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Pelleting Diets Impairs TRAMP Prostate Carcinogenesis

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Abstract

Diets rich in soy products may reduce the risk of prostate cancer (PCa). Daidzein, the major isoflavone present in soy germ, can be metabolized by the gut microbiota into equol. The effects of daidzein and equol on PCa have not been well studied. The objective of this study was to investigate the effect of feeding 2% soy germ, 92 ppm daidzein, or 88 ppm equol diets on the progression of PCa in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. 3-week old male C57BL/6 X FVB TRAMP mice were weaned from our breeding colony and immediately acclimated to an AIN-93G control diet for one week. At 4 weeks of age, mice (n = 30 per diet group) were randomized to one of four pelleted study diets until 18 weeks of age. Unexpectedly, we did not detect any statistical differences in cancer incidence between diets. We suggest that these results are due to the physical attributes of the pelleted diets in the current study. Mice fed pelleted diets had reduced food intake and significantly decreased body weights ($p < 0.001$) compared to previous studies. A reduction in food intake is known to reduce cancer incidence in a number of cancer models and is likely to have contributed to the decrease in expected cancer incidence in the current study. In conclusion, we suggest that the hardness of the diets pellets could result in a decreased cancer incidence in TRAMP mice.

Keywords

Diet, Pelleted, Cancer, Soy, TRAMP

1. Introduction

Over the last 30 years, evidence that a diet rich in soy products may be protective against PCa, has been growing. Soy products contain an array of bioactive compounds including saponins, lignans, and the isoflavones genistein, daidzein, and

glycitein. Intake of isoflavones varies geographically. Consumption of isoflavone-rich foods such as tofu, tempeh, and miso is common in Asian countries where dietary intakes of isoflavones have been estimated to range from 15 mg/day in China to 26 - 54 mg/day in Japan, while the average intake of isoflavones in Western countries is estimated at 3 mg/day [1] [2].

Our lab recently demonstrated that consumption of a 2% soy germ diet reduced the incidence of PCa in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model by 34% compared to the control diets [3]. Soy germ, the hypocotyl of the soybean, has a radically different isoflavone profile than whole soybeans. It contains considerably higher levels of daidzein and glycitein and relatively low levels of genistein when compared to whole soybeans. Soy germ is currently utilized in dietary supplements, and food scientists have been investigating ways to incorporate it into functional foods for disease prevention [4] [5] [6] [7]. Therefore, it would be beneficial to determine the anticancer effects of soy germ and its mechanisms of action.

Genistein, the predominant isoflavone in soy products, has been investigated for its anti-proliferative, antioxidant, and chemopreventive properties in cell, animal, and human models [8] [9]. Although genistein has been associated with a protection against several cancers and other chronic diseases, there is a need to investigate the effects of other commonly-consumed isoflavones, daidzein, glycitein, and their metabolites.

Epidemiological studies suggest a reduced risk of PCa in men who consume soy products [10], and this effect appears to be especially pronounced in men who are "equol producers" (>20 nM serum equol) [11]. Daidzein can be metabolized by the gut microbiota into the weak estrogen-like compound, equol. It is not well understood if equol production is responsible for the protective effect observed with soy intake. Equol has been shown to have a higher binding affinity for estrogen receptor-β (ERβ) than daidzein [12]. Equol has also been shown *in vitro* and *in vivo* to bind 5α-dihydroxytestosterone (DHT), thereby inhibiting its binding to the androgen receptor modulating androgen signaling in the prostate [13]. This is significant because equol's ability to be a selective estrogen modulator as well as an androgen antagonist makes it a promising target to study for hormonally-driven cancers like PCa.

In TRAMP mice, we previously demonstrated that prostatic equol was 39 times higher than genistein and 3 times higher than daidzein in mice consuming soy germ [3]. The elevated concentration of equol in the prostate suggests that equol was a critical contributor to the anticarcinogenic effects of soy germ found in the TRAMP model [3]. If the benefits from soy germ are primarily attributed to equol production, then an estimated 70% - 75% of the Western population would not benefit from soy germ consumption as it is estimated that only 25% - 30% of the populations in Western countries are equol "producers" compared to up to 80% of individuals in China, Japan, and South Korea [14]. Limited *in vivo* studies have directly examined feeding equol and the incidence of PCa in rodents. Due to the previously observed protective effect of soy germ, we designed

this study to follow up on the results from Zuniga *et al.* and compare the effects of soy germ, daidzein, and equol in prostate carcinogenesis [3]. We hypothesized that equol was primarily responsible for the protective effect of soy germ, and that the soy germ, daidzein, and equol-fed mice would have reduced cancer incidence compared to the control group.

