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Increased aggressive behavior and decreased affiliative behavior in adult male monkeys after long-term consumption of diets rich in soy protein and isoflavones

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Abstract

Estrogen produced by aromatization of gonadal androgen has an important facilitative role in male-typical aggressive behavior that is mediated through its interaction with estrogen receptors (ER) in the brain. Isoflavones found in soybeans and soy-based dietary supplements bind ER and have dose- and tissue-dependent effects on estrogen-mediated responses. Yet, effects of isoflavone-rich diets on social and aggressive behavior have not been studied. We studied the effects of long-term (15 months) consumption of diets rich in soy isoflavones on spontaneous social behavior among adult male cynomolgus macaques (*Macaca fascicularis*) (n = 44) living in nine stable social groups. There were three experimental conditions which differed only by the source of dietary protein: casein and lactalbumin (no isoflavones), soy protein isolate containing 0.94 mg isoflavones/g protein, and soy protein isolate containing 1.88 mg isoflavones/g protein. In the monkeys fed the higher amount of isoflavones, frequencies of intense aggressive (67% higher) and submissive (203% higher) behavior were elevated relative to monkeys fed the control diet (P's < 0.05). In addition, the proportion of time spent by these monkeys in physical contact with other monkeys was reduced by 68%, time spent in proximity to other monkeys was reduced 50%, and time spent alone was increased 30% (P's < 0.02). There were no effects of treatment on serum testosterone or estradiol concentrations or the response of plasma testosterone to exogenous gonadotropin-releasing hormone (GnRH). The results indicate that long-term consumption of a diet rich in soy isoflavones can have marked influences on patterns of aggressive and social behavior.

Keywords: Estrogen; Androgen; Aggression; Social behavior; Monkeys; Soy protein; Soy isoflavones

Introduction

There is abundant evidence that estrogens produced by the aromatization of testosterone and acting through neural estrogen receptors (ER) have a critical role in facilitating aggressive behavior in male rodents (see a review by Simon, 2002). Studies with "knockout" mice have advanced our understanding of ER function in aggression, with recent results indicating that ER alpha (ERα) is obligatory for full behavioral expression while ER beta (ERβ) may function primarily as a negative modulator of ERα, at least during puberty and early adulthood (Nomura et al., 2002; Pfaff et al., 2002). Evidence also exists for an important role of estrogens in maintaining normal social and aggressive behavior in nonhuman primates (Michael and Zumpe, 1993; Michael et al., 1987, 1995). More recently, studies focusing on neural mechanisms linking estrogens and mood have identified a prominent role for ERβ in the modulation of serotonergic neuronal function in the rhesus midbrain (see a review by Bethea et al., 2002a). Given the recognized effects of serotonin in aggression across a range of species, these findings likely have important implications for the regulation of agonistic behavior in primates.

The isoflavones present in soybeans and many soy-rich nutritional supplements bind estrogen receptors and produce dose- and tissue-dependent estrogenic effects (Dixon and Ferreira, 2002; Jefferson et al., 2002; Mueller, 2002). Although the effects of isoflavones on social and aggressive behavior in males have not been studied, a commercially

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available isoflavone supplement containing genistein and daidzein reduced the ability of estradiol plus progesterone treatment to promote lordosis behavior in female rats (Patisaul et al., 2001a). It was suggested that this effect was due to interference with estrogenic regulation of gene expression in the periventricular and ventromedial nuclei. These findings, supported by additional results from gene expression assays as well as studies of central and peripheral estrogen-dependent responses, indicate that soy isoflavones can alter gene transcription and physiological responses. These effects of phytoestrogens appear to be produced primarily through interactions with ERβ, the ER subtype that preferentially binds this class of estrogens (An et al., 2001; Jefferson et al., 2002; Kuiper et al., 1997).

In the present study, the effects of extended consumption of isoflavone-rich diets on agonistic and social behaviors of male cynomolgus monkeys were determined. The results showed that the high-isoflavone diet resulted in increased levels of agonistic behavior and decreased expression of affiliative behaviors relative to males that consumed the control or low isoflavone diets.

Methods

Animals and diets

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Wake Forest University School of Medicine. Animal facilities are fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

The study population was 44 male cynomolgus monkeys imported as adults from Indonesia (Institute Pertanian Bogor). These animals were randomly selected for behavioral assessments from a larger population (n = 91) of monkeys assigned to a study of the effects of dietary soy protein isolate on experimentally induced atherosclerosis (primary outcome variable) and multiple secondary outcome variables.

