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February 18, 2008

Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, rm. 1061
Rockville, MD 20852.

RE: Docket No. 2007N-0464

The Weston A. Price Foundation, a non-profit nutrition education foundation with offices in Washington DC, is pleased to submit these comments and petition pursuant to the Federal Food, Drug and Cosmetic Act (FFDCA). The Foundation urges the US Food and Drug Administration to amend the Final Rule Re Food Labeling: Health Claims; Soy Protein and Heart Disease, which became effective October 19, 1999. Foods containing soy protein should not carry a heart disease health claim.

Under section 403 (4) (3) (B) (i) of the Federal Food, Drug and Cosmetic Act, FDA can authorize a health claim only if the standard of significant scientific agreement is met. The data submitted with this petition establishes a lack of consensus among experts, qualified by scientific training and experience, about claims that soy protein prevents heart disease or even lowers cholesterol.

In this petition, we will establish the fact that this standard has not been met and that the benefits of soy are putative and unproven for the following reasons:

- The totality of the scientific evidence on soy protein and heart disease is contradictory and inconsistent.
- Studies published since 1999 undermine the conclusions drawn from key studies evaluated by the FDA when it approved the health claim in 1999.
- Recent studies show that soy can actually contribute to or cause heart disease, including endothelial damage (especially in women), heart arrhythmias and cardiomyopathy, an increasingly prevalent condition that afflicts 1 in 500 Americans.

We have included as part of this petition extended commentary, summaries of important journal articles, quotations from qualified researchers and complete references to publicly available studies, all of which raise questions about and/ or disprove the validity of the currently allowed soy protein/heart disease health claim.

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Our petition includes three sections in which we rebut and refute the evidence used to establish the 1999 soy/ heart disease health claim:

I. PRELIMINARY REQUIREMENTS: We establish the fact that the soy protein health claim failed to conform to the requirements of 21 CFR 101.14 (b). Furthermore, soy protein products are widely available in the food supply today but were not in common use in food prior to 1958 and soy protein isolated has not received GRAS (Generally Recognized As Safe) status.

II. SCIENTIFIC EVIDENCE: Our review of the scientific literature on soy and heart disease indicates that soy protein does not reliably lower cholesterol, does not lower homocysteine, does not prevent heart disease and may cause, contribute to or accelerate the development of heart disease.

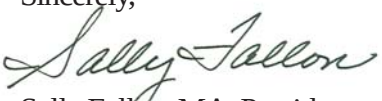
III. CONCLUSION: We document longstanding concerns in the scientific community – including experts at the FDA's own Laboratory for Toxicological Research, the National Center for Environmental Health Sciences, the Israeli Health Ministry, the French Food Agency and the German Institute for Risk Assessment – about soy's possible role in carcinogenesis, thyroid disease, reproductive health problems (including infertility) and other health problems.

In the light of the January 2006 Science Advisory published by the American Heart Association, we ask that the FDA give careful consideration to this petition. Although the AHA originally supported the FDA health claim, the AHA expert committee's subsequent examination of the evidence led the AHA to conclude that soy protein does not reliably lower cholesterol and does not prevent heart disease. The U.S. Agency for Healthcare Reform and Quality has also examined the evidence, and the Agency concluded in 2005 in a 245-page report that soy products may exert a small benefit on LDL and triglyceride levels, but the effects may be of small clinical effect in individuals.

We maintain that the FDA in its mandated role as America's foremost consumer protection agency has a duty to the American public to amend the Final Rule and thereby disallow use of a health claim regarding soy protein and heart disease health and to require all food manufacturers to cease and desist using this claim in their advertising and packaging.

Finally, we request a public hearing on these issues.

Sincerely,



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SECTION A:

PRELIMINARY REQUIREMENTS:
INELIGIBILITY OF SOY PROTEIN FOR A HEALTH CLAIM

Soy protein isolate has never received GRAS (Generally Recognized As safe) status as an additive to food. Unlike other GRAS substances in use prior to 1958, soy protein isolate was not originally developed as a food but as an industrial product to bind and seal paper products. It therefore does not qualify as a product having a long history of safe use in the food supply.

Soy protein isolate contains a number of toxins and carcinogens introduced into the product by the high temperatures, high pressures and chemicals used in its manufacture. In 1979, the Select Committee of GRAS Substances (SCOGS) examined safety issues pertaining to the manufacture of soy protein isolate and recommended the establishment of acceptable levels of the carcinogens nitrite and nitrosamines and the toxic amino acid lysinoalanine in order to avoid future problems. To this date, the FDA has not established safe levels of these toxins and no agency is monitoring levels of these substances in edible food. The SCOGS committee determined that 150 mg per day of soy protein was the maximum safe dose, an amount is far less than the 25 grams per day of soy protein recommended in the currently allowed soy/heart disease health claim.[1]

The approval of the soy/heart health claim in 1999 helped establish the image of soy as heart healthy and increased consumption of soy protein in the United States from an average of 0.78 g per day in 1999 to 2.23 grams per day in 2004. As a direct result of the health claim, many people who would not otherwise choose soy have consciously added more soy foods carrying the heart disease health claim to their diets. Many of these people consume amounts well in excess of the average. Populations at greatest risk are infants on soy formula, vegetarians and vegans who consume soy as both meat and dairy replacements and adults self medicating with soy foods because of their trust in the FDA-approved claim that, in so doing, they will prevent heart disease.

In its Final Ruling on the 1999 soy protein/heart health claim, the FDA chose to disregard the SCOGS's committee's warning and dismiss the dangers of nitrites, nitrosamines and lysinalanines, merely stating that good manufacturing practices are and should be employed. The fact is that good manufacturing processes are not subject to oversight and today's soy products do contain dangerous levels of these toxic and carcinogenic substances.

Scientists have known since 1937 that nitrosamines damage the liver; and since 1956 that nitrosamines are mutagens and carcinogens.[2,3] Nitrates occur naturally in vegetables, water and many foods and beverages, including those containing soy. Nitrate is harmless until reduced to nitrite, which occurs through the processing methods used to manufacture soy protein isolate (SPI), casein and other fractionated food products. Nitrites are very reactive chemically and lead to nitrosamine formation in processed foods. Preformed nitrosamines are especially likely to be found in soy protein isolates and other soy products that have undergone acid washes, flame drying or other high temperature spray-drying processes. USDA studies from the 1980s showed that soy protein isolate contained almost twice the nitrite levels contained in other soy protein products. They also found in soy protein levels of 1.5 parts per billion of a potent nitrosamine known as N-nitrosodimethylamine (NDMA).[4]

The California Environmental Protection Agency Office of Environmental Health Hazard Assessment has established safe levels for nitrosamines ranging from 40 ng per day for NDMA to 80 ug per day for the relatively weak nitrosamine N-nitrosodiphenlamine. The soy industry has stated that intakes of soy protein at the level of 25 grams per day and higher are reasonable, prudent and present no safety concerns. Indeed, in the petition to the FDA that led to the currently allowed soy/heart disease health claim, Protein Technologies International (PTI) promoted 100 grams per day of soy protein as healthful. Levels of nitrosamines vary from batch to batch, but if we accept the USDA finding of 1.4 parts per billion, people eating 100 grams per day of soy protein clearly could easily exceed safe limits. Furthermore, the safe levels have been defined for a 70 kg adult male, levels that might be toxic for adult women, teenagers, children and infants.[5]

Lysinoalanine is a cross-linked amino acid that is produced when the essential amino acid lysine is subjected to strong alkaline treatments. Soyfood processors use alkali because it helps them transform soybeans into soy milk, tofu, textured vegetable protein, soy protein isolate, soy protein concentrate and other products quickly and profitably. Only old-fashioned, fermented soy products or precipitated tofu made at home or in small, cottage-type industries can claim to be lysinolanine-free." [6,7]

Ghulam Sarvar, PhD, of the Nutrition Research Division of the Banting Research Centre in Ottawa, writes: The data suggested that LAL (lysinoalanine), an unnatural amino acid derivative formed during processing of foods, may produce adverse effects on growth, protein digestibility, protein quality and mineral bioavailability and utilization. The antinutritional effects of LAL may be

more pronounced in sole-source foods such as infant formulas and formulated liquid diets which have been reported to contain significant amounts (up to 2400 ppm of LAL in the protein) of LAL"[8]

The highest levels of lysinoalanines occur in soy protein isolates manufactured for use as sizing and coating adhesives for paper and paper-bound products. Such products are produced at high alkaline pH levels. Rats fed soy proteins processed using similar high alkali baths have suffered kidney damage, specifically increased organ weights, lesions and kidney stones. The industry claims that soy proteins intended for human consumption are safer because they are extracted at a pH level below 9, but a look at new processes receiving patents reveals that keeping alkaline levels low is not a high priority for much of the food-processing industry.[9-11] A recently patented process invented to deflavor soy milk, flour, concentrates and isolates involves adjusting the pH to levels ranging from 9 to 12.[12] A high pH makes it possible to dissolve the soy proteins and release the beany flavors through a special ultrafiltrated membranous exhaust system. Soy industry publications and processing manuals have repeatedly stated that soy's beaniness is a major deterrent to consumer acceptance and profitability and that it must find failproof and economically feasible ways to turn beany tasting soybeans into bland soy ingredients.

Clearly these facts do not support the assertion in FDA's Final Rule that good manufacturing practices are and should be employed. Furthermore, we would like to remind the FDA of the language it used regarding GRAS status in the Proposed Rule, Food Labeling: Health Claims: Soy protein and Coronary Heart Disease (63 FR 62977). FDA tentatively concludes that the petitioner has provided evidence that satisfies the requirement in §101.14 (b)(3)(ii) that use of soy protein at the levels necessary to justify the claim is safe and lawful. We believe that the word tentatively indicates that the FDA recognizes the fact that the GRAS issue has not been resolved. Nearly 30 years after the Select Committee on GRAS substances (SCOGS) voiced concerns about lysinoalanine, nitrites and nitrosamines, needed safety studies remain to be carried out.

It is evident that the soy industry knows that soy protein isolate ingredients would not be legitimately eligible for GRAS status. Protein Technologies International's original petition to the FDA -- submitted May 4, 1998 -- proposed defining soy protein concentrate and soy protein isolate as soy flour because the procedures used to convert vegetable flours to vegetable protein concentrates and isolates were commonplace in various sectors of the grain industry, such as corn processing, well before 1958. PTI therefore concluded that soy protein isolate and soy protein concentrate should be considered no different from soy flour.

Although FDA did not take issue with this self-determination of GRAS status in 1999, we submit that FDA should reconsider this decision. Soy flour is very different from the soy protein isolates and concentrates now penetrating the market. Soy flour is a low-tech product with a comparatively long -- though minor -- history of use in the food supply. Soy flour has been most heavily consumed as part of wartime rations, vegetarian fare and to stave off hunger caused by poverty, famine or natural disasters. It has no standard of identity as the industry uses natural, full-fat soy flours, raw enzyme active flours used as bleaching agents and crumb color enhancers, toasted soy flours and defatted and low-fat soy flours.[13-15]

In contrast, soy protein concentrate and soy protein isolate are high tech products that are precisely manufactured under industrial conditions in chemical factories, not kitchens. Soy protein concentrate (SPC) comes from defatted soy flakes, consists of 70 percent protein and retains most of the soybean's fiber. It is made by precipitating the solids with aqueous acid, aqueous alcohol, moist heat and/or organic solvents. These immobilize the protein, which is then removed along with some of the soy carbohydrates, isoflavones and salt residue. Different processing methods favored by different manufacturers affect the quality of the protein, the levels of the antinutrients and toxic residues, solubility, emulsifying ability and texture. Two types of SPC are in general use. Textured soy concentrate a subtype of textured soy protein (TSP) is put through an extruder and turned into the flakes, chunks and granules of ersatz meat. Functional soy protein is used by food processors in the binding phase of production to guarantee firmness, cohesion and juiciness. Food processors often combine forms of SPC to form soy protein products.[16-18]

Soy protein isolates are a component of numerous products sold in today's stores, including energy bars, shake powders, pasta sauces, burgers and hot dogs. SPI is also the major ingredient in most of today's soy infant formulas. Consisting of 90 to 92 percent protein, SPI is a highly refined product processed to remove off flavors, beany tastes and flatulence producing compounds and to improve digestibility. The manufacture of SPI is a complicated, high-tech procedure in which vitamin, mineral and protein quality are sacrificed. Indeed soy isolates increase the body's requirements for vitamins E, K, D and B12. Among the minerals, phosphorous is poorly utilized, and calcium, magnesium, manganese, molybdenum, copper, iron and especially zinc deficiencies appear in animals fed SPI as the primary source of protein in their diets. Soy protein isolates are also more deficient in sulfur-containing amino acids than other soy protein products.[19-21]

Both soy protein isolate and soy protein concentrate contain glutamate, a potent excitatory neurotransmitter, although the FDA has no requirement to disclose its actual concentration." [22, 23]

Although the manufacturing process varies and some companies hold patents on key elements of the process, the basic procedure begins with defatted soybean meal,which is mixed with a caustic alkaline solution to remove the fiber, then washed in an acid solution to coagulate the protein. The protein curds are then dipped into yet another alkaline solution and spray dried at high temperatures.[24,25]

Toxicologists, endocrinologists and other expert scientists have questioned the safety of soy protein because of the known presence of antinutrients (protease inhibitors, phytates, lectins, saponins and oxalates) as well as the plant hormones known as phytoestrogens. A large body of research exists documenting these hazards, refuting industry claims that there are no known safety hazards associated with soy protein. Specific issues related to protease inhibitors and the development of heart disease will be discussed in depth later in this petition. Given the numerous reports of antinutrients, toxins and carcinogens in modern soy products, they cannot be assumed safe. We therefore conclude that the FDA is not authorized to allow a health claim for soy and heart disease.

