Energy Intake and Prostate Tumor Growth, Angiogenesis, and Vascular Endothelial Growth Factor Expression

Purna Mukherjee, Alexander V. Sotnikov, Heather J. Mangian, Jin-Rong Zhou, Willard J. Visek, Steven K. Clinton

Background: A sedentary lifestyle coupled with excessive energy intake is speculated to be a factor associated with increased incidence of prostate cancer. We have investigated the effects of energy intake on prostate tumor growth in experimental animals. Methods: Two transplantable prostate tumor models, i.e., the androgen-dependent Dunning R3327-H adenocarcinoma in rats and the androgen-sensitive LNCaP human carcinoma in severe combined immunodeficient mice, were studied. R3327-H tumor growth and relevant tumor biomarkers (proliferation index, apoptosis [programmed cell death], microvesSEL density, and vascular endothelial growth factor [VEGF] expression) were compared in ad libitum fed control rats, ad libitum fed castrated rats, and groups restricted in energy intake by 20% or 40%. A second set of experiments involving both tumor models examined tumor growth in ad libitum fed rats or in animals whose energy intake was restricted by 30% using three different methods, i.e., total diet restriction, carbohydrate restriction, or lipid restriction. All P values are two-sided. Results: R3327-H tumors were smaller in energy-restricted or castrated rats than in control rats (P<.001). Tumors from energy-restricted rats exhibited changes in tumor architecture characterized by increased stroma and more homogeneous and smaller glands. In castrated rats, the tumor proliferation index was reduced (P<.001), whereas apoptosis was increased in both energy-restricted (P<.001) and castrated (P<.001) rats. Tumor microvesSEL density and VEGF expression were reduced by energy restriction and castration (P<.003 versus control). Restriction of energy intake by reduction of carbohydrate intake, lipid intake, or total diet produced a similar inhibition of growth of R3327-H or LNCaP tumors. These effects were associated with reduced circulating insulin-like growth factor-I. Conclusions: Our observations are consistent with the hypothesis that energy restriction reduces prostate tumor growth by inhibiting tumor angiogenesis. Furthermore, dietary fat concentration does not influence prostate tumor growth when energy intake is reduced. [J Natl Cancer Inst 1999;91:512–23]

The wide variation in prostate cancer incidence between nations (1) and the increase in risk with migration from low- to high-incidence countries (2,3) implicate environmental variables, such as diet, as important etiologic factors. Prostate cancer detection and death rates are greater in nations with a population having a sedentary lifestyle and an affluent dietary pattern characterized by energy-dense diets composed of highly processed foods rich in refined carbohydrates, meats, and dairy products but a low proportion of energy derived from fruits, vegetables, and whole-grain foods. The reductionist scientific approach has led most investigators to search for specific components of the affluent dietary pattern that promote the prostate cancer cascade. Although no definitive causal or protective associations have been identified for specific nutrients or dietary factors, several hypotheses have emerged from investigations in humans and laboratory models and are the focus of continued research (4–8).

We have focused our present investigations on the role of energy intake in rodent prostate carcinogenesis for several reasons. Increasing rates of obesity, reflecting a disequilibrium of energy balance, are characteristic of nations where prostate cancer rates are high (9–12). The relationships between prostate cancer and energy intake, physical activity, anthropometrics, and the many genetic and exogenous factors that modulate their interactions are just beginning to be investigated (4,5,13–15). In parallel with the increasing obesity seen in the U.S. population (16), laboratory rodents used in carcinogenesis studies have been selected in recent decades by commercial suppliers to grow faster and reach mature weight more quickly for economic reasons (17). Typical laboratory housing for rodents used in cancer studies provides limited physical activity and unrestricted access to food (ad libitum). These circumstances promote adiposity, higher risk of spontaneous cancer, diminished life span, and increased sensitivity to known carcinogens (17). The role of energy balance in prostate carcinogenesis thus far has received limited attention in laboratory models (8).

Laboratory animal models encompassing the diverse characteristics of human prostate tumorigenesis do not exist. We have selected two prostate tumor models that reflect several specific and relevant features for our investigations: the Dunning R3327-H adenocarcinoma in rats and the LNCaP human carcinoma in severe combined immunodeficient (SCID) mice. The androgen-dependent Dunning R3327-H transplantable prostate adenocarcinoma was originally derived from a spontaneous lesion in a Copenhagen rat. The tumor is composed of ducts and numerous proliferative acini, similar to those of moderately dif-
differentiated human prostate carcinoma (18). The slow-growing R3327-H tumor is very sensitive to androgens (19,20), as is true for most newly diagnosed human prostate cancers. We chose to substantiate our findings from the R3327-H model with the androgen-sensitive, poorly differentiated LNCaP human prostate carcinoma grown as xenografts in SCID mice (21). In addition to exploring the dose-dependent effects of diet restriction on tumor growth, we compared the growth of prostate tumors when energy restriction was accomplished by total diet restriction or the withdrawal of equivalent amounts of energy as carbohydrate or lipid.

The characterization of biomarkers sensitive to dietary interventions is critical for planning intervention studies designed to test dietary hypotheses concerning prostate cancer prevention and as an adjuvant to other therapeutic interventions. We hypothesize that energy restriction acts on hormonal, growth factor, and cytokine networks mediating interactions between the growing tumor and the vasculature. Folkman and colleagues (22) have proposed that progressive tumor growth requires angiogenesis, a process that ensures continued delivery of oxygen and nutrients and the removal of metabolic waste products. Furthermore, they (22–24) have suggested that tumor cell proliferation rates and apoptosis are inversely associated and intimately interrelated with the efficiency of the tumor vasculature. We therefore explored the effects of energy restriction on prostate tumor growth and the interrelationships between tumor proliferation, apoptosis, and angiogenesis. Although a growing array of mediators is being characterized as modulators of angiogenesis, we have focused on circulating insulin-like growth factor-I (IGF-I) (25–28) and tissue vascular endothelial growth factor (VEGF) (29–33) as potential mediators of dietary effects on prostate tumor growth.

Materials and Methods

Animals and Housing

Male Fisher × Copenhagen F1 rats were provided by Dr. Norman Altman of the Papanicolaou Cancer Institute, University of Miami (Miami, FL). They were housed in individual stainless-steel, wire-bottomed cages in rooms maintained at 22 °C ± 1 °C with 14 hours of fluorescent lighting per 24-hour period. Male SCID mice (Taconic, Germantown, MD) at 10 weeks of age were maintained in plastic shoebox cages with autoclaved bedding and filtered air with 12 hours of darkness daily at an ambient air temperature of 22 °C ± 2 °C. The care and use of the laboratory animals followed guidelines set forth by the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals (revised September 1986). All animal procedures were reviewed and approved by the Dana-Farber Cancer Institute Animal Care and Use Committee or the University of Illinois Laboratory Animal Care Advisory Committee.

