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Binding of Steroid Hormones In Vitro by Water-Insoluble Dietary Fiber

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Abstract

BINDING OF STEROID HORMONES IN VITRO BY WATER-INSOLUBLE DIETARY FIBER

by

Crystal G. Whitten

Previous work conducted in our laboratory has shown that steroid hormones bind to natural (e.g., brans, oat hulls) and purified (e.g., cellulose, lignin) fiber components. To explore this relationship further, we assessed the in vitro binding of estrone, estradiol-17 β , and testosterone to a water-insoluble fiber fraction obtained from composite food samples of 18 Seventh-day Adventist (SDA) nonvegetarian (NV), 20 SDA lacto-ovovegetarian (LOV), and 15 SDA vegan (V) men. Three-day dietary food records and 72-hr food composites were obtained contemporaneously from each subject. The LOV and V subjects consumed significantly more crude, dietary and water-insoluble fiber than the NV subjects. Water-insoluble fiber bound an average of 73% of all estrogens tested, and 56% of the testosterone. Binding levels of testosterone were significantly higher in the Vs than in the LOVs and omnivores. Additionally, there was a significant relationship, for all groups combined, between water-insoluble fiber intakes and binding of estrone and

testosterone. These results suggest that diet may modify hormonal status, possibly through binding of steroid hormones by dietary fiber, leading to altered enterohepatic circulation of these hormones.

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BINDING OF STEROID HORMONES IN VITRO BY
WATER-INSOLUBLE DIETARY FIBER

by

Crystal G. Whitten

A Thesis in partial fulfillment
of the requirements for the Degree Master of Science
in Nutrition

December 1987

Each person whose signature appears below certifies that this thesis in his opinion is adequate in scope and quality as a thesis for the degree Master of Science.

_____, Chairman
Terry D. Shultz, Assistant Professor of Biochemistry and
Associate Professor of Nutrition.

David C. Nieman, Associate Professor of Health Promotion and
Education, and Associate Professor of Nutrition.

U.D. Register, Professor of Nutrition and Biochemistry.

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INTRODUCTION

The low risk of cancer in Seventh-day Adventists (SDAs) has been observed by a number of investigators (1-10). In comparing California SDAs and non-SDAs, Phillips (5) found that SDA males had a standardized prostate cancer mortality rate approximately 30% that of males in the general population. This suggests that some aspect of the SDA lifestyle, perhaps vegetarianism, may be associated with the lower risk.

Natural and purified fibers have been shown to bind unconjugated and conjugated steroid hormones in vitro. The extent of binding is a function of the type of fiber used and the particular steroid studied (11). The extent to which steroid hormones are bound by dietary components may interfere with enterohepatic circulation and absorption (12-17), thus affecting circulating hormone levels. Although dietary fiber is not known to be degraded by mammalian enzymes, it may be digested to varying degrees by microflora of the digestive tract. This digestibility is variable and dependent on the source of plant fiber (18, 19). Several dietary fiber components are degraded to only a small extent (e.g., hemicellulose, cellulose, and lignin), and it is reasonable to suggest that fiber binds steroid hormones in the gut, thus possibly reducing their involvement in the etiology of prostatic cancer (13). To assess the possible role of dietary fiber, experiments were designed to determine the

extent to which water-insoluble fiber residues from the diets of nonvegetarian and vegetarian men bound steroid hormones.

MATERIALS AND METHODS

Subject Selection

The nonvegetarian and lacto-ovovegetarian subjects were recruited from the Loma Linda University community, while the vegans were students and staff members of an SDA college in Northern California (Weimar Institute, Weimar, CA). Participants were individually interviewed and completed a personal and medical history questionnaire in order to determine their health history and current health status. The study was explained to potential participants and each subject signed an informed consent before acceptance. The protocol was approved by the University's Committee on Human Studies.

