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# Low Dietary Soy Isoflavonoids Increase Hippocampal Spine Synapse Density in Ovariectomized Rats

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## Abstract

High dietary intake of plant estrogens (phytoestrogens) can affect brain structure and function. The effects of phytoestrogen intake within the range of normal animal and human dietary consumption, however, remain uncertain. The aim of the present study was to determine the effects of the isoflavonoids present in a standard low phytoestrogen laboratory rat chow on spine synapse density in the stratum radiatum of area CA1 of the hippocampus.

Weanling rats (22 days old) were fed either standard chow (Teklad 2018), a nutritionally comparable diet without soy (Teklad 2016) or a custom diet containing Teklad 2016 supplemented with the principal soy isoflavonoids, daidzein and genistein, for 40 days. Rats were ovariectomized at 54 days of age. Eight days later, spine synapse density on the apical dendrites of hippocampal pyramidal neurons in the stratum radiatum of area CA1 was measured by electron microscopic stereological analysis. Animals maintained on Teklad 2016 exhibited an approximately 60% lower CA1 spine synapse density than animals consuming Teklad 2018. Replacing genistein and daidzein in Teklad 2016 returned synapse density to levels indistinguishable from those in animals on Teklad 2018.

These results indicate that the isoflavonoids in a standard laboratory rat diet exert significant effects on spine synapse density in the CA1 region of the hippocampus. Since changes in spine synapse density in this region of the hippocampus have been linked to cognitive performance and mood state, these data suggest that even relatively low daily consumption of soy phytoestrogens may be sufficient to influence hippocampal function.

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**Keywords**

Phytoestrogen; isoflavonoid; hippocampus; CA1; dendritic spine synapses

## INTRODUCTION

A number of studies have suggested that women may benefit from eating foods rich in phytoestrogens, or taking soy-based supplements (which contain the isoflavonoids genistein and daidzein) to minimize the symptoms associated with the loss of ovarian estrogens at menopause, including depression and cognitive dysfunction (Franco et al., 2016; Pitkin, 2012; Soni et al., 2014). Work in laboratory animals supports this hypothesis, demonstrating estrogen-like effects of isoflavonoids in rats (Lephart et al., 2002; Luine et al., 2006; Lund et al., 2001; Pisani et al., 2012). However, not all the reported effects on cognitive function have been positive, in either animals (Neese et al., 2012) or humans (Henderson et al., 2012; Soni et al., 2014; St John et al., 2014; Zhao and Brinton, 2007). This may in part be because isoflavonoids do not have the same receptor specificity as the principal mammalian estrogen, estradiol. While estradiol acts through at least three different receptor systems [estrogen receptor (ER)α, ERβ and the G-protein coupled transmembrane estrogen receptor GPR30 (Prossnitz and Barton, 2011)], genistein and daidzein are partially selective for ERβ and GPR30 (Kajta et al., 2013; Thomas and Dong, 2006) and have lower affinity for ERα (Casanova et al., 1999).

In addition to their potential use as a dietary supplement, phytoestrogens are also important in laboratory animal studies when phytoestrogens and other xenobiotic estrogens (Brown, Setchell, 2001) may be present in the food supply. Many commercial animal foods contain soy meal, contributing significant quantities of isoflavonoids, including genistein and daidzein. Studies comparing the effect of high and low phytoestrogen diets suggest that these compounds exert significant estrogenic effects (Lee et al., 2009; Lund et al., 2001; Patisaul and Jefferson, 2010; Whitten et al., 2002), raising the potential for confounding effects in studies of endocrine function and behavior (Jensen and Ritskes-Hoitinga, 2007; Thigpen et al., 2013; Thigpen et al., 2004). However, since some of these studies used diets with a relatively high isoflavonoid content (>300mg/kg; Bu et al., 2005; Lee et al., 2009; Lund et al., 2001) it remains uncertain whether similar effects may also occur in animals fed using standard diets, with isoflavonoid contents in the 100–300 mg/kg range (Jensen and Ritskes-Hoitinga, 2007). Interpretation of the data is also complicated by the fact that substituting other foodstuffs for phytoestrogen-rich components in the diet may potentially have effects related to their nutritional value, rather than their activity as estrogens.

The present study was performed to determine whether the phytoestrogens present in a standard rat chow influence the density of spine synapses in area CA1 of the hippocampus. Spine and spine synapse formation in this region of the brain are highly sensitive to estrogen and have been linked to estradiol-induced changes in both cognitive performance (Frankfurt and Luine, 2015; Phan et al., 2011) and mood state (Hajszan and MacLusky, 2006; Hajszan et al., 2005, 2010). We compared spine synapse density in ovariectomized rats fed either Teklad 2018 (a widely used rodent diet with reduced alfalfa and soy content); a nutritionally

comparable soy-free diet (Teklad 2016); or Teklad 2016 supplemented with genistein and daidzein to levels similar to those present in Teklad 2018. The results suggest that even relatively low levels of dietary isoflavonoids may have positive effects on hippocampal neuroplasticity.

