Effects of dietary fat and fiber on plasma and urine androgens and estrogens in men: a controlled feeding study[1,2]

Joanne F Dorgan, Joseph T Judd, Christopher Longcope, Charles Brown, Arthur Schatzkin, Beverly A Clevidence, William S Campbell, Padmanabhan P Nair, Charlene Franz, Lisa Kahle, and Philip R Taylor

ABSTRACT We conducted a controlled feeding study to evaluate the effects of fat and fiber consumption on plasma and urine sex hormones in men. The study had a crossover design and included 43 healthy men aged 19–56 y. Men were initially randomly assigned to either a low-fat, high-fiber or high-fat, low-fiber diet for 10 wk and after a 2-wk washout period crossed over to the other diet. The energy content of diets was varied to maintain constant body weight but averaged ≈13.3 MJ (3170 kcal)/d on both diets. The low-fat diet provided 18.8% of energy from fat with a ratio of polyunsaturated to saturated fat (P:S) of 1.3, whereas the high-fat diet provided 41.0% of energy from fat with a P:S of 0.6. Total dietary fiber consumption from the low- and high-fat diets averaged 4.6 and 2.0 $g \cdot MJ^{-1} \cdot d^{-1}$, respectively. Mean plasma concentrations of total and sex-hormone-binding-globulin (SHBG)-bound testosterone were 13% and 15% higher, respectively, on the high-fat, low-fiber diet and the difference from the low-fat, high-fiber diet was significant for the SHBG-bound fraction ($P = 0.04$). Men's daily urinary excretion of testosterone also was 13% higher with the high-fat, low-fiber diet than with the low-fat, high-fiber diet ($P = 0.01$). Conversely, their urinary excretion of estradiol and estrone and their 2-hydroxy metabolites were 12–28% lower with the high-fat, low-fiber diet ($P \leq 0.01$). Results of this study suggest that diet may alter endogenous sex hormone metabolism in men. *Am J Clin Nutr* 1996;64: 850–5.

KEY WORDS Diet, dietary fats, dietary fiber, estrogen, androgens

INTRODUCTION

Diet, particularly fat consumption, has been implicated as a risk factor for prostate cancer. Prostate cancer mortality is higher in countries where per capita consumption of animal fat is elevated (1), and high-fat diets have been associated with an increased risk of prostate cancer in several case-control and cohort studies (2–9). In the Health Professionals Follow-up Study, a prospective study of 51 529 men, the relative risk for advanced prostate cancer was 1.79 (95% CI = 1.04, 3.07) for those in the highest compared with the lowest quintile of fat intake, and the association was primarily due to animal fat consumption (8).

Prostate cancer is a hormone-dependent cancer and a current hypothesis is that diet modifies prostate cancer risk through an effect on the sex hormones (10, 11). Male vegetarians have been reported to have lower plasma testosterone and estradiol concentrations than omnivores (12), and dietary fat and fiber have been correlated with sex hormone concentrations in several studies (12–14). Specific diet-hormone relations reported in men include positive correlations of testosterone with polyunsaturated fat (13) and dihydrotestosterone with vegetable fat consumption (14) and inverse correlations of testosterone and estradiol with fiber intake (12). However, comparisons between vegetarians and omnivores and correlations of specific dietary components with hormones from different studies have been inconsistent (12–17). Moreover, in three diet intervention studies (18–20), serum or urine testosterone levels were depressed with the low-fat, high-fiber or vegetarian diet, but findings for other hormones were inconsistent.

In 1986 the National Cancer Institute and Beltsville Human Nutrition Research Center, Agriculture Research Service, conducted a controlled feeding study in men to evaluate the effect of modifying dietary fat and fiber intakes on several indexes potentially related to cancer or atherosclerosis, including plasma lipoproteins (21), prostaglandins (22), fecal mutagens, and hormones. As part of this study, we evaluated the effect of these dietary components on plasma and urine androgens and estrogens.

[1] From the Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, MD; the Diet and Human Performance Laboratory, Beltsville Human Nutrition Research Center, ARS, US Department of Agriculture, Beltsville, MD; the Departments of Obstetrics and Gynecology and Medicine, University of Massachusetts Medical School, Worcester, MA; and Information Management Services, Inc, Silver Spring, MD.

