-rich foods did result in better control of tumour growth while causing minimal effects on bone health.

CARDIOVASCULAR DISEASE

C.R. Sirtori (Niguarda Hospital, Milan, Italy) discussed the particular protocols of various studies that have measured the cholesterol lowering effect of soy protein, and the mechanism of cholesterol lowering. The mechanism differs from that of statins, which act on cholesterol biosynthesis, whereas soy protein stimulates LDL-receptor activity. It is now well-established that the cholesterol lowering effect of soy is not due to the presence of phytoestrogens, but to active soy peptides and it is possible that in the future tablets containing soy-protein derived peptides will be available for cholesterol reduction.

M. Lovati (University of Milano, Italy) discussed studies with a synthetic peptide corresponding to a sequence that differs between the α - and α' - subunits of 7S soy globulin. This globulin and the α' - subunit have been previously shown to positively modulate LDL-receptor activity and to reduce triglyceride synthesis in rats fed a cholesterol-rich diet.

In an in vitro study, the synthetic peptide was found to have similar activity to the α' - subunit on a modulator of LDL-receptor

activity. This was followed-up with an *in vivo* study in which the synthetic peptide (5 mg/kg body wt) was administered for 14 days to casein-cholesterol-fed rats. Administration of the peptide resulted in 25% lower plasma cholesterol levels and 28% lower triglyceride levels compared with control rats fed the casein-cholesterol diet only.

These results give support to the future aim of using bioactive peptides as a potential tool in the management of hypercholesterolemic patients. Novel soy cultivars with a high content of bioactive peptides may have potential for development as functional foods to enable a population approach to reducing plasma lipid risk factors for cardiovascular disease.

C.D. Gardner (Stanford University, California, USA) described a randomised controlled trial comparing low-fat dairy milk with two different types of soymilk (one made with whole soy beans and the other with soy protein isolate) on plasma lipids in hypercholesterolemic adults (LDL-cholesterol 160-220 mg/dL).

The 28 subjects (aged 30-65 years) had low Framingham risk scores (<10%) and were not on cholesterol-lowering medication. The cross-over trial consisted of three 4-week dietary phases each separated by a 4-week wash-out. The interventions consisted of consuming enough milk to provide 25 g protein/day preferably divided into three portions per day.

The results showed a 5% reduction in LDL-cholesterol for both soymilks, compared to low-fat dairy milk, but there was no significant difference between the two different soymilks. No differences were observed on plasma HDL-cholesterol or triglycerides.

Approximately a third of the subjects were equol producers but this sample size was too small to draw any conclusions for analysis by equol status.

J.W. Anderson (University of Kentucky, USA) began by discussing his 1995 meta-analysis of 38 studies investigating a cholesterol-lowering effect of soy foods. This suggested an overall reduction in LDL-cholesterol of 12.9%. Though many studies published since 1995 have reported beneficial changes in lipoproteins with soy consumption, the reductions have generally been less impressive than the meta-analysis would suggest. Hence details of a further meta-analysis of 55 trials with a non-soy control published since 1995 were presented.

Studies have been conducted in adult males and females, and the average baseline LDL-cholesterol was 152 mg/dL (range 89-199 mg/dL). The new analysis has shown that soy protein consumption (averaging 44 g/day for an average of 5 weeks) is associated with an 8.5% reduction in LDL-cholesterol, and a net change (i.e. less the placebo effect) of 4.5%. Sub-group analysis shows that consuming soy protein once per day is less effective than consuming soy protein at least twice per day. A similar magnitude of effect is seen in males and females and there does not seem to be any difference between pre- and postmenopausal women.

The meta-analysis also shows that soy protein has beneficial

effects on plasma HDL-cholesterol and triglyceride levels.

In 16 studies testing soy incorporated into muffins and biscuits the reductions in LDL were less impressive than studies using soymilk, tofu and soy nuts. Since soy peptides are thought to be responsible for much of the cholesterol lowering effect, this may be explained by fragmentation of the most active hypocholesterolemic peptides with baking.

Prof Anderson concluded that consuming soy protein significantly reduces LDL-cholesterol. The effect is seen within two weeks and is sustained for at least 16 weeks. LDL-cholesterol reduction is seen at all baseline levels of plasma LDL (from 91-247 mg/dL).

