ELSEVIER

Developmental Brain Research 126 (2001) 217–221

DEVELOPMENTAL BRAIN RESEARCH

www.elsevier.com/locate/bres

Short communication

Maternal and perinatal brain aromatase: effects of dietary soy phytoestrogens

K. Scott Weber[a], Kenneth D.R. Setchell[b], Edwin D. Lephart[a,*]

[a] *633 WIDB, Neuroscience Center, Brigham Young University, Provo, UT 84602, USA*
[b] *Clinical Mass Spectrometry, Children's Hospital Medical Center, Cincinnati, OH 45229, USA*

Accepted 8 November 2000

Abstract

Phytoestrogens are extensively investigated for their potential to prevent many hormone-dependent cancers and age-related diseases, however little is known about their effects in brain. Brain aromatase and plasma phytoestrogen levels were determined in Sprague–Dawley rats fed a phytoestrogen-rich diet during pregnancy/lactation. Ingested phytoestrogens cross the placenta and become concentrated in maternal milk as evident from high infantile plasma concentrations. Dietary phytoestrogens, however, do not alter brain aromatase during pregnancy/lactation or perinatal development.

Theme: Development and regeneration

Topic: Nutritional and prenatal factors

Keywords: Aromatase; Brain; Phytoestrogen; Maternal; Fetal; Infantile; Rat

Phytoestrogens are biologically active plant-derived non-steroidal estrogen-like molecules found in many foods but particularly in legumes, whole-grains, flax and most soy products [1,21]. These estrogen-like molecules selectively bind to estrogen receptors and particularly with ERβ [12]. Phytoestrogens have been the focus of much attention because of their potential to protect against many age-related diseases including, cardiovascular disease and osteoporosis and hormone-dependent cancers (e.g., breast and prostate cancer) [1,11,17,21]. However, there is little known about the effects of phytoestrogens on the central nervous system (CNS) even though isoflavones have been shown to be transported into brain tissue [15].

We recently reported that soy diets containing moderate or high levels of phytoestrogens significantly reduced hypothalamic calcium-binding proteins (calbindin and calretinin), during prenatal development and in adulthood [15,24], suggesting a potentially higher vulnerability to neurotoxicity or programmed cell death [15,24].

Furthermore, the major androgen-metabolizing enzyme, aromatase cytochrome P450 (P450 arom) plays a critical role in influencing the genesis of sexually dimorphic brain structures during perinatal development by converting testicular derived testosterone to estrogen in situ within certain brain structures [14]. The local formation of estrogens (from androgen precursors) via brain aromatase also modulates neuroendocrine functions and regulates sexual behavior in hypothalamic regions of the brain [14]. These biological parameters represent important elements in the structure/function relationship of the genesis of the rat CNS perinatally and the morphometric and functional characteristics of hypothalamic brain areas postnatally [14]. It is known that phytoestrogens inhibit aromatase enzyme activity in peripheral endocrine tissue sites during postnatal development (in vitro) [1,11,17]. However, the effect of phytoestrogens on brain aromatase has never previously been examined during perinatal development [14].

To determine if phytoestrogens influence maternal, fetal or infantile brain aromatase, we examined aromatase enzyme activity in the medial basal hypothalamic and preoptic area (mbh–poa) from Sprague–Dawley rats that were fed a high phytoestrogen-containing diet and compared this with a control group fed a diet low in phytoestrogens.

*Corresponding author. Tel. +1-801-378-8970; fax: +1-801-378-1349.
E-mail address: edwin_lephart@byu.edu (E.D. Lephart).

