Dietary Fat and Protein Intake Differ in Modulation of Prostate Tumor Growth, Prolactin Secretion and Metabolism, and Prostate Gland Prolactin Binding Capacity in Rats[1,2]

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ABSTRACT The combined effects of dietary fat and protein concentration on prostate tumor growth and endocrine homeostasis were evaluated in male rats. A 2 × 2 factorial experiment examined the effects of protein (5 and 20% of energy as casein) and fat (10 and 40% of energy as corn oil) on the growth of the Dunning R3327-H transplantable prostate adenocarcinoma in Copenhagen × Fisher F_1rats. Rats fed protein-restricted diets for 20 wk exhibited lower energy intakes, final body weights and tumor growth rates. Weanling male Sprague-Dawley rats fed protein-restricted diets for 4 wk had serum concentrations of prolactin, growth hormone and testosterone which were 68, 17 and 85% of controls, respectively. After 16 wk of feeding, there were no effects of dietary protein on serum hormone concentrations despite reduced energy intake and body weight. The metabolic clearance rate of serum prolactin was lower in rats fed the low protein diets for 4 or 16 wk; however, no differences were noted when adjusted for body weight. In vivo studies employing intravenously injected ^{125}I-labeled prolactin revealed slight alterations in the metabolism of circulating prolactin monomer or binding to serum proteins in protein-restricted rats. The maximal binding capacity of prolactin receptors on the prostate membrane fraction was 42% lower in rats fed diets restricted in protein despite normal serum hormone concentrations at 16 wk. Dietary fat had no effect on tumor growth or prolactin homeostasis although a slightly greater serum testosterone was noted in rats fed high fat diets. In contrast, restriction of dietary protein caused significant changes in energy intake, serum hormone concentrations, prolactin metabolism, prostatic prolactin binding capacity and prostate tumor growth rates. These studies support the hypothesis that dietary protein and energy intake, particularly during periods of rapid growth and development, may alter prostate biology and modulate the risk of future prostate cancer progression. J. Nutr. 127: 225–237, 1997.

KEY WORDS: • *prostate cancer* • *dietary fat* • *dietary protein* • *prolactin* • *rats*

Prostate cancer is the second leading cause of cancer-associated mortality in American men, accounting for ~40,000 deaths and 244,000 new cases per year (Wingo et al. 1995). Incidence rates for prostate cancer show ~100-fold differences among nations and geographic locations (Parkin et al. 1993, Wingo et al. 1995). Although genetic factors may contribute to these differences, the increases in incidence within economically developed nations over time (Whelan et al. 1990) and in populations migrating from low- to high-risk areas (Haenszel and Kurihara 1968, Shimizu et al. 1991, Staszewski and Haenszel 1965) suggest a central role for environmental factors. Diet and nutritional factors are frequently suggested as major contributors to this variation (Clinton 1993). Descriptive epidemiologic studies have reported associations between components of diets characteristic of affluent populations, such as fat and animal products, and the mortality from prostate cancer (Armstrong and Doll 1975, Blair and Fraumeni 1978, Carroll and Kohr 1975). Case-control studies have provided little additional insight into the relationship between diet and prostate cancer because of the limitations of retrospective dietary assessment methods in diseased individuals, the dietary homogeneity among study participants and the limited number of subjects in most studies (Graham et al. 1983, Kaul et al. 1987, Mettlin et al. 1989, Ohno et al. 1988, Ross et al. 1983, West et al. 1991). The few prospective studies of prostate cancer risk have identified some associations with an affluent diet that will require confirmation and reinforcement from additional cohorts (Hirayama 1979, Hsing et al. 1990, Mills et al. 1989,

[1] Supported by the Public Health Service, National Institutes of Health, National Cancer Institute, Grant Nos. KO7 CA01680 to S. K. Clinton and CA 23326, CA 29629 to W. J. Visek.

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Manuscript received 4 April 1996. Initial review completed 18 April 1996. Revision accepted 8 October 1996.

Snowdon et al. 1984). Factors most frequently, but not uniformly, cited as contributing to risk are dietary lipids, particularly saturated fats from animal products (Blair and Fraumeni 1978, Gann et al. 1994, Giovannucci et al. 1993, Graham et al. 1983, Heshmat et al. 1985, Kolonel et al. 1988, Ross et al. 1987, Talamini et al. 1986, West et al. 1991, Whittemore et al. 1995).

The lack of well-characterized rodent models of prostate carcinogenesis has inhibited the thorough evaluation of dietary lipids and the initiation and promotion of prostate cancer. Several spontaneous prostate cancers in rats have been maintained by serial transplantation (Issacs and Coffey 1979) and allow examination of dietary effects on the growth rates of established prostatic cancer. We have previously observed that essential fatty acid deficiency inhibited the growth rate of the slow-growing, well-differentiated, hormone-sensitive R3327-H Dunning prostatic adenocarcinoma (Clinton et al. 1988). Others, using androgen stimulation in a carcinogen-induced prostate cancer model have not shown a clear enhancement of tumorigenesis by dietary fat concentration (Pollard and Luckert 1985, Pour et al. 1991). A recent study using immune-deficient mice bearing the human LNCaP prostate carcinoma showed that those fed a diet containing 40% energy from fat exhibited more rapid tumor growth rates and higher serum prostatic specific antigen than mice fed <20% energy from lipid (Wang et al. 1995).

The majority of the world's population reside in developing countries undergoing socioeconomic transition. Many of these populations exhibit a low incidence of age-adjusted prostate cancer risk (Wingo et al. 1995) and may consume diets that are occasionally marginal in protein concentration or contain proteins of low biological value. We hypothesize that inadequate protein nutrition during childhood and adolescence may have profound and lasting effects on endocrine homeostasis and prostate biology, leading to an overall reduction in prostate cancer risk. To our knowledge, the effects of dietary protein on experimental prostate tumorigenesis have not been examined. The present study examined the effects of dietary protein on the growth rate of the Dunning R3327-H prostate tumor and possible interactions with dietary fat.

The endocrine system, through the coordinated secretion of a diverse array of hormones, integrates host nutritional status with complex interorgan metabolic processes to optimize host growth, development and function. Changes in endocrine status are frequently proposed as the link between diet and prostate cancer risk. The androgen dependency of normal and malignant prostate tissue, established over 50 years ago, provided the foundation for current endocrine therapy of prostate cancer and hypotheses concerning the critical role of androgens in the pathogenesis of prostate malignancy (Huggins and Clark 1940, Huggins and Hodges 1941). Much less is known about the role of prolactin in prostate growth, differentiation and function, but several lines of evidence suggest that prolactin may also be an important hormone (Costello and Franklin 1994). Early data from Huggins and Russell (1946) showed that prostate glands undergo more profound atrophy following hypophysectomy than following castration alone. Hypophysectomy was subsequently found to reduce the uptake of infused radiolabeled testosterone in the rat ventral prostate, providing a basis for the interactions between pituitary hormones and androgens (Lawrence and Landau 1965). A more specific role for prolactin was suggested with the observation of synergy between purified prolactin and androgens in stimulating prostatic growth and function (Grayhack and Lebowitz 1967, Grayhack et al. 1955). A permissive effect of prolactin on androgen-stimulated prostatic growth was further demonstrated with studies employing prolactin-secreting pituitary isografts (Holland and Lee 1980). Similar studies showed that prolactin-secreting isografts increase in vivo uptake, distribution and clearance of labeled androgens by rat prostates (Prins and Lee 1982). Direct effects of prolactin on the prostate were demonstrated using prostate organ culture (Lasnitzki 1972) and human prostate cancer cell lines in vitro (Janssen et al. 1996). Prolactin receptors were subsequently found on prostatic epithelial cells (Aragona and Friesen 1975, Barkley et al. 1977, Witorsch 1979). The observation that the binding of prolactin to the prostate is androgen regulated suggested another mechanism for their interaction (Charreau et al. 1977, Kledzik et al. 1976). The development of specific monoclonal antibodies directed against epitopes of the prolactin receptor led to in vivo studies showing that antibody administration prevents prolactin-induced epithelial proliferation in the prostate (Sissom et al. 1988). These studies illustrate the crucial and interactive effects of prolactin and androgens in modulating prostate function.

