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Brain Research Bulletin 65 (2005) 219–223

BRAIN RESEARCH BULLETIN

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Effects of dietary phytoestrogens on core body temperature during the estrous cycle and pregnancy

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Abstract

Phytoestrogens have received increased investigative attention due to their potential protective effects in connection to age-related diseases and hormone-dependent cancers. Phytoestrogens appear to be an effective treatment during perimenopause where symptoms, such as hot flashes are reduced. However, little is known about the influence of phytoestrogens on core body temperature during various hormonal conditions. This study examined the effects of dietary phytoestrogens on core body temperature during estrous cycles or pregnancy by feeding Long–Evans rats either a diet rich in phytoestrogens (Phyto-600) versus a diet relatively low in phytoestrogens (Phyto-free). Independent of treatments, body temperature was highest at proestrus and declined during estrus and diestrus. Moreover, the consumption of the Phyto-600 diet moderately decreased body temperature during proestrus, estrus and diestrus versus Phyto-free-fed animals. During pregnancy, independent of treatments, core body temperature decreased as a function of increasing gestational length. Phyto-600-fed rats displayed significantly decreased body temperatures (by approximately 0.5 °C) from gestation days 6 to 19, compared to Phyto-free values. The results from this study indicate that consumption of dietary phytoestrogens alters the neuroendocrine mechanism of core body temperature regulation that may help explain, in part, the beneficial effects of phytoestrogens for hot flashes.

Keywords: Soy; Isoflavones; Estrogens; Neuroendocrine metabolism; Body temperature regulation; Rat

1. Introduction

Sex hormones affect a variety of physiological and behavior functions and progesterone has long been known to elevate body temperature [6] by influencing the firing patterns of hypothalamic preoptic thermosensitive neurons [21,26]. Conversely, estrogen is known to decrease body temperature [21,26] by apparently re-setting thermoregulatory mechanisms, such as increasing the sweating threshold in postmenopausal women with hot flashes [5]. In rodents, body temperature increases during proestrus, when estrogen and progesterone concentrations are highest, while during estrus there is a drop in body temperature [7,16,17].

During pregnancy, body temperature decreases shortly before parturition in rats [4] that is characteristic of a number of other species, including rabbits and sheep that display a similar pattern [11,20]. It has been reported in rats near the end of pregnancy that this decrease in body temperature results from a re-setting of the hypothalamic thermoregulatory neurons [3].

In reference to steroid hormone action, phytoestrogens (plant estrogen-like molecules) are non-steroid derived structures that are comparable to 17β-estradiol, including an aromatic A-ring with hydroxyl groups positioned in the same plane at a distance similar to 17β-estradiol. Phytoestrogens have the ability to bind mammalian estrogen receptors (ER) with a greater affinity for ERβ versus ERα [1,8,12]. However, in general, phytoestrogens are less potent than endogenous steroidal estrogens, such as 17β-estradiol and their hormonal action varies between species and routes of administration [15,29]. Phytoestrogens differ not only in their binding affinities for the ER, but also in their potential to increase the rate of receptor binding to the ERE. EC(50)α, the con-

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doi:10.1016/j.brainresbull.2005.01.008

centrations to induce an increase in the binding response of ERα to ERE by 50% as compared to unliganded ER, are: 17β-estradiol (0.03 μM) > equol (3.5 μM) > genistein (15 μM) > daidzein (>300 μM). EC(50)β are: 17β-estradiol (0.01 μM) > genistein (0.03 μM) > daidzein (0.35 μM) > equol (0.4 μM). The ratios of EC(50)α/EC(50)β are for 17β-estradiol, 3; coumestrol, 8; equol, 8.8; genistein, 500; daidzein > 850 [9].

Of all the studies examining the effects of phytoestrogens, most investigative attention has examined age-related diseases (i.e. cardiovascular and osteoporosis) and hormone-dependent cancers, with a few reports covering the influence of phytoestrogens on perimenopausal symptoms like hot flashes [1,8,13,18,19,24,25,29]. Therefore, since phytoestrogens can alter neuroendocrine parameters [13], this study examined the effects of dietary phytoestrogens on core body temperature during the estrous cycle and pregnancy by feeding Long–Evans rats, either a diet rich in phytoestrogens (Phyto-600) or a diet relatively low in phytoestrogens (Phyto-free).