2. Materials and Methods

2.1. Animal Methods

The University of Illinois Laboratory Animal Care Advisory Committee approved all animal procedures. Female and male heterozygous TRAMP (C57BL/6) mice from our colony were bred with FVB/NJ mice to obtain (TRAMP × FVB/NJ)F_1 offspring for the study. A minimum of 28 mice per diet group were projected to be needed to reach statistical significance by power analysis ($\alpha = 0.05$, $\beta = 0.8$) for cancer incidence and two extra mice were added to each diet group to account for the occasional unforeseen death of study animals. Mice were genotyped via PCR-based DNA (Sigma-Aldrich, Saint Louis, MO) screening using established methods [3]. Offspring were weaned at 3 weeks of age, individually housed in shoebox cages under controlled conditions (12 h light/dark cycle, 22˚C, 60% humidity) and acclimated to a pelleted, modified semi-purified AIN-93G diet for one week. The modified AIN-93G included corn oil instead of standard soybean oil as the fat source (Table 1). At 4 weeks of age, mice were

Table 1. Composition of experimental diets.

	g/100 g total diet			
	Control (Modified AIN-93G)	2% Soy Germ[a]	Daidzein[b]	Equol[c]
Cornstarch	39.7	39.8	39.7	39.7
Casein	20	19	20	20
Maltodextrin	13.2	13.2	13.2	13.2
Sucrose	10	10	10	10
Fiber[d]	5	4.3	5	5
Mineral Mix[e]	3.5	3.5	3.5	3.5
Vitamin Mix[f]	1	1	1	1
L-Cystine	0.3	0.3	0.3	0.3
Choline Bitrate	0.25	0.25	0.25	0.25
Corn Oil	7	6.5	7	7
TBHQ, antioxidant	0.0014	0.0014	0.0014	0.0014
Soy Germ[g]	0	2	0	0
Daidzein	0	0	0.0092	0
Equol	0	0	0	0.0088

[a]2% Soy Germ diet contains 71 ppm daidzein equivalents, 68 ppm glycitein equivalents, and 35 ppm genistein equivalents. [b]Daidzein diet contains 92 ppm daidzein equivalents. [c]Equol diet contains 88 ppm equol equivalents. [d]Non-nutritive cellulose. [e]AIN-93G-MX formation. [f]AIN-93G-VX formation. [g]Frutarom Soy-Life® Complex Micro.

randomized to consume one of four experimental diets: AIN-93G control, AIN-93G + 2% soy germ, AIN-93G + 82 ppm daidzein, or AIN-93G + 88 ppm equol. The daidzein diet included 92 ppm of purified daidzein to match levels found in a 2% soy germ diet. Equol was matched to daidzein equivalents found in a 2% soy germ diet with the assumption that 100% of the daidzein would be metabolized to equol by the highly efficient microbiota in the mouse [15]. Non-transgenic control littermates (n = 10 per diet group) were included in the study to confirm the effects of the transgene.

Mice were weighed weekly and individual food intake was measured when fresh food was provided weekly. At 18 weeks of age, mice were asphyxiated by CO_2 and blood was collected via cardiac puncture. Serum was separated from the red blood cell volume following centrifugation at 4˚C for 10 minutes, aliquoted, frozen, and stored at −80˚C for future analyses. The prostate was micro-dissected into individual lobes (anterior, ventral, and dorso-lateral). One half of each prostate lobe was fixed overnight in 10% phosphate-buffered formalin and then transferred to 70% ethanol for histological evaluation. All animals were thoroughly examined for gross metastases by trained research staff during the necropsy. A section of liver, lungs, and lymph nodes were fixed in 10% phosphate-buffered formalin and transferred to 70% ethanol after 24 hours for evaluation of micro-metastases. Liver, testes, spleen, gonadal adipose, and one half of each prostate lobe were flash frozen in liquid nitrogen and stored at −80˚C.

