DIETARY APPROACHES FOR ATHEROSCLEROSIS PREVENTION: VEGETABLE PROTEIN

This sponsored symposium, held as part of the XIV International Symposium on Atherosclerosis in Rome (June 21, 2006) focused on various beneficial aspects of soy consumption. Prof. C.R. Sirtori (Milan, Italy) and Prof Widhalm (Vienna,Austria) chaired the event . Prof. Sirtori explained in his opening comments that soy protein is effective in reducing cholesterolaemia.

The first speaker, **Prof. G. De Backer** (Ghent, Belgium), gave a presentation entitled *Healthy food choices recommended in the European guidelines on CVD prevention: how about the implementation in clinical practice?* Dietary Guidelines for cardiovascular disease (CVD) prevention, such as those of the Third European Joint Task Force of European and Other Societies published in 2003, are an essential part of clinical strategies to prevent CVD. The impact of diet on CVD risk has positive effects on blood lipids, and also on other markers, including benefits on caloric balance, blood pressure, glucose metabolism, blood clotting and antioxidant activity.

The general guidelines are that:

- Food choices should be varied
- Energy intake should be adjusted to maintain ideal body weight
- Consumption of fruit and vegetables, wholegrain cereals, low fat products should be encouraged
- Consumption of oily fish and omega-3 fatty acid should be recommended
- Salt intake should be limited to 5-6g per day
- Total fat should not exceed 30% energy
- Saturated fat should not be greater than 1/3rd of total fat intake, and
- Saturated fat should be replaced partly by complex carbohydrate, partly by monounsaturated fats, and partly by polyunsaturated fats in an isocaloric diet.

While these recommendations provide a general dietary framework it is important that local cultural needs in different European countries are also taken into account. The dietary guidelines should also be adjusted to suit the particular clinical needs of individual patients.

Since surveys of daily practice show an unacceptable gap between dietary intakes and the recommendations, an implementation plan is warranted. The EUROASPIRE II survey (Lancet 2001, 357: 995-1001) set out to determine whether patients with CHD are being effectively managed by lifestyle and drugs. The study was conducted in 15 different centres and of the total survey sample, 59% had already reduced their calorie intake at interview, but there was wide variation between countries. Despite this, 80% of patients were still overweight at the time of interview. Similarly 87% said they had already reduced dietary fat intake and 36% had changed the type of fat they eat. However, at the time of interview 58% of the sample had total cholesterol >5mmol/L(193mg/dl). The full results of the EUROASPIRE III survey will be available at the end of 2006.

EUROACTION, a European Society of Cardiology (ESC) demonstration project in preventive cardiology undertaken in 1060 patients with established disease in hospital and primary care, found that 24% of participants were using saturated fats for spreading and 13% were using saturated fats for for frying, only 45% were eating above 400g fruit and vegetables a day, 46% almost never ate oily fish while only 3% ate oily fish more than 3 times per week. Following a 16-week intervention, there was an 18% increase in those meeting the recommended saturated fat intake (<10% energy), 71% were eating above 400g fruit and vegetables per day, and 58% of the participants were eating 20g fish per day. Overall the results of the EUROASPIRE and EUROACTION surveys show that dietary recommendations are not being translated or implemented as health professionals would like.

A further question is how to implement guidelines for the large majority of people at risk of developing heart disease in the general population, which is more difficult to answer. Information from the World Health Organization (WHO) shows that 21 of 26 countries fail to meet the goal for <30% energy from total fat, and the general population in almost all EU countries is far from meeting this target. A particular concern is that countries such as Bulgaria and Italy that traditionally had a reasonable dietary profile in the general population, have shown increased consumption from saturated fat over the last 30 years.

Reasons why dietary recommendations are not translated or implemented as health professionals would like, include barriers for patients, for the health care system, and for physicians. A more professional and multidisciplinary approach is therefore needed to implementing dietary changes, involving health care professionals and the food industry among others.

In the question and answer session, it was discussed that while the dietary recommendations are directly focused on adults, we should also start as early as possibly with guidelines for children.

The second talk was given by **Dr M. Lovati** (Milan, Italy) who has been working in the field for a number of years. In her talk, *Vegetable proteins: mechanism for cholesterol reduction* Dr Lovati explained that soy proteins are an important dietary tool for cholesterol lowering, especially for patients with high baseline cholesterol levels. The mechanism for this effect is up-regulation of the LDL receptor on liver cells. Soy protein is heterogeneous and the major storage globulins in soy are 11S and 7S. Studies have investigated which globulin is involved in cholesterol lowering, and whether it is a soy protein, or a specific peptide that modulates LDL-receptor activity.

In vitro studies have shown that the 7S soy globulin is responsible for up-regulation of the LDL receptor and *in vitro* and *in vivo* studies have demonstrated that the purified __ sub-unit of the 7S soy globulin positively modulates LDL receptor activity. Rats fed a hypercholeserolemic diet and treated with 7S globulin or the __ subunit have a dose-dependent decrease in plasma cholesterol and triglycerides, and the highest doses produce the same level of cholesterol reduction as clofibrate(a drug which is used in the treatment of hypercholesterolemia).