The evidence described above is the basis of our hypothesis that dietary protein and lipids may modulate prostate biology and risk of cancer via alterations in the activity of pituitary hormones and androgens. We therefore evaluated the combined effects of dietary protein and fat intake on the growth rate of the hormone-sensitive Dunning R3327H well-differentiated adenocarcinoma of the prostate. We subsequently evaluated the effects of identical diets on serum hormone concentrations and pursued a detailed evaluation of prolactin secretion, metabolism and binding to the prostate gland. The hormone studies are designed to evaluate the effects of diet during the period of weaning though sexual maturity in the rat, which is when the prostate undergoes maximal growth and development. We propose that endocrine homeostasis during this critical period in prostate development has a long-term effect on the prostate cancer cascade.

MATERIALS AND METHODS

Animals and housing. Weanling male Copenhagen × Fisher F_1rats (Papanicolaou Cancer Research Institute, Miami, FL) were used in the cancer study, and weanling male Sprague-Dawley rats (Harlan Industries, Madison, WI) were used in the endocrine experiments. Rats were individually caged in stainless steel wire-bottomed cages maintained in rooms with 12 h of darkness daily (1800–0600 h) and an ambient air temperature of 22 ± 2°C. The care and use of laboratory animals followed guidelines set forth by the University of Illinois and the Public Health Service Policy on Humane Care and Use of Laboratory Animals, revised September 1986.

Diets. Four diets were formulated following the AIN 76 recommendations (AIN 1977 and 1980) with varied fat and protein concentrations (**Table 1**). Protein was substituted on an equal weight and energy basis for carbohydrate. dl-Methionine supplementation was proportional to casein content. Fat was substituted for an equivalent amount of energy from carbohydrate. The content of minerals, vitamins, protein, choline and cellulose was adjusted to maintain constant nutrient-to-energy ratios across dietary fat treatments. Diets were prepared monthly (Research Diets, New Brunswick, NJ or Teklad, Madison, WI), analyzed for protein (Shahinian and Reinhold 1971) and fat (Folch et al. 1957) at the time of delivery, and stored at 4°C. Fresh diet was provided three times per week (between 1000 and 1200 h) and total intake was recorded for each rat.

Prostate cancer study. The Dunning R3327-H prostate adenocarcinoma was received from the Papanicolaou Cancer Research Institute as bilateral subcutaneous implants in the flanks of a Copenhagen × Fisher F_1male rat (Dunning 1963). The implants were allowed to reach a volume of 2–3 cm^3 before removal for transplantation of 65- to 75-mg portions to bilateral subcutaneous sites in the flanks of 48 recipient rats. The recipients, randomly assigned to their respective diets at weaning and fed for 4 wk before tumor implantation, contin-

TABLE 1

The composition and nutrient density of the diets

Component	Low fat Low protein	Low fat High protein	High fat Low protein	High fat High protein
	g			
Casein[1]	5.00	20.00	5.95	23.81
DL-Methionine	0.08	0.30	0.09	0.34
Corn oil	4.00	4.00	20.00	20.00
Dextrin	54.15	44.00	41.55	29.52
Sucrose	27.07	22.00	20.85	14.77
Minerals[2]	3.50	3.50	4.17	4.17
Vitamins[2]	1.00	1.00	1.20	1.20
Choline[3]	0.20	0.20	0.24	0.24
Cellulose	5.00	5.00	5.95	5.95
Total	100.00	100.00	100.00	100.00
Approximate *kJ/g*[4]	15.8	15.4	18.7	18.3
% energy from protein	5	20	5	20
% energy from lipid	10	10	40	40

[1] 87–89% protein.
[2] AIN-76A mineral and vitamin mixtures (American Institute of Nutrition 1977 and 1980).
[3] Choline provided as choline bitartrate.
[4] Calculations based upon estimated metabolizable energy of 16.7 kJ/g of protein and carbohydrate and 37.7 kJ/g of lipid. The concentrations of minerals, vitamins and fiber were adjusted to maintain a constant ratio to energy.

ued their assigned diet for an additional 16 wk. Tumor measurements in centimeters were obtained every 4 wk and volumes estimated using the formula: (length × width × height) × 0.5236 = volume in cm^3. After 16 wk of tumor growth, the rats were killed and their tumors were removed and weighed.

Serum and pituitary hormone study. One hundred twenty-eight male Sprague-Dawley rats were randomized among the four dietary treatments (32 rats per diet) at weaning. After 4 or 16 wk of feeding, 16 rats per diet were killed for serum and pituitary hormone assays. Four rats per diet were decapitated at 0400, 1000, 1600 and 2000 h for collection of trunk blood and anterior pituitaries. Decapitations, with minimal stress, were completed within 10 s after removal of the rats from their cages. Blood samples were allowed to clot at 4°C for 4 h. The serum was separated by centrifugation and stored at −20°C until assayed. Each anterior pituitary, removed immediately after decapitation, was stored at −20°C in 1 mL of 0.01 mol/L PBS, pH 7.4, until assayed. Pituitaries were homogenized with a Potter-Elvehjem glass homogenizer with the hormone concentrations of the supernatant quantitated after centrifugation at 375 × *g* for 10 min.

Prolactin and growth hormone were measured by RIA with reagents supplied by the National Institutes of Health (Bethesda, MD). ^{125}I (New England Nuclear, Boston, MA) was used for radio-iodination of rat prolactin. The primary antibody (rabbit) was NIAMDD-anti-rat-PRL-S-8. Goat anti-rabbit IgG (Research Products International, Elk Grove Village, IL) was used as a second antibody. The amount of prolactin was expressed relative to NIAMDD-rat-PRL-RP2. Rat growth hormone for iodination was NIAMDD-rat-GH-I-4, and rat growth hormone antiserum (monkey) was NIAMDD-anti-rGH-S-4. The amount of growth hormone was expressed relative to NIADDKD-rat-GH-RP-1. Testosterone was quantitated by RIA (Clinton et al. 1988, Falvo and Nalbandov 1974, Falvo et al. 1974).