Prostate Adenocarcinoma Tumor Cell Lines

The Dunning R3327-H adenocarcinoma was transported from the Papanicolaou Cancer Research Institute as bilateral subcutaneous implants in the flank donor rats. The tumors were allowed to reach 2.5 cm in diameter and were harvested by sterile techniques. Tumor slices approximately 6×5×2 mm and weighing 73 mg ± 3 mg (mean ± standard deviation [SD]) were prepared. The slices were transplanted to bilateral subcutaneous sites in the flanks of the anesthetized recipient rats that were then randomly assigned to experimental groups. When the most rapidly growing tumors reached diameters of approximately 2.5 cm, the experiments were terminated, and the tumor tissue was harvested for histopathologic studies.

The LNCaP human prostate adenocarcinoma cell line obtained from the American Type Culture Collection (Manassas, VA) was maintained at 37 °C and in 5% CO2, with Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum, penicillin (60,000 U/L), streptomycin (60 mg/L), and L-glutamine (2.4 mM). All cell culture materials were purchased from Sigma Chemical Co. (St. Louis, MO). LNCaP tumors were initially established in SCID mice by subcutaneous inoculation of 2 × 106 LNCaP cells into the mice, and they were subsequently maintained in the laboratory by serial transplantation. LNCaP tumors, approximately 1 cm in diameter, from donor mice were harvested under sterile conditions and dissociated with a TeFlon homogenizer in sterile phosphate-buffered saline (PBS). Cells were washed, counted, and immediately inoculated into recipient mice at 2 × 106 viable cells (viability determined by trypsin blue dye exclusion) in 0.1 mL of sterile PBS. Mice were then randomly assigned to dietary treatments. The study was terminated after 35 days, and the tumor tissue was harvested for histopathologic evaluation.

Diet Restriction and Dunning R3327-H Tumorigenesis in Rats

Rats were randomly assigned (10 per group) to one of the following four treatment groups: 1) ad libitum fed, hormonally intact, 2) total diet restriction of 20%, 3) total diet restriction of 40%, or 4) ad libitum fed and castrated at the time of tumor transplantation. All rats were fed theAIN76A diet (Research Diets, Inc., New Brunswick, NJ) (34,55). Total diet restriction is technically easy and maintains a constant ratio of nutrients to energy in all treatment groups. Diets were stored at 4 °C, and fresh food was provided daily. Dietary treatments began on the day after tumor transplantation and were continued throughout the experiment. All animals had continual access to distilled water. The average daily feed intake for the ad libitum fed rats was determined for each 7-day period, and the two restricted groups were provided 80% (20% restriction) and 60% (40% restriction) of that quantity on a daily basis. Calipers were used to quantify largest tumor diameter on a weekly basis. At 16 weeks after transplantation, all rats were killed and their tumors were weighed. Samples of each tumor were fixed in formalin for histologic evaluation.

Energy Restriction Versus Diet Restriction and Dunning R3327-H Tumorigenesis in Rats

The inhibition of tumor growth when total diet is restricted may be due to reduced energy intake and may be accentuated by the lower intake of essential amino acids, lipids, or certain vitamins and minerals. In contrast, restriction of energy alone by selective removal of fat or carbohydrate calories from the diet allowed ad libitum fed controls and the restricted animals to consume the same amounts of protein, vitamins, and minerals. In this experiment, rats were randomly assigned (eight per group) to one of five treatment groups: 1) ad libitum fed controls, 2) rats subjected to restriction of energy intake by 30% from lipid only, 3) rats subjected to restriction of energy intake by 30% from carbohydrate only, 4) rats subjected to total diet restriction of 30%, or 5) ad libitum fed rats castrated at the time of tumor transplantation. Diets for studies comparing differing methods of restriction (Table 1) are based on the American Institute of Nutrition recommendations and were prepared according to our formulations by Research Diets, Inc. (New Brunswick, NJ). At 16 weeks after transplantation, all rats were killed and their tumors were weighed. Samples of each tumor were fixed in formalin for histologic evaluation.

Energy Restriction Versus Diet Restriction and LNCaP Tumorigenesis in Mice

Adult male SCID mice (eight per group) were subcutaneously inoculated with tumor cells and were randomly assigned to one of the following four treatment groups: 1) ad libitum fed controls, 2) mice whose energy intake was restricted by 30% from lipid only, 3) mice whose energy intake was restricted by 30% from carbohydrate only, or 4) mice whose total diet was restricted by 30%. At 4 weeks after inoculation, all mice were killed and their tumors were weighed. Samples of each tumor were fixed in formalin for histologic evaluation.

Histology and Morphometry

Formalin-fixed tumor specimens were embedded in paraffin and sectioned at 5 μm thickness. Hematoxylin–eosin-stained slides were examined by light microscopy (Olympus BHTU, Tokyo, Japan) for characterization of tumor morphology and cytologic features. Low-power images of whole-tumor sections were digitized at 2700 dots per inch by use of a 35-mm scanner (Polaroid SprintScan 35; Polaroid Corp., Cambridge, MA) and PathScan Enabler (Meyer Instruments, Houston, TX). The digitized images were used to compute tumor density, a value representing the extent to which the tissue elements are compact. We determined tissue density by measuring total tumor area and quantitating the...
**Factor VIII Staining and Microvessel Quantitation**

After deparaffinization, rehydration, and washing, sections were incubated with trypsin at 37 °C for 30 minutes, quenched with 0.3% H₂O₂–methanol for 30 minutes, and blocked with 10% normal goat serum in buffer (100 mL 0.01 M phosphate buffer and 0.9% sodium chloride [pH 7.4], with 1.0 g bovine serum albumin and 0.1 mL Tween 20 [PBA]). The sections were treated with a rabbit polyclonal antibody directed against human factor VIII-related antigen (Dako Corp., Carpinteria, CA; 1:100 dilution with PBA), followed by a biotinylated anti-rabbit immunoglobulin G (IgG) at 1:100 dilution (Vector Laboratories, Inc., Burlingame, CA). The sections were then treated with avidin–biotin complex followed by 3-3′-diaminobenzidine as substrate for staining according to the manufacturer’s directions ( Vectastain Elite ABC Kit; Vector Laboratories, Inc.). The sections were then treated with rabbit polyclonal VEGF antiserum (Dako Corp., Carpinteria, CA; 1:20 dilution) in blocking solution overnight at 4 °C, followed by 3-3′-diaminobenzidine substrate, counterstained with methyl green, and mounted. Positive and negative control slides were used for comparison. Substitution of TdT with distilled water served as a negative control. Three representative areas lacking necrosis were selected for each slide without knowledge of the treatment group, and both apoptotic cells and tumor cells were counted by use of light microscopy at a 400-fold magnification. The apoptotic index (AI) was expressed as percentage, AI (%) = A × 100/(A + C), where A = apoptotic cells and C = counterstained, unlabeled cells.