2. Materials and methods

2.1. Animals

Eight female and six male Long–Evans rats at 50 days of age were purchased from Charles River Laboratories (Wilmington, MA, USA). These animals were caged individually and housed in the Brigham Young University Bio-Ag vivarium and maintained on a light/dark schedule (lights on 06:00–19:00 h). Animal usage/protocol for this study was approved by the Institute of Animal Care and Use Committee at Brigham Young University.

2.2. Treatment diets

We have determined that the total phytoestrogen level in the diet from the supplier ranges from approximately 200 to 300 parts per million (ppm). Upon arrival, the animals were allowed ad libitum access to either a commercially available diet with high phytoestrogen levels (Harlan Teklad Rodent Diet 8604, Madison, WI, USA, containing 600 μg of phytoestrogens/gram of diet (or approximately 600 ppm)), referred to hereafter as the Phyto-600 diet or a custom (plant-based) phytoestrogen-'reduced' diet (or approximately 10–15 ppm of total phytoestrogens), referred to hereafter as the Phyto-free diet, obtained from Ziegler Bros. (Gardner, PA, USA) and water [2,13,14,28]. The major biologically active molecules possessing estrogen-like actions in these diets are isoflavones (Table 1). The content/nutrient composition of these diets has been described in detail previously [28]. The data presented in the table do not include minor isoflavone components and by previous quantification the Phyto-600 diet contains approximately 600 μg/g diet or 600 ppm. The diets were balanced and matched for equivalent percentage content of protein, carbohydrate, fat, amino acids, vitamins and minerals, etc.

The core body temperature (estrous cycle) was measured at 90 days of age, after the rats had been on these diets for 6 weeks. After 2 more weeks on the diet treatments, the males and females (by diet treatment) were mated, and core body temperature during pregnancy was quantified. Circulating phytoestrogen (isoflavone) serum levels from rats maintained on these diets (lifelong) have been reported previously by our laboratory using GC/MS analysis [13,14,28].

2.3. Core body temperature

Core body temperature was monitored by radio telemetry by implanting a small electronic chip under the skin external to the left thoracic cavity (near) just above the heart that measured and transmitted core body temperature (±0.1 °C) to a notebook-sensor monitor (BioMedic Data Systems Inc., Seaford, DE, USA) within a second and repeated measurements were made throughout the day and/or the duration of the experiments.

2.4. Experiment I: Core body temperature during the estrous cycle

Vaginal smears were taken for two full estrous cycles, and core body temperatures were measured (at 9:00 a.m., 12:00 p.m. and 3:00 p.m. light phase and 6:00 p.m., and 9:00 p.m. dark phase of the light/dark cycle daily). Temperatures at proestrous, estrous and diestrous phases of the cycle were recorded and compared (there were no significant differences between dietrus and metestrus values, so these levels were combined into one group designated as diestrus). Finally, there were no significant alterations in cycle length or pattern of the animals on the diet treatments.

2.5. Experiment II: Core body temperature during pregnancy

After experiment I, all the rats were mated with male rats within the same diet treatment groups. Temperatures were taken at 3:00 p.m. light phase or at 9:00 p.m. dark phase of the light/dark cycle, daily from gestation day (GD) 6 through GD 19. (Due to concerns about impairing the onset of parturition, measurements ended at GD 19 and all rats delivered normally on GD 21 or GD 22.)

Table 1
Concentration of individual isoflavones (μg/g) in the treatment diets

Diets	Daidzin	Daidzein	Genistin	Genistein	Glycitin	Total
Phyto-600	198.6	9.9	281.5	9.1	46.8	545.9
Phyto-free	ND*	ND	ND	ND	ND	ND

The data presented in this table do not include minor isoflavone components and by previously quantification the phyto-600 diet contains approximately 600 μg/g or 600 ppm.

* None detected (below the limits of HPLC detection, less than 0.5 μg/g).

2.6. Statistical analysis

All the data were expressed as mean ± S.E.M. and analyzed by the statistical analysis system (SAS). The data were tested by repeated measures based mixed-model analysis and considered significantly different at $p < 0.05$.