Metabolic clearance rate (MCR). Eighty male Sprague-Dawley rats were randomly assigned among the four dietary treatments (20 per group). After 4 or 16 wk of feeding, the MCR of prolactin was determined on 10 rats per diet. Each rat received a single intraperitoneal injection of 2-Br-α-ergocryptine (CB-154, 0.5 mg/kg, Sandoz, East Hanover, NJ) dissolved in ethanol-saline at 0800 h to suppress endogenous prolactin release (Shaar and Clemens 1972). Two hours later, each rat was anesthetized (ketamine-HCl, Bristol Laboratories, Syracuse, NY) and a jugular catheter implanted. Thirty minutes later, a single intravenous bolus of purified rat prolactin (25 μg/kg) dissolved in saline was administered. Blood samples were collected via the jugular catheters at time 0 and at 5, 10, 15, 30 and 60 min after prolactin injection; the serum prolactin concentration was measured using the immunoassay described above. Blood volume was replaced with normal saline at each sampling time. The MCR of prolactin was calculated by a numerical procedure (Normand and Fortier 1970) using the following equation:

$$MCR = R_s / \int_o^a X(dt)$$

where R_s is the injected dose, X is the serum concentration at a given time after injection and dt is the time interval between samples. The integral $\int_o^a X(dt)$ was calculated to give the area under the hormone disappearance curve. The estimated secretion rate was calculated by multiplying the MCR by the mean basal endogenous prolactin concentration determined in the parallel study described above.

Prolactin metabolism. Eight weanling male Sprague-Dawley rats were randomly assigned to each diet and fed for 16 wk. All procedures were performed between 0800 and 1200 h. Rats were given a single peritoneal injection of 2-Br-α-ergocryptine as described above at 0800 h to suppress endogenous prolactin release. At 1000 h, a jugular catheter was inserted in each rat after administration of anesthesia (ketamine-HCl, Bristol). Approximately 30 min thereafter, each rat received 0.2 mL of a monomeric prolactin mixture which contained 0.5 μg of immunoreactive prolactin and 25 ng of ^{125}I-prolactin tracer (Sinha et al. 1979). Rat prolactin (NIAMDKD-rat-PRL-I-5) was iodinated with ^{125}I at room temperature using the chloramine-T method (5.0 μg prolactin, 37 kBq ^{125}I, 20 μg chloramine-T in 0.05 mol/L phosphate, pH 7.4 for 20 s followed by 250 μg sodium metabisulfite) (Greenwood et al. 1963). After completion of the reaction, 100 μg of unlabeled rat prolactin in 400 μL of 0.01 mol/L PBS, pH 7.6, was added to the iodination vial. The entire mixture was chromatographed on a Sephadex G-100 column (1.5 × 30 cm.) and 70 1-mL aliquots of eluate were collected and counted. The contents of the tube with the highest radioactivity and the two adjacent tubes were pooled and diluted with saline for injection.

One-milliliter blood samples were collected at 10 and 20 min after injection of the labeled prolactin and the serum was isolated for chromatography. Blood volume was replaced with sterile saline after the 10 min blood collection. Five hundred microliters of serum from each sample was chromatographed on a Sephadex G-100 column using PBS with 2 g/L bovine serum albumin, pH 7.6 as the eluting buffer (Sinha and Baxter 1979a). High molecular weight blue dextran (2.5 mg/mL, Pharmacia, Uppsala, Sweden) was added to each column

TABLE 2

The combined effects of dietary protein and fat concentration on body weight, energy intake and the final weight of Dunning R3327-H transplanted prostate adenocarcinomas in Copenhagen × Fisher F_1 rats[1]

Dietary protein	Dietary fat	Final body weight	Energy Intake	Tumor weight	Tumor weight
% energy		g	kJ/d[2]	g	g/100 g body weight
5	10	221	200	2.01	0.93
5	40	242	204	2.37	0.98
20	10	409	254	6.15	1.50
20	40	418	262	6.18	1.48
Pooled SEM		17	25	0.80	0.26
Statistics[3]					
Protein		$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.05$
Fat		NS	NS	NS	NS
Interactions		NS	NS	NS	NS

[1] Twelve rats were assigned to each diet at 4 wk of age and received bilateral subcutaneous tumor implants weighting 70 ± 5 mg at 8 wk of age. All rats were necropsied 20 wk after the initiation of dietary treatment. Values represent the mean ± SEM.
[2] To convert kJ/d to kcal/d divide by 4.16.
[3] Statistical analysis expressed as main effects of protein or fat or their interactions. NS means not significant at $P > 0.05$.

to mark the void volume and 70 1-mL fractions were collected and analyzed for radioactivity and immunoreactive prolactin by RIA as above. Because ^{125}I was used to label the prolactin injected, the radioactive antigen for prolactin assays was prepared with ^{131}I to allow discrimination from the fraction of ^{125}I-labeled prolactin. The areas of the peaks for absolute radioactivity and immunoreactivity were calculated and expressed as the percentage of total activity. The radioactivity in the free iodide peak was not included in the calculation of total prolactin.

Prolactin binding capacity. Four treatment groups of 16 rats were fed their respective diets for 16 wk as described in the above hormone study. Within the context of a large dietary study, it was not feasible to quantitate the prolactin receptor number or affinity characteristics by Scatchard analysis. We therefore devised a more convenient assay which provided a single value representing maximal prolactin binding capacity of the isolated prostate membranes at saturating concentrations of ligand. With this assay, it was possible to evaluate a large number of samples and estimate prostate gland cell membrane binding capacity as a prelude to future investigations which may describe in greater detail the effects of diet on prolactin receptor number and affinity. Rats were killed by decapitation, and their multiple-lobed prostate was removed, minced and placed into buffer (25 mmol/L Tris-HCl, 10 mmol/L $CaCl_2$, 300 mmol/L sucrose, pH 7.4) at 4°C. The tissues were homogenized and centrifuged at 800 × *g* to remove the nuclear fraction; the remaining supernatant was centrifuged at 27,000 × *g* for 20 min to obtain a membrane pellet which was resuspended in buffer for the prolactin receptor assays. Ovine prolactin was iodinated with ^{125}I (New England Nuclear) by a modification of the chloramine-T procedure of Hunter and Greenwood (Sinha and Baxter 1979). The mass of I^{125}-prolactin used in each assay was quantified by determining the specific activity according to the procedure of Shiu and Friesen (1976). The binding of prolactin to prostatic membranes was determined in duplicate for assay mixtures (0.5 mL final volume) containing 100 mg of membrane protein, buffer (0.025 mol/L Tris-HCl, pH 7.6 containing 10 mmol/L $MgCl_2$ and 1 g/L bovine serum albumin) and ^{125}I-prolactin, in the presence and absence of excess unlabeled ovine prolactin, to define nonspecific binding. The assays were terminated by the addition of ice-cold buffer and centrifuged. The pellets were counted in the gamma counter, and the amount specifically bound was calculated. The amount of ^{125}I-prolactin used in these assays was based upon preliminary studies using increasing concentrations of labeled ligand to define the amount providing maximal ligand binding. In addition, the affinity of the ^{125}I-prolactin was determined by incubating with increasing amounts of membrane protein until a plateau of binding was reached which was defined as maximum bindability. All calculations were corrected for maximum bindability of the labeled prolactin.