**VEGF Immunohistochemistry**

After deparaffinization, rehydration, and washing, slides were immersed in 0.01 M citrate buffer (pH 6.0), heated in a microwave oven to boiling for 5–6 minutes, and cooled in buffer at room temperature. After quenching was performed, the following steps were taken to determine VEGF immunoreactivity: 
- (a) The sections were first blocked with 10% normal horse serum in PBA for 30 minutes; 
- (b) the sections were treated with rabbit polyclonal VEGF antiserum (Calbiochem, La Jolla, CA) as primary antibody at a 1:20 dilution in blocking solution overnight at 4 °C; 
- (c) the sections were then treated with biotinylated anti-rabbit IgG at a 1:100 dilution (Vectastain Elite ABC Kit) for 30 minutes; 
- (d) the sections were then treated with avidin–biotin complex following the manufacturer’s instruction (Vector Laboratories, Inc.); 
- (e) VEGF immunoreactivity was then visualized by 3-3′-diaminobenzidine tetrahydrochloride (Vector Laboratories, Inc.); and 
- (f) after being washed with water, sections were counterstained with hematoxylin for 30 seconds.

**In Situ Apoptotic Cell Detection**

To detect apoptotic cells, we used the ApopTag in situ detection kit (Oncor, Inc., Gaithersburg, MD) and followed the manufacturer’s procedures with minor modifications (36); this procedure is also known as terminal deoxynucleotidyltransferase (TdT)-mediated deoxyuridinemonophosphate-biotin nick-end labeling (TUNEL). In brief, after deparaffinization, rehydration, and washing in PBS, sections were treated with 20 µg/mL of proteinase K for 15 minutes at room temperature and washed again. Endogenous peroxidase activities in sections were quenched with 3% H₂O₂ in PBS for 5 minutes. The 3′ hydroxy DNA strand breaks were enzymatically labeled with digoxigenin nucleotide via TdT and incubated for 1 hour at 37 °C, and the reaction was terminated with a stop buffer according to the manufacturer’s directions (Oncor, Inc.). Sections were then incubated with anti-digoxigenin peroxidase for 30 minutes at room temperature, washed, stained with 3-3′-diaminobenzidine substrate, counterstained with methyl green, and mounted. Positive and negative control slides were used for comparison. Substitution of TdT with distilled water served as a negative control. Three representative areas lacking necrosis were selected for each slide without knowledge of the treatment group, and both apoptotic cells and tumor cells were counted by use of light microscopy at a 400-fold magnification. The apoptotic index (AI) was expressed as percentage, AI (%) = A × 100/(A + C), where A = apoptotic cells and C = counterstained, unlabeled cells.

**Table 1. Percentage composition (grams of each compound per 100 g of diet) and nutrient density of diets used in rodent prostate cancer studies**

<table>
<thead>
<tr>
<th></th>
<th>Diet 1: ad libitum fed controls, castrated animals, or total diet restriction</th>
<th>Diet 2: energy-restricted animals (low-fat, high-carbohydrate diet)</th>
<th>Diet 3: energy-restricted animals (high-fat, low-carbohydrate diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grams per 100 g of diet</strong></td>
<td><strong>Protein</strong>* 16.95</td>
<td>19.95</td>
<td>25.64</td>
</tr>
<tr>
<td></td>
<td><strong>tν-Methionine</strong> 0.34</td>
<td>0.40</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td><strong>Sucrose</strong> 17.17</td>
<td>20.21</td>
<td>8.89</td>
</tr>
<tr>
<td></td>
<td><strong>Corn starch</strong> 34.23</td>
<td>40.32</td>
<td>17.60</td>
</tr>
<tr>
<td></td>
<td><strong>Lipid†</strong> 20.33</td>
<td>6.21</td>
<td>30.78</td>
</tr>
<tr>
<td></td>
<td><strong>Minerals‡</strong> 3.95</td>
<td>4.66</td>
<td>5.98</td>
</tr>
<tr>
<td></td>
<td><strong>Choline</strong> 0.25</td>
<td>0.27</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td><strong>Vitamins‡</strong> 1.13</td>
<td>1.33</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td><strong>Cellulose</strong> 5.65</td>
<td>6.65</td>
<td>8.55</td>
</tr>
<tr>
<td><strong>Total grams</strong></td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td><strong>kcal/g§</strong></td>
<td>4.5</td>
<td>3.7</td>
<td>4.8</td>
</tr>
<tr>
<td>**% of total energy</td>
<td></td>
<td>**</td>
<td><strong>Protein</strong> 13</td>
</tr>
<tr>
<td></td>
<td><strong>Carbohydrate</strong> 45</td>
<td>65</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td><strong>Fat</strong> 40</td>
<td>15</td>
<td>58</td>
</tr>
</tbody>
</table>

*Protein was provided as isolated soy protein. †Lipid was provided as striped soybean oil. ‡American Institute of Nutrition formulations of vitamin and mineral mixes. §Physiologic fuel values for protein, carbohydrate, and fat at 4, 4, and 9 kcal/g, respectively, were used in calculating the caloric content.

**Proliferation Index**

To determine the proliferation index, we calculated the fraction of cells with proliferating cell nuclear antigen (PCNA) staining. After deparaffinization, rehydration, and washing, sections were soaked in 10 m phosphate buffer and 0.9% sodium chloride [pH 7.4], with 1.0 g bovine serum albumin and 0.1 mL Tween 20 (PBA). The sections were treated with rabbit polyclonal antibody directed against human factor VIII-related antigen (Dako Corp., Carpinteria, CA; 1:100 dilution with PBA), followed by a biotinylated anti-rabbit immunoglobulin G (IgG) at 1:100 dilution (Vector Laboratories, Inc., Burlingame, CA). The sections were then treated with avidin–biotin complex followed by 3-3′-diaminobenzidine as substrate for staining according to the manufacturer’s directions (Vectastain Elite ABC Kit; Vector Laboratories, Inc.). The sections were then rinsed three times between all steps with PBS (100 mL 0.01 M phosphate buffer and 0.9% sodium chloride) after being washed with water, sections were counterstained with hematoxylin for 30 seconds. After dehydration and clearing (xylene), the slides were mounted. Unless otherwise stated, all incubations were completed by use of image analysis software (KS400; Kontron Elektronik, Munich, Germany).
treatment group. After linearization and 1-hour warm-up of the imaging work- 
station (Roche Pathology Workstation, RIAS, Elon College, SC), six high-power 
fields per tissue section were acquired by use of a digital camera (ProgRes 3012; 
Kontron Elektronics) and the light microscope (Olympus BHTU). Each high-
power field was shadow corrected and minimally processed to eliminate com-
promised areas that could bias the analysis, such as artifacts generated during 
processing or staining. True-color image analysis was utilized to segment 
VEGF-labeled cells and counterstained cells for calculating labeling index based 
on the following formula: labeling index (%) = L × 100/(L + C), where L = 
 labeled cells and C = counterstained, unlabeled cells. Nonspecific antibody 
staining in the lumina of tumor glands was digitally removed for preparation of 
representative figures.