Animals were fed for 4 months an isoflavone-free atherogenic control diet (Table 1) and were randomized to treatment groups using a stratified randomization scheme so that groups were well matched for total plasma cholesterol and high-density lipoprotein (HDL) cholesterol concentrations measured at month 4. The three treatment groups differed only by the source of dietary protein. The diets are summarized in Table 1. Group 1 was fed a casein/lactalbumin-based diet (no isoflavones). Group 2 was fed a soy protein-based diet containing 0.94 mg total isoflavones/g protein. Group 3 was fed a soy protein-based diet containing 1.88 mg total isoflavones/g protein. Each group received diet in amounts calculated to provide 150 cal/kg of body weight daily and received water ad libitum by automatic watering devices. The treatment period was 15 months.

Table 1
Composition of experimental diets

Ingredient[a]	Control (isoflavone-free)	Low isoflavone	High isoflavone
Casein	8.50		
Lactalbumin	8.50		
Soy protein blend[b]		8.55	17.45
Soy protein-IF[c]		8.55	
DL-methionine		0.30	0.30
Wheat flour, self-rising	35.76	35.76	35.76
Dextrin	9.00	8.50	8.47
Sucrose	7.00	7.00	7.00
Alphacel	8.00	8.03	8.01
Lard	5.00	5.00	5.00
Beef tallow	4.00	4.10	4.00
Butter, lightly salted	3.10	3.17	3.10
Safflower oil (linoleic)	3.00	3.00	2.99
Crystalline cholesterol	0.092	0.092	0.092
Completed vitamin mix[d]	2.50	2.50	2.50
Mod. #2 Ausman–Hayes[e]	5.00	5.00	5.00
Calcium carbonate	0.400	0.295	0.238
Calcium phosphate, monobasic	0.150	0.151	0.090

[a] g/kg dry weight.
[b] IB1.2 UN 30CA, Lot 038A-01: provided by Protein Technologies International, St. Louis, MO. Contains 1.08 mg genistein, 0.78 mg daidzein, 0.17 mg glycitein per gram soy protein isolate.
[c] FXP H0140, Lot MS320000317: provided by Protein Technologies International; contains 0.03 mg genistein, 0.01 mg daidzein, 0.01 mg glycitein per gram soy protein.
[d] Complete vitamin mixture (WFUSM formula) obtained from Harlan, Madison, WI.
[e] Mineral mix obtained from Harlan Teklad, Madison, WI. All calcium phosphate tribasic and potassium phosphate dibasic removed from mineral mixture and replaced with potassium carbonate and dextrin.

Animals were housed in social groups of four or five individuals in identical pens measuring 20 × 8 × 10 ft. All animals within each social group were fed the same diet, that is, were assigned to the same treatment group. The housing units have windows and, therefore, animals experienced natural light–dark cycles. Monkeys housed in this way typically form social status hierarchies in which some individuals (“dominants”) predictably defeat others (“subordinates”) in aggressive encounters.

Behavioral observations

Behavioral data were recorded with the Observer® system (Noldus Information Technology) and included the actions (instantaneous acts measured as a rate/hour/monkey) identified in Table 2 and these behavioral states (measured as a percentage of total time spent/monkey): body contact with another monkey, in proximity to another monkey, grooming another monkey, being groomed, playing and alone. Each observation for a social group consisted of a 30-min, continuous-event sample during which all occurrences of the specified behavioral actions and behavioral states of the monkeys were recorded. The behavioral actions are dyadic events and were recorded as such. Hence, an instance in which animal A grabbed animal B which then fled would be recorded as a “grab” initiated by A and a

Table 2
Behavioral acts

Intense aggression	Mild aggression	Intense submission	Mild submission
Bite	Open mouth threat	Crouch	Lip smack
Charge	Stare threat	Flee	Fear grimace
Grapple	Displace another monkey	Scream	Present perineum
Slap			Move away
Grab			
Push			

"flee" initiated by B. Furthermore, during these sampling periods, all animals were assigned to one of the three mutually exclusive behavioral states describing the spacing of individuals ("close", "alone", "body contact"). That is, all behavioral acts (e.g., fighting, playing) occur while animals were also either within touching distance ("close"), in body contact, or not within touching distance ("alone") of another individual. Observations were made on each social group three times per week for 8 weeks during the pretreatment period and again for 8 weeks at the end of 15 months of treatment.

