Journal of the American Association for Laboratory Animal Science
Vol 45, No 4
July 2006
Pages 20–23

Concentrations of Isoflavones in Macaques Consuming Standard Laboratory Monkey Diet

Fawn C Stroud,[1,*] Susan E Appt,[2] Mark E Wilson,[1] Adrian A Franke,[3] Michael R Adams,[2] and Jay R Kaplan[2]

The soy isoflavones genistein and daidzein, as well as the daidzein metabolite equol, have structural similarities to mammalian estrogens and bind with varying affinity to both known subtypes of the estrogen receptor. Consequently, prospective studies in both humans and animals have begun to evaluate the potential effects of isoflavones on estrogen receptor-mediated phenomena. However, many diets of laboratory-housed animals derive their protein from soy and thus likely contain substantial quantities of isoflavones. Exposing experimental subjects to these isoflavones via such diets could confound studies, particularly those evaluating the effects of estrogen or estrogen-like ligands. The aim of this study was to compare the levels of circulating concentrations of isoflavones and their metabolites in monkeys fed either a soy-free diet, a soy-based diet providing 130 mg of isoflavone (daidzein, genistein, and glycitein aglycon equivalents) daily, or a commercially available 'chow' diet containing an unspecified amount of soybean meal. Animals consuming the commercial diet had serum concentrations of daidzein, genistein, and glycitein that were significantly higher than those of animals fed a soy-free diet but similar to those of monkeys fed a soy-based diet formulated to be high in isoflavones. Notably, animals fed the commercial diet also had serum equol concentrations that were similar to or, in some cases, in excess of serum concentrations in the animals fed the soy diet. These data argue for the use of soy-free diets in studies investigating estrogenic effects on physiologic or behavioral endpoints.

Phytoestrogens are compounds found in plants that are important for plant growth (nodulation) and for protection of plants from environmental stress and ultraviolet radiation.[31] Soy isoflavones are probably the best known of the phytoestrogens because of the prominence of soybeans and soy-derived supplements in some human diets and because of the biologic activity and health benefits often attributed to these compounds. Specifically, the soy isoflavones genistein and daidzein (as well as the daidzein metabolite equol, produced by the gut flora of some humans and most species of animals) have structural similarities to mammalian estrogens and bind to both the α and, preferentially, β estrogen receptors.[12,19,25,32-34,40] Thus, soy isoflavones show biologic activity in vitro and in vivo, even in human studies.[3,4,26,37] The resulting effects are both estrogenic and anti-estrogenic, depending on tissue, dose, and concentrations of circulating endogenous estrogens.[7] Furthermore, although soy isoflavones bind and activate estrogen receptors, genistein is also a tyrosine kinase inhibitor,[38] a potent antioxidant in vitro,[15,16,29] and an ion channel modulator.[11,21] With regard to health, the habitual consumption of isoflavone-rich diets has been associated epidemiologically with reduced incidence of many chronic diseases.[2,3,20]

These characteristics of isoflavones have made them the subject of considerable research interest. Less well appreciated, however, is the fact that many experimental subjects are often exposed inadvertently to high doses of isoflavones because the manufacturers of commercial animal diet typically derive a large proportion of the protein comprising these diets from soybeans. For example, mice and rats consuming commercially produced rodent diets have steady-state serum isoflavone concentrations in excess of 2000 ng/ml, several orders of magnitude greater than endogenous estrogen concentrations.[6] This degree of isoflavone exposure could well confound a wide range of experiments addressing not only estrogen receptor action but also a broader array of biological effects (for example, antioxidant and tyrosine kinase activity) associated with phytoestrogens.[6] Any such effects undoubtedly extend to anthropoid primates, such as the macaque monkey species often used as human surrogates in biomedical research. Here we compare circulating concentrations of isoflavones, isoflavone metabolites, and equol among cohorts of macaques consuming 1 of 3 diets: 1) specifically formulated, high in soy isoflavones; 2) specifically formulated, devoid of soy and soy isoflavones; or 3) commercially available, soy-derived. The objective was to determine the extent to which consumption of a commonly used commercially available diet might expose laboratory monkeys to isoflavones.