IGF-I Analysis

Blood was collected into EDTA-coated tubes from the orbital venous plexus 
of individual mice anesthetized with metofane before the tumor was harvested. 
Mice were fasted for 3 hours before their blood was collected, and plasma was 
isolated by centrifugation at room temperature for 10 minutes at 375 g and was 
stored at −70 °C until assayed. IGF-I concentrations were measured by radio-
immunoassay (Nichols Institutes Diagnostics, Capistrano, CA) with purified 
plasma-derived IGF-I as the standard.

Statistical Analysis

Food intake, body weight, tumor size, and quantitative measurements based 
on tumor histologic sections were analyzed initially by analysis of variance 
(ANOVA) (37) followed by Scheffé’s test (38) or Fisher’s protected least-
significant difference (37) to calculate two-sided pairwise comparisons among 
treatment groups by use of Statview 4.5 (Abacus Concepts, Inc., Berkeley, CA) 
and Power Macintosh computers (Apple Computer, Cupertino, CA).

RESULTS

Diet Restriction and Dunning R3327-H Tumorigenesis in Rats

All data regarding food intake are summarized in Fig. 1. The 
ad libitum fed rats consumed between 55 and 65 kcal/day for the 
duration of the experiment (Fig. 1, A). The castrated rats showed 
a gradual and progressive reduction in energy intake that was 
approximately equivalent to a 17% restriction over the duration 
of the experiment (P<.01 versus ad libitum fed rats). The final 
body weight (Fig. 1, B) was reduced by 14% (P<.01) and 30% 
(P<.01) compared with that of the ad libitum fed rats (400 ± 37 
g (mean ± SD)) for rats fed 20% (346 g ± 12 g) and 40% (281 
g ± 10 g) total diet restriction, respectively. Castrated males 
weighed 19% (324 g ± 20 g) less (P<.01) than ad libitum fed, 
hormonally intact rats. The tumor diameter (Fig. 1, C) in hormo-
nally intact rats (2.2 cm ± 0.4 cm, mean ± SD) was reduced 
74% in castrated rats (0.6 cm ± 0.1 cm; P<.001), 39% in rats 
restricted by 40% total diet restriction (1.3 cm ± 0.5 cm; P<.01), 
and 24% in rats having 20% total diet restriction (1.7 cm ± 0.4 
cm; P<.05). No significant difference in tumor diameter was 
detected between the two diet-restricted groups, although the tumor 
diameters of both groups were different from the tumor 
diameter of the castrated group (P<.001). The final tumor weight 
measured at necropsy was 8.9 g ± 2.3 g (mean ± SD) in ad 
libitum fed, hormonally intact rats and was reduced by 98% with 
castration (0.2 g ± 0.1 g; P<.001), 76% by 40% energy restric-
tion (3.4 g ± 2.4 g; P<.001), and 62% by 20% energy restric-
tion (2.1 g ± 1.2 g; P<.01). No significant difference was detected in 
final tumor weights between rats fed 20% restricted or 40% 
restricted diets, although the final tumor weights in both groups 
were different from those of castrated animals (P<.001 for each 
comparison). All of the above data were analyzed initially by 
ANOVA (37) followed by Fisher’s protected least-significant 
difference (37) to calculate two-sided pairwise comparisons 
among treatment groups.

Energy Restriction Versus Diet Restriction and Dunning 
R3327-H Tumorigenesis in Rats

The average daily intake of energy, food, and selected nutri-
tent fractions for the rats fed the various diets is shown in Table 
2. The hormonally intact and ad libitum fed rats consumed be-
tween 61 and 69 kcal/day for the duration of the experiment 
(Fig. 2, A). Castrated males showed a gradual reduction in en-
ergy intake during the experiment (49 kcal/day by week 16) that 
averaged 16% less than hormonally intact, ad libitum fed rats for 
the total experiment (P<.001). Diets were provided to the three 
restricted groups each day and consumed without waste. Castr-
ated males (357 g ± 14 g, mean ± SD) weighed 21% less than
hormonally intact and ad libitum fed rats (453 g ± 39 g) (Fig. 2, B) by the end of the study (P<0.01). Each type of energy restriction produced nearly identical changes in body weight (Fig. 2, B). The final body weights for rats restricted in fat (329 g ± 13 g), in carbohydrate (334 g ± 19 g), or in total diet (330 g ± 19 g) intake were 27%, 26%, and 27% less than those of ad libitum fed rats, respectively (P<0.001, for each comparison). The tumor diameter (Fig. 2, C) in ad libitum fed control rats was 2.8 cm ± 0.8 cm and was reduced by 90% in castrated rats (0.2 cm ± 0.3 cm, mean ± SD; P<0.0001), by 26% in rats restricted by lipid calories (2.0 cm ± 0.6 cm; P<0.05), by 36% in rats restricted by carbohydrate calories (1.7 cm ± 0.9 cm; P<0.008), and by 39% in rats having total diet restriction (1.6 cm ± 0.6 cm; P<0.005). No statistically significant difference in tumor diameter was detected between the three diet-restricted groups, although all restricted groups exhibited tumor diameters that were different from those of castrated rats (P<0.0003). The final tumor weight in castrated males (0.2 g ± 0.2 g, mean ± SD) was reduced by 99% compared with that in hormonally intact and ad libitum fed rats (18.8 g ± 9.0 g; P<0.0001). Restriction of energy intake as fat, carbohydrate, or total diet resulted in final tumor weights that were reduced by 37% (11.8 g ± 7.6 g; P<0.05), 62% (7.1 g ± 5.2 g; P<0.002), and 60% (7.5 g ± 5.9 g; P<0.002), respectively, compared with final tumor weights in ad libitum fed rats. Among the groups subjected to the three types of restriction, there was no statistically significant difference, although the mean tumor diameter, calculated tumor volume, or final tumor weight were consistently greater for the lipid-restricted group than for the other two restricted groups. All of the above data were analyzed initially by ANOVA (37) followed by Fisher’s protected least-significant difference (37) to calculate two-sided pairwise comparisons among treatment groups.