Finally, the FDA-approved soy/heart health claim has indirectly served to put men, women and children with soy allergies at risk . Soy is now one of the top eight allergens, a fact acknowledged by the Food Allergen Labeling and Consumer Protection Act (S. 741) that went into effect January 2006. In fact, soy allergies are increasing, may already be in the top six and some experts predict they will soon be in the top four. Many allergy experts believe that the increased use of soy protein ingredients in food products -- encouraged in part by the positive image given to soy by the FDA-approved soy/heart health claim -- has increased exposure and the potential for sensitization. Soy proteins are now incorporated into more than 60 percent of the commercial recipes for baked goods, canned, packaged and other processed foods. This hidden soy poses a clear danger to allergy sufferers, who may experience symptoms that range from mild to life threatening, involving, the gastrointestinal, cutaneous and respiratory systems.[26-32]

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SECTION II: A
THE EFFECT OF SOY PROTEIN ON TOTAL AND LDL CHOLESTEROL

NO STANDARD OF SIGNIFICANT SCIENTIFIC AGREEMENT HAS BEEN MET

The FDA soy/heart health claim is predicated upon the assumption that 25 grams of soy protein have been demonstrated to have a consistent, clinically significant effect on total and LDL cholesterol. However, a thorough review of the literature on soy and cholesterol including studies published since the Final Ruling in 1999 -- indicates that no standard of significant scientific agreement has been met. The data on soy and cholesterol are inconsistent and contradictory at best, with some studies showing that soy can lower total and/or LDL-cholesterol and other studies showing that it can raise or have no effect on total and/or LDL cholesterol.

In January 2006, the American Heart Association (AHA) announced in its journal *Circulation* that soy foods are unlikely to prevent heart disease and have little effect on cholesterol levels. The AHA issued its scientific advisory following a thorough review of the knowledge base on soy protein and cardiovascular disease (CVD) and careful evaluation of 22 studies by the AHA Nutrition Committee. Excerpts come from the original journal article (*emphasis ours*).

> Sachs, FM, Lichtenstein A, et al. Soy protein, isoflavones and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee. C*irculation*, 2006, 113, 7, 1023-44.
>
> Soy protein and isoflavones (phytoestrogens) have gained considerable attention for their potential role in improving risk factors for cardiovascular disease. This scientific advisory assesses the more recent work published on soy protein and its component isoflavones. In the majority of 22 randomized trials, isolated soy protein with isoflavones, as compared with milk or other proteins, decreased LDL cholesterol concentrations; the average effect was approximately 3%. This reduction is very small relative to the large amount of soy protein tested in these studies, averaging 50 g, about half the usual total daily protein intake. No significant effects on HDL cholesterol, triglycerides, lipoprotein(a), or blood pressure were evident. Among 19 studies of soy isoflavones, the average effect on LDL cholesterol and other lipid risk factors was nil. . . . Thus, *earlier research indicating that soy protein has clinically important favorable effects as compared with other proteins has not been confirmed. . . . .A separate analysis of soy isoflavones, the plant estrogens found in soy protein, showed no effect on cholesterol or other lipids . . .*

The US Agency for Healthcare Research and Quality has also thoroughly reviewed the literature on soy and heart disease. In a 245-page report issued in August 2005, the Agency concluded

that much of the research carried out on soy is inconclusive, that soy products appear to exert a small benefit on LDL cholesterol and triglycerides but that those effects may be of small clinical effect in individuals . . . . : Excerpts from the report below indicate that the standard of scientific agreement has not been met and the FDA should not allow a soy/heart disease health claim.

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.

There is a great deal of heterogeneity of effects found on lipoprotein and triglyceride levels. None of the factors we evaluated, including population, quality, applicability, soy isoflavone dose, soy protein dose, or baseline lipidemia level satisfactorily explained the heterogeneity. Overall, the majority of studies reported small to moderate effects on the lipids, despite a wide range of net effects for total cholesterol, LDL, and triglycerides. With few exceptions, studies consistently reported a small benefit on HDL. While we cannot exclude the possibility of publication bias (negative studies being less likely to be published) as an explanation for the effect of soy on LDL, there was no clear evidence that negative trials were missing. However, the clinical heterogeneity of the trials makes this analysis difficult. Since most studies reported multiple outcomes, including lipids, it is possible that publication bias is less likely among these studies. It is also probably less likely that negative trials for HDL and triglycerides have not been published, unless the effect on LDL (and other outcomes) was also negative.

Given the large amount of heterogeneity and inadequate reporting, particularly related to soy protein and isoflavone dose, many questions remain as to whether specific soy products in adequate doses may be of benefit in specific populations. Further, well-conducted studies are needed to clarify the effect of soy dose on lipid parameters and to determine whether soy components other than protein or isoflavones may be responsible for the lipid effects seen.
Total Cholesterol: A total of 61 studies reported data on the effect of consumption of soy products on total cholesterol levels. The median net change compared to control found was approximately 6 mg/dL (or 2.5%) with a wide range of effects, from 33 to +7 mg/dL (12% to +4%). Across studies, there were no discernable differences in effect based on baseline total cholesterol, soy protein consumption, soy isoflavone consumption, soy incorporated into diet or as supplement, or population (post-menopausal women, pre-menopausal women, men). However, 2 studies reported greater net effect of soy in subjects with more severely elevated lipids. Most studies that directly compared different doses of soy protein or soy isoflavones found no significant difference in effect, although results were mixed. Most studies that also directly compared effect in men and women found no difference.

Low Density Lipoprotein: A total of 52 studies reported data on the effect of consumption of soy products on LDL levels. A wide range of effects were reported, ranging from 32 to +13 mg/dL (or 21% to +9%). While few studies found a statistically significant benefit of soy consumption, meta-analysis across the diverse studies yielded a statistically significant net change of 5 (95% CI 8 to 3) mg/dL (roughly 3%). Across studies, there is possible evidence that the beneficial effect of soy products increases with increasing baseline LDL, particularly among studies where mean baseline LDL was greater than 130 mg/dL; although these associations were not statistically significant. Similarly, there is possible evidence of an

> association between higher soy protein dose and greater net reduction in LDL; however, only in the sub-analysis of studies with elevated baseline LDL was this association statistically significant. When studies with minimal doses of soy protein (<10 g/day) were omitted, the association was non-significant. No association was found between soy isoflavone dose and net effect. Qualitative analysis across all studies revealed no other associations between net change and other variables, including differences among soy products . . . soy incorporated into diet or as supplement, or population (post-menopausal women, pre-menopausal women, men). The 3 studies that compared effect to baseline LDL level came to conflicting conclusions. Most studies that directly compared different doses of soy protein or soy isoflavones found no significant difference in effect, although results were mixed. Most studies that also directly compared effect in men and women found no difference.

A key study used by the FDA to arrive at its decision to approve the 1999 health claim was a meta-analysis funded by the petitioner, Protein Technologies International. Subsequent research has shown this study to be deeply flawed; even its lead researcher James W. Anderson, MD, has stated that most studies since 1995 have reported less impressive results.

> Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *NEJM,* 1995, 333, 5, 276-282.

Anderson et al concluded This meta-analysis of 38 studies indicates that the consumption of soy protein is associated with significant decreases in serum cholesterol, LDL cholesterol and triglyceride concentrations and with a nonsignificant increase in serum HDL cholesterol concentrations. More accurately, the authors offered some proof that substituting soy protein for animal protein would bring about a 7 to 20 percent lowering of cholesterol among hypercholesterolemic individuals with levels over 260 mg/dl but that soy would do little or nothing for individuals whose cholesterol was lower than 250 mg/dl. In other words, soy protein is not likely to lower the cholesterol levels of the average American and should not be the subject of an FDA-approved health claim intended to lower cholesterol levels in the general population.

Anderson reported that soy protein tended to have less effect on LDL cholesterol in trials in which the participants were eating a low-fat and low cholesterol diet as compared with a more usual higher-fat and higher-cholesterol diet. The American Heart Association (AHA), however, disagreed, reporting in the January 17, 2006 issue of *Circulation* that the effect on LDL of soy protein or isoflavones does not appear to be modulated by the saturated fat and cholesterol content of the diet.

The US Agency for Healthcare Reform and Quality also criticized this study, noting in its

August 2005 report, that Anderson's meta-analysis used looser inclusion criteria, including non-randomized trials, studies of children, very small sample sizes, and short intervention durations. Their findings were highly affected by several non-randomized trials and . . . and a study that lacked randomization to a non-soy control.

Finally, on November 1, 2005, at the Sixth International Symposium on the Role of Soy in the Prevention and Treatment of Chronic Disease in Chicago, Anderson reported, Since our 1995 meta-analysis, most studies have reported less impressive alterations in serum lipoproteins than the 12.9% decreases in LDL-c then reported for soy foods. Anderson further announced that soy protein extraction or baking may fragment the most active hypocholsterolemic peptides. If true, as per Section C of the Final Rule Nature of the Food Eligible to Bear the Claim, FDA should enforce the removal of labels from any products in the marketplace bearing the soy/heart disease health claim in which processing methods have fragmented the most active hypocholesterolemic peptides.

As per findings of the American Heart Association, US Agency for Healthcare Research and Quality and Anderson's own admissions, no standard of scientific agreement was been met on this key study used by the FDA in 1999 when it approved a health claim based on the cholesterol-lowering potential of soy protein.

Recently, Sirtori et al confirmed Anderson's findings re cholesterol lowering by soy in hypercholesterolemic individuals. Excerpts are from the original journal article.

Sirtori CW, Eberinil I, Arnold A. Hypocholesterolaemic effects of soya proteins: results of recent studies are predictable from the Anderson meta-analysis data.. *Br J Nutr*, 2007, 97, 816 822.

. . . We investigated whether, by applying the same criteria used in the Anderson meta-analysis, i.e. evaluation of the net cholesterol change compared with baseline plasma cholesterol level, we could detect a prediction model allowing future studies on soya products to be evaluated in terms of their cholesterol-reducing potential. This allowed us to prepare a nomogram that clearly and visually confirmed what was already distinctly stated in the Anderson meta-analysis: that the plasma choles terol response to soya protein is not linear, but rather correlates to the square of baseline cholesterol level. There is apparently a threshold level that needs to be reached before a significant reduction in plasma cholesterol occurs, and the cholesterol response is far more dramatic in individuals with the highest cholesterolaemias. In brief, any study carried out on individuals with cholesterol levels below 240 250 mg/dl will most likely lead to minimal (probably clinically insignificant) cholesterol reductions. This was already evident from the earliest clinical study (Sirtori et al. 1977), carried out on inpatients given a complete substitution of animal proteins with soya proteins. In this, in spite of the similarity and obligatory adherence to the dietary regimen for all patients, individuals with cholesterol levels in the range 240 250 mg/dl (Fig. 2 in the original paper) showed only

minimal reductions. A somewhat similar finding was reported by Bakhit et al. (1994), when evaluating the effects of adding 25 g/d soya proteins (the final daily amount recommended by the US Food and Drug Administration) to the diet of individuals with varying baseline plasma cholesterol levels. In this series, the threshold for cholesterol reduction compared with no reduction, or possibly a cholesterol increase was indicated at approximately 220 mg/dl. In this study, definite reductions occurred in subjects with cholesterolaemias of around 240 mg/dl. It may be worthy of note in spite of publication of the Anderson meta-analysis, more than one third of the recent studies quoted (thirteen of thirty-three) were based on patients with initial cholesterol levels below 240 mg/dl.

