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Low-Fat High-Fiber Diet Decreased Serum and Urine Androgens in Men

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To validate our hypothesis that reduction in dietary fat may result in changes in androgen metabolism, 39 middle-aged, white, healthy men (50–60 yr of age) were studied while they were consuming their usual high-fat, low-fiber diet and after 8 wk modulation to an isocaloric low-fat, high-fiber diet. Mean body weight decreased by 1 kg, whereas total caloric intake, energy expenditure, and activity index were not changed. After diet modulation, mean serum testosterone (T) concentration fell ($P < 0.0001$), accompanied by small but significant decreases in serum free T ($P = 0.0045$), 5α-dihydrotestosterone ($P = 0.0053$), and adrenal androgens (androstendione, $P = 0.0135$; dehydroepiandrosterone sulfate, $P = 0.0011$). Serum estradiol and SHBG showed smaller decreases. Parallel decreases in urinary excretion of some testicular and adrenal androgens were demonstrated. Metabolic clearance rates of T were not changed, and production rates for T showed a downward trend while on low-fat diet modulation. We conclude that reduction in dietary fat intake (and increase in fiber) results in 12% consistent lowering of circulating androgen levels without changing the clearance. **(*J Clin Endocrinol Metab* 90: 3550–3559, 2005)**

EPIDEMIOLOGICAL STUDIES USING case-control and cohort studies showed a link between dietary fat, in particular saturated fat (from animal fat and red meat) and cancer of the prostate (1–7). A higher risk of developing prostate cancer was associated with higher plasma levels of α-linolenic acid and oleic acid independent of high meat or dairy intake (8, 9). The mechanisms by which dietary fat influences the risk of prostate cancer are not known; tissue and/or serum androgen levels have been suggested as possible mediators (1).

Androgens are essential for the growth and development of the prostate gland. In nude mice carrying transplanted androgen-dependent human prostate cancer PC82 cell line, treatment with testosterone (T) resulted in tumor growth; conversely, reducing serum androgens to very low levels prevented tumor growth (10, 11). Clinical studies show that androgen ablation or antiandrogen therapy is associated with prostate cancer remission and improvement in metastatic prostate cancer (12). Despite these observations, epidemiological studies addressing the relationship between serum androgen (and estrogen) levels and prostate cancer risk produced controversial data (13, 14). In either case-control or prospective studies, when blood samples obtained from prostate cancer patients were compared with those in healthy subjects, most failed to show consistent or significant differences in serum sex hormone levels (14–30). A recent metaanalysis of cohort/nested case-control studies that performed adjustment for all measured serum hormones, age, and body mass index (BMI) demonstrated that men whose serum total T levels were in the highest quartile were 2.34 times more likely to develop prostate cancer than those in the lowest quartile (31). The lack of differences in serum androgen levels between patients with prostate cancer and controls reported in these studies may be related to the small number of patients studied, methodological difficulties, the problems of selecting appropriate control subjects, the timing of the blood samples, failure to reflect tissue compartment differences in androgen metabolism, and differences in the stages of the patients with prostate cancer (14). Most importantly, it was known that a single measurement of serum androgen level did not necessarily reflect the integrated androgen levels, their metabolic products, and/or androgen actions in the prostate (32). Meikle *et al.* (21) found that basal sex hormone levels were similar, whereas the metabolic clearance rate for T (MCR_T) and the production rate for T (PR_T) were significantly higher in patients with prostate cancer than in controls. These data suggest that alterations in MCR_T and PR_T may occur without demonstrable changes in serum T in prostate cancer patients.

In designing this study, we hypothesized that decreasing fat and increasing fiber intake would alter androgen metabolism and production. We anticipate that any decrease in androgens would be very small without leading to any

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Abbreviations: 5α-A-3α,17β-diol, 5α-Androstanediol-3α,17β-diol; 5β-A-3β,17β-diol, 5β-androstane-3β,17β-diol; Andr, androsterone; BMI, body mass index; CV, coefficient of variation; d_0T, serum unlabeled T; d_3T, trideuterated T; DHEA-S, dehydroepiandrosterone sulfate; DHT, 5α-dihydrotestosterone; E_2, estradiol; Epi-T, epitestosterone; Etio, etiocholanolone; G, glucuronide; GC-MS, gas chromatography-mass spectrometry; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MCR_T, metabolic clearance rate for T; LC-MS-MS, liquid chromatography-tandem mass spectrometry; PR_T, production rate for T; PSA, prostate-specific antigen; T, testosterone.

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symptoms or signs of androgen deficiency. The changes in androgens during low fat intake may also be reflected within the prostate, creating an unfavorable environment for prostate cancer growth and development. In the present study, we conducted dietary fat and fiber modulation in 39 healthy middle-aged men and showed that decreasing fat and increasing fiber in the diet resulted in decreases in circulating and urinary excretion of most testicular and adrenal androgens without changing significantly the clearance rates of T, the major androgen in the male.

Subjects and Methods

Subjects

We recruited 41 non-Hispanic white subjects into the study. Only non-Hispanic whites were studied to minimize the reported effects of ethnicity on androgen levels, especially 5α-reduced androgens, and metabolism (33–36). The subjects were between 50 and 63 yr old (54.2 ± 0.5 yr, mean ± SE). They had no significant medical histories; normal physical examinations, blood counts, serum chemistry, and liver function tests; and serum prostate-specific antigen (PSA) levels were less than 4 ng/ml. Their mean BMI was 27.8 ± 0.6 kg/m^2 at baseline. Before entry into the study, all subjects were screened by the General Clinical Research Center (GCRC) dietitians and completed a 4-d food record to ensure that they were consuming a high-fat (>30% calories as fat) and low-fiber (<20 g fiber/d) diet. Only two of the 41 subjects did not complete the study, due to personal reasons. The data were reported on the 39 subjects that completed the study. There were no serious adverse events reported during the study. The study was approved by the Institutional Review Board, and each subject signed a written consent form.

Study design (Fig. 1)

The first 19 subjects completed a unidirectional diet modulation from their customary high-fat (baseline) diet to a low-fat diet for 8 wk. They were studied at baseline and then 8 wk after the low-fat diet. The low-fat, high-fiber diet was provided by the Harbor-UCLA GCRC dietitians, with a 7-d menu for 8 wk. The next 20 subjects were randomized for a bidirectional diet modulation study. Thirteen subjects proceeded from baseline (high-fat) to a low-fat diet for 8 wk and then back to their customary high-fat diet for 8 wk. These 13 subjects were studied at the three time points (baseline, low-fat, and then high-fat), and an additional 2 wk were added between changing from low-fat back to their customary high-fat diet. Seven subjects were studied at baseline (high-fat), then after another 8 wk of their customary high-fat diet, and then after modulation to a low-fat diet for 8 wk (baseline, high-fat, low-fat) (Fig. 1).