0165-3806/01/$ – see front matter
PII: S0165-3806(00)00138-3

The low phytoestrogen diet (Phyto-low) was obtained from Ziegler Bros. (Gardner, PA, USA) that was balanced and matched for equivalent percentage of protein, carbohydrate and fat to that of the commercially available phytoestrogen-containing diet (Phyto-high) (Harlan-Teklad, Madison, WI, USA). The concentration and type(s) of phytoestrogens in the two diets were analyzed in duplicate by reverse-phase high pressure liquid chromatography (HPLC), as detailed elsewhere [4,19]. The Phyto-high diet had a total of 603 microg/g of phytoestrogens consisting mainly of daidzin, genistin and glycitin while the phytoestrogen content in the Phyto-low diet was below the limits of detection, as reported previously from our laboratory [15].

Sprague–Dawley (30-day-old) female rats were obtained from Simonsen Lab. (Gilroy, CA, USA) and housed in a controlled environment. Some animals were placed on the Phyto-high diet ($n=10$) whereas, the remaining animals were placed on the Phyto-low diet ($n=13$). At 80 days of age, the female rats were mated with breeder male rats. The animals remained on their assigned diets throughout gestation and lactation. At gestation days 16.5 and 20.5 and on postnatal day 3.5 maternal, fetal and neonatal brain tissue samples were collected. Also, fetal and neonatal body weight [in grams (g)$\pm$0.001 g] and anogenital distance (AGD) [in millimeters (mm)$\pm$0.1 mm; using microcalipers] were measured at the time of tissue collection to determine if the phytoestrogen diets had any influence on these parameters [24]. Each animal was marked, tracked and confirmation of the sex of each animal was performed by dissecting the abdominopelvic region under a microscope and observing the presence or absence of the testicular organs in the pelvic cavity, as previously performed in our laboratory [24].

Trunk blood was sampled from the fetal, infantile or maternal animals at the time brain tissue was collected representing 3 to 5 litters per collection (i.e., during late gestation or early postnatal development). Plasma was prepared then stored at -20°C until assayed. The concentration and type(s) of phytoestrogens were analyzed from pooled (by treatment) plasma samples by gas chromatography/mass spectrometry using standard methods with internal controls to validate the assay, as outlined previously [22]. The assay precision for the individual isoflavones was 6–10%, coefficient of variation. To provide a comparison, plasma phytoestrogen levels were determined in adult male Sprague–Dawley rats fed the same Phyto-high and Phyto-low diets. The obtained values were expressed in nanograms per ml.

For all of the animals assayed, the medial basal hypothalamic and preoptic (mbh–poa) brain tissue site was dissected using landmark boundaries, as detailed in previous reports from our laboratory [14,15,25]. The isolated mbh–poa tissue samples were incubated in 200 μl of DMEM with a saturating concentration of [1β-^{3}H]testosterone (at 2.5 μM; DuPont/New England Nuclear, Boston, MA, USA), as substrate for 1 h. Using standard assay procedures [15,25], the rates of aromatase activity in each tissue sample were determined by the 'tritiated water' release assay where aromatization of the substrate was isolated from the aqueous phase of the reaction mixture. The protein content of each tissue fragment was determined by the method of Lowry et al. [16]. Aromatase activity rates were expressed in fmol/h (of incubation)/mg protein.

The data derived from this study was tested by a standard statistical model=sex by treatment (and sex/treatment interaction) via analysis of variance (ANOVA), followed by pair wise comparisons (via Neumam–Kuels) to detect significant differences between males vs. females or the treatment groups ($\alpha=P<0.05$). Plasma phytoestrogens levels were analyzed by ANOVA, followed by pair wise comparisons for fetal, infantile, maternal and adult male values ($\alpha=P<0.05$).

During late pregnancy or early lactation, phytoestrogen levels in maternal plasma were significantly higher in the Phyto-high vs. Phyto-low groups at gestation day (GD) 16.5 and 20.5 or postnatal day (PND) 3.5 (Table 1). To provide a comparison, adult male rats fed the Phyto-high diet displayed higher phytoestrogen levels (but were within a similar range) to that of the maternal Phyto-high values (Table 1). In fetal (GD 20.5) or infantile (PND 3.5) plasma samples, phytoestrogen levels reflected the maternal profiles where significantly higher values were recorded in the Phyto-high vs. the Phyto-low groups (Table 1).