Statistical analysis. The effects of dietary fat and protein on tumor growth were evaluated by two-way ANOVA, with Scheffé's multiple-comparison method (Scheffé 1953) used subsequently to test differences among the four dietary groups using Statview 4.01 (Abacus Concepts, Berkeley, CA). The serum hormone concentrations, pituitary hormone contents and prolactin receptor data were analyzed by ANOVA with dietary fat and protein concentration, sampling time and their interactions in the statistical models following the General

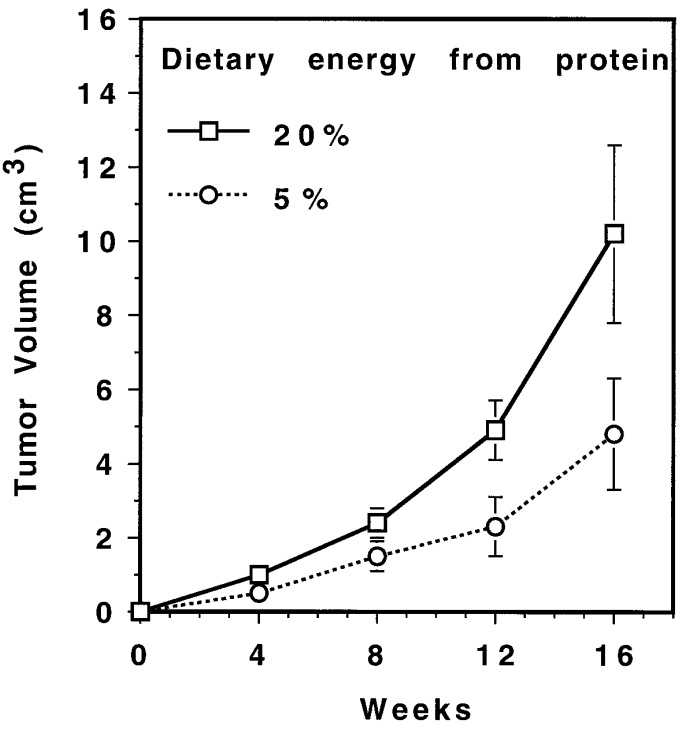

FIGURE 1 The main effects of diets containing protein at 5 or 20% of energy on the growth rate of the Dunning R3327-H prostatic adenocarcinoma in Copenhagen × Fisher F_1male rats. Tumor volumes were significantly lower in rats fed the low protein diets ($P < 0.05$). There were no effects of dietary fat concentration or interactions with dietary protein on tumor volume (data not shown). Values represent means ± SEM, $n = 24$.

TABLE 3

The combined effects of dietary protein and fat concentration on body weight and energy intake of weanling male Sprague-Dawley rats after 4 and 16 wk of feeding[1]

Dietary protein	Dietary fat	Body weight[1]		Energy intake[1,2]	
		4 wk	16 wk	4 wk	16 wk
% energy		*g*		*kJ/d*	
5	10	101	210	154	212
5	40	106	223	154	216
20	10	236	419	245	270
20	40	252	456	249	287
Pooled SEM		3	7	5	8
Statistics[3]					
Protein		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Fat		NS	$P < 0.05$	NS	NS
Interaction		NS	NS	NS	NS

[1] Means represent data from 16 rats. Dietary treatments were initiated at 23 d of age with initial body weights of 40–60 g.
[2] To convert kJ/d to kcal/d divide by 4.16.
[3] Statistical analysis expressed as main effects of protein or fat or their interactions. NS means not significant at $P > 0.05$.

Linear Models procedure of the Statistical Analysis System (SAS Institute, Cary, NC). The metabolic clearance rate was similarly evaluated without sampling time in the model. For the prolactin metabolism study, the proportions of prolactin in each peak at 10 and 20 min were analyzed separately for radioactivity and by immunoassay, using repeated measures ANOVA that included dietary fat and protein concentration, sampling time and their interactions in the statistical models (SAS Institute).

RESULTS

Prostate tumor growth. Copenhagen × Fisher F_1 rats fed dietary corn oil providing 10 or 40% of energy showed no significant differences in energy intake, body weight, tumor growth rates or final tumor weight (**Table 2**). In contrast, rats fed diets containing 5% of energy as protein consumed 31% less total energy ($P < 0.001$) and averaged 45% less in final body weight ($P < 0.001$) compared with those consuming a diet adequate in protein. Palpable tumor volumes were significantly lower in those fed low protein diets ($P < 0.05$). The main effects of dietary protein are shown in **Figure 1**. Rats fed diets restricted in protein exhibited a significant reduction in prostate tumor weight at necropsy ($P < 0.01$) even after adjusting for differences in body size ($P < 0.05$) (Table 2). There were no significant interactions between dietary fat and protein on weight gain, energy intake, tumor volume or final tumor weight.

Serum and pituitary hormone study. Mean body weights of Sprague-Dawley rats fed the low protein diets for 4 wk were 42% of those fed normal protein diets ($P < 0.001$) (**Table 3**). Energy intakes for rats fed the low protein diets were 62% of those fed 20% energy as protein ($P < 0.001$). In contrast, dietary fat concentration had no significant effect on energy intake after 4 wk of feeding, although rats fed high fat diets weighed slightly more than those fed corn oil at 10% of energy ($P < 0.05$). After 16 wk, rats fed low protein diets weighed 49% of those fed normal protein diets ($P < 0.001$). Corresponding energy intake was 75% of that measured for rats fed 20% of energy as protein ($P < 0.001$). The average body weight for rats fed diets containing fat at 40% of energy was 7.5% ($P < 0.05$) greater after 16 wk of feeding than observed for those fed 10% of energy as fat.

Serum and pituitary hormone concentrations were determined at 0400, 1000, 1600 and 2000 h after 4 and 16 wk of feeding. Serum prolactin concentrations were greater at 1600 and 2000 h ($P < 0.001$) than at other time intervals in growing male rats (data not shown), reflecting the normal diurnal variation in prolactin secretion. Because no significant interactions between sampling time and diet were found, the data were pooled across sampling times and presented in **Tables 4** and **5**.

Rats fed low protein diets showed decreases of 32% in serum prolactin ($P < 0.05$), 47% in pituitary weight ($P < 0.001$), 50% in pituitary protein ($P < 0.001$), and 40% in pituitary prolactin compared with those fed normal protein after 4 wk of feeding (Tables 4 and 5). After 16 wk, no difference in serum prolactin was noted, although rats fed low protein diets exhibited 48% lower pituitary weight ($P < 0.001$), 36% less pituitary protein ($P < 0.001$) and 34% lower pituitary prolactin ($P < 0.001$). Because dietary protein has a profound effect on body weight, the pituitary prolactin content was also expressed per unit of final body weight (**Fig. 2**) and a relative increase in the amount of prolactin in the pituitary of rats fed protein-restricted diets for 4 or 16 wk was observed. We observed no effect of fat on serum or pituitary prolactin after 4 or 16 wk of feeding.

Rats fed 5% energy as protein had serum growth hormone concentrations averaging only 17% of those fed adequate protein after 4 wk ($P < 0.001$), although no significant effects were noted at 16 wk (Table 4). After 4 or 16 wk of feeding, pituitary growth hormone content (Table 5) was significantly lower in rats fed low protein diets ($P < 0.0001$). As expected, a significant portion of the decrease in pituitary growth hormone content can be accounted for by the smaller size of the pituitary gland, associated with the reduced body mass in rats fed a protein- and energy-restricted diet. However, we observed a relative decrease in pituitary growth hormone content even when the data were expressed as total pituitary growth hormone per unit body weight at 4 wk. The alteration in pituitary growth hormone content per unit body weight normalized by 16 wk (**Fig. 3**).