Serum testosterone and estradiol

At the end of the 4-month pretreatment period and the 15-month treatment period, animals were sedated with ketamine and blood was collected for the determination of serum total testosterone, free testosterone, and total estradiol. Also, serum was collected 15 and 45 min following an intravenous injection of 40 μg gonadotropin-releasing hormone (GnRH) (National Pituitary Program) and analyzed for total testosterone. For estradiol assays, serum (0.5 ml) was first extracted by addition of ethyl ether (4 ml) followed by vortexing for 5 min. The aqueous layer was then frozen in a dry ice/isopropanol bath and the organic phase was decanted. Extracts were dried and reconstituted with the zero standard serum from the radioimmunoassay kit (DSL 4800, ultrasensitive estradiol, Diagnostics Systems Laboratories, Webster, TX, USA). Serum total and free testosterone were determined on unextracted samples using solid phase Coat-a-Count radioimmunoassays (Diagnostics Products Corporation, Los Angeles, CA, USA). Coefficients of variation for the individual assays were as follows: estradiol, 7.9%; total testosterone, 9.6% and free testosterone, 6.8%.

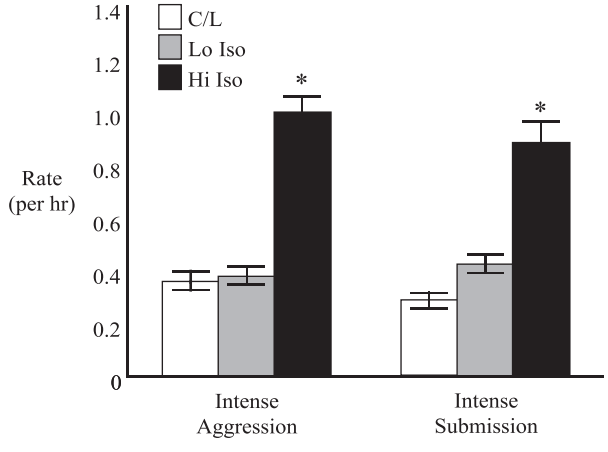

Fig. 1. Frequencies (mean ± SEM) of episodes of intense aggression and submission among male cynomolgus monkeys fed an isoflavone-free casein and lactalbumin-based diet (C/L) ($n = 14$), a diet based in soy protein isolate containing 0.94 mg/g of isoflavone (Lo Iso) ($n = 15$), and a diet based in soy protein isolate containing 1.88 mg/g isoflavone (Hi Iso) ($n = 15$). *$P < 0.05$ relative to C/L group.

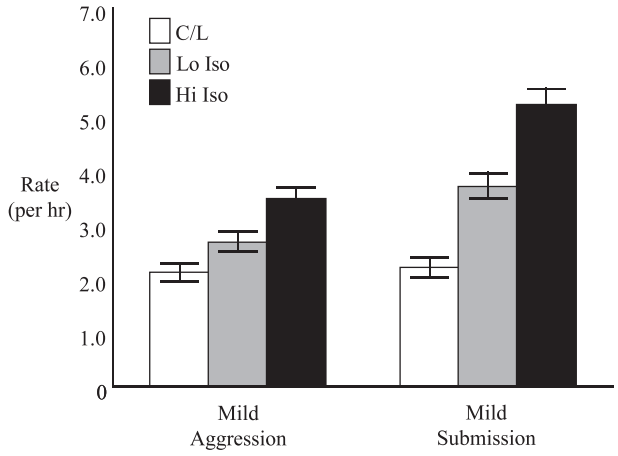

Fig. 2. Frequencies (mean ± SEM) of episodes of mild aggression and submission among male cynomolgus monkeys fed an isoflavone-free casein and lactalbumin-based diet (C/L) ($n = 14$), a diet based in soy protein isolate containing 0.94 mg/g of isoflavone (Lo Iso) ($n = 15$) and a diet based in soy protein isolate containing 1.88 mg/g isoflavone (Hi Iso) ($n = 15$). Although there was a tendency toward a main effect of treatment on mild submission ($P < 0.07$), differences among groups were not significant statistically.