[2] Address reprint requests to JF Dorgan, CPSA, DCPC, National Cancer Institute, Executive Plaza North, Room 211, 6130 Executive Boulevard, Bethesda, MD 20892-7326. E-mail: dorganj@dcpcepn.nci.nih.gov.

Received February 15, 1996.

Accepted for publication July 25, 1996.

SUBJECTS AND METHODS

Healthy male volunteers aged 19–56 y from the Beltsville, MD, area who met the following criteria were recruited for the controlled feeding study in 1986: *1*) no history of diabetes, cancer, or cardiovascular, kidney, or chronic gastrointestinal disease; *2*) no medication use other than an occasional analgesic; *3*) a weight-for-height 80–130% of the desirable value based on 1983 Metropolitan Life Insurance tables (23); *4*) normal results from a physical examination, including a complete blood count and biochemical profile; and *5*) no adherence to a vegetarian diet in the past year. The research with human volunteers was approved by the Institutional Review Boards of Georgetown University and the National Cancer Institute. Informed consent was obtained from all men before enrollment.

The high-fat, low-fiber diet was designed to provide ≈40% of energy from fat, with a ratio of polyunsaturated to saturated fat (P:S) of 0.5, and 2.0 g total dietary fiber/MJ. The low-fat, high-fiber diet was designed to provide < 20% of energy from fat, a P:S of 1.2, and 4.6 g total dietary fiber/MJ. For both diets, approximately one-third of the fiber was to come from each of the fruit and vegetable, cereal, and legume groups. The study used a crossover design. Participants were paired by age, smoking status, and body mass index, and one man from each pair was randomly assigned to each diet. After 10 wk on the diet and a 2-wk washout period, participants were crossed over to the other diet for 10 wk.

All meals were prepared in the Human Study Facility at the Beltsville Human Nutrition Research Center. On weekdays subjects ate breakfast and dinner in the dining room at this facility and lunches were provided at breakfast for consumption later in the day at work or at home. On weekends subjects ate prepackaged meals at home. No foods other than those provided by the study were permitted except tea and coffee. Sweeteners and other additives to these beverages were limited to those provided by the study, and quantities used were recorded by participants. Salt was allowed ad libitum and consumption was estimated by using tared salt shakers. Water of known mineral content was provided to participants. Alcohol and vitamin and mineral supplements were prohibited.

Men were weighed on each weekday and the energy content of the diets was varied in 1.7-MJ (400-kcal) increments to maintain constant body weight. Diets provided all known nutrients in amounts to meet recommended dietary allowances (24). Nutrient composition was calculated by using US Department of Agriculture (USDA) food-composition data together with data from the food industry, the Nutrition Coordinating Center at the University of Minnesota, and analyzed values. A 7-d menu cycle was used and composites of each menu were analyzed to confirm the nutrient composition.

Blood was collected between 0600 and 0900 after a 12-h fast on 1 d during the last week of each diet phase of the controlled diet study. A 24-h urine sample was also collected on every day of the final week and aliquots proportional to the total daily volume from each day were pooled to create a single urine specimen for each dietary period. Urine was collected on ice and kept cold until measured and apportioned for storage. All plasma and urine specimens were stored at −80 °C or lower until shipped to the laboratory on dry ice for hormone analyses, which were performed between 1988 and 1989.

Testosterone, dihydrotestosterone, estrone, and estradiol in plasma were measured by radioimmunoassay after solvent extraction and celite chromatography (25). Androstanediol glucuronide was measured by radioimmunoassay as described by Horton et al (26–28). Dehydroepiandrosterone sulfate (DHEAS) was measured with a radioimmunoassay kit (ICN-Biomedical, Costa Mesa, CA) and SHBG was measured with an immunoradiometric assay kit (Farmos Group Ltd, Oulunsalo, Finland). The percentages of unbound and albumin-bound testosterone and estradiol were measured by using centrifugal ultrafiltration (29, 30), and percentages of SHBG-bound hormones were calculated. Urinary testosterone glucuronide was measured with a radioimmunoassay kit (ICN-Biomedical) and our results were similar to those reported for normal males by Doberne and New (31) and Tresguerres et al (32) after hydrolysis with β-glucuronidase. The urinary estrogens were measured by radioimmunoassay after β-glucuronidase hydrolysis (33) and LH-20 sephadex chromatography (Pharmacia LKB Biotechnology, Piscataway, NJ) as described (34). Urinary 2-hydroxyestrone and 2-hydroxyestradiol were measured by radioimmunoassay according to the methods of Chattoraj et al (35).

