SECTION II: B
THE EFFECT OF SOY PROTEIN ON HOMOCYSTEINE

In 1999 FDA approved a soy/heart health claim on the premise that soy protein lowers total and LDL cholesterol. The FDA gave no consideration to soy protein's effect on other cardiovascular risk factors.

A considerable body evidence suggests that homocysteine level is a far better marker of heart disease risk than cholesterol.[1-5] We submit that it is improper for FDA is to allow a claim for soy protein being heart healthy unless it also has shown a consistent and significant effect on the lowering of homocysteine levels. No such effect has been found.

In 2005 the US Agency for Healthcare Research and Quality released a report showing that no definitive conclusions could be drawn regarding soy's effect on homocysteine levels. An excerpt from the 245-page agency report is below:

> US Agency for Healthcare Research and Quality. *Effects of Soy on Health Outcomes*
> . Evidence Report/Technology Assessment , Number 126, Prepared by Tufts-New England Medical Center Evidence-based Practice Center, Boston, MA. August 2005.
>
> Only five studies of moderate to poor quality reported data on the effect of consumption of soy products on homocysteine levels. Overall, across studies, there were no discernable differences in effect based on baseline levels, soy protein consumption, soy isoflavone consumption, soy incorporated into diet or as supplement, or population (post-menopausal women, pre-menopausal women, men). Four studies reported greater net effect of soy on homocysteine levels compared to controls. Given the small number of studies no definite conclusions can be made on the beneficial effect of soy protein consumption on this CVD risk factor.

It is important to point out that most studies on soy and homocysteine are deeply flawed because of the routine use of casein as the control. This was the case in four out of five of the studies reviewed above and is true for most of the studies published since. Casein is a fractionated milk protein product with elevated methionine levels and extremely low levels of the amino acid cysteine. This stimulates the body to make cysteine through the toxic intermediary homocysteine. We also know that in humans, methoinine loading can lead to a rapid increase in plasma homocysteine levels.[6]

The strong likelihood that casein will raise homocysteine levels compared to soy protein makes it an extremely poor control in terms of evaluating soy protein's effect on homocysteine levels.[7]

Casein is a poor protein high in methionine and low in cysteine. Soy is a poor protein low in methionine and higher in cysteine. The fact that soy protein does not have a consistently and demonstrably better effect on homocysteine levels compared to casein indicates that it is a very poor quality protein indeed.

In the 2007 study excerpted below, soy performed even worse than the casein control in a variety of categories, including homocysteine.

> Anderson JW, Fuller J, Patterson K, Blair R, Tabor A. Soy compared to casein meal replacement shakes with energy-restricted diets for obese women: randomized controlled trial. *Metabolism.* 2007 Feb;56(2):280-8.
>
> The purpose of the present study was to evaluate the weight-loss efficacy and changes in body composition, waist circumference, blood pressure, and levels of plasma glucose, insulin, serum lipids, C-reactive protein, and homocysteine from consumption of either 3 soy shakes or 3 casein shakes daily as part of a 16-week, energy-restricted diet for obese women. Forty-three women with body mass index values of 30 to 40 kg/m(2) were randomized to intensive dietary interventions using either casein (n = 21) or soy (n = 22) shakes. Subjects were instructed to consume 3 shakes, 1 prepackaged entrée, and 5 servings of fruits or vegetables daily to achieve an energy intake of 4.5 to 5.0 MJ/d. . . . Body fat losses were 23.7% +/- 2.0% for casein and 21.8% +/- 2.4% for soy and did not differ significantly. Both study groups lost significant amounts of weight with a highly structured behavioral program incorporating 4 meal replacements and vegetables and fruits. Differences in weight loss and body composition changes between casein and soy treatments were not significant.
>
> Serum homocysteine levels increased in both groups and were significantly increased at 8 weeks with soy, but there were no significant differences between treatments.

