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Phytosterol feeding induces alteration in testosterone metabolism in rat tissues

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The objective of the present study was to examine the metabolism of testosterone in rat tissues as influenced by dietary phytosterols. Testosterone metabolism includes reductions to more active metabolites or aromatization to estrogen. Both higher levels of androgens and estrogens are implicated as risk factors in the development of prostate cancer. Tissues studied included liver, testis, and prostate. Feeding 2% phytosterols with 0.2% cholic acid to rats for 22 days resulted in a 33% reduction in serum testosterone compared with controls, which received only 0.2% cholic acid in the diet. 5-α-Reductase was reduced by 41 to 44% and 33% in the liver and prostate, respectively. No effect of phytosterols was observed in the testis. Only aromatase activity of the prostate was reduced by 55% upon feeding phytosterols. It was concluded that dietary phytosterols may reduce the risk of prostate cancer by lowering the activities of the enzymes of testosterone metabolism. (J. Nutr. Biochem. 9: 712–717, 1998) © *Elsevier Science Inc. 1998*

Keywords: testosterone; prostate cancer; 5-α-reductase; aromatase; β-sitosterol; liver; prostate; testis

Introduction

Prostate cancer is the second leading cancer in men in Western societies. Epidemiologic studies suggest that dietary fat plays a role in the mortality from the disease.[1] Societies that consume high levels of fat have a higher death rate from the disease compared with those that consume lower fat levels.[2] We recently showed that the type of fat, namely sterols, also may be important in the development of the disease.[3] β-Sitosterol, the main plant sterol (phytosterol; PS), inhibits the growth of LNCaP, a human prostate cancer cell line, when compared with cholesterol.[3] These findings may explain the lower mortality from prostate cancer in vegetarians and in societies that consume little fat and have a mostly vegetarian diet.

Work from our laboratory indicates that some of the PS, such as β-sitosterol and campesterol, can be detected in some rat tissues upon feeding.[4] We also reported an inhibition of growth in HT-29 cells, a human colon cancer cell line in culture by β-sitosterol.[5] In vivo studies indicate that PS feeding results in lower incidence of chemically-induced colon cancer in mice[6] and lower cell proliferation in rat colon mucosa,[7] which is considered to be a risk factor in colon cancer. The mechanism by which PS offers protection from cancer is not fully understood. Recently, we identified one of the mechanisms by which PS may inhibit cell growth: stimulation of the sphingomyelin cycle by PS in HT-29 cells.[8] In the present study we investigated the role of PS in the metabolism of testosterone, the main male sex hormone, in certain tissues. The hormone can be activated by reduction to dihydrotestosterone (DHT) and other reduced metabolites or by conversion to estrogen by aromatization. The end products of these two pathways are implicated in the development of prostate cancer.[9,10] The tissues examined included liver, testis, and ventral prostate because these tissues play a role in the development of prostate cancer. The liver is the most active tissue in testosterone metabolism, the testis is the main site of testosterone synthesis, and prostate is the target tissue.

Materials and methods

Materials

^{3}H-Testosterone [1,2,6,7-^{3}H(N)] (3145 Gbq (85 Ci)/mmol) and ^{3}H androstenedione [1β-^{3}H(N)] 906.5 Gbq (24.5 Ci)/mmol) were purchased from Dupont NEN (Boston, MA USA). Nonradioactive steroids (testosterone, androstenedione, DHT, androstanediol, androstandione, and androsterone), NADPH, dithiothreitol (DDT), calcium sulfate, dextran, ethylenediamine tetraacetic acid (EDTA),

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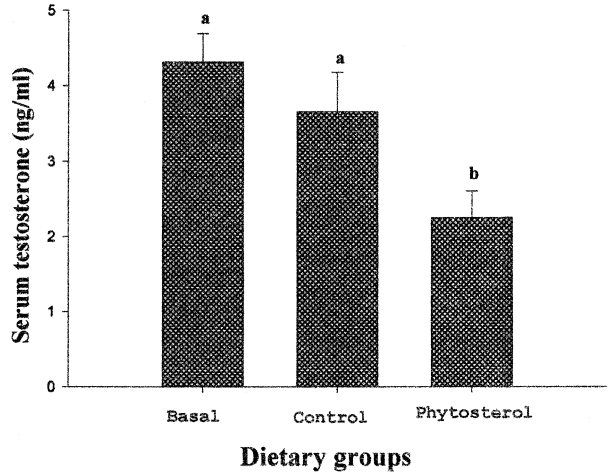

Figure 1 Effect of dietary phytosterol (PS) on rat serum testosterone concentrations. Bars (average ± SEM of three to six samples) with different letters (a,b) are significantly ($P < 0.05$) different. The control diet contained a basal diet and 0.2% cholic acid. The PS diet contained a basal diet plus 0.2% cholic acid and 2% PS mix.

glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were obtained from Sigma (St. Louis, MO USA). Other chemicals were obtained from Fisher Scientific (Fair Lawn, NJ USA).