3. Results

3.1. Experiment I: Core body temperatures during the estrous cycle

During a 24 h period, core body temperatures for both diet treatment groups displayed relatively high values at 9:00 p.m., decreased to their lowest levels at 3:00 p.m. during the light phase of the light/dark cycle, then returned to their highest levels at 9:00 p.m. during the dark phase of the light/dark cycle (Fig. 1A (proestrus), B (estrus) and C (diestrus)). Across the diet treatment groups at 9:00 p.m., core body temperatures were highest during proestrus (≈38.6 °C), decreased slightly during estrus (≈38.3 °C) and remained at their lowest levels during diestrus (≈38.1 °C). There were no significant differences in core body temperatures between the Phyto-free versus Phyto-600 groups at any time points during any phases of the estrous cycle (of a 24 h interval). However, the greatest divergence between the groups occurred at 3:00 p.m., when temperatures were reduced by approximately 0.5 °C in Phyto-600 versus Phyto-free-fed animals ($n = 8$ per group) during proestrus, estrus and diestrus (see Fig. 1A–C). When all temperatures across the testing interval were analyzed during estrus, as a group Phyto-600-fed animals showed significantly lower temperatures compared to Phyto-free values ($p < 0.05$).

3.2. Experiment II: Core body temperature during pregnancy

Overall, rats on both Phyto-600 and Phyto-free diets displayed decreasing core body temperatures as a function of increasing gestation length during both light and dark periods from GD 6 to GD 19 (Fig. 2A and B). For example, from GD 6 during the light period (3:00 p.m.), independent of treatment, body temperatures decreased an average of 1 °C (from 37.6 to 36.6 °C) to GD 19, while during the dark period (9:00 p.m.), values decreased from 38.0 °C at GD 6 to 37.4 °C at GD 19. Within either diet group, core body temperatures were higher during the dark period (at 9:00 p.m.) of the light/dark cycle, when rats are most active, compared to the light period (at 3:00 p.m.). In general, maternal temperature was significantly lower (by approximately 0.5 °C) at both 3:00 and 9:00 p.m. in Phyto-600-fed rats compared to Phyto-free-fed rats throughout pregnancy (from GD 6 to GD 19, $n = 8$ per group).