## METHODS

### Animals

Female Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were kept in individual cages on a 12-h light, 12-h dark cycle and provided with unlimited access to water and food. Animal protocols used in this study were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory animals and approved by the Institutional Animal Care and Use Committee of Yale University. The complete experimental protocol is summarized in Table 1. Rats arrived at the age of 22 days and were housed until the age of 54 days, when they were sexually mature (first estrus occurs in this strain of rats at 33–34 days of age; (Brown et al., 1994). The animals were maintained during the 40 day period of study with minimal experimental manipulation, to minimize potential interference from stress which is known to interfere with both pubertal development (Holder and Blaustein, 2014) and the effects of estrogen on hippocampal neuroplasticity (Frick et al., 2004; Scharfman et al., 2007).

The experiment was performed on three groups of rats, each consisting of 4 animals. Rats in the control group were fed a normal soy meal-containing diet (Teklad 2018; Envigo Research Indianapolis, Indiana, USA). In this diet, isoflavonoid contents (daidzein + genistein aglycone equivalents) range from 150–250mg/kg, which is in the mid-range of isoflavonoid contents of commercial soy-containing laboratory rodent diets (Jensen and Ritskes-Hoitinga, 2007). The second group of rats (phytoestrogen reduced) was fed a nutritionally comparable diet (Teklad 2016) containing no soybean meal, in which isoflavonoid levels typically are below 20mg/kg. Rats in the third experimental group were fed a special diet based on Teklad 2016, but with 220mg/kg of pure isoflavonoids (112 mg/kg genistein and 108 mg/kg daidzein) mixed into the food at the time of milling. The content of isoflavones in the diet was provided by the manufacturer, based on data generated by Dr. Patricia Murphy at Iowa State University using previously described chromatographic procedures (Wang, Murphy, 1994). The basic nutrient contents of the non-supplemented Teklad 2016 and 2018 diets are summarized in Table 2.

The animals were fed the test diets, with food and water ad libitum, from the age of 22 days until the end of the experiment, 40 days later. At 54 days of age, they were anesthetized with 4ml/kg b.wt. of a mixture of ketamine (25mg/ml), xylazine (1.3mg/ml), and acepromazine (0.25mg/ml), and then ovariectomized (OVX). Following ovariectomy, the animals were allowed to recover for a further 8 days. At 62 days of age, under deep ether anaesthesia, the vascular system was perfused via the left ventricle with 50–80 ml of heparinized saline followed by 200ml of fixative containing 4% paraformaldehyde, 0.1% glutaraldehyde in 0.1M, pH 7.4 phosphate buffer (PB). Following perfusion, selected of the brain areas were dissected out and immersed in the same fixative for an additional 24 hours. One hundred micron thick sections were prepared on a vibratome perpendicular to the longitudinal axis of

the hippocampal formation, dehydrated in graded series of ethanol (70% contained 1% uranyl acetate) and flat embedded in Durcupan.

### Determination of Spine synapse density

Spine synapses were counted in all animal groups according to our standard protocol, using unbiased stereological methods (Leranth et al., 2004). Briefly, to assess possible changes in tissue volume, a correction factor was calculated assuming that the hormonal treatments did not alter the total number of pyramidal neurons (Rusakov et al., 1997). In all hippocampi, ten disector pairs (pairs of adjacent 2μm toluidine blue-stained semithin sections mounted on slides) were sampled and analyzed using the technique of Braendgaard and Gundersen (1986). The pyramidal cell density value (D) was calculated using a formula: D = N/sT, where N is the mean disector score across all sampling windows, T is the thickness of the sections (2μm), and s stands for the length of the window. Based on these values, a dimensionless volume correction factor kv was calculated: kv = D/D1, where D1 is the mean density across the groups of hippocampi. Thereafter, using the toluidine blue-stained semithin sections as guides, each block was trimmed to contain the same area, located between the middle and distal portion of the stratum radiatum. Pairs of consecutive serial ultrathin sections (“reference” and “look-up”) were cut from the vibratome sections taken from all parts of the hippocampus along its longitudinal axis. The section pairs were collected on Formvar coated single-slot grids. Digitized images were taken at a magnification of 11,000× in a Tecnai 12 transmission electron microscope furnished with an AMT Advantage 4.00 HR/HR-B CCD camera system. Identical regions in reference and look-up sections were identified using landmarks (myelinated fibers, large dendrites, or blood vessels) that did not change appreciably between neighboring sections because of their size. Digitized electron micrographs were printed out, coded, and the code was not broken until the analysis was completed. Only those spine synapses were counted that were present in the reference section, but not in the look-up section (Fig. 1a,b). To increase the efficiency of counting, the analysis was performed treating each reference section as a look-up section, and vice versa. The measured synaptic density values were divided by the volume correction factor kv, providing a synaptic density estimate normalized with respect to the density of pyramidal cells and possible changes in hippocampal volume. Average spine synapse density values for each animal were used to calculate mean synapse densities (± SEM) for each treatment group.

### Statistical analysis

Data are presented as means +/− SEM. One way ANOVA followed by the Scheffé post-hoc test for comparison of individual group means was used to analyze results. Analyses were performed using Statview (SAS institute Inc. Cary, North Carolina). Statistical significance was set at $p<0.05$ (two-tailed).