Animals and controls

The tissue samples obtained for this study were from previous experiments.[7] In brief, 18 Sprague-Dawley male rats (Harlan Industries, Indianapolis, IN USA) weighing 240 to 270 g were randomly assigned to three groups; one group received a basal diet, the control group received the basal diet plus 0.2% cholic acid, and the PS group received 0.2% cholic acid plus 2% PS mixture (Sigma). The PS mixture contained 56% β-sitosterol, 28% campesterol, 10% stigmasterol, and 6% dihydrobrassicasterol. PS were added at the expense of sucrose. The diet composition was previously published.[7] The ingredients of the diets were purchased from U.S. Biochemical (Cleveland, OH USA) and National Casein (Riverton, VA USA). Cholic acid was added to stimulate the absorption of sterols. Soybean oil with the antioxidant tertbutylhydroquinone was purchased from Dyets, Inc. (Bethlehem, PA USA). Rats were fed the diets ad libitum and had free access to water for 22 days. There was no effect of bile acids or PS on growth, food intake, and food efficiency in these rats.[7]

Methods

Testosterone measurement. Total serum testosterone (testosterone plus DHT) was measured using an enzyme-linked immunosorbent assay (ELISA) kit obtained from Neogen Corp. (Lexington, KY USA).

Preparation of tissue microsomes. Tissues were homogenized in 50 mM Tris buffer (pH 7.0) and centrifuged at 10,000 × g for 20 minutes at 4°C. The resulting supernatant was centrifuged at 105,000 × g for 60 minutes to pellet the microsomes. Microsomes were suspended in homogenization buffer and stored at −80°C for enzyme analysis.

Measurement of 5 α-reductase activity. 5-α-Reductase activity was determined by measuring the conversion of testosterone to its reduced metabolites.[11] Reactions were initiated by the addition of microsomes (5 μg protein) to incubation media containing 10 mM dithiothreitol, 1 mM NADPH, 25 μM testosterone, and 0.1 μCi ^{3}H-testosterone in a total volume of 150 μL. The assay was carried out at 37°C for 15 minutes and was terminated by adding 2 mL ethyl acetate containing carrier steroids. After centrifugation at 1,000 × g for 5 minutes, an aliquot of the upper phase was dried under nitrogen. Steroids were dissolved in ethyl acetate and separated by TLC using dichloromethane-diethyl ether (11:1, v/v) as a developing system.[12] After drying, plates were redeveloped in the same system. After air drying, the plates were sprayed with 1% $CeSO_4$–10% H_2SO_4 solution and heated at 110°C for 5 to 10 minutes. For identification, authentic steroid standards were run on the same plate. The areas corresponding to the steroids were scraped into scintillation vials (Ecolume, ICN Biochemicals, Irvine, CA USA) added, and the radioactivity measured. Assay blanks without microsomal enzymes were run parallel to the samples to correct for nonenzymatic breakdown of testosterone.

Measurement of aromatase activity. Aromatase activity of rat liver, testis, and ventral prostate was determined according to the procedure of Lephart and Simpson,[13] which is based on measuring the ^3H_2O released upon aromatization of androstenedione. Microsomes were incubated in buffer containing 1 mM NADPH, 10 mM glucose 6-phosphate, and 0.35 units of glucose 6-phosphatase dehyrogenase, 150 μM androstenedione, and 0.5 μCi [^{3}H]-androstenedione. The incubation was carried out at 37°C for 2 hours for liver and 3 hours for testis and prostate. The reaction was terminated by adding 1.75 mL chloroform and 1 mL of H_2O. One milliliter of the upper phase was transferred to test tubes along with 1 mL of 5% charcoal and 0.5% dextran. After mixing, the tubes were centrifuged and an aliquot of the upper phase was taken for liquid scintillation counting using Ecolite (ICN Radiochemicals). All values were corrected by running nonenzymatic blanks.

Protein measurement. Protein was measured using the method of Bradford[14] with reagents obtained from Bio-Rad (Richmond, CA USA) and bovine serum albumin as standard.