About 10–14 d after their diet assessment, they were admitted to the GCRC for their androgen metabolism study. During this baseline period

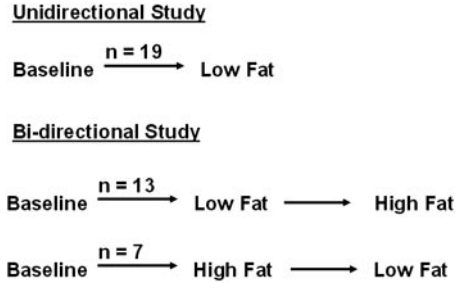

FIG. 1. Flow chart of the study. Thirty-nine subjects completed the study, of which 19 subjects were studied at baseline and then after unidirectional diet modulation to low-fat diet; 13 were studied at baseline, after diet modulation to low-fat diet for 8 wk, and then after taking their customary high-fat diet for 8 wk; and seven were studied at baseline, studied again after 8 wk of customary high-fat diet, and after diet modulation to a low-fat diet for 8 wk.

at the GCRC, they were fed a high-fat, low-fiber diet mimicking their customary diet. During the low-fat diet modulation, the subjects were fed an isocaloric weight-maintaining diet with less than 15% of total calories as fat, 20% from protein, and 65–70% from carbohydrates and 25–35 g fiber/d. Only the low-fat meals were prepared and packaged by the GCRC kitchen and picked up by the volunteers during their weekly visits. The subjects continued to be fed this low-fat diet during their GCRC admission for the low-fat period studies. The high-fat diet in the second paradigm was not provided by the GCRC kitchen, and the subjects were asked to eat their usual customary diet similar to that (baseline) before entry into the study. During their 4-d stay at the GCRC, at the end of the high-fat diet period, they were fed food mimicking their customary diet. During the period after the baseline period, when the subjects were taking their low-fat diet or customary high-fat diet, the subjects returned to the GCRC at weekly intervals, where the dietitians met with them to ensure compliance and adjusted caloric intake to maintain weight when necessary. Given that a 3500-kcal deficit over 7 d would produce a loss of about 0.5 kg, we adjusted calories by changing food portions accordingly when weight decreased by at least 0.5 kg. Because the sodium content of the protocol diet was much less than the participant's usual diet, some weight loss (water loss) occurred within the first week of diet initiation. Therefore, adjustments in calories were made only after study participants had consumed the diet for 2 wk. Body weight and serum lipid profile were determined weekly. In addition to keeping 4-d food records at the end of 4 and 8 wk of their high-fat period (customary diet), unannounced 24-h food recalls were conducted by the dietitians on a weekly basis to ensure a high level of compliance. All food records were analyzed by the Minnesota-based Nutrient Data System.

The subjects were admitted to the GCRC for 4 d during their baseline period and at the end of 8 wk of low-fat or high-fat diet periods. At each study period, the following were performed: after 1 d of acclimatization, on d 2 and 3, six 8-h urine samples for androgen metabolites were collected, starting from 0800 h on d 2. On d 3, at 0800 h, a baseline blood sample was drawn for measurements of T, free T, 5α-dihydrotestosterone (DHT), 5α-androstanediol-3α,17β-diol (5α-A-3α,17β-diol)-glucuronide (G), androstenedione, dehydroepiandrosterone sulfate (DHEA-S), estradiol (E_2), LH, FSH, and SHBG. After an overnight fast, at 0800 h on d 4, baseline serum for hormones as above and quantitative measurement of fatty acids composition were obtained. The subjects were then started on trideuterated T (d_3T) infusion for 12 h, for the determination of MCR_T and PR_T, as previously described (37). The dose of stable isotope-labeled T (d_3T) infused was calculated as approximately 10% of the PR_T, such that the physiological homeostasis would not be disturbed (data from our laboratory showed that T infusion at this dose did not alter the pulsatile secretion of gonadotropins or change the secretion of T). At 0800 h, a priming dose equivalent to about 10% of the infusion dose was given (approximately 35 μg/1.7 m^2), followed by a constant infusion, for 12 h, of 350 μg/1.7 m^2 of d_3T. The labeled T was administered in 0.5% ethanol in normal saline via a constant-rate infusion pump. Plasma samples (50 ml) were drawn at 4, 5, 6, and 12 h after the start of the infusion for measurement of serum hormones. Serum d_3T and serum unlabeled T (d_0T) were quantitated by liquid chromatography-tadem mass spectrometry (LC-MS-MS), and serum total and free T and other hormones were measured by RIA. During the infusion, aliquots of the infused d_3T were collected from the infusion tubing about every 4 h for analyses to determine the concentration of d_3T in the infusate, measured both as d_3T by LC-MS-MS and as total T by RIA, which could detect the nanogram levels of d_3T in the infusate. Preliminary data showed that steady-state levels of T were achieved in plasma between 4 and 5 h. The subjects remained supine until after the blood sample at 6 h after start of infusion was drawn. The patient returned to the supine position from the 10th to the 12th hour of infusion. MCR_T was calculated by the formula: MCR_T = amount of d3T infused per hour/concentration of d3T in the serum and multiplied by 24 h to express as liters per day. PR_T was then calculated from the formula: $PR_T = MCR_T \times$ serum T concentration and expressed in milligrams per day. The average serum d3T and T concentrations, at 5 and 6 h after the start of the infusion, was used to calculate the MCR_T and PR_T (37). On d 5, a timed 2-h urine sample was collected for the baseline measurement of total energy expenditure, and a blood sample was drawn to determine the natural abundance of ^{2}H and ^{18}O before administering doubly labeled water (0.15 g H2^{18}O and 0.3 g ^2H_2O/kg body weight mixed with water). After 3 h, another serum sample was collected for the estimation of total

body water. The subjects were then instructed to collect urine samples, from 0800 to 1000 h on each day over the next 10 d, for the estimation of total energy expenditure (38, 39).