These results surprised and disappointed the researchers who chose to omit mention of soy's comparatively poor performance from the abstract. In the body of the paper, they wrote It is possible that the intensity of the intervention . . . may have minimized differences between casein and soy effects. We submit that the intensity was indeed the source of the problem; fed three shakes per day, the subjects consumed very little food that could have helped them compensate for the amino-acid deficiencies of either the soy or the casein in the shakes.

Some researchers claim that soy protein's low methionine content should be regarded as an asset because it might be the key to its purported homocysteine-lowering benefits. [8] However, the research does not support the idea that soy's low level of methionine is beneficial. The FDA requires manufacturers to add this essential amino acid to soy infant formula and manufacturers routinely add it

to soy-based animal feeds to ensure adequate growth. In adults, methionine deficient diets and altered methionine metabolism have been linked to compromised immunity, atherosclerosis and malignancies.[9-15]

Rather than improve homocysteine levels, methionine deficiencies can lead to reduced SAM s-adenosyl methionine) synthesis, which, in turn, might raise levels of homocysteine. [16] Diets containing soy protein isolates proved atherogenic to Cebus monkeys, but feeding supplemental methionine to them prevented atherogenesis, probably because of reduced plasma levels of homocysteine due to increased SAM synthesis.[15,16]

Similarly, the study of retired school teachers excerpted below found that higher levels of methionine were associated with less coronary artery disease and greater clearance of homocysteine from the blood.

> Stolzenberg-Solomon RZ, Miller Er et al. Association of dietary protein intake and coffee consumption with serumhomocysteine concentrations in an odler population . *Am J Clin Nutr,* 1999, 69, 467-475.
>
> . . . increased dietary protein intake was associated with lower fasting tHcy concentrations and greater coffee consumption with higher fasting tHcy concentrations . . . The mechanism behind the inverse relation between protein intake and fasting tHcy concentrations is speculative. Dietary methionine is correlated with dietary protein. Because oral methionine loading increases tHcy concentrations , we initially hypothesized that dietary protein would be positively associated with tHcy. Note that methionine loading represents a short-term, extreme situation, however, in which one amino acid, methionine, is metabolized through the homocysteine pathway. In contrast, protein intake, as examined in this study,represents long-term consumption. Short- and long-term changes in protein intake can alter protein catabolism . In addition,high-protein foods contain other amino acids and nutrients that could influence tHcy.
>
> In animals, a high methionine intake induces more efficient catabolism of homocysteine through activation of homocysteine-catabolizing enzymes. It has been shown that the transsulfuration and, to a lesser extent, the methionine regeneration pathways are activated in the livers of animals fed excessive amounts of methionine . Finkelstein and Martin believed that serine and betaine were the limiting factors for their respective reactions with excess methionine Andersson et al found no changes in results of a post methionine load test, methionine clearance, or tHcy concentration after excess methionine was added to 6 human subjects usual diets for 13 d. This study, however, had limited power because of small numbers, did not control for differences in the participants usual diets or energy intake, and limited the feeding of the additional methionine to the 2 wk before the test.
>
> High protein intakes might have beneficial physiologic effects. Preagricultural humans evolved on a diet high in animal protein (37%), low in fat (22%) and high in fruits and vegetables (41% carbohydrate) Recently, in the Nurses Health Study, higher methionine intake was prospectively associated with less coronary artery disease 95% CI: 0.65; 1.03) independent of

dietary folate, other cardiovascular risk factors and vitamin B-12 intake (relative risk: 1.09; 5% CI: 0.82, 1.44). If high-protein diets are not limited in serine or choline, it is biologically plausible that these 2 pathways could be increasing tHcy clearance from the blood and possibly increasing survival.