The impact of the phytoestrogen diets on fetal or infantile body weights and anogenital distances (AGD) was determined (Table 2). Due to the small size and fragile condition of the fetus at GD 16.5, male and female body weights were combined and AGDs were not measured. In this regard, there were no significant differences between the treatment groups for body weight at GD 16.5. At GD 20.5, male and female body weights were significantly lower in the offspring of dams fed the Phyto-high vs. the Phyto-low group (Table 2). Also, male prenatal AGD values at 20.5 were significantly less in the Phyto-high vs. Phyto-low treated animals. However, when the AGD measurements were standardized (by dividing this value by the animal's body weight), for both male and female parameters AGD/body weight ratios were significantly greater in the Phyto-high vs. the Phyto-low values (Table 2). A similar finding was observed at PND 3.5, where AGD values in females receiving the Phyto-high diet via their mother's milk were significantly higher than those exposed to a Phyto-low diet (Table 2). Although, when AGD values were standardized by body weight there were no significant differences between the treatment groups at PND 3.5.

When maternal brain (mbh–poa) aromatase patterns were examined there were no significant changes in the profile during the perinatal interval and no significant

Table 1
Phytoestrogen concentrations in plasma samples from fetal, infantile, maternal and adult male rats on phytoestrogen diets[a]

	Phyto-high (ng/ml)				Phyto-low (ng/ml)			
	Genistein	Daidzein	Equol	Total	Genistein	Daidzein	Equol	Total
Fetal								
G.D 16.5	NA	NA	NA	NA	NA	NA	NA	NA
G.D. 20.5	106.39	67.53*	51.58*	225.50*	80.11	17.89	1.23	99.23
Infantile								
PND 3.5	234.85*	341.13*	161.85*	737.83*	61.02	17.78	5.46	84.26
Maternal								
G.D 16.5	366.37*	279.94*	643.56*	1289.87*	50.47	27.49	11.14	89.11
G.D. 20.5	232.31*	193.80*	578.12*	1004.24*	50.83	24.18	21.78	96.78
PND 3.5	441.22*	339.46*	906.22*	1686.90*	66.01	51.62	17.00	134.64
Adult male								
100–105 Days old	420.95*	390.27*	932.37*	1744.00*	74.90	35.51	14.48	125.00

[a] Pooled plasma samples (fetal, infantile and maternal values represent 3–5 litters per diet treatment group. While the adult male values represent $n=8$ per diet treatment group). Phytoestrogen concentrations were measured by gas chromatography/mass spectrometry [22]. Phytoestrogen-rich (Phyto-high) and Phytoestrogen-low (Phyto-low) diets are balanced and matched for equivalent percentage content of protein, carbohydrate, and fat. The Phyto-high diet has 600 μg of isoflavones per gram of diet while the Phyto-low diet isoflavone levels are below HPLC detection.
[b] *Phyto-high values are significantly higher than Phyto-low values.

differences between the Phyto-high vs. the Phyto-low groups (Fig. 1A) were detected during late pregnancy (GD 16.5 and 20.5) or early lactation (PND 3.5). Furthermore, when late fetal or infantile mbh–poa aromatase levels were assayed, regardless of treatments the enzyme activity was at moderate levels at GD 16.5, increased at GD 20.5, then decreased back to moderate levels at PND 3.5 (Fig. 1B). For fetal or infantile mbh–poa aromatase there were no significant differences between the treatment groups nor were sex differences in mbh–poa aromatase activity observed (within or across the treatment groups, Fig. 1B).