Statistical analysis

To reduce skewness and equalize group variances, data underwent square root transformation before analysis. Data were analyzed using one-way analysis of variance and

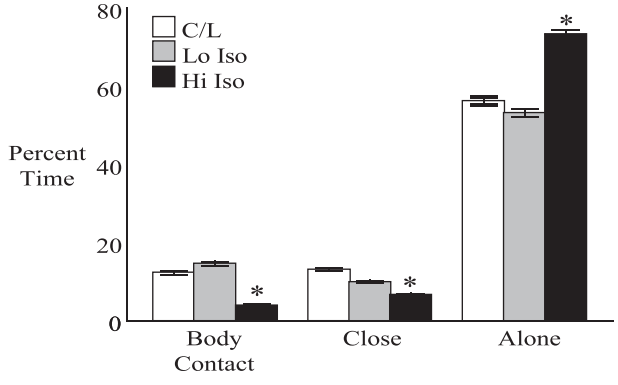

Fig. 3. Percentage of time spent (mean ± SEM) close to another monkey, in body contact with another monkey and alone among male cynomolgus monkeys fed an isoflavone-free casein and lactalbumin-based diet (C/L) ($n = 14$), a diet based in soy protein isolate containing 0.94 mg/g of isoflavone (Lo Iso) ($n = 15$), and a diet based in soy protein isolate containing 1.88 mg/g isoflavone (Hi Iso) ($n = 15$). *$P < 0.02$ relative to C/L group.

Table 3
Serum estradiol and testosterone concentrations (mean ± SEM)

	Control (n = 14)	Low isoflavone (n = 15)	High isoflavone (n = 15)
Baseline total testosterone (ng/dl)	324 ± 73	199 ± 32*	213 ± 47*
Treatment total testosterone (ng/dl)	387 ± 88	396 ± 104	292 ± 52
Baseline free testosterone (pg/ml)	3.99 ± 1.00	2.12 ± 0.35	2.55 ± 0.78
Treatment free testosterone (pg/ml)	3.47 ± 1.72	4.89 ± 1.59	3.95 ± 1.26
Baseline estradiol (pg/ml)	6.9 ± 0.7	7.4 ± 0.7	6.6 ± 0.7
Treatment estradiol (pg/ml)	9.1 ± 0.7	10.0 ± 1.3	8.4 ± 0.7
Testosterone response to GnRH-baseline period (ng/dl)	1173 ± 151	1393 ± 188	1385 ± 209
Testosterone response to GnRH-treatment period (ng/dl)	1397 ± 148	1476 ± 233	1416 ± 169

* $P < 0.05$ relative to control group.

covariance with the pretreatment outcome variable of interest as the covariate. Post hoc testing was done using two-tailed t tests, adjusted for multiple comparisons.

Results

There was a main effect of treatment on rates of intense aggression [$F(2,38) = 3.25$; $P < 0.05$] and submission [$F(2,38) = 3.30$; $P < 0.05$]. Intense aggressive acts were 67% more frequent and intense submissive acts were 203% more frequent among monkeys fed the high-isoflavone diet relative to those fed the control diet (Fig. 1).

Although differences in mild aggression [$F(2,38) = 0.72$; $P > 0.4$] and submission [$F(2,38) = 2.80$; $P < 0.07$] related to treatment were not statistically significant, there was a tendency towards a higher rate of mild forms of submission among monkeys fed the high-isoflavone diet relative to those fed the control diet (Fig. 2).

There were main effects of treatment on percentage of time spent alone [$F(2,38) = 7.72$; $P < 0.002$], in body contact with another monkey [$F(2,38) = 11.23$; $P < 0.0002$] and near to another monkey [$F(2,38) = 4.87$; $P < 0.02$]. Monkeys fed the high-isoflavone diet spent 68% less time in body contact with other monkeys, 50% less time in proximity to other monkeys, and 30% more time alone than monkeys fed the control diet (Fig. 3).

There were no effects of treatment on serum estradiol, total testosterone, free testosterone, and testosterone response to exogenous GnRH [all F's(2,38) < 1.0; P's > 0.4] (Table 3).

Discussion

Although considerable attention has been directed at the potentially beneficial effects of isoflavones in reducing the risk of various cancers, osteoporosis, cardiovascular disease, and postmenopausal symptoms (Brynin, 2002; Clarkson et al., 2001; Dai et al., 2002; Davis et al., 1999; Dixon and Ferreira, 2002; Goldwyn et al., 2000; Peeters et al., 2003), less effort has been invested in characterizing neurobehavorial effects and the mechanism of action of these phytoestrogens in the brain. To the best of our knowledge, the present study is the first to demonstrate that long-term consumption of isoflavones can alter patterns of agonistic and social behavior in primates. The results demonstrated that the higher dose of isoflavone (1.88 mg total isoflavones/g protein) produced significant effects on sex hormone-sensitive behaviors. Adult male cynomolgus monkeys that consumed this diet displayed elevated levels of agonistic behavior relative to controls, including severe forms of aggression and submission. These animals also spent significantly less time engaging in affiliative behavior and more time alone. The focus on changes in agonistic behavior is based on previous results with male cynomolgus monkeys that showed that decreases in affiliation and social contact were secondary to increased displays of aggression (Botchin et al., 1993; Kaplan et al., 1994).