In conclusion we found a strong inverse correlation between protein intake and serum 1n tHcy concentrations in older persons. In addition, we found independent positive relations between in tHcy and . . . prestudy use of supplemental B vitamins

Soy protein is also likely to raise homocysteine levels because the cysteine is either biounavailable or damaged by modern processing methods. Much of the cysteine contained in soybeans is bound up in the cysteine protease inhibitors, which include the trypsin inhibitors, cystatins and soyacystatins. Because protease inhibitors are stubbornly resistant to heat treatments and other modern processing methods, soybean cysteine is not readily available compared to other proteins.[17-26] Compounding the problem, polyunsaturated oil residues leftover from the soy protein extraction processes create epoxides that are not only capable of poisoning L-cysteine but all other thiol substances in the body. [27-30] Cysteine itself can be rapidly oxidized and irreparably damaged during the manufacturing process when exposed to atmospheric oxygen and an alkaline pH (above about 7.5 to 8)[31] With such damage through treatments and exposures, it is not surprising that soy is such a poor source of cysteine.

Cysteine is also damaged by chemical processing at high temperatures and intense pressures used to eliminate soy's beany flavor (which does not appeal to most consumers) and inactivate the antinutritional factors such as oligosaccharides and protease inhibitors (which cause flatulence and other forms of digestive distress).[32-35] These treatments -- especially those involving acidic chloride salts produced in cycling between acidic and basic treatments -- leach carcinogenic metals (nickel, cobalt and chromium) from the stainless steel vats into the soy protein products, where they bind tenaciously to any available cysteine. High pH exacerbates the problems of metal binding to soy protein and peptides by causing the alkaline hydrolysis (disproprotionation) of cystine disulfides to their sulfenate and thiolate ions. Because thiolate ions are likely to be oxidized further in atmospheric oxygen and because of their tenacious binding with toxic metals, they are unlikely to return to the disulfide form with the elimination of water when the pH is lowered.[36,37]

Elevated homocysteine levels are a likely consequence of soy protein's low level of bioavailable cysteine. It has been known for decades that whenever the body attempts to replace depleted or *unavailable* levels of cysteine, it does so even if from limiting amounts of methionine, but mammalian systems do so through the toxic intermediary metabolite, homocysteine. Accumulated metal toxins in

the body from the processing of foods and environmental exposures can contribute to failure of this pathway by binding and interfering with homocysteine's conversion, thereby causing it to accumulate metabolically.[38, 39.] Accumulating metal toxins may even co-precipitate with and concentrate homocysteine in vulnerable areas of the body causing arterial plaque, neoplasia, tumors and a variety of other metabolic imbalances.[40] The metals known to bind thiols the most tightly include some of the most potent known carcinogens. However, copper, iron, manganese and other metals that are nutritious or otherwise beneficial to the body in small amounts are also associated with cancer and other diseases when found at excessive levels and co-accumulating with homocysteine.[41,42]

Recently, a new, related threat has emerged. With the extensive use of antibiotics, resistant pathogenic organisms have developed. Several pathogens have been reported to divert methyl groups in order to methylate mercury or other toxic metals. When methylated, mercury is far more toxic, has far greater affinity for fatty tissues and is far more difficult to remove from the body. [43-45] Under normal circumstances, the body would use these methyl groups to regenerate methionine from homocysteine, to remove any inhibition of cysteine biochemistry by homocysteine, or to perform critical methylating reactions involving S-adenosyl-methionine (SAM).[46]

Cysteine is also critical for the vitalethine/monooxygenase receptor/humoral immunity pathways through which people respond to infections, cancer and other immune challenges. When dietary methionine and cysteine are marginalized as in soy protein -- only homocysteine may be *available*, and vitalethine may not be produced. Through its thiolactone-enol tautomer, homocysteine probably directly poisons vitaletheine's sulfenic acid, thereby uncoupling the ability of vitalethine s monooxygenase receptor to catalytically reactivate vitaletheine to its sulfenic acid and essentially uncoupling virtually all sulfur-dependent regulatory control in the body. This metabolic poisoning is chemically exacerbated by metal toxicity (which afflicts most of the American population), especially through homocysteine s thiolactone that is poised to react with vitaletheine s thioperoxide, and is especially problematic for people also suffering from methionine and cysteine deficiencies (as in people who overly consume soy protein with its load of protease inhibitors). Unfortunately, even when cysteine can reportedly be made *available*, and to increase in liver (in response to soy feedings), poisonings with copper, cadmium, and mercury, [47] and presumably other metals that bind thiols tightly like those accumulating in highly processed foods (*e.g.*, nickel, cobalt, and chromium from carcinogenic stainless steel,[48] can still imbalance sulfur biochemistry by shifting away from the control of the vitalethine/monooxygenase receptor/humoral immunity pathways, and into the more reducing environments (*e.g.*, glutathione, albeit S-blocked by metal toxins,[49] favoring cholesterol biosynthesis, isoprenlylation and oncogene expression, cell-mediated/lymphokine-dependent