Methods

Animals. Subjects were 6-y-old female rhesus monkeys (*Macaca mulatta*; n = 10), housed at the Yerkes National Primate Research Center of Emory University and approximately 9-y-old female (n = 89) and male (n = 61) cynomolgus monkeys (*M. fascicularis*) initially imported from the Indonesian Primate Center (Bogor, Indonesia) and housed at Wake Forest University School of Medicine. The rhesus females were ovariectomized and lived in small social units as described previously.[39] The cynomolgus monkeys were reproductively intact but were similarly housed in small social groups.[18] The protocol and all procedures used in the investigations involving these animals were approved by the appropriate institutional animal care and use committee in accordance with the Animal Welfare Act and the US Department of Health and Human Services *Guide for Care and Use of Laboratory Animals*.[28] Both research facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Diets. Rhesus monkeys were fed standard monkey chow (Jumbo Monkey Diet 5037, Purina Mills, St Louis, MO). This diet provides 18%, 13%, and 69% of calories from protein, fat, and carbohydrates, respectively. The diet contains an unspecified

Received: 28 Nov 2005. Revision requested: 10 Feb 2006. Accepted: 10 Feb 2006.
[1]*Yerkes National Primate Research Center, Emory University, Atlanta, Georgia;* [2]*Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, North Carolina;* [3]*Cancer Research Center of Hawaii, Honolulu, Hawaii.*
[*]*Corresponding author. Email: fstroud@rmy.emory.edu*

Table 1. Experimental groups

Species	Sex	N	Diet
Rhesus monkey	Female, ovariectomized	10	Purina 5037
Cynomolgus monkey	Female	43	High-isoflavone, soy-based
Cynomolgus monkey	Female	46	Soy-free
Cynomolgus monkey	Male	31	High-isoflavone, soy-based
Cynomolgus monkey	Male	30	Soy-free

amount of dehulled soybean meal and ground soybean hulls. The cynomolgus monkeys consumed either a soy-free diet in which protein was derived from casein and lactalbumin (n = 46 females, 30 males) or a diet in which protein was derived from soy (n = 43 females, 31 males). The soy-based and soy-free diets were isocaloric, and both derived 19%, 44%, and 37% from protein, fat, and carbohydrates, respectively.[1] In the soy-based diet, all protein was derived from SUPRO 670-HG (Solae, St Louis, MO), which contains on average 1.105 mg genistein, 0.365 mg daidzein, and 0.08 mg glycitein/g soy protein isolate (expressed in aglycone units). The soy-based diet provided an isoflavone dose approximately equivalent to 130 mg/day for human beings, based on an average intake of 1800 cal for women and 2000 cal for men. Monkeys were fed 120 kcal of diet per kg body weight and therefore consumed approximately 8.6 mg isoflavone per kg body weight. In addition to being isocaloric, the soy-based and soy-free diets were formulated to be comparable for cholesterol (0.20 mg/kcal), calcium (830 mg/1800 kcal), and phosphorus (820 mg/1800 kcal). This high-fat, atherogenic diet was fed as a part of a study assessing soy effects on the development of cardiovascular disease.[18]

The rhesus monkeys had been consuming the standard monkey diet since they were weaned some 5.5 y earlier, a diet that also was consumed by their mothers. The cynomolgus monkeys had been consuming the soy-based and soy-free diets for at least 2 y, with unknown prior soy exposure. All animals were fed ad libitum twice daily, and the rhesus monkeys were supplemented with fresh fruit, vegetables, and/or cereal daily. Some of these supplemental foods items given to the rhesus monkeys occasionally were apples, pears, onions, and sunflower seeds, and these contain phytoestrogens of the lignans class.[27] However, the monkeys typically only ate a single piece of the supplemental fruit or vegetable. For purposes of this study, we will focus on the isoflavone class of phytoestrogens. There were thus 5 comparison conditions, which are described in Table 1.