### Table 2. Average daily intake of energy, food, and selected nutrient fractions from the various treatment groups in experiments designed to compare different types of energy restriction used for studies of prostate tumor growth in rats and mice

<table>
<thead>
<tr>
<th>Experiment and treatment group</th>
<th>Energy intake, kcal/day</th>
<th>Food intake, g/day</th>
<th>Protein intake, g/day</th>
<th>Fat intake, g/day</th>
<th>Carbohydrate intake, g/day</th>
<th>Vitamin intake, mg/day</th>
<th>Mineral intake, mg/day</th>
<th>Fiber intake, mg/day</th>
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<tbody>
<tr>
<td>R3327-H tumor in rats</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum fed</td>
<td>63.4</td>
<td>14.00</td>
<td>2.37</td>
<td>2.85</td>
<td>7.20</td>
<td>0.16</td>
<td>0.55</td>
<td>0.79</td>
</tr>
<tr>
<td>Total diet restriction*,†</td>
<td>44.8</td>
<td>9.89</td>
<td>1.68</td>
<td>2.01</td>
<td>5.08</td>
<td>0.11</td>
<td>0.39</td>
<td>0.56</td>
</tr>
<tr>
<td>Lipid restriction*,‡</td>
<td>44.8</td>
<td>12.01</td>
<td>2.39</td>
<td>0.75</td>
<td>7.26</td>
<td>0.16</td>
<td>0.56</td>
<td>0.80</td>
</tr>
<tr>
<td>Carbohydrate restriction*,§</td>
<td>44.8</td>
<td>9.33</td>
<td>2.39</td>
<td>2.87</td>
<td>2.47</td>
<td>0.16</td>
<td>0.56</td>
<td>0.80</td>
</tr>
<tr>
<td>Ad libitum fed, castrated</td>
<td>53.8</td>
<td>11.88</td>
<td>2.01</td>
<td>2.42</td>
<td>6.11</td>
<td>0.13</td>
<td>0.47</td>
<td>0.67</td>
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<tr>
<td>LNCaP tumor in mice</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum fed</td>
<td>15.5</td>
<td>3.42</td>
<td>0.58</td>
<td>0.70</td>
<td>1.76</td>
<td>0.039</td>
<td>0.135</td>
<td>0.193</td>
</tr>
<tr>
<td>Total diet restriction*,¶</td>
<td>11.1</td>
<td>2.45</td>
<td>0.50</td>
<td>0.70</td>
<td>1.26</td>
<td>0.028</td>
<td>0.097</td>
<td>0.138</td>
</tr>
<tr>
<td>Lipid restriction*,¶</td>
<td>11.1</td>
<td>2.96</td>
<td>0.59</td>
<td>0.18</td>
<td>1.79</td>
<td>0.039</td>
<td>0.137</td>
<td>0.197</td>
</tr>
<tr>
<td>Carbohydrate restriction*,¶</td>
<td>11.1</td>
<td>2.32</td>
<td>0.59</td>
<td>0.71</td>
<td>0.62</td>
<td>0.039</td>
<td>0.138</td>
<td>0.198</td>
</tr>
</tbody>
</table>

*Rats in each of the restricted treatment groups were fed daily an amount of their respective diets equal to 70% of the energy intake (30% restriction) of the ad libitum fed group. After food consumption and wastage were measured, the restricted rats bearing the Dunning R3327-H prostate tumor consumed 29% less food than that consumed by ad libitum fed controls when averaged for the entire experiment.

†Total diet restriction provides a proportional decrease in the intake of all diet components (see Table 1); therefore, the ratio of each component to other constituents remains constant between the restricted and ad libitum fed group.

‡Restriction of calories derived from lipid results in a 74%–75% reduction in fat intake while maintaining identical intake of protein, carbohydrate, vitamins, minerals, and fiber as that in the ad libitum fed group.

§Restriction of calories from carbohydrate results in a 65%–66% reduction in carbohydrate while maintaining identical consumption of other components as that in the ad libitum fed group.

¶Mice in each of the restricted treatment groups were fed daily an amount of their respective diets equal to 70% of the energy intake (30% restriction) of the ad libitum fed group. After food consumption and wastage were measured, the restricted severe combined immunodeficient mice bearing the human LNCaP prostate carcinoma consumed 28% less food than that consumed by their controls when averaged for the entire experiment.

### Energy Restriction Versus Diet Restriction and LNCaP Tumor Growth in SCID Mice

Energy intake averaged 15.4 kcal/day ± 1.1 kcal/day (mean ± SD) for ad libitum fed mice and 11.1 kcal/day ± 0.0 kcal/day for the three restricted groups, representing an average reduction in energy intake of 28.1% for the experiment (Fig. 3, A). The final body weights (Fig. 3, B) for mice restricted in fat (19.6 g ± 0.7 g, mean ± SD), carbohydrate (20.4 g ± 1.3 g), or total diet (19.6 g ± 0.9 g) were 17%, 13%, and 12% lower, respectively, than those for the ad libitum fed mice (23.4 g ± 1.8 g) (P<0.001, for each comparison). The tumor diameter at necropsy (Fig. 3, C) was 2.6 cm ± 0.9 cm (mean ± SD) in controls and was reduced by 31% in mice restricted in lipid calories (1.8 cm ± 0.4 cm; P<0.02), by 44% in those restricted in carbohydrate calories (1.5 cm ± 0.5 cm; P<0.002), and by 48% in rats with total diet restriction (1.4 cm ± 0.6 cm; P<0.001). No significant difference in tumor diameter was detected between the three diet-restricted groups. The calculated tumor volumes were 2.1 cm³ ± 0.7 cm³ (mean ± SD) in ad libitum fed mice and 1.4 cm³ ± 0.3 cm³ in mice restricted in fat calories (P<0.02), 1.2 cm³ ± 0.4 cm³ in those restricted in carbohydrate calories (P<0.002), and 1.1 cm³ ± 0.5 cm³ in total diet-restricted mice (P<0.002). Tumor weights were not obtained because of the invasion of the local tissues and precluded reliable dissection for accurate quantitation. All of the above data were analyzed initially by ANOVA (37) followed by Scheffé’s test (38) to calculate two-sided pairwise comparisons among treatment groups.

### Dunning Tumor Histology and Morphometry

The results of our histopathologic analysis showed no major differences between animals restricted by 20% and those restricted by 40% of energy or restricted by different methods of
restriction. Therefore, the following descriptive observations are relevant to all restricted groups. The cross-sectional diameters of prostate tumors from control rats were larger than those of the tumors from castrated rats, whereas tumors from diet-restricted rats (all types of restriction) had cross-sectional diameters intermediate in size (Fig. 4, A, B, and C). We initially used light microscopy to examine hematoxylin–eosin-stained sections of the Dunning R3327-H tumors (Fig. 4, D, E, and F). Tumors from hormonally intact and ad libitum fed rats resembled a moderately differentiated human prostate adenocarcinoma exhibiting numerous glands and duct-like structures of variable and irregular shape with large luminal volumes containing secretions (Fig. 4, D). The glands were surrounded by a loose connective tissue defined by faint or nonstaining stromal elements. A rich supply of vascular structures of varying size containing red blood cells was readily apparent within the tumor stroma of hematoxylin–eosin-stained sections from ad libitum fed rats. These characteristics differed considerably in tumors from castrated rats (Fig. 4, F). The glandular elements were small and very uniform in shape, with glands and ducts closely packed, back-to-back, and separated by a layer of dense stromal matrix. The entire tumor from a castrated rat was surrounded by a prominent fibrous connective tissue capsule that was poorly developed in the tumors from hormonally intact and ad libitum fed rats. Tumors from castrated rats also typically had dense septa dividing the tumor into lobules. Vascular elements were virtually undetectable under low-power light microscopy within the tumors from the castrated animals and were observed only within or near the

**Fig. 2.** Energy intake (A), body weight (B), and tumor diameter (C) in rats bearing the Dunning R3327-H prostate adenocarcinoma that were randomly assigned to one of five treatment groups: 1) ad libitum fed (control), 2) castration and ad libitum intake, 3) 30% energy restriction from lipid calories, 4) 30% energy restriction from carbohydrate calories, or 5) 30% energy restriction by reduction of total diet intake. Castration reduced energy intake by 16% (two-sided P<.001 versus control) (A). Energy restriction by each method produced identical reductions in growth rate (two-sided P<.001 for each restricted group versus control), and the data were pooled for purposes of clarity in graphic presentation (B). Castration reduced final body weight by 21% (two-sided P<.01 versus control). Final tumor diameter (C) was reduced by 26% (two-sided P<.05), 36% (two-sided P<.008), 39% (two-sided P<.005), and 90% (two-sided P<.0001) for rats restricted in lipid calories, restricted in carbohydrate calories, restricted in total diet, or castrated and ad libitum fed, respectively. Data points represent the mean values (n = 8), and error bars show the 95% confidence interval for selected points.