inflammation and proliferation, and ultimately cancerous and atherosclerotic neoplasia, plaques, and calcifications [50,51]

Yet another mechanism by which soy protein might increase homocysteine is through thyroid depression, a well-documented effect.[52-60] In addition to contributing to atherogenesis, arrhythmias, atrial fibrillation, PVCs and other heart disease risk markers, low thyroid status impacts homocysteine levels.

> Orzechowska-Pawilojc A, Sworczak K, Lewczuk A, Babinska A Homocysteine, folate and cobalamin levels in hypothyroid women before and after treatment. *Endocr J.* 2007 54, 3, 471-476.
>
> Thyroid status influences the plasma tHcy. Free triiodothyronine and next free thyroxine have the greatest negative influence. This would account for hyperhomocysteinemia in the hypothyroid state and premature atherogenesis."

> Mayer O Jr, Simon J, Filipovský J, Plásková M, Pikner R. Hypothyroidism in coronary heart disease and its relation to selected risk factors. *Vasc Health Risk Manag.* 2006, 2, 4, 499-506.
>
> . .. Hypothyroid subjects had higher total homocysteine in both genders . . .. Hypothyroid females had higher total and LDL cholesterol, and were more often treated for diabetes. CONCLUSIONS: HT was found highly prevalent in patient with clinical coronary heart disease, mainly in females, and was associated with several cardiovascular risk factors.

> Evrengul H, Tanriverdi H et al. Interaction of plastma homocysteine and thyroid hormone concentrations in the pathogenesis of the slow coronary flow phenomenon. *Cardiology,* 2007, 108, 3, 186-192.
>
> BACKGROUND AND OBJECTIVE: The slow coronary flow (SCF) phenomenon is an angiographic observation and a well-recognized clinical entity characterized by delayed opacification of vessels in a normal coronary angiogram due to reasons yet unclear. Thyroid hormones exert significant effects on plasma homocysteine (Hcy) levels and microvascular resistance. Recently, several investigators have consistently reported that elevation of the plasma Hcy level can severely disturb vascular endothelial function and play a role in the pathogenesis of SCF. Accordingly, we investigated the levels of plasma Hcy and thyroid hormones and their relationship in patients with SCF. CONCLUSION: fT3 levels were decreased and plasma Hcy levels were increased significantly in patients with SCF as compared to controls. This finding suggests that thyroid hormones and/or (?) a possible disturbance in their metabolism may be responsible for the elevated levels of plasma Hcy in patients with SCF and may play a role in the pathogenesis of the SCF phenomenon.

* * * * *

Phytates in soy might decrease homocysteine levels, but the limited evidence available suggests that this does not occur in people consuming soy protein products with their full complement of isoflavones. This detail is significant because the FDA soy/heart health claim is for standard, isoflavone-containing soy protein products. The study showing the effect of phytates involved a ***special*** soy protein product in which the ***isoflavones had been removed***.[61]

* * * * *

The homocysteine theory of arteriosclerosis is based on evidence that elevation of blood homocysteine concentrations is a major contributing factor in cardiovascular disease. Homocysteine may become elevated as the result of dietary, genetic, metabolic, hormonal, or toxic factors. Dietary deficiency of vitamin B-6 and folic acid and absorptive deficiency of vitamin B-12, which result from traditional food processing or abnormal absorption of B vitamins, are important factors in causing elevations in blood homocysteine.[62]