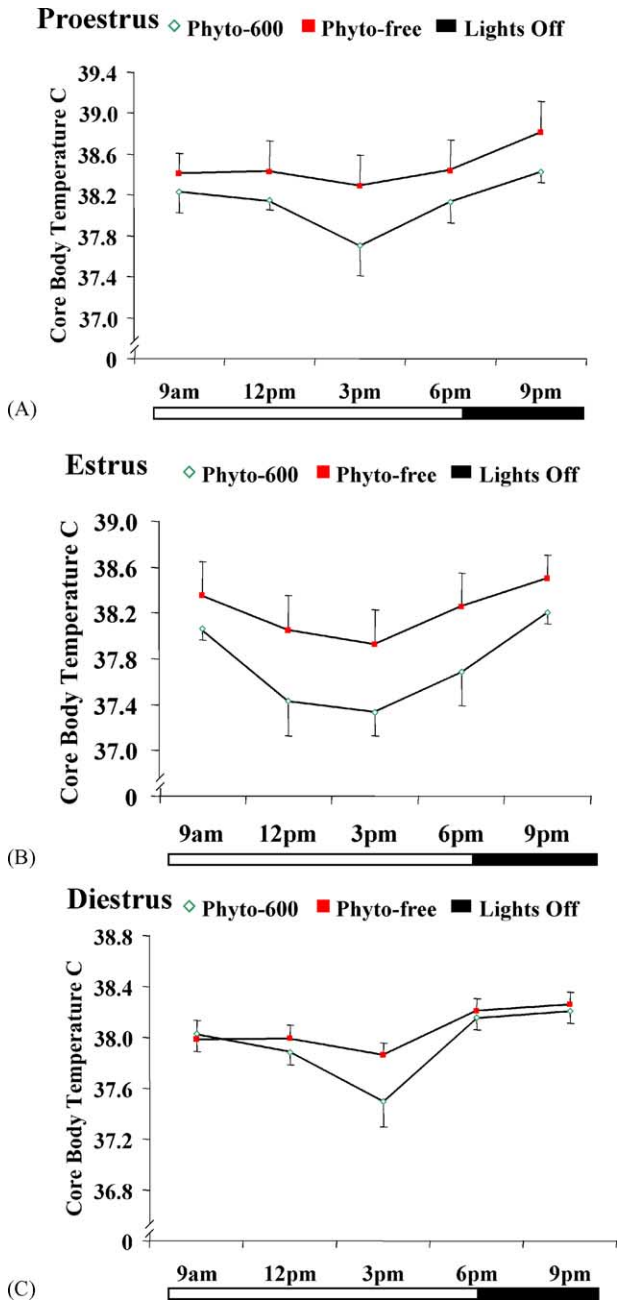

Fig. 1. Core body temperatures of adult rats fed phytoestrogen-rich (Phyto-600) vs. a Phyto-free diet during proestrus (A), estrus (B) and diestrus (C). In general, Phyto-600-fed animals displayed a slight (and non-significant) decrease in core body temperatures during the estrous cycle vs. Phyto-free-fed animals (this was especially evident during the estrous phase of the cycle; $n = 8$ animals per group). When all temperatures across the testing period were analyzed during estrus, as a group Phyto-600-fed animals showed significantly lower temperatures compared to Phyto-free values ($p < 0.05$).

4. Discussion

This study examined the influence of dietary phytoestrogens during the estrous cycle and pregnancy in Long–Evans rats. The impetus for this investigation was due in part to the potential health benefits of dietary phytoestrogens

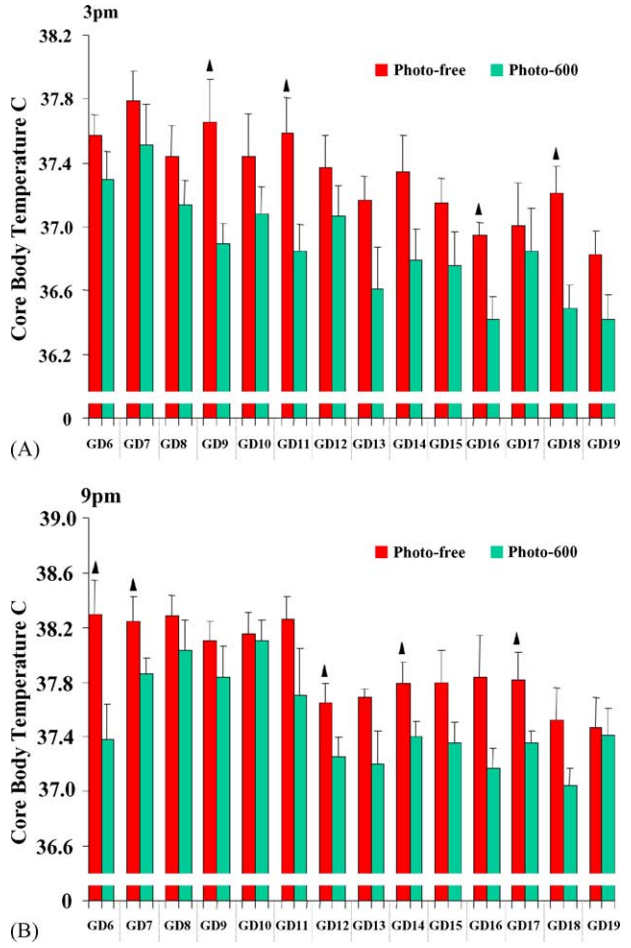

Fig. 2. Daily core body temperatures of adult rats fed a phytoestrogen-rich (Phyto-600) vs. a Phyto-free diet during gestation at 3 p.m., near the end of the light phase of the light/dark cycle (A), and at 9:00 p.m., near the beginning of the dark phase (B). In general, maternal body temperature was significantly lower (by approximately 0.5 °C) at both 3:00 and 9:00 p.m. in Phyto-600-fed rats compared to Phyto-free-fed rats throughout pregnancy (GD 6–GD 19; $n = 8$ per group); (▲) significantly increased core body temperatures in Phyto-free-fed animals vs. Phyto-600-fed animals (for a given day of gestation), $p < 0.05$.

(specifically isoflavones), especially in regard to diminishing hot flashes associated with perimenopause in humans [1,8,13,19,22,29] and to examine body temperatures in an animal model during endogenous fluctuating hormonal states.