Materials and methods

Serum hormone assays. Serum T levels were determined, as previously described, by specific RIA (40, 41) using reagents obtained from MP-Biomedical (Costa Mesa, CA). The serum was extracted by ethylacetate and hexane before assay. The cross-reactivities of the antiserum used in the T RIA were 2.0% for DHT, 2.3% for androstenedione, 0.8% for 5α-A-3α,17β-diol, 0.6% for etiocholanolone (Etio), and less than 0.01% for all other steroids tested. The lower limit of quantitation of serum T measured by this assay was 0.87 nmol/liter (0.25 ng/ml). The accuracy (recovery) of the T assay, determined by spiking steroid free serum with 0.87, 1.73, 3.47, 11, 34.7, and 52 nmol/liter T, was 114, 118, 109, 94, 92, and 92%, respectively (mean, 104%). The normal adult male range in this laboratory was 10.33–36.17 nmol/liter (2.98–10.43 ng/ml). Serum free T concentrations were measured by an overnight equilibrium dialysis and direct assay of T concentration in the dialysate by RIA. Serum DHT was measured by RIA after potassium permanganate treatment of the sample followed by extraction. The methods and reagents of the DHT assay were provided by Diagnostic Systems Laboratories, Inc. (Webster, TX). The cross-reactivities of the antiserum used in the RIA for DHT were 6.5% for 3β-androstanediol, 1.2% for 5α-A-3α,17β-diol, 0.4% for 5α-A-3α,17β-diol-G, 0.4% for T (after potassium permanganate treatment and extraction), and less than 0.01 for other steroids tested. This low cross-reactivity against T was further confirmed by spiking steroid free serum with 35 nmol/liter (1000 ng/dl) of T and taking the samples through the DHT assay. The result, even on spiking with over 35 nmol/liter of T, was measured as less than 0.1 nmol/liter DHT. The lower limit of quantitation of serum DHT in this assay was 0.43 nmol/liter. All values below this value were reported as 0.43 nmol/liter. The mean accuracy (recovery) of the DHT assay, determined by spiking steroid free serum with amounts of DHT varying from 0.43–9 nmol/liter was 101% (range, 83–114%). The adult male range in our laboratory was 1.06–6.66 nmol/liter (30.7–193.2 ng/dl). Serum 5α-A-3α,17β-diol-G was measured by RIA using reagents from Diagnostic Systems Laboratories, Inc. The cross-reactivities of the antiserum used were 1.2% for DHT-G, 0.9% for T-G, and negligible for all the other steroids tested. The mean recovery of steroid free serum spiked with 5α-A-3α,17β-diol-G varied between 93–103% from 5.4 nmol/liter (2.5 ng/ml) to 214 nmol/liter (100 ng/ml). The adult male reference range was 6–37 nmol/liter (3.0–17.3 ng/ml). Serum E2, androstenedione, and dehydroepiandrosterone levels were measured by direct RIAs, without extraction, with reagents from Diagnostic Systems Laboratories, Inc. Normal ranges for adult men were established in our laboratory. The intraassay coefficients of variation (CVs) of the steroid assays varied between 7 and 9%, and interassay CVs varied between 8 and 15%, respectively, except for the free T assay (intraassay CV, 12.9%; and interassay CV, 21.5%) (40, 41). Serum SHBG, FSH, and LH levels were measured by highly sensitive and specific fluroimmunometric assays with reagents provided by Delfia (Wallac, Inc., Gaithersburg, MD). Serum IGF-I levels were measured by kits manufactured by Nichols Institute Diagnostics (San Juan Capistrano, CA), and IGF-II and IGFBP-3 by kits from Diagnostic Systems Laboratories, Inc., respectively. The intra- and interassay CVs were less than 5 and 12%, respectively. All these assays were validated in the Harbor-UCLA Endocrine Research laboratory and recently reported (40, 41). All samples drawn from a subject during each of the study periods were measured in the same assay. The samples from the first 19 subjects were assayed in a batch when the unidirectional study was completed, and then the next 20 subjects participating in the bidirectional study were assayed as a second batch when all subjects completed the study, approximately 1.5 yr later.

Urinary androgens measured by gas chromatography-mass spectrometry (GC-MS) analysis. Six endogenous steroids were quantitated by GC-MS at the UCLA Olympic Laboratory: T, epitestosterone (Epi-T), androsterone (Andr), Etio, 5α-androstane-3α,17β-diol (5α-A-3α,17β-diol), and 5β-androstane-3β,17β-diol (5β-A-3β,17β-diol) (42, 43). The internal standards d_3T and d_4Etio were added to the urine samples that were extracted over a solid-phase extraction cartridge. The eluate was then deconjugated with G, followed by the formation of the trimethylsilyl derivative before GC-MS analysis. The peak height ratio of T, Epi-T, 5α-A-3α,17β-diol, 5β-A-3β,17β-diol to d_3-T, and Andr and Etio to d_4-Etio were calculated, regressed against the concentration of the steroid, and the amounts of steroid in the samples were obtained from the regression equations. For the T analysis, the mean slope for 11 batches of samples was 0.0241 (CV, 10.2%). Urine creatinine was measured by an assay that uses liquid chromatography and is both highly specific for creatinine (44) and unaffected by interferences that might afflict the more commonly applied alkaline picric acid method.

LC-MS-MS assay for d_3-labeled T. We developed a stable isotope method at the UCLA Olympic Laboratory designed to quantitate d_3T in serum with a low limit of detection suitable for the estimation of MCR_T and PR_T after constant infusion of d_3T. The details of the LC-MS-MS assay, which has been previously described (45), also quantitated d_0T. The advantages of the LC-MS-MS approach include simplified sample preparation (underivatized steroids can be analyzed directly), high recovery, improved signal to noise ratio, and less difficulty with interferences due to MS-MS technology (46). A LC-10A binary pump LC (Shimadzu Scientific Instruments, Columbia, MD) equipped with a Series 200 autosampler (Applied Biosytems, Foster City, CA), coupled to an Applied Biosystem-Sciex API 300 triple quadrupole mass spectrometer (Applied Biosystems-Sciex, Thornhill, Ontario, Canada), and equipped with an APCI interface was used to perform the analysis.

Measurement of serum lipids. Serum lipids were measured by the Clinical Biochemistry Laboratory of the Harbor-UCLA Medical Center. The total cholesterol method was based on three coupled enzymatic steps using cholesterol esterase, cholesterol oxidase, and horseradish peroxidase. Serum direct high-density lipoprotein (HDL) cholesterol was determined by using a selective detergent that solubilized only the HDL lipoprotein particles and released the HDL cholesterol to react with the enzymatic reagents for cholesterol. For samples with serum triglyceride levels less than 400 mg/dl (4.5 mmol/liter), the low-density lipoprotein (LDL) cholesterol was estimated indirectly by using the Friedwald-Levy formula. For samples with serum triglyceride levels more than 400 mg/dl (4.5 mmol/liter), all non-LDL cholesterol particles and their cholesterol contents were removed first by a selective detergent, then a second detergent solubilized the remaining LDL particles and released the LDL cholesterol to react with the enzymatic reagents for cholesterol. The serum triglyceride method was based on three coupled enzymatic steps using glycerol kinase, glycerophosphate oxidase, and horseradish peroxidase. The total, HDL, and LDL cholesterol, as well as the triglyceride methods and their reagent kits, were based on the Beckman Synchron LX System (Beckman Coulter, Inc., Brea, CA).

Quantitative measurement of total free fatty acid. Fatty acid analysis was determined by a modification of the method of Lepage and Roy at the Clinical Nutrition Research Unit at UCLA (47). Fatty acid methyl esters were separated and quantified on an HP5890A Series II gas-liquid chromatograph (Hewlett-Packard Co., Avondale, PA) fitted with a Model 7673A automatic split injection system with a 100-sample tray, electronic pressure control, and flame ionization detector. The integrator was programmed with the theoretical relative response factors of fatty acids from a methyl esters external standard (NuChek Prep, Elysian, MN) and was programmed to convert raw data to corrected peak areas. The stored results were converted to a spreadsheet format. Quantification was based on the internal standard, heptadecanoic acid free fatty acid (NuChek Prep), which was added to the samples before processing. The fatty acid methyl esters identified include: C6:0, C8:0, C10:0, C12:0, C14:0, C14:ln-5, C16:0, C16:ln-7, C18:0, C18:ln-9(cis), C18:ln-9(trans), C18:2n-6(cis), C18:2n-6(trans), C18:3n-3, C18:3n-6, C20:0, C20:ln-9, C20:2n-9, C20:3n-6, C22:0, C20:4n-6, C22:ln-9, C20–5n-3, C24:0, C24:ln-9, C22:5n-3, C22:6n-3.