2.2. Diet Formulation and Isoflavone Analysis

Experimental diets were pelleted and prepared as a custom AIN-93G formulation by Harlan Laboratories (Madison, WI). Diets were balanced for protein, carbohydrates, fat, energy, and fiber, provided 3.8 Kcal/g, and were stored at 4˚C in the dark. Experimental diet compositions are presented in **Table 1**. Diets were pelleted to ensure proper mixing and distribution of the soy germ, daidzein, and equol. Soy germ was analyzed for daidzein isoflavone equivalents. Isoflavone contents of the final experimental diets were analyzed by the National Center for Toxicological Research in Jefferson, AR using HPLC-UV. Briefly, 500 mg of diet was crushed and ground into a powder and placed in a 15 mL centrifuge tube. 2.5 mL of 80% methanol:water (80:20, v/v) was added to each tube. Samples were vortexed and then sonicated for 30 minutes. The supernatant was removed following centrifugation and placed into a 10 mL volumetric flask. The above methanol:water addition, vortex, sonication, and centrifugation was performed 3 - 5 more times and the supernatants were combined for each sample. The combined supernatant was filtered prior to HPLC analysis. Isoflavone analysis of prepared diets is presented in **Table 2**.

2.3. Serum Isoflavone Analysis

Serum isoflavones were analyzed by the National Center for Toxicological Research in Jefferson, AR using HPLC-UV [16]. The limits of detection of genistein, daidzein, and equol were 0.004, 0.002, and 0.03 μM respectively.

Table 2. Isoflavone analyses of the prepared diet (ppm).

Diet	AIN-93G + 2% Soy Germ (ppm)	AIN-93G + 92 ppm Daidzein (ppm)	AIN-93G + 88 ppm Equol (ppm)
Genistein	35	0	0
Daidzein	71	90	0
Glycitein	68	0	0
Equol	0	0	80

Table 2 shows the isoflavone analyses in the diets. Genistein, daidzein, glycitein, and equol were detected in the soy germ diet. Only daidzein and equol were detected in their respective diets. Values are the mean of n = 5.

2.4. Histopathology

Formalin-fixed, paraffin-embedded prostate sections were stained with hematoxylin and eosin (H&E) and blindly evaluated by a pathologist using an established and published grading scheme [17]. Each lobe of the prostate was evaluated for the most severe lesion and the most common lesion in each lobe. Additionally, sections of liver and lungs from mice with gross tumors and visible lymph node metastases were evaluated for the presence of micro-metastases. Results were compared using Fisher's exact test between treatment groups and the control.

2.5. Immunohistochemistry

Immunohistochemistry for proliferating cell nuclear antigen (PCNA) and cleaved caspase-3 (CC3) were performed on paraffin-embedded (4 μm) fixed sections of the dorso-lateral prostate lobe according to a previously published protocols [18] [19]. Briefly, slides were placed in a decloaking chamber and treated in a citrate buffer (pH 6.0) for 30 seconds at 125˚C and 10 seconds at 90˚C for antigen retrieval. In a BioGenex i6000 Automated Staining System (BioGenex, San Ramon, CA), endogenous peroxide was quenched with a 3% H_2O_2 solution for 15 minutes, slides were blocked with Power Block™ (BioGenex) for 10 minutes, avidin blocked for 15 minutes, and then incubated with rabbit anti-proliferating cell nuclear antigen (PCNA) antibody (Abcam, Cambridge, MA) or rabbit anti-cleaved caspase-3 (Cell Signaling, Danvers, MA) for 30 minutes and visualized using a Super Sensitive™ Link-Label IHC Detection System (BioGenex). Slides were stained with DAB and counterstained with hematoxylin. Mouse small intestinal tissue was used as the positive control for PCNA and mouse thymus tissue was used as the positive control for cleaved caspase-3. Negative controls were generated by omitting the primary antibody. Stained slides were scanned with a NanoZoomer 2.0-HT digital slide scanner (Hamamatsu, Bridgewater, NJ) with Olympus Uplansapo 20× objective at 40X digital zoom, resulting in 0.23 μm resolution. Images were captured with NDP view software (Hamamatsu). One image from the dorso-lateral lobe was blindly quantified and the proliferative index (PI) percentage (PCNA positive-stained nuclei/total nuclei counted ×100) and apoptotic index (AI) percentage (number of activated caspase-3 cells/otal nuclei counted ×100) calculated. These indices

were established by counting at least 1000 randomly selected cells from each image.

