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Estrogens and phytoestrogens: brain plasticity of sexually dimorphic brain volumes☆

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Abstract

Sexually dimorphic brain volumes (sexually dimorphic nucleus of the preoptic area (SDN-POA) and anteroventral periventricular (AVPV) nucleus) are influenced by estrogens. Phytoestrogens, derived from plants (especially soy products), are molecules structurally and functionally similar to estradiol. The purpose of this study was to examine: the consumption of phytoestrogen (using a phytoestrogen-rich (Phyto-600) versus a phytoestrogen-free (Phyto-free)) diets from conception to adulthood (or changing the diets during adulthood) and characterizing (a) circulating plasma phytoestrogen levels, (b) testosterone levels in males, (c) sexually dimorphic brain volumes (i.e. the SDN-POA and AVPV) and (d) the presence of apoptotic cells in these brain structures in Long-Evans rats. Phyto-600 fed animals displayed total serum phytoestrogens levels 37-fold higher compared to Phyto-free values. Circulating testosterone levels were not significantly altered by the diets. Female SDN-POA volumes were not altered by the diets. Whereas, males fed a Phyto-free diet displayed decreased SDN-POA volumes compared to male Phyto-600 values. Females fed the Phyto-600 diet displayed larger AVPV volumes compared to males on the same diet or females on the Phyto-free diet. Males fed the Phyto-free diet had the largest AVPV values compared to Phyto-600 fed males. When the SDN-POA region was examined in lifelong Phyto-free fed males, apoptotic cells were present versus males fed the Phyto-600 diet and in the AVPV region the opposite results were obtained. In summary, consumption of dietary phytoestrogens (estrogen mimics) can alter hormone-sensitive hypothalamic brain volumes in rodents during adulthood.
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Keywords: Phytoestrogens; SDN-POA; AVPV; Rat; Soy; Hormones

1. Introduction

1.1. Estrogens-brain structure and function

Estrogens are known to (a) regulate certain dimorphic brain structures (proliferation and migration of neurons) during various developmental intervals [1–3], (b) protect against neurodegenerative diseases, such as Alzheimer's disease and stroke [4–8] , and (c) influence learning, memory, the stress response, pain perception, mood, reproductive endocrine function and sexual behavior [4–11].

1.1.1. Phytoestrogens

Phytoestrogens, derived from plants (especially soy products), are molecules structurally and functionally similar to estradiol [12–16]. Soy is the major protein source in natural-ingredient commercially available animal diets (phytoestrogens range from 200 to 800 μg/g) [14,17,18]. Phytoestrogens have gained recognition as protective agents against age-related diseases (cardiovascular and osteoporosis) and hormone-dependent cancers (e.g. breast and prostate cancers) [12,14–16,19]. These estrogen mimics bind estrogen receptors ($\beta > \alpha$) [16,20] and this characteristic apparently plays a role in their health promoting effects. However, the influence of phytoestrogens on brain structure and function remains to be elucidated.

1.1.2. Sexually dimorphic brain structures

Two hypothalamic brain regions are sexually dimorphic in rats (the sexually dimorphic nucleus of the preoptic area (SDN-POA) and anteroventral periventricular (AVPV) nucleus) that are under the influence of steroid hormones and estrogen-like molecules during perinatal and postnatal development [1–3,21,22]. The SDN-POA volume is 2–5 times larger in males compared to females and this brain

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region regulates sexual behavior and gonadotrophin levels [1,3,23–25]. The AVPV region is approximately two times larger in adult females compared to males and regulates LHRH via expressing receptors for ovarian hormones [22]. While SDN-POA parameters are thought to develop during perinatally, AVPV characteristics change as late as 60–80 days postbirth and, more recent data suggests that both structures are more plastic during postnatal development than previously thought [3,21,22,26,27].

1.1.3. Phytoestrogens and sexually dimorphic brain structures

In two separate studies, the administration of genistein, the most studied biologically active phytoestrogen, during neonatal development to gonadectomized female rats significantly increased SDN-POA volumes [28–30]. We previously reported that dietary phytoestrogens significantly change sexually dimorphic brain volumes in adult rats [31,32]. For example, changing adult male rats from a phytoestrogen-rich diet to a phytoestrogen-free diet sex-reversed SDN-POA volumes characteristics (i.e. volumes significantly decreased in phytoestrogen-free fed males) [32]. In another study, when AVPV volumes were examined a similar sex-reverse effect was observed during postnatal development when the phytoestrogen diets were changed (e.g. AVPV volumes significantly increased in phytoestrogen-free fed males) [31]. In light of the estrogenic nature of phytoestrogens and having studied animals fed a phytoestrogen-rich diet (from conception) that were changed to a phytoestrogen-free diet during adulthood we endeavored to repeat this diet treatment design along with an additional treatment of feeding animals a phytoestrogen-free diet from conception that were then changed to a phytoestrogen-rich diet in adulthood.

Therefore, the design of this study was to examine: (1) lifelong dietary consumption (from conception to time of tissue collection) of soy phytoestrogens (using a phytoestrogen-rich versus a phytoestrogen-free diet) or (2) how changing the dietary consumption of phytoestrogens, during adulthood, influence (a) circulating plasma phytoestrogen levels, (b) testosterone levels in males, (c) sexually dimorphic brain volumes (i.e. the SDN-POA and AVPV) and (d) the presence of apoptotic cells in these sexually dimorphic brain structures in Long-Evans rats.