A few studies have shown that consuming soy reduces hot flashes in postmenopausal women [23,27]. The decrease in body temperatures (during the estrous cycle and pregnancy) with soy consumption in the present study may have a similar mechanism to that of estrogens, altering the temperature threshold [5]. Presumably, the isoflavones act via ERβ to decrease body temperatures [5,10].

In general, in agreement with previous studies, our results in cycling or pregnant rats showed that core body temperatures during the dark phase of the light/dark cycle is higher when rodents are most active compared to the light phase [7,16,17]. Independent of the diet treatments, the present results during the estrous cycle are very similar to those of Marrone et al. [16], where body temperatures were highest during proestrus and declined until diestrus. Additionally, thermoregulatory patterns of core body temperature during pregnancy declined as gestation length increased, especially near term [4]. The role of steroids, specifically estrogen-decreasing and progesterone-increasing core body temperature via hypothalamic preoptic thermosensitive neurons is implicated in these findings [21,26]. However, when estrogen-like molecules (phytoestrogens) consumed via the diet were examined, the alterations on core body temperature during the estrous cycle and pregnancy were similar to the hormonal actions of endogenous estrogens. Notably, in Phyto-600-fed animals body temperatures during the estrous cycle were reduced slightly compared to Phyto-free-fed rats, and throughout gestation, there was a significant decline in body temperatures during both the dark and light phases of the light/dark cycle in Phyto-600 versus Phyto-free animals. This suggests that the high abundance of circulating isoflavones in Phyto-600-fed rats possessing estrogenic biochemical properties [1,8,9,13,19,22,28,29] act presumably via the ER system [9,15] within the hypothalamus to significantly reduce body temperature comparable to that of endogenous estrogens.

In this regard, our findings parallel one animal study that examined the effects of soy phytoestrogens decreasing tail-skin temperatures of ovariectomized rats [22]. We have further investigated this aspect of core body and skin temperatures' regulation by dietary phytoestrogens in male rats (unpublished studies). From these studies, in general, tail-skin temperature decreases in a dose-dependent manner as a function of increasing isoflavone concentrations when four different phytoestrogen diets were used. Additionally, core body temperatures were significantly decreased during a 24 h interval similar to that seen in female rats reported in the present study.

Since isoflavones possess a high affinity for ERβ versus ERα [9,13,19,24], it is interesting to speculate that the distribution and abundance of these ER subtypes within the hypothalamus may play a role in thermoregulation. Furthermore, other factors, such as progesterone receptor expression [26] may act in connection with endogenous estrogens and/or dietary phytoestrogens in altering homeostatic mechanisms for temperature control during natural conditions of fluctuating hormonal status.

Therefore, in summary, the results derived from this study indicate that consumption of dietary phytoestrogens alters the neuroendocrine mechanism of core body temperature regulation that may help explain, in part, the beneficial effects of phytoestrogens as an effective treatment for hot flashes via their estrogen-like hormone action.

Acknowledgements

This work was supported, in part, by grants from the USDA (2002-00798; EDL), the BYU Research Office (21-

223566; EDL) and The BYU Dean's Graduate Fellowship in Neuroscience (LB).