For each analyte, plasma and urine samples from the same man were analyzed in the same batch to remove the effects of between-batch variability in hormone assays. Within-batch CVs of plasma hormone measurements in replicate quality-control samples averaged 8.2% for estrone, 5.7% for estradiol, 11.8% for testosterone, 13.7% for dihydrotestosterone, and 8.9% for DHEAS. The within-batch CV for the percentage free and albumin-bound estradiol and testosterone were all < 10% except for free estradiol, which was 10.8%. In urine the within-batch CV for glucuronides averaged 16.0% for estrone, 6.8% for estradiol, 20.8% for estriol, and 9.2% for testosterone. For the catechols, the within-batch CV averaged 9.4% for 2-hydroxyestrone and 12.0% for 2-hydroxyestradiol.

Because distributions of plasma and urine hormones were not normal, geometric means were used to describe the data. Methods proposed by Fleiss (36) were used to evaluate carryover and diet effects. To determine whether carryover from period 1 to period 2 was the same regardless of initial diet, for each hormone the sum of levels from both periods for men initially randomly assigned to the high-fat, low-fiber diet were compared with the sum for men initially randomly assigned to the low-fat, high-fiber diet by using a Wilcoxon rank-sum test. Because there was no evidence of differential carryover for any hormone at a significance level of ≤ 0.10, carryover was ignored when the effect of diet was evaluated. The diet effect was estimated by the difference between hormone levels after the high-fat, low-fiber and low-fat, high-fiber diets regardless of diet order. The significance of the diet effect was tested by comparing the difference in hormone levels between periods 1 and 2 for men initially randomly assigned to the high-fat, low-fiber diet with the same difference for men initially randomly assigned to the low-fat, high-fiber diet using a t test if the distribution of differences was normally distributed and a Wilcoxon rank-sum test otherwise. All analyses were performed by using SAS statistical software (37).

RESULTS

Of the 45 men randomly assigned to the controlled diet study, 43 completed both phases. Their mean (± SD) age was 33.8 ± 9.2 y and their median body weights at baseline and at the end of the controlled feeding study, respectively, were 79.6 kg (range: 58.2–122.2 kg) and 79.0 kg (range: 60.2–115.8 kg), which were not significantly different. Seven (16%) of the men were black and 10 (23%) smoked cigarettes.

The nutrient composition of the actual diets consumed during each study period is shown in **Table 1**. Energy intake was ≈13.3 MJ (3170 kcal)/d on both the high-fat, low-fiber and low-fat, high-fiber diets. The median daily percentage of energy from fat was 41.0% (range: 38.6–42.0%) with the high-fat diet and 18.8% (range: 17.4–20.5%) with the low-fat diet. Type of fat also differed between diets; the median P:S was 0.6 (range: 0.5–0.7) for the high-fat diet compared with 1.3 (range: 1.0–1.6) for the low-fat diet. Total dietary fiber intake from the low-fat diet was more than twice that from the high-fat diet.

Hormones in plasma and urine specimens collected at the end of each period of the controlled diet study and differences between the high-fat, low-fiber and low-fat, high-fiber diets are shown in **Tables 2** and **3**. Plasma concentrations of androgens tended to be elevated with the high-fat, low-fiber diet; geometric mean concentrations of total and SHBG-bound testosterone were 13% and 15% higher, respectively, compared with the low-fat, high-fiber diet and for SHBG-bound testosterone the mean difference was significant ($P = 0.04$). Men's daily excretion of testosterone glucuronide was also 13% higher when the high-fat, low-fiber diet was consumed than when the low-fat, high-fiber diet was consumed, and the mean difference was significant ($P = 0.01$). We also evaluated the effect of diet on the ratio of dihydrotestosterone to testosterone in plasma and found that the ratio was essentially unchanged after the low-fat, high-fiber diet compared with that after the high-fat, low-fiber diet; the mean difference was −0.01 (95% CI = −0.02, 0.01).