## RESULTS

Since the phytoestrogen content of soy meal can vary substantially from batch to batch (Heindel and vom Saal, 2008; Lee et al., 2003; Thigpen et al., 2007), we evaluated data provided by the manufacturer for the total isoflavonoid content of different batches of the

diets, to verify that the feed used in assessing effects on synapse density was representative. Seven samples were evaluated at three monthly intervals, to assess the degree of variability in isoflavonoid concentrations. All seven samples of Teklad 2018 contained approximately 200–250 mg of total isoflavonoids/kg (mean 231.9 +/− 8.8 mg/kg; Fig 2). Regression analysis revealed a significant positive correlation between the phytoestrogen content of the 7 batches of Teklad 2018 analyzed and the total phytoestrogen concentrations in the soy bean meal used to prepare the diets (r=0.935 p=0.002; see legend to Fig. 2 for individual soy meal contents), consistent with the hypothesis that the variation in dietary phytoestrogen content resulted from variations in the soy meal component. The principal isoflavonoids identified were the β-glycoside derivatives of genistein (~ 54% by weight) and daidzein (~ 40% by weight), consistent with previous observations on the composition of soy meal (Wang and Murphy, 1994). Only two samples of Teklad 2016 contained measurable amounts of isoflavonoids (mean 3.28+/− 2.34 mg/kg; Fig 2). The final batches received (#7; isoflavonoid contents Teklad 2016: undetectable; Teklad 2018: 233mg/kg; Fig. 2) were representative of the series of diets analyzed and were used to evaluate effects on CA1 spine synapse density.

At the time of perfusion for evaluation of spine synapse density, body weights did not differ significantly between the treatment groups (data not shown). Differences in CA1 spine synapse density in animals fed each of the three diets are shown in Figs 3 and 4. Animals fed on an isoflavonoid-containing diet exhibited higher spine synapse densities than those on unmodified Teklad 2016. The difference in synapse density was apparent even on individual pairs of ultrathin sections, more synapses being observed on micrographs from animals fed Teklad 2016 supplemented with genistein and daidzein (Fig. 3b) compared to animals fed Teklad 2016 without added phytoestrogen (Fig. 3a). Quantitative stereological analysis revealed that animals maintained on the regular Teklad 2018 diet had a more than twofold higher spine synapse density in the CA1 region than animals raised on soy-free Teklad 2016 chow (Fig 4). Addition of 112 mg/kg genistein and 108 mg/kg daidzein to Teklad 2016, to replace the principal isoflavonoids found in Teklad 2018, resulted in synapse densities within the same range as in the animals fed Teklad 2018 (Fig. 4).

## DISCUSSION

Hippocampal spine synapse formation has been linked to changes in behavior, including effects on cognitive performance (Frankfurt and Luine, 2015) and mood state (Hajszan and MacLusky, 2006; Hajszan et al., 2005, 2010). The present data indicate that even in a standard laboratory rat chow, the dietary soy phytoestrogen content is sufficient to result in a more than twofold higher CA1 spine synapse density in OVX female rats, compared to soy-free animals. The low spine synapse density observed in rats on the soy free diet is similar to values previously reported in female rats maintained on Teklad 2018 after treatment with high doses of bisphenol-A, an environmental chemical that inhibits estrogen-induced spine synapse formation (Leranth et al., 2008; MacLusky et al., 2005), consistent with the hypothesis that the higher synapse density observed in animals fed Teklad 2018 reflects the estrogenic activity of the phytoestrogens in the diet. These data are consistent with the previous observations of Luine *et al* (2006), who observed higher hippocampal spine densities in OVX rats maintained for 9 weeks on a high soy diet (Purina LabDiet 5001),

compared to animals fed Teklad 2016. Although we did not directly compare the effects of the different diets to those of estradiol administration, previous work using similar analytical techniques in CD Sprague-Dawley rats has demonstrated that the spine synapse density observed in CA1 after a maximal dose of estradiol is approximately 1.2–1.3 synapses/$\mu m^2$ (MacLusky et al., 2005a, b). Thus, the effect of adding genistein and daidzein to Teklad 2016, while statistically significant, probably represents less than half of the maximal increase in spine synapse density observed after treatment with estradiol. Direct comparison between the effects of increasing doses of isoflavonoids and estradiol on CA1 spine synapse density will be required to determine whether this interpretation is correct.

## Potential Mechanisms

The effects of the natural circulating estrogen, estradiol, on spine and spine synapse formation in the brain are believed to involve three main receptors: ERα, ERβ and GPR30 (Bean et al., 2014; Gabor et al., 2015; Jelks et al., 2007; Szymczak et al., 2006). It seems reasonable to assume that the effects of genistein and daidzein on CA1 spine synapse density may involve these same receptor systems. However, interpretation of the available data in terms of specific mechanisms is complicated, both by the broad range of receptor binding activities exhibited by the isoflavonoids as well as by *in vivo* conversion of these compounds to biologically active metabolites.