2. Methods

2.1. Animals

Long-Evans male and female rats (10 per sex at 50 days old) were purchased from Charles River Laboratories (Wilmington, MA, USA) for breeding. These animals were caged individually and housed in the Brigham Young University Bio-Ag vivarium and maintained on a 10 h dark 14 h light schedule (lights on 14.00–04.00 h). The animals and methods of this study were approved by the institute of animal care and use committee (IACUC) at Brigham Young University.

2.2. Treatment-diets

Upon arrival all 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 per gram of diet; referred to hereafter as the Phyto-600 diet, or a custom phytoestrogen-free diet; referred to hereafter as the Phyto-free diet, obtained from Ziegler Bros. (Gardner, PA, USA) and water. In the Phyto-free diet, the phytoestrogen concentrations were below the detectable limits of HPLC analysis [32]. The content and nutrient composition of these diets are described in detail elsewhere [32,33]. The diets were balanced and matched for equivalent percentage content of protein, carbohydrate, fat, amino acids, vitamins and minerals, etc. Circulating phytoestrogen serum levels of rats maintained on these diets (lifelong) have been reported previously by our laboratory using GC/MS analysis [32,34,35]. The animals were time mated within their respective diets so that the offspring of these pairings would be exposed solely to either the Phyto-600 or Phyto-free diet.

2.3. Diet treatment design

The animals used in this experiment are the offspring of the females described above. The animals ($n = 16$–20 by sex and diet) were weaned at 30 days of age by sex and placed into colony cages (four per cage) and remained on their original diets (i.e. Phyto-600 or Phyto-free). At 40–45 days old, animals were singly housed and remained on their assigned diet treatments. At 75 days of age, one-half of the animals ($n = 8$–10, by sex) were switched to the opposite diet (i.e. Phyto-600 ⇒ Phyto-free or Phyto-free ⇒ Phyto-600) (see Fig. 1). Therefore, one-half of the animals were exposed to the diet treatments lifelong (from conception to time of sacrifice), while, the other half of the animals were exposed to one diet until postnatal day 75 then changed to the opposite diet until time of sacrifice at 120 days of age (or exposed short-term for approximately 45 days following the diet change during adulthood) (see Fig. 1). This resulted in four different diet treatments by sex (i.e. Phyto-600 ⇒ Phyto-600 (600-600); Phyto-600 ⇒ Phyto-free (600-free)—Protocol A; and Phyto-free ⇒ Phyto-free (free-free); Phyto-free ⇒ Phyto-600 (free-600)—Protocol B). At 120 days of age, all animals were sacrificed (first being anesthetized with Ketamine and xylazine (0.1 ml/100 g body weight)). Blood was collected from the inferior vena cava from all animals for analysis of phytoestrogen and testosterone levels (in males).

2.4. Serum phytoestrogen levels

Serum phytoestrogen concentrations and type(s) were analyzed by sex and diet treatments via gas chromato-

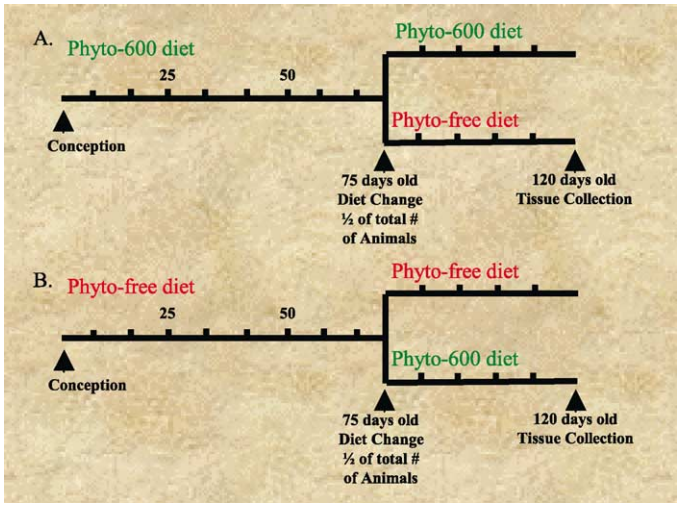

Fig. 1. Diet treatment protocols. One-half of the animals were exposed to the diet treatments lifelong (from conception to time of sacrifice), while, the other half of the animals were exposed to one diet until postnatal day 75 then changed to the opposite diet until time of sacrifice at 120 days of age (or exposed short-term for approximately 45 days following the diet change during adulthood). This resulted in four different diet treatments by sex (i.e. Protocol A—Phyto-600 ⇒ Phyto-600 (600-600); Phyto-600 ⇒ Phyto-free (600-free); Protocol B— Phyto-free ⇒ Phyto-free (free-free); Phyto-free ⇒ Phyto-600 (free-600)).

graphy/mass spectrometry (GC/MS). This was performed by liquid–solid extraction and liquid gel chromatographic techniques to isolate the phytoestrogen fractions using standards (internal controls) to validate the assay [36].

2.5. Serum testosterone levels

Testosterone serum levels were measured, in males, using a standard radioimmunoassay kit (Active Testosterone RIA) purchased from Diagnostic Systems Laboratories, Inc. (Webster, TX, USA). All samples were tested (in duplicate) in a single assay and the intra-assay coefficient of variance: 7%.

2.6. SDN-POA and AVPV morphometric analysis

After blood samples were collected, all animals were transcardially perfused with isotonic saline and then with 10% formaldehyde. The brains were removed and stored in formaldehyde for approximately 1 week to fix the tissue. The brains were then sectioned at 50 μm using a series 1000 cryostat (Zeiss, Thornwood, NY, USA) and stained with Thionin. With the aid of a microprojector (McBain Instruments, Los Angeles, CA, USA) at 10× magnification the SDN-POA and AVPV sections were traced by two investigators without knowledge of the diet treatment or sex of the brain samples. The areas of each tracing were then quantified with a BioQuant scanner (R&M Biometrics, Nashville, TN, USA) using a Dell PC optiplex GX1 and the SDN-POA and AVPV volumes were averaged and calculated using established methods [24,31,32,37].