Analyses of isoflavones. Serum samples were collected on a single occasion from each monkey for analyses of circulating isoflavone concentrations. Animals were fed in the morning, and blood samples were collected in the afternoon, 4 h after feeding, via conscious bleeds in rhesus monkeys and with ketamine HCl (15 mg/kg) in cynomolgus monkeys. Blood was processed immediately, and the serum was kept frozen and protected from light until analysis. Serum isoflavones were analyzed by liquid chromatography photo-diode array electrospray mass spectrometry through slight modification of a previously established method[14] to include equol in the panel of isoflavonoids (genistein, dihydrogenistein, daidzein, dihydrodaidzein, glycitein, and O-desmethylangolensin) and isotopically labeled internal standards.[9,13] Detection limits previously were found to be 1 to 15 nM, depending on the analyte, and interassay coefficients of variations were 8% to 22% at levels less than 20 nM, 7% to 14% at 20 nM to 100 nM, and 3% to 12% at levels exceeding 100 nM.

Statistical analysis. With use of a statistical software program (SPSS version 11.0, SPSS, Chicago, IL), data were expressed as mean ± standard error of the mean and were analyzed by 1-way analysis of variance. Differences between the 5 condition groups were evaluated by Newman–Kuels post hoc tests. Values for which $P < 0.05$ were considered significantly different.

Results

As expected, serum levels of isoflavones were low in both male and female cynomolgus monkeys fed soy-free diet compared with those fed an experimental diet containing soy (Table 2). However, serum isoflavone concentrations were also significantly elevated in rhesus monkey females fed the commercially available monkey chow in comparison to those seen in the animals fed the soy-free diets.

The absolute concentration of the isoflavones and their metabolites varied between the females fed chow and both the male and female soy-diet groups. For example, levels of genistein and its metabolite dihydrogenistein were significantly ($P \leq 0.001$) higher in animals fed soy-based diet than in female monkeys fed chow. Importantly, however, serum concentrations of these compounds were also significantly ($P \leq 0.003$) higher in chow-consuming females compared with monkeys on the soy-free diet (Table 2). Glycitein concentrations were significantly higher ($P \leq 0.001$) in chow-fed compared with soy-fed animals. However, daidzein levels were significantly higher in soy-fed males compared with either soy- ($P \leq 0.001$) or chow-fed ($P \leq 0.001$) females; this difference was reflected in the similar serum pattern of the daidzein metabolite, dihydrodaidzein (Table 2). In contrast, the daidzein metabolite O-desmethylangolensin was significantly higher in chow-fed females compared with soy-consuming animals. Notably, levels of equol, the main daidzein metabolite with estrogenic activity, was also elevated significantly ($P = 0.019$) in chow-fed females, to levels similar to those of soy-consuming males and higher than that of soy-fed females (Table 2).

In terms of total unmetabolized soy isoflavones (daidzein, genistein, and glycitein combined), serum concentrations were significantly lower in animals that received the soy-free diet compared with soy- ($P \leq 0.001$) or chow-fed ($P \leq 0.001$) females (Table 2). Among these groups, unmetabolized isoflavones were significantly higher in soy males than in soy- ($P = 0.026$) and chow-fed ($P = 0.029$) females, which did not differ. Total isoflavone metabolite concentrations also were significantly decreased ($P \leq 0.001$) in animals fed the soy-free diet. However, total metabolite concentrations were significantly ($P = 0.035$) higher in chow-fed females compared with females that received the soy-based diet, with males statistically similar to soy-fed females ($P = 0.128$) and chow-fed females ($P = 0.201$) (Table 2). Equol was the predominant metabolite in animals fed either the soy-based diet or the commercial diet, ranging from 85% ± 3% in males given the soy-based diet, 89% ± 2% in females on the soy-based diet, and 90% ± 2% in chow-consuming females. In terms of total isoflavonoid levels, equol accounted for 51% ± 4% in males given the soy-based diet, 62% ± 2% in females on the soy-based diet, and 70% ± 3% in chow-consuming females.