The LDL receptor is regulated by a sophisticated system of membrane bound transcriptional factors. Does the __ sub-unit interfere with this mechanism?

Studies with cell cultures investigating the effect of soy protein on SREBP-1 (sterol regulatory element binding protein-1) modulation have shown that the __ sub-unit of the 7S globulin causes a marked increase in SREBP-1 in membranes and nuclei. For the __ sub-unit of the 7S globulin to exert its effects *in vivo*, it must be released by enzymatic breakdown of soy protein, enter the enterocytes, then enter the blood stream and reach the LDL receptor.

In a study to test which peptides are active, the effect of a synthetic peptide corresponding to a sequence between the _ and __ subunits from the 7S soy globulin on cell cholesterol homeostasis was investigated. In an *in vitro* experiment, cells exposed to the synthetic peptide showed an increased uptake of LDL and of LDL degradation compared with non-exposed cells. A dose dependent increase in the SREBP-2 mRNA and LDL-receptor mRNA was also observed. In an *in vivo* study, feeding the synthetic peptide to casein-cholesterol fed rats for 14 days resulted in lower plasma cholesterol and triglyceride levels, compared with control rats. These data provide evidence that the __ sub-unit from the 7S soy globulin and its synthetic peptide are responsible for the direct up-regulation of LDL receptors via the SREBP-2 pathway.

In the future it may be possible to select the breeding of novel soy cultivars with high amounts of bioactive peptides for cholesterol lowering.

In the question and answer session, it was discussed that there is not yet sufficient of the purified __ subunit from the 7S soy globulin to undertake clinical research.

Prof. S. Kersten (Wageningen, The Netherlands) discussed *Soy and impact on peroxisome proliferator activated receptors (PPARs).* PPARs are part of the nuclear hormone receptors family that play an important role in the regulation of metabolic processes. They bind specific ligands, and regulate gene transcription by binding to DNA.

There are three different PPARs, and they each regulate various metabolic processes and are characterized by a distinct tissue expression profile:

- PPAR-_ is expressed in liver, heart and small intestine. It is involved in metabolism of lipid, glucose and amino acids, and the inflammatory response.
- PPAR-_ is expressed in adipose tissue and colon and activates lipid and glucose metabolism, is an important regulator of cell cycle control, and the inflammatory response.
- PPAR-_/_ is ubiquitous and activates the fatty acid oxidation pathway in muscle, production of VLDL in liver and wound healing in the skin.

Since the binding pocket of PPARs is very large, PPARs can be activated by various compounds:

- Ligands of PPAR-_ include eicosanoids, PUFA, phytanic acid and fibrates.
- Ligands of PPAR-_ include eicosanoids (PGJ2), PUFA and glitazones.
- Ligands of PPAR-_/_ include PUFA and synthetic agonists.

The fatty acids and isoflavones in soy are both potential activators of PPARs. Polyunsaturated fatty acids, present at high concentrations in soy, have been demonstrated to bind and activate PPARs both *in vitro* and *in vivo*. Soy provides linoleic (18:2) and _-linolenic (18:3) fatty acids, which are connected with numerous health benefits. For example _-linolenic acid has been shown to improve vascular function and cardiac arrhythmia and to have anti-inflammatory effects.

Binding to PPARs is one of the proposed mechanisms for the potential therapeutic effect of isoflavones. PPAR-_ stimulates anabolic processes such as adipogenesis, lipogenesis and glucose uptake. Genistein stimulates adipogenesis at high concentrations and it has been shown that at increasing concentrations genistein can activate the PPAR-_ receptor. Genistein and daidzein can also activate PPAR-_, which is involved in catabolic processes such as fatty acid oxidation, and daidzein can activate PPAR-_/_, although the activation effect is more modest than with PPAR-_.

While isoflavones in soy are able to bind and activate PPARS both *in vitro* and *in vivo* it is unclear to what extent isoflavones can simulate gene expression via PPAR-_ *in vivo*.

In his talk *Soy & phytoestrogens: beyond lipid lowering, anti-oxidant, anti-inflammatory effects* **Prof. T. Sanders** (London, UK) explained that the context of the overall diet is important when considering benefits of soy since the consumption of soy products results in increases in plasma phytoestrogen concentrations, to levels where physiological effects may be expected. How much soy people eat is therefore relevant - for example in Japan, Korea and China typical intakes are 15g to 30g per day. Recent data from China show that substitution of vegetable for animal protein is associated with raised blood pressure.

Soy protein has anti-atherogenic properties compared with casein and research has shown that these effects are reduced with alcohol extracted soy protein. The anti-atherogenic properties cannot entirely be explained by the effects on serum lipids alone. Other potential mechanisms include anti-inflammatory effects and antioxidant effects that conserve endothelial function. Both of these effects may occur at lower levels of intake than required for lipid lowering.

