Reproductive Biology and Endocrinology

BioMed Central

Research

Androgen receptor expression in the rat prostate is down-regulated by dietary phytoestrogens

Trent D Lund*[1], Daniel J Munson[1], Herman Adlercreutz[2], Robert J Handa[1] and Edwin D Lephart[3]

Address: [1]Department of Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA, [2]Institute for Preventive Medicine, Nutrition, and Cancer Folkhälsan Research Center, and Division of Clinical Chemistry, Biomedicum, University of Helsinki, Finland and [3]The Neuroscience Center and Department of Physiology and Developmental Biology, Brigham Young University, Provo, Utah, USA

Email: Trent D Lund* - tlund@colostate.edu; Daniel J Munson - daniel.munson@colostate.edu; Herman Adlercreutz - herman.adlercreutz@helsinki.fi; Robert J Handa - robert.handa@colostate.edu; Edwin D Lephart - Edwin_Lephart@byu.edu

* Corresponding author

Published: 16 January 2004

Reproductive Biology and Endocrinology 2004, **2**:5

Received: 14 November 2003
Accepted: 16 January 2004

This article is available from: http://www.rbej.com/content/2/1/5

Abstract

Background: It is well established that the growth of the prostate gland is a hormone-dependent phenomenon involving both androgenic and estrogenic control. Proliferation of prostate cells is, at least in part, under control of estrogen receptor beta (ER-beta). Phytoestrogens bind ER-beta with high affinity and therefore may have antiproliferative effects in the prostate.

Methods: The prostates of male Long-Evans rats fed a diet high in phytoestrogens (Phyto-600) or very low levels of phytoestrogens (Phyto-free) were analyzed to determine the impact of dietary phytoestrogens on prostate weight and androgen receptor (AR) expression in the prostate.

Results: Dietary phytoestrogens significantly decreased post-pubertal prostate weight gain in Phyto-600 vs Phyto-free fed males. Additionally, dietary phytoestrogens (Phyto-600) decreased AR expression in the prostate as determined by in situ hybridization.

Conclusions: Soy phytoestrogens, present in diet, alter prostate growth presumably by binding ER-beta and subsequently reducing AR expression within the prostate.

Introduction

Despite decades of research concerning the endocrine and molecular events controlling prostate growth, our understanding of this process is far from complete. By far the most studied and well-characterized prostate growth pathway, which remains a target for controlling malignant growth of the prostate, is its androgenic dependency [1]. Although the prostate is highly dependent on 5α-reduced androgens for growth, estrogens can also control normal gland function and may serve to control pathological growth [2-4]. Within the prostate, estrogen receptor beta (ERβ) has been shown to be the most prevalent ER [2,4]. Prostatic ERβ specifically binds 5α-androstane-3β, 17β-diol (3βAdiol), a DHT metabolite and the predominant endogenous estrogen in prostate tissue [2,3]. Furthermore, 3βAdiol, via its binding to ERβ, can regulate prostate AR gene expression and serves as an anti-proliferative agent [2-4].

It is of interest to note that ERβ possesses a relative binding affinity (RBA), for several steroid hormones, which is different from ERα. Importantly, the RBA of a number of

phytoestrogens is several fold-greater for ERβ than for ERα [5-7]. Phytoestrogens have received increased investigative attention due to numerous reports of potential protective action against hormone-dependent cancers (i.e., breast and prostate cancer) [8-11]. Of the phytoestrogens, human consumption of isoflavones has the largest impact due to its availability and variety in food products containing soy. Of the isoflavones, genistein and daidzein (particularly its intestinal metabolite equol) are thought to be the most potent estrogens and thus have attracted the most attention [9,10,12]. The phenolic ring structures of these isoflavones enable these compounds to bind ER and mimic estrogen with higher affinity for ERβ [7] than for ERα [5-7].

Previous studies have shown that prostate weight is decreased in male rats fed a diet high in phytoestrogens when compared to males fed a low phytoestrogen diet [13-15]. As a possible mechanism for prostate reduction in these studies we examined the effects of dietary soy phytoestrogens on prostate AR mRNA expression.