Serum testosterone concentrations were 15% lower in rats fed the low protein diets compared with controls ($P < 0.001$) after 4 wk of feeding, whereas no effect of dietary protein was observed at 16 wk (Table 4). Rats fed high corn oil diets exhibited 11% greater mean concentrations of serum testoster-

TABLE 4

The combined effects of dietary protein and fat on serum prolactin, growth hormone and testosterone in male Sprague-Dawley rats after 4 and 16 wk of feeding[1]

		Prolactin		Growth hormone		Testosterone	
Dietary protein	Dietary fat	4 wk	16 wk	4 wk	16 wk	4 wk	16 wk
% energy		*ng/mL*					
5	10	8.9	12.5	43.1	87.3	3.0	3.4
5	40	9.8	17.0	26.3	102.6	3.2	3.7
20	10	12.9	12.8	200.9	102.6	3.7	3.4
20	40	14.7	13.9	201.1	102.0	3.6	4.1
Pooled SEM		1.9	2.0	30.1	28.3	0.1	0.2
Statistics[2]							
Protein		$P < 0.05$	NS	$P < 0.001$	NS	$P < 0.001$	NS
Fat		NS	NS	NS	NS	NS	$P < 0.05$
Interaction		NS	NS	NS	NS	NS	NS

[1] Data represent the means for 16 rats pooled across 4 times of killing: 0400, 1000, 1600 and 2000 h.
[2] Statistical evaluation is expressed as main effects of protein or fat or their interactions. NS means not significant at $P > 0.05$.

one ($P < 0.05$) after 16 wk, although no significant differences were observed at 4 wk.

In addition to the hormone studies presented in Table 4, we also evaluated serum prolactin, growth hormone and testosterone in the Copenhagen × Fisher F_1male rats bearing the R3327-H prostate adenocarcinoma (Table 2, Fig. 1). As observed in the Sprague-Dawley male rats, we found no effect of dietary protein on the concentrations of these hormones after 20 wk of dietary treatment and 16 wk after tumor implantation.

Prolactin clearance and secretion. The injected prolactin disappeared from the circulation of rats at a multiexponential rate (**Fig. 4**). Low protein diets were associated with a lower prolactin MCR ($P < 0.001$), but no significant dietary effects were detected when data were adjusted for body weight (**Table 6**). The calculated prolactin secretion rates, corrected for body weight, were reduced with protein-restricted diets fed for 4 wk ($P<0.05$), whereas no differences were seen at 16 wk (**Fig. 5**). Dietary fat concentration was not associated with any significant change in MCR or estimated secretion rate.

Prolactin metabolism. The radiolabeled and purified rat prolactin used for intravenous injection was homogeneous and showed one chromatographic peak (**Fig. 6**). However, at 10 and 20 min after intravenous injection, three radiolabeled components were found in the serum. **Figure 7** shows a representative chromatogram from individual rats fed dietary protein at 5 or 20% of energy. The first peak, termed "big" prolactin, eluted with the blue dextran marker. The second peak appeared in the effluent at a position equal to that of the purified and labeled monomeric form of injected prolactin. The third component was free iodine, arising from deiodination of the labeled prolactin. No immunoreactivity was detected by RIA in the fractions covered by the third radioactive peak. The size of the free-iodine peak increased with sampling time as expected (**Table 7**). "Big" prolactin comprised 19.6% of total radioactivity in the circulation at 10 min after injection and increased to 24.4% at 20 min ($P < 0.01$). At each sampling time, rats fed diets low in protein showed a greater proportion of radioactivity in the monomeric form, although this relationship was not significant ($P = 0.10$) (Table 7, Fig. 7). "Big" prolactin detected by immunoassay comprised 36.4% of total prolactin in the circulation at 10 min after injection and increased to 44.9% by 20 min ($P < 0.001$). At each sampling

TABLE 5

The combined effects of dietary protein and fat on pituitary weight and the content of protein, prolactin and growth hormone in male Sprague-Dawley rats after 4 or 16 wk of feeding[1]

		Pituitary weight		Pituitary protein		Pituitary prolactin		Pituitary growth hormone	
Dietary protien	Dietary fat	4 wk	16 wk	4 wk	16 wk	4 wk	16 wk	4 wk	16 wk
% energy		*mg*				*μg*			
5	10	4.5	6.1	0.34	0.52	2.2	3.6	0.15	0.47
5	40	4.5	6.5	0.30	0.52	2.2	5.0	0.17	0.46
20	10	8.4	10.0	0.69	0.81	3.6	6.6	0.54	0.89
20	40	8.8	10.4	0.62	0.81	3.6	6.4	0.50	0.88
Pooled SEM		0.2	0.2	0.02	0.02	0.2	0.4	0.03	0.04
Statistics[2]									
Protein		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.0001$	$P < 0.0001$
Fat		NS	NS	NS	NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	NS	NS	NS	NS

[1] Data represent the mean for a total of 16 rats pooled across 4 times of killing: 0400, 1000, 1600, and 2000 h.
[2] Statistical evaluation is expressed as main effects of protein or fat and their interactions. NS means not significant at $P > 0.05$.

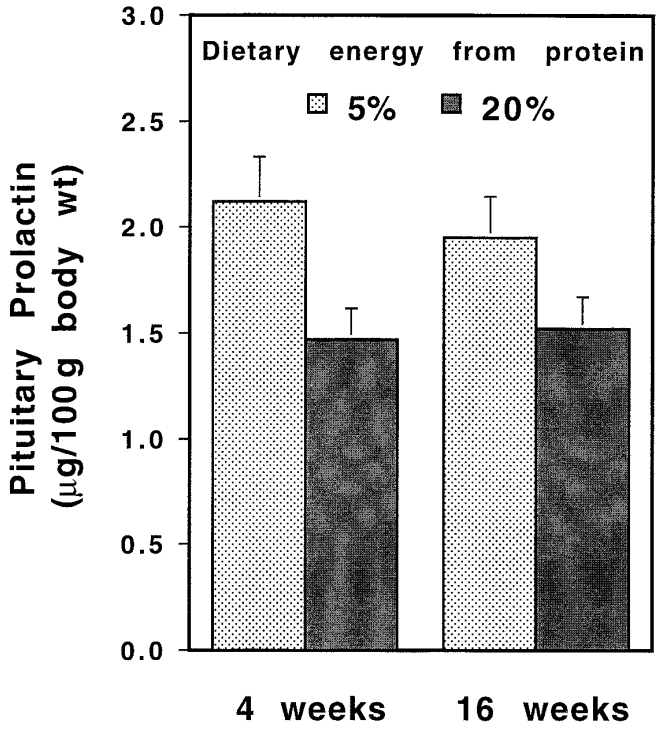

FIGURE 2 The main effects of dietary protein at 5 or 20% of energy on total pituitary prolactin adjusted for differences in body weight in male Sprague-Dawley rats. Pituitary prolactin was greater in those fed diets low in protein for 4 ($P < 0.01$) or 16 wk ($P < 0.05$). Values represent means ± SEM, $n = 16$.