Table 1 Effect of dietary phytosterols on the metabolism of testosterone in the liver*

	Metabolites				
Dietary group	DHT	5-α-A	Δ4	A	Diols
Control	1,163 ± 199—a	11 ± 6—a	1,455 ± 76—a	1,029 ± 133—a	1,048 ± 210—a
Phytosterols	640 ± 59—b	53 ± 31—a	778 ± 123—b	879 ± 120—a	344 ± 92—b

*Values (pmol/mg/hr) are the average ± SEM of four samples.
Values with different letters in one column are significantly ($P < 0.05$) different.
DHT–5-α-dihydrotestosterone. 5-α-A–5-α-androstanedione. Δ4–androstenedione. A–androsterone. Diols–the sum of 3α- and 3β-androstanediols.

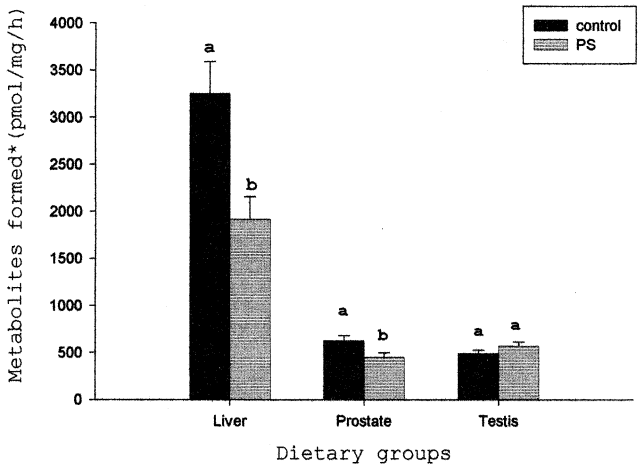

Figure 2 Effect of dietary phytosterol (PS) on microsomal 5-α-reductase activity in rat liver, testis, and prostate. Bars (average ± SEM of four to five samples) with different letters (a,b) in the same tissue are significantly ($P < 0.05$) different. Metabolites formed include DHT, androstandiols, androstandione, and androsterone.

Results

Effect of dietary PS on serum testosterone

There was no difference between the levels of testosterone in sera of animals fed the basal diet and the control diet (basal diet plus 0.2% cholic acid) (*Figure 1*). PS feeding significantly reduced the plasma testosterone by 33 to 48% of the basal and the control diets.

Effects of dietary PS on 5-α-reductase activity in rat tissues

Because the presence of cholic acid in the diet did not influence serum testosterone levels, studies of enzyme activities for 5-α-reductase and aromatase were conducted only on the control, which included 0.2% cholic acid and the PSE groups. PS feeding resulted in a 44% reduction in 5-α-reductase in the liver based on DHT data alone or 41% based on the summation of total 5-α-reduced metabolites (*Table 1* and *Figure 2*). Androstenedione is not considered a 5-α-reduced metabolite. This effect of dietary PS was absent in the testis (*Table 2*). The effect of PS on 5-α-reductase activity in the prostate was only detected in androsterone (*Table 3*). The reduction was 33% compared with the control diet group. If all reduced testosterone metabolites were considered, the reduction would be 55%. It is noteworthy that we were not able to detect androstandiols in the prostatic tissue.

Effects of PS on tissue aromatase

The only effect of PS on tissue aromatase was restricted to the prostate (*Table 4* and *Figure 3*). PS feeding reduced prostate aromatase activity by 57% compared with the controls.

Discussion

The objective of this study was to determine the effect of dietary PS on the activities of 5-α-reductase and aromatase enzymes of several rat tissues. The observation that prostate cancer does not develop in men castrated prior to puberty,[15] and is not reported in 5-α-reductase deficient males,[16] suggests that suppression of DHT levels with 5-α-reductase inhibitors might be useful in preventing the development of prostate cancer in high-risk groups.[17]

The present study investigated the effect of dietary PS on the activity of 5-α-reductase enzyme in rat liver, testis, and prostate. The liver is a major sterol metabolizing organ in the body and the testis and prostate are the main site of synthesis and a target tissue, respectively. Data from this study showed that dietary PS significantly ($P < 0.05$) reduces 5-α-reductase activity in rat liver microsomes. The 5-α-DHT formed from testosterone in the PS group was 44% lower and the total of 5-α-reduced metabolites of testosterone was 41% lower than that of the control group.

Studies (reviewed by Prahalada et al.[18]) reported that inhibition of 5-α-reductase by finasteride in men results in decreased levels of DHT in the circulation and in the prostate.