Discussion

As expected, the animals that received a soy-free diet had

Table 2. Concentration (mean ± standard error of the mean) of isoflavonoids in the serum of female and male cynomolgus monkeys fed soy-free and soy-based diets and in the serum of female rhesus monkeys fed a standard commercial (Purina 5037) monkey diet

	Soy-free diet		Soy-based diet			
Isoflavone (ng/ml)	Female	Male	Female	Male	Standard commercial diet	$F_{(4, 155)}$
Diadzein	10 ± 3[c]	4 ± 1[c]	129 ± 16[b]	221 ± 23[a]	143 ± 22[b]	43.91
Genistein	5 ± 2[d]	4 ± 2[d]	123 ± 15[b]	256 ± 35[a]	56 ± 12[c]	36.53
Glycitein	0[d]	1 ± 1[d]	18 ± 2[c]	35 ± 4[b]	52 ± 16[a]	33.76
Equol	8 ± 3[c]	8 ± 5[c]	546 ± 45[b]	614 ± 57[a]	729 ± 77[a]	72.24
Dihydrodaidzein	8 ± 4[c]	3 ± 1[c]	36 ± 7[b]	52 ± 9[a]	25 ± 5[b]	10.54
Dihydrogenistein	2 ± 1[c]	1 ± 1[c]	23 ± 7[a]	28 ± 7[a]	4 ± 1[b]	6.51
O-desmethylangolensin	1 ± 1[c]	1 ± 1[c]	15 ± 6[b]	11 ± 5[b]	47 ± 15[a]	6.69
Total unmetabolized isoflavonoid	15 ± 4[c]	9 ± 3[c]	277 ± 30[b]	512 ± 51[a]	250 ± 46[b]	54.69
Total metabolized isoflavonoid	18 ± 7[c]	13 ± 6[c]	622 ± 56[b]	705 ± 64[a,b]	806 ± 80[a]	63.50

The results of 1-way analysis of variance (F statistic) are shown for each isoflavonoid. For each isoflavonoid, groups with different superscripts are significantly different from each other ($P < 0.05$; note that an F value with 4, 155 degrees of freedom that is at least 2.77 has a P of less than or equal to 0.05).

virtually no isoflavones in their plasma whereas the soy-fed monkeys, both males and gonadally intact females, had substantial amounts. Notably, males that ate a soy-based diet had higher plasma concentrations of almost every isoflavone and metabolite than did females, despite consuming an identical diet. Chow-fed females had plasma isoflavone concentrations that generally were comparable to those of soy-fed males and females and substantially higher than those of the monkeys on the soy-free diet. Finally, both the absolute and relative concentrations of equol were greater in chow-fed females than in soy-fed monkeys of either sex.

Most studies investigating the health effects of phytoestrogens in either human or nonhuman subjects use an isoflavone aglycon-equivalent dose between 25 and 90 mg/day. The estimated per capita intake for Japanese eating a traditional diet is probably no more than 50 mg isoflavones daily.[23] The animals that consumed the soy diet here were provided approximately 2.5 times the Japanese median daily isoflavone intake, considering differences in metabolic rates between humans and monkeys. This animal dose approximated the range sometimes used in human clinical studies. Several studies report human equol producers have plasma equol concentrations ranging from 0 to 130 nmol/l, depending on the type of diet consumed.[5] In other reports, the highest serum levels of isoflavones in humans fed soy were 4.7 ng/ml (genistein) and 3.9 mg/ml (daidzein), whereas concentrations of equol were too low to be detected.[36] These levels are similar to those found in male and female monkeys receiving a soy-free diet and much lower than those in the soy- or chow-fed monkeys. On the basis of the observations presented here, monkeys consuming a typical, commercial chow diet enriched with fruits and vegetables actually have an isoflavone exposure equivalent to that used in experiments designed to demonstrate the benefits as well as potential adverse effects of soy. The fact that soy has been shown experimentally to have effects on behavior, endogenous hormones, fertility, and carcinogenesis at this level of exposure indicates that experiments involving commercial chow-fed monkeys could inadvertently be confounded by high levels of isoflavones.[24]