**Fig. 3.** Energy intake (A), body weight (B), and tumor diameter (C) in male severe combined immunodeficient mice bearing the LNCaP human prostate adenocarcinoma that were randomly assigned to one of four treatment groups: 1) ad libitum fed, 2) 30% energy restriction from lipid calories, 3) 30% energy restriction from carbohydrate calories, or 4) 30% energy restriction by reduction of total diet intake. The actual overall energy restriction for the three groups was 28.1% for the 35-day experiment. Final body weights were reduced by 17%, 13%, and 12% in mice fed diets restricted by 30% in lipid calories, carbohydrate calories, or total diet, respectively (two-sided P<.001). Tumor diameter was reduced by 31% (two-sided P<.02), 44% (two-sided P<.002), and 48% (two-sided P<.001) in mice fed diets restricted by 30% in lipid calories, 30% in carbohydrate calories, or 30% in total diet, respectively. Data points show the mean values (n = 8), and error bars show the 95% confidence interval for selected points.
surrounding capsule. Prostate tumors from rats consuming restricted diets exhibited a tumor architecture that was different from that of the ad libitum, a rat fed at 30% total diet restriction, and a castrated rat, respectively. Tumor size is consistent with data shown in Fig. 2, C. Panels D, E, and F: hematoxylin–eosin-stained sections of prostate tumors from a rat fed ad libitum, a rat fed at 30% total diet restriction, and a castrated rat, respectively. Tumor sections from ad libitum fed rats show heterogeneously sized glands and ducts with extensive intraluminal secretions. Numerous large and small vascular channels are readily observed. The space between glands and ducts is composed of a loose connective tissue matrix. Tumors from energy-restricted rats show smaller and more homogeneous glands with less conspicuous luminal secretions and with fewer identifiable vascular structures. The matrix is more cellular and dense. Tumors from castrated rats show numerous, small homogeneous glands separated by a thin rim of dense matrix. Panels G, H, and I: computerized images generated to show the areas composed of red blood cells within vascular channels of sections shown in D, E, and F, respectively. Examination of high-power fields of tissue obtained from ad libitum fed rats revealed several large vessels and a large number of microvessels. In contrast, only rare sections of tumors from rats fed energy-restricted diets contained large vessels, and the number of microvessels was reduced. Tumors from castrated animals showed few vascular structures containing red blood cells. Calculations from multiple sections show a mean ± standard deviation (SD) for vascular area of 2.6% ± 0.2% for ad libitum fed rats, 0.3% ± 0.2% for energy-restricted rats, and <0.1% (below resolution) for castrated rats. Panels J, K, and L: immunohistochemical staining for vascular endothelial growth factor (VEGF) at low magnification, revealing the extensive staining of the glandular elements of the tumors from ad libitum fed rats in J, while K shows the only focus of staining observed in any tumor section from rats fed energy-restricted diets. Tumors from castrated rats showed no evidence of VEGF staining. Image analysis indicates VEGF staining in 24.5% ± 7.5% (mean ± SD) of the tumor area in ad libitum fed rats compared with 0.4% ± 0.8% for energy-restricted rats and no staining in tumors from castrated rats. Panels M, N, and O: a high-power image illustrating the intense immunohistochemical localization of VEGF to the intracellular compartment of the glandular cells from an ad libitum fed rat (M) and less intense staining in the small focus in a tumor from a rat fed an energy-restricted diet (N). We did not observe VEGF staining in any tumor section from castrated rats.

restricted rats had a greater density quantitated at 0.7 ± 0.1 (pooled for all types of restriction), whereas the tumors from castrated rats had the highest density at 0.9 ± 0.1. We did not detect any significant difference in tumor histology between rats fed diets restricted by 20% or 40% of energy or between tumors from rats fed diets restricted at 30% by different methods.

We also used computerized image analysis to quantitate vascular channels containing red blood cells identified in hematoxylin–eosin-stained sections, which we refer to as the blood vessel density ratio. Representative images of tumor tissue from hormonally intact and ad libitum fed rats, diet-restricted rats, and castrated rats containing a vascular space filled with red blood cells are shown in Fig. 4, G, H, and I. The fraction of prostate tumor tissue containing vascular elements was 2.6% ± 0.1% (mean ± SD) for ad libitum fed rats versus 0.3% ± 0.1% in the diet-restricted groups (pooled). In contrast, it was not possible to accurately quantitate vessels in tumors of castrated animals on the basis of red blood cell detection with the use of image analysis, since the few vessels were small and below the satisfactory resolution (<0.1%). All of the above data were analyzed...
initially by ANOVA (37) followed by Scheffé’s test (38) to calculate two-sided pairwise comparisons among treatment groups.

**LNCaP Tumor Histology and Morphometry**

We used light microscopy to examine the LNCaP tumors that appeared as a continuous sheet of anaplastic cells that would have been classified as high-grade or poorly differentiated tumors if observed in a human biopsy specimen (images not shown). The tumors showed virtually no evidence of gland formation. The LNCaP tumors showed some areas of necrosis, as is typically observed in rapidly growing transplantable tumors. Blood vessels were present but were smaller and more homogeneous in the LNCaP tumor than in the Dunning tumor. Little stroma or matrix was observed by light microscopy in the LNCaP tumor. All of the above data were analyzed initially by ANOVA (37) followed by Scheffé’s test (38) to calculate two-sided pairwise comparisons among treatment groups.

**Proliferation and Apoptosis**

We quantitated the proliferation and apoptosis in R3327-H prostate tumor samples from both experiments. The data in Table 3 are derived from *ad libitum* fed rats, castrated rats, and rats restricted at 20% or 40% of total energy. We pooled the data for the restricted groups, since no significant differences in tumor proliferation or apoptosis were observed between rats subjected to different levels of energy restriction or different methods of caloric restriction. The PCNA labeling index was reduced 54% in castrated rats compared with *ad libitum* fed rats (*P*<.0001), whereas no significant effect was noted with energy restriction. The apoptotic index more than doubled with castration (*P*<.001) or total dietary restriction (*P*<.001). The above results were confirmed by the analysis of tumors obtained from the second R3327-H experiment in which energy restriction was accomplished by three different approaches (data not shown). LNCaP tumors in mice also showed no changes in proliferation index with restriction, but the increase in apoptotic index was more than twofold (*P*<.0007). As we observed in the Dunning R3327-H rat study, we detected no significant difference in the effect on proliferation or the proliferation index between the different methods of energy restriction (data not shown). All of the above data were analyzed initially by ANOVA (37) followed by Scheffé’s test (38) to calculate two-sided pairwise comparisons among treatment groups.