The experimental groups were comprised of healthy adult Caucasian male SDA nonvegetarians (NV), lacto-ovovegetarians (LOV), and vegans (V). The NV group consisted of 18 men, ranging in age from 21 to 34 yr; the LOVs, 20 men from 21 to 34 yr; and the Vs, 15 men from 23 to 36 yr. Subjects were classified as vegetarians if they ate meat, fish, or poultry no more than twice a month and had followed this dietary regimen for at least one year. The LOV and V subjects had been vegetarians for an average of 23 (range: 12-30) and 4 (range: 1-7) years, respectively. The NVs had eaten meat all their lives. On average they consumed meat, fish, or poultry six times per week. All participants were healthy men within 20% of ideal body weight with no apparent

metabolic disorders as assessed by their medical histories. Additionally, the subjects had no weight change of more than 4.5 kg during the past year.

Anthropometric Measurements

Weight (W) and height (H) were determined by the use of a calibrated physician's scale and a wall bound steel measuring tape. The body mass index (W/H^2) was calculated as an estimate of obesity (20,21).

Dietary Assessment

All subjects were individually instructed in keeping a three-day dietary record. Instructions were provided with the record sheets and included procedures for reporting intake, estimating portions and describing food combinations. Records were examined by the investigator to make certain that all relevant information was included with adequate description of the foods recorded. Foods were coded for computerized analysis of energy, carbohydrate, and crude fiber. The nutrient data bank was one developed by the Ohio State University Hospital and School of Allied Medical Professions (22). Dietary fiber was calculated separately (23-25). Diets were recorded for weekdays in a majority of instances.

Food Sample Collection

Duplicate food portions were collected by each subject on consecutive days over each 24-hr, three-day dietary record period. Portions were duplicated as precisely as possible by visual, volume and weight measurements. Subjects were instructed as to the importance of maintaining their usual food habits. After each duplicate portion was collected, its container was stored by the participant in his refrigerator until it was collected the next morning by the investigator and transported directly to the laboratory. Contents were weighed, homogenized in deionized water (25% w/v), and aliquots stored at -20°C.

Water-insoluble Fiber Preparation

The water-insoluble fiber fraction from each subject's 24-hr food collection was determined by a modified Hellendoorn technique as described by Asp and Johansson (26) with slight modifications. Twenty g of homogenized sample of each 24-hr dietary intake were initially lipid-extracted four times with 100 ml of ethyl ether and washed with 30 ml of water. The fiber residue was then re-weighed to obtain the fat-free weight and transferred to 250 ml Erlenmeyer flasks containing 50 ml of water and boiled for 15 min. After cooling to room temperature, pepsin

(3,750 IU/g food) was added and the pH adjusted to 2.0. Flasks were then covered with parafilm and incubated in a shaking water bath for 18 hr at 40°C. After incubation, 50 ml of 0.1 M NaH_2PO_4 was added to each flask and the pH adjusted to 6.8. Amylase (6,250 IU/g food) was next added and the samples were incubated in a shaking water bath for one hr at 40°C. Following incubation, the pH of the hydrolysates was adjusted to 4.5 and centrifuged at 1,000 x g for 30 min. The supernatant was removed and the fiber residue transferred to 50 ml glass centrifuge tubes and washed three times with 120 ml of water and twice with 40 ml of acetone. The water-insoluble fiber was then transferred to foil-lined weighing boats and dried in an oven overnight at 70°C. Water-insoluble fiber samples were weighed and stored in vials in a desiccator until final preparation for the fiber binding assay. All water and organic solvents were of high-performance liquid chromatography (HPLC) grade. Pepsin and amylase were purchased from Sigma Chemical Co., St. Louis, MO. HPLC grade ethyl ether and acetone were obtained from Fisher Scientific, Tustin, CA.

Fiber Binding Analysis

Prior to the binding analysis, the lipid-extracted and enzyme hydrolyzed residue was ground in a mortar and pestle and then passed through a Tyler Standard 175 micron (42 mesh) sieve [W.S. Tyler Co, Cleveland, OH] in order to obtain an homogeneous fiber particle size. Steroid binding capacity of the water-