Trends were inconsistent for the plasma estrogens, and none of the differences examined were significant. However, men's excretion of glucuronides of estradiol and estrone and their 2-hydroxy metabolites were all 12–28% lower after the high-fat, low-fiber diet ($P \leq 0.01$).

DISCUSSION

In this controlled feeding study plasma androgens in a single blood draw tended to be elevated when men ate a high-fat, low-fiber compared with a low-fat, high-fiber diet for 10 wk. With the high-fat, low-fiber diet, amounts of testosterone glucuronide were also greater and estradiol and estrone glucuronide amounts were lower in 24-h urine samples collected over 1 wk and pooled.

Howie and Shultz (12) reported lower plasma testosterone and estradiol concentrations in male vegetarians than in meat eaters, but the majority of studies that compared plasma androgens and estrogens in vegetarians and omnivores did not detect differences (13, 15–17). Vegetarians were reported to have elevated SHBG concentrations in two studies (13, 16), and in one (16) the ratio of testosterone to SHBG was depressed, suggesting that vegetarians may have less non-SHBG-bound, or bioavailable, testosterone compared with omnivores. In the study by Howie and Shultz (12), plasma testosterone and estradiol concentrations were inversely correlated with dietary fiber ingestion. Key et al (13) on the other hand found positive correlations of testosterone with polyunsaturated fat intake and SHBG with total, saturated, and polyunsaturated fat intakes.

Twenty-four–hour excretions of the androgens DHEAS, androsterone, and etiocholanolone, and the estrogens estradiol, estrone, and estriol were significantly lower in middle-aged South African blacks, who customarily follow a vegetarian diet compared with North American blacks eating meat (18). When the North Americans were fed a diet without any meat or meat products, their 24-h urine excretion of androgens and estrogens decreased significantly, and when the South Africans were switched to a Western diet including meat, their urine output of these hormones increased. Diet-hormone relations were age-dependent, however, and when South African blacks aged ≥ 60 y ate meat, their urine estrogens and androgens and serum androgens were lower than when they consumed a vegetarian diet (18, 38).

Because vegetarians could differ from omnivores on characteristics other than diet that influence hormone concentrations, Raben et al (20) investigated the effect of consuming a vegetarian diet on serum hormones in a controlled study. Eight men were fed a lactoovovegetarian diet and a mixed-meat diet

TABLE 1
Medians and ranges of daily nutrient consumption by diet

	High-fat, low-fiber diet		Low-fat, high-fiber diet	
	Median	Range	Median	Range
Energy (MJ)	13.2	10.2–18.5	13.3	10.0–18.5
Protein (% of energy)	14.8	13.0–18.1	17.1	16.0–17.9
Carbohydrate (% of energy)	45.3	43.6–48.8	67.5	66.0–68.7
Total fat (% of energy)	41.0	38.6–42.0	18.8	17.4–20.5
Saturated fat (% of energy)	14.7	13.9–15.4	4.4	3.7–5.2
Linoleic acid (% of energy)	8.1	6.6–8.8	5.3	4.1–6.3
Oleic acid (% of energy)	14.1	12.1–16.1	6.4	5.5–7.4
P:S[1]	0.6	0.5–0.7	1.3	1.0–1.6
Cholesterol (mg/MJ)	45.1	44.1–46.3	18.2	17.5–18.6
Total dietary fiber (g/MJ)	2.0	2.0–2.0	4.6	4.6–4.6

[1] Ratio of polyunsaturated to saturated fat.