Previous work (Qu et al., 2013; Sanchez-Andrade, Kendrick, 2011; Witty et al., 2012) has demonstrated that ERα mediated mechanisms contribute substantially to estrogen-mediated regulation of CA1 spines and cognitive behavior. Phan *et al* (Phan et al., 2011) observed rapid potentiation of social recognition, object recognition and object placement learning, as well as an increase in CA1 spine density, after administration of the highly specific ERα agonist propyl pyrazole triol (PPT). These effects were not reproduced by the selective ERβ agonist diarylpropionitrile (DPN). Genistein and daidzein also have higher relative binding affinities for ERβ than for ERα (Casanova et al., 1999); but in other respects they differ significantly from DPN. While DPN has approximately 70 fold higher affinity for ERα than for ERβ (Meyers et al., 2001), genistein is less selective: the ratio of ERβ to ERα affinity for genistein is approximately 16 (Muthyala et al., 2004). Daidzien is extensively converted by gut bacteria to S-equol (Setchell et al., 2005), a compound with binding affinities for ERα and ERβ comparable to those of genistein (Muthyala et al., 2004), but different biological activity with respect to the activation of cellular estrogen response pathways (Carreau et al., 2009; Liu et al., 2014). Thus, variations in the extent of metabolism of dietary isoflavonoids to equol could contribute significantly to their biological effects. GPR30 may also play an important role in mediating responses to isoflavonoid administration. This receptor associates with the dendritic spine scaffolding protein PSD-95 and has been implicated in dendritic spine formation (Akama et al., 2013). GPR30 has a relatively high binding affinity for genistein (Thomas, Dong, 2006) and also appears to be activated by daidzein at low micromolar concentrations (Kajta et al., 2013). Since GPR30 activation increases CA1 spine density and enhancement of hippocampal synaptic transmission (Ervin et al., 2015; Gabor et al., 2015; Kumar et al., 2015), the ability of the isoflavonoids to bind to this receptor suggests that GPR30-mediated responses could contribute to their effects on hippocampal spine synapse density. Taken together, these data

suggest that the phytoestrogens present in soy meal may exert their effects on hippocampal spine synapse density via several complementary mechanisms, involving ERα and ERβ as well as GPR30. The extent to which each of these specific mechanisms contributes to the responses remains to be determined.

## Implications for animal and human behavior

Increases in spine synapse density on the dendrites of pyramidal neurons in CA1 have been postulated to contribute to the enhancement of cognitive function observed after estrogen exposure (Frankfurt, Luine, 2015). Although most studies of the effects of soy-containing diets have used substantially higher phytoestrogen doses than those employed here, the available data are consistent with the hypothesis that induction of spine synapse formation by soy isoflavonoids may enhance memory. Teklad Rodent Diet 8604 has been reported to enhance visuospatial memory in female rats, compared to animals fed a phytoestrogen-free diet (Lund et al., 2001). Diet 8604 contains approximately 3 times as much isoflavonoid (600 ppm) as the diet used in the present study (Bu et al., 2005). Luine *et al* (Luine et al., 2006) reported improved performance on a test of spatial memory (object placement) in rats maintained on Purina LabDiet 5001, which contains approximately 810mg/kg isoflavones (Brown and Setchell, 2001), compared to Teklad 2016. Similarly, Lee *et al* (2009) found that inclusion of 526.9mg/kg soy extract into the diet for 16 weeks improved the performance of OVX rats in the Morris Water Maze.

Whether these data can be extrapolated to humans remains uncertain, for two reasons. First, there are significant species differences in phytoestrogen metabolism. Rats and humans differ greatly in the amount of equol biosynthesis. In Sprague-Dawley rats consuming a diet containing soy phytoestrogens, approximately 77% of the circulating isoflavonoids was found to be equol. By contrast, in women consuming a diet containing a soy protein isolate, in whom daidzein and genistein represented more than 80% of the circulating isoflavones, equol was undetectable (Gu et al., 2006). Species differences in the extent of isoflavonoid conversion to Sequol could thus have significant effects on its biological activity. Differences in the further catabolism of the soy phytoestrogens could also contribute, because rats and mice appear to conjugate isoflavones less efficiently than humans (Setchell et al., 2011).

Second, dietary isoflavonoid intake is typically higher in laboratory animals than in humans. Although the dose of phytoestrogens used in the present study is in the low to mid-range of studies examining the estrogenicity of these compounds in rodents (Lee et al., 2009; Luine et al., 2006; Lund et al., 2001), it is still considerably greater than typical human dietary consumption rates. At the end of the present experiment, average daily food intake was 18–20g/d, consistent with literature values for Teklad 2018 in young adult female rats (Rodrigues et al., 2009). At 62 days of age, when the animals in all three treatment groups weighed 190–200g, their daily isoflavonoid intake was thus approximately 10–12 mg/kg each of daidzein and genistein. The highest natural human dietary phytoestrogen intake is found in people consuming traditional South Asian diets, in whom isoflavonoid consumption may be as high as 25–100mg/d (i.e. approximately 0.5 – 2 mg/kg body weight daily (Bakker, 2004; Mei et al., 2001). People on Western diets typically consume

considerably less. The isoflavonoid intakes in the present study are thus at least 10 times higher, on a dose/kg body weight basis, than in people consuming a relatively soy-rich diet. Although, as noted above, extrapolation between species is difficult because of species differences in isoflavonoid metabolism, dose levels may be one of the reasons why animal studies have demonstrated positive cognitive effects of dietary phytoestrogen exposure (Lee et al., 2009; Luine et al., 2006; Lund et al., 2001), while human clinical studies have yielded more variable results (Henderson et al., 2012; Soni et al., 2014; St John et al., 2014; Zhao, Brinton, 2007). The only human population in which isoflavonoid consumption rates approach those of animal studies comprises people consuming soy extracts, which may deliver as much as several hundred mg of isoflavonoids a day (Pop et al., 2008); but here again pharmacokinetic factors may preclude direct comparisons based purely on isoflavonoid dose (Gardner et al., 2009). Preliminary studies have suggested that supplementation with purified soy isoflavones may improve cognitive performance, in both men and women (Gleason et al., 2009; Thorp et al., 2009). However, these studies were based on relatively small subject numbers and it remains uncertain whether soy isoflavone supplementation has beneficial effects, in larger human populations (Clarkson et al., 2011).