Plasma carotenoids, tocopherols, and retinol. The carotenoids (α-carotene, β-carotene, cryptoxanthin, lycopene), d-α-tocopherol, d-γ-tocopherol, and retinol as all-trans retinol) were measured using a modification of the HPLC method by Epler *et al.* (48). After thawing, the EDTA plasma was deproteinated with an equal volume of ethanol solution containing tocopherol acetate as internal standard and butylated hydroxytoluene as antioxidant. The mixture was extracted twice with hexane. The combined hexane extracts were evaporated under a stream of nitrogen. The residue was dissolved in a mixture of ethanol and ethylacetate (1:1,

vol:vol). The analysis was performed on an Agilent 1050 HPLC with multiple wavelength detector (Agilent Technologies, Wilmington, DE). A Baker-bond narrow-pore C18 column, 20 × 4.6 cm (J. T. Baker, Inc., Phillipsburg, NJ), with Vydac C18 guard column (Alltech Associates, Inc., Deerfield, IL) was used. Tocopherols were detected at 295 nm, carotenoids at 445 nm, and retinol at 325 nm. The CVs for intraassay pool plasma sample were 7.4 for lutein, 8.7 for retinol, 8.8 for α-tocopherol, 10.3 for β-carotene, 10.6 for γ-tocopherol, 11.5 for β-cryptoxanthine, 12.2 for lycopene, and 14.2 for α-carotene.

Total energy expenditure by method of doubling labeled water. The measurement of the ratio of ^2H to ^{18}O enrichment in urine was by isotope ratio mass spectrometry at the Harbor-UCLA Biomedical Mass Spectrometry Core Facility. Briefly, the ^{18}O enrichment in water was measured from CO_2 after equilibrating the oxygen isotopes between urine and CO_2 gas placed into a vacutainer. For measurement of ^2H enrichment in water, urine was first distilled to remove contaminating substances and then reduced to hydrogen gas by zinc catalyst reaction in a sealed quartz tube. The ^2H content in hydrogen gas was measured by isotope ratio mass spectrometry. These data of the ^{18}O and ^2H abundances were expressed in parts per million. The decay curves for ^2H and ^{18}O in body water (urine) were constructed. The respiratory exchange ratio, determined by indirect calorimetry (or a respiratory quotient of food), was used to estimate respiratory quotient for the purpose of calculating energy expenditure from the calculated carbon dioxide production derived from the doubly labeled water method (38, 39).

Statistical analyses

The primary outcome variables for this study were the differences between measurements of serum and urine androgens, MCR_T and PR_T, before and after diet modulation. The variables were transformed if necessary to ensure they met the assumptions of normal distribution for valid statistical analysis. The distribution of the serum hormones had been established in a large sample of normal subjects in our laboratory, and these results were used for the data in this study. For all other variables, the distributions were tested using the Shapiro-Wilk test on the raw and transformed data. All urinary androgens were log-transformed for statistical analyses. For simplicity of presentation, the graphs and tables present statistics for untransformed data. The analysis was focused on changes within each individual subject; therefore, we used paired *t* tests to determine whether there was a significant change. Repeated-measures ANOVA covarying for order of diet was used to determine whether the bidirectional studies with intervening diets affected the paired comparisons. Nonparametric tests, such as Wilcoxon's rank sum test and Freedman's ANOVA, were used when the distributions could not be transformed to meet normality. With 39 subjects, we had power to detect changes of 0.56 SD or greater. We note that Hamalainen *et al.* (49) showed a decrease in serum T of 0.56 SD in 30 healthy males placed on a low-fat diet for 6 wk, and Rosenthal *et al.* (50) showed a decrease in E_2 of more than 1 SD in 21 older males on a low-fat/high-fiber diet.

The initial statistical analyses indicated that all serum and urine hormones measured showed no significant change between baseline and high-fat diet periods, irrespective of whether the subjects were on baseline to low-fat for the unidirectional study or baseline to low-fat to high-fat or baseline to high-fat to low-fat diet sequence for the bidirectional study. Moreover, differences between baseline and low-fat periods were not affected by an intervening high-fat period in the baseline to high-fat to low-fat diet group. Thus, for clarity of presentation, we will discuss in detail only the differences between the baseline (high-fat) and the low-fat periods, treating all 39 subjects who completed the study as a single group. The bidirectional study results on serum androgens will be presented in a single table. Results are shown as mean ± SEM.

Results

Diet composition

Table 1 shows the diet composition of the 39 subjects before (baseline) and during the 8 wk of low-fat diet modulation or taking their customary high-fat diet. During the low-fat period, the calorie intake was unchanged, but the fat intake was decreased from a customary intake of 111.7 ± 5.3 to 40.1 ± 1.3 g/d. With the low-fat diet, α-linoleic acid (18:2) was reduced from 18.0 ± 1.2 to 8.0 ± 0.4 g/d and α-linolenic acid (18:3) from 1.77 ± 0.11 to 0.68 ± 0.05 g/d.

Plasma retinol, lutein, α- and γ-tocopherol, α- and β-carotene, and lycopenes

When the diet was modulated from high-fat baseline to low-fat experimental diet, there were significant increases in cryptoxanthin (baseline, 0.065 ± 0.006; low-fat, 0.095 ± 0.008 μmol/liter; $P < 0.0001$), lutein (baseline, 0.13 ± 0.01; low-fat, 0.17 ± 0.01 μmol/liter; $P < 0.0001$), α-carotene (baseline, 0.065 ± 0.008; low-fat, 0.088 ± 0.011 μmol/liter; $P < 0.0002$), and β-carotene (baseline, 0.22 ± 0.04; low-fat, 0.29 ± 0.04 μmol/liter; $P = 0.027$ and significant decrease in γ-tocopherol (baseline, 4.97 ± 0.47; low-fat, 3.84 ± 0.47 μmol/liter; $P = 0.0004$). α-Tocopherol, retinol, and lycopene levels did not show significant changes with diet modulation.

Effect of diet modulation on weight and body composition

The caloric intake of the subjects was adjusted when there were weight changes of over 0.5 kg/wk. Mean body weight while on baseline customary diet was 86.2 ± 3.1 kg, which decreased significantly to 85.1 ± 2.1 kg after 8 wk of low-fat diet ($P = 0.002$) despite the caloric intake adjustment. The BMI also decreased from 27.8 ± 0.6 to 26.5 ± 1.1 kg/m^2 after low-fat diet ($P = 0.0033$). The body composition parameters measured by dual-energy x-ray absorptiometry (Fig. 2) showed a significant decrease in body mass ($P = 0.016$) that was due mainly to the decrease in body fat ($P = 0.05$). The percent body water measured by deuterated water was 51.0 ± 1.4 before and 51.4 ± 1.1 after low-fat diet, which was not statistically different.