References

[1] H. Adlercreutz, W. Mazur, Phyto-oestrogens and western diseases, Ann. Med. 29 (1997) 95–120.
[2] 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. Agric. Food Chem. 41 (1993) 1961–1967.
[3] H.L. Eliason, J.E. Fewell, Thermoregulatory control during pregnancy and lactation in rats, J. Appl. Physiol. 83 (1997) 837–844.
[4] J.E. Fewell, Body temperature regulation in rats near term of pregnancy, Can. J. Physiol. Pharmacol. 73 (1995) 364–368.
[5] R.R. Freedman, C.M. Blacker, Estrogen raises the sweating threshold in postmenopausal women with hot flashes, Fertil. Steril. 77 (2000) 487–490.
[6] S. Isreal, O. Schneller, The thermogenic property of progesterone, Fertil. Steril. 1 (1950) 53–64.
[7] S. Kent, M. Hurd, E. Satinoff, Body temperature and wheel-running in rats over the estrous cycle, Physiol. Behav. 3 (1968) 29–33.
[8] D.C. Knight, J.A. Eden, A review of the clinical effects of phytoestrogens, Obstet. Gynecol. 87 (1996) 897–904.
[9] D. Kostelac, G. Rechkemmer, K. Briviba, Phytoestrogens modulate binding response of estrogen receptors alpha and beta to the estrogen response element, J. Agric. Food Chem. 51 (2003) 7632–7635.
[10] G.G. Kuiper, B. Carlson, K. Grandien, E. Enmark, J. Haggblad, S. Nilsson, J.A. Gustafsson, Comparison of the ligand binding specificity and transcript tissue distritution of estrogen receptors alpha and beta, Endocrinology 138 (1997) 863–870.
[11] H.P. Laburn, D. Mitchell, K. Goelst, Fetal and maternal body temperatures measured by radiotelemetry in near-term sheep during thermal stress, J. Appl. Physiol. 72 (1992) 894–900.
[12] G. Leclerq, J.C. Heuson, Physiological and pharmacological effects of estrogens in breast cancer, Biochim. et Biophys. Acta 560 (1979) 427–455.
[13] E.D. Lephart, T.D. West, K.S. Weber, R.W. Rhees, K.D.R. Setchell, H. Adlercreutz, T.D. Lund, Neurobehavioral effects of dietary soy phytoestrogens, Neurotox Treat. 24 (2002) 1–12.
[14] E.D. Lephart, H. Adlercreutz, T.D. Lund, Dietary soy phytoestrogens effects on brain structure and aromatase in Long–Evans rats, Neuroreport 12 (2001) 3451–3455.
[15] L. Markiewicz, J. Garey, H. Adlercreutz, E. Gurpide, In vitro bioassays of nonsteroidal phytoestrogens, J. Steroid Biochem. Mol. Biol. 45 (1993) 399–405.
[16] B.L. Marrone, R.T. Gentry, G.N. Wade, Gonadal hormones and body temperature in rats: effects of estrous cycles, castration and steroid replacement, Physiol. Behav. 17 (1976) 419–425.
[17] J. McLean, W. Coleman, Temperature variation during the estrous cycle: active vs. restricted rats, Psychol. Sci. 22 (1971) 179–180.
[18] R.J. Miksicek, Commonly occurring plant flavonoids have estrogenic activity, Mol. Pharmacol. 44 (1993) 37–43.
[19] A.L. Murkies, G. Wilcox, S.R. Davis, Clinical review 92: phytoestrogens, J. Clin. Endocrinol. Metab. 83 (1998) 297–303.
[20] E.F. Naccarato, W.S. Hunter, Brain and deep abdominal temperatures during induced fever in pregnant rabbits, Am. J. Physiol. 245 (1983) R421–R425.
[21] T. Nakayama, M. Suzuki, N. Ishizuka, Action of progesterone on preoptic thermosensitive neurones, Nature 258 (1975) 80.
[22] Y. Pan, M.S. Anthony, M. Binns, T.B. Clarkson, A comparison of oral micronized estradiol with soy phytoestrogen effects on tail-skin temperatures of ovariectomized rats, Menopause 8 (2001) 171–174.
[23] R. Russo, R.R. Corosu, The clinical use of preparation based on phyto-oestrogens in the treatment of menopausal disorders, Acta Biomed. Ateneo Parmense 74 (2003) 137–143.
[24] K.D.R. Setchell, Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones, Am. J. Clin. Nutr. 68 (1998) 1333S–1346S.
[25] K.D.R. Setchell, A. Cassidy, Dietary isoflavones: biological effects and relevance to human health, J. Nutr. 129 (1999) 758S–767S.
[26] C.L. Tsai, K. Kanousue, K. Matsumura, Effects of estradiol treatment on responses of rat preoptic warm sensitive neurons to progesterone in vitro, Neurosci. Lett. 136 (1992) 23–26.
[27] A. Vincent, L.A. Fitzpatrick, Soy isoflavones: are they useful in menopause? Mayo Clin. Proc. 75 (2000) 1174–1184.
[28] K.S. Weber, K.D.R. Setchell, D.M. Stocco, E.D. Lephart, Dietary soy-phytoestrogens decrease testosterone levels and prostate weight, without altering LH, prostate 5α-reductase or testicular StAR levels in adult male Sprague–Dawley rats, J. Endocrinol. 170 (2001) 591–599.
[29] P.L. Whitten, H.B. Patisaul, Cross-species and interassay comparisons of phytoestrogen action, Environ. Health Perspect. 109 (Suppl. 1) (2001) 5–20.