An isoflavone-induced increase in agonistic behavior mediated through ERβ appears to be the most parsimonious explanation for the observed behavioral effects. Support for this position comes from several observations. These include the significantly higher affinity of soy isoflavones for ERβ relative to ERα and multiple observations demonstrating that phytoestrogen–ERβ complexes exhibit weaker transactivation functions compared to 17-β estradiol (E_2)–ERβ complexes (An et al., 2001; Jefferson et al., 2002; Kuiper et al., 1997; Yi et al., 2002). We believe the "weaker agonist" property of phytoestrogens acting through ERβ is central to the changes in agonistic behavior. Multiple processes may contribute to the increased agonism, primarily as a function of whether target cells express ERβ alone or if there is co-localization with ERα.

The argument for an isoflavone-induced enhancement of agonistic behavior mediated through ERβ is tied to several lines of evidence. First, there is the well-documented ability of estradiol to facilitate the display of aggression and other reproductive behaviors in males, including primates (see reviews by Michael and Zumpe, 1993; Simon, 2002). Second, studies of uterine proliferation in ERβ knockout mice and investigations of estrogenic regulation of cyclin D1 gene expression demonstrated that ERβ is an inhibitory modulator of ERα. Interestingly, recent investigations of aggression in ER knockout mice also were consistent with these findings. More specifically, Nomura et al. (2002) found that ERβ knockouts were more aggressive at puberty and early in adulthood then WT males. It was suggested that

ERβ is a negative modulator at these developmental stages while ERα is essential for full expression of agonistic behavior. The consistency between these findings and those noted in immature mouse uterus is notable and reinforces the concept of ERβ as a negative modulator of ERα.

In limbic system regions that are part of the neuroanatomical substrate for inter-male aggression, (reviewed in Simon, 2002), a substantial portion of target neurons for estrogen express both forms of ER based on in situ hybridization and, more recently, immunocytochemical findings (Gundlah et al., 2001; Mitra et al., 2002; Shughrue and Merchenthaler, 2001; Shughrue et al., 1998). In these cells, two ERβ-mediated mechanisms can be proposed as contributors to the increased agonism observed in males consuming the high-isoflavone diet. One involves the reduced transactivation function of phytoestrogen–ERβ vs. E_2–ERβ complexes (Jefferson et al., 2002). As noted earlier, studies of cell proliferation in immature mouse uterus and the regulation of cyclin D1 gene expression have shown that E_2–ERβ complexes negatively modulate ERα-induced effects (Liu et al., 2002; Weihua et al., 2000). In the present study, the high-isoflavone diet may have produced levels of genistein, daidzein, and equol (the primary metabolite of daidzein in rodents and primates) that resulted in competition for E_2 binding to ERβ, diminishing the inhibitory influence of ERβ. A second, more speculative mechanism involves changes in the function of ERα/ERβ heterodimers in the presence of sufficient concentrations of soy isoflavones. In vitro studies have shown the formation of α/β heterodimers that retain DNA binding ability (Pace et al., 1997; Petterson et al., 1997). While the function of these heterodimers has not been determined, it may be that soy isoflavone binding to ERβ reduced inhibitory effects on gene expression regulated by the β component of the heterodimer. In both cases, weakening the presumptive inhibitory effect of ERβ in regions such as the medial preoptic area, bed nucleus of the stria terminalis, and medial amygdala would result in enhanced ERα function and, as a consequence, increased agonistic behavior. Studies are needed to assess these potential mechanisms.

The third potential mechanism for contributions of phytoestrogen–ERβ complexes to the increased agonistic behavior involves effects in target cells that express only ERβ. The modulation of serotonergic tone in the rhesus monkey provides an example of this hypothesized process. In nonhuman primates, only ERβ are found in 5-HT neurons (Bethea et al., 2002b; Gundlah et al., 2001). Estradiol normally acts in these cells to enhance serotonergic tone by increasing tryptophan hydroxylase synthesis and decreasing 5-HT transporter expression (Lu and Bethea, 2002; Lu et al., 2003). The level of isoflavones from the high-dose diet could result in competition for ERβ binding. Because genistein, daidzein, and equol are weaker agonists and exhibit reduced transactivation effects compared to estradiol (An et al., 2001; Jefferson et al., 2002; Yi et al., 2002), a consequence of the high-isoflavone diet would be reduced serotonergic function, which is associated with increased agonistic behavior in mammals from rodents to humans (Simon, 2002).