TABLE 2
Geometric mean (95% CI) concentrations and mean (95% CI) differences in plasma sex-hormone-binding globulin (SHBG), androgens, and estrogens by diet

	High-fat, low-fiber diet		Low-fat, high-fiber diet		High-fat diet − low-fat diet		
	Mean	95% CI	Mean	95% CI	Difference[1]	95% CI	P^2
SHBG (nmol/L)	19.2	16.9, 21.6	18.5	16.2, 21.2	1.2	−0.6, 2.9	0.33
Androgens							
Testosterone (nmol/L)							
Total	13.3	11.6, 15.3	11.8	10.1, 13.8	1.6	−0.2, 3.5	0.10
Free	0.31	0.26, 0.36	0.33	0.23, 0.28	0.03	−0.02, 0.08	0.27
Albumin-bound	4.4	3.8, 5.2	4.1	3.4, 4.9	0.4	−0.4, 1.1	0.37
SHBG-bound	8.2	7.0, 9.7	7.1	6.0, 8.5	1.2	0.1, 2.4	0.04
Dihydrotestosterone (nmol/L)	1.2	1.1, 1.4	1.1	0.9, 1.3	0.09	−0.04, 0.22	0.20
DHEAS (μmol/L)	9.3	8.2, 10.5	8.8	7.6, 10.1	0.4	−0.6, 1.4	0.10
Androstanediol glucuronide (nmol/L)	1.7	1.5, 1.9	1.7	1.5, 1.9	0.1	−0.1, 0.2	0.99
Estrogens							
Estrone (pmol/L)	154.1	140.3, 170.0	148.0	133.8, 164.7	4.8	−6.3, 16.0	0.28
Estradiol (pmol/L)							
Total	99.4	87.9, 112.6	104.6	90.9, 120.3	−6.8	−18.7, 5.0	0.31
Free	2.2	2.0, 2.5	2.4	2.1, 2.8	−0.3	−0.6, 0.1	0.15
Albumin-bound	32.9	28.8, 37.4	36.3	30.7, 42.7	−1.7	−6.1, 2.3	0.12
SHBG-bound	63.4	55.7, 72.2	64.9	56.5, 74.1	−2.9	−10.3, 4.4	0.46

[1] Mean differences of untransformed values after removal of outliers: total estradiol (n = 1), free estradiol (n = 1), albumin-bound estradiol (n = 3). DHEAS, dehydroepiandrosterone sulfate.

[2] P value for test of H_o: difference = 0 when using all observations based on a t test when differences were normally distributed (total, free, albumin-bound, and SHBG-bound testosterone; dihydrotestosterone; and SHBG-bound estradiol) and a Wilcoxon rank-sum test when differences were not normally distributed (SHBG, DHEAS, androstanediol glucuronide, estrone, and total, free, and albumin-bound estradiol).

each for 6 wk. The diets were isoenergetic and ≈28% of energy was derived from fat on both diets. The P:S, however, was > 1 with the vegetarian diet compared with ≈0.5 with the meat diet and the fiber content of the vegetarian diet was approximately twice that of the meat diet. Prediet serum concentrations of testosterone were comparable and decreased significantly by 35% after the vegetarian diet but not after the meat diet. Differences in other androgens and estrogens were also reported, but these were attributed to dissimilarities in baseline concentrations.

Hamalainen et al (19) studied the effect of modifying dietary fat on serum sex hormones in 30 healthy, free-living men 40–49-y old. Serum sex hormones were measured after a 2-wk period when men consumed their usual diets, which provided 40% of energy as fat with a P:S of 0.15 and again after a 6-wk intervention period when men consumed isoenergetic diets that provided 25% of energy as fat with a P:S of 1.22. After the intervention, men had significantly lower serum concentrations of androstenedione and total and free testosterone. However, concentrations of dihydrotestosterone, DHEAS, estradiol, and estrone did not differ between the two periods.

Reed (39) failed to detect a difference in total testosterone in six men following isoenergetic diets providing 20 and 100 g fat/d. Because SHBG concentrations increased, however, free testosterone decreased after the low-fat diet.

Our finding of greater daily urine testosterone excretion in men consuming a high-fat, low-fiber diet relative to men consuming a low-fat, high-fiber diet is consistent with the findings of most controlled feeding studies. We also observed elevated plasma testosterone concentrations when men ate the high-fat, low-fiber diet, but the difference from concentrations in men who consumed the low-fat, high-fiber diet was significant only

TABLE 3
Geometric mean (95% CI) amounts and mean (95% CI) differences in daily urine hormones by diet