Z. Faridi (Yale Griffin Prevention Research Center, Connecticut, USA) described a double-blind, randomized, placebo-controlled cross-over trial in 25 healthy postmenopausal women that aimed to test the effects of 25 g soy isoflavone protein/day and/or 20 g soy lecithin/day on endothelial function. Whilst favourable effects on plasma lipids were observed, improvement in endothelial function was not confirmed, with no statistically significant differences between the treatment groups.

IMMUNE FUNCTION

It is suggested that following the menopause immune function may be compromised due to the falling concentrations of estrogen, an immune-modulating hormone, and to other effects of aging. Hence soy isoflavones, which act as phytoestrogens, may offer potential immunological benefits to women at this life stage.

T. Ryan Borchers (Washington State University, USA) described a 16-week double-blind, randomized, placebo-controlled trial in 52 postmenopausal women aged 50-65 years that aimed to investigate the effects of 70 mg soy isoflavones/day, from either soymilk or taken as a supplement, on markers of immunity and oxidative stress and compared with cows' milk.

The study consisted of three experimental groups – (1) the control group (n=19) consumed 706 ml cows' milk/day plus a placebo supplement, (2) the soymilk group (n=18) consumed 706 ml soymilk/day providing 71.6 mg isoflavones, plus a placebo supplement, and (3) the soy supplement group (n=15) consumed 706 ml cows' milk/day plus 70 mg soy isoflavones in a supplement. The subjects were highly educated, were light exercisers, and were not on HRT. They were instructed to exclude other foods high in isoflavones.

Both of the soy isoflavone interventions were associated with increased β -cell populations ($P<0.05$), increased concentrations of plasma interferon-gamma (IFN- γ) ($P<0.05$), and with decreased concentrations of an oxidative marker of DNA damage (8-hydroxy-2-deoxy-guanosine; $P<0.05$). There was no effect on measures of plasma C-reactive protein, tumor necrosis factor-alpha, interleukin-2, or urinary 8-isoprostane.

The positive effects observed are suggestive of protective effects of soy isoflavones over a 16-week period against DNA damage in postmenopausal women and suggest the potential to offer immunological benefit.

The prevalence of food allergy in children is increasing. Peanut allergy is particularly concerning as it is often life-long and life-threatening, and there is currently no therapy. **T. Zhang (Mount Sinai School of Medicine, New York, USA)** described a study to investigate the potential effects of ImmuSoy in peanut-allergic mice. ImmuSoy is a koji fungus (*Aspergillus oryzae*) and lactobacteria soybean fermentation product based on traditional Japanese fermentation technology.

Following peanut sensitisation (10 weeks), peanut allergic mice (n=8-10/group) were fed (1) chow containing ImmuSoy (0.5% or 1%), (2) chow containing lactobacillus rhamnosus GG (LGG) (0.5%), or (3) regular chow (control group) for 4 weeks. Following challenge with peanut, all control mice developed anaphylaxis compared with 50% and 25% of low-dose and high-dose ImmuSoy fed mice, respectively, and with 80% of LGG-fed mice.

Mice fed ImmuSoy also showed significant reductions in plasma histamine and serum IgE levels compared with control mice ($P<0.05$), with no significant differences observed for the LGG-fed mice. It was suggested that ImmuSoy might be a potential novel therapy for peanut allergy.

DIABETES

Research from animal studies and in diabetic subjects suggests that soy consumption can improve glycemic control, though not all studies are consistent. In addition there are few data on such effects in healthy adults.

S Ho (Chinese University of Hong Kong) described a 1-year randomized controlled trial in 173 community-based postmenopausal Chinese women aged 48-62 years, designed to assess the effect of 0 (n=58), 40 (n=62) or 80 (n=54) mg isoflavones/day on bone changes, but that also measured fasting serum glucose at baseline and after 1 year. Habitual intake of soy foods was assessed using a food frequency questionnaire.

The mean intake of soy protein was 5.3g/day and along with changes in BMI and baseline glucose, soy protein intake was significantly correlated with changes in fasting glucose seen over the year of the trial. In addition, for women with an initial fasting glucose over the median (92.1 mg/dL) compared with below the median, habitual soy protein intake was significantly and inversely associated with both the absolute and the percent change in fasting glucose.