An alternative explanation for the increased agonism seen in the males consuming the high-isoflavone diet is increased impulsivity. This concept represents an extension of the phytoestrogen–ERβ-mediated decrease in serotonergic tone described above. Findings with nonhuman primates and humans that have associated reduced serotonin metabolites in cerebrospinal fluid and/or a blunted response to fenfluramine challenge with elevated levels of impulsive or inappropriate aggression provide support for this position (e.g., Coccaro et al., 1998; Fairbanks et al., 2001; Higley et al., 1996; Manuck et al., 1998).

The suggested processes underlying the increased levels of agonistic behavior have focused on ERβ. These models were advanced while recognizing that soy isoflavones bind to ERα and that one group has suggested that genistein may act as an ERα antagonist in ovarectomized female rats (Patisaul et al., 2001b). The view that phytoestrogen–ERα complexes did not contribute to the observed increases in agonistic behavior is supported by multiple observations. Among these are data showing that isoflavones exhibit a 7- to 20-fold lower binding affinity for ERα relative to ERβ (Kuiper et al., 1997, 1998; Makela et al., 1999) and that at serum concentrations produced in the present study, soy isoflavones are ERβ selective agonists of transcriptional activation and repression (An et al., 2001). In addition, results with ERα knockout mice indicate that this receptor subtype appears to be essential for full expression of aggressive behaviors (Pfaff et al., 2002). Because isoflavones are weaker agonists than estradiol (Jefferson et al., 2002), physiologically significant interactions with ERα would be expected to result in decreased agonistic behavior. Clearly, this did not occur as substantially elevated levels of agonism were observed in males that consumed the high-isoflavone diet.

The finding of increased agonism coupled with decreased social behavior represents a potentially adverse effect of the consumption of an isoflavone-rich diet. Although the effects were dose-dependent, they were not accompanied by changes in serum gonadal hormone levels or the pituitary-gonadal response to GnRH. The interpretation of serum total testosterone data is complicated somewhat by the existence of a difference between groups during the baseline (pretreatment) period. However, there was no difference between groups in serum total testosterone during the treatment period, even when they were adjusted for these pretreatment differences using analysis of covariance. Furthermore, there were no differences in plasma estradiol or free testosterone either before or after treatment These finding indicate that soy isoflavones can exert neurobehavioral effects at concentrations below those needed to produce alterations in other physiological endpoints, reinforcing the concept of cell- and response-specific effects of phytoestrogens. While numerous studies have indicated the

existence of beneficial effects of dietary soy isoflavones with respect to decreased risk of prostate and breast cancer, cardiovascular disease, and other conditions (Brynin, 2002; Clarkson et al., 2001; Davis et al., 1999; Dixon and Ferreira, 2002; Goldwyn et al., 2000; Peeters et al., 2003), the present findings suggest that careful attention will be required to balance beneficial and potentially adverse effects. Clearly, there is need to extend investigations to other estrogen-dependent behaviors and to further characterize the different cellular mechanisms that mediate the effects of phytoestrogens in the brain.

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References

An, J., Tzagarakis-Foster, C., Scharschmidt, T.C., Lomri, N., Leitman, D.C., 2001. Estrogen receptor β-selective transcriptional activity and recruitment of coregulators by phytoestrogens. J. Biol. Chem. 276, 17808–17814.

Bethea, C.L., Lu, N.Z., Gundlah, C., Streicher, J.M., 2002a. Diverse actions of ovarian steroids in the serotonin neural system. Front. Neuroendocrinol. 23, 41–100.

Bethea, C.L., Mirkes, S.J., Su, A., Michelson, D., 2002b. Effects of oral estrogen, raloxifene and arzoxifene on gene expression in serotonin neurons of macaques. Psychoneuroendocrinology 27, 431–445.

Botchin, M.B., Kaplan, J.R., Manuck, S.B., Mann, J.J., 1993. Low versus high prolactin responders to fenfluramine challenge: marker of behavioral differences in adult male cynomolgus macaques. Neuropsychopharmacology 9, 93–99.

Brynin, R., 2002. Soy and its isoflavones: a review of their effects on bone density. Altern. Med. Rev. 7, 317–327.