	High-fat, low-fiber diet		Low-fat, high-fiber diet		High-fat diet − low-fat diet		
	Mean	95% CI	Mean	95% CI	Difference[1]	95% CI	P^2
Glucuronides							
Testosterone (nmol/d)	161.3	124.6, 207.4	143.0	112.5, 180.8	26.1	9.6, 42.6	0.01
Estrone (nmol/d)	11.1	9.8, 12.6	13.8	12.3, 15.6	−2.4	−3.4, −1.3	0.0002
Estradiol (nmol/d)	3.8	3.4, 4.3	4.3	3.7, 4.9	−0.6	−1.0, −0.2	0.008
Estriol (nmol/d)	6.8	5.5, 8.3	6.8	5.5, 8.4	0.3	−0.7, 1.4	0.57
Catechols							
2-hydroxyestrone (nmol/d)	71.8	65.1, 78.6	85.2	76.4, 94.1	−14.6	−21.6, −7.5	0.0003
2-hydroxyestradiol (nmol/d)	9.3	8.1, 10.7	12.9	11.5, 14.4	−3.4	−4.8, −2.0	<0.0001

[1] Mean differences of untransformed values after removal of outliers: testosterone (n = 2), estrone (n = 1), estriol (n = 1).

[2] P value for test of H_o: difference = 0 when using all observations based on a t test when differences were normally distributed (estradiol, 2-hydroxyestrone, 2-hydroxyestradiol) and a Wilcoxon rank-sum test when differences were not normally distributed (testosterone, estrone, and estriol).

for the fraction that was SHBG-bound. The lack of significance for plasma total testosterone may have been due to within-person variation in hormone concentrations. Plasma testosterone has been reported to exhibit considerable diurnal variation, falling 42% from morning until night in one study (40). Furthermore, because of short-term fluctuations in blood concentrations, Goldzieher et al (41) estimated that a testosterone value from a single blood draw would be within 20% of the underlying mean only 68% of the time. Testosterone amounts in 24-h urine specimens collected over 1 wk and pooled, therefore, are probably a more valid assessment than are concentrations in a single-morning blood draw collected after an overnight fast.

We observed lower daily urine estradiol and estrone glucuronide excretion in men after consumption of a high-fat, low-fiber diet. Hill et al (18) also reported lower urine estrogens in South African black men aged ≥ 60 y who ate a diet with meat rather than their usual vegetarian diet. In younger men aged < 55 y, the opposite effect was observed, indicating that age modified the relation of diet and urine hormones. The men in our study were 19–56 y of age; when we analyzed the data separately for those aged ≤ 30 y and for those aged > 30 y, both groups had lower urine estrogens after consuming the high-fat, low-fiber diet.

Because the major source of estrogen in men is the peripheral aromatization of androgens, our finding of an increase in urinary excretion of testosterone but a slight decrease in the excretion of estrone and estradiol after a high-fat low-fiber diet was unexpected. However, it is possible that this was a result of a decrease in the peripheral aromatization of androgens that offset the slight increase in androgen amounts. Although peripheral aromatization of androgens is influenced by body weight (42), the men's weight was stable throughout the study. No data are available on the effect of dietary fat and fiber on peripheral aromatization.

The major urinary metabolites of estradiol are the catechols (2-hydroxyestradiol and 2-hydroxyestrone) and 16α-hydroxyestrone. Although controversial (43), 16α-hydroxyestrone has been implicated in mammary carcinogenesis (44) and could potentially play a role at different sites. We observed decreased excretion of catechol estrogens after a high-fat, low-fiber diet. Although we did not measure urinary 16α-hydroxyestrone, we did measure estriol, which was unaffected by diet in our study. Among women, Longcope et al (45) found that consumption of a high-fat diet resulted in decreased excretion of catechol estrogens and significantly increased excretion of 16α-hydroxyestrone and its metabolite estriol. Adlercreutz et al (43), however, reported no difference in urinary of 2-hydroxyestrone between omnivorous and vegetarian women, although their dietary fat and fiber intakes differed significantly. Additional studies are needed to clarify whether discrepancies in the effects of dietary fat and fiber on the pathways of estrogen metabolism reflect a sex difference and/or are due to differences in study design.

In summary, results of this controlled feeding study suggest that dietary fat and fiber may affect sex hormone metabolism in men in a way that may influence prostate cancer risk.