It was calculated that soy protein accounted for 14.1% ($P=0.002$) of the variation in absolute and 12.8% ($P=0.003$) of the variation in the percent changes of fasting glucose. A daily increase of 10g soy protein was associated with a 9.3% decrease in fasting glucose ($P<0.001$) in the women with higher initial fasting glucose.

These interesting results suggest that soyfood intake can improve glycemic control and insulin resistance in healthy women, particu-

larly with higher fasting glucose measures. Longer term trials using different doses of protein and isoflavones, and conducted in men and other population sub-groups, are now warranted to further test this hypothesis.

Y. Yamori (Mukogawa Women's University, Japan) discussed a randomized, cross-over controlled study investigating the metabolic effects of consuming a soy rich vs a control diet in healthy firefighter trainees aged 18-25 years. The soy diet consisted of common Japanese products such as soymilk and tofu, and contained 29.4g soy protein/day compared with 8.6g soy protein/day in the control diet.

Of the 43 subjects who completed the study, soy consumption was associated with a significant reduction in systolic and diastolic blood pressure, fasting glucose, BMI and body fat. The control diet was not associated with any significant changes in these parameters. Significant inter-diet differences were also noted for changes in the baseline levels of fasting glucose measurement and for HOMA-IR. These results suggest that regular soy intake may have beneficial effects on insulin resistance in young healthy men, but further studies are needed to confirm these findings.

MENOPAUSAL SYMPTOMS

Studies investigating the efficacy of soy isoflavones on menopausal symptoms have frequently used retrospective assessment of symptoms, and scales for the psychometric tests that may not be sufficiently sensitive to measure effects due to soy. Hence methodological issues may partly explain the inconsistent findings in this area.

C Hill (University of Leeds UK) discussed a 12-month trial that she has undertaken using a methodologically rigorous approach. The subjects were 21 British postmenopausal women aged 50-65 years, with a BMI of 19-30 and with no current use of HRT. Overall the subjects were not a severely symptomatic sample. The study was a prospective double-blind, randomized, placebo-controlled cross-over trial comparing a treatment period of 100mg soy isoflavone supplement/day (aglycone weight) for 8 weeks against 8-weeks placebo. Self-reported compliance indicated that 98% of the supplements were taken.

No significant difference was observed between the soy isoflavone-supplemented and placebo phases of the study on the total menopausal symptom score. However significant reductions were observed with isoflavone supplementation for the frequency of feeling 'easily stirred up' (excitable), the severity of early morning awakenings and sleeplessness, and by 4 weeks for the frequency of sleep problems.

Daily frequency and the severity of hot flushes were also significantly reduced across all weeks of soy isoflavone supplementation, with greater efficacy of treatment in women experiencing more than five hot flushes per day. Increases in circulating levels of estrogen were significantly correlated with the reduction in hot flushes.

It was determined that a third of the subjects were equal producers. No differences were observed with equal status, though the

number of subjects may have been too small to determine any statistically significant differences.

Hill concludes that this methodologically sound study validates the findings from earlier studies that used less rigorous methodology.

P. Williamson-Hughes (Archer Daniels Midland Company, Decatur USA) described a critical review undertaken to determine whether the observed effects, or indeed lack of effects, of isoflavone intake on hot flush symptoms could be attributed to specific isoflavones. This information is important because, though several reviews and meta-analyses have evaluated the clinical effects of soy isoflavones in general, the study treatments have often contained a range of isoflavone sources and molecules. Differentiating between the chemical identities of individual isoflavones allows for more precision in measuring the effects and interpreting the results.

A literature search was undertaken to identify studies using semi-pure soy isoflavone extracts administered as dietary supplements and compared with a control group. Studies using other herbals, soy foods, soy protein powders, red clover isoflavones or combination therapies were not included in the analysis. This process resulted in 11 studies, with a total of 378 subjects with an average of 5 hot flushes daily. Total aglycone (equivalent weight) intake ranged from 30-114 mg/day, while intake of genistein ranged from 5 to 75 mg/day. The papers were then divided into two sub-sets with six studies providing above 10 mg genistein per treatment (total n=188) and the remaining five studies (total n=167) providing supplements high in daidzein or formononetin and with low genistin/genistein content.