Phytoestrogens appear to protect against hormone-dependent and age-related diseases [1,2,11,17,20,21], however, few studies have examined the effects of phytoestrogens, in a food matrix, in brain. This study specifically characterized the effect of phytoestrogens on the brain androgen-metabolizing enzyme, aromatase because this enzyme plays a pivitol role in the neurogenesis of sexually dimorphic brain structures and regulatory aspects in neuroendocrine function [14,18].

Validation of the content and bioavailability of the phytoestrogens concentrated in the food matrix was demonstrated by significantly higher plasma phytoestrogen levels in animals fed the Phyto-high vs. the Phyto-low

Table 2
Body weight and anogenital distance (AGD) of perinatal rats fed phytoestrogen-rich (phyto-high) or phytoestrogen-low (phyto-low) diets[a]

	GD 16.5		GD 20.5				PND 3.5			
	Phyto-high M and F	Phyto-low M and F	Phyto-high		Phyto-low		Phyto-high		Phyto-low	
			M	F	M	F	M	F	M	F
n	40	33	34	30	30	28	16	23	17	17
Body Wt. (g)	0.591 (0.012)	0.581 (0.014)	2.67 (0.035)	2.55 (0.047)	3.16[c] (0.061)	2.99[c] (0.057)	7.49 (0.320)	7.29 (0.186)	8.10 (0.471)	7.70 (0.423)
AGD (mm)	NA[b]	NA	3.7 (0.07)	2.5 (0.07)	4.0[d] (0.08)	2.6 (0.07)	5.2 (0.10)	3.2[f] (0.05)	5.0 (0.14)	3.0 (0.10)
AGD/Body Wt.	NA	NA	1.39[e] (0.024)	0.98[e] (0.024)	1.29 (0.027)	0.87 (0.031)	0.71 (0.030)	0.45 (0.013)	0.65 (0.052)	0.40 (0.023)

[a] Data derived from the perinatal animals are expressed as the mean (±s.e.m.) for body weight (body wt.), AGD, and AGD/Body Wt. GD, Gestation day; PND, Postnatal day; M, males; F, females.
[b] NA, not assayed (due to fragile fetal tissue).
[c] Significantly greater Phyto-low male or female body weights compared to Phyto-high male or female values.
[d] Significantly greater Phyto-low male AGDs compared to Phyto-high male AGD values.
[e] Significantly greater Phyto-high male or female AGD/Body weight ratios compared to Phyto-low male or female values.
[f] Significantly greater Phyto-high female AGDs compared to Phyto-low female AGD values.