In summary, we observed a robust difference in CA1 spine synapse density between rats maintained on a standard rat diet (Teklad 2018) and animals on an isoflavonoid-free diet (Teklad 2016). This difference was eliminated when genistein and daidzein were added to Teklad 2016 at concentrations similar to those found in Teklad 2018. Given the natural variation that occurs in the isoflavonoid content of soy meal-containing laboratory animal diets (Heindel and vom Saal, 2008; Lee et al., 2003; Thigpen et al., 2007), these data indicate that careful control of dietary isoflavonoid intake should be exercized in studies examining the relationship between hippocampal synaptic plasticity and behaviors that involve hippocampal function.

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## Highlights

- Effects of isoflavonoids on spine synapse density were examined in area CA1 of the rat hippocampus
- Feeding a diet containing daidzein and genistein for 40 days doubled CA1 spine synapse density
- Dietary isoflavonoid intake should be controlled in studies of hippocampal structure and function

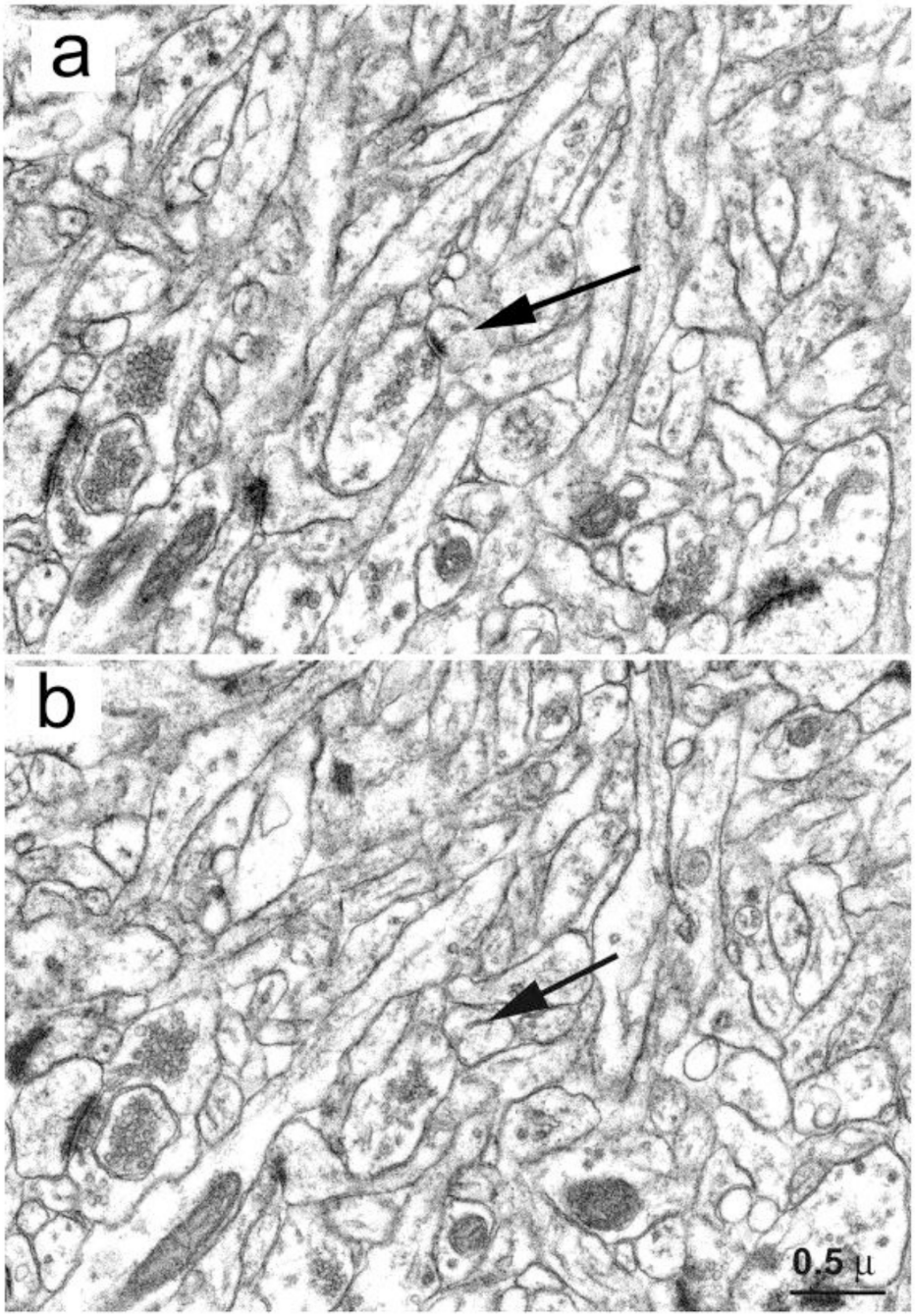


**Figure 1.**

Electron micrographs show matching areas on two consecutive, serial sections from the stratum radiatum of the CA1 area. (a) and (b) show the look-up and reference sections, respectively. Only those spine synapses were counted that were seen just in one of the section pairs (arrows). Scale bar (lower right, panel b) = 0.5µm.