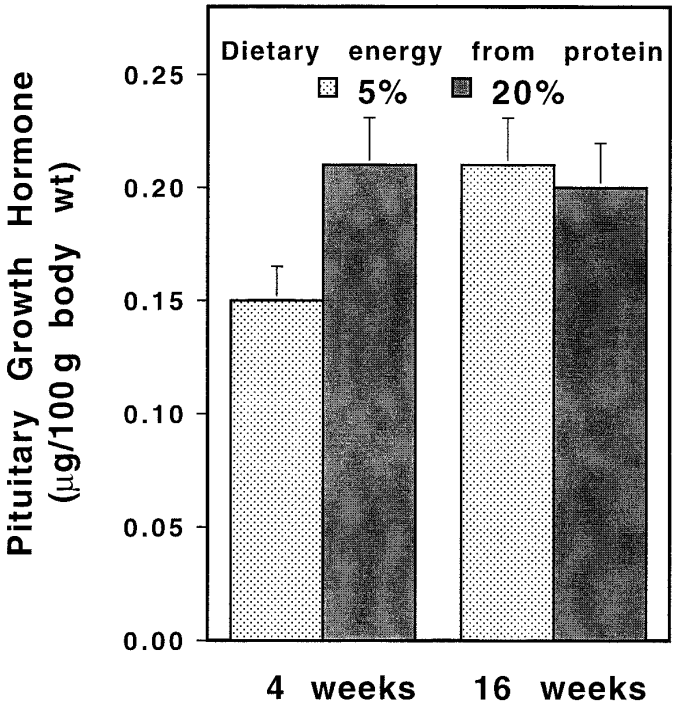

FIGURE 3 The main effects of dietary protein at 5 or 20% of energy on total pituitary growth hormone adjusted for differences in body weight in male Sprague-Dawley rats. Pituitary growth hormone was lower in rats fed diets low in protein for 4 wk ($P < 0.01$), but not at 16 wk. Values represent means ± SEM, $n = 16$.

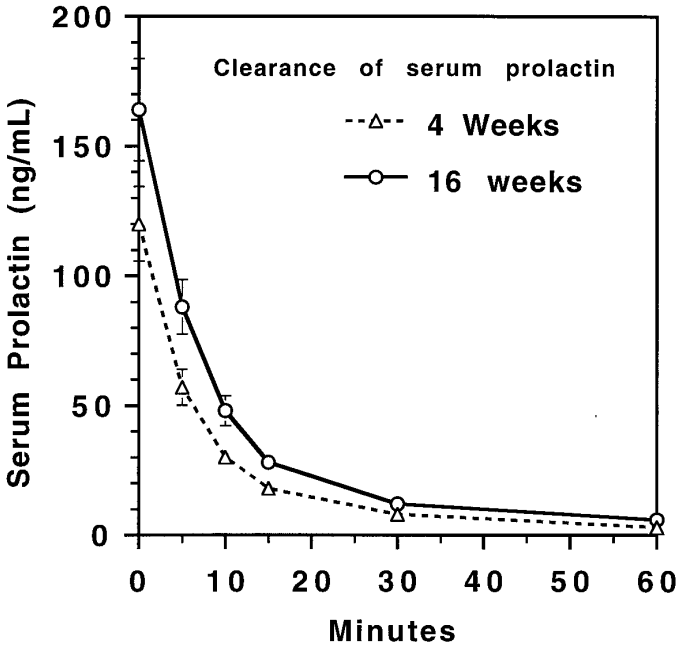

FIGURE 4 Disappearance of immunoreactive prolactin from the sera of male Sprague-Dawley rats after a single intravenous injection of rat prolactin (25 μg/kg body weight). Values represent the pooled means ± SEM for 40 rats (10 rats fed each of the four diets) for 4 or 16 wk after weaning. The data were used to generate clearance rates which are shown in Table 6.

time, rats fed protein-restricted diets showed a greater proportion of prolactin in the monomeric form using the immunoassay ($P < 0.001$). No effects of dietary fat were observed on the metabolism of prolactin. Prolactin metabolism was not measured in rats fed their diets for 4 wk. "Big" prolactin may represent a family of prolactin forms including dimers and several complexes of prolactin with serum binding proteins.

Prolactin binding capacity. Prostatic membranes from rats fed the low protein diet exhibited only 58% of the total prolactin binding capacity of membranes isolated from rats fed normal protein for 16 wk ($P < 0.01$) (**Fig. 8**). We did not examine prolactin binding capacity in rats fed their diets for 4 wk. The binding of prolactin to isolated prostate membrane fractions was not influenced by dietary fat intake and there were no interactions with dietary protein.

DISCUSSION

Dietary protein, lipid, and energy intake as modulators of prostate carcinogenesis. Our studies demonstrate that dietary protein restriction reduced the growth rate of the Dunning R3327-H transplantable prostate adenocarcinoma in rats. Diets restricted in protein, deficient in essential amino acids, or imbalanced in amino acid content have profound effects on metabolism and somatic growth, which is due in part to the associated depression in absolute energy intake (Mitchell 1927 and 1928, Rose 1927). Restrictions of energy intake have been shown to inhibit carcinogenesis and tumor growth rates consistently in numerous animal models (Albanes 1987, Weindruch and Walford 1988). It is probable that the inhibition of prostate tumor growth observed in our study was due to a combination of biological effects related to both protein and energy restriction. It is of interest that we observed a significant reduction in final tumor weight in protein/energy-restricted rats even after adjusting for the change in body weight. How-

TABLE 6

The combined effects of dietary protein and fat on the metabolic clearance rate of exogenously administered prolactin in Sprague-Dawley rats fed their respective diets for 4 or 16 wk[1]

		Metabolic clearance rate at 4 wk[2]		Metabolic clearance rate at 16 wk[3]	
Dietary protein	Dietary fat	mL/min	mL × min^{-1} × 100 g body wt^{-1}	mL/min	mL × min^{-1} × 100 g body wt^{-1}
% energy					
5	10	3.0	3.1	5.3	1.0
5	40	3.5	3.3	4.3	0.8
20	10	7.3	2.9	7.8	0.9
20	40	7.0	2.5	6.7	1.5
Pooled SEM		0.6	0.4	0.7	0.2
Statistics[4]					
Protein		$P < 0.001$	NS	$P < 0.001$	NS
Fat		NS	NS	NS	NS
Interaction		NS	NS	NS	NS

[1] Data represent the means for 10 rats.
[2] Mean body weights at 4 wk were as follows: low fat and low protein, 102 g; low fat and high protein, 257 g; high fat and low protein, 113 g; and high fat and high protein, 287 g.
[3] The mean body weights at 16 wk were as follows: low fat and low protein, 279 g; low fat and high protein, 434 g; high fat and low protein, 251 g; and high fat and high protein, 450 g.
[4] Statistical evaluation expressed as main effects of protein or fat or their interactions. NS means not significant at $P > 0.05$.