insoluble dietary fiber was determined as described by Shultz and Howie (11). Binding was carried out by mixing 50 mg of binding substance with similar substrate quantities (0.043-0.057 μCi ; 0.89-0.95 pmol/incubation) of [^3H]estrone (0.95 pmol), [^3H]estradiol-17 β (0.94 pmol), and [^3H]testosterone (0.89 pmol) in 5 ml of 0.1 M phosphate buffer (pH 7.0) in a 25 ml stoppered Erlenmeyer flask. After incubation at 37°C for 2 hr, the reaction mixture was centrifuged at 12,000 x g for 10 min. One ml of supernatant was then transferred to 15 ml of BetaPhase counting fluor (InterChem Enterprises, Inc., San Diego, CA) and analyzed for radioactivity by liquid scintillation spectrometry in a Beckman Liquid Scintillation Counter (Model LS-7500). Counting efficiency for ^3H was approximately 36%. The total supernatant radioactivity was a measure of unbound steroid. All analyses were carried out in triplicate. Estrone and estradiol-17 β interassay variabilities were 2% for both (n=7). Testosterone interassay variability was 2% (n=8). The [6,7- ^3H]estrone and estradiol-17 β (60 Ci/mmol), and [1 β 2 β - ^3H]testosterone (49 Ci/mmol) were purchased from New England Nuclear, Boston, MA. Prior to use, all radiochemicals were checked for purity by thin-layer chromatography (27-29).

Statistical Analyses

Anthropometric measurements, dietary nutrient intakes, water-insoluble fiber and binding capacities were analyzed by use

of Duncan's multiple range comparison test of means.

Correlations between individual variables were examined by linear regression analysis (Pearson Correlation Coefficient).

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL). Statistical tests were considered to be significant at the $P < 0.05$ level.

RESULTS

Collection of the three-day dietary records and food composites were spread uniformly through April to July, 1986. Subjects from each group were studied throughout the time period.

The omnivorous and vegetarian participants differed significantly with respect to age, weight, height and body mass index (Table 1). The Vs were older ($P < 0.01$ for both) and weighed less ($P < 0.05$ for both) than the NVs and LOVs. Body mass index measurements were significantly greater for the NV than LOV and V men. The LOVs were taller than the NVs and Vs ($P < 0.01$ for both).

Table 2 presents three-day nutrient intakes for the three groups. Dietary intakes of energy were significantly higher for the LOV ($P < 0.05$) and V ($P < 0.01$) groups than the NVs. Carbohydrate consumption by the LOV and V men was significantly higher than the NV subjects ($P < 0.05$, $P < 0.01$), probably due to the higher grain and legume intake of the vegetarians. The Vs consumed significantly more energy ($P < 0.05$) and had higher carbohydrate intakes ($P < 0.01$) than did the LOVs. As expected, the two vegetarian groups (LOV and V) also consumed significantly more crude and dietary fiber ($P < 0.01$ for both groups and fiber measurements) than did the omnivores. Vegans consumed significantly more crude and dietary fiber than the LOVs. The crude and dietary fiber intakes of the combined groups (NV, LOV, and V) were significantly related ($r = 0.77$, $P < 0.001$).

Water-insoluble dietary fiber intakes of the NV, LOV and V groups as determined from 24-hr food collections are presented in Figure 1. Compared with the NVs, the LOVs had a 32% ($P<0.01$) and the Vs a 50% ($P<0.01$) higher level of water-insoluble fiber, while the Vs had the highest intake. In addition, simple correlation analysis of the combined groups revealed significant relationships between crude and dietary fiber and water-insoluble fiber intakes ($r=0.62$, $P<0.001$; $r=0.82$, $P<0.001$, respectively).

The percentages and relative amounts (pmol/incubation) of each steroid bound by water-insoluble fiber are shown in Table 3. Estrone and estradiol-17 β bound more of the water-insoluble fiber than did testosterone. Ability to bind hormones ranged from 53% (testosterone) to 75% (estrone). Binding of testosterone by the dietary water-insoluble fiber fraction was significantly higher for the V group than for the NVs and LOVs ($P<0.05$, $P<0.01$, respectively). However, binding levels of estrone and estradiol-17 β did not differ significantly between groups.