2.6. Serum Testosterone

Serum testosterone was measured in TRAMP mice fed control (n = 10), soy germ (n = 10), daidzein (n = 10), and equol (n = 10) diets. Serum testosterone was extracted from 150 μL of serum using di-ethyl ether. After the ether evaporated, a hexane and methanol extraction was used to extract the testosterone from other serum lipids for analyses. Serum testosterone was evaluated using Coat-A-Count total testosterone radioimmunoassay (Siemens TKTT2, Los Angeles, CA).

2.7. Statistical Analyses

Statistical analyses were completed with SAS software (version 9.2; SAS Institute, Cary, NC, USA). Body weight, food intake, serum isoflavones, serum, testosterone, and proliferation and apoptosis indexes were compared among treatments by analysis of variance (ANOVA) when the assumptions of ANOVA were met, and followed by post-hoc Tukey-Kramers studentized range test with $\alpha = 0.05$; when the assumptions of ANOVA were not met, the Wilcoxon and Kruskal-Wallis non-parametric test was chosen. Differences in cancer incidence by histopathology and diet group were analyzed using Fisher's exact test.

3. Results

3.1. Weight Gain and Feed Intake

Body weight and food intake were measured weekly and recorded. Food intake was not significantly different between groups (Table 3). Average food intake was similar to other studies of TRAMP mice consuming pelleted diets [20] [21], but 50% less than powdered diets fed by Zuniga *et al.* [3]. Body weights were not significantly different between groups. Organ weights of the urogenital tract, liver, lungs, testes, gonadal adipose, spleen, ventral prostate, anterior prostate, and dorso-lateral prostate were not different between groups (data not shown).

Table 3. TRAMP body weights and food intake.

	AIN-93G Control	AIN-93G + 2% Soy Germ	AIN-93G + 92 ppm Daidzein	AIN-93G + 88 ppm Equol
Beginning Body Weight (g)	20.2 ± 3.7	19.4 ± 3.6	20.3 ± 3.8	20.6 ± 3.8
Ending Body Weight (g)	27.3 ± 5	27 ± 5	27.7 ± 5	28 ± 5
Average Food Intake (g/day)	2.3 ± 0.4	2.3 ± 0.4	2.3 ± 0.4	2.2 ± 0.4
n	30	29	30	30

Table 3 shows that there is no difference between body weights and food intakes between diet groups in this study. Values are the mean ± SEM.

3.2. Serum Isoflavones

Genistein, daidzein, and equol were below the limit of detection in the AIN-93G control-fed mice. Genistein was only detected in mice consuming soy germ. Daidzein was only detected in the serum of mice fed soy germ or the daidzein diet and was significantly different between the two groups ($p = 0.048$) (Table 4). Equol was detected in all the mouse serums from experimental diet groups except for the control diet and was not statistically different between groups. Differences in serum isoflavones between diets were analyzed using ANOVA and the Tukey-Kramer studentized range test ($\alpha = 0.05$).

3.3. Histopathology

There were no differences in overall combined cancer incidence between diet groups (Figure 1). Mice fed the control, soy germ, daidzein, and equol diets had a 24%, 31%, 20%, and 28% incidence of cancer overall respectively. There was also no difference in cancer incidence by diet between anterior, ventral, or dorso-lateral lobes individually (data not shown). Furthermore, we were not able to detect any differences between pathology scores between diet groups (Table 5).

Table 4. TRAMP serum isoflavone analyses.

	Serum (μM)			
Diet	Control	2% Soy Germ	Daidzein	Equol
Genistein	ND	0.11 ± 0.05	ND	ND
Daidzein	ND	0.44 ± 0.27[a]	0.17 ± 0.18[b]	ND
Equol	ND	2.67 ± 1.92	1.44 ± 0.46	3.37 ± 2.12

Table 4 shows serum levels of isoflavones between diet groups. A significant difference was found in the level of serum daidzein between the soy germ-fed and the daidzein-fed groups. Values are Mean ± SEM. ND = Not detected in the serum. Superscript letters in the same row indicate a significant difference between groups ($p = 0.048$). $n = 7$ per group.