The absolute concentration of the isoflavones and their metabolites varied between the chow animals and those fed the soy diets. This variation could reflect differences in the concentration of isoflavones in the diet. Furthermore, the current study admittedly is limited by the use of different species, reproductive conditions, and housing locations and the fact that samples for the rhesus monkeys were taken on only 1 occasion. It also is not known whether animals differed in the amount of food that was ingested. It would be particularly important to determine whether rhesus monkeys reliably produce relatively more equol than do cynomolgus monkeys when both are consuming similar amounts of the same isoflavone-containing diets and whether equivalent male–female differences in response to dietary isoflavones occur in both species.

Reports indicate that variability in equol excretion in human subjects is likely due to differences in the intestinal metabolism of the flavonoid daidzein by *Bacteroides ovatus* spp., *Ruminococcus productus* spp., and *Streptoccocus intermedius* spp.[35] Previous work in cynomolgus monkeys supports this suggestion, as pretreatment with antibiotics substantially reduces equol production.[5] Furthermore, we have shown elsewhere that male monkeys reliably exhibited higher plasma isoflavone concentrations than did females when both were chronically fed the same diets.[17] Current data have indicated that some of the possible factors that may influence the bioavailability of soy isoflavones are food matrix, gut microflora, and gender.[8] In the present study, serum concentrations of all isoflavones and metabolites except O-desmethylangolensin were higher in males than females consuming the same diet. Although we cannot rule out a gender-associated difference in the quantity of the soy diet consumed, a sex effect in genistein concentrations occurs in rats fed the same dose, with males having higher circulating levels compared to females possibly because of a faster rate of biotransformation and clearance in the liver of male rats and retention of parent compounds in the sex organs of female rats.[10]

Finally, and most importantly, the substantially higher isoflavone values for chow-fed monkeys compared with those consuming a diet deriving all protein from casein and lactalbumin argues that these data are not due to artifact or measurement error. We conclude that the serum levels of biologically active isoflavones, particularly equol, genistein and daidzein are high in animals fed a nonhuman primate commercially available diet. These data should compel experimenters to reconsider the use of these diets in studies investigating estrogenic effects on peripheral tissues[2] as well as neuroendocrine and behavioral endpoints.[22,30] Indeed, given the pleiotropic effects of estrogen, studies should use soy-free diets for animals so as not to confound or mask results. Soy-free diets are commercially available, and the need to use these diets becomes increasingly apparent when studies such as this are performed.

Acknowledgments

This analysis was supported in part by grants HL 079421 (JRK), HL 045666 (JRK), HD38917 (MEW), and RR00165 (YNPRC). The isoflavone analyses were supported by funds from grant CA71789 (AAF). Dr Appt was supported in part by grant T32 RR 07009.