2.7. Detection of apoptosis in SDN-POA and AVPV brain regions

Using a similar technique to Chung et al. [38], the male brain sections were examined for apoptotic cell nuclei (identified by intense staining due to condensation of fragmented nuclear material) at 400× magnification. Specifically, only the central portion of male SDN-POA or the AVPV areas were examined and evidence of apoptosis could be detected using this analysis in order to correlate changes in brain cell number and appearance of apoptosis with alterations in nuclear volumes in the sexually dimorphic regions.

2.8. Statistical analysis

The data derived from these experiments were tested by analysis of variance (ANOVA) using SPSS statistical software, followed by Bonferroni post hoc comparisons to detect significant differences between sexes and diet treatment groups ($\alpha = P < 0.05$).

3. Results

3.1. Circulating serum phytoestrogen levels by diet treatment

Fig. 2(A–D) illustrates the levels and types of phytoestrogens from animals fed the Phyto-600 (lifelong (600-600) or short-term (free-600)) versus the Phyto-free (lifelong (free-free) or short-term (600-free)) diets by sex. Regardless of whether male or female rats were fed the Phyto-600 diet lifelong or if the diet was switched from a Phyto-free

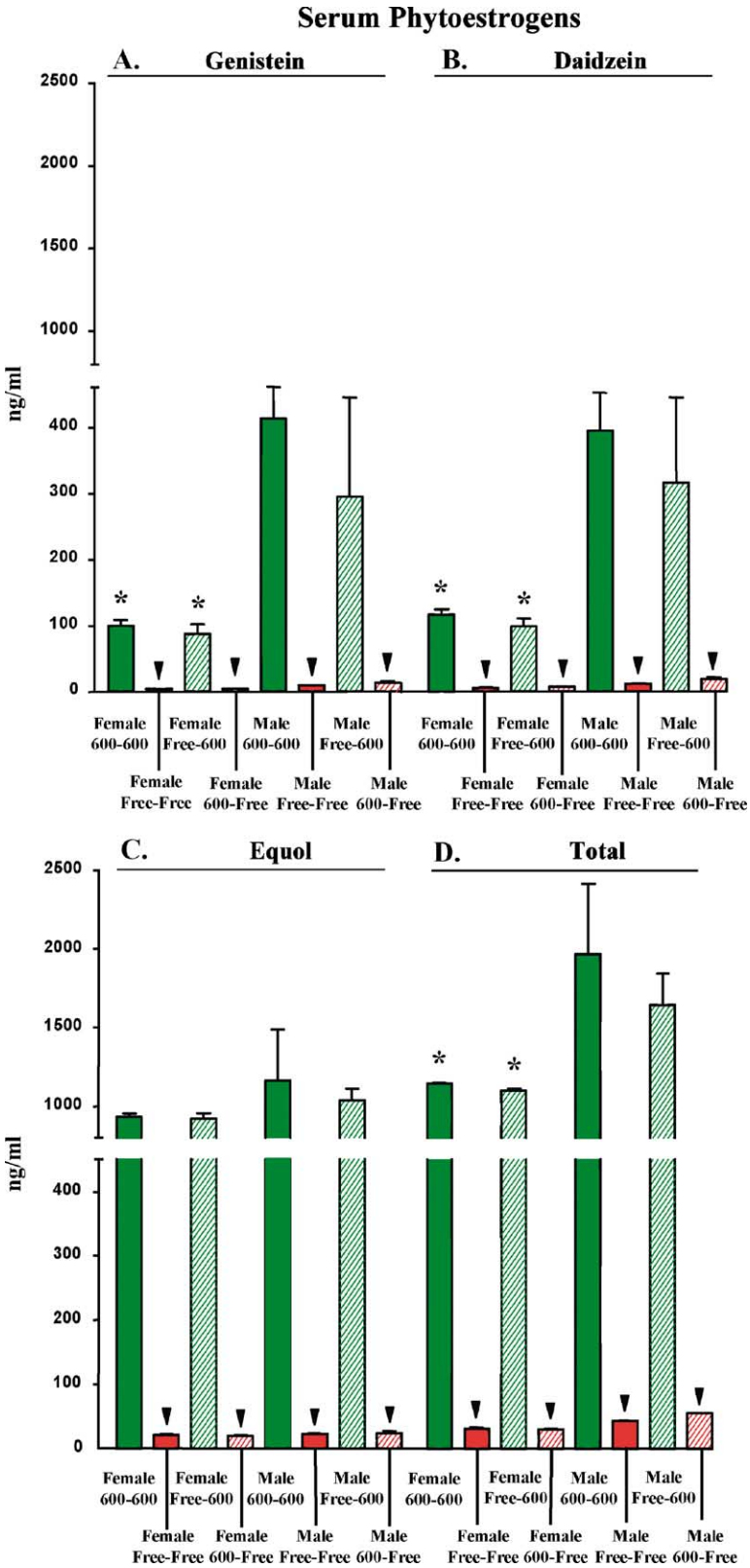

Fig. 2. Circulating serum phytoestrogen levels by diet treatments in ng/ml. Phytoestrogen analysis was performed by GC/MS. (A) Genistein levels by sex and diet treatments. (B) Daidzein levels by sex and diet treatments. (C) Equol levels (a known metabolite of daidzein) by sex and diet treatments. (D) Total serum phytoestrogen levels by sex and diet treatments. Symbol (∗): significantly lower genistein, daidzein or total phytoestrogen levels in female 600-600 and female free-600 animals compared to male 600-600 and male free-600 values. (▼) Significantly lower genistein, daidzein, equol or total phytoestrogen levels in females or males fed the free-free or 600-free diets compared to females or males fed the 600-600 or free-600 diets.