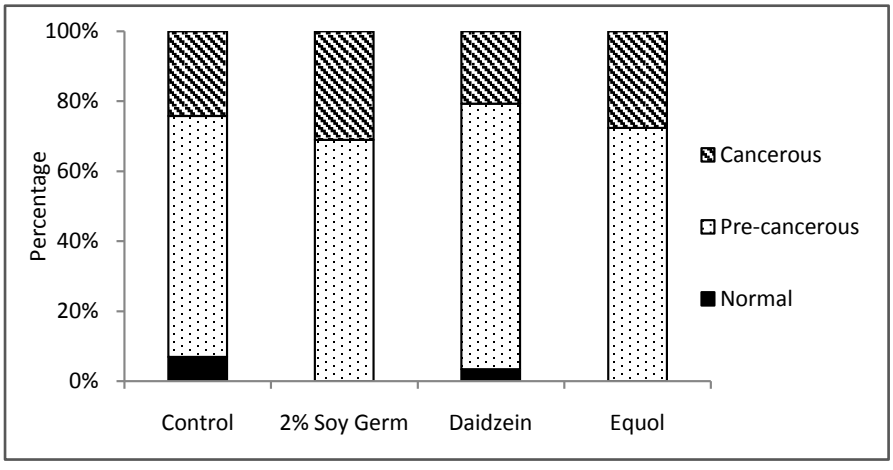

Figure 1. Overall prostate cancer incidence in TRAMP mice. (In Figure 1, different patterned bars represent the distribution of cancer severity among diets. No differences were present between the experimental diets and the control or between experimental diets. n = 29 - 30/diet group).

3.4. Immunohistochemistry

There was no statistical difference between AI (Figure 2) or PI (Figure 3) between diet groups in prostates with HGP.

3.5. Serum Testosterone

There were no statistical differences in serum testosterone levels between groups (Figure 4). Furthermore, we examined serum testosterone levels in wild-type non-transgenic mice (n = 5/diet) to determine the impact of our diets on serum testosterone in a non-cancer model. There were no differences between groups, though the daidzein group was numerically lower than the other diet groups (Figure 5).

4. Discussion

Due to previous findings in our laboratory [3], we hypothesized that diets incorporating 2% soy germ, daidzein, or equol would equally reduce the incidence

Table 5. TRAMP histopathology results as a percentage of total prostatic lesions by diet results are the incidence of each stage of pathology and overall incidence (sum of WD-PD) within dietary groups.

		PIN				Adenocarcinoma			Prostate Cancer (WD-PD)
TRAMP									
Diet	n	NP	LG	MG	HG	PLL	WD	MD	PD	
AIN-93G Control	29	7%	0%	21%	41%	7%	3%	10%	10%	24%
AIN-93G + 2% Soy Germ	29	0%	3%	3%	55%	7%	3%	0%	28%	31%
AIN-93G + 82 ppm Daidzein	29	3%	7%	3%	66%	0%	3%	7%	10%	20%
AIN-93G + 88 ppm Equol	29	0%	0%	3%	59%	10%	3%	17%	7%	28%

NP = Normal Prostate, LG = low grade PIN, MG = moderate grade PIN, HG = high-grade PIN, PLL = phyllode-like lesions, WD = well differentiated, MD = moderately differentiated, PD = poorly differentiated.

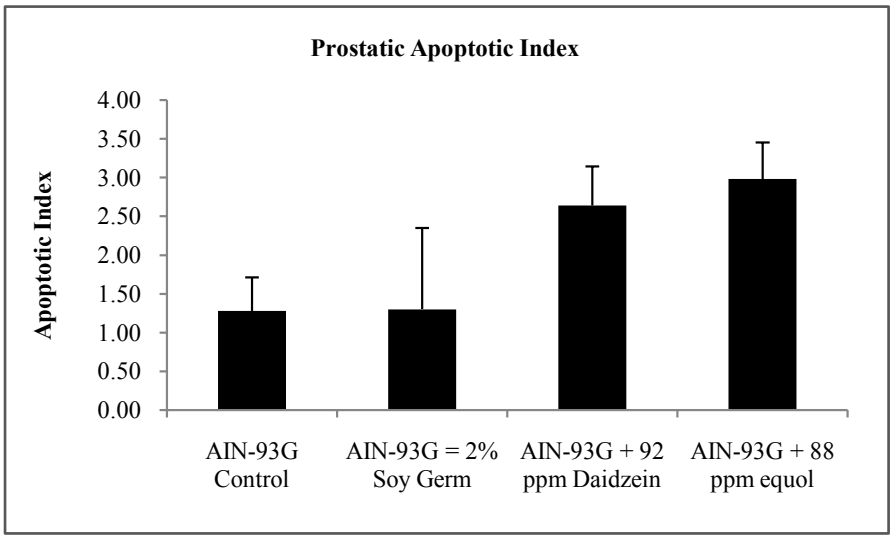

Figure 2. TRAMP prostatic apoptotic index. (Values are means ± SEM. n = 5/diet groups).