Materials and Methods

Animals

Ten 50 day-old Long-Evans females were purchased from Charles River Laboratories (Wilmington, MA, USA) for breeding. These animals and subsequent offspring were caged individually in hanging wire mess cages (nesting material provided) housed in the Brigham Young University Bio-Ag vivarium and maintained on a 10-hour dark 14-hour light schedule (lights on 1400-0400). The Animal Care and Use Committee (IACUC) at Brigham Young University approved the animals and methods for these studies. 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 micrograms of phytoestrogens/gram of diet (Phyto-600), or a custom diet with very low phytoestrogen levels (Phyto-free), obtained from Ziegler Bros. (Gardner, PA, USA) and water. For the Phyto-free diet, the phytoestrogen concentrations were below the detectable limits of HPLC analysis [14]. The content and nutrient composition of these diets are described in detail elsewhere [14]. At 80–85 days of age, the females were time mated with young male breeders (75–80 days of age) within their respective diet treatments so that the offspring of these pairings would be exposed solely to either the Phyto-600 or Phyto-free diet. Twenty-seven days following birth, the animals were weaned and randomly separated by sex into colony cages (4 animals per cage, only males were used in the studies presented here). The animals were fed the same diet as their mother (Phyto-600 or Phyto-free). At 40 days of age, animals were singly caged and remained on the assigned diet treatments. At 33, 55 and 75 days of age animals were sacrificed and ventral prostates were collected, dissected free of fat, weighed and frozen for later analysis.

In Situ Hybridization

In order to compare changes in receptor within the prostate of 75 day olds we measured AR expression using in situ hybridization as described elsewhere [16]. Briefly, prostates were sectioned at 16 μm on a Leitz 1720 digital cryostat, thaw mounted onto Superfrost plus slides (VWR Scientific, West Chester, PA) and stored at -80 C until assayed. For assay, tissue sections were thawed at room temperature, fixed with 10% formaldehyde, acetylated with 0.25% acetic anhydride, dehydrated in graded alcohols, and air-dried. Sections were incubated in a hybridization solution (50% formamide, 0.60 M NaCl, 0.02 M Tris, 0.01 M EDTA, 10% dextran sulfate, 2 X Denhart's solution, 50 mM dithiothreitol, 0.2% SDS, 100 mg/ml salmon sperm DNA, 500 mg/ml total yeast RNA, and 50 mg/ml yeast transfer RNA) containing the radiolabeled cRNA at a concentration of 2×10^7 cpm/ml at 60°C or 37°C respectively overnight. After hybridization, the slides were rinsed in 2 X SSC (standard saline citrate). For *in situ* hybridization of the cRNA probe, nonhybridized RNA was digested with 30 mg/ml ribonuclease A for 30 min at 37°C. The final wash stringency was 0.1 X SSC at 60°C. For autoradiographic detection of hybridization, slides were exposed to autoradiographic film for 10 days.

Image Analysis

The density of hybridization signal per fixed area was counted using a video camera (Sony XC-77; Tokyo, Japan) connected to a Nikon (Melville, NY) lens and a Dell computer utilizing Scion Image (Frederick, MD) software to detect differences in density expression. Four measurements were taken from each of five different sections from each animal, comparing labeled nuclei with unlabeled control nuclei. The means of the five sections were averaged together to give the mean value for that animal. Background was determined for each section and then subtracted from the density value for that section by taking a density measurement outside of the labeled area.

Prostate Phytoestrogen Levels

Daidzein, genistein and equol levels were determined using time-resolved fluoroimmunoassay (TR-FIA) in tissues isolated from the prostate using standard methods based on previously published and validated methods [17,18]. In brief prostate samples were lyophilized (pooled by treatment). Following addition of 300 μl of water and mixing, the samples were left at room temperature for 10 min, and thereafter carefully mixed and sonicated for 10 min. Tritiated estradiol glucuronide was added as an internal standard and 700 μl of methanol were mixed with samples. Fatty material was precipitated overnight at -20°C. The samples were then centrifuged

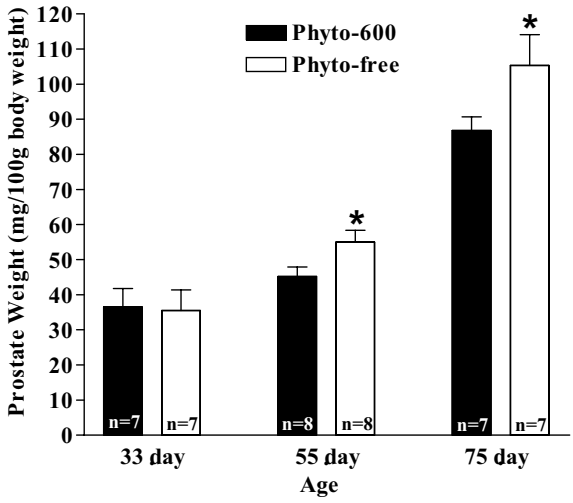

Figure 1
Effects of Dietary Phytoestrogens on Prostate Weight in Male Long-Evans Rats fed either a phytoestrogen-rich (Phyto-600) or a phytoestrogen-free (Phyto-Free) diet. At 55 and 75 days-old (but not 33) Phyto-Free-fed male prostate weights (*) were significantly greater compared to Phyto-600-fed male values. Bars represent mean (± SEM).