to a Phyto-600 diet during adulthood, these animals displayed significantly higher levels of genistein, dadizein and equol (a metabolite of daidzein and presumably genistein [35]) compared to animals fed the Phyto-free diet lifelong or in male and female rats switched to the Phyto-free diet (from the Phyto-600 diet) in adulthood. Equol was the major circulating phytoestrogen (see Fig. 2C), whereas, genistein and daidzein represented lower percentages of the total circulating phytoestrogens (see Fig. 2A and B). In Phyto-600 males (600-600 or free-600), serum genistein and daidzein levels were significantly higher than Phyto-600 fed females (600-600 or free-600; see Fig. 2A and B), although, the equol concentrations in Phyto-600 (600-600 or free-600) males did not differ significantly from that of female values (600-600 or free-600; see Fig. 2C). The diet treatments in Phyto-600 fed animals (600-600 or free-600, regardless of sex) displayed total serum phytoestrogens levels approximately 37-fold higher compared to that of Phyto-free (free-free or 600-free) fed animals (male Phyto-600 (600-600 and free-600) mean = 1807 versus male Phyto-free (free-free and 600-free) mean = 49 ng/ml and female Phyto-600 (600-600 and free-600) mean = 1125 versus female Phyto-free (free-free and 600-free) mean = 30 ng/ml) (see Fig. 2D).

3.2. Testosterone levels are not altered by phytoestrogens in Long-Evans male rats

When serum testosterone levels were determined, there were no significant differences detected among the diet treatments in male rats (diets: (600-600 = 2.4 ± 0.5; free-600 = 2.7 ± 0.8; free-free = 2.9 ± 0.7 and 600-free = 3.1 ± 0.5 ng/ml (mean ± S.E.M.).

3.3. Dietary change in phytoestrogen consumption during adulthood alters brain morphology

3.3.1. SDN-POA volumes

Using a dietary protocol displayed in Fig. 1 above, in the present study, we repeated diet protocol A along with performing the opposite experiment, namely, diet protocol B. As shown in Fig. 3(A-C) SDN-POA volumes are displayed by sex and diet treatments. In general, female SDN-POA volumes were not significantly altered by the diet treatments (Fig. 3A). However, female Phyto-free lifelong (free-free) SDN-POA volumes versus Phyto-free ⇒ Phyto-600 (free-600) value comparisons, approached significance ($P < 0.08$).

Confirming our previous results, male SDN-POA volumes were significantly influenced by the diet treatments (Fig. 3B). Males fed the Phyto-600 diet lifelong (600-600) or males fed the Phyto-free diet then switched to the Phyto-600 diet (free-600) displayed the largest SDN-POA volumes. This suggests that male rats initially fed a Phyto-free diet but then switched to a Phyto-600 diet (free-600) in adulthood significantly increased SDN-POA volumes comparable to males fed a Phyto-600 diet lifelong (600-600) (Fig. 3B). However, when male rats were fed a Phyto-free diet lifelong (free-free) or if males were initially fed the Phyto-600 diet but subsequently switched to the Phyto-free diet (600-free), then SDN-POA volumes significantly decreased by approximately 1/3 compared to Phyto-600 lifelong (600-600) or Phyto-free ⇒ Phyto-600 (free-600) male values (Fig. 3B). This data set suggests that male rats initially fed a Phyto-600 diet but then switched to a Phyto-free diet during adulthood significantly decreased SDN-POA volumes.

For SDN-POA volumes characteristics examining data sets between sex, males displayed, regardless of within or across diet treatment comparisons, significantly larger SDN-POA volumes compared to female values (Fig. 3C).

3.3.2. AVPV volumes

When AVPV characteristics were examined as influenced by the dietary protocols, again significant volume alterations were observed (Fig. 4A–C). In females, the largest AVPV volumes were seen in females fed the Phyto-600 diet lifelong (600-600) or Phyto-free animals switched to the Phyto-600 diet (free-600) (Fig. 4A). Conversely, AVPV volumes significantly decreased (by approximately 25%) in Phyto-free fed females ((lifelong, free-free) or Phyto-600 animals switched to the Phyto-free (600-free) diet) compared to Phyto-600 ((lifelong, 600-600) or Phyto-free ⇒ Phyto-600 (free-600)) values (Fig. 4A). These data suggest that in females, a maximal increase or decrease in AVPV volumes can be achieved by changing the phytoestrogen content of the diet, even during young adulthood.

In males, if a diet switch was instituted (either Phyto-600 to Phyto-free (600-free) or Phyto-free to Phyto-600 (free-600)) during young adulthood then these animals displayed intermediate AVPV volumes between the largest values seen in Phyto-free lifelong (free-free) fed males, versus the smallest AVPV values, in Phyto-600 lifelong (600-600) fed males (Fig. 4B). Opposite to the female results, the male data suggest a pattern similar to a dose-dependent effect of dietary phytoestrogens on AVPV volumes.

Male versus female data sets revealed significantly larger female versus male AVPV volumes in female 600-600 or free-600 compared to male 600-600 or free-600 values (Fig. 4C). Female free-free versus male free-free diet treatments showed a sex-reversed pattern for AVPV volumes. When female 600-free versus male 600-free AVPV volumes were examined numerically higher male versus female values were observed (similar in pattern to that of the female (free-free) versus male (free-free) data above), however, this difference was not statistically significant (Fig. 4C).