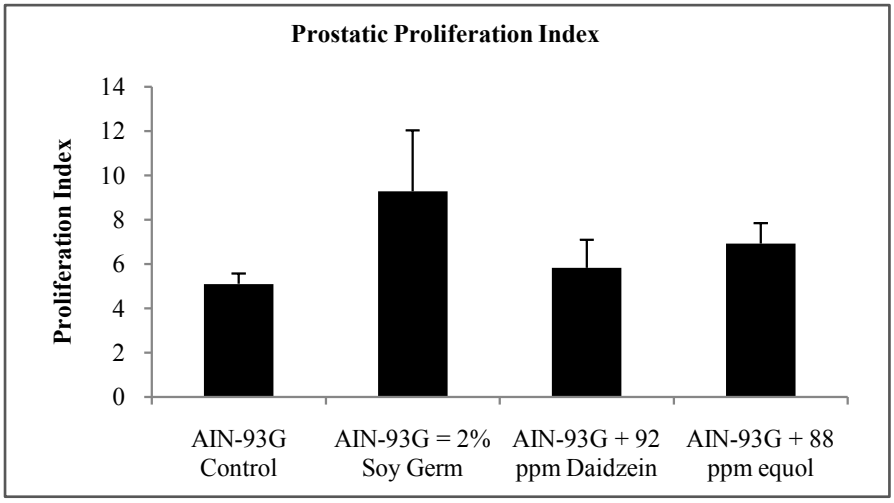

Figure 3. TRAMP proliferation index. (Values are means ± SEM. n = 5/diet group).

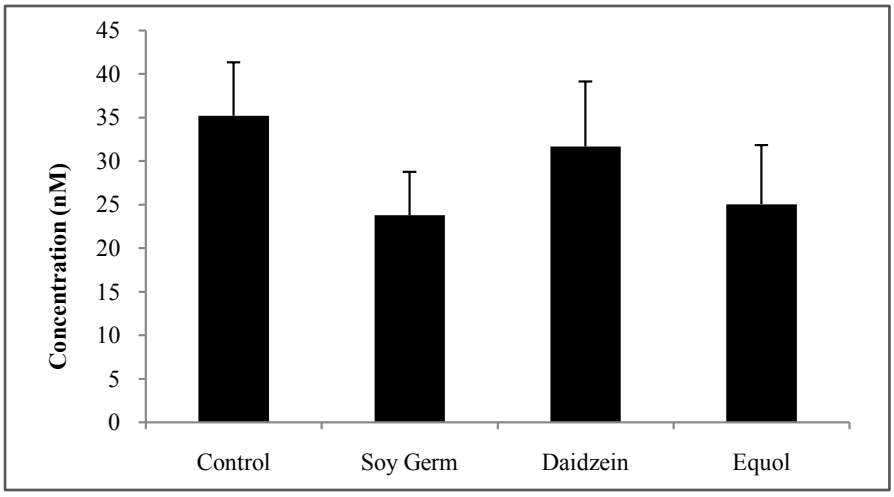

Figure 4. TRAMP serum testosterone. (**Figure 4** shows serum levels of testosterone in TRAMP mice. There were no significant differences detected between diet groups. Values represent mean ± SEM. n = 10/diet group).