As pertains to the more marked cholesterol reductions occurring in hypercholesterolaemic individuals, the threshold seems to be around 280 300 mg/dl (Anderson et al. 1995; Sirtori et al. 1998). If the basic mechanism of cholesterol reduction, i.e. LDL-receptor upregulation, the object of a series of reports by our group (Lovati et al. 1987, Duranti et al. 2004) and confirmed by other investigators (Baum et al. 1998), is accepted, it then becomes reasonable to conclude that this mechanism is most likely to be effective in carriers of an LDL-receptor deficiency status (Brown & Goldstein, 2004), i.e. those with more severe hypercholesterolaemias, compared with moderately hypercholesterolaemic individuals.

The findings of both Anderson *et al* and Sirtori *et al* indicate that if soy protein lowers cholesterol, it is most likely to occur in those with hypercholesterolaemia and defective LDL-receptors. This affects a very limited portion of the U.S. population and should not impact general recommendations. The current FDA soy/heart health claim misleads the public by giving the impression that soy lowers LDL and total cholesterol in the general population.

Finally, we wish to point out that most studies on soy and cholesterol are deeply flawed because of the routine use of casein as the control. Casein is a fractionated milk protein product that is high in methionine and seriously deficient in cysteine. Research at the Faculty of Agriculture, Shizuoka University, Japan, has shown that -- compared to other proteins -- casein will significantly raise total cholesterol levels and lower HDL levels:

Sugiyama K, Ohkawa S, Muramatsu K. Relationship between amino acid composition of diet and plasma cholesterol level in growing rats fed a high cholesterol diet .*J Nutr Sci Vitaminol* (Tokyo), 1986, 2, 4, 413-433.

The effects of dietary sulfur-containing amino acids and glycine on plasma cholesterol level were studied in rats fed amino acid mixture diets containing cholesterol. The relationship between the amino acid composition of dietary proteins and plasma cholesterol levels was also investigated in rats fed diets containing various kinds of protein such as casein, egg albumin,

pork protein, fish protein, corn gluten, wheat gluten and soy protein. Feeding the amino acid mixture corresponding to casein led to an approximately two-fold level of plasma total cholesterol as compared with feeding the amino acid mixture corresponding to wheat gluten. It was possible to reduce the plasma cholesterol of rats fed the amino acid mixture of the casein type by increasing the proportion of cystine in the total sulfur amino acids. Inversely, the deprivation of cystine resulted in an enhancement of the plasma cholesterol of rats fed the gluten type amino acid mixture. Glycine had a tendency to resist increases in the plasma cholesterol level. A significant negative correlation was noted between plasma cholesterol levels and the content of cystine in intact dietary proteins. The results suggest that the differential effect of dietary proteins on plasma cholesterol level is mainly associated with sulfur-containing amino acids included in the protein, regardless of whether it is of animal or plant origin.

Sugiyama K, Muramatsu K.J Significance of the amino acid composition of dietary protein in the regulation of plasma cholesterol. *Nutr Sci Vitaminol (*Tokyo). 1990, 36 Suppl 2:S105-10.

Three approaches were employed to identify the amino acid residue(s) that is responsible for the different effects of dietary proteins on the plasma cholesterol level in rats fed cholesterol-enriched diets. 1) Experiments on the effects of individual amino acids added to a 25% casein diet showed that sulfur-containing amino acids have the most potent effects on the plasma cholesterol level. Under the dietary conditions used, methionine significantly increased the level of plasma cholesterol while cystine decreased it. It was found that glycine can prevent the methionine-induced enhancement of plasma cholesterol. 2) There was a significant negative correlation between cystine content of dietary proteins and plasma cholesterol levels when animals were fed 7 kinds of animal and plant proteins. 3) Experiments with amino acid mixtures varying in methionine, cystine, and glycine content showed that diets high in methionine and low in cystine and glycine content tend to increase the plasma cholesterol level and diets of opposite amino acid content tend to decrease the plasma cholesterol level. From these results, it was suggested that sulfur-containing amino acids and glycine in dietary proteins are responsible, at least in part, for the alteration of plasma cholesterol level by dietary proteins.

Therefore casein is an extremely poor control in terms of evaluating soy protein's effect on cholesterol. The fact that soy protein does not have a consistently and demonstrably better effect on cholesterol compared to casein indicates that it is a very poor cholesterol reducer indeed and unfit to be the subject of an FDA-approved cholesterol reducing heart health claim.

* * * * *

MECHANISM FOR CHOLESTEROL LOWERING: NO ASSURANCE OF SAFETY

In addition, an FDA health claim for soy and heart disease is improper because research has not conclusively identified the mechanism for cholesterol lowering or provided adequate assurance of safety in terms of its overall effect on the body.

To date, the most accepted explanation for soy protein's cholesterol lowering potential if and when cholesterol lowering occurs posits stimulation of LDL-receptor activity, causing alterations in LDL receptor quantity and/or activities, as presented in the studies cited below. We will establish in this petition that this mechanism may serve as compensation for a soy-dependent increase in bile acid excretion. As this increase in bile acid excretion would be accompanied by losses of fat-soluble thyroid and steroid hormones, and of fat-soluble vitamins, cholesterol-lowering is achieved at a steep price. The key studies are as follows. Excerpts come from the journal articles.

Potter, SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J Nutr*, 1995, 125, 606S-611S.

and

Potter SM. Soy protein and serum lipids. *Curr Opin Lipidol.* 1996, 7, 4, 260-264.

Evdence exists indicating that substitution of soy for animal protein reduces both total and LDL-cholesterol concentrations in humans. There are a number of biologically active compounds associated with soy protein; however, the precise mechanism and the component(s) of soy responsible have not been fully established. Some studies suggest that, when soy protein is fed, cholesterol absorption or bile acid reabsorption, or both, is impaired. This is observed in some animal species such as rabbits and rats but not in humans, nor when amino acids replace intact soy protein. Other workers have proposed that changes in endocrine status are responsible, however, this again has not been observed in humans. Increases in LDL receptor activity in both animals and humans have been reported after ingestion of soy protein or various extracts of soy, or both. Furthermore, the soybean isoflavone genistein may inhibit lesion and thrombus formation via inhibition of second messengers.

Lapré JA, West CE, Lovati MR, Sirtori CR, Beynen AC. Dietary animal proteins and cholesterol metabolism in rats. *Int J Vitam Nutr Res.* 1989;59(1):93-100.

The effects on cholesterol metabolism in rats of diets containing various animal proteins or soy protein were studied. The animal proteins tested were casein, whey protein, fish protein, hemoglobin, plasma proteins, ovalbumin, egg-yolk protein, beef protein and chicken-meat protein. The semi-purified diets were isonitrogenous and balanced for residual fat and cholesterol in the protein preparations. The nature of the dietary protein had no effect on serum

cholesterol concentration. Group mean liver cholesterol concentration was increased and fecal excretion of bile acids was decreased by all animal proteins when compared with soy protein. This study suggests that carefully balancing diets for components other than protein in the protein preparations prevents protein effects on serum cholesterol in rats but not on liver cholesterol and bile acid excretion.

Wright SM, Salter AM. Effects of soy protein on plasma cholesterol and bile acid excretion in hamsters.*Comp Biochem Physiol B Biochem Mol Biol*. 1998 Feb;119(2):247-54.

The effect of dietary casein and soy protein on lipoprotein metabolism was compared in the Golden Syrian hamster (Mesocricetus auratus). Total plasma cholesterol was similar in animals fed either protein, but significant differences were seen in lipoprotein profile. In animals fed soy protein, cholesterol concentrations were lower in very-low-density lipoproteins (VLDL) but higher in low-density and high-density lipoproteins, compared with those fed casein. Significant differences were also seen depending on the nutritional state of the animals. In casein-fed hamsters, total plasma triacylglycerol and chylomicron + VLDL cholesterol and triacylglycerol were significantly higher when blood was collected during feeding, compared with animals that had been fasted overnight. By contrast, no significant change was seen in animals on the soy protein diet. This suggests that either intestinally derived lipoproteins are more rapidly cleared on the soy protein diet or that soy inhibits feeding-induced VLDL secretion. Fecal bile acid excretion was higher in the soy protein group and there was a significant correlation between soy intake and bile acid excretion. Hepatic cholesterol decreased as the amount of soy protein consumed increased, suggesting that it is this pool of cholesterol that is used to replace the excreted bile acids. No significant difference was seen in plasma insulin or glucagon between hamsters fed the two proteins. Plasma triiodothyronine concentrations were, however, significantly higher and thyroxine concentrations lower in the soy protein-fed animals. This study shows specific effects of dietary proteins on plasma lipoprotein concentrations dependent on nutritional status of the animal.

Lovati MR, Manzoni C, Gianazza E, Arnoldi A, Kurowska E, Carroll KK, Sirtori CR. Soy protein peptides regulate cholesterol homeostasis in Hep G2 cells. *J Nutr*. 2000, 130, 10, 543-9.

Cells exposed to Croksoy(R)70 enzyme digestion products showed a more marked up-regulation of LDL receptors vs. controls, compared with vs. Hep G2 cells incubated with undigested Croksoy(R)70. Among soy-derived products, only the 7S globulin inhibited apo B secretion and (14)C-acetate incorporation when tested in Hep G2 cells at a concentration of 1.0 g/L. These findings support the hypothesis that if one or more peptides can reach the liver after intestinal digestion, they may elicit a cholesterol-lowering effect. Moreover, the protein moiety, devoid of isoflavone components, is likely to be responsible for this major biochemical effect of soy protein.

Sirtori CR, Lovati MR, Manzoni C, Monetti M, Pazzucconi F, Gatti E. Soy and cholesterol reduction: clinical experience. *J Nutr.* 1995, 125(3 Suppl) 598S-605S

. . . . in man, similar to experimental animals, soy protein may in some way up-regulate LDL receptors depressed by hypercholesterolemia or by dietary cholesterol administration.

Lovati MR, Manzoni C, Canavesi A, Sirtori M, Vaccarino V, Marchi M, Gaddi G, Sirtori CR. Soybean protein diet increases low density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients.*J Clin Invest.* 1987, 80, 50, 1498-502.

The soybean diet regimen dramatically affected the degradation of LDL in mononuclear cells. Degradation was increased 16-fold vs. the basal activity and 8-fold compared with the standard low lipid diet with animal proteins. There was, however, no clear relationship between the reduction of total and LDL-cholesterolemia and the increased LDL degradation. These findings confirm similar data previously obtained in cholesterol-fed rats and suggest that some factor/s, most likely of a protein nature, may regulate the expression of lipoprotein receptors in peripheral cells, particularly when receptor activity is suppressed by experimental diets and/or spontaneous hypercholesterolemia.

Homocysteine levels were not determined in this study. In animals and humans, dietary methionine deficiency causes hyperhomocysteinemia by decreased synthesis of adenosyl methionine and dysregulation of homocysteine metabolism.[1] Hyperhomocysteinemia affects LDL by causing aggregation of LDL particles and increased uptake by mononuclear cells. [2]

Cho S-J, Juillerat MA, Lee C-H. Cholesterol lowering mechanism of soy protein hydrolysate. *Journal of Agricultural and Food Chemistry*, 2008, 55, 26, 10599-10604.

Numerous attempts have been made to find the mechanism and component of the cholesterol lowering activity of soybean. In this study, it was proved that the peptides in soybean protein hydrolysate (SPH) made by certain proteases have a hypocholesterolemic effect. Among the mechanisms suggested, that is, blockage of bile acid and/or cholesterol absorption, inhibition of cholesterol synthesis, and stimulation of low-density lipoprotein receptor (LDL-R) transcription, SPH appeared to stimulate LDL-R transcription. When Hep T9A4 cells were incubated with soy protein hydrolysates by using the proteases from Bacillus amyloliquefaciens FSE-68, LDL-R transcription was strongly stimulated, but the other mechanisms were not affected. Among the six types of SPH, F1-15, hydrolyzed with the neutral protease to a degree of hydrolysis (DH) of 15%, showed the highest LDL-R transcription. The fractions of molecular weight of 200 and 3000 Da showed LDL-R transcription stimulating activity. The bioactivity is due to soybean peptides because the ethanol extract of soybean protein which contains isoflavones does not stimulate LDL-R transcription. In conclusion, dietary up-regulation of LDL-R transcription by soybean may be consequent to an enhanced catabolism or a reduced synthesis of intracellular cholesterol. Therefore, we suggest that soy peptides can effectively stimulate LDL-R transcription in the human liver cell line and reduce blood cholesterol levels.