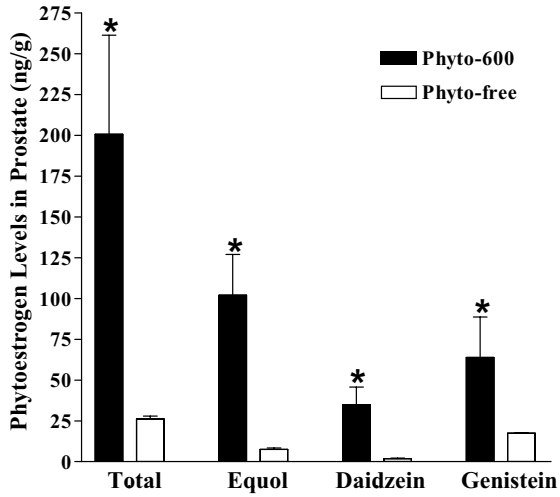

Figure 2
Prostate phytoestrogen content in male Long-Evans rats fed either the Phyto-600 or Phyto-free diet. Phytoestrogen levels are expressed in nanograms/gram (ng/g). Total = the sum of the three main phytoestrogens (equol, daidzein, and genistein). * Animals fed the Phyto-600 diet had significantly higher total, equol, daidzein and genistein levels compared to males fed the Phyto-free diet ($p < 0.05$). Bars represent mean (± SEM).

(3500 rpm) for 10 min at -10°C and the supernatant was decanted into another tube. The procedure was repeated by adding an additional 1 ml, ice-cold 70% methanol, mixing, centrifugating in the cold, and decanting the supernatant into the tube containing the extract. Methanol was evaporated under a stream of nitrogen until only water remained. The samples were extracted once with 2.5 ml of n-hexane, which removed the remaining fat (the remaining n-hexane was removed with nitrogen). Helix Pomatia enzyme was purified with activated charcoal and used for enzymatic hydrolysis of the phytoestrogen conjugates. The hydrolysis (2 h at 60°C) was carried out using ascorbic acid in 0.15 M acetate buffer. The samples were then extracted twice with 3 ml of diethylether, freezing separated the phases and the ether fractions were combined and evaporated. After adding 300 µl of TRIS buffer pH 7.76 (TR-FIA buffer) a sample was taken for measurement of recovery in a β-counter. Equol was determined in the same way genistein and daidzein, using an antiserum to 4'-O-carboxymethyl-equol-bovine serum albumin and the Europium label was synthesized using the same derivative of equol. The intra-assay CV determined, from a pool of rat prostates, varied from 3.2 to 4.1%.

Statistical analysis
All data were analyzed by one-way analysis of variance using StatView statistical software (Cary, NC). $P < 0.05$ was considered significant.

Results
Phytoestrogens within diet decrease prostate weight
The effects of dietary phytoestrogens on prostate weights in pre-, early and young adult age male rats is shown in Figure 1. Prostate weight was significantly increased in 55 ($p = 0.040$) and 75 ($p = 0.003$) (but not 33 ($p = 0.87$) days old, males exposed to the Phyto-free diet (~10–15%) compared to animals fed the Phyto-600 diet.

Phytoestrogens are found within prostate
Prostate phytoestrogen levels are displayed in Figure 2. In general Phyto-600 fed males displayed significantly higher total phytoestrogen levels compared to Phyto-free fed males ($p = 0.0078$). Furthermore, levels of equol ($p = 0.0021$), daidzein ($p = 0.028$) and genistein ($p = 0.014$) were all significantly increased in the ventral prostates of Phyto-600 vs Phyto-free fed males.