Fortification of the US food supply with folic acid in 1998, as mandated by the US Food and Drug Administration, was associated with a further decline in mortality from vascular disease, presumably because of increased blood folate levels and decreased blood homocysteine in the population. However, the currently allowed soy/heart disease health claim has had the potential for worsening the homocysteine situation because soy protein products do not naturally contain any B12. In fact there is evidence that soy protein isolates increase the body's requirements for B12.[63-65] Vitamin B12 at the level of 400 - 1,000 mcg per day is needed to facilitate the conversion of homocysteine back to methionine,thereby improving homocysteine levels. Vitamin B12 functions as a cofactor for methionine synthase, the enzyme that catalyzes the remethylation of homocysteine to form methionine.[66]

In summary, soy protein is a product devoid of B12 and reportedly can even increase the body's requirements for B12. FDA-mandated B12 fortification might reduce soy protein's contribution to elevated homocysteine levels by providing the key nutrient (vitamin B12) required for converting it back to methionine, but fortification alone cannot make soy protein a “heart healthy” substance for the myriad reasons discussed above and elsewhere in this petition. These issues include but are not limited

to the following: compromised availability of cysteine, cystine and methionine; the incomplete digestion of soy protein due to the action of protease inhibitors and other factors; and the toxic accumulations of ornithine and metal toxins which result from the processing of soy protein. After ingestion soy protein products create, or have been associated with, increased HMG-Coenzyme A reductase activity along with bile acid synthesis and secretion, thyroid disruption including decreases in T4 and increases in T3, steroid hormone imbalances, and dangerous accumulations of homocysteine, especially homocysteine thiolactone. Because many of these soy protein-induced changes have been associated with cancer, thyroid and steroid hormone disruption, humoral immune suppression, thymus atrophy, and cardiovascular disease such as atherosclerosis, soy protein clearly does not merit a health claim. We therefore request that FDA amend the "Final Rule Re Food Labeling: Health Claims; Soy Protein and Heart Disease" to disallow the heart disease health claim for soy protein.

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ENDNOTES

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plasticised PVC films for applications such as wrapping food and covering food for re-heating in a microwave oven. Levels of ESBO in fresh retail meat samples wrapped in film ranged from less than 1 to 4 mg/kg, but were higher (max. 22 mg/kg) in retail cooked meat. Migration into sandwiches and rolls from 'take-away' outlets ranged from less than 1 to 27 mg/kg depending on factors such as the type of filling and the length of the contact time prior to analysis. The levels of migration of ESBO into cheese and cakes were consistent with previous experience with plasticiser migration--direct contact with fatty surfaces leading to the highest levels. When the film was used for microwave cooking in direct contact with food, levels of ESBO from 5 to 85 mg/kg were observed, whereas when the film was employed only as a splash cover for reheating foods, ESBO levels ranged from 0.1 to 16 mg/kg. For a variety of aby foods there was no significant difference in ESBO levels between foods packaged in glass jars with PVC gaskets and foods in cans containing ESBO in the can lacquer. In both cases ESBO levels were low, ranging from less than 0.1 to 7.6 mg/kg. It is not clear for these retail samples, if the low levels observed (average 1.9 mg/kg) result solely from migration or contain some contribution from naturally occurring epoxides.

31. http://www.vitaletherapeutics.org/vtlcsmal.htm reaction
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33. Dudásová S, Grancicová E. Influence of casein and soy flour proteins on aminoacid content in the liver of experimental animals. *Physiol Res*. 1992, 41, 6, 411-6. Research Institute of Human Nutrition, Bratislava. We have observed a significantly increased content of fats and decreased content of proteins in the liver of experimental rats fed a diet supplemented with 25% casein proteins in comparison with the application of de-fatted soy flour. Casein proteins have a higher content of methionine in relation to cystine than baked soy flour. But the soy diet in contrast to the casein diet has a high content of free amino acids which are not present in casein at all: aspartic acid, asparagine, alpha-aminoadipic acid, methionine, norleucine, lysine, phenylalanine, beta-alanine, ethanolamine, histidine, proline, gamma-aminobutyric acid, taurine. Differences in free valine, alanine, arginine, glycine, ornithine and cysteic acid are also significant. The content of free amino acids in the liver of experimental animals fed a soy diet is high in the content of cystine, cystathionine, ornithine, beta-aminoisobutyric acid, beta-alanine, gamma-aminobutyric acid, leucine. We have also found accumulation of methionine, glycine, alpha-aminobutyric acid, taurine and citrulline in free aminoacids from the liver of animals fed a casein diet. Citrulline and glycine in free aminoacids from the liver of animals fed a soy