The most recent study by Cho et al has been widely publicized as finally offering a viable theory of how soy can lower cholesterol. As we have shown, many researchers over the past two decades have concluded that soy lowers cholesterol by stimulation or other effects on the LDL receptor sites. We submit that it would be wrong to draw extended conclusions from the Cho study as it is only an *in vitro* study and the researchers themselves are cautious, noting that their results suggest and that further experiments are required. In addition, in this study researchers tested soy peptides from which isoflavones were removed. The FDA health claim applies to standard soy protein products, which contain intact isoflavones.

More importantly, whether addressing the conclusions related to soy protein stimulation of LDL receptor sites drawn by Cho *et al* or earlier researchers, we must address the possible dangers of altering liver function homeostasis. Lowering cholesterol in the LDL or VLDL may serve as compensation for a soy-dependent-increase in bile acid excretion with the possibility of concomitant losses of fat-soluble thyroid and steroid hormones, and of fat-soluble vitamins. Indeed some of these undesirable side effects are noted in the studies cited above.

Increased breakdown of LDL via LDL-receptor sites could represent bodily compensation for the losses in thyroid hormone production and utilization. Such thyroid hormone perturbation has been widely reported in the scientific literature, including the work of leading scientists at the FDA's National Laboratory for Toxicological Research.[3-5] Without this compensatory salvage pathway for thyroid hormones, humans might not have developed any tolerance at all for soy as a food source. In other words, instead of making thyroid hormones and activating them in a tightly regulated way, excess soy protein consumption could spur the body to degrade LDL just to enhance bile acid production for salvaging existing thyroid hormones from the alimentary tract.

We maintain that this substitute mechanism for regulating thyroid hormones by a salvage operations cannot be presumed safe, much less confer health benefits. Soy-dependent activation of any bile salvage pathways for thyroid hormones could end 1) any specific, peroxide-independent, NADPH-dependent iodination in thyroid tissues leading to the controlled biosynthesis of thyroid hormone [4, 5] and 2) the controlled activation of same by the thiol-dependent deiodinase that converts thyroxine (T4) into 3,5,3 -triiodothyronine (T3).[8-10]

Increasing bile acid production increases the likelihood that two products of hepatic cholesterol's metabolism, cholate[11] and deoxycholate, will putrefy to the bile acid metabolites

apocholate[12] and 3-methylcholanthrene,[13] both of which are known to cause cancer. This is especially true when constipation is also an issue, as is consistently the case with at least 12.8 percent of the American people.[14] Cancer patients and other patients undergoing pain therapy also frequently experience constipation as a common adverse side effect of opioid drugs.[15]

Enhanced, bile-mediated recycling of thyroid hormones also could affect and even contribute to the enterohepatic retention of fat-soluble toxins, including cancer promoting excesses of estrogens, testosterones and their metabolites (such as DHT associated with prostate cancer),[16] all of which the healthy body unaffected by excessive soy protein consumption normally would excrete. Other studies implicate dietary factors other than just soy-derived in profoundly influencing cholesterol, LDL, HDL, and triglyceride levels.[17] For example, the availability of the amino acids L-cystine and L-cysteine, and the quality and quantity of fat in the diet can greatly influence factors associated with cardiovascular disease.[18] Significantly, many fats can radically influence responses to dietary soy, such as the enterohepatic recirculation of soy components and metabolites.[19] Thus it is difficult if not impossible to predict health outcomes connected with soy consumption in the general population.

Soy protein-induced lowering of cholesterol through any increase in the production of bile acids (*e.g., deoxycholate*) also could severely compromise normal levels of the monooxygenase receptor for vitalethine, a fairly recently discovered, endogenous regulator of key metabolic pathways.[20] Vitalethine is critical to the body's ability to ward off and fight cancers through humoral immunity.[21,22] Vitalethine is made from *available* dietary L-cysteine and pantothenic acid (vitamin B5). [Cysteine in soybeans has limited bioavailability as discussed in the homocysteine section of this petition.]

Vitalethine is also critical to thyroid function. Together with its monooxygenase receptor, vitalethine is needed for iodination of proteinaceous tyrosine residues, a process critical to the specific and controlled formation of thyroid hormones.[23-25] Significantly, the monooxygenase receptor for vitalethine is especially labile in the presence of deoxycholate. When protected by NADPH in the absence of deoxycholate, this monooxygenase activity reversibly down-regulates the over-production of mevalonate catalyzed by HMG-CoA reductase. This is a key to the enzyme-coupled regulation of critical isoprenylation reactions needed for biosyntheses of cholesterol and of heart-healthy Coenzyme Q(s).

Vitalethine, its monooxygenase receptor, and naturally-produced cysteamine may also help maintain important peptidyl, proteinaceous, and mixed disulfides like those necessary for the proper functioning and consequent regulation of insulin, serum albumin, antibodies, and many, if not all, branch-point enzymes.[26]

Therefore, soy-dependent activation of any bile salvage pathways for thyroid hormones could suppress the enzyme regulations needed for :

- The biosynthesis of thyroid hormones [27]
- The controlled activation of thyroid hormones, including the thio-dependent deiodinase conversion of T4 into T3[28-30]
- Humoral immunity[21,22, 31-37]
- Thymic cell apoptosis and thymus atrophy [21,22 32-37]
- The induction of HMG-Co-enzyme A reductase and cholesterol biosynthesis[38-40]
- The production of heart healthy coenzyme Q10 [41,42]
- Cancer oncogene expression[31,32]
- Sugar metabolism, including genetic predisposition to diabetes.[31, 43]

Given this long list of documented risks, we maintain that any possible benefits from soy protein's cholesterol lowering are far outweighed by the risks.

* * * * *

The FDA soy/heart disease health claim does not specifically target infants and is not used on labels by manufacturers of soy infant formula, but the image of soy as heart healthy has encouraged many parents and pediatricians to recommend soy formula. The study below indicates that cholesterol lowering would put infants fed soy formula at special risk. In infants, cholesterol is critical for proper development of the brain and nervous system. Unlike breast milk and dairy formulas, soy formula is already devoid of this needed substance, and any cholesterol-lowering properties the formula may have would further reduce the pool of available cholesterol needed for neurological development.

Potter JM, Nestel PJ. Greater bile acid excretion with soy bean than with cow milk in infants. *Am J Clin Nutr,* 1976, 29, 5. 546-551.

The excretion of fecal sterols and bile acids was measured in five infants from the 1st week of life to 2 or 3 months of age as the composition of their diet was changed from cow milk to soy bean milk. Bile acid excretion, adjusted for body weight, was initially lower during the 1st than during the 3rd week, when it reached adult values. The average excretion of bile acids was 6.8 mg/kg per day with soy bean milk and 3.6 mg/kg per day with cow milk. Net sterol excretion (total sterol output minus cholesterol intake) was also twice as high with soy bean milk and probably reflected enhancement of cholesterol re-excretion as well as of synthesis since the cholesterol content of soy beans is nil. However, net sterol excretion remained higher with soy

bean than with cow milk even when egg yolk cholesterol was added to the soy bean milk. It is concluded that the substitution of soy bean milk for cow milk, which lowered the plasma cholesterol in all infants (even in the presence of dietary cholesterol) leads to an increase in bile acids and probably also in cholesterol excretion in young infants.

* * * * *

In conclusion, we submit that the long-term clinical effects of soy protein-induced cholesterol lowering on lipoprotein reductions and alterations of mononuclear cell LDL receptors, messenger RNA concentrations, and other selected cardiovascular and other health risk factors are not completely known. Accordingly, we cannot presume they are beneficial or safe for infants, children and adults. In addition, evidence for soy protein's adverse effects on thyroid function, humoral immunity and the potential to cause, contribute to or accelerate the growth of cancer provides serious cause for concern. Later in this document we will address soy's effect on other known cardiovascular risk factors including homocysteine, C-reactive protein, blood pressure and endothelial function.

* * * * *

The FDA might also consider mounting evidence that cholesterol is not a reliable marker of heart disease risk and that lipid lowering therapy whether by nutraceuticals or pharmaceuticals -- is not necessarily beneficial or even safe.

Hayward RA, Hofer TP, Vijan S. .Narrative review: lack of evidence for recommended low-density lipoprotein treatment targets: a solvable problem. *Ann Intern Med.* 2006, 3, 145, 7, 520-530.

Current clinical evidence does not demonstrate that titrating lipid therapy to achieve proposed low LDL cholesterol levels is beneficial or safe."

We submit that lipid therapy by soy protein food products does not reliably lower cholesterol, -- and when cholesterol lowering does occur -- has not been proven beneficial nor even safe. We therefore request the FDA to amend the Final Rule Re Food Labeling: Health Claims; Soy Protein and Heart Disease to disallow the heart disease health claim.

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ENDNOTES

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8. Leonard JL, Rosenberg IN, Subcellular distribution of thyroxine 5'-deiodinase in the rat kidney: a plasma membrane location, *Endocrinology*, 1978, 103, 1, 274-280.
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10. Visser, T. J. , Mechanism of inhibition of iodothyronine-5'- deiodinase by thioureylenes and sulfite, *Biochim. Biophys. Acta*, 1980, 611, 371-378.
11. Baijal, P. K., et al. Tumor-enhancing effects of cholic acid are exerted on the early stages of colon carcinogenesis via induction of aberrant crypt foci with an enhanced growth phenotype. *Can J Physiol Pharmacol*. 1998, 76, 12, 1095-1102. Department of Foods and Nutrition, University of Manitoba, Winnipeg, Canada.The objective of the study was to establish whether cholic acid (CHA) enhanced colonic tumor incidence in the early phase of carcinogenesis. Male, Sprague-Dawley rats (n = 180) were injected twice with azoxymethane (AOM) (15 mg x kg(-1) body weight x week(-1), s.c., given 1 week apart). Following the first AOM injection, animals were randomly assigned to two groups, control AIN-93G diet (CON) or control diet containing 0.2% CHA by weight (CHA). Three weeks after the first injection, 20 animals (10

animals/group) were killed and aberrant crypt foci (ACF) were enumerated. The remaining animals were further subdivided and animals randomly assigned to CON or CHA diets, creating four treatments: CON-CON, CON-CHA, CHA-CHA, and CHA-CON. After 3, 12, and 20 weeks (following the first carcinogen injection), the animals were killed and the number and crypt multiplicity of ACF enumerated. Macroscopic tumors were evaluated at week 20. Total ACF were not different between groups. Average crypt multiplicity and medium (4-6 crypts/focus) and large (> or = 7 crypts/focus) ACF were greater in CHA-CHA and CHA-CON compared with CON-CON and CON-CHA ($p < 0.01$). Transient exposure to CHA (CHA-CON) was sufficient to induce development of ACF with an accelerated growth phenotype and elicit a tumor-enhancing effect. CHA-CHA had the highest tumor incidence (82.8%, $p < 0.05$) followed by CHA-CON (56.7%, $p < 0.05$), and tumor multiplicity and number of tumors per rat in CHA-CON were similar to CHA-CHA (2.29 and 1.3 versus 2.33 and 1.9, respectively). Delayed intervention with CHA (CON-CHA) produced a tumor outcome similar to CON-CON (31 and 30%, respectively), it did not enhance colonic tumor incidence. Taken collectively these results suggest cholic acid was effective in enhancing colon carcinogenesis during early phases and ineffective in post-initiation phases.