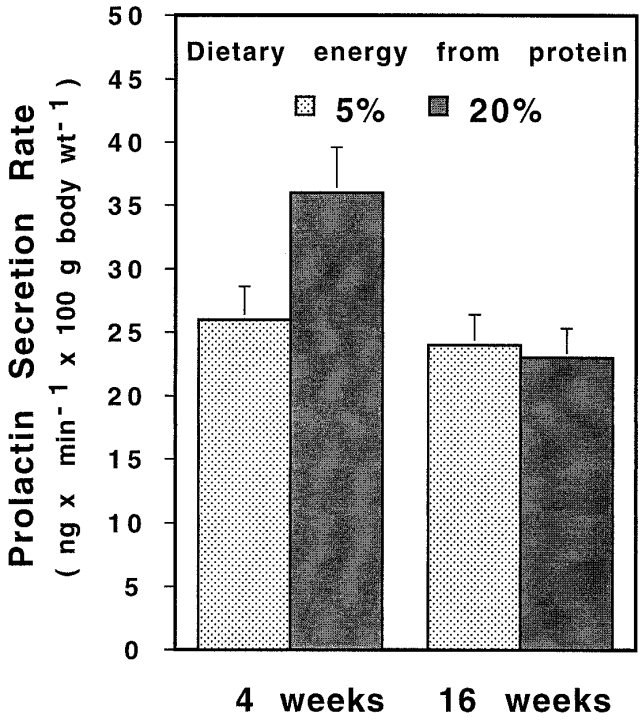

FIGURE 5 The main effects of dietary protein at 5 and 20% of energy on the estimated secretion rate of pituitary prolactin in male Sprague-Dawley rats. Secretion rate (ng × min^{-1} × 100 g body wt^{-1}) was calculated based upon the measurement of serum prolactin concentrations (Table 4) and the metabolic clearance rate of prolactin (Table 6). Rats fed diets restricted in protein exhibited a reduction in calculated prolactin secretion by the pituitary after 4 wk of feeding. Because the secretion rate is calculated based upon two variables already evaluated by our statistical program, we refrained from additional testing. The data are presented for descriptive purposes and represent means ± SEM, $n = 16$.

ever, energy intake per gram of body weight actually was greater in the protein-restricted group.

The vast majority of male children, adolescents and adults in affluent nations consume diets adequate in protein concentration and quality. However, males in developing nations may experience periods when dietary protein intake is inadequate, especially during childhood and adolescence when requirements are greater. It is possible that early dietary patterns may alter prostate development, endocrine profiles and other host processes that have long-term effects on prostate cancer risk. Human prostate cancer develops through a multistep process, characterized by a long latency period during which the evolving cancer accumulates multiple genetic defects and by progressive loss of autocrine, paracrine and endocrine growth control. The long latent period of human prostate cancer also provides opportunities for dietary factors to intervene at various stages to lower or enhance risk of progression to a clinically significant lesion. Our observation that the growth of an established transplantable prostate tumor can be modulated by dietary protein and energy intake suggests that later stages of prostate tumorigenesis are also sensitive to dietary interventions. It is important to realize, however, that our studies employed a well-differentiated, slow-growing, and androgen-sensitive tumor. The significant findings observed with this tumor model may not be reproduced in a study employing a poorly differentiated, anaplastic, and hormone-resistant prostate tumor.

Dietary fat and prostate cancer. Our studies showed no overall effect of dietary fat concentration or interactions with protein-energy intake on the growth rate of the Dunning R3327-H transplantable prostate carcinoma. We have previously reported that dietary fat intake as corn oil reduced the growth rate of prostate tumors only when total fat was reduced to concentrations which supplied marginal amounts of essential fatty acids (Clinton et al. 1988). The earlier study suggested that a minimal supply of essential fatty acids is necessary for optimal growth of established tumors. A more recent study, using immune-deficient nude mice bearing the LNCaP human prostate adenocarcinoma, showed a more rapid growth rate in

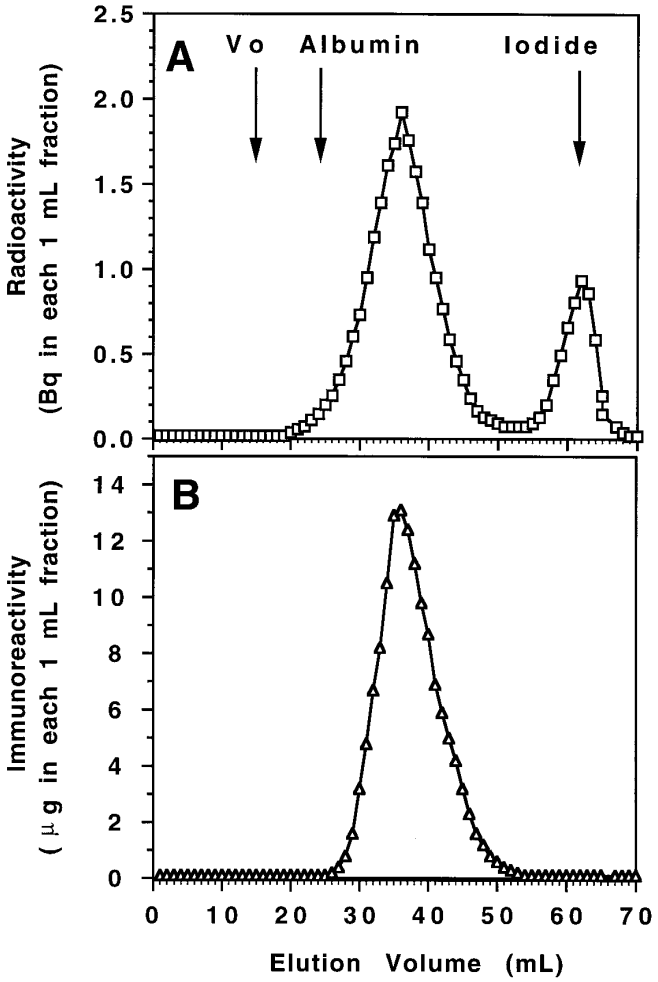

FIGURE 6 Chromatographic profile on Sephadex G-100 of ^{125}I-labeled rat prolactin for intravenous injection into male rats fed diets varying in fat and protein concentration for 16 wk. The column was equilibrated and eluted at room temperature with phosphosaline buffer, pH 7.6, containing 2 g/L bovine serum albumin. Arrows indicate the positions of the void volume (V_o), peaks of human serum albumin and free ^{125}I. The immunoreactivity is expressed as the amount of prolactin detected by RIA in each 1-mL fraction eluted from the column (*panel A*). Similarly, the radioactivity is expressed as the counts in the same 1-mL fractions (*panel B*). The contents of the tube with the highest radioactivity and the two adjacent tubes were pooled and diluted for injection. No free iodine was observed in the injected material.

mice fed dietary corn oil at 40.5% of the diet compared with 21.2% or less (Wang et al. 1995). Clearly, our understanding of the role of fatty acids and lipids in prostate cancer is only beginning to emerge.

Dietary effects on serum and pituitary prolactin. We observed that serum prolactin was reduced in protein/energy-malnourished rats during a period when rapid prostate growth would be expected. However, the abnormality in serum prolactin normalized by 16 wk despite the continuation of dietary treatments and significant differences in energy intake and body weight. This observation indicates that changes in serum prolactin homeostasis resulting from dietary insult are most profound when the host is undergoing most rapid growth. Perhaps human studies of diet, hormones and prostate carcinogenesis should focus on adolescent males. Our study further suggests that measurements of serum hormones such as prolactin, testosterone or growth hormone may not be sensitive indicators of protein/energy nutrition after adult body size and weight have been achieved, even when final adult anthropometrics are significantly different.