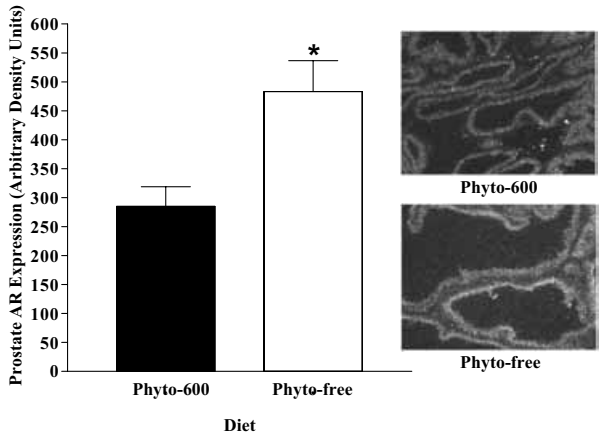

Figure 3
In situ hybridization was used to detect prostate androgen receptor (AR) mRNA expression in male Long-Evans rats fed the Phyto-600 or Phyto-free diet. * AR expression was significantly decreased in the prostates of Phyto-600 fed males compared to Phyto-free fed males. Arbitrary units refer to density of AR mRNA expression. Bars represent mean (± SEM). Dark field micrograph at 100× magnification are included for comparison purposes.

Dietary phytoestrogens decrease AR expression in the Prostate
AR mRNA expression in the prostate was significantly decreased ($p = 0.04$) in males fed the Phyto-600 vs males fed the Phyto-free diet. These data are presented graphically in Figure 3.

Discussion
Growth of the prostate is a hormone-mediated phenomenon regulated by both androgens and estrogens [1-4]. Within the prostate, ERβ has been shown to be an important player [2,4] in regulating hormone-dependent morphological alterations. Moreover, the DHT metabolite 3βAdiol, is the predominant endogenous estrogen in rodent prostate [2,3]. 3βAdiol, via binding to ERβ, regulates prostate AR gene expression and serves as an antiproliferative agent [2-4] by decreasing the influence of androgen action. Recent finding demonstrate that neonatal exposure to estrogens interrupts normal prostate development [19]. Down-regulation of AR protein was found to occur immediately following neonatal exposure to estradiol benzoate and then persist through adulthood indicating a permanent imprint on the ability of the prostate to express normal AR levels [19]. Furthermore, this exposure to estrogen and subsequent down-regulation of AR protein levels, identified in the ventral prostate gland, looks to be via an acceleration of AR degradation, which is mediated through the proteosome pathway [19].

In the studies presented here, we have identified that dietary soy-derived phytoestrogens, perhaps in a manner similar to 3βAdiol, decreased prostate weight and down-regulated AR expression in the prostate. Previous research by Fritz et al. [20] identified a similar reduction in AR in rats fed genistein. Furthermore, in these studies exposure to genistein in the diet, starting at conception resulted in not only down-regulated AR, but also ERα and -β mRNA expression in the dorsolateral prostate in a dose-dependent manner [20] a finding they also identified in genistein fed adults [20]. Since phytoestrogens bind ERβ with a high RBA [5-7] and because ERβ regulates prostate growth by down regulating AR expression, it is possible that the reduction in prostate weight (and AR expression) seen in the present study are a result of high circulating phytoestrogens from diet and subsequently high steady state levels of phytoestrogens in prostate tissue. Since the changes in prostate weight was only present following the initiation of puberty our data suggest that the differences in prostate weight are the result of the reduction in AR expression and resulting decrease in androgenic stimulation. It is interesting to note that previous research from our lab has shown that dietary phytoestrogens have no effect of circulating testosterone or estrogen levels [13]. The effect of phytoestrogens on prostate growth may be direct via binding ERβ [20]. However, there may be an indirect consequence of this binding and subsequent reduction in AR expression in the prostate that may involve other metabolites of phytoestrogens that are not completely understood or alternative pathways. For example, dietary soy products have been shown to inhibit experimental prostate tumor growth through a combination of direct effects on tumor cells such as blocking cell cycle progression at the G2-M phases and enhancing DNA fragmentation [21]. Furthermore, genistein's action as a tyrosine kinase inhibitor cannot be ruled out as a possible means by which the phytoestrogens in diet are affecting prostate weight and AR expression [22]. However, no matter the mechanism of action these data help to more firmly establish the broad implications for prostate health in the dietary applications of phytoestrogens, as reported by other investigators [8-10,12,13,15,20-22].

Acknowledgements
This research was supported in part by USDA grant 2002-00798 (EDL) and NIH grant NS39951 (RJH).