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The carcinogen 3-methylcholanthrene can be produced from deoxycholic acid and is postulated by some investigators to play a role in the pathogenesis of colon carcinoma. The small quantities of this compound which could be carcinogenic have been difficult to measure in feces because of many potentially interfering compounds. Using 3-[6-14C]methylcholanthrene as an internal standard, petroleum ether extraction, C-18 SepPak separation, preparative high performance liquid chromatography, and gas-liquid chromatography-mass spectrometry with selected ion monitoring, we developed an assay capable of detecting less than 35 ng of 3-methylcholanthrene per gram of stool. Application of this technique to stools of five patients with colon carcinoma and two normal controls revealed no detectable 3-methylcholanthrene in any stool sample. This negative result was confirmed by incubating radiolabeled cholic acid in fecal homogenates. Although greater than 90% of this radiolabeled bile acid was converted to deoxycholic acid, none of the radioactivity was found in the thin-layer chromatography fraction corresponding to 3-methylcholanthrene. These observations provide evidence against a role for

3-methylcholanthrene in pathogenesis of human colon carcinoma. Similar assays could be used for analysis of other carcinogens in stool samples. In-Tele-Health © 2006 (from Hyperhealth Pro CD-ROM)

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15. *http://www.wikipedia.com/wiki/Opioid* Constipation: this develops in 99% of patients on opioids and since tolerance to this problem does not develop, nearly all patients on opioids will need a laxative . . .
16. Lewis JG, Nakajin S, Ohno S, Warnock A, Florkowski CM, Elder PA. Circulating levels of isoflavones and markers of 5alpha-reductase activity are higher in Japanese compared with New Zealand males: what is the role of circulating steroids in prostate disease? *Steroids.* 2005, 15, 70, 14, 974-979. Steroid & Immunobiochemistry Laboratory, Canterbury Health Laboratories, P.O. Box 151, Christchurch 8001, New Zealand. john.lewis@cdhb.govt.nz. Epidemiological evidence implicates dietary isoflavone intake as protective against prostate disease. A putative mechanism is attenuated circulating androgen levels in male populations consuming an isoflavone rich diet. We investigated this hypothesis by collecting plasma from 60 Japanese and 60 New Zealand males aged between 21 and 31 years each consuming their traditional diets. We measured plasma testosterone, dihydrotestosterone (DHT), androstenedione, dehydroepiandrosterone sulfate (DHEAS), the combined levels of androsterone sulfate and epiandrosterone sulfate (AoS/epiAoS), sex hormone-binding globulin, and cortisol and corticosteroid-binding globulin as well as the isoflavones genistein and equol. Plasma genistein and equol levels were several times higher in Japanese males as would be expected from an isoflavone rich diet. However, androstenedione, DHEAS, calculated free testosterone and paradoxically markers of 5alpha-reductase, DHT and AoS/epiAoS were all also significantly higher in Japanese rather than the New Zealand male counterparts. All other comparisons were not significant. Plasma DHT and DHEAS correlated positively with plasma equol and plasma AoS/epiAoS correlated positively with genistein levels. Taken together the results suggest that, rather than reduced levels of steroidogenesis, Japanese males may have increased 5alpha-reductase activity and possibly altered 17beta OH steroid dehydrogenase activity. Significantly the positive association between isoflavones levels and 5alpha-steroids is counter-intuitive to isoflavone intake offering prostate protection, unless this is postulated to occur through other mechanisms.

17. Sugiyama K, Ohkawa S, Muramatsu K. Relationship between amino acid composition of diet and plasma cholesterol level in growing rats fed a high cholesterol diet..*J Nutr Sci Vitaminol* (Tokyo). 1986, 32, 4, 413-23. The effects of dietary sulfur-containing amino acids and glycine on plasma cholesterol level were studied in rats fed amino acid mixture diets containing cholesterol. The relationship between the amino acid composition of dietary proteins and plasma cholesterol levels was also investigated in rats fed diets containing various kinds of protein such as casein, egg albumin, pork protein, fish protein, corn gluten, wheat gluten and soy protein. Feeding the amino acid mixture corresponding to casein led to an approximately two-fold level of plasma total cholesterol as compared with feeding the amino acid mixture corresponding to wheat gluten. It was possible to reduce the plasma cholesterol of rats fed the amino acid mixture of the casein type by increasing the proportion of cystine in the total sulfur amino acids. Inversely, the deprivation of cystine resulted in an enhancement of the plasma cholesterol of rats fed the gluten type amino acid mixture. Glycine had a tendency to resist increases in the plasma cholesterol level. A significant negative correlation was noted between plasma cholesterol levels and the content of cystine in intact dietary proteins. The results suggest that the differential effect of dietary proteins on plasma cholesterol level is mainly associated with sulfur-containing amino acids included in the protein, regardless of whether it is of animal or plant origin.
18. Bays H, Stein EA. Pharmacotherapy for dyslipidaemia--current therapies and future agents. *Expert Opin Pharmacother.* 2003, 4, 11, 1901-2938. L-MARC Research Center, 3288 Illinois Avenue, Louisville, KY 40213, USA. HbaysMD@aol.com. Current lipid-altering agents that lower low density lipoprotein cholesterol (LDL-C) primarily through increased hepatic LDL receptor activity include statins, bile acid sequestrants/resins and cholesterol absorption inhibitors such as ezetimibe, plant stanols/sterols, polyphenols, as well as nutraceuticals such as oat bran, psyllium and soy proteins; those currently in development include newer statins, phytostanol analogues, squalene synthase inhibitors, bile acid transport inhibitors and SREBP cleavage-activating protein (SCAP) activating ligands. Other current agents that affect lipid metabolism include nicotinic acid (niacin), acipimox, high-dose fish oils, antioxidants and policosanol, whilst those in development include microsomal triglyceride transfer protein (MTP) inhibitors, acylcoenzyme A: cholesterol acyltransferase (ACAT) inhibitors, gemcabene, lifibrol, pantothenic acid analogues, nicotinic acid-receptor agonists, anti-inflammatory agents (such as Lp-PLA(2) antagonists and AGI1067) and functional oils. Current agents that affect nuclear receptors include PPAR-alpha and -gamma agonists, while in development are newer

PPAR-alpha, -gamma and -delta agonists, as well as dual PPAR-alpha/gamma and 'pan' PPAR-alpha/gamma/delta agonists. Liver X receptor (LXR), farnesoid X receptor (FXR) and sterol-regulatory element binding protein (SREBP) are also nuclear receptor targets of investigational agents. Agents in development also may affect high density lipoprotein cholesterol (HDL-C) blood levels or flux and include cholesteryl ester transfer protein (CETP) inhibitors (such as torcetrapib), CETP vaccines, various HDL 'therapies' and upregulators of ATP-binding cassette transporter (ABC) A1, lecithin cholesterol acyltransferase (LCAT) and scavenger receptor class B Type 1 (SRB1), as well as synthetic apolipoprotein (Apo)E-related peptides. Fixed-dose combination lipid-altering drugs are currently available such as extended-release niacin/lovastatin, whilst atorvastatin/amlodipine, ezetimibe/simvastatin, atorvastatin/CETP inhibitor, statin/PPAR agonist, extended-release niacin/simvastatin and pravastatin/aspirin are under development. Finally, current and future lipid-altering drugs may include anti-obesity agents which could favourably affect lipid levels.

19. Adlercreutz H, Höckerstedt K, Bannwart C, Bloigu S, Hämäläinen E, Fotsis T, Ollus A . Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *J Steroid Biochem.* 1987, 27, 4-6, 1135-44. Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, Finland. A brief account of our present knowledge on the enterohepatic metabolism of estrogens and on the origin, metabolism and biological effects of mammalian lignans and phytoestrogens is undertaken. Furthermore, recently published results on the effects of dietary fiber, fat and carbohydrates on estrogen metabolism are reviewed. New preliminary results are presented on quantitative assays of lignans and phytoestrogens in urine of women belonging to various dietary and population groups and in a group of chimpanzees. The highest values of lignans and phytoestrogens were found in the non-human primates, and in macrobiotic, lactovegetarian and Japanese women, all groups considered having a low risk for the development of breast and other hormone-dependent cancer. New results on correlations between intake of various fibers, lignan and phytoestrogen excretion and plasma levels of estrogens, free testosterone and SHBG in women are presented. There is a significant positive correlation between the intake of fiber and urinary excretion of lignans and phytoestrogens, and the concentration of plasma SHBG. Fiber intake and urinary excretion of lignans and equol correlated negatively with plasma percentage free estradiol. Enterolactone excretion correlated negatively with plasma free testosterone. It is concluded that dietary macro- and micronutrients seem to play an important role in estrogen metabolism.

20. http://www.vitaletherapeutics.org/nomncltr.htm, www.vitaletherapeutics.org/vtlhmgmo.htm and http://www.vitaletherapeutics.org/vtlmoflo.htm
21. Knight, G. D., Laubscher, K. H., Fore, M. L., Clark, D. A., and Scallen, T. J. Vitalethine modulates erythropoiesis and neoplasia. *Cancer Res*, 1994, 54: 5623-5635.
22. Knight, G. D., Mann, P. L., Laubscher, K. H., Scallen, T. J. Seemingly diverse activities of beta-alethine. *Cancer Res, 1994,* 54: 5636-5642.
23. http://www.vitaletherapetuics.org/ipreface.htm
24. DeGroot, L. J. and F. Carvalho, Iodide binding in thyroid cellular fractions, *J. Biol. Chem, 1960, 235, 5,* 1390-1397.
25. DeGroot, LJ, Davis AM Studies on the biosynthesis of iodotyrosines: a soluble thyroidal iodide-peroxidase tyrosine-iodinase system, *Endocrinol,* 1962, 70, 492-504.
26. Ziegler, DM, Poulsen LL, Protein disulfide bond synthesis: a possible intracellular mechanism, *Trends Biochem Sci.* 1977, 2, 79-81.
27. http://www.vitaletherapeutics.org/ipreface.htm
28. Chopra IJ. Sulfhydryl groups and the monodeiodination of thyroxine to triiodothyronine. *Science.* 1978, 24, 199, 1431, 904-906. Sulfhydryl reagents exert a profound influence on the monodeiodination of thyroxine to triiodothyronine by rat and sheep tissues in vitro. A marked dithiothreitol-induced increase in the monodeiodination by fetal sheep liver homogenates suggests that the characteristically low conversion in fetal tissues is related more to the status of sulfhydryl groups than to a deficiency of the monodeiodinating enzyme.
29. Chopra IJ. Alterations in monodeiodination of iodothyronines in the fasting rat: effects of reduced nonprotein sulfhydryl groups and hypothyroidism. *Metabolism.* 1980, 29, 2, 161-167.
30. Persky VW, Turyk ME, Wang L, Freels S, Chatterton R Jr, Barnes S, Erdman J Jr, Sepkovic DW, Bradlow HL, Potter S. Effect of soy protein on endogenous hormones in postmenopausal women. *Am J Clin Nutr.* 2002, 75, 1, 145-153. Erratum in: *Am J Clin Nutr* 2002, 76, 3, 695. Division of Epidemiology and Biostatistics, the School of Public Health, the University of Illinois at Chicago, USA. vwpersky@uic.edu BACKGROUND: The long-term clinical effects of soy protein containing various concentrations of isoflavones on endogenous hormones are unknown. OBJECTIVE: We examined the effects of ingestion of soy protein containing various concentrations of isoflavones on hormone values in postmenopausal women. DESIGN: Seventy-three hypercholesterolemic, free-living, postmenopausal women participated in a 6-mo double-blind trial in which 40 g protein as part of a National Cholesterol Education Program Step I diet was provided as casein from nonfat dry milk (control), isolated soy protein (ISP)

containing 56 mg isoflavones (ISP56), or ISP containing 90 mg isoflavones (ISP90). Endogenous hormone concentrations were measured at baseline and at 3 and 6 mo. RESULTS: The concentration of thyroxine and the free thyroxine index were higher in the ISP56 group, and the concentration of thyroid-stimulating hormone was higher in the ISP90 group than in the control group at 3 and 6 mo ($P < 0.05$). Triiodothyronine was significantly higher in the ISP90 group only at 6 mo. Thyroxine, free thyroxine index, and thyroid-stimulating hormone at 6 mo were inversely associated with measures of baseline estrogenicity. No significant differences were found for endogenous estrogens, cortisol, dehydroepiandrosterone sulfate, insulin, glucagon, or follicle-stimulating hormone after baseline hormone values were controlled for. CONCLUSIONS: This study does not provide evidence that long-term ingestion of soy protein alters steroid hormone values, but it suggests that soy protein may have small effects on thyroid hormone values that are unlikely to be clinically important. The thyroid effects are, however, consistent with previous findings in animals