We postulated that the reduction in serum prolactin with protein/energy restriction was due to changes in secretion rate, MCR or perhaps both. We observed that the effects of diet on serum prolactin seem to be more closely related to secretion rates rather than an alteration in clearance from the circulation. Pituitary prolactin content expressed per milligram of pituitary protein or as total pituitary prolactin per unit body weight was significantly increased in protein/energy-restricted rats, suggesting that prolactin synthesis in the pituitary was not

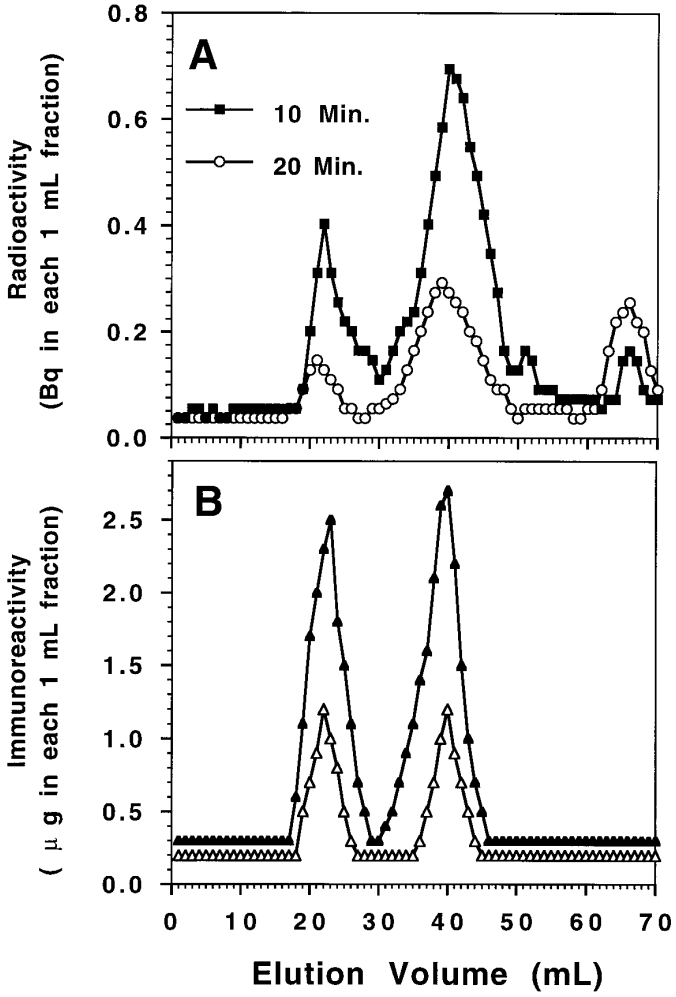

FIGURE 7 Representative prolactin profile following Sephadex G-100 chromatography of the sera from a rat at 10 and 20 min after injection with monomeric ^{125}I-labeled rat prolactin. The data are from a rat fed 20% of energy from protein and 10% of energy from lipid. Total radioactivity and RIA were completed on each 1-mL fraction eluted from the column. The first peak which eluted soon after the void volume corresponds to the protein bound, dimeric or polymeric forms of circulating prolactin produced in vivo after administration of monomeric prolactin. The second component eluted at the position corresponding to the administered monomeric form of prolactin. The peak between fraction 60 and 70 (60 and 70 mL) corresponds to free iodide resulting from the deiodination of ^{125}I-labeled prolactin and shows no immunoreactivity. Profiles were obtained from eight rats receiving each of the four dietary treatments. Averaged over all rats, radioactivity data show that ~85% of the prolactin was in the monomeric form at 10 min and 81% at 20 min. In contrast, monomeric prolactin accounted for ~58% of the immunoassay detectable prolactin at 10 min, and 63% at 20 min.

TABLE 7

Percentage of the area covered by the large or monomeric prolactin as determined by radioactivity counting and RIA at 10 and 20 min after administration of ^{125}I-prolactin in male Sprague-Dawley rats fed diets varying in protein and fat for 16 wk[1]

Time	Dietary protein	Dietary fat	Radioactivity large	Radioactivity monomeric	Radioimmunoassay large	Radioimmunoassay monomeric
	% energy		%			
10 min	5	10	19.1	80.9	33.6	66.4
	5	40	17.1	82.9	32.6	67.4
	20	10	21.1	78.9	37.9	62.1
	20	40	21.2	78.8	41.3	58.7
20 min	5	10	23.8	76.2	42.7	57.3
	5	40	21.9	78.1	40.2	59.8
	20	10	29.0	71.0	50.6	49.4
	20	40	23.0	77.0	45.9	54.1
Pooled SEM			1.5	1.5	1.9	1.9
Statistics[2]						
Protein			NS[3]	NS[3]	$P < 0.01$	$P < 0.01$
Fat			NS	NS	NS	NS
Time			$P < 0.01$	$P < 0.01$	$P < 0.001$	$P < 0.001$
Interaction			NS	NS	NS	NS

[1] Data represent the mean percentage of the total area covered by the ''large'' and ''monomeric'' prolactin for 7 or 8 rats.
[2] Statistical evaluation is expressed as main effects of protein, fat, time after prolactin administration or their interactions. NS means not significant at $P < 0.05$.
[3] Statistical evaluation indicated a trend for dietary protein with $P < 0.10$.

limiting. We speculate that the increased pituitary prolactin resulted from an inhibition of posttranslational regulation of prolactin processing and secretion. The mechanisms underlying this observation are unknown but may involve an alteration in the balance between critical signals from the neurointermediate lobe and median eminence of the hypothalamus to stimulate or inhibit prolactin release. In addition, it is possible that dietary factors may also modulate the recruitment of pituitary mammotropes into the secretory pool or their responsiveness to prolactin-releasing stimuli. A more complete understanding of the neuroendocrine processes modulated by protein and energy to govern dynamic synthesis and release of prolactin is necessary.

Diet and the structural heterogeneity of serum prolactin. We observed that the conversion of exogenously administered monomeric prolactin to higher molecular weight forms was slightly, but significantly, reduced in protein/energy-restricted rats. In our study, endogenous prolactin secretion has been pharmacologically suppressed, and the structural heterogeneity develops in the circulation exclusively from the exogenously administered monomeric prolactin. One explanation may be that serum prolactin binds to circulating proteins which alter its migration on Sephadex columns (Sinha 1992). Perhaps dietary protein restriction reduces the concentration of a circulating prolactin binding protein and thereby reduces formation of a higher molecular weight complex. Another possibility is that a portion of prolactin circulates as a dimer or larger molecular weight oligomer. Overall, our observations in male rats are similar to those reported for mice (Sinha and Baxter 1979a and 1979b). Dietary fat over the range of 10–40% of energy had no significant effect on the circulating forms of prolactin. This observation is similar to that observed in female rats fed diets varying in fat concentration and source (Clinton et al. 1995).

At present, we can only speculate on how diet can mediate the in vivo conversion of monomeric prolactin to larger forms. In addition to the changes in prolactin structure or binding to other proteins that may occur in the circulation, it is clear that structural heterogeneity results from complex transcriptional and posttranslational processes involved in secretion from the pituitary in different physiologic states. It is probable, although unproven, that diet and nutrition can also influence these processes and thereby alter prolactin bioactivity. The molecular structures of prolactin found in vivo may be very diverse. It is possible that prolactin exists as oligomers as well as variously proteolytically cleaved, deamidated, phosphorylated and glycosylated forms (Sinha 1992, Walker 1994). Additional prolactin-like molecules encoded by distinct genes and perhaps additional forms derived from the differential splicing of mRNA from the known prolactin gene add to the complexity. Posttranscriptional changes involving phosphorylation are critical regulatory controls. The nonphosphorylated and phosphorylated monomer forms show differential release from the pituitary in response to different physiologic signals and have different activities in target tissues (Sinha 1992, Walker 1994). It is intriguing that the nonphosphorylated and phosphorylated forms are recognized to varying degrees by prolactin antisera and that they differ markedly in their biological activity. The more acidic forms are the least antibody reactive and are reported to