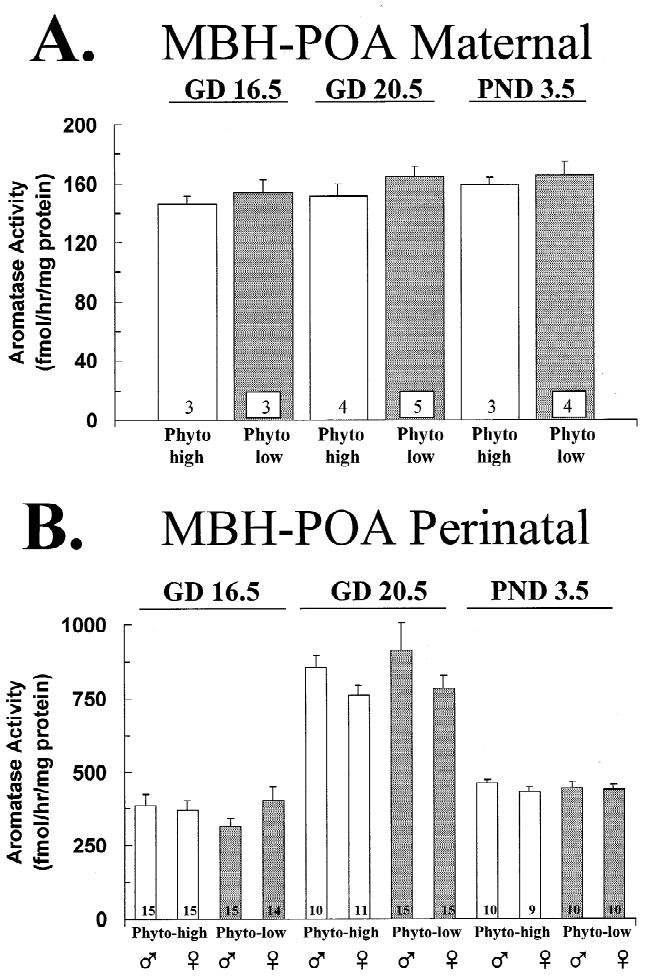

Fig. 1. (A) Maternal medial basal hypothalamic–preoptic area (mbh–poa) aromatase levels at gestation day (GD) 16.6, 20.5 and postnatal day (PND) 3.5 in animals fed the phytoestrogen-rich (Phyto-high) and phytoestrogen-low (Phyto-low) diets. The number at the base of each bar represents the number of animals assayed. There were no significant differences in maternal brain aromatase between the treatment groups tested during late pregnancy or early lactation. (B) Fetal and infantile medial basal hypothalamic–preoptic area (mbh–poa) aromatase levels at gestation day (GD) 16.6, 20.5 and postnatal day (PND) 3.5 in animals fed the phytoestrogen-rich (Phyto-high) and phytoestrogen-low (Phyto-low) diets. The number at the base of each bar represents the number of animals assayed. There were no significant differences in fetal or infantile brain aromatase between the treatment groups or across sex during perinatal development.

diets. The high plasma phytoestrogen levels represent a model of the 'Asian' diet, whereas, low plasma phytoestrogen levels in animals fed the phytoestrogen-low diet are similar to what is observed in humans consuming a 'Western' diet [1]. Furthermore, the passage of phytoestrogens into brain has been reported previously [15]. However, as seen in the present study, there appears to be a lower production of circulating phytoestrogens during late pregnancy in maternal rats compared to the levels displayed by adult male animals fed the same Phyto-high diet. This may reflect a difference in phytoestrogen metabolism during late pregnancy or the increased circulatory volume during late pregnancy might have a diluting effect on the ingested phytoestrogens. Finally, the significantly higher fetal plasma isoflavone levels (at GD 20.5; @ 2.2-fold higher) clearly confirm the transplacental passage of phytoestrogens to the fetal compartment. Also, the infantile (at PND 3.5 @ 8.8-fold higher) plasma phytoestrogen levels (compared to Phyto-low values) suggest the concentrating of phytoestrogens in maternal milk that is ingested during early postnatal development.

The significant reduction in fetal body weights observed in the Phyto-high group represents an estrogenic influence on this parameter. Previous studies have shown a consistent decrease in body weight due to estrogen hormones [3]. The mechanism by which estrogenic molecules reduce body weight is unknown. However, estrogens are known to increase leptin production in rats [23]. On the other hand, based upon preliminary findings from our laboratory, the significant decrease in the fetal Phyto-high body weights cannot be account for by a reduction in food or water intake by the maternal animals fed the Phyto-high diet, since we observed no significant difference between the treatment groups for consumption during pregnancy or lactation in this study.

The anogenital distance (AGD) parameter represents an androgen hormone sensitive measurement [24]. In this regard, the significantly smaller AGDs of prenatal Phyto-high animals suggests that body weight may have a greater impact on this dependent variable and/or a lack of blocking androgen hormonal action on external genital development. However, when AGD was standardized by body weight, Phyto-high AGD/body weight ratios were significantly higher than the prenatal Phyto-low values. While this presents a complex picture of modulating steroid hormonal action for this parameter, these data are in agreement with that presented by other investigators [9] and the influence of phytoestrogens may be similar to the selective estrogen receptor modulator (SERM), raloxifene that has both agonist and antagonist effects [8].