31. Knight GD, Laubscher KH., Fore ML., Clark DA, Scallen, T. J. Vitalethine modulates erythropoiesis and neoplasia. *Cancer Res,*1994, 54: 5623-5635.
32. Knight,GD., Mann PL., Laubscher KH, Scallen T J. Seemingly diverse activities of beta-alethine. *Cancer Res,* 1994, 54: 5636-5642.
33. Bounous G, Kongshavn PA, Gold P. The immunoenhancing property of dietary whey protein concentrate. *Clin Invest Med*. 1988, 11, 4, 271-278. Montreal General Hospital Research Institute, Quebec, Canada. The plaque-forming cell response to sheep red blood cells was found to be enhanced in mice fed a formula diet containing 20 g lactalbumin/100 g diet in comparison to mice fed equivalent formula diets of similar nutritional efficiency containing 20 g/100 g diet of either casein, soy, wheat or corn protein, egg albumin, beef or fish protein, Spirulina maxima, or Scenedesmus protein, or Purina mouse chow. This effect was manifest after 2 weeks and persisted for at least 8 weeks of dietary treatment. Mixing lactalbumin with either casein or soy protein in a 20 g protein/100 g diet formula significantly enhanced the immune response in comparison to that of mice fed diets containing 20% soy protein or casein.
34. Cooke PS, Selvaraj V, Yellayi S. Genistein, estrogen receptors, and the acquired immune response. *J Nutr.* 2006, 136, 3, 704-708. Department of Veterinary Biosciences, University of Illinois, Urbana, 61802, USA. p-cooke@uiuc.edu Estrogen regulates thymic development and immune function. Despite the critical role of estrogens in inducing thymic involution and modulating immune responses, the mechanism of this effect is unclear. Similarly, humans and animals are exposed to increasing amounts of the estrogenic soy isoflavone genistein in the diet,

but whether genistein can induce immune changes has not been definitively established. We reported previously that genistein induces thymic atrophy in mice, and decreases both humoral and cell-mediated immunity. These thymic effects of genistein occur via estrogen receptor (ER)-mediated and non-ER-mediated pathways. Genistein injections produced the most pronounced effects, but dietary administration to mice that produced serum genistein concentrations similar to those reported in human infants consuming soy formula also had demonstrable effects. Microarray analysis of the effects of estradiol and genistein on neonatal thymus indicated that estradiol affected genes involved in transcription, apoptosis, cell cycle, and thymic development and function; genistein had similar effects on many estradiol target genes, but also had unique actions not replicated by estradiol. Despite extensive work showing inhibitory effects of genistein on immunity, other rodent studies reported that genistein or other phytoestrogens stimulate various aspects of immune function. Although the present data strongly indicate that genistein can regulate immune function, possibly at physiologic concentrations, further work is required to definitively establish overall thymic and immune effects of genistein and soy, which may vary with age, species, and specific end point.

35. Yellayi S, Naaz A, Szewczykowski MA, Sato T, Woods JA, Chang J, Segre M, Allred CD, Helferich WG, Cooke PS. The phytoestrogen genistein induces thymic and immune changes: a human health concern? *Proc Natl Acad Sci* U S A. 2002, 28, 99, 11, 7616-7621. Department of Veterinary Biosciences, University of Illinois, Urbana, IL 61802, USA. Use of soy-based infant formulas and soy/isoflavone supplements has aroused concern because of potential estrogenic effects of the soy isoflavones genistein and daidzein. Here we show that s.c. genistein injections in ovariectomized adult mice produced dose-responsive decreases in thymic weight of up to 80%. Genistein's thymic effects occurred through both estrogen receptor (ER) and non-ER-mediated mechanisms, as the genistein effects on thymus were only partially blocked by the ER antagonist ICI 182,780. Genistein decreased thymocyte numbers up to 86% and doubled apoptosis, indicating that the mechanism of the genistein effect on loss of thymocytes is caused in part by increased apoptosis. Genistein injection caused decreases in relative percentages of thymic CD4(+)CD8(-) and double-positive CD4(+)CD8(+) thymocytes, providing evidence that genistein may affect early thymocyte maturation and the maturation of the CD4(+)CD8(-) helper T cell lineage. Decreases in the relative percentages of CD4(+)CD8(-) thymocytes were accompanied by decreases in relative percentages of splenic CD4(+)CD8(-) cells and a systemic lymphocytopenia. In addition, genistein produced suppression of humoral immunity. Genistein injected at 8 mg/kg per day produced serum genistein levels comparable to

those reported in soy-fed human infants, and this dose caused significant thymic and immune changes in mice. Critically, dietary genistein at concentrations that produced serum genistein levels substantially less than those in soy-fed infants produced marked thymic atrophy. These results raise the possibility that serum genistein concentrations found in soy-fed infants may be capable of producing thymic and immune abnormalities, as suggested by previous reports of immune impairments in soy-fed human infants.

36. Zhang R, Li Y, Wang W. Enhancement of immune function in mice fed high doses of soy daidzein. *Nutr Cancer.* 1997, 29, 1, 24-28. Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing, China. High soy consumption leading to high exposures of soy isoflavones has been associated with a reduced risk of cancers at many sites. As part of a study focusing on the chemopreventive mechanisms, we have investigated the modulating effects of daidzein, a prominent and more bioavailable isoflavone in soy foods, on murine immune function. Swiss mice were fed daidzein at various doses daily for seven consecutive days. At high doses (20 and 40 mg/kg), daidzein exerted a stimulatory effect on nonspecific immunity, as shown by increases in the phagocytic response of peritoneal macrophages and thymus weight, in a dose-dependent manner. Augmentation of spleen immunoglobulin M-producing cells against sheep red blood cells demonstrated an-activation of humoral immunity. Enhanced cell-mediated immunity was also observed as increases in lymphocyte proportion of peripheral blood. However, no significant immunoregulatory effect was found when mice were fed 10 mg/kg daidzein. These results demonstrate for the first time that daidzein at high doses enhances several immunologic functions and suggest a novel approach to understanding the mechanism(s) by which soy foods may contribute to observed cancer prevention.
37. Blalock TL, Thaxton JP, Garlich JD. Humoral immunity in chicks experiencing marginal vitamin B-6 deficiency. *J Nutr.* 1984, 114, 2, 312-322. An economical vitamin B-6-deficient ration that was palatable to broiler chickens was prepared and fed to 1-day-old chicks. The experimental ration was a typical soy-glucose ration. Vitamin B-6 was removed by washing the soybean meal with water. Microbiological analysis revealed that the washed ration contained 0.45 mg vitamin B-6 per kilogram. Experimental rations were formulated to contain 0.5, 1.0 and 3.0 mg supplemental pyridoxine x HCl per kilogram of ration. These supplemental levels produced the following total pyridoxine concentrations in the diet: 0.95, 1.48 and 3.18 mg pyridoxine x HCl activity per kilogram of diet. Chicks were grown to 7 weeks of age and characteristic vitamin B-6 deficiency signs were quantitated and/or observed. Notable signs in

chicks receiving 0.5 mg added vitamin B-6 were increased mortality, decreased body weight gain and increased incidence of abnormal leg conformation. The humoral immune system of broiler chicks that were moderately deficient in vitamin B-6 was investigated. Marginal pyridoxine deficiency caused significant reduction in antibody levels to sheep red blood cells (SRBC) and relative levels of IgM and IgG during the peak and degradation phases of the primary response. During the hyperimmune response total anti-SRBC levels were not affected; however, relative levels of IgM and IgG were lowered.

38. Guder W, Nolte I, Wieland O. The influence of thyroid hormones on beta-hydroxy-beta-methylglutaryl-coenzyme A reductase of rat liver. *Eur J Biochem.* 1968, 4, 2, 273-278.
39. Oppenheimer JH. Thyroid hormone action at the cellular level. *Science.* 1979, 9, 203, 4384, 971-979. A large body of circumstantial evidence suggests that the basic unit of thyroid hormone action is the triiodothyronine nuclear receptor complex. This complex stimulates the formation, directly or indirectly, of a diversity of messenger RNA (mRNA) sequences. A generalized increase in mRNA as well as a disproportionate increase in a limited number of RNA sequences have been demonstrated. Regulation of thyroid hormone effects may be carried out largely at a local cellular level. Highly selective alterations in sensitivity to the triiodothyronine nuclear receptor complex may occur at specific target genes. Metabolic factors and hormones participate in such regulation. In a given tissue, alterations in the total number of receptor sites has not been shown to be useful as an index of thyroid hormone response, and local modulation of the response to the triiodothyronine receptor complex by a variety of factors other than triiodothyronine may be carried out at a postreceptor level.
40. Sirtori CR, Galli G, Lovati MR, Carrara P, Bosisio E, Kienle MG. Effects of dietary proteins on the regulation of liver lipoprotein receptors in rats. *J Nutr.* 1984, 114, 8, 493-500. Female rats fed a 1.2% cholesterol diet with animal proteins (casein) develop a significant hypercholesterolemia, with a marked increase of very low density lipoprotein (VLDL)-associated cholesterol. Substitution of soy proteins for casein in the diet counteracts the increase of both total and VLDL cholesterol. Studies of liver receptor activity were carried out with both casein and soybean-cholesterol diets, to define the site of action of soy proteins. Binding of a cholesterol-rich lipoprotein fraction (beta-VLDL) to hepatic membranes is normal when a soybean-cholesterol diet is administered, and markedly reduced with casein-cholesterol. The activity of receptor-linked enzymes, HMG-CoA reductase, cholesterol 7 alpha-hydroxylase and acyl-CoA:cholesterol O-acyltransferase (ACATase), is differently affected by the two diets. HMG-CoA reductase activity is reduced by both diets with, however, significantly higher

enzyme activities in the soybean-cholesterol-fed group. Both 7 alpha-hydroxylase and ACATase activity levels are significantly raised by casein-cholesterol but are in a normal range with soybean-cholesterol. These findings suggest that the hepatic receptor regulation of cholesterol metabolism is differently affected by animal and vegetable proteins in the diet.

41. Moyad MA. The placebo effect and randomized trials: analysis of alternative medicine. *Urol Clin North Am*. 2002, 29, 1, 135-155.
42. Platt R. Current concepts in optimum nutrition for cardiovascular disease. *Prev Cardiol*. 2000, 3, 2, 83-87.
43. Gilbert HF. Molecular and cellular aspects of thiol-disulfide exchange. *Adv Enzymol Relat Areas Mol Biol.* 1990, 63, 69-172.

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.

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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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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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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.

60. Bicíková M, Tallová J, Stanická S, Hill M, Vondra K, Hampl R. Levels of testosterone, allopregnanolone and homocysteine in severe hypothyroidism. *Clin Chem Lab Med.* 2002, 40, 10, 1024-1027. Institute of Endocrinology, Prague, Czech Republic. mbiciko@endo.cz.
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.

61. Hussein WI, Green R, Jacobsen DW, Faiman C. Normalization of hyperhomocysteinemia with L-thyroxine in hypothyroidism. *Ann Intern Med.* 1999, 7, 131, 5, 348-51. Comment in: *Ann Intern Med.* 2000, 18, 132, 8, 677. The Cleveland Clinic Foundation, Ohio 44195, USA. BACKGROUND: Hyperhomocysteinemia is an independent risk factor for coronary, peripheral, and cerebrovascular disease. Elevated plasma homocysteine levels were described in a preliminary report on primary hypothyroidism. OBJECTIVE: To determine whether restoration of euthyroidism by L-thyroxine replacement therapy would reduce or normalize plasma homocysteine levels. DESIGN: Prospective cohort study. SETTING: Outpatient endocrinology department of a tertiary center. PATIENTS: 14 patients (10 women and 4 men; 25 to 77 years of age): 4 with newly diagnosed chronic (Hashimoto) hypothyroidism and 10 who had been rendered acutely hypothyroid (thyroid-stimulating hormone level > 25 mU/L) by total thyroidectomy for thyroid carcinoma. MEASUREMENTS: Total plasma homocysteine levels were measured at baseline and 3 to 9 months later, after euthyroidism had been attained by L-thyroxine replacement therapy. RESULTS: Median baseline plasma homocysteine levels in both sexes (women, 11.65 micromol/L [range, 7.2 to 26.5 micromol/L]; men, 15.1 micromol/L [range, 14.1 to 16.3 micromol/L]) were higher (P = 0.002) than those in healthy female (n = 35) and male (n = 36) volunteers (women, 7.52 micromol/L [range, 4.3 to 14.0 micromol/L]; men, 8.72 micromol/L [range, 5.94 to 14.98 micromol/L]). Eight patients (57%) had baseline plasma homocysteine levels that exceeded the upper limit of sex-specific reference ranges. Upon attainment of euthyroidism, all patients had a diminution in plasma homocysteine levels. The median overall change of -5.5 micromol/L (range, -15.4 to -1.8 micromol/L) corresponds to a difference of -44% (range, -58% to -13%) (P < 0.001). Homocysteine levels returned to normal in 7 of the 8 patients with elevated pretreatment values. CONCLUSIONS: Hypothyroidism may be a treatable cause of hyperhomocysteinemia, and elevated plasma homocysteine levels may be an independent risk factor for the accelerated atherosclerosis seen in primary hypothyroidism.