Effect on serum lipids and plasma fatty acids

Serum total cholesterol ($P = 0.0001$), LDL cholesterol ($P = 0.0001$), and HDL cholesterol ($P = 0.0003$) levels showed significant decreases from baseline with low-fat diet modulation (Fig. 2). Serum triglyceride levels showed a significant increase from baseline (1.80 ± 0.14 mmol/liter) during diet

TABLE 1. Dietary composition at baseline (high fat) and in low-fat and high-fat diets

	n	kcal/d	Fat	SFA	PUFA	MUFA	Fiber (g/d)
			(% kcal)				
Baseline (high-fat)	39	2627 ± 104	37.9 ± 1.0	13.2 ± 0.5	7.1 ± 0.3	14.6 ± 0.4	19.0 ± 0.7
Low-fat	39	2597 ± 70	13.9 ± 0.3	4.9 ± 0.3	3.1 ± 0.1	5.6 ± 0.3	31.7 ± 0.7
High-fat	20	2317 ± 114	33.3 ± 1.2	10.9 ± 0.6	6.1 ± 0.3	12.5 ± 0.8	21.7 ± 1.5

SFA, Saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

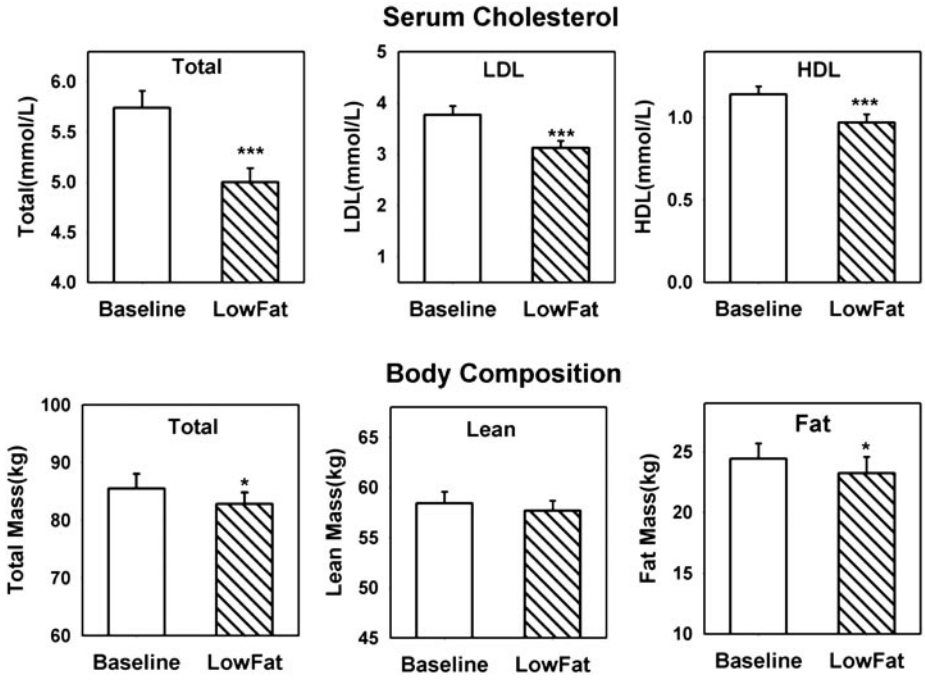

FIG. 2. *Upper panels*, Significant decreases in mean serum total, LDL, and HDL cholesterol levels; *lower panels*, significant decreases in mean total and fat but not lean mass (assessed by dual-energy x-ray absorptiometry) in the 39 subjects after low-fat diet modulation (*, $P < 0.05$; ***, $P < 0.001$).

fat reduction (low-fat diet, 2.11 ± 0.20 mmol/liter; $P = 0.0006$). Quantitative measurements of fatty acids showed significant decreases in C18:2, linoleic acid (baseline, 3623 ± 118; low-fat, 2876 ± 143 μmol/liter; $P < 0.0001$) and C22:0 (baseline, 52 ± 4; low-fat, 37 ± 3 μmol/liter; $P < 0.0001$) and increase in C16:1 (baseline, 239 ± 20; low-fat, 376 ± 30 μmol/liter; $P < 0.0001$).

Changes in serum hormone levels

With the change of diet from a high-fat (baseline) to a low-fat diet, small, but significant, reductions in mean serum total T, free T, DHT, and 5α-A-3α,17β-diol-G levels were demonstrated (Fig. 3). Serum hormone levels were measured on five different time points on the fourth day at the GCRC at baseline and 8 wk after taking a low-fat diet. The mean of the average serum total T concentration over the day (measured by RIA) fell 12%, from 16.9 ± 0.9 to 14.9 ± 0.6 nmol/liter ($P = 0.0001$). Corroborating the RIA data, d_0T (measured by LC-MS-MS) also significantly decreased (baseline, 16.0 ± 2.6; low-fat, 14.9 ± 0.8 nmol/liter; $P = 0.036$). Similarly, serum DHT levels decreased from 1.87 ± 0.12 to 1.70 ± 0.1 nmol/liter ($P = 0.0053$), and 5α-A-3α,17β-diol-G levels fell from 14.0 ± 1.0 to 11.6 ± 1.0 nmol/liter ($P = 0.0001$). Serum androstenedione (baseline, 3.91 ± 0.25; low-fat, 3.66 ± 0.25 nmol/liter; $P = 0.0135$) and DHEA-S (baseline, 4.24 ± 0.33; low-fat, 3.69 ± 0.27 μmol/liter; $P = 0.0011$), representing secretory and/or metabolic products of androgens of both testicular and adrenal origins, were also significantly decreased after low-fat diet. Serum free T levels also were lower by 10% when the subjects were on low-fat diet (0.18 ± 0.01 nmol/liter) than on customary high-fat diet (0.20 ± 0.01 nmol/liter; $P = 0.0045$). Serum SHBG levels decreased slightly (8%) but significantly (baseline, 46.4 ± 3.7 nmol/liter; low-fat, 42.6 ± 2.9 nmol/liter; $P = 0.0105$). This decrease may be partially responsible for the more marked decrease in serum total T than free T levels and could influence the MCR_T as discussed below. Serum E_2 levels decreased very slightly with diet modulation (baseline, 87.7 ± 3.0 pmol/liter; low-fat, 83.8 ± 2.9 pmol/liter; $P = 0.0290$) (Fig. 2). Mean serum FSH and LH both decreased slightly but significantly (FSH: baseline, 5.8 ± 1.3 IU/liter; low-fat, 5.3 ± 1.2 IU/liter; $P = 0.0065$; LH: baseline, 4.4 ± 0.8 IU/liter; low-fat, 4.0 ± 0.8 IU/liter; $P = 0.0401$) with dietary manipulation.

