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.
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> 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.

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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.
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> . . . 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.

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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.

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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.

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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.

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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

1. Ingenbleek Y, Young VR. The essentiality of sulfur is closely related to nitrogen metabolism: a clue to hyperhomocysteinamia. *Nutr Res Rev* 2004,17, 135-151.

2. Naruszewicz M, Mirkewicz E, Olszewski AJ, McCully KS: Thiolation of low-density lipoproteins by homocysteine thiolactone causes increased aggregation of interaction with cultured macrophages. *Nutr Metab Cardiovasc Dis* 1994,4,70-77.

3. Doerge DR. Inhibition of thyroid peroxidase by dietary flavonoids, *Chem Res Toxicol*, 1996, 9, 16-23.

4. Divi RL, Chang HC, Doerge DR. Anti-thyroid isoflavones from soybean. *Biochem Pharmacol*, 1997, 54, 1087-1096.

5. Chang HC, Doerge DR. Dietary genistein inactivates rat thyroid peroxidase in vivo without an apparent hypothyroid effect. *Toxicol Appl Pharmacol,* 2000, 168, 224-252.

6. http://www.vitaletherapeutics.org/ipreface.htm

7. http://www.vitaletherapeutics.org/disrtatn.htm

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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.

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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

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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.

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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.

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