3.4. Apoptosis in SDN-POA and AVPV brain regions by diet treatments

Since the greatest changes in brain volumes by diet treatments were observed in males further analysis was performed only in these samples. When hypothalamic brain

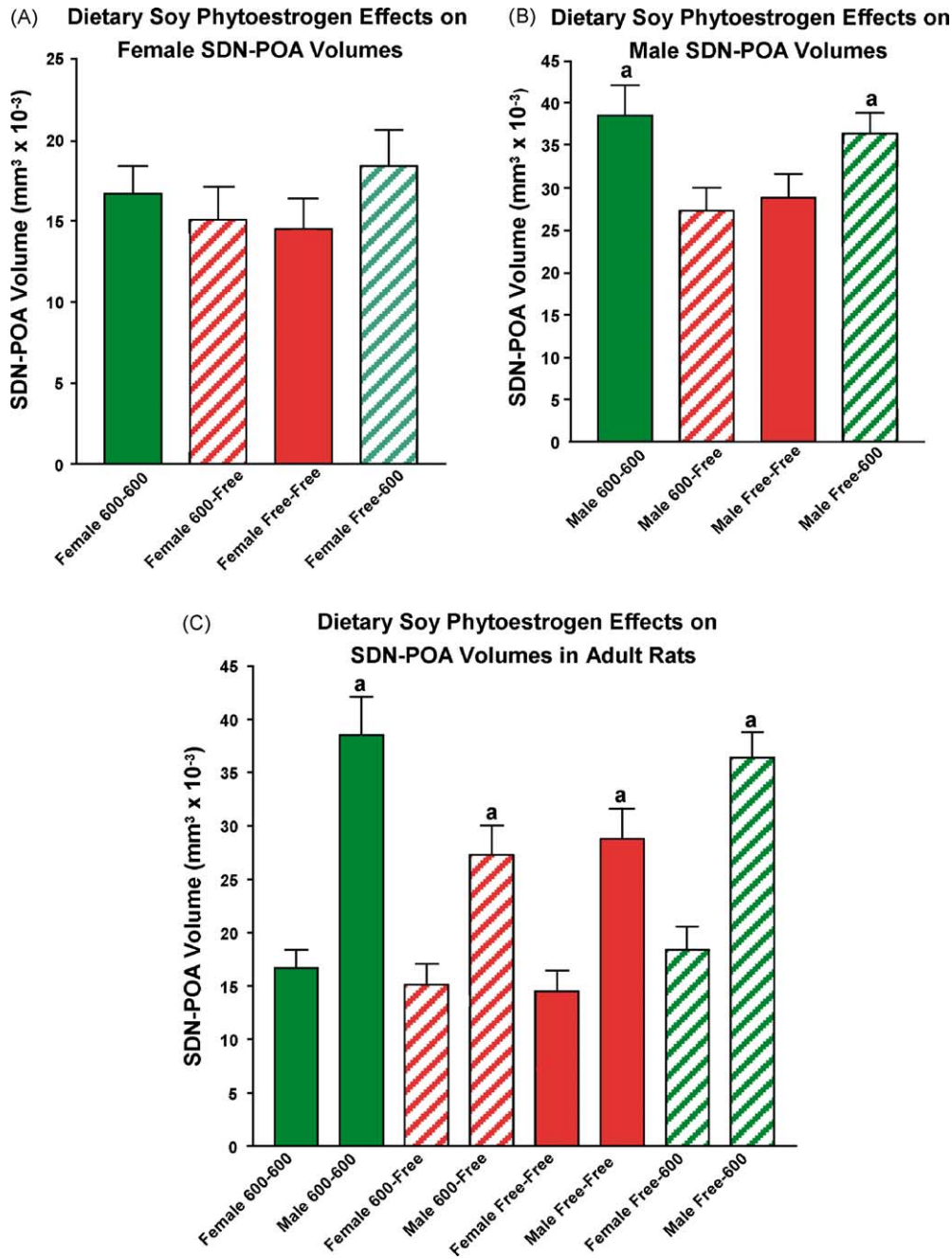

Fig. 3. Dietary soy phytoestrogen effects on the sexually dimorphic nucleus (SDN) of the preoptic area (POA) volumes. (A) The influence of dietary phytoestrogens on female SDN-POA volumes. No significant alterations were detected. (B) The influence of dietary phytoestrogens on male SDN-POA volumes: (a) male 600-600 or male free-600 fed animals displayed significantly larger volumes compared to male 600-free or male free-free fed values. (C) The influence of dietary phytoestrogens on male vs. female SDN-POA volumes: (a) all male SDN-POA volumes (regardless of diet treatment) were significantly larger than all female SDN-POA volumes (regardless of diet treatment).

sections from lifelong Phyto-600 (600-600) and lifelong Phyto-free (free-free) fed male rats were compared (Fig. 5), the largest SDN-POA volumes with robust cells were seen in the lifelong 600-600 animals, which also displayed few if any apoptotic cells (Fig. 5, top left-hand panel). Whereas, when the central portion of the SDN-POA region was examined in lifelong Phyto-free (free-free) fed males, apoptotic cells were present and the appearance of the cells were less robust (see arrows in Fig. 5, top right-hand panel) compared to cells obtained from Phyto-600 (600-600) fed males (top left-hand panel). In examining AVPV characteristics the opposite results were obtained across the diet treatments. Notably, males fed the Phyto-free diet lifelong (free-free) displayed robust neurons without the appearance of apoptotic cells (bottom right-hand panel), while Phyto-600 (600-600) treated males displayed apoptotic cells in the