The mechanisms by which PS influence the activities of 5-α-reductase and aromatase is not clear from these studies; however, some can be proposed. One of the possible mechanisms is that PS may be incorporated in the membranes and thus affect the fluidity of membranes that house the enzymes. Several studies demonstrated the sensitivity of several membrane bound enzymes to membrane fluidity.[19] Another possibility is that PS may compete with testosterone and its metabolites for the active sites of these enzymes. In addition, PS may modulate the expression of these enzymes. Work is in progress in our laboratory to investigate these possibilities.

In the present study we found that dietary PS significantly ($P < 0.05$) lowered serum total testosterone by 48% compared with a basal diet and 33% when compared with a

Table 2 Effect of dietary phytosterols on the metabolism of testosterone in the testis*

	Metabolites				
Dietary group	DHT	5-α-A	Δ4	A	Diols
Control	44 ± 15—a	12 ± 3—a	315 ± 55—a	128 ± 38—a	305 ± 27—a
Phytosterols	40 ± 8—a	14 ± 2—a	346 ± 20—a	119 ± 32—a	398 ± 42—a

*Values (pmol/mg/hr) are the average ± SEM of four to five samples.
Values with the same letters in one column are significantly ($P < 0.05$) different.
DHT–5-α-dihydrotestosterone. 5-α-A–5-α-androstanedione. Δ4–androstenedione. A–androsterone. Diols–the sum of 3α- and 3β-androstanediols.

Table 3 Effect of dietary phytosterols on the metabolism of testosterone in the prostrate*

Dietary group	Metabolites			
	DHT	5-α-A	Δ4	A†
Control	50 ± 11—a	49 ± 13—a	13 ± 10—a	528 ± 51—a
Phytosterols	78 ± 15—a	21 ± 13—a	24 ± 11—a	353 ± 35—b

*Values (pmol/mg/hr) are the average ± SEM of four to five samples.
†Androstanediols were not detected in prostate microsomes.
Values with different letters in one column are significantly ($P < 0.05$) different.
DHT–5-α-dihydrotestosterone. 5-α-A–5-α-androstanedione. Δ4–androstenedione. A–androsterone. Diols–the sum of 3α- and 3β-androstanediols.

cholic acid diet (control). Testosterone is the major male sex steroid hormone in the blood; it constitutes 95% of the total.[20] The reduction in plasma testosterone in animals fed PS does not go hand in hand with the observed reduction in the activities of 5-α-reductase and aromatase in liver and prostate, or even with the lack of effect of PS on the activities of these enzymes in the testis, the major site for testosterone synthesis. The level of testosterone in the blood is determined by the balance between synthesis and elimination through conjugation with glucuronides and sulfates, and thus PS could influence these pathways. More research is needed in this area.

Although DHT, a potent androgen resulting from the 5-α-reduction of testosterone, is clearly important in regulating prostatic cell growth, epidemiologic evidence of the association between circulating androgen levels and prostate cancer is controversial. Some studies[21–23] reported that there was an association between serum testosterone level, but not DHT, and the risk of prostate cancer. On the other hand, other studies[24–26] reported no association between prostate cancer and total circulating testosterone.

Dietary PS also reduced 5-α-reductase activity in rat prostate. The total reduced metabolites of testosterone in the PS group was 55% lower than that of the control group. However, the amount of 5-α-DHT formed in vitro was not significantly different. Some studies reported that in human prostate DHT is rapidly reduced to androstanediols.[12,27,28] In the present study, androstanediols were not detected in rat prostate. The data showed that DHT is converted to androstandione and then metabolized further to androsterone. In support of the present finding, a study conducted by Liang et al.[29] reported that androstandiols were not detected in rat prostate. This discrepancy could be explained by the difference in assay methods used in these studies. In addition, in the present study rat microsomal preparations of ventral prostate was used whereas others used the whole rat prostate tissue.[29]

Table 4 Effect of dietary phytosterols on aromatase activity of liver, testis, and prostate*

Dietary group	Liver	Testis	Prostate
Control	78.4 ± 11.5—a	16.5 ± 1.8—a	10.1 ± 2.4—a
Phytosterol	69.6 ± 6.9—a	17.8 ± 0.9—a	4.3 ± 1.0—b

*Values (pmol/mg/hr) are the average ± SEM of four to six samples.
Values with different letters in one column are significantly ($P < 0.05$) different.