Clarkson, T.B., Anthony, M.S., Morgan, T.M., 2001. Inhibition of postmenopausal atherosclerosis progression: a comparison of the effects of conjugated equine estrogens and soy phytoestrogens. J. Clin. Endocrinol. Metab. 86, 41–47.

Coccaro, E.F., Kavoussi, R.J., Hauger, R.L., Cooper, T.B., Ferris, C.F., 1998. Cerebrospinal fluid vasopressin levels: correlates with aggression and serotonin function in personality-disordered subjects. Arch. Gen. Psychiatry 55, 708–714.

Dai, Q., Franke, A.A., Jin, R., Shu, X.O., Hebert, J.R., Custer, L.J., Cheng, J., Gao, Y.T., Zheng, W., 2002. Urinary excretion of phytoestrogens and risk of breast cancer among Chinese women in Shanghai. Cancer Epidemiol., Biomarkers Prev. 11, 815–821.

Davis, S.R., Dalais, F.S., Simpson, E.R., Murkies, A.L., 1999. Phytoestrogens in health and disease. Recent Prog. Horm. Res. 54, 185–210.

Dixon, R.A., Ferreira, D., 2002. Genistein. Phytochemistry 60, 205–211.

Fairbanks, L.A., Melega, W.P., Jorgensen, M.J., Kaplan, J.R., McGuire, M.T., 2001. Social impulsivity inversely associated with CSF 5-HIAA and fluoxetine exposure in vervet monkeys. Neuropsychopharmacology 24, 370–378.

Goldwyn, S., Lazinsky, A., Wei, H., 2000. Promotion of health by soy isoflavones; efficacy, benefit and safety concerns. Drug Metab. Drug Interact. 17, 261–289.

Gundlah, C., Lu, N.Z., Mirkes, S.J., Bethea, C.L., 2001. Estrogen receptor beta (ERbeta) mRNA and protein in serotonin neurons of macaques. Brain Res., Mol. Brain Res. 91, 14–22.

Higley, J.D., Mehlman, P.T., Poland, R.E., Taub, D.M., Vickers, J., Suomi, S.J., Linnoila, M., 1996. CSF testosterone and 5-HIAA correlate with different types of aggressive behaviors. Biol. Psychiatry 40, 1067–1082.

Jefferson, W.N., Padilla-Banks, E., Clark, G., Newbold, R.R., 2002. Assessing estrogenic activity of phytochemicals using transcriptional activation and immature mouse uterotrophic responses. J. Chromatogr. 777, 179–189.

Kaplan, J.R., Shively, C.A., Fontenot, M.B., Morgan, T.M., Howell, S.M., Manuck, S.B., Muldoon, M.F., Mann, J.J., 1994. Demonstration of an association among dietary cholesterol, central serotonergic activity, and social behavior in monkeys. Psychosom. Med. 56, 479–484.

Kuiper, G.G.J.M., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., Gustafsson, J., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138, 863–870.

Kuiper, G.G.J.M., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., van der Burg, B., Gustaffson, J., 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139, 4252–4263.

Liu, M.M., Albanese, C., Anderson, C.M., Hilty, K., Webb, P., Uht, R.M., Price Jr., R.H., Pestell, R.G., Kushner, P.J., 2002. Opposing action of estrogen receptors α and β on cyclin D1 gene expression. Biol. Chem. 277, 2453–24360.

Lu, N.Z., Bethea, C.L., 2002. Ovarian steroid regulation of 5-HT1A receptor binding and G protein activation in female monkeys. Neuropsychopharmacology 27, 12–24.

Lu, N.Z., Eshleman, A.J., Janowsky, A., Bethea, C.L., 2003. Ovarian steroid regulation of serotonin reuptake transporter (SERT) binding, distribution, and function in female macaques. Mol. Psychiatry 8, 353–360.

Makela, S., Savolainen, H., Aavik, E., Myllarniemi, M., Strauss, L., Taskinen, E., Gustafsson, J.A., Hayry, P., 1999. Differentiation between vasculoprotective and uterotrophic effects of ligands with different binding affinities to estrogen receptors alpha and beta. Proc. Natl. Acad. Sci. U. S. A. 96 (12), 7077–7082.

Manuck, S.B., Flory, J.D., McCafferey, J.M., Matthews, K.A., Mann, J.J., Muldoon, M.F., 1998. Aggression, impulsivity and central nervous system serotonergic responsivity in a nonpatient sample. Neuropsychopharmacology 19, 287–299.