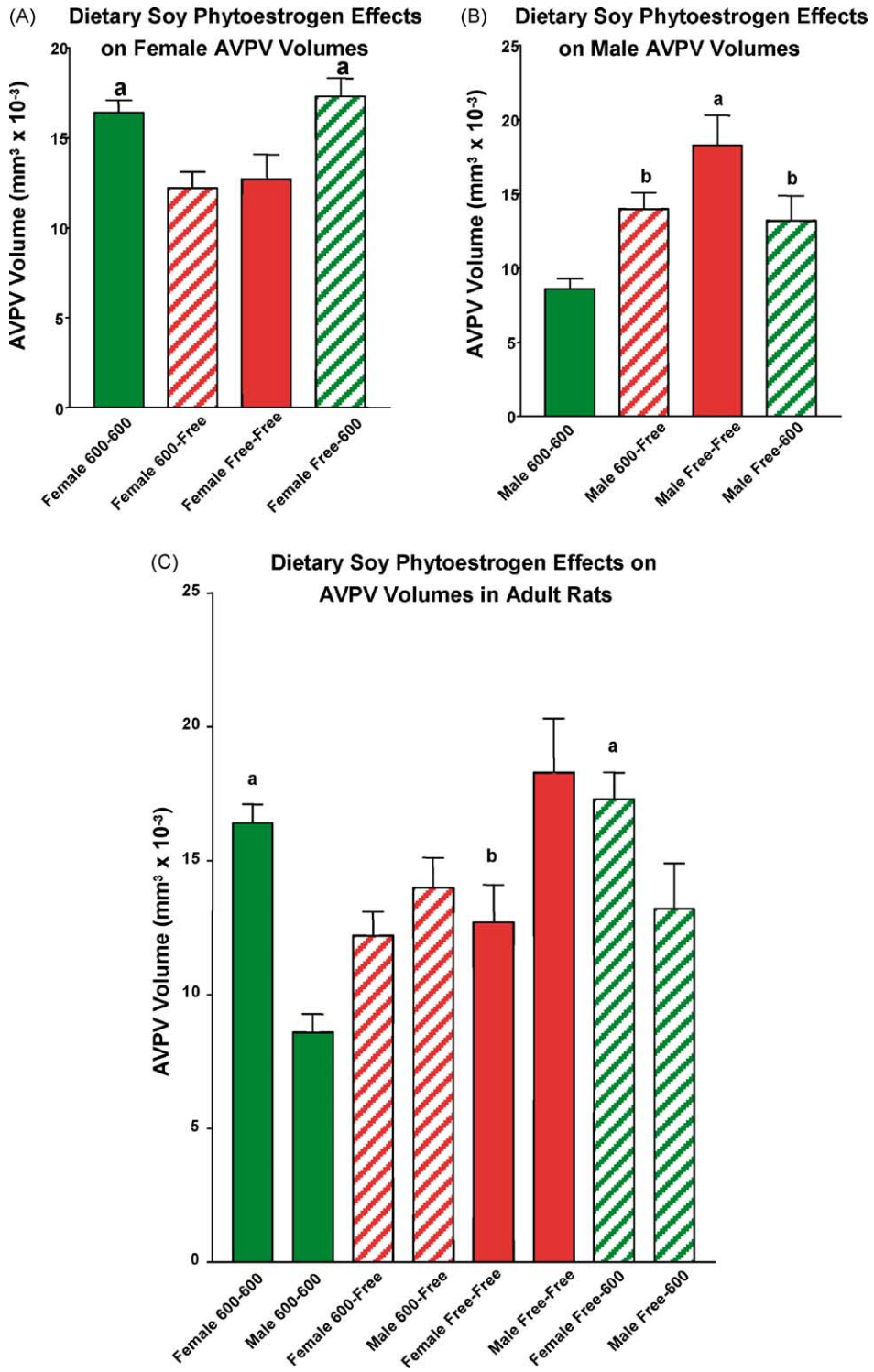

Fig. 4. Dietary soy phytoestrogen effects on the anteroventral periventricular (AVPV) nucleus volumes. (A) The influence of dietary phytoestrogens on female AVPV volumes: (a) female 600-600 or female free-600 animals displayed significantly larger AVPV volumes compared to female 600-free or female free-free values. (B) The influence of dietary phytoestrogens on male AVPV volumes: (a) male free-free fed animals displayed significantly larger volumes compared to all other diet treatment groups; (b) male 600-free or male free-600 fed animals displayed significantly larger volumes compared to male 600-600 values. (C) The influence of dietary phytoestrogens on male vs. female AVPV volumes: (a) female 600-600 or female free-600 animals displayed significantly larger volumes compared to male 600-600 or male free-600 values; (b) female free-free animals displayed significantly smaller volumes compared to male free-free values.

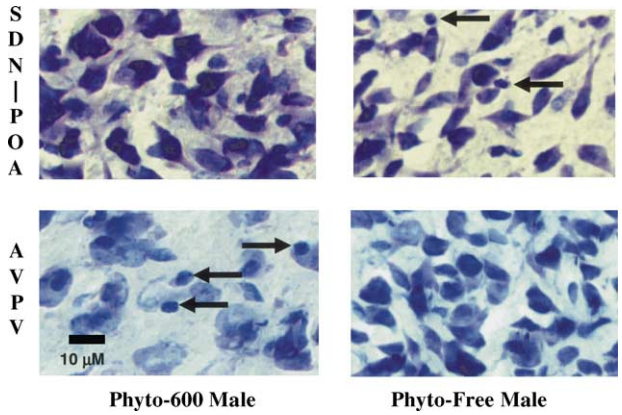

Fig. 5. Apoptosis in SDN-POA and AVPV brain regions by diet treatments. Representative photomicrographs of the central portion of the SDN-POA or AVPV regions. Apoptotic cell nuclei are indicated by the arrows shown in: (1) the top right-hand panel from the SDN-POA of male rats fed a phytoestrogen-free (free-free) diet lifelong vs. animals fed a phytoestrogen-rich (600-600) diet lifelong (top left-hand panel) and (2) the bottom left-hand panel from the AVPV of male rats fed a phytoestrogen-rich (600-600) diet lifelong vs. animals fed a phytoestrogen-free (free-free) diet.