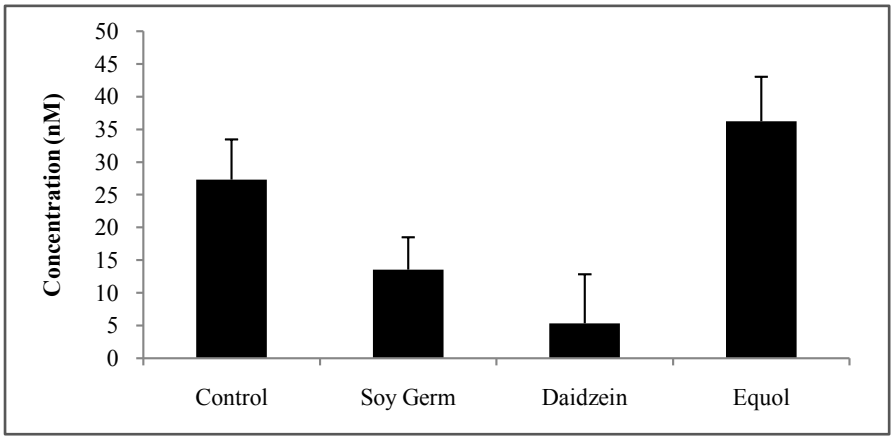

Figure 5. Wild-type serum testosterone. (**Figure 5** shows serum levels of testosterone in wild-type mice. There were no significant differences detected between diet groups. Values represent mean ± SEM. n = 10/diet group).

of PCa in TRAMP mice compared to the control diet. We further hypothesized that the mechanism would be due to the isoflavone metabolite, equol. Contrary to our hypothesis, we did not detect any dietary differences in cancer incidence. Mice in the control group only had a 24% incidence of cancer whereas in a previous study in our lab, mice in the control group had a 100% incidence of cancer at the same 18-week time point [3]. Despite using the same animal model, rodent colony, animal facility, sacrifice age, lot of soy germ, and animal procedures, we were not able to repeat the previous findings [3]. Similar levels of isoflavones were detected in the serum and diets from both studies. The only difference, aside from specific diets used, was the physical format that the diet was provided to the rodents. Zuniga *et al.* fed rodents a powdered version of the AIN-93G-based diets, while the current study provided the similarly-formulated diets in pelleted form [3].

The diet's physical form resulted in drastically different food intakes between the studies. Zuniga *et al.* reported that mice consumed 5.7 grams/day while in the current study only 2.3 grams/day were consumed [3]. It should be noted that measurement of powdered food intake is less precise than with pelleted food intake, as powdered food tends to be distributed in bedding and in the cage, so at best, we can only get an estimate of powdered food intake in these studies. Nonetheless, pelleted food intake in the current study was consistent with other studies of TRAMP mice consuming pelleted diets [20] [21]. The differences in food intake resulted in significantly different body weights between the two studies (**Figure 6**). Mice fed pelleted diets in the current study were significantly lighter than mice fed powdered diets in the previous study as early as 8 weeks of age ($p < 0.001$) and continuing until animals were sacrificed at 18 weeks of age (**Figure 6**) ($p < 0.001$).

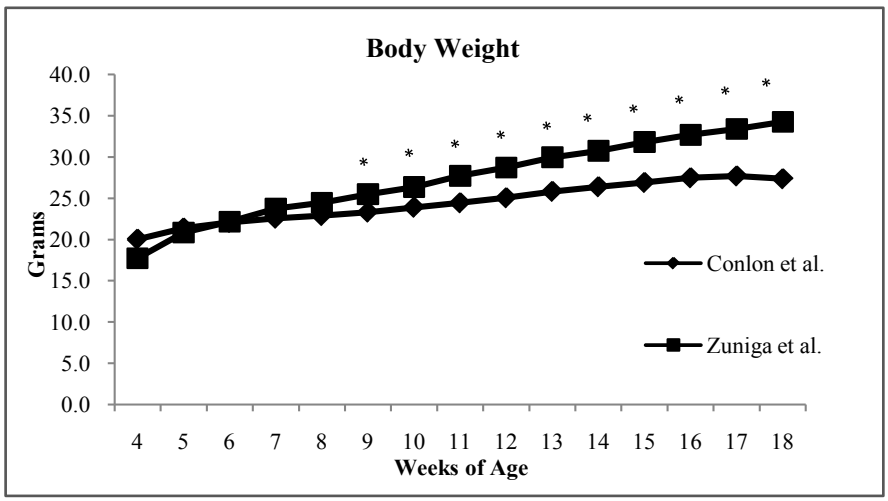

Figure 6. TRAMP body weight comparison between Zuniga *et al.* and Conlon *et al.* (**Figure 6** is a graphical representation of body weights between the current study and a previous study by Zuniga *et al.* [3]. *Represents a significant difference ($\alpha < 0.05$) between Zuniga *et al.* and the current study, Conlon *et al.* at specific time points. Values represent the mean body weight across all diets at each week of age. Both studies n = 29 - 31 were weighed at each time point).