**Microvessel Density and VEGF Expression**

Factor VIII staining and quantitation of vessel density (Table 3) in Dunning tumors revealed an approximately 62% reduction in vascularity from energy restriction compared with that in *ad libitum* fed rats (*P*<.003). Vessel density in castrated animals was profoundly reduced (*P*<.0001), and occasional small vessels were noted within the tumor and some larger vessels were observed in the periphery of the tumor infiltrating from the dense stromal capsule. Similarly, we observed an approximately 49% reduction in microvessel quantitation as a result of energy restriction in the LNCaP tumor (*P*<.04). All of the above data were analyzed initially by ANOVA (37) followed by Scheffé’s test (38) to calculate two-sided pairwise comparisons among treatment groups.

VEGF staining in Dunning R3327-H tumors from *ad libitum* fed rats was 24.5% ± 7.5% (mean ± SD) of cells (Table 3). Staining was primarily intracellular and most intense in the glandular elements of the tumor (Fig. 4, J through O). VEGF staining was observed in only one small cluster of glands in a slide from tumors obtained from energy-restricted rats (0.4% ± 0.8%; *P*<.0001 versus *ad libitum* fed rats). Furthermore, no detectable staining was observed in Dunning tumors from castrated rats (0.0% ± 0.0%; *P*<.0001 versus *ad libitum* fed). Staining in consecutive sections showed identical patterns, which further suggested specificity of detection for VEGF expression that occurs in large patches of the Dunning tumor, as was observed in human specimens (39–41). All of the above data were analyzed initially by ANOVA (37) followed by Scheffé’s test (38) to calculate two-sided pairwise comparisons among treatment groups.

VEGF expression in LNCaP tumors was very low with less than 5% of the tumor area from all rats showing positive staining and qualitatively very different from the Dunning tumor (images not shown). In contrast to the large areas of VEGF expression in the Dunning tumor, staining in the LNCaP tumor was by individual cells, approximately one in 30. The LNCaP cells expressing VEGF exhibited cytologic changes suggestive of cell death

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proliferation index, %¶</th>
<th>Apoptotic index, %¶</th>
<th>Microvessel density, vessels per high-power field¶</th>
<th>VEGF expression, % positive cells¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunning R3327-H</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ad libitum</em> fed rats</td>
<td>50 ± 6*</td>
<td>0.9 ± 0.6*</td>
<td>9.7 ± 3.4*</td>
<td>24.5 ± 7.5*</td>
</tr>
<tr>
<td><em>Ad libitum</em> fed, castrated rats</td>
<td>23 ± 2b</td>
<td>2.0 ± 0.6b</td>
<td>&lt;1b</td>
<td>0.0 ± 0.0b</td>
</tr>
<tr>
<td>Energy-restricted rats</td>
<td>44 ± 7*</td>
<td>2.2 ± 0.3b</td>
<td>3.7 ± 0.6b</td>
<td>0.4 ± 0.8b</td>
</tr>
<tr>
<td>LNCaP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ad libitum</em> fed mice</td>
<td>75 ± 6a</td>
<td>5.4 ± 1.6a</td>
<td>8.0 ± 3.1a</td>
<td>3.1 ± 2.8a</td>
</tr>
<tr>
<td>Energy-restricted mice</td>
<td>65 ± 12a</td>
<td>11.1 ± 2.4a</td>
<td>4.1 ± 2.0a</td>
<td>4.8 ± 5.8a</td>
</tr>
</tbody>
</table>

*PCNA = proliferating cell nuclear antigen.
†TUNEL = terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate-biotin nick-end labeling.
‡VEGF = vascular endothelial growth factor.
§Male Fisher × Copenhagen F1 rats were used for studies with the Dunning R3327-H rat prostate tumor model, whereas male severe combined immunodeficient mice were used for studies with the human LNCaP prostate tumor model.
¶Data represent means ± standard deviation. Data represent results from independent experiments with the Dunning R3327-H or LNCaP tumor lines. The values with different superscripts for each parameter for each tumor model are statistically significantly different. See text for two-sided *P* values for specific comparisons.
with vacuolated cytoplasm or dissolution of the cell. These observations suggest that LNCaP cells do not express VEGF under baseline conditions in vivo but may activate the gene during apoptosis or death from toxic effects. Changes in diet did not alter the low baseline VEGF expression in the LNCaP model. All of the above data were analyzed initially by ANOVA (37) followed by Scheffe’s test (38) to calculate two-sided pairwise comparisons among treatment groups.

**Plasma IGF-I**

Blood samples were obtained from SCID mice bearing the LNCaP tumors at the time of necropsy, and IGF-I was evaluated by immunoradiometric assay. Circulating IGF-I concentrations (mean ± SD) in ad libitum fed mice were 339 ng/mL ± 59 ng/mL, in contrast to 125 ng/mL ± 44 ng/mL in mice having total diet restriction (P<.0001 versus ad libitum fed), 160 ng/mL ± 35 ng/mL in mice restricted in fat calories (P<.0001 versus ad libitum fed), and 114 ng/mL ± 26 ng/mL in mice restricted in carbohydrate calories (P<.0001 versus ad libitum fed, P<.05 versus fat restriction). All of the above data were analyzed initially by ANOVA (37) followed by Scheffé’s test (38) to calculate two-sided pairwise comparisons among treatment groups.

**DISCUSSION**

Our studies illustrate several novel findings concerning the interrelationships between diet, biomarkers, and prostate tumor growth: (a) Energy intake modulates the growth of prostate tumors, as seen in two animal models exhibiting dissimilar histopathologic and biologic features; (b) dietary fat over a wide range of intake fails to modulate prostate tumor growth under conditions of dietary energy restriction; (c) energy restriction alters prostate tumor morphology consistent with a less aggressive phenotype; (d) energy restriction is associated with increased prostate tumor cell apoptosis with little effect on the proliferation index; (e) energy restriction reduces prostate tumor angiogenesis; (f) VEGF expression is inhibited by energy restriction in the rat model; and (g) circulating IGF-I concentrations are reduced by energy restriction in the mouse model. These observations may be of significance in planning future laboratory and human studies for establishing successful prostate cancer prevention programs or dietary interventions in patients as an adjunct to standard medical management.