The lack of effect of dietary phytoestrogens on maternal and perinatal (fetal and infantile) brain aromatase has not been reported previously. Interest to examine brain aromatase stems from the known inhibition of aromatase in peripheral endocrine tissue sites by phytoestrogens [1,10]. Furthermore, investigators have observed changes in sexually dimorphic brain structures (e.g., the sexually dimorphic nucleus of the preoptic area of the hypothalamus) when pharmacologic concentrations of genisten were injected in rats, however circulating phytoestrogen levels were not measured in those studies [5,6]. It has also been demonstrated that phytoestrogens pass the blood–brain barrier in postnatal animals [15] and that dietary derived phytoestrogens alter brain calcium-binding protein levels during prenatal and postnatal development [15,24].

In the present study, maternal, fetal or infantile mbh–poa aromatase levels were within a similar range and profile to that noted by our laboratory and other inves-

tigators [7,13,14]. While there were significantly higher plasma phytoestrogen levels in the Phyto-high vs. the Phyto-low fed maternal and perinatal male and female animals (during late pregnancy and early lactation) maternal, fetal or infantile mbh–poa aromatase levels were not altered by the diet treatments. These results suggest that changes in the sexually dimorphic brain structures observed by previous investigators may not be due to alterations in aromatase mbh–poa levels but may be the result of: (1) modifications in brain calcium-binding proteins regulating programmed cell death during genesis of the CNS [15,24]; or (2) changes in the plasma concentrations of androgens that serve as substrate(s) for the P450 aromatase enzyme in brain.

In summary, studies implicating phytoestrogens providing health benefits are increasing each year. However, the influence of phytoestrogens on brain development and function are unknown. This study addressed this insufficiency, in part, by examining brain aromatase in maternal and perinatal rats. The findings of this study suggest that: (1) phytoestrogens cross the placenta and are concentrated in maternal milk as evident in the infantile plasma levels and, (2) in the mbh–poa brain region (unlike peripheral endocrine tissues) dietary phytoestrogens do not alter aromatase enzyme levels during perinatal development in male and female or in maternal rats during late pregnancy or early lactation.

Acknowledgements

This research work was supported, in part, by grants from the National Science Foundation, IBN-9507972 (to EDL), and the BYU Bio-Ag College, 11-223566-9600-13811 (to EDL).