Regression analyses were conducted between hormonal binding parameters and water-insoluble fiber intake averages. Comparison of the percentages and amounts of estrone, estradiol-17 β , and testosterone bound with the water-insoluble fiber intakes of each of the three groups revealed no significant relationships. However, when the three groups were combined, significant relationships were found between estrone and testosterone percentages and concentration binding levels (data not shown) and water-insoluble fiber intakes [$r=0.30$, $P<0.05$ for both; $r=0.32$,

$P < 0.05$ for both; Figures 2 and 3]. Comparison, by linear regression analysis, of the combined group's estradiol-17 β with the water-insoluble fiber intake by linear regression analysis approached significance ($r = 0.24$, $P < 0.08$ for both percentages and amounts).

DISCUSSION

Crude and dietary fiber intakes as well as water-insoluble fiber levels of the three groups were similar to those previously reported for nonvegetarian and vegetarian men (13,26,30,31). Dietary fiber was measured by two distinct and independent methods. A three-day dietary intake record estimated total dietary fiber, whereas a chemical determination assessed water-insoluble fiber only. Although water-insoluble fiber is a constituent of total dietary fiber, the results showed mean values of the former to be greater than those of the latter. This may be explained by (a) the wide statistical variation of both determinations, or (b) the different methodologies employed for their determination.

We feel that the gravimetrically defined water-insoluble fiber values are valid and representative within the constraint of somewhat broad statistical variation. In addition, Kurup et al. (32) reported that chemically defined water-insoluble fiber components (i.e., neutral detergent fiber, hemicellulose, lignin, and cellulose) comprised between 50% to 70% of the total dietary fiber in study groups comparable to ours.

The water-insoluble fiber sequestered unconjugated estrogen and testosterone to different degrees. However, the insoluble fiber appeared to bind significant proportions of the estrone, estradiol-17 β , and testosterone. The binding ability of water-insoluble fiber remained moderately high, averaging 67% for all

the steroids. Shultz and Howie (11) previously observed enhanced adsorption by wheat, oat, and corn brans, oat hulls, cellulose, and lignin by the less hydrophilic, mono- and dihydroxy steroids, thus tending to support the theory of hydrophobic fiber binding for bile salts [compounds structurally related to steroid hormones] as proposed by Eastwood and Hamilton (33). Several investigators (11,33-35) have reported that lignin may be the principal component responsible for binding bile acids and steroid hormones.

Recent work in our laboratories has been directed toward identifying which specific dietary fiber fractions are important in the binding of steroid hormones. Preliminary unpublished observations reveal that lignin correlates most closely to the binding (by percentage and amount) of estrone, estradiol-17 β and testosterone. This finding was most evident for the V group (estrone: $r=0.56$, $P<0.025$; estradiol-17 β : $r=0.68$, $P<0.005$; and testosterone: $r=0.13$, N.S.), but also held true for the three groups combined (estrone: $r=0.25$, $P<0.025$; estradiol-17 β : $r=0.24$, $P<0.05$; and testosterone: $r=0.25$, $P<0.025$). No significant relationships were found for the binding of neutral detergent fiber, hemicellulose, or cellulose.

The findings of this study tend to support the results of Shultz and Howie (11), who found that lignin exhibited substantial steroid-binding properties. Consequently, lignin may be the key structural component of water-insoluble fiber residue most responsible for steroid binding.

Epidemiologic studies have demonstrated an association between dietary nutrients, especially fat and fiber, and the incidence of hormone-dependent cancers, such as prostate and breast (36-39). Recent studies indicate that diet may modify sex-hormone metabolism in both men and women (12-14,40-42). Indeed, male and female nonvegetarians and vegetarians, known to be at different risks for prostate and breast cancers, were found to exhibit different plasma levels of unconjugated androgens and estrogens (12-14,40-42). Goldin et al. (12) reported that food fiber increases the intestinal clearance of estrogen in premenopausal women. They found a significant negative correlation between fiber intake and blood estrogens and a positive correlation between fiber intake and fecal estrogen suggesting that food fiber increases clearance and fecal excretion. Blood estrogen levels were lower in the vegetarians than in the omnivorous women. Howie and Shultz (13) showed that plasma levels of testosterone and estradiol-17 β were lower in male SDA vegetarians than in nonvegetarians. The vegetarians were consuming more crude and dietary fiber than the omnivores. Additionally, the present finding of an association between dietary water-insoluble fiber and steroid binding supports the hypotheses of both Goldin et al. (12) and Howie and Shultz (13) that estrogen and androgen metabolism may be related to dietary intake by altering the enterohepatic circulation of these steroids through fecal excretion (15-17). Dietary fiber has been suggested to play a similar role in the fecal excretion of bile

acids (43-45), another group of metabolites structurally related to steroid hormones which undergo enterohepatic circulation.