protein supplement were not recorded. Our investigations have shown that the application of a soy diet enriched with cystine acts protectively on methionine and that methionine is preferentially utilized for protein synthesis. The catabolic pathway of methionine prevails in animals on a casein diet.

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38. Allred MC, MacDonald JL Determination of sulfur amino acids and tryptophan in foods and food and feed ingredients: collaborative study. *J Assoc Off Anal Chem.* 1988 May-Jun;71(3): 603-6. Ralston Purina Co., Central Research Services, St. Louis, MO 63164. Samples of 4 foods, 1 animal feed, isolated soy protein, and beta-lactoglobulin were analyzed by 9 laboratories to determine concentrations of cysteine as cysteic acid, methionine as methionine sulfone, and tryptophan. Sulfur amino acids were determined by AOAC method 43.A08-43.A13 for food and feed ingredients, in which samples are oxidized with performic acid before protein hydrolysis with 6N HCl. Tryptophan was determined after protein hydrolysis with 4.2N NaOH. In both methods, free amino acids were separated by ion-exchange or reverse-phase chromatography. Each laboratory was provided with detailed methods and with sealed vials containing solutions of standards. Samples were analyzed in duplicate, and variation between laboratories was determined. Coefficients of variation between laboratories for the 6 samples ranged from 5.50 to 11.8% for methionine as methionine sulfoxide, 8.59 to 17.3% for cysteine as cysteic acid, and 3.87 to 16.1% for tryptophan. Amino acid recoveries were determined by analysis of beta-lactoglobulin and were based on expected levels of each amino acid obtained from amino acid sequence data. The mean recovery of cysteine was 97% with a range of 88-119%. For methionine, mean recovery was 98% (range 89-115%) and for tryptophan, 85% (range 59-102%). Method 43.A08-43.A13 for food and feed ingredients has been adopted official first action for determination of cysteine and methionine in processed foods. The alkaline hydrolysis method has been adopted official first action for determination of tryptophan in foods and food and feed ingredients.

39. Jocelyn, P.C. *Biochemistry of the SH Group*, pp. 1-46, 100-136, 163-278, 337-349. London Academic Press, 1972).

40. *Ibid*.

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Methylation events play a critical role in the ability of growth factors to promote normal development. Neurodevelopmental toxins, such as ethanol and heavy metals, interrupt growth factor signaling, raising the possibility that they might exert adverse effects on methylation. We found that insulin-like growth factor-1 (IGF-1)- and dopamine-stimulated methionine synthase (MS) activity and folate-dependent methylation of phospholipids in SH-SY5Y human neuroblastoma cells, via a PI3-kinase- and MAP-kinase-dependent mechanism. The stimulation of this pathway increased DNA methylation, while its inhibition increased methylation-sensitive gene expression. Ethanol potently interfered with IGF-1 activation of MS and blocked its effect on DNA methylation, whereas it did not inhibit the effects of dopamine. Metal ions potently affected IGF-1 and dopamine-stimulated MS activity, as well as folate-dependent phospholipid methylation: Cu(2+) promoted enzyme activity and methylation, while Cu(+), Pb(2+), Hg(2+) and Al(3+) were inhibitory. The ethylmercury-containing preservative thimerosal inhibited both IGF-1- and dopamine-stimulated methylation with an IC(50) of 1 nM and eliminated MS activity. Our findings outline a novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation. The potent inhibition of this pathway by ethanol, lead, mercury, aluminum and thimerosal suggests that it may be an important target of neurodevelopmental toxins.