central AVPV region (see arrows in Fig. 5, bottom left-hand panel). Central AVPV neurons from Phyto-600 (600-600) males showed a comparable pattern (i.e. similar in shape and number) to the central region of the SDN-POA of males fed the Phyto-free diet lifelong (free-free). This corresponds to the significantly decreased sexually dimorphic volumes seen in these animals compared to male volume parameters fed the opposite diet treatments (i.e. for Phyto-free (free-free) fed male AVPV values and Phyto-600 (600-600) fed male SDN-POA values, respectively).

4. Discussion

The present findings are similar to previous data reported from our laboratory employing these diets [31,32]. Namely, phytoestrogen diet content reflects circulating plasma levels, which in turn predicts phytoestrogen entry into brain and regional differences in various brain regions that correspond, in general, with the distribution and abundance of ER-β [39]. Specifically, in this study, serum phytoestrogen levels were significantly increased by approximately 37-fold in Phyto-600 (lifelong (600-600) or dietary switched (free-600)) fed animals (in the 1–2 μM range similar to that of humans on an Asian soy diet [19]) compared to Phyto-free (lifelong (free-free) or dietary switched (600-free)) values, regardless of the initial diet treatments the rats were exposed to. Notably, the major circulating phytoestrogen was equol with genistein and daidzein representing lower levels of the estrogen-like molecules.

We and other investigators have shown that these estrogen mimics influence body weight, food/water intake, locomotor activity and anxiety levels, learning and memory, body temperature, neuroendocrine function, puberty onset and prostate weight [30,32,33,35,39–45]. Furthermore, testosterone levels were not altered by the diet treatments, confirming earlier data employing only diet protocol A [32]. This confirmation of dietary phytoestrogens not influencing testosterone levels that in turn serves as substrate for the aromatase enzyme in brain suggests that substrate availability is unaltered. In fact, we have previously shown that brain aromatase (in a series of studies) is not significantly influenced by dietary phytoestrogens [31,34,35]. It is reasonable to suggest that the probable action of dietary phytoestrogens is at estrogen receptors where they act as selective estrogen receptor modulators (SERMS) [9,16]. Thus, phytoestrogens are similar in action to that of tamoxifen or raloxifene where they mimic the effects of estrogens at some receptor sites, but block estrogen's hormone action at other sites in a tissue-specific manner [9,16,46]. This is especially intriguing to consider since phytoestrogens demonstrate differential affinities for ERβ versus ERα and the varying expression of ER subtypes throughout different brain regions [47,48].

Previous investigators have demonstrated that genistein injected into gonadectomized female rats significantly increased SDN-POA volumes [28,29], however, in intact female rats genistein treatment resulted in a non-significant decrease in SDN-POA volumes [30]. This suggests that phytoestrogens influence sexually dimorphic volumes that are dependent on the basal estrogen hormonal status of the organism [35]. We have also recently shown that dietary phytoestrogens significantly alter sexually dimorphic brain volumes, in adult rats in a gender-dependent manner, and the results from this study employing protocol A are similar to our previous results [31,32]. Furthermore, when diet protocol B was tested the SDN-POA and AVPV volumes were significantly altered regardless of whether male or female animals were on the diet treatments lifelong or if the diet treatments were switched during adulthood demonstrating the plasticity of these sexually dimorphic brain areas (see summary Table 1). Previous investigators have demonstrated that SDN-POA and AVPV brain volumes are sensitive to hormonal influences or affected by sexual activity during postnatal development [3,21,27,49,50]. This suggests that hormone-dependent brain structures are more plastic in adulthood than previously thought. In this regard, neurogenesis has been shown in human and rodent brains studies and, in fact, in avain brain areas controlling vocal song production express seasonal plasticity with increases in volume and cell number during reproductive periods and decreases in these parameters during non-breeding intervals [51–55]. In the present study, greater changes in AVPV volumes were seen with the diet treatments compared to the SDN-POA region and more robust alterations in brain nuclear volumes were observed in males versus females. This may be due to the AVPV developmental time course of hormonal influences that modulate cellular and hence volumetric characteristics that occur into the postnatal period [3,22,37,56].

Table 1
Hypothalamic sexually dimorphic volumes influenced by diet treatments and sex

(A) **SDN-POA Volumes: Sex comparisons within Diet Treatment**

Sex	Diet Treatments			
Females vs. Males	600-600 ∧ 600-600	600-Free ∧ 600-Free	Free-Free ∧ Free-Free	Free-600 ∧ Free-600

(B) **AVPV volumes: Sex comparisons within Diet Treatments**

Sex	Diet Treatments			
Females vs. Males	600-600 ∨ 600-600	600-Free ≯≪ 600-Free	Free-Free ∧ Free-Free	Free-600 ∨ Free-600

(C) **SDN-POA Volumes: Diet treatments within Sex**

Sex	Diet Treatments						
Females	600-600	≅	600-Free	≅	Free-Free	≅	Free-600
Males	600-600	>	600-Free	≅	Free-Free	<	Free-600