References

1. **Adams MR, Golden DL, Williams JK, Franke AA, Register TC, Kaplan JR.** 2005. Soy protein containing isoflavones reduces the size of atherosclerotic plaques without affecting coronary artery reactivity in adult male monkeys. J Nutr **135:**2852–2856.
2. **Adlercreutz H, Heinonen SM, Penalvo-Garcia J.** 2004. Phytoestrogens, cancer, and coronary heart disease. Biofactors **22:**229–236.
3. **Adlercreutz H, Mazur W.** 1997. Phyto-oestrogens and Western diseases. Ann Med **29:**95–120.
4. **Beaglehole R.** 1990. International trends in coronary heart disease mortality, morbidity, and risk factors. Epidemiol Rev **12:**1–15.
5. **Blair RM, Appt SE, Franke AA, Clarkson TB.** 2003. Treatment with antibiotics reduces plasma equol concentration in cynomolgus monkeys (*Macaca fascicularis*). J Nutr **133:**2262–2267.
6. **Brown NM, Setchell KD.** 2001. Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones. Lab Invest **81:**735–747.
7. **Cassidy A, Bingham S, Setchell KD.** 1994. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr **60:**333–340.
8. **Cassidy A, Brown JE, Hawdon A, Faughnan MS, King LJ, Millward J, Zimmer-Nechemias L, Wolfe B, Setchell KD.** 2006. Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. J Nutr **136:**45–51.
9. **Cline JM, Franke AA, Register TC, Golden DL, Adams MR.** 2004. Effects of dietary isoflavone aglycones on the reproductive tract of male and female mice. Toxicol Pathol **32:**91–99.
10. **Coldham NG, Sauer MJ.** 2000. Pharmacokinetics of [(14)C]genistein in the rat: gender-related differences, potential mechanisms of biological action, and implications for human health. Toxicol Appl Pharmacol **164:**206–215.
11. **Figtree GA, Griffiths H, Lu YQ, Webb CM, MacLeod K, Collins P.** 2000. Plant-derived estrogens relax coronary arteries in vitro by a calcium antagonistic mechanism. J Am Coll Cardiol **35:**1977–1985.
12. **Fournier DB, Erdman JW Jr, Gordon GB.** 1998. Soy, its components, and cancer prevention: a review of the in vitro, animal, and human data. Cancer Epidemiol Biomarkers Prev **7:**1055–1065.
13. **Franke AA, Custer LJ, Hundahl SA.** 2004. Determinants for urinary and plasma isoflavones in humans after soy intake. Nutr Cancer **50:**141–154.
14. **Franke AA, Custer LJ, Wilkens LR, Le Marchand LL, Nomura AM, Goodman MT, Kolonel LN.** 2002. Liquid chromatographic-photodiode array mass spectrometric analysis of dietary phytoestrogens from human urine and blood. J Chromatogr B Analyt Technol Biomed Life Sci **777:**45–59.
15. **Fritz WA, Coward L, Wang J, Lamartiniere CA.** 1998. Dietary genistein: perinatal mammary cancer prevention, bioavailability, and toxicity testing in the rat. Carcinogenesis **19:**2151–2158.
16. **Jenkins DJ, Kendall CW, Connelly PW, Jackson CJ, Parker T, Faulkner D, Vidgen E.** 2002. Effects of high- and low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. Metabolism **51:**919–924.
17. **Kaplan JR, Adams M, Simon N, Wagner J, Franke AA.** 2005. Monkeys exhibit sex differences in behavioral and physiological responses to a high-isoflavone, soy-based diet, p 20 [abstract]. 6th International Symposium on the Role of Soy in Preventing and Treating Chronic Disease; 2005 Oct 30–Nov 2; Chicago. Champaign (IL): American Oil Chemists' Society.
18. **Kaplan JR, Manuck SB, Anthony MS, Clarkson TB.** 2002. Premenopausal social status and hormone exposure predict postmenopausal atherosclerosis in female monkeys. Obstet Gynecol **99:**381–388.
19. **Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA.** 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology **138:**863–870.
20. **Lephart ED, Setchell KD, Lund TD.** 2005. Phytoestrogens: hormonal action and brain plasticity. Brain Res Bull **65:**193–198.