To our knowledge, these studies are the first to characterize the effects of energy intake on prostate tumorigenesis in experimental models. Restriction of total dietary consumption or the limitation of energy intake by selective reductions in the consumption of fat or carbohydrate calories has been shown to inhibit the incidence or growth of tumors having diverse histologic origins (42–44). Our studies report that energy intake ad libitum enhances the growth rate of rat and growth of transplantable human and rat prostate tumors illustrating diverse histologic, biologic, and molecular characteristics. The Dunning R3327-H adenocarcinoma, a slow-growing, well-differentiated to moderately differentiated, androgen-dependent tumor, mimics many of the features observed in newly diagnosed, localized human prostate cancer. In contrast, the human LNCaP line is a rapidly growing anaplastic (poorly differentiated) prostate cancer with no evidence of glandular formation in vivo. Our studies with the Dunning model clearly showed that restriction of energy intake can significantly inhibit tumor growth without inducing malnutrition in the host. The adult rats with the highest degree of energy restriction (40%) continued to show a gradual increase in body weight. The main difference between the restricted and the ad libitum fed rats is the scarcity of carcass adipose tissue stores, which was readily apparent at necropsy (although body composition was not measured).

Our studies also provide insight into the interrelationship between energy intake, dietary fat concentration, and prostate tumorigenesis. Previous studies have shown that prostate tumor growth is inhibited by essential fatty acid deficiency (45) as well as by diets rich in omega-3 fatty acids (46), whereas it is stimulated by linoleic acid (47) or high-fat diets (48). Comparing different types of energy restriction required that we use a high-fat diet providing 40% of energy as the ad libitum fed treatment group. This afforded the opportunity to reduce energy intake by restricting either fat or carbohydrate calories by 30%. It is interesting that restriction of fat calories, carbohydrate calories, or total diet provided 15%, 57%, and 40% of energy from fat, respectively. However, all types of energy restriction produced a significant and similar reduction in prostate tumor growth. Our studies revealed no evidence that high-fat diets promote prostate tumor growth under energy-restricted conditions. Prior studies (48) showing that high-fat diets enhance prostate tumor growth in rodents were completed under conditions of ad libitum feeding. Our studies indicate that the effect of dietary fat concentration on prostate tumor growth may depend on total energy intake as was suggested in studies with other experimental tumor models (49–53).

An obstacle to translational research in nutrition and prostate cancer prevention or to the application of nutrition as an adjuvant to therapy is the lack of surrogate biomarkers relevant to both dietary intervention and prostate cancer progression. The biomarkers examined in our study may be useful individually but, more importantly, they may be useful as a pattern. Folkman and colleagues (22) have observed that biomarkers of tumor angiogenesis are predictably associated with a specific pattern of changes in proliferation and apoptosis. For example, antiangiogenic growth factors and cytokines typically reduce tumor microvessel density in parallel with increased apoptosis and little effect on proliferation rates (23,24,54,55). We observed a twofold to threefold reduction in microvessel density, increased apoptosis, and minimal change in PCNA labeling index with energy restriction in our studies with the Dunning or LNCaP tumor models. Our studies suggest that energy restriction produces a pattern of biomarker changes identical to that observed following institution of antiangiogenic therapy.

Our results are consistent with a hypothesis that energy restriction inhibits prostate tumor growth via a shift in the balance between angiogenic and antiangiogenic factors (22). Solid tumor growth beyond a volume of 2–3 mm³ cannot be sustained by diffusion and depends on establishment of a vascular network for supplying nutrients and removing metabolic waste products (56,57). Enhanced tumor angiogenesis reflects the summation of many signaling processes between the tumor cells, matrix, and host vascular cells. The critical mediators are the angiogenic and antiangiogenic growth factors or hormones found in the circulation and tumor microenvironment (57). We propose that nutritional status directly or indirectly influences interactions between tumor cells and local vasculature by changing the expression of angiogenic growth factors. We evaluated this hypothesis by immunohistochemically examining the expression
of VEGF. VEGF is one of the most potent angiogenic factors known, enhancing endothelial cell proliferation and formation of new vessels (29). Furthermore, VEGF enhances vessel permeability, which may thereby provide an improved supply of nutrients to the tumor (29). VEGF expression by tumor cells in vitro is enhanced by glucose deficiency and hypoxia (29–32). With regard to the human prostate, immunoreactive VEGF or messenger RNA is constantly detected in the cancer specimens, whereas VEGF expression is variable in benign prostate hyperplasia and normal prostate tissue (39,41). VEGF expression has also been documented in prostate cancer cell lines, and the DU-145 line has been reported to exhibit the highest expression, the PC3 line intermediate expression, and the LNCaP line the lowest expression (33,39,40). Using an antibody against a VEGF epitope that is common to all isoforms, we examined VEGF expression in the Dunning and LNCaP tumors in vivo. In rats having ad libitum access to food, prostate tumors showed a high intracellular cytoplasmic expression of VEGF, particularly in the cells lining glandular and duct-like structures, as was reported for human prostate cancer (39,41). Furthermore, areas of positive staining occurred in a patchwork pattern throughout the tumors from ad libitum fed rats, a characteristic feature observed in human prostate cancer specimens suggesting that regulation may vary between specific microenvironments within the tumor, perhaps as a result of local changes in oxygenation or nutrient metabolism. We observed a striking inhibition of VEGF expression with diet and energy restriction in the Dunning model, with only a single glandular focus of staining detected in only one tumor specimen. A different picture emerged with the LNCaP tumor grown in vivo; this tumor exhibited little baseline VEGF staining, with image analysis detecting only 5% of the total area exhibiting positive staining. Furthermore, VEGF expression in the LNCaP tumor was associated with cells that appeared to be undergoing cytolysis or apoptosis. On the basis of these observations, we propose that VEGF is one of possibly many regulators of tumor neoangiogenesis altered by diet restriction to change the balance from a proangiogenic to an antiangiogenic microenvironment. However, our studies also indicate that diet-induced alterations in angiogenesis in the LNCaP prostate tumor model are not dependent on VEGF and that there must be other contributory factors.

We propose that another factor linking dietary intake with prostate tumor angiogenesis is IGF-I. We observed that circulating IGF-I is significantly reduced by diet or energy restriction in rats bearing prostate tumors, as was previously reported in studies of leukemia, breast cancer, and bladder cancer (52,58,59). A recent study of prospectively collected blood samples in a human cohort (25) showed that higher concentrations of circulating IGF-I were associated with increased risk of prostate cancer. IGF-I is mitogenic for normal and malignant prostate tumor cells (26). IGF-I receptors are present in prostate tissue, and expression is reduced by 5α-reductase inhibitors (27). IGF-I is also considered to be an angiogenic growth factor, stimulating endothelial cell proliferation, migration, and tube formation in vitro (28). IGF-I also has been implicated in promoting angiogenesis and collateral neovascularization following ischemic injury (60). The reduction in circulating IGF-I by dietary interventions represents a potential mechanism whereby prostate tumor angiogenesis may be reduced with an attenuation of tumor growth and reduced risk of metastases.

We used androgen deprivation by castration as an interven-

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NOTES

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