References

[1] H. Adlercreutz, W. Mazur, Phyto-oestrogens and Western diseases, Ann. Med. 29 (1997) 95–120.
[2] H. Adlercreutz, W. Mazur, P. Bartels, V. Elomaa, S. Watanabe, K. Wahala, M. Landstrom, E. Lundin, A. Bergh, J.E. Damber, P. Aman, A. Widmark, A. Johansson, J.X. Zhang, G. Hallmans, Phytoestrogens and prostate disease, J. Nutr. 130 (2000) 658S–659S.
[3] M.E. Brewster, W.R. Anderson, R. Pop, Effect of sustained estradiol release in the intact male rat: correlation of estradiol serum levels with actions on body weight, serum testosterone, and peripheral androgen-dependent tissues, Physiol. Behav. 61 (1997) 225–229.
[4] L. Coward, N.C. Barnes, K.D.R. Setchell, S. Barnes, Genistein, daidzein, and their β-glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets, J. Agr. Food Chem. 41 (1993) 1961–1967.
[5] K.A. Faber, C.L. Hughes Jr, The effect of neonatal exposure to diethylstilbestrol, genistein, and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat, Biol. Reprod. 45 (1991) 649–653.
[6] K.A. Faber, C.L. Hughes Jr, Dose–response characteristics of neonatal exposure to genistein on pituitary responsiveness to gonadotropin releasing hormone and volume of the sexually dimorphic nucleus of the preoptic area (SDN–POA) in postpubertal castrated female rats, Reprod. Toxicol. 7 (1993) 35–39.
[7] F.W. George, S.R. Ojeda, Changes in aromatase activity in the rat brain during embryonic, neonatal, and infantile development, Endocrinol. 111 (1982) 522–529.
[8] T. Hol, M.B. Cox, H.U. Bryant, M.W. Draper, Selective estrogen receptor modulators and postmenopausal women's health, J. Womens Health 6 (1997) 523–531.
[9] C.L. Hughes Jr., W. Foster, L. Platt, S. Chan, S. Thompson, S. Hubbard, A. DuBose, L. Tyrey, Midgestation intrauterine exposure of the human fetus to dietary isoflavones in North America: How does this exposure compared to animal studies in late gestation and lactation that alter developmental endpoints, Internat. Soy Symp. 3 (1999) 28–29.
[10] A.R. Ibrahim, Y.J. Abul-Hajj, Aromatase inhibition by flavonoids, J. Steroid Biochem. Mol. Biol. 37 (1990) 257–260.
[11] D.C. Knight, J.A. Eden, A review of the clinical effects of phytoestrogens, Obstet. Gynec. 87 (1996) 897–904.
[12] G.G. Kuiper, B. Carlsson, K. Grandien, E. Enmark, J. Haggblad, S. Nilsson, J.A. Gustafsson, Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta, Endocrinology 138 (1997) 863–870.
[13] D.R. Ladle, N.A. Jacobson, E.D. Lephart, Hypothalamic aromatase cytochrome P450 and 5α-reductase enzyme activities in pregnant and female rats, Life Sci. 61 (1997) 2017–2026.
[14] E.D. Lephart, A review of brain aromatase cytochrome P450, Brain Res. Rev. 22 (1996) 1–26.
[15] E.D. Lephart, J.M. Thompson, K.D.R. Setchell, H. Adlercreutz, K.S. Weber, Phytoestrogens decrease brain calcium-binding proteins but do not alter hypothalamic androgen metabolizing enzymes in adult male rats, Brain Res. 859 (2000) 123–131.
[16] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein determination with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[17] A.L. Murkies, G. Wilcox, S.R. Davis, Clinical review 92: Phytoestrogens, J. Clin. Endo. Metab. 83 (1998) 297–303.
[18] F. Naftolin, Brain aromatization of androgens, J. Reprod. Med. 39 (1994) 257–261.
[19] K.D.R. Setchell, M.B. Welsh, C.K. Lim, High-performance liquid chromatographic analysis of phytoestrogens in soy protein preparations with ultraviolet, electrochemical and thermospray mass spectrometric detection, J. Chromatogr. 386 (1987) 315–323.
[20] K.D.R. Setchell, Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones, Am. J. Clin. Nutr. 68 (1998) 1333S–1346S.
[21] K.D.R. Setchell, A. Cassidy, Dietary isoflavones: biological effects and relevance to human health, J. Nutr. 129 (1999) 758S–767S.
[22] K.D.R. Setchell, L. Zimmer-Nechemias, J. Cai, J.E. Heubi, Isoflavone content of infant formulas and the metabolic fate of these phytoestrogens in early life, Lancet 350 (1997) 23–27.
[23] H. Shimizu, Y. Shimomura, Y. Nakanishi, T. Futawatari, K. Ohtani, N. Sato, M. Mori, Estrogen increases in vivo leptin production in rats and human subjects, J. Endocrinol. 154 (1997) 285–292.
[24] H. Taylor, E.M. Quintero, A.M. Iacopino, E.D. Lephart, Phytoestrogens alter hypothalamic calbindin-D28k levels during prenatal development, Dev. Brain Res. 114 (1999) 277–281.
[25] K.S. Weber, N.A. Jacobson, K.D.R. Setchell, E.D. Lephart, Brain aromatase and 5alpha-reductase, regulatory behaviors and testosterone levels in adult rats on phytoestrogen diets, Proc. Soc. Exp. Biol. Med. 221 (1999) 131–135.