Our findings show for the first time that binding of steroid hormones by dietary water-insoluble fiber is related to the actual fiber content of the diet. However, further studies are warranted to provide additional information on the mechanism whereby specific dietary components may alter sex-hormone production and metabolism.

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Table 1. Demographic and anthropometric characteristics of the Seventh-day Adventist (SDA) nonvegetarian (NV), lacto-ovovegetarian (LOV), and vegan (V) men.

Parameter	NV (n=18)	LOV (n=20)	V (n=15)
Age (yr)	25.5±3.8 ^{†, #}	25.7±3.2 ^{\$}	29.5±4.5 ^{#, \$}
Height (cm)	178.2±6.9 [#]	186.0±7.1 ^{#, \$}	179.3±6.3 ^{\$}
Weight (kg)	75.2±8.1 ^{**}	73.9±8.9 ^{††}	67.2±9.4 ^{**, ††}
Body Mass Index*	23.6±1.6 ^{#, \$}	21.3±2.2 [#]	20.8±1.8 ^{\$}

* Weight (kg)/height (m²).

[†] Mean ± SD.

^{#, \$} P<0.01; ^{**}, ^{††} P<0.05: values sharing the same superscript in a row differ significantly from each other.

Table 2. Three-day dietary intake averages of the NV, LOV, and V men.

Nutrient	NV (n=18)	LOV (n=20)	V (n=15)
Energy (kcal)	2,061±356 ^{*,†} , #	2,489±615 [†] , \$	2,915±739 [#] , \$
Carbohydrate (g)	266±64 [†] , #	351±87 [†] , **	475±135 [#] , **
Crude Fiber (g/1000kcal)	2±1 [#] , **	4±2 [#] , ††	6±1 ^{**} , ††
Dietary Fiber (g/1000kcal)	8±3 [#] , **	14±5 [#] , ††	18±4 ^{**} , ††

* Mean ± SD.

†, \$ P<0.05; #, **, †† P<0.01: values sharing the same superscript in a row differ significantly from each other.

Table 3. Binding of steroid hormones by the dietary water-insoluble fiber fraction.

Hormone	NV (n=18)	LOV (n=20)	V (n=15)
Estrone	74.5±4.4* [0.66±0.04] [†]	72.7±3.6 [0.65±0.03]	74.7±1.7 [0.66±0.02]
Estradiol-17β	73.5±4.4 [0.69±0.04]	71.1±4.1 [0.67±0.04]	72.2±2.2 [0.68±0.02]
Testosterone	55.8±5.6 [#] [0.49±0.05] [#]	53.1±4.4 ^{\$} [0.47±0.04] ^{\$}	59.2±4.1 ^{#,\$} [0.53±0.04] ^{#,\$}

* Mean ± SD, percent bound; individual binding capacities were determined from composite 72-hr samples.

[†] Amount of steroid bound [pmol bound/incubation] stated in brackets.

[#] P<0.05; ^{\$} P<0.01: values sharing the same superscript in a row differ significantly from each other.

Figure Legends

Figure 1. Water-insoluble fraction (Mean $\pm$ SD) of dietary fiber in the nonvegetarian (NV), lacto-ovovegetarian (LOV), and vegan (V) groups. Group values were derived from the mean of three separate determinations per individual. Significant differences: NV vs. LOV, NV vs. V, and LOV vs. V, $P < 0.01$.

Figure 2. Correlation of the combined groups [NV(●), LOV(○), and V(▲)] percentage estrone binding levels with water-insoluble fiber intakes. The points represent individual mean values about the regression line.

Figure 3. Correlation of the combined groups [NV(●), LOV(○), and V(▲)] percentage testosterone binding levels with water-insoluble fiber intakes. See Figure 2 for further details.

Figure 1

Figure 2

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Figure 3