Changes in urinary androgens

The changes in 24-h urinary excretion of testicular and adrenal androgens measured by GC-MS are shown in Table 2. The urinary levels of androgens were based on two 24-h urine collections and measured by GC-MS at the UCLA-Olympic Laboratory. The results corroborated, in general, the changes in serum levels of androgens. Urinary excretion of Epi-T, Andr, Etio, and 5α-A-3α,17β-diol were significantly decreased after 8 wk of a low-fat diet. However, urinary excretion of T and 5β-A-3α,17β-diol also followed the same decreasing trend but just failed to reach statistical significance.

Effects of diet modulation on MCR_T and PR_T

These data were obtained after continuous d_3T infusion to reach steady-state in each subject before and after 8 wk of diet modulation. Serum enrichment of d_3T was quantitated with LC-MS-MS. At steady-state, serum and infusate samples obtained at multiple time points were measured by both RIA and LC-MS-MS to determine the T concentration. The results showed that reducing fat did not significantly alter the MCR_T derived from RIA measurements (baseline, 826 ± 43; low-fat, 779 ± 39 liter/d; P = 0.35) or from LC-MS-MS (baseline, 755 ± 42; low-fat, 780 ± 43 liter/d; P = 0.46). In 31 of 39 subjects, PR_T decreased after low fat modulation when serum

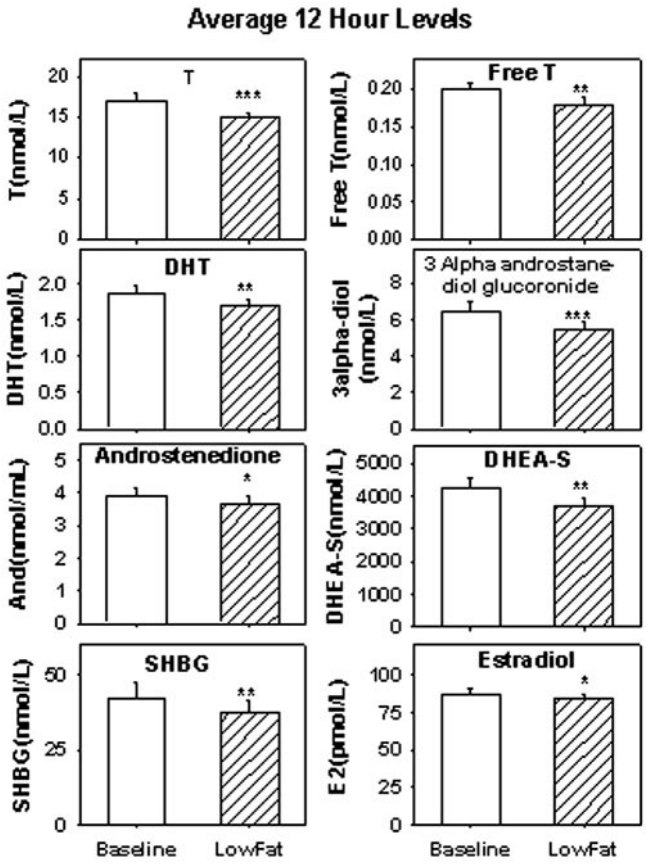

FIG. 3. Mean serum T, free T, DHT, 5α-A-3α,17β-diol-G, androstenedione, DHEA-S, SHBG, and E_2 over 12 h in the 39 subjects at baseline and after low-fat diet (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ when compared with baseline).

and infusate total T levels were measured by RIA (baseline, 4.02 ± 0.25; low-fat, 3.23 ± 0.19 mg/d; P = 0.0007). However, when serum d_0T and infusate d_3T estimated by LC-MS-MS were used to calculate PR_T, the mean PR_T showed a trend to decrease but failed to reach statistical significance during the low-fat period (baseline, 3.25 ± 0.20; low-fat, 3.03 ± 0.15 liter/d; $P = 0.52$).

Changes in serum IGFs

Serum IGF I (baseline, 298 ± 23; low-fat, 337 ± 24 ng/ml), IGF II (baseline, 483 ± 18; low-fat, 501 ± 16 ng/ml), and IGF BP-3 (baseline, 3.0 ± 0.1; low-fat, 3.1 ± 0.1 ng/ml) levels did not significantly change with low-fat diet modulation.

Energy expenditure and activity index

The subjects' weekly activity before and during diet modulation was monitored by an activities questionnaire (51). The calculated scores were not significantly changed before and during diet modulation. The total energy expenditure estimated by doubly labeled water administration and measured by isotope ratio-mass spectroscopy was not significantly different before (2787 ± 167 kcal/d) and after (2919 ± 176 kcal/d) diet modulation.

Serum PSA

The mean serum PSA was 1.16 ± 0.13 ng/ml at baseline and was not significantly changed (1.07 ± 0.14 ng/ml) after low-fat diet.

Bidirectional diet modulation

Of the 39 subjects, 20 subjects completed the bidirectional studies (13 subjects in the baseline to low-fat to high-fat group and seven subjects in the baseline to high-fat to low-fat group; Fig. 1). Serum hormone concentrations were not significantly different between baseline (high fat) and when the subjects were placed on the high-fat diet, irrespective of whether the subjects were on baseline to low-fat to high-fat diet or baseline to high-fat to low-fat diet sequence. Moreover, differences between baseline and low-fat periods were not affected by an intervening high-fat diet period. Because of the small number of subjects in the bidirectional study (baseline to low-fat to high-fat, n = 13 and baseline to high-fat to low-fat, n = 7) and the samples for serum total and free T assays from the 19 subjects in the unidirectional study were done as different batches from the 20 that participated in the bidirectional study, the baseline serum total and free T levels presented in Table 3 in these subgroups appeared to be lower than the entire group of 39 subjects. Logarithmic transformed mean baseline serum T concentrations in the 19 subjects that underwent the unidirectional study were marginally higher than the 13 subjects ($P = 0.031$) that underwent baseline to low to high group but were not statistically different from the seven subjects ($P = 0.074$) that underwent baseline to high to low. Despite the apparent differences in baseline concentration, mean serum total T level was significantly lower during the low-fat period ($P = 0.0298$) compared with baseline in the baseline to low to high sequence. Similarly, the mean serum total T was lower in the low-fat period ($P = 0.009$) compared with levels during the high-fat period (equivalent to a second baseline as the subjects were taking their customary high-fat diets) in the baseline to high to low-fat sequence subgroups (Table 3). The baseline serum free T concentrations were lower in the bidirectional study subjects compared with the 19 subjects in the unidirectional ($P = 0.001$). This could be due to the larger interassay difference in the free T assay because the samples were done as different batches. Because of the higher assay variation and the small number of sub-

TABLE 2. Urinary androgens at baseline and during low-fat diet

	T (nmol/24 h)	Epi-T (nmol/24 h)	Andr (μmol/24 h)	Etio (μmol/24 h)	5α-A-3α, 17β-diol (nmol/24 h)	5β-A-3α, 17β-diol (nmol/24 h)
Baseline	100 ± 11	121 ± 10	7.29 ± 0.62	6.88 ± 0.63	199 ± 20	548 ± 66
Low-fat	93 ± 11	105 ± 10	6.15 ± 0.42	6.12 ± 0.59	175 ± 17	509 ± 66
P^a	0.065	0.0002	0.001	0.015	0.004	0.061

[a] The statistical analyses on this and subsequent tables were performed on transformed data when necessary; for simplicity, means ± SEM values are given.