Michael, R.P., Zumpe, D., 1993. A review of factors influencing the sexual and aggressive behavior of macaques. Am. J. Primatol. 30, 213–241.

Michael, R.P., Bonsall, R.W., Zumpe, D., 1987. Testosterone and its metabolites in male cynomolgus monkeys (*Macaca fascicularis*): behavior and biochemistry. Physiol. Behav. 40, 527–537.

Michael, R.P., Clancy, A., Zumpe, D., 1995. Distribution of androgen receptor-like immuno-reactivity in the brains of cynomolgus monkeys. J. Neuroendocrinol. 7, 713–719.

Mitra, S.W., Hoskin, E., Yudkovitz, J., Pear, L., Wilkinson, H.A., Hayashi, S., Pfaff, D.W., Ogawa, S., Rohrer, S.P., Schaeffer, J.M., McEwen, B.S., Alves, S.E., 2002. Immunolocalization of estrogen receptor β in the mouse brain: comparison with estrogen receptor. Endocrinology 144, 2055–2067.

Mueller, S.O., 2002. Overview of in vitro tools to assess the estrogenic and antiestrogenic activity of phytoestrogens. J. Chromatogr., B: Biomed. Sci. Appl. 777, 155–165.

Nomura, M., Durbak, L., Chan, J., Smithies, O., Gustafsson, J., Korach, K.S., Pfaff, D.W., Ogawa, S., 2002. Genotype/age interactions on aggressive behavior in gonadally intact estrogen receptor β knockout (βERKO) male mice. Horm. Behav. 41, 288–296.

Pace, P., Taylor, J., Suntharalingam, S., Coomes, R.C., Ali, S., 1997. Human estrogen receptor β binds DNA in a manner similar to and dimerizes with estrogen receptor α. J. Biol. Chem. 272, 25832–25838.

Patisaul, H.B., Melby, M., Whitten, P.L., Young, L.J., 2001a. Genistein affects ERβ- but not ERα-dependent gene expression in the hypothalamus. Endocrinology 143, 2189–2197.

Patisaul, H.B., Dindo, M., Whitten, P.L., Young, L.J., 2001b. Soy isoflavone supplements antagonize reproductive behavior and estrogen receptor α- and β-dependent gene expression in the brain. Endocrinology 142, 2946–2952.

Peeters, P.H., Keinan-Boker, L., van der Schouw, Y.T., Grobbee, D.E., 2003. Phytoestrogens and breast cancer risk. Review of the epidemiological evidence. Breast Cancer Res. Treat. 77, 171–183.

Petterson, K., Grandien, K., Kuiper, G.G.J.M., Gustafsson, J., 1997. Mouse estrogen receptor β forms estrogen response element-binding heterodimers with estrogen receptor α. Mol. Endocrinol. 11, 1486–1496.

Pfaff, D., Ogawa, S., Kia, K., Vasudevan, N., Krebs, C., Frohlich, J., Kow, L., 2002. Genetic mechanisms in neural and hormonal controls over female reproductive behavior. In: Pfaff, D., Arnold, A., Etgen, A., Fahrbach, B., Rubin, R. (Eds.), Hormones, the Brain, and Behavior. Academic Press, San Diego, pp. 441–510.

Shughrue, P.J., Merchenthaler, I., 2001. Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. J. Comp. Neurol. 436, 64–81.

Shughrue, P.J., Scrimo, P.J., Merchenthaler, I., 1998. Evidence for the colocalization of estrogen receptor-β mRNA and estrogen receptor-α immunoreactivity in neurons of the rat forebrain. Endocrinology 139, 5267–5270.

Simon, N.G., 2002. Hormonal processes in the development and expression of aggressive behavior. In: Pfaff, D., Arnold, A., Etgen, A., Fahrbach, S., Rubin, R. (Eds.), Hormones, Brain and Behavior. Academic Press, San Diego, pp. 39–85.

Weihua, Z., Saji, S., Makinen, S., Cheng, G., Jensen, E.V., Warner, M., Gustafsson, J., 2000. Estrogen receptor (ER) β, a modulator of ERα in the uterus. Proc. Natl. Acad. Sci. 97, 5936–5941.

Yi, P., Driscoll, M.D., Huang, J., Bhagat, S., Hilf, R., Bambara, R.A., Muyan, M., 2002. The effects of estrogen-responsive element- and ligand-induced structural changes on the recruitment of cofactors and transcriptional responses by ERα and ERβ. Mol. Endocrinol. 16, 674–693.