REFERENCES

1. Rose DP, Boyar AP, Wynder EL. International comparisons of mortality rates for cancer of the breast, ovary, prostate, and colon, and per capita food consumption. Cancer 1986;58:2363–71.
2. Graham S, Haughey B, Marshall J, et al. Diet in the epidemiology of carcinoma of the prostate gland. J Natl Cancer Inst 1983;70:687–92.
3. Ross RK, Shimizu H, Paganini-Hill A, Honda G, Henderson BE. Case-control studies of prostate cancer in blacks and whites in Southern California. J Natl Cancer Inst 1987;78:869–74.
4. Kolonel L, Yoshizawa CN, Hankin JH. Diet and prostatic cancer: a case-control study in Hawaii. Am J Epidemiol 1988;127:999–1012.
5. West DW, Slattery ML, Robison LM, French TK, Mahoney AW. Adult dietary intake and prostate cancer risk in Utah: a case-control study with special emphasis on aggressive tumors. Cancer Causes Control 1991;2:85–94.
6. Bravo MP, Castellanos E, del Rey Calero J. Dietary factors and prostatic cancer. Urol Int 1991;46:163–6.
7. Whittemore AS, Kolonel LN, Wu AH, et al. Prostate cancer in relation to diet, physical activity, and body size in blacks, whites, and Asians in the United States and Canada. J Natl Cancer Inst 1995;87:652–61.
8. Giovannucci E, Rimm EB, Colditz GA, et al. A prospective study of dietary fat and risk of prostate cancer. J Natl Cancer Inst 1993;85:1571–9.
9. Le Marchand L, Kolonel LN, Wilkens LR, Myers BC, Hirohata T. Animal fat consumption and prostate cancer: a prospective study in Hawaii. Epidemiology 1994;5:276–82.
10. Pienta K, Esper PS. Is dietary fat a risk factor for prostate cancer? J Natl Cancer Inst 1993;85:1538–40.
11. Ross RK, Henderson BE. Do diet and androgens alter prostate cancer risk via a common etiologic pathway? J Natl Cancer Inst 1994;86:252–4.
12. Howie BJ, Shultz TD. Dietary and hormonal interrelationships among vegetarian Seventh-Day Adventists and nonvegetarian men. Am J Clin Nutr 1985;42:127–34.
13. Key TJA, Roe L, Thorogood M, Moore JW, Clark GMG, Wang DY. Testosterone, sex hormone-binding globulin, calculated free testosterone, and oestradiol in male vegans and omnivores. Br J Nutr 1990;64:111–9.
14. Field AE, Colditz GA, Willett WC, Longcope C, McKinlay JB. The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. J Clin Endocrinol Metab 1994;79:1310–6.
15. Deslypere JP, Vermeulen A. Leydig cell function in normal men: effect of age, life-style, residence, diet, and activity. J Clin Endocrinol Metab 1984;59:955–62.
16. Belanger A, Locong A, Noel C, et al. Influence of diet on plasma steroid and sex plasma binding globulin levels in adult men. J Steroid Biochem 1989;32:829–33.
17. Pusateri DJ, Roth WT, Ross JK, Shultz TD. Dietary and hormonal evaluation of men at different risks for prostate cancer: plasma and fecal hormone-nutrient interrelationships. Am J Clin Nutr 1990;51:371–7.
18. Hill P, Wynder EL, Garbaczewski L, Garnes H, Walker ARP. Diet and urinary steroids in black and white North American men and black South African men. Cancer Res 1979;39:5101–5.
19. Hamalainen EK, Adlercreutz H, Puska P, Pietinen P. Diet and serum sex hormones in healthy men. J Steroid Biochem 1984;20:459–64.
20. Raben A, Kiens B, Richter EA, et al. Serum sex hormones and endurance performance after a lactoovovegetarian and a mixed diet. Med Sci Sports Exerc 1992;24:1290–7.
21. Clevidence BA, Judd JT, Schatzkin A, et al. Plasma lipid and lipoprotein concentrations of men consuming a low-fat, high-fiber diet. Am J Clin Nutr 1992;55:689–94.
22. Ferretti A, Judd JT, Taylor PR, Schatzkin A, Brown C. Modulating influence of dietary lipid intake on the prostaglandin system in adult men. Lipids 1989;24:419–22.