TABLE 3. Serum androgens in the subjects in the bidirectional diet modulation study

Parameters	Baseline → low-fat → high-fat (n = 13)			Baseline → high-fat → low-fat (n = 7)		
Total T (nmol/liter)	15.2 ± 1.7	13.4 ± 1.9[a]	13.6 ± 1.1	15.0 ± 1.8	15.8 ± 1.5	13.7 ± 1.7[b]
Free T (nmol/liter)	0.16 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
DHT (nmol/liter)	1.7 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.1	1.6 ± 0.1	1.4 ± 0.1
5α-A-3α, 17β-diol G (nmol/liter)	15.1 ± 1.9	13.2 ± 2.1[a]	13.8 ± 1.5	14.1 ± 2.5	14.0 ± 2.3	10.7 ± 1.4
SHBG (nmol/liter)	50.2 ± 6.29	45.7 ± 5.3[a]	43.8 ± 5.6	40.5 ± 6.5	44.1 ± 7.0	46.8 ± 7.1
E_2 (pmol/liter)	87.4 ± 6.6	81.9 ± 5.7	84.0 ± 5.0	79.6 ± 6.7	77.3 ± 6.4	73.6 ± 7.4
Androstenedione (nmol/liter)	3.4 ± 0.3	3.3 ± 0.4	3.3 ± 0.3	2.5 ± 0.3	2.6 ± 0.4	2.3 ± 0.4
DHEA-S (μmol/liter)	4.7 ± 0.7	4.0 ± 0.5[a,b]	5.2 ± 0.8	4.1 ± 0.8	4.2 ± 0.7	3.2 ± 0.5
LH (IU/liter)	6.4 ± 2.3	5.8 ± 2.2	5.6 ± 2.0	2.8 ± 0.6	3.2 ± 0.6	2.9 ± 0.7
FSH (IU/liter)	8.1 ± 3.9	7.2 ± 3.4	7.8 ± 3.9	4.3 ± 1.0	4.3 ± 1.0	4.0 ± 1.0

[a] Significantly different from baseline.
[b] Significantly different from high-fat diet.

jects, serum free T did not show significant changes with the bidirectional diet modulation. Table 3 shows that, in the limited number of 13 subjects who underwent the sequence of baseline to low-fat to high-fat diet, mean serum 5α-A-3α,17β-diol G ($P = 0.0116$) and SHBG ($P = 0.0187$) concentrations decreased from baseline to low-fat diet and remained low 8 wk after the subjects returned to their customary high-fat diet. Serum DHEA-S decreased when the subjects changed from baseline to a low-fat diet ($P = 0.0344$ when compared with baseline) and rose when the subjects returned to their high-fat diet ($P = 0.0381$ when compared with low-fat period).

Discussion

In the present study, we showed that diet modulation from a high-fat (>30% calories as fat and low-fiber < 20 g/d) to a low-fat diet (about 15% calories as fat and 25–30 g/d of fiber) significantly lowered serum concentrations of testicular (total and free T, DHT, 5α-A-3α,17β-diol-G) and adrenal androgens (androstenedione and DHEA-S). Although serum E_2 was also significantly reduced, the changes were quantitatively small. Though serum concentrations of both gonadotropins were significantly decreased, these changes were very small and may not represent clinically significant suppression of the hypothalamic-pituitary axis with diet modulation. The lower circulating levels of androgens were accompanied by significantly reduced urinary excretion of most of the testicular and adrenal androgens except urinary T excretion. The reason why urinary T excretion was not decreased with low-fat diet modulation was not clear and may be related to the conversion of T to other androgen metabolites, many of which were shown to be lower with low-fat diet. Serum SHBG showed a small decrease with low-fat diet. This decrease in SHBG did not affect clearance of T, because there were no significant group changes in MCR_T. We reasoned that if the mean serum T levels are decreased and the mean MCR_T levels are not changed, then the production rates would be expected to decrease in these subjects. Using the total T measurements in serum and infusate by RIA, the PR_T was significantly decreased, by about 12%, as anticipated. PR_T, estimated using d_0T in the serum and d_3T in the infusate, measured by LC-MS-MS, failed to reach significance because in some subjects, the MCR_T increased more and the serum d_0T was decreased less than the RIA data. The reason for the small differences in PR_T using two different estimates of serum T is not clear. This could be due, for example, to assay variability and interference by other substances in the RIA. There were exceptions to the overall lack of group effect on MCR; in six of 39 subjects, there was substantial increase in MCR_T (consistent in both RIA and LC-MS-MS data); and in all of these subjects, serum SHBG levels fell with low fat modulation, whereas their PR_T showed increases. In this subgroup, more than one factor may have been responsible for the reduction of serum T. These changes in sex steroids were associated with minimal weight loss (1.2 kg) and no change in energy expenditure. Thus, decreases in androgen levels were unlikely to be related to weight loss or changes in energy expenditure. Similar weight loss was also reported in an isocaloric, low-fat diet modulation study reported by Hamalainen *et al.* (49).

Earlier diet fat modulation studies in Black and white North American men and Black South African men showed that while eating their customary low-fat diets, Black South African men had lower levels of urinary androgens and estrogens than North American Black or white men on higher-fat diets. The urinary androgen and estrogen content increased significantly in Black South African men fed a Western diet, and urinary sex steroid decreased in Black North American men fed a vegetarian diet (52). Prior studies comparing hormone levels in men taking a vegetarian *vs.* an omnivorous diet reported no differences in serum T and E2 concentrations, but the calculated free T levels were reported to be slightly lower in some studies but not in others (53–55). Similar to our study, Hamalainen *et al.* (49) studied the effect of changing the customary Western diet (40% fat) to an isocaloric experimental diet with low fat (25%) for 6 wk in 30 normal men. Significant reductions in serum total and free T and androstenedione and a small, but nonsignificant, reduction in serum E_2 were observed during dietary manipulation. Another study in the United States (50) measured serum T, E_2, and lipid levels in 21 men after modulation to a high fiber, low-fat diet (<10% fat) under residential conditions for 26 d. Serum E_2 levels were significantly reduced, but serum T levels were unchanged. In a more recent controlled feeding study, 43 healthy men were randomly assigned to a low-fat, high-fiber (18.8% energy from fat and 4.6 g/MJ·d fiber) or high-fat, low-fiber (41% energy from fat and 2.0 g/MJ·d fiber) diet for 10 wk and, after a 2-wk washout period, crossed over to the other diet. The mean serum total and SHBG-bound T and urinary excretion of T were 13–15% higher on a high-fat,

low-fiber diet; and urinary estrogens, conversely, were 12–28% lower (56). Our current report represents a comprehensive evaluation of serum and urinary testicular and adrenal androgens as well as the MCR and PR_T before and after controlled feeding of a low-fat, high-fiber diet. Our data indicate that lowering diet fat without reducing weight causes a reduction in serum T, free T, DHT, and 5α-A-3α,17β-diol G, as well as androstenedione and DHEA-S in the male and a trend suggesting decreased endogenous production of T.

