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*

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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.

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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.

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