62. Nedrebø BG, Ericsson UB, Nygård O,Refsum H, Ueland PM, Aakvaag A, Aanderud S, Lien EA. Plasma total homocysteine levels in hyperthyroid and hypothyroid patients. *Metabolism.* 998, 47, 1, 89-93. Department of Internal Medicine, University Hospital of Bergen, Norway. We found a higher plasma concentration of total homocysteine (tHcy), an independent risk factor for cardiovascular disease, in patients with hypothyroidism (mean, 16.3 micromol/L; 95% confidence interval [CI], 14.7 to 17.9 micromol/L) than in healthy controls (mean, 10.5 micromol/L; 95% CI, 10.1 to 10.9 micromol/L). The tHcy level of hyperthyroid

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.

63. Hanson LN, Engelman HM, Alekel DL, Schalinske KL, Kohut ML, Reddy MB. Effects of soy isoflavones and phytate on homocysteine, C-reactive protein, and iron status in postmenopausal women. *Am J Clin Nutr.* 2006 Oct;84(4):774-780.
64. McCully KS. Homocysteine, vitamins, and vascular disease prevention. *Am J Clin Nutr.* 2007 86, 5, 1563S-8S.
65. Rackis JJ Biological and physiological factors in soybeans. *J Am Chem Soc*, 1974, 51,161A-169A.
66. Visser A, Thomas A. Review: Soya protein products their processing, functionality and application aspects. *Food Rev Inter,* 1987, 3, 1&2, 20.
67. Life Sciences Research Office, SCOGs-101. Evaluation of the health aspects of soy protein isolates as food ingredients, 1979. Bureau of Foods, Food and Drug Administration. FASEB.
68. Smith, Allan K. Circle, Sidney J. *Soybeans: Chemistry and Technology.* Volume 1 Proteins (Westport, CT, Avi , 1972. p. 188).

SECTION II: C

EFFECTS OF SOY PROTEIN ON OTHER CARDIOVASCULAR RISK FACTORS, ARRHYTHMIAS AND CARDIOMYOPATHY

In its 245-page report on soy and health outcomes, the US Agency for Healthcare Research and Quality found insufficient evidence to recommend soy for improving cardiovascular risk factors, including HDL, triglycerides, lipoprotein (a), c-reactive protein, endothelial function, systemic arterial compliance, oxidized LDL or blood pressure. Excerpts below are from the agency's report.

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.

HIGH DENSITY LIPOPROTEIN: A total of 56 studies reported data on the effect of consumption of soy products on HDLlevels. The median net change compared to control found was +1 mg/dL. This estimate was in agreement with the meta-analysis estimate of +0.6 (95% CI 0.5, +1.8) mg/dL, which was not statistically significant. With only 2 exceptions, all studies reported a net effect on HDL of less than 10 percent, with an even distribution between net increases and net decreases or zero effect. Across studies, there were no consistent differences in effect based on baseline HDL, soy protein consumption, soy isoflavone consumption, soy incorporated into diet or as supplement, or population (post-menopausal women, pre-menopausal women, men). A possible association between baseline HDL and net change was found; although this association disappeared with the exclusion of 2 outlier studies. Studies that directly compared different baseline degree of abnormal lipids, doses of soy protein or soy isoflavones, or populations found no significant difference in effect.

TRIGLYCERIDES: A total of 54 studies reported data on the effect of consumption of soy products on triglyceride levels. The median net change compared to control found was approximately 3 mg/dL (or 2%), although a wide range of effects were reported, ranging from 49 to +66 mg/dL (49% to +31%). Meta-analysis estimated a significant net effect of 8 (95% CI 11, 5) mg/dL. Meta-regression revealed a possible association between increased mean baseline triglyceride level and greater net reduction in triglycerides. Neither isoflavone or soy protein dose was associated with net effect on triglycerides. Within specific studies that investigated these possible associations, though, most studies found no associations. There was no evident association with whether soy was incorporated into diet or as supplement, or based on population (post-menopausal women, pre-menopausal women, men).

LIPOPROTEIN (A) The large majority of studies reported non significant changes in Lp(a) from baseline after soy intervention. Among 18 studies, 2 found a net decrease of at least 4 mg/dL (or a statistically significant decrease) in Lp(a) concentration, 4 found a net increase of at least 4 mg/dL (or a statistically significant increase), and 12 found no effect. Only 3 studies

reported significant or near significant net changes in Lp(a) after soy protein consumption compared to controls. Nilausen 1999109 (Table 29) reported a non-significant change in Lp(a) among men consuming soy, but a significant decrease in Lp(a) among controls consuming caseinate; this resulted in a statistically significant net increase in Lp(a). Teede 200177 (Table 30) found a substantially greater, statistically significant, increase in Lp(a) among men and post-menopausal women supplemented with soy product compared to casein. Dent 200182 (Table 30), reported a marginally significant net decrease in median Lp(a) among hypercholesterolemic peri-menopausal women supplemented with soy product with and without isoflavone compared to whey protein.

C-REACTIVE PROTEIN: No study found a significant effect of soy protein consumption on CRP level. Two studies reported trends towards increases in CRP levels from baseline among women after soy intervention, but these effects were non-significant compared to controls. However, the rise in CRP levels was not seen in the sub-analysis of men.

ENDOTHELIAL FUNCTION: Nine randomized trials and 1 cohort study of generally poor to moderate quality and limited applicability investigated the effect of isolated soy protein or pure soy isoflavones on endothelial-dependent function. Overall, limited evidence suggests a possible small improvement in endothelial-dependent function with consumption of soy products by post-menopausal women. However, 1 of 2 studies of men reported a significant worsening of function with soy consumption. There is insufficient evidence regarding different types or doses of soy products to compare their relative effectiveness.

SYTEMIC ARTERIAL COMPLIANCE: Three randomized trials of generally poor to moderate quality and limited applicability investigated the effect of soy protein or pure soy isoflavones on systemic arterial compliance. Overall, limited evidence suggests a possible small improvement in systemic arterial compliance with consumption of soy products by men and post-menopausal women. There is insufficient evidence regarding different types or doses of soy products to compare their relative effectiveness.

LDL OXIDATION: Nine randomized trials and 1 cohort study of generally poor quality and moderate to good applicability investigated the effect of soy protein or pure soy isoflavones on LDL oxidation. Overall, evidence suggests a possible improvement in LDL oxidation with consumption of soy products by men or women. However, 1 of 2 studies of men and women found a significant worsening of LDL oxidation with soy consumption. There is insufficient evidence regarding different types or doses of soy products to compare their relative effectiveness.

BLOOD PRESSURE: A total of 22 studies with mostly moderate quality reported data on the effect of consumption of soy products on systolic and diastolic BP. Overall, soy consumption does not appear to affect BP level. Across studies there were no discernable differences in effect based on baseline BP, soy protein consumption, soy isoflavone consumption, soy incorporated into diet or as supplement, or population (post-menopausal women, pre-menopausal women, men).

Clearly, soy protein does not attain scientific agreement on its effect on these cardiovascular risk factors. In fact, soy protein could have a detrimental effect on these cardiovascular risk factors. We include excerpts from several studies showing adverse effects below (*emphases ours.*):

Teede JH, Dalais FS et al. Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. *J Clin Endocrinol Metab,* 2001, 86, 7, 3053-3060.

To address the cardiovascular effects of dietary soy containing phytoestrogens, we measured blood pressure (BP), lipids, vascular function (systemic arterial compliance and pulse wave velocity), and endothelial function (flow-mediated vasodilation) in a randomized, double-blind trial. Two hundred thirteen healthy subjects (108 men and 105 postmenopausal women), 50-75 yr old, received either soy protein isolate (40 g soy protein, 118 mg isoflavones) or casein placebo for 3 months. There were 34 withdrawals (16%), with 179 subjects (96 men and 83 women) completing the protocol. After intervention in the soy group, compared with casein placebo, urinary phytoestrogens increased, accompanied by a significant fall in BP reflected by the BP model ($P < 0.01$) encompassing mean change (+/-SEM) in systolic (-7.5 +/- 1.2 vs. -3.6 +/- 1.1 mm Hg, $P < 0.05$), diastolic (-4.3 +/- 0.8 vs. -1.9 +/- 0.7 mm Hg, $P < 0.05$), and mean BP (-5.5 +/- 1 vs. -0.9 +/- 1 mm Hg, $P < 0.008$). In the lipid model, soy induced greater changes, compared with placebo ($P < 0.001$). On individual analysis, significant contributors included a reduction in the low- to high-density lipoprotein ratio (-0.33 +/- 0.1 vs. 0.04 +/- 0.1 mmol/L, $P < 0.05$) and triglycerides (-0.2 +/- 0.05 vs. -0.01 +/- 0.05 mol/L, $P < 0.05$) and an increase in Lp(a) lipoprotein (+/- 95% confidence interval) [42 (range, 17-67) vs. 4 (range, -22-31) mg/L, $P < 0.05$], whereas total, low-density lipoprotein, and high-density lipoprotein cholesterol improved in both groups; but no treatment effect was demonstrated. The arterial functional model demonstrated no difference between groups; although again, overall function improved in both groups. On individual analysis, peripheral PWV (reflecting peripheral vascular resistance) improved with soy ($P < 0.01$), whereas flow-mediated vasodilation (reflecting endothelial function) declined (in males only), compared with casein placebo ($P < 0.02$). No effect of treatment on the hypothalamic-pituitary-gonadal axis was noted in males or females.

In normotensive men and postmenopausal women, soy improved BP and lipids but, overall, did not improve vascular function. Potential adverse effects were noted, with a decline in endothelial function (in males only) and an increase in Lp(a). Further research in hypertensive and hyperlipidemic populations is needed.

Kreijkamp-Kaspers S, Kok L, et al. Randomized controlled trial of the effects of soy protein containing isoflavones on vascular function in postmenopausal women. *Am J Clin Nutr.* 2005 Jan;81(1):189-95

BACKGROUND: The incidence of cardiovascular disease increases after menopause, possibly because of the decline in estrogen. Soy protein, a rich source of estrogen-like isoflavones, is hypothesized to improve vascular function. OBJECTIVE: The objective of this study was to

investigate whether supplementation with soy protein, a rich source of estrogen-like isoflavones, improves vascular function. DESIGN: We performed a 12-mo double-blind randomized trial to compare the effects of soy protein containing 99 mg isoflavones/d (aglycone weights) with those of milk protein (placebo) on blood pressure and endothelial function in 202 postmenopausal women aged 60-75 y. RESULTS: Changes in endothelial function during the intervention were not significantly different between the soy and the placebo groups. After the intervention, systolic blood pressure increased in the soy group significantly more than it did in the placebo group; the difference in change was 4.3 mm Hg (95% CI: 0.3, 8.4 mm Hg; P = 0.04) for systolic blood pressure, but only 2.0 mm Hg (95% CI: -0.74, 4.71 mm Hg; P = 0.15) for diastolic blood pressure. In the soy group only, systolic and diastolic blood pressure decreased and endothelial function improved in the equol producers, whereas systolic and diastolic blood pressure increased and endothelial function deteriorated in the equol nonproducers.

CONCLUSIONS:*The results of this trial do not support the hypothesis that soy protein containing isoflavones have beneficial effects on vascular function in older postmenopausal women. Whether certain subgroups of women (eg, equol producers) do benefit from the intervention remains to be elucidated.*

Nilausen K, Meinertz H. Lipoprotein(a) and dietary proteins: casein lowers lipoprotein(a) concentrations as compared with soy protein. *Am J Clin Nutr*. 1999, 69, 3, 419-25.