23. Metropolitan Life Insurance Company. 1983 Height and weight tables. Stat Bull Metropol Insur Co 1983;64:2–9.
24. National Research Council. Recommended dietary allowances. 9th ed. Washington, DC: National Academy of Sciences, 1980.
25. Longcope C, Franz C, Morello C, Baker R, Johnston CC. Steroid and gonadotropin levels in women during the peri-menopausal years. Maturitas 1986;8:189–96.
26. Horton R, Hawks D, Lobo R. 3α,17β-androstanediol glucuronide in plasma: a marker of androgen action in idiopathic hirsutism. J Clin Invest 1982;69:1203–6.
27. Barberia J, Pages L, Horton R. Measurement of androstanediol in plasma in a radioimmunoassay using celite column chromatography. Fertil Steril 1976;27:1101–4.
28. Horton R, Endres D, Galmarini M. Ideal conditions for hydrolysis of androstane-3α,17β-diol glucuronide in plasma. J Clin Endocrinol Metab 1986;62:22–7.
29. Longcope C, Femino A, Johnston JO. Androgen and estrogen dynamics in the female baboon (*Papio anubis*). J Steroid Biochem 1988;31:195–200.
30. Longcope C, Hui SL, Johnston CC. Free estradiol, free testosterone and sex hormone binding globulin in peri-menopausal women. J Clin Endocrinol Metab 1987;64:513–8.
31. Doberne Y, New MI. Urinary androstanediol and testosterone in adults. J Clin Endocrinol Metab 1976;42:152–4.
32. Tresguerres JA, Lisboa BP, Tamm J. A simple radioimmunoassay for the measurement of testosterone glucosiduronate in unextracted urine. Steroids 1976;28:13–23.
33. Longcope C. Methods and results of aromatization studies in vivo. Cancer Res 1982;42(suppl):3307s–11s.
34. Rotti K, Stevens J, Watson D, Longcope C. Estriol concentrations in plasma of normal, non-pregnant women. Steroids 1975;25:807–16.
35. Chattoraj SC, Fanous AS, Cecchini D, Lowe EW. A radioimmunoassay method for urinary catechol estrogens. Steroids 1978;31:375–91.
36. Fleiss JL. The design and analysis of clinical experiments. New York: John Wiley and Sons, 1986:263–90.
37. SAS Institute, Inc. SAS user's guide, version 5. Cary, NC: SAS Institute Inc, 1985.
38. Hill P, Wynder EL, Garbaczewski L, Walker ARP. Effect of diet on plasma and urinary hormones in South African black men with prostatic cancer. Cancer Res 1982;42:3864–9.
39. Reed MJ, Cheng RW, Simmonds M, Richmond W, James VHT. Dietary lipids: an additional regulator of plasma levels of sex hormone binding globulin. J Clin Endocrinol Metab 1987;64:1083–5.
40. Rose RM, Kreuz LE, Holaday JW, Sulak KJ, Johnson CE. Diurnal variation of plasma testosterone and cortisol. J Clin Endocrinol Metab 1972;54:177–8.
41. Goldzieher JW, Dozier TS, Smith KD, Steinberger E. Improving the diagnostic reliability of rapidly fluctuating plasma hormone levels by optimized multiple-sampling techniques. J Clin Endocrinol Metab 1976;43:824–30.
42. Simpson ER, Merrill JC, Alexander J, Hollub AJ, Graham-Lorence S, Mendelson CR. Regulation of estrogen biosynthesis by human adipose cells. Endocrine Rev 1989;10:136–48.
43. Adlercreutz H, Fotsis T, Hockerstedt K, et al. Diet and urinary estrogen profile in premenopausal omnivorous and vegetarian women and in premenopausal women with breast cancer. J Steroid Biochem 1989;34:527–30.
44. Fishman J, Telang NT. The role of estrogen in mammary carcinogenesis. Ann NY Acad Sci 1995;768:91–100.
45. Longcope C, Gorbach S, Goldin B, et al. The effect of a low fat diet on estrogen metabolism. J Clin Endocrinol Metab 1987;64:1246–50.