The associations between diet format and obesity have been examined extensively in rodent models [22] [23] [24]. Ford *et al*. described the association between food consumption and diet hardness in mice, and observed that increasing the hardness of food, negatively impacted growth and food intake [25]. Additionally, rats fed soft pellets developed obesity faster than their counterparts fed hard pellets [26]. Desmarchelier *et al*. examined the impact of feeding a control, high-fat, and Western diet to mice in either pelleted or powdered form [24]. The results of that study indicated that regardless of diet composition, all mice fed powdered diets had similar weight gains [24]. This suggests that the texture and hardness of the food, rather than the nutrient composition, promoted hyperphagia and obesity [24]. Furthermore, in another study, long-term ingestion of powdered food induced hyperglycemia and systemic illness, increased adrenal gland activity, and increased blood pressure in mice [22]. This could be a result of easier access to powdered foods (bowls of food in the cage vs. food provided above the cage in a rack), ingestion of powdered food from fur while cleaning, and/or faster rates of absorption of powdered food [22]. Together these findings suggest that the dietary format of food impacts the growth, development, and systemic health of healthy rodents. A diet in powdered format that contributes to excessive weight gain and systemic illness may promote carcinogenesis in a transgenic mouse model of PCa while pelleted diets might delay the onset of carcinogenesis.

It has been previously shown that TRAMP mice fed a high-fat Western diet have accelerated tumor growth and tumor burden compared to the control-fed mice [27]. Alternatively, caloric restriction has been shown to reduce PCa incidence and progression in TRAMP mice [20] [28]. A 20% dietary restriction beginning at 7 weeks of age resulted in significant reductions in PCa incidence at 11 and 20 weeks of age in TRAMP mice [29]. Additionally, TRAMP mice who were intermittently or chronically calorically restricted had a decreased incidence of PCa [20]. While in the current study, the mice were not purposely calorically restricted and were allowed access to their food *ad libitum*, they consumed nearly 50% fewer grams of food/day than mice fed powdered food and weighed significantly less than mice fed powdered food [3]. This reduction in food intake most likely accounted for the reduction in cancer incidence observed.

However, changes in caloric intake and energy balance and expenditure also alters cancer incidence in TRAMP mice. While caloric restriction alone has been shown to reduce cancer incidence in the TRAMP model, changes in energy balance and excess caloric retention contribute to cancer progression. Mice housed at 22˚C expend more energy and have lower body mass than mice housed closer to their thermoneutral zone (30˚C - 35˚C) [30]. Interestingly, when mice are housed a 22˚C, they consume 30% more calories, but have less body mass than mice housed at 27˚C [30]. This suggests that mice consume more food to compensate for thermoregulatory demands, but that energy balance plays a role beyond food intake. The use of pelleted diets in the current study and the tem-

perature mice were housed at (22˚C) combined to produce an environment where mice had to expend energy to consume food (standing/reaching for pellets) and expend energy to maintain thermoregulation, ultimately keeping their body weight lower. Mice in prior studies in our lab fed powdered diets slept in their food bowls, consumed diet when grooming, and ultimately gained more body weight than mice in the current study [3]. These results suggest that pelleted diets fed *ad libitum* resulted in decreased body weight and decreased cancer incidence compared to mice fed powdered diets *ad libitum* [3]. The decrease in body weight was likely a contributing factor to the decrease in expected cancer incidence in all dietary treatment groups.

In this study, cancer incidence was remarkably lower in the TRAMP model than previous studies in our lab [3] [31]. The reduction in cancer incidence may be explained by a change in dietary format from powdered to pelleted diets. The combination of feeding pelleted diets and housing mice individually below their thermonuetral zone may have shifted energy balance in TRAMP mice to only eat enough for maintenance and growth. Future studies utilizing pelleted diets might detect differences between dietary treatments by prolonging the sacrifice age further than 18 weeks to allow for cancer development or switching back to powdered diets. Despite null results regarding cancer incidence, we observed a significant effect of daidzein and equol on serum cytokines in mice with prostate tumors. This may suggest that daidzein and equol diets, but not a whole soy germ diet, may have anti-inflammatory properties in advanced stage PCa. Future studies in TRAMP mice should examine serum cytokine levels as well as prostatic and tumoral mRNA expression of cytokines to confirm these findings.

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