The average intake of genistein in the first sub-set of studies was 40.8 mg/day and the range of intake was wider than in the second sub-set of studies, for which the average genistein intake was 6.6 mg/day. Overall, the studies with a high intake of genistein showed a significant improvement in hot flush symptoms, whereas only one of the studies with a low intake of genistein showed any beneficial effect on hot flush symptoms. The results suggest that supplements with a higher genistein ratio and with a minimum threshold for genistein of about 10-15 mg genistein/day and unrelated to the total amount of isoflavones, are more efficacious in reducing hot flush symptoms than other soy isoflavone supplements.

This finding is important since it indicates the importance of discriminating between the individual isoflavones, and that failure to do this can potentially lead to misleading results.

UD Rohr (AHS, Vienna) discussed an in vivo trial comparing tibolone, a compound used in postmenopausal hormone therapy, with isoflavone supplements. Eleven perimenopausal and 36 postmenopausal women received 80 mg isoflavones/day for 3 months, while 24 postmenopausal women received 2.5 mg tibolone/day for 6 months. In in vitro tests isoflavones showed a preference for binding to ER- β , while tibolone, which is metabolised into 3 further active compounds, showed an affinity for ER α .

The PAP-smear test to control for inflammation and cancer risk was conducted after 3-months of taking either tibolone or iso-

flavones. Isoflavones reduced the number of cell counts in the vaginal PAP-smear test for both perimenopausal and postmenopausal women, while tibolone increased the cell counts in postmenopausal women.

Since hypertrophic effects are believed to be facilitated by ER- α and suppressed by ER- β , the ER- β affinity of isoflavones could explain the results of the PAP-smear test in the isoflavone-supplemented groups. The ER- α affinity of tibolone may be correlated to the increased number of cells in the PAP-smear test observed in the tibolone group.

OSTEOPOROSIS

Some studies in postmenopausal women have reported an association between soy consumption and reduced loss of bone density. **F Squadrito (University of Messina, Sicily)** presented the 1-year results from a recent double-blind, randomized, placebo-controlled trial investigating the effect of genistein on bone loss, cardiovascular risk prevention and climacteric symptoms of the menopause.

The aim of the study is to confirm previously reported beneficial effects of genistein in a 2-year study with a larger population of postmenopausal women. Just under 400 postmenopausal women (mean age approximately 54.5 years, mean BMI approximately 24.5 and with a bone mineral density at the femoral neck of <0.8 g/cm²) were randomly assigned to either placebo or genistein groups.

After 1-year, treatment with 54 mg genistein/day (n=198) showed significant beneficial changes in bone resorption and bone formation markers while there was no effect in the placebo group (n=191). The genistein group also showed increased bone mineral density in the femur and lumbar spine compared with baseline, again with no changes seen in the placebo group.

Positive effects of genistein were also reported on cardiovascular disease risk factors. Compared with placebo, 12 months of genistein treatment significantly decreased fasting glucose and insulin levels, decreased insulin resistance, and reduced fibrinogen levels.

Compared with placebo, genistein was also associated with a reduction from baseline of both the number and severity of hot flushes. At baseline subjects were experiencing on average 4.5 hot flushes a day and this was reduced in the genistein group by 21.2% at 1 month, 41.7% at 3 months, 40.3% at 6 months and 45.1% at 12 months, compared with only a small effect in the placebo group. Severity of hot flushes was reduced in the genistein group by 10.6% at 1 month, 20.5% at 3 months, 22.4% at 6 months and 29.7% at 12 months. No significant changes were detected in endometrial thickness with genistein compared with placebo, suggesting the safety of genistein for postmenopausal women.

It will be interesting to see if these positive results still hold after completion of the full 2-year study.

F. Lovrien (Sioux Valley Hospital, South Dakota, USA) presented details of a 2-year randomized trial undertaken in women 1 to 5 years postmenopause. The trial compared the effects of 40 g daily soy protein (2x20 g/day), with or without HRT against 40 g casein (2x20 g/day), with or without HRT. The soy protein provided 88 mg aglycone isoflavones (48 mg genistein, 32 mg daidzein and 8 mg glycitein) and all women also received 1400 mg calcium/day.