(D) **AVPV Volumes: Diet Treatments within Sex**

Sex	Diet Treatments						
Females	600-600	>	600-Free	≅	Free-Free	<	Free-600
Males	600-600	≪*	600-Free	<	Free-Free	>	Free-600

(A) Male SDN-POA volumes (within each diet treatment) were significantly larger than female values. (B) Female vs. male AVPV volumes within each diet treatment varied dependent upon the terminal diet the animals were exposed to. Females fed the 600-600 or free-600 diets displayed significantly larger AVPV volumes compared to male values within the same diet treatments. Whereas, males fed the free-free diet displayed significantly larger AVPV volumes compared to females values within the same diet treatment. Finally, males fed the 600-free diets displayed similar volumes measurements (≅) to that of female fed the same 600-free diets. (C) All females SDN-POA values were similar (≅) across the diet treatments, while male volumes were dependent upon the terminal diet the animals were exposed to (i.e. male 600-600 or male free-600 values were significantly larger compared to male 600-free or male free-free fed volumes and the latter two groups displayed similar (≅) volume measurements to one another). (D) Female AVPV volumes were dependent upon the terminal diets the animals were exposed to (i.e. 600-600 or free-600 volumes were significantly larger compared to animals on the 600-free or free-free diets and the latter two groups displayed similar (≅) measurements to one another). Male AVPV volumes were also dependent upon the terminal diets the animals were exposed to, however, a greater impact of the diet treatments was seen compared to female values (e.g. male 600-600 fed males displayed the smaller AVPV volumes (<<*), whereas, male free-free fed animals displayed the largest AVPV volumes, with male 600-free and male free-600 values being similar to one another).

When the largest changes in the sexually dimorphic hypothalamic volumes of male rats were compared by diet treatments a striking correspondence was seen when volume cell densities and the appearance of apoptotic cells were examined. It is reasonable to consider two main factors in examining these findings: (1) the differential effect of estrogenic hormone action in the SDN-POA versus the AVPV and, (2) the estrogen-like agonist influence of dietary phytoestrogens in males. It is clear that estrogen's action in the SDN-POA of males results in larger volumes compared to females and estrogen apparently has that opposite effect in the AVPV where female volumes are larger than male values [3,56,57]. The more robust effect of dietary phytoestrogens observed in males where the presence of the estrogen-like molecules resulting in larger neuron cell diameters suggests a protective effect within the SDN-POA [27,48,58]. Conversely, dietary phytoestrogens may activate an apoptotic mechanism by a Fas-ligand system via ERβ within the hypothalamic region that may be involved in the regulation of AVPV volumes in Phyto-600 versus Phyto-free fed male rats [59]. Support for the changes in AVPV volumes comes from studies showing that the phytoestrogen, genistein, causes programmed cell death in cortical tissue [60]. This represents a complex picture of regulating estrogenic hormone action within the brain and we are currently attempting to determine whether dietary phytoestrogens act as estrogenic, antiestrogenic and/or antiandrogenic molecules via receptor binding and in vivo physiological studies that would assist us in revealing this complex image.

The increased use and availability of phytoestrogen-rich consumables (via soy foods) and soy-derived nutritional supplements in the human diet suggest beneficial health effects not only for the protection against cardiovascular disease and certain cancers but neuroendocrine disorders, such as, hot flashes [9,12,13,16,61]. The implications derived from the present results to human brain health issues are unknown. The time course of neural development, influence of hormonal agents and plasticity of brain nuclear volumes are different between rodents and humans [2,3,37,62,63] and in the case of the human model many of these factors are not as well studied compared to the rodent model. For example, abundant data exists for the development (and known characteristics) of the SDN-POA in the rat compared to the interstitial nuclei of the anterior hypothalamus in humans [1,3,62,63]. However, in connection to brain plasticity an LH surge (under hormonal manipulation) cannot be obtained in male rats, whereas, in monkeys or humans this gonadotrophic neuroendocrine response is attainable, suggesting a more malleable neuro-hormonal circuitry [64,65]. Furthermore, global changes have been documented in male (but not female) human brains of individuals consuming soy-rich diets where increased dementia, ventricular size and decreased brain weights were observed [66]. Although, circulating phytoestrogen levels were not determined, nor was a direct link of consuming soy-foods (tofu) with neurodegeneration established in this study. Moreover, studies in rodents suggest that consuming a diet rich in phytoestrogens enhances visual spatial memory (VSM) in females, but inhibits VSM in males [39,43]. Studies, from our laboratory, manipulating the hormonal status of rats prenatally provide a potential mechanism by which phytoestrogens influence VSM performance [67]. Also, other investigators present evidence that phytoestrogens, in different animals models, may protect against Alzhemier's disease [58,68].

From the data base(s) currently available that have examined the effects of dietary phytoestrogens in brain, it is evident that: (1) this is a relatively new focal area for basic and clinical research studies and, (2) the preliminary results obtained to date are far from clear in portraying common biological themes/mechanisms. This challenging picture is most likely due to the hormonal SERM-like action of phytoestrogens and the varying distribution of ER subtypes throughout the brain [44,45]. Given the practical limitations of examining at the structural and functional levels the effects of dietary phytoestrogens in brain, it is important to test these estrogen mimics in animal models in order to establish comparative data sets that will then elucidate phytoestrogen hormone action and identify safety and potentially beneficial health issues.

In summary, consumption of dietary phytoestrogens can change hormone-sensitive hypothalamic brain volumes in rodents even during adulthood with more robust effects observed in males versus females. The data demonstrates the plasticity of these brain regions by molecules that mimic or inhibit estrogen hormone action via ER subtypes. Further research is warranted to determine the effects of phytoestrogens and the mechanisms by which these estrogen mimic act in altering brain structure and function.

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