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and cadmium ions. Copper ions exhibited mainly two effects that were different from those of mercury and cadmium ions. They lowered the reduced fractions of thiols and increased the release of homocysteine into the medium, whereas mercury and cadmium ions mainly influenced the metabolism of glutathione by increasing its synthesis. Even 0.1 micromol/l of copper ions increased the release of homocysteine in HeLa cell lines. An increased cellular concentration of glutathione and an increased release of glutathione into the medium were observed after addition of mercury and cadmium ions at a concentration of 1 micromol/l, which is just above the toxicity limit in human blood. The different cell lines varied in some respects in their response to the addition of metal ions. Cadmium ions had no effect on thiol metabolism in endothelial cell lines, and copper ions did not significantly increase the release of homocysteine into the medium in hepatoma cell lines. Furthermore, the metabolism of thiols during basal conditions (without the addition of metal ions) differed somewhat in the three cell lines investigated. One example is the low amount of extracellular glutathione in hepatoma cell lines, which probably was due to its rapid degradation to cysteinylglycine by gamma-glutamyl-transpeptidase.

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54. Doerge DR, Sheehan DM. Goitrogenic and estrogenic activity of soy isoflavones. Environ Health Perspect. 2002 Jun;110 Suppl 3:349-353. Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, Arkansas, USA. Soy is known to produce estrogenic isoflavones. Here, we briefly review the evidence for binding of isoflavones to the estrogen receptor, in vivo estrogenicity and developmental toxicity, and estrogen developmental carcinogenesis in rats. Genistein, the major soy isoflavone, also has a frank estrogenic effect in women. We then focus on evidence from animal and human studies suggesting a link between soy consumption and goiter, an activity independent of estrogenicity. Iodine deficiency greatly increases soy antithyroid effects, whereas iodine supplementation is protective. Thus, soy effects on the thyroid involve the critical relationship between iodine status and thyroid function. In rats consuming genistein-fortified diets, genistein was measured in the thyroid at levels that produced dose-dependent and significant inactivation of rat and human thyroid peroxidase (TPO) in vitro. Furthermore, rat TPO activity was dose-dependently reduced by up to 80%. Although these effects are clear and reproducible, other measures of thyroid function in vivo (serum levels of triiodothyronine, thyroxine, and thyroid-stimulating hormone; thyroid weight; and thyroid histopathology) were all normal. Additional factors appear necessary for soy to cause overt thyroid toxicity. These clearly include iodine deficiency but may also include additional soy components, other defects of hormone synthesis, or additional goitrogenic dietary factors. Although safety testing of natural products, including soy products, is not required, the possibility that widely consumed soy products may cause harm in the human population via either or both estrogenic and goitrogenic activities is of concern. Rigorous, high-quality experimental and human research into soy toxicity is the best way to address these concerns. Similar studies in wildlife populations are also appropriate.

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56. Mariotti S, Cambuli VM. Cardiovascular risk in elderly hypothyroid patients. *Thyroid.* 2007 17, 11, 1067-73.Endocrinology Department of Medical Sciences M. Aresu, University of Cagliari, Policlinico Universitario di Monserrato, Monserrato, Cagliari, Italy. mariotti@pacs.unica.it Overt hypothyroidism (OH) and subclinical hypothyroidism (SH) are frequently found in the elderly. OH is associated with several functional cardiovascular abnormalities and increased risk of atherosclerosis resulting from hypertension associated to