Our data indicated no observable effect of dietary PS on 5-α-reductase activity in rat testis. This was not unexpected because in general the activity of 5-α-reductase in the testis is relatively low.[30] It is known that the testis is not a target organ of androgens.

Studies have shown a significant improvement of symptoms in patients with benign prostatic hyperplasia (BPH) who were clinically treated with PS.[31] However, the mechanism was not clearly understood. The present study suggests that PS may alter prostatic cell proliferation by inhibiting 5-α-reductase activity. A previous study[32] demonstrated that PS inhibits 5-α-reductase activity in the epithelium and stroma of human BPH by 15% and 10%, respectively. An association between 5-α-reductase and the risk of prostate cancer has been reported in some studies.[17–33]

Data obtained from the present study also demonstrates that different organs metabolize testosterone differently. In the liver, the major metabolite of testosterone is androstandione, followed by DHT, androstandiols, and androsterone. 5-α-Androstandione was found in relatively small amounts. In the prostate, the major metabolite of testosterone metabolism is androsterone, followed by DHT. Androstandione and 5-α-androstandione were found in small amounts. No androstandiols were detected. In the testis, the major metabolite of testosterone is androstandione, followed by androstandiols and androsterone. DHT and 5-α-androstandione were found in small amounts.

Although aromatase activity was detected in rat liver, testis, and prostate microsomes, dietary PS significantly reduced only prostatic aromatase. Kaburagi et al.[34] reported that aromatization of androgen to estrogen occurs in human prostate. They found that aromatase activity tended to be higher in BPH than in normal prostate homogenates. Stone et al.[35] reported that aromatization occurred in prostatic tissues of patients with prostate cancer. Data obtained from the present study showed a reduction of 58% of aromatase activity in rat prostate achieved by dietary PS feeding. Because high levels of both androgen and estrogen are considered risk factors for the development of prostate cancer,[35,36] the present data may suggest a role of dietary PS in the treatment and prevention of BPH and prostate cancer by inhibiting aromatization of androgen to estrogen in prostatic cells.

Aromatase inhibitors, atamestane, and 4-hydroxy-andro-

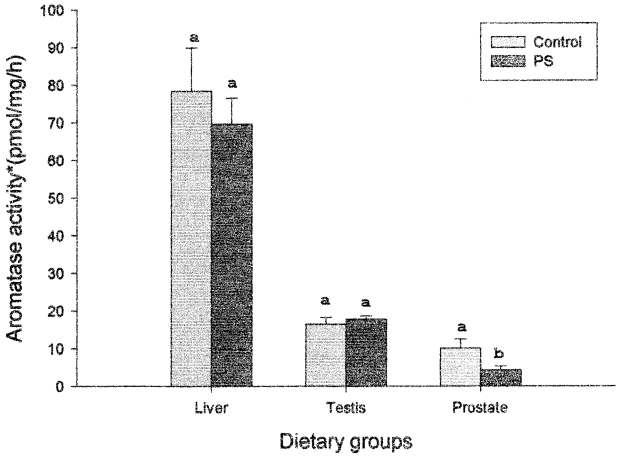

Figure 3 Effect of dietary phytosterol (PS) supplementation on aromatase activity in liver, testis, and prostate microsomes. Bars (average ± SEM of four to seven samples) with different letters (a,b) in the same tissue are significantly ($P < 0.05$) different. Aromatase activity was measured by measuring ^3H_2O released during aromatization of androstenedione.

standione (4-OHAD) have been evaluated for their effect on BPH and prostate cancer patients. Schweikert et al.[35] demonstrated a significant improvement in symptoms of BPH after treatment with atamestane 200 mg daily for 3 months. Stone et al.[10] have shown that estrogen formation was inhibited by 4-OHAD in a dose-dependent manner in both BPH and prostate cancer tissues.

The present study suggests that dietary PS may alter androgen metabolism by inhibiting 5-α-reductase and aromatase activity. Thus, it may explain the previous finding that vegetarians have lower serum testosterone and 17-β-estradiol levels than nonvegetarians.[36] Furthermore, it may also partially explain the role of dietary habits in the risk of prostate cancer. It has been demonstrated that vegetarians have a lower risk of hormone-dependent cancer than nonvegetarians.[37] A lower mortality rate of prostate cancer in Asian countries than in Western countries has been demonstrated in other epidemiologic studies.[38]

In conclusion, the combined effect of dietary PS on testosterone metabolism observed in terms of its reduction of serum testosterone and the activities of the main two enzymes in some tissues suggest beneficial effect of ingestion of PS to reduce the risk of prostate cancer.

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