21. **Liew R, Macleod KT, Collins P.** 2003. Novel stimulatory actions of the phytoestrogen genistein: effects on the gain of cardiac excitation-contraction coupling. FASEB J **17:**1307–1309.
22. **Lund TD, Munson DJ, Haldy ME, Setchell KD, Lephart ED, Handa RJ.** 2004. Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. Biol Reprod **70:**1188–1195.
23. **Messina M.** 1995. Regarding the safety of soy. N Z Med J **108:**301.
24. **Monfort SL, Thompson MA, Czekala NM, Kasman LH, Shackleton CH, Lasley BL.** 1984. Identification of a non-steroidal estrogen, equol, in the urine of pregnant macaques: correlation with steroidal estrogen excretion. J Steroid Biochem **20:**869–876.
25. **Morito K, Hirose T, Kinjo J, Hirakawa T, Okawa M, Nohara T, Ogawa S, Inoue S, Muramatsu M, Masamune Y.** 2001. Interaction of phytoestrogens with estrogen receptors alpha and beta. Biol Pharm Bull **24:**351–356.
26. **Mueller SO, Simon S, Chae K, Metzler M, Korach KS.** 2004. Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor alpha (ERalpha) and ERbeta in human cells. Toxicol Sci **80:**14–25.
27. **Murkies A.** 1998. Phytoestrogens—what is the current knowledge? Aust Fam Physician **27(1 Suppl):**S47–S51.
28. **National Research Council.** 1996. Guide for the care and use of laboratory animals, 6th ed. Washington (DC): National Academy Press.
29. **Patel RP, Boersma BJ, Crawford JH, Hogg N, Kirk M, Kalyanaraman B, Parks DA, Barnes S, Darley-Usmar V.** 2001. Antioxidant mechanisms of isoflavones in lipid systems: paradoxical effects of peroxyl radical scavenging. Free Radic Biol Med **31:**1570–1581.
30. **Patisaul HB.** 2005. Phytoestrogen action in the adult and developing brain. J Neuroendocrinol **17:**57–64.
31. **Ruiz-Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell G, Rice-Evans CA.** 1997. Antioxidant activity of phytoestrogenic isoflavones. Free Radical Res **26:**63–70.
32. **Setchell KD, Brown NM, Lydeking-Olsen E.** 2002. The clinical importance of the metabolite equol: a clue to the effectiveness of soy and its isoflavones. J Nutr **132:**3577–3584.
33. **Setchell KD, Clerici C, Lephart ED, Cole SJ, Heenan C, Castellani D, Wolfe BE, Nechemias-Zimmer L, Brown NM, Lund TD, Handa RJ, Heubi JE.** 2005. S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. Am J Clin Nutr **81:**1072–1079.
34. **Setchell KD, Lydeking-Olsen E.** 2003. Dietary phytoestrogens and their effect on bone: evidence from in vitro and in vivo, human observational, and dietary intervention studies. Am J Clin Nutr **78:**593S–609S.
35. **Ueno T, Uchiyama S, Kikuchi N.** 2002. The role of intestinal bacteria on biological effects of soy isoflavones in humans. J Nutr **132:**594.
36. **Valentin-Blasini L, Blount BC, Caudill SP, Needham LL.** 2003. Urinary and serum concentrations of seven phytoestrogens in a human reference population subset. J Expo Anal Environ Epidemiol **13:**276–282.
37. **Vitolins MZ, Quandt SA, Bell RA, Arcury TA, Case LD.** 2002. Quality of diets consumed by older rural adults. J Rural Health **18:**49–56.
38. **Wijetunge S, Lymn JS, Hughes AD.** 2000. Effects of protein tyrosine kinase inhibitors on voltage-operated calcium channel currents in vascular smooth muscle cells and pp60(c-src) kinase activity. Br J Pharmacol **129:**1347–1354.
39. **Wilson ME, Fisher J, Chikazawa K, Yoda R, Legendre A, Mook D, Gould KG.** 2003. Leptin administration increases nocturnal concentrations of luteinizing hormone and growth hormone in juvenile female rhesus monkeys. J Clin Endocrinol Metab **88:**4874–4883.
40. **Zhan S, Ho SC.** 2005. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr **81:**397–408.