atherogenic lipid profile. Other potential atherogenic factors involved in OH are increased circulating C-reactive protein and homocysteine, increased arterial stiffness, endothelial dysfunction, and altered coagulation parameters. Similar (although mild) cardiovascular abnormalities are present in SH. Since all these abnormalities regress with levothyroxine (L-T4) administration, the cardiovascular benefits of replacement therapy in OH are not questionable, independently from the patient's age or the presence of coexisting cardiovascular disease. On the other hand, in spite of a very large number of studies, no consensus has been reached so far about the actual cardiovascular and/or general health impact of SH, and different recommendations have been recently made about screening and treatment of this condition. Although divergent results have been obtained in several epidemiological studies, recent meta-analyses provide evidence for a slight but significant increase of coronary heart disease (CHD) risk in SH. However, no agreement has been reached in favor or against active screening and/or treatment of mild thyroid failure. Moreover, L-T4 therapy is discouraged in aged subjects, because the increased oxygen consumption consequent to thyroid hormone administration could be dangerous, especially in the presence of coexisting CHD. In keeping with this concept are recent data showing reduced mortality risk in untreated mild hypothyroid subjects aged >85 years, suggesting that some degree of decreased thyroid activity at the tissue level might have favorable effects in the oldest-old. However, the effects of subtle thyroid dysfunction may be different in different age ranges. Since the main studies supporting a role for SH as a risk factor for atherosclerosis, cardiovascular disease, and all-cause mortality have been carried out in populations aged > or =55-60 years, mild thyroid failure could concur to increased cardiovascular risk in middle-aged and "young elderly" subjects, while being devoid of detrimental effects and possibly protective in the oldest-old. Further studies are needed to confirm this hypothesis.

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Hypothyroidism is associated with premature atherosclerosis and cardiovascular disease. Recently, total homocysteine (tHcy) and C-reactive protein (CRP) emerged as additional cardiovascular risk factors. We first investigated CRP and tHcy in different severities of primary hypothyroidism and in a second study we evaluated the effect of L-thyroxine treatment in patients with subclinical hypothyroidism (SCH) in a double-blind, placebo-controlled trial. One hundred and twenty-four hypothyroid patients (63 with subclinical, 61 with overt hypothyroidism, OH) and 40 euthyroid controls were evaluated. CRP was measured using a latex-based high sensitivity immunoassay; tHcy was determined by a fluorescence polarization immunoassay. tHcy values were significantly elevated in OH (P=0.01). In SCH tHcy levels were not augmented as compared to controls. CRP values were significantly increased in OH (P=0.016) and SCH (P=0.022) as compared to controls. In a univariate analysis tHcy correlated significantly with fT4, vitamin B12, folic acid and creatinine levels. In multiple regression analysis only fT4 (beta=0.33) had a significant effect on tHcy. CRP did not correlate with thyroid hormones. In SCH, L-T4 replacement had no significant effect on either tHcy or CRP levels. This is the first paper to show that CRP values increase with progressive thyroid failure and may count as an additional risk factor for the development of coronary heart disease in hypothyroid patients. In contrast to overt disease, only CRP, but not tHcy values, are affected in SCH, yet without significant improvement after L-thyroxine therapy.

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Hypothyroidism is very often associated with cardiovascular diseases and neurological complications. Recently, homocysteine has been studied as an independent risk factor for atherosclerosis which negatively affects vascular endothelial cells. Because homocysteine metabolism is related to thyroid and steroid hormones, we studied these relationships in severe hypothyroidism and in euthyroid state. Homocysteine, testosterone and allopregnanolone concentrations were measured in the fasting plasma from 16 women who underwent total thyroidectomy, and who were either hypothyroid or euthyroid. Although all women used oral contraceptives, they were not protected against hyperhomocysteinemia during hypothyroid state. With the normalization of thyroid hormone concentrations homocysteine levels decreased to normal levels. There was a positive correlation between testosterone and homocysteine in the euthyroid state which suggests that not only estrogens but also androgen state should be considered in future studies on homocysteine.

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patients did not differ significantly from that of the controls. Serum creatinine was higher in hypothyroid patients and lower in hyperthyroid patients than in controls, whereas serum folate was higher in hyperthyroid patients compared with the two other groups. In multivariate analysis, these differences did not explain the higher tHcy concentration in hypothyroidism. We confirmed the observation of elevated serum cholesterol in hypothyroidism, which together with the hyperhomocysteinemia may contribute to an accelerated atherogenesis in these patients.

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