OBJECTIVE: We compared the effects of dietary soy protein and casein on plasma Lp(a) concentrations. DESIGN: Nine normolipidemic men were studied initially while consuming their habitual, self-selected diets, and then, in a crossover design, while consuming 2 liquid-formula diets containing either casein or soy protein. The dietary periods lasted 45 d (n = 7) or 33 d (n = 2). Fasting total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerol, and Lp(a) concentrations were measured throughout. RESULTS: After 30 d of each diet, the mean concentration of Lp(a) was not significantly different after the soy-protein and self-selected diets. However, Lp(a) decreased by an average of 50% (P < 0.001) after the casein diet as compared with concentrations after both the soy-protein and self-selected diets. Two weeks after subjects switched from the self-selected to the soy-protein diet, Lp(a) increased by 20% (P = 0.065), but subsequently decreased to baseline. In contrast, the switch to the casein diet did not cause an increase in Lp(a), but instead a continuing decrease in mean concentrations to 65% below baseline (P < 0.0002). Total cholesterol, LDL cholesterol, and HDL cholesterol were significantly lower > or =30 d after both the casein and soy-protein diets than after the self-selected diet (P < 0.001). HDL cholesterol was 11% higher after the soy-protein diet than after the casein diet (P < 0.002), but LDL cholesterol, total cholesterol, and triacylglycerol were not significantly different after the casein and soy-protein diets. CONCLUSION: *These findings indicate that soy protein may have an Lp(a)-raising effect, potentially detrimental to its use in antiatherogenic diets.*

Pepine CJ, von Mering GO, et al. Phytoestrogens and coronary microvascular function in women with suspected myocardial ischemia: a report from the Women's Ischemia Syndrome Evaluation (WISE) Study. *J Womens Health* (Larchmt). 2007 May;16(4):481-8.

AIMS: Soy phytoestrogens are popular, but information on their coronary effects in patients with suspected ischemic heart disease is limited. Accordingly, we investigated the relationship between blood phytoestrogen levels and coronary reactivity in women with suspected myocardial ischemia referred for coronary angiography. reactivity variables and daidzein or endogenous estrogen. METHODS: Coronary flow velocity reserve (CFVR) and volumetric flow reserve (VFR) to adenosine (ADO) and nitroglycerin (NTG) (nonendothelial-dependent responses) and acetylcholine (ACH) (endothelial-dependent response) were assessed in 106 women from the Women's Ischemia Syndrome Evaluation (WISE). Blood phytoestrogen (daidzein and genistein) and estrogen (estradiol) levels were correlated with coronary reactivity measures. RESULTS: Participants were mostly postmenopausal (79%), mean age 56 years, and 24% had obstructive coronary artery disease (CAD) at angiography. Genistein blood levels were negatively correlated with nonendothelial-dependent coronary flow responses. The highest genistein tertile (>6.1 ng/mL) had a CFVR of 2.1 +/- 0.5 (mean +/- SD) and VFRADO of 1.0 +/- 0.6, and both were significantly (p= 0.0001) lower compared with the other genistein tertiles combined. Similar associations were noted for CFVR(NTG) and VFR(NTG) (p = 0.03 and p = 0.01, respectively). The highest genistein tertile was associated with lower CFVR(ACH) compared with the other tertiles (p = 0.03). In multivariable modeling, blood genistein levels were significant independent predictors of coronary flow responses to ADO. There were no significant correlations between coronary reactivity variables and daidzein or endogenous estrogen. CONCLUSIONS: *In women with suspected myocardial ischemia, higher genistein blood levels are associated with impaired nonendothelial-dependent and endothelial-dependent coronary microvascular function.*

Pepine et al studied the effect of phytoestrogens not soy protein on microsvascular health. The currently allowed soy/heart health claim concerns soy protein. However, *all* soy protein products naturally contain isoflavones, often at high levels. As such, studies showing the detrimental effects of soy isoflavones are relevant. The study excerpted below indicates that soy genistein could cause heart arrhythmias:

Chiang CE, Luk HN, Chen LL, Wang TM, Ding PY.Genistein inhibits the inward rectifying potassium current in guinea pig ventricular myocytes. *J Biomed Sci*. 2002, 9, 4 321-326.

Genistein is an isoflavone with potent inhibitory activity on protein tyrosine kinase. Previous studies have shown that genistein has additional effects, among which the direct blocking effects on various ionic channels have recently been disclosed. Using whole-cell voltage clamp and current clamp techniques, we demonstrate that micromolar concentrations of genistein dose-dependently and reversibly inhibit the inward rectifying K(+) current, and depolarize the resting membrane potential, resulting in abnormal automaticity in guinea pig ventricular myocytes. Interestingly, another potent tyrosine kinase inhibitor, tyrphostin 51, did not produce the same inhibitory effect, while the inactive analogue of genistein, daidzein, had a similar blocking

effect. *We suggest that genistein directly blocks the inward rectifying K(+) current in ventricular myocytes, and one should be cautious of its pro-arrhythmic effect in clinical use*

* * * * *

Finally, we cite research from the University of Colorado showing that soy worsens cardiomyopathy, an increasingly prevalent heart condition that affects 1 in 500 Americans and is the leading cause of death in young athletes.

Stauffer BL, Konhilas JP, Luczak ED, Leinwand LA. Soy diet worsens heart disease in mice.J Clin Invest. 2006, 116, 1, 209-216.

We report that dietary modification from a soy-based diet to a casein-based diet radically improves disease indicators and cardiac function in a transgenic mouse model of hypertrophic cardiomyopathy. *On a soy diet, males with a mutation in the alpha-myosin heavy chain gene progress to dilation and heart failure. However, males fed a casein diet no longer deteriorate to severe, dilated cardiomyopathy*. Remarkably, their LV size and contractile function are preserved. Further, this diet prevents a number of pathologic indicators in males, including fibrosis, induction of beta-myosin heavy chain, inactivation of glycogen synthase kinase 3beta (GSK3beta), and caspase-3 activation.

Luckey SW, Mansoori J, Fair K, Antos CL, Olson EN, Leinwand LA.Blocking cardiac growth in hypertrophic cardiomyopathy induces cardiac dysfunction and decreased survival only in males. *Am J Physiol Heart Circ Physiol.* 2007, 292, 2, H838-45.

Mutations in myosin heavy chain (MyHC) can cause hypertrophic cardiomyopathy (HCM) that is characterized by hypertrophy, histopathology, contractile dysfunction, and sudden death. The signaling pathways involved in the pathology of HCM have not been elucidated, and an unresolved question is whether blocking hypertrophic growth in HCM may be maladaptive or beneficial. To address these questions, a mouse model of HCM was crossed with an antihypertrophic mouse model of constitutive activated glycogen synthase kinase-3beta (caGSK-3beta). Active GSK-3beta blocked cardiac hypertrophy in both male and female HCM mice. However, doubly transgenic males (HCM/GSK-3beta) demonstrated depressed contractile function, reduced sarcoplasmic (endo) reticulum Ca(2+)-ATPase (SERCA) expression, elevated atrial natriuretic factor (ANF) expression, and premature death. In contrast, female HCM/GSK-3beta double transgenic mice exhibited similar cardiac histology, function, and survival to their female HCM littermates. *Remarkably, dietary modification from a soy-based diet to a casein-based diet significantly improved survival in HCM/GSK-3beta males.* These findings indicate that activation of GSK-3beta is sufficient to limit cardiac growth in this HCM model and the consequence of caGSK-3beta was sexually dimorphic. Furthermore, these results show that blocking hypertrophy by active GSK-3beta in this HCM model is not therapeutic.

* * * * *

We submit that it is improper for the FDA to allow a soy/heart disease health claim when many studies show soy's potential to cause heart disease. 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.

* * * * *

III: CONCLUSION

In Section I of this petition, we noted that soy protein isolate has not earned GRAS status and for this reason cannot properly be the subject of a health claim. In Section II, we showed that the totality of publicly available scientific evidence does not support the premise that soy protein prevents heart disease or even that it lowers total or LDL-cholesterol levels. Furthermore, many respected scientists have warned about studies showing that soy protein can contribute to the development of heart disease.

In 1998, scientists from the FDA's Laboratory of Toxicological Research in Jefferson, Arkansas, voiced opposition to the soy protein/heart health claim. Daniel Sheehan, PhD, Director of the Estrogen Base Program, Division of Genetic and Reproductive Technology, and Daniel Doerge, PhD, Division of Biochemical Toxicology, wrote a seven-page letter to the FDA, excerpted below:

> We oppose this health claim because there is abundant evidence that some of the isoflavones found in soy, including genistein and equol, a metabolite of daidzein, demonstrate toxicity in estrogen sensitive tissues and in the thyroid. This is true for a number of species , including humans. Additionally, the adverse effects in humans occur in several tissues and, apparently, by several distinct mechanisms. . .
>
> While isoflavones may have beneficial effects at some ages or circumstances, this cannot be assumed to be true at all ages. Isoflavones are like other estrogens in that they are two-edged swords, conferring both benefits and risks. The health labeling of soy protein isolate for foods needs to be considered just as would the addition of any estrogen or goitrogen to foods, which are bad ideas. . .
>
> Estrogenic and goitrogenic drugs are regulated by FDA, and are taken under a physician's care. Patients are informed of risks, and are monitored by their physicians for evidence of toxicity. There are no similar safeguards in place for foods, so the public will be put at potential risk from soy isoflavones in soy protein isolate without adequate warning and information.

Irvin E. Liener, PhD, professor emeritus at the University of Minnesota and a leading expert and textbook writer on protease inhibitors and other antinutritional factors in soybeans, also wrote the FDA in 1998 to express his specific concerns about trypsin inhibitors and the FDA's failure to have thoroughly examined USDA and other significant research on this subject. His letter concluded:

> Trypsin inhibitors do in fact pose a potential risk to humans when soy protein is incorporated into the diet.

Since 1999, other top US government scientists have published warnings about the dangers of soy protein and its phytoestrogenic constituents. We would particularly like to remind the FDA of work carried out at the molecular toxicology laboratory at the National Institute of Environmental Health Sciences (NIEHS) in Triangle Park, North Carolina. Retha Newbold's team at NIEHS has spent more than 25 years investigating endocrine disruption caused by the soy estrogen genistein, DES and other environmental estrogens and reported on those findings at symposia and in prestigious peer-reviewed journals. After publication of one such study in the January 2006 issue of *Biology of Reproduction*, NIEHS director Dr. David Schwartz commented, Although we are not entirely certain about how these animal studies on genistein translate to the human population, there is some reason to be cautious.

The findings of scientists at both the FDA's National Laboratory for Toxicological Research and National Institute of Environmental Health Sciences -- which clearly demonstrate the risks of soy protein and its phytoestrogenic constituents genistein and daidzein -- provide a mandate to the FDA to rescind the heart health claim for soy protein.

Finally, we would like to draw the FDA's attention to the health advisories issued by three foreign governments about these and other safety issues surrounding the consumption of soy protein.

In 2005, the Israeli Health Ministry warned its citizens that babies should not receive soy formula, that children age 18 and under should consume soy foods or soy milk no more than once per day to a maximum of three times per week and that adults should exercise caution because of adverse effects on fertility and increased breast cancer risk. The Israeli Ministry based its advice upon the conclusions reached by a 13-member committee of nutritionists, oncologists, toxicologists, pediatricians and other specialists who spent more than a year examining the evidence. The committee concluded that the estrogen-like plant hormones in soy can cause adverse effects on the human body, including cancer promotion and reproductive problems. They strongly urged that consumption of soy foods be minimized until absolutely safety has been proven.

In 2006, the French Food Agency (AFSSA) announced tough new regulations that will soon require manufacturers to improve the safety of soy infant formula and to put warning labels on packages of soy foods and soy milk. The new regulations followed an extensive investigation

culminating in the requirement that manufacturers remove the estrogenic isoflavones from soy infant formula down to 1 ppm and to include warning labels on packages of soy foods and soy milk that will alert consumers of the risks for children under three, children with hypothyroidism and women who have been diagnosed with or have a family history of breast cancer.

In 2007, the German Federal Institute for Risk Assessment warned that babies should not be given soy infant formula without clear, concrete medical reasons and that adults should be wary of excess soy food and soy supplement consumption because soy isoflavones offer no proven health benefits and may pose health risks. Professor Dr. Andreas Hensel, President of the Federal Institute for Risk Assessment (BfR), expressed concerns about the marketing of soy foods and isoflavone supplements to menopausal women and doubts about the claimed advantages of the supplements for heart, bone and breast health.

In conclusion, the precautionary principle mandates that FDA rescind the health claim for soy protein, especially in the light of the Israeli, French and German governments' warning advisories. The FDA, in its mandated role as America's foremost consumer protection agency, has a duty to the American public to amend the Final Rule to disallow the heart disease health claim for soy protein and to require all soy food manufacturers currently using it to cease and desist.

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