After 2 years soy protein was associated with a significant reduction in bone loss from the spine ($p < 0.05$), measured by DEXA. The change in the soy group with no HRT ($n=28$) was 0.02% compared with baseline while the change in the casein group with no HRT ($n=20$) was 2.73% compared with baseline. Similar changes in bone mineral density were seen for the soy and casein groups with HRT. However there were no significant differences between the groups in bone loss from the hip after 1 or 2 years, which may be explained by a slower rate of change in bone density at the hip. This suggests that long-term dietary supplementation with soy can reduce bone loss in postmenopausal women, but may be site specific or may be an even longer-term effect.

A 2-year randomized double-blind trial to investigate the effect of soy protein with or without isoflavones on bone mineral density in just over 100 postmenopausal women aged 55-72 years was described by **J.C. Gallagher (Creighton University Medical School, Omaha NE, USA)**. The four groups consisted of (1) 25 g soy protein with 90 mg isoflavones (as aglycone equivalent)/day, (2) 25 g soy protein with <5 mg isoflavone/day, (3) 25 g total milk protein including casein and whey/day, and (4) a non-protein control. The three treatment groups also received 500 mg calcium and 125IU vitamin D/day.

While the unadjusted preliminary analyses indicate no significant differences in the rate of bone loss (measured by DEXA) between the three treatment groups, beneficial changes were observed for the effect of soy on blood lipids. Soy was found to reduce LDL-cholesterol and to increase HDL-cholesterol (both favourable changes) and soy, but not milk protein, improved the LDL/HDL-cholesterol ratio. Interestingly soy with isoflavones had the greater effect on improving the LDL/HDL-cholesterol ratio.

WEIGHT CONTROL

Meal replacements are one option for eliciting weight loss and potentially may also be useful adjuncts to lifestyle changes for weight maintenance. Soy protein has been shown to have specific effects on fat, glucose and lipid metabolism and to promote weight loss in animals. Hence an interesting area of investigation is the comparison of soy-protein-based and casein-based meals replacements.

J.W. Anderson (University of Kentucky, USA) discussed a 16-week randomized controlled trial of weight loss in obese women (BMI 30-40, with no evidence of diabetes and not taking weight loss medication). The trial was designed to investigate the effects of intensive lifestyle intervention led by a dietitian and to compare soy protein- and casein-based meal replacements. The soy protein group ($n=17$) received 62 g soy protein/day containing

147 mg isoflavones/day (aglycone equivalent weight), while the casein group ($n=18$) received approximately 68 g casein/day. Total energy intake ranged from 1050 to 1200 kcal/day across both groups.

Weight losses of 17 pounds (7.7 kg) at 8 weeks and 28 pounds (12.7 kg) at 16 weeks were significant compared with baseline, but there were no statistically significant differences between the groups. Similarly, total body fat losses averaging 23% and visceral fat losses averaging 29% were not significant between the groups. Subjects consuming soy had a greater reduction in LDL-cholesterol compared with the casein group but the difference was not statistically significant at 16 weeks.

J.W. Erdman (University of Illinois, USA) updated delegates on a 2-day forum held earlier in the year at the University of Illinois in which a scientific panel discussed the current state of knowledge regarding the management of obesity and related diseases, and the potential role for soy and its components. In addition to its lipid lowering effects, emerging evidence suggests that soy may beneficially affect adipogenesis and may be beneficial for diabetics.

With regard to weight control the panel concluded that portion control and meal replacement can work, and that it is necessary to clarify the discernible scientific benefits of soy-based products. Advertising needs to be appropriate and consumers need guidance and reinforcement. Current gaps in the science include long-term effects of soy on sustained weight loss, the effects of soy on total body fat loss and regional fat loss, and the mechanisms of action.

CONCLUSIONS

The symposium highlighted the vast amount of research being undertaken, across an ever-widening range of areas, to investigate the health effects of soy. The results of the on-going longer-term human trials in areas such as ischaemic heart disease and osteoporosis are keenly awaited, in the hope that they will provide more conclusive and supportive data on the health benefits of soy.

Dit congresverslag is u aangeboden door Alpro Soja Nederland BV.

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