References
1. Marker PC, Donjacour AA, Dahiya R, Cunha GR: **Hormonal, cellular, and molecular control of prostatic development.** *Dev Biol* 2003, **253:**165-174.
2. Weihua Z, Lathe R, Warner M, Gustafsson JA: **An endocrine pathway in the prostate, ERβ, AR, 5α-androstane-3β, 17β-diol, and CYP7B1, regulates prostate growth.** *PNAS* 2002, **99:**13589-13594.
3. Weihua Z, Makela S, Andersson LC, Salmi S, Webster JI, Jensen EV, Nilsson S, Warner M, Gustafsson JA: **A role for estrogen receptor**

β **in the regulation of growth of the ventral prostate.** *PNAS* 2001, **98:**6330-6335.
4. **International Prostate Health Council Study Group Estrogen and prostatic disease.** *The Prostate* 2000, **45:**87-100.
5. Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA: **Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta.** *Endocrinology* 1999, **138:**863-870.
6. Kuiper GG, Lemmen JM, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA: **Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta.** *Endocrinology* 1999, **139:**4252-4263.
7. Petersen DN, Tkalcevic GT, Koza-Taylor PH, Turi TG, Brown TA: **Identification of estrogen receptor β2, a functional variant of estrogen receptor β expressed in normal rat tissues.** *Endocrinology* 1998, **139:**1082-1092.
8. Adlercreutz H: **Evolution, nutrition, intestinal microflora, and prevention of cancer: a hypothesis.** *Proc Soc Exp Bio Med* 1997, **217:**241-246.
9. Adlercreutz H, Mazur W: **Phyto-estrogens and western diets.** *Ann Med* 1997, **29:**95-120.
10. Setchell KDR, Cassidy A: **Dietary isoflavones: biological effects and relevance to human health.** *J Nutr* 1999, **129:**7585-7675.
11. Coward L, Barnes NC, Setchell KDR, Barnes S: **Genistein, daidzein, and their glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets.** *J Agr Food Chem* 1993, **41:**1961-1967.
12. Kurzer MS, Xu X: **Dietary phytoestrogens.** *Annu Rev Nutr* 1997, **17:**353-381.
13. Lephart ED, West TW, Weber KS, Rhees RW, Setchell KD, Adlercreutz H, Lund TD: **Neurobehavioral effects of dietary soy phytoestrogens.** *Neurotoxicol Teratol* 2002, **24:**5-16.
14. Weber KS, Setchell KDR, Stocco DM, Lephart ED: **Dietary soy-phytoestrogens decrease testosterone levels and prostate weight, without altering LH, prostate 5α-reductase or testicular StAR levels in adult male Sprague-Dawley rats.** *J Endocrinol* 2001, **170:**591-599.
15. Whitten PL, Patisaul HB: **Cross-species and interassay comparisons of phytoestrogen action.** *Environ Health Perspect* 2001, **109(Suppl 1):**5-20.
16. Kerr JE, Allore RJ, Beck SG, Handa RJ: **Distribution and hormonal regulation of androgen receptor (AR) and AR messenger ribonucleic acid in the rat hippocampus.** *Endocrinology* 1995, **136:**3213-3221.
17. Wang GJ, Lapcik O, Hampl R, Uehara M, Al-Maharik N, Stumpf K, Mikola H, Wahala K, Adlercreutz H: **Time-resolved fluoroimmunoassay of plasma daidzein and genistein.** *Steroids* 2000, **65:**339-348.
18. Uehara M, Lapcik O, Hampl R, AlMaharik N, Makela T, Wahala K, Mikola H, Adlercreutz H: **Rapid analysis of phytoestrogens in human urine by time-resolved fluoroimmunoassay.** *J Steroid Biochem Mol Biol* 2000, **72:**273-282.
19. Woodham C, Birch L, Prins GS: **Neonatal estrogen down-regulates prostatic androgen receptor through a proteosome-mediated protein degradation pathway.** *Endocrinology* 2003, **144:**4841-4850.
20. Fritz WA, Wang J, Eltoum IE, Lamartiniere CA: **Dietary genistein down-regulates androgen and estrogen receptor expression in the rat prostate.** *Mol Cell Endocrinol* 2002, **186:**89-99.
21. Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK: **Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice.** *J Nutr* 1999, **129:**1628-1635.
22. Reinikainen P, Palvimo JJ, Janne OA: **Effects of mitogens on androgen receptor-mediated transactivation.** *Endocrinology* 1996, **137:**4351-4357.