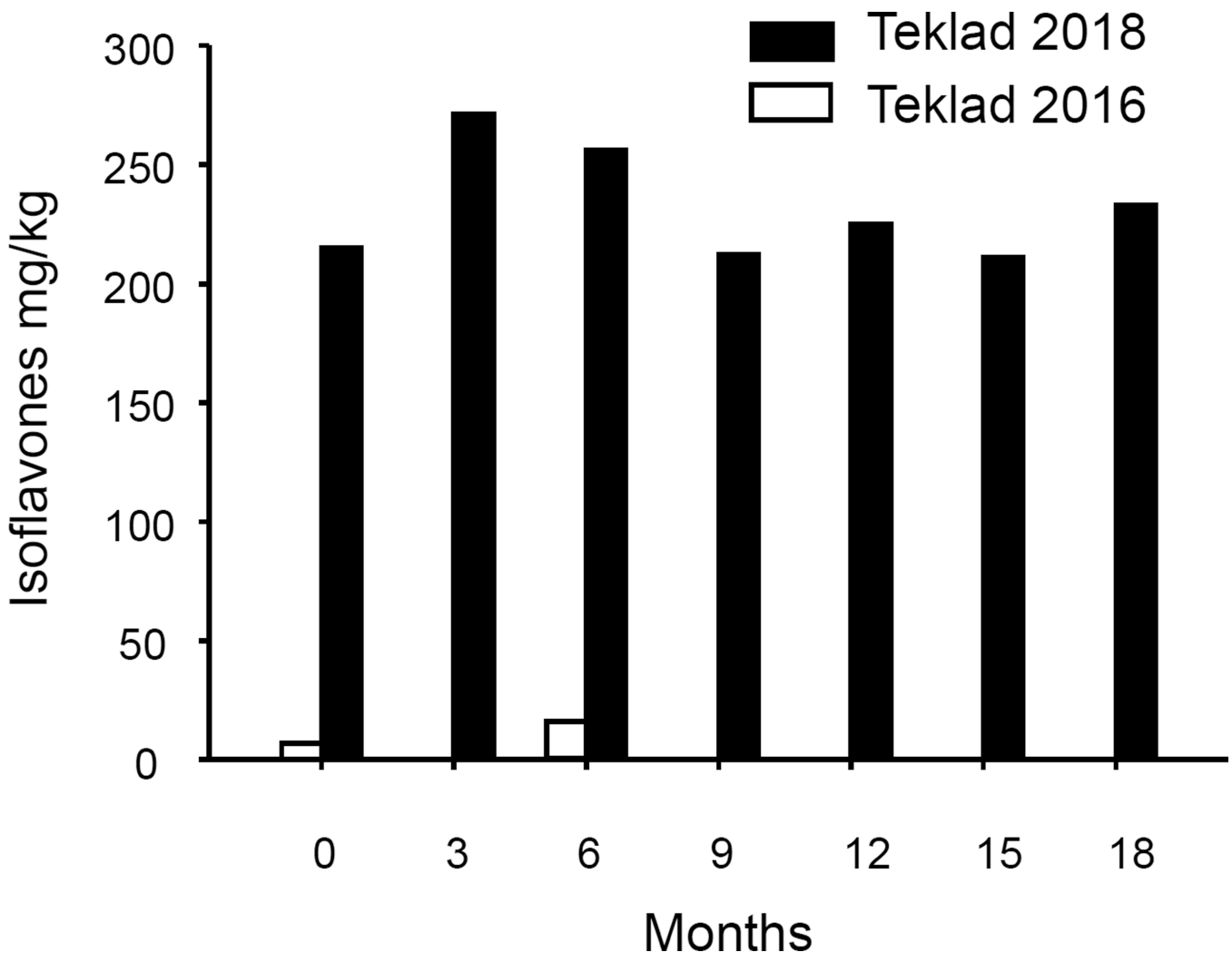


**Figure 2.**

Isoflavonoid content of the Teklad 2018 and Teklad 2016 diets. Batches of each preparation were assayed at three month intervals to establish the reproducibility of total isoflavonoid content. Isoflavonoid content was determined as previously described (Wang and Murphy, 1994). All seven samples of Teklad 2018 contained approximately 200–250 mg of total isoflavonoids/kg (231.9 +/− 8.8 mg/kg), reflecting the isoflavonoid contents of the soy meal used in manufacturing the diets (1.72, 2.20, 2.27, 1.65, 1.91, 1.66 and 1.79 g/kg, respectively, for the seven batches analyzed). Only two samples of Teklad 2016 contained measurable amounts of isoflavonoids (3.28+/− 2.34 mg/kg).