We noted a significant decrease in the mean concentration of SHBG while the subjects were fed the low-fat diet. Our findings are similar to previous controlled feeding studies (49, 56) where a low-fat diet decreased SHBG levels though not to a significant level. SHBG-bound T fractions were lower during low-fat diet in the study reported by Dorgan *et al.* (56). In our study, the lower SHBG levels can account only partially for the 12% decrease in total serum T, because free T, measured independently using equilibrium dialysis and direct assay of the dialysate, also showed a 10% decrease. In other cross-sectional studies, dietary intake of fiber had a positive correlation with SHBG levels in women (57) and in men (58). In the latter epidemiological study, the positive correlation between fiber intake and SHBG concentrations persisted after controlling for age, BMI, T, and E2 levels. The results from these cross-sectional studies were consistent with the results in the study reported by Jin *et al.* (36), where the Chinese in China had high SHBG levels (possible lower bioactive T), which were inversely related to prostate volume. SHBG levels fell when the Chinese migrated to Australia, presumably due to acculturation to the Western lifestyle and changes in their diet components. Similarly, vegetarians, presumably consuming diets with higher fiber content, had higher serum SHBG levels, which resulted in lower calculated measures of free T (54, 55). When food records were analyzed, Key *et al.* (55) reported that serum SHBG was positively correlated with dietary fat and saturated fatty acids. In addition to diet, age and obesity are related to SHBG concentrations. Thus, serum SHBG concentrations in controlled feeding studies such as ours and that reported by Hamalainen *et al.* (49) and Dorgan *et al.* (56) might be influenced more by the strikingly lower fat intake than by the small increase in fiber intake in the diet.

The compliance during diet modulation in our study was enhanced by the weekly reporting to the dietitians. Adherence to the diet was also reflected in significant decreases in serum total, HDL, and LDL cholesterol and linolenic acid (18:2) levels. The changes in qualitative fatty acids were similar to those reported in a low-fat diet modulation study in women (59). In addition, the changes in serum lutein and α- and β-carotenoids were consistent with the change in dietary components required to produce the low-fat, high-fiber diet consumed by our subjects. During the study, an isocaloric diet was fed. Despite attempts to maintain constant weight by adjusting food portions when the subject's weight changed, a very small decrease in weight (about 1.2 kg) still occurred during the low-fat period. There was no change in activity of the total energy expenditure from baseline to the end of diet modulation.

To demonstrate that the effect of low fat on androgen and its metabolites was not partially caused by changes in lifestyle resulting from enrolling in a study and repeated encounters with the dietitians and medical professionals, a bidirectional study was performed in a subset of 20 subjects. The results indicated that there were no significant differences between the baseline (customary high-fat) and the high-fat period. Moreover, the differences in hormone levels between baseline and low-fat period were not influenced by an intervening high-fat period. When the subjects were restudied after returning to their customary higher-fat diet for 8 wk, their total caloric intake was lower than at baseline, and some of the androgens levels (serum T and 5α-A-3α,17β-diol) remained lower than baseline levels. This could be due to the inability of the subjects to return immediately to a diet with the same amount of calories and fat; alternatively, the 8-wk period was insufficient for serum androgens levels and production rates to return to baseline.

The mechanisms by which dietary fat intake modulates androgen metabolism are not clear. High-fat diets usually contain low fiber. Fiber may reduce reabsorption of steroid hormones excreted through the biliary tract by changing the activities of steroid hydrolysis enzymes or the qualitative microflora of the gut. This would result in a decrease in serum androgen levels without affecting the production rate. Lower levels of cholesterol (lipoproteins) in the body may provide less cholesterol to the inner mitochondrial membrane or decrease the mobilization of cholesterol by the acute steroidogenic regulatory protein for subsequent steroidogenesis. This may result in the small 12% decrease in total serum T as shown in this study. This small decrease in serum T may not be adequate to activate the gonadotrophs to result in an increased LH secretion through the negative feedback mechanism. Alternatively, dietary modulation might influence the hypothalamic axis and decrease gonadotropin secretion. Alterations in fatty acid content or quality may alter also steroid metabolism, *e.g.* inhibition of 5α-reductase activity by γ-linolenic acid and other aliphatic unsaturated fatty acids (49, 60). High-fat-containing meals may very acutely lower serum total and free T levels without altering LH, suggesting that changes in fatty acids may directly modulate T production by the testis (61).

We measured serum IGF-I, IGF-II, and IGFBP-3 in the subjects because recent studies suggest that high serum IGF-I and low serum IGF-II and IGFBP-3 might predict higher risk of developing prostate cancer, especially advanced cancer (62–69). Though it is not clear whether IGFs have a causal relationship with prostate cancer risk or are simply a biomarker of disease, IGF-I and IGFBP-3 have also been implicated to directly affect prostate cancer cell growth and apoptosis *in vitro* (70–72). Moreover, there are studies showing that raised IGF-I levels and low IGFBP-3 levels are associated with high dietary intake of dairy products and inversely related to high vegetable consumption (73–81). Others suggest that high-fat diet may work through the IGF axis to lower SHBG and increase free androgen levels (82). In our study, there were no significant changes in IGF-I, IGF-II, and IGFBP 3 levels with low-fat diet in a controlled feeding paradigm; and thus, results did not support the relationship between IGF-I and serum androgens during diet fat and fiber modulation.

We conclude from our study that diet modulation from a

high-fat, low-fiber typical Western diet to a low-fat, high-fiber diet significantly reduced serum androgens and some urinary androgens with a decreasing trend in PR_T. We speculate that changes observed in circulating androgens with a low-fat diet may have a chronic effect on intraprostatic androgen levels or metabolism. Thus, we hypothesize that low-fat diet modulates intraprostatic androgen levels, metabolism, and action either directly or indirectly by interacting with oncogenes, growth factors, or carcinogens, providing an unfavorable environment for the growth and development of prostate cancer. We cannot rule out an additional effect of low-fat diet on delivery of androgens (*i.e.* lowered SHBG levels) to the prostate cells, altered transport of androgens from the extracellular to intracellular milieu of the prostate cell, or alterations in intracellular androgen metabolism. Studies are ongoing in our center to test the hypothesis that diet fat modulation would significantly alter the microenvironment of the prostate by decreasing intraprostatic androgen levels and changing the activities of the 5α-reductase, 3-hydroxy-steroid oxireductase, and 17β-hydroxysteroid dehydrogenase in the prostate gland.

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