The phytoestrogens genistein and daidzein and the metabolite equol are antagonists and partial agonists for the estrogen receptor-_ (ER-_) and ER-_. Genistein is also an inhibitor of tyrosine kinase and has been demonstrated to be an antioxidant *in vivo*. Equol is produced from daidzein by intestinal bacteria in about a third of people, and is more estrogenic than genistein. Studies in animals have demonstrated dietary soy isoflavone-induced increases in antioxidant and eNOS gene expression, while life-long exposure of rats to a soy-based diet increased plasma isoflavone levels and resulted in increased sensitivity of mesenteric arteries to acetyl choline, which suggests increased production of NO.

Whereas estrogens beneficially lower LDL cholesterol, raise HDL cholesterol, and increase endothelial NO production, they are also associated with a number of adverse effects including an increase in procoagulant activity, a decrease in fibrinolytic activity and an increase in C-reactive protein. In contrast soy phytoestrogen consumption does not have the undesirable effects seen with oral estrogens on markers of procoagulant activity in young men and women, or in post-menopausal women. Results from the randomised, double-blind ISOHEART trial in postmenopausal women show that treatment with isoflavone lowered C-reactive protein after 4 weeks of the 8-week study in subjects with initial values >1mg/L.

While endothelium-dependent vasodilation in the forearm vasculature is induced by infused genistein, showing a similar effect to that of 17-_-estrodiol, there is no effect with daidzein. Genistein also potentiates the endothelium-dependent vasodilator acetylcholine.

Large changes with soy on flow mediation have been observed but the results are very variable – some of these differences are due to use of different doses and different test materials. While real effects are suspected, more research is needed in this area, and also to determine the influence of the changes on disease risk.

Decreased lipid oxidation has been demonstrated *in vivo*, following consumption of 15g intact soy protein, compared with a similar amount of alcohol extracted soy protein. Consuming intact soy protein foods appears to decrease markers of oxidative damage and may have beneficial effects on endothelial function.

Finally, **Prof. K. Widhalm** (Vienna, Austria) in a presentation entitled *Safety & efficacy of soy in prevention of atherosclerosis in children* discussed data in adolescents that show LDL cholesterol levels to be clearly related to the percentage of the aorta with fatty streaks. However, there is uncertainty as to when the prevention of premature CVD should start in children with familial hypercholesterolemia (FH) and polygenic hypercholesterolemia (PH).

Prof Widhalm has therefore been investigating the long-term effects of soy in such children, as data for long-term additional benefits of soy protein are sparse. In phase 1 of the trial, subjects (n=23) followed a reduced and modified fat diet for 3 months and in phase 2 of the trial (n=16) Alpro soy products were substituted for animal protein with the aim of providing 0.5kg soy protein/kg body weight. Subjects that entered the trial had either FH (LDL -cholesterol >155mg/dl, total cholesterol >270mg/dl, TG<100 mg/dl or positive gene analysis), or PH (LDL >130mg/dl) and were not on lipid lowering medication. The average age of subjects included in the trial was 7.2 years, range 5-20 years – i.e. a very young sample.

Dietary records confirmed that the subjects were eating sufficient soy protein during phase 2 of the trial. Saturated fat intake was reduced from 45% of total fat at baseline, to 36.8% after phase 1 and to 30.7% after phase 2 (p<0.0001) and polyunsaturated fat intake increased from 19% at baseline to 25.7% after phase 1 and 33.8% after phase 2

($p<0.001$). Monounsaturated fat intake remained steady ranging around 37-35% of total fat intake.

While the phase 1 diet resulted in a significant 13% reduction in serum total cholesterol ($p<0.0001$), a significant 12% reduction in LDL-cholesterol ($p<0.0001$), and a 10% reduction in apolipoprotein B (($p<0.05$) the phase 2 soy diet reduced total cholesterol by a further 8% ($p<0.01$), reduced LDL cholesterol by a further 8% ($p<0.01$) and reduced apolipoprotein-B by a further 21% ($p<0.001$).
Prof Widhalm referred to the many questions surrounding FH, including how and when patients should be screened and what markers should be used, when treatment should be started, what goals of treatment are appropriate, whether children with a gene defect should be treated more aggressively, how intensive dietary therapy should be, and when drug treatment should be started.

Prof Widhalm concluded that substituting soy for animal protein is of additional benefit to a low fat diet and may be a useful dietary tool for the prevention of early vascular disease in children and adolescents with FH and PH. The results of his study suggest that in many children drug treatment can be postponed by strict dietary management.

In the question and answer session the potential benefit of a portfolio diet investigating the joint effect of plant stanols, soy protein and reduced fat intake, on lowering cholesterol levels was discussed. It was suggested that a randomised-controlled trial should be undertaken.

Overall the speakers presented some promising data and provided an informative update on some of the more recent work on various aspects of soy, and its potential health benefits.

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