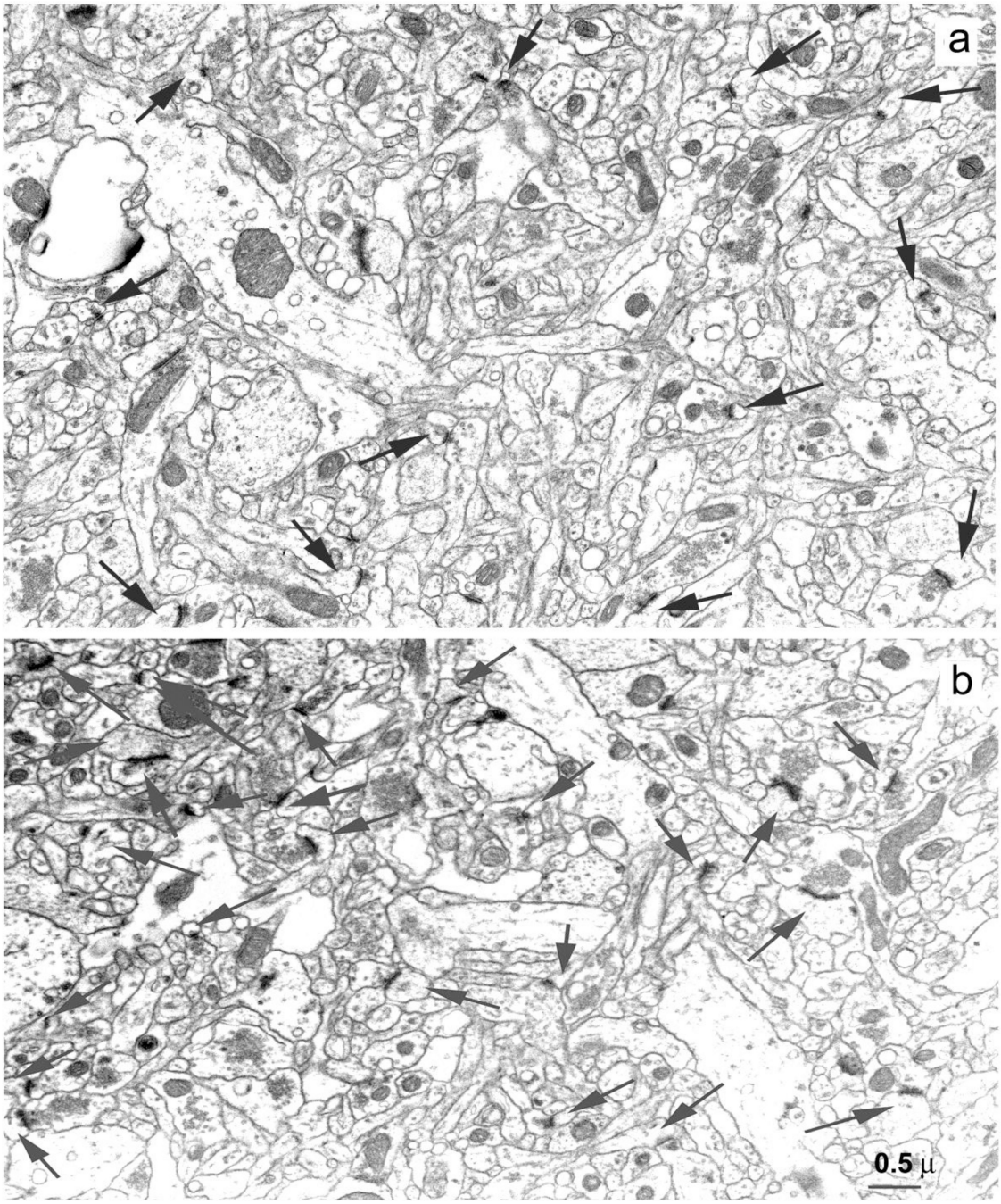


**Figure 3.**

Electron micrographs from the stratum radiatum of the CA1 area of rats maintained on Teklad 2016 diet (a) or Teklad 2016 supplemented with 112 mg/kg genistein and 108 mg/kg daidzein (b). Arrowheads point to spine synapses. Note the greater synapse density in the section from an animal supplemented with genistein and daidzein. Scale bar (lower right, panel b) = 0.5µm.

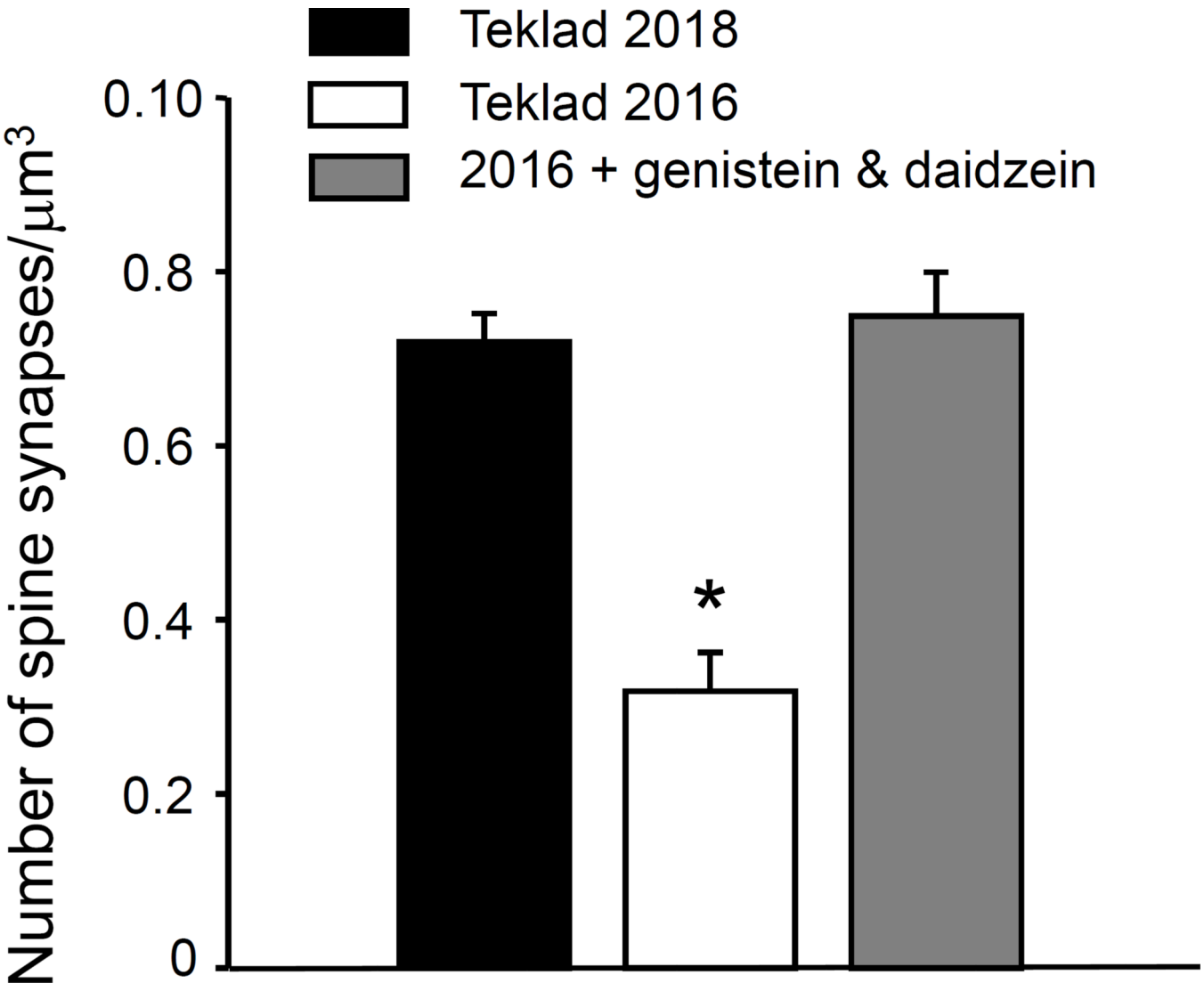


**Figure 4.**

Pyramidal cell dendritic spine synapse densities in the CA1 stratum radiatum of OVX rats maintained between the ages of 22 and 62 days on each of the three test diets. Results represent the means + SEM of the average synapse densities determined in four animals per treatment group. Black bar: control Teklad 2018 diet; white bar: Soy-free Teklad 2016 diet; grey bar: Teklad 2016 supplemented with 112 mg/kg genistein and 108 mg/kg daidzein. * Significantly different from data from rats maintained on either Teklad 2018 or Teklad 2016 supplemented with genistein and daidzein (Scheffé test; $p<0.05$ level).

## Table 1

| Weaning Diets introduced | OVX | CA1 Spine Density assessment | # of animals |
|---|---|---|---|
| 22 days | 54 days | 62 days | |
| Diet compositions: | | | |
| Group 1 Teklad 2018 total isoflavonoids 232 mg/kg | | | 4 |
| Group 2 Teklad 2016 (isoflavonoids undetectable) | | | 4 |
| Group 3 Teklad 2016 + 112 mg/kg genistein & 108 mg/kg daidzein | | | 4 |

Summary of the experimental protocol. Animals were received from the supplier and immediately switched onto one of the three test diets. After ovariectomy (OVX) at 54 days, the animals were maintained on their regular schedule for a further 8 days, and then perfused for CA1 spine synapse density assessment.

## Table 2

| Macronutrient Content | Units | Teklad 2018 | Teklad 2016 |
|---|---|---|---|
| Crude Protein | % | 18.6 | 16.4 |
| Fat | % | 6.2 | 4 |
| Crude Fiber | % | 3.5 | 3.3 |
| Ash | % | 5.3 | 4.9 |
| Insoluble Fiber | % | 14.7 | 15.2 |
| Carbohydrate (available) | % | 44.2 | 48.5 |
| Energy Density | kcal/g (kJ/g) | 3.1/(13.0) | 3.0/(12.6) |
| Calories from Protein | % | 24 | 22 |
| Calories from Fat | % | 18 | 12 |
| Calories from Carbohydrate | % | 58 | 66 |

Macronutrient contents of the two diets used in this study. Details of the other components of these two diets are provided on the manufacturer's Web sites: (http://www.envigo.com/resources/data-sheets/2018-datasheet-0915.pdf; http://www.envigo.com/resources/data-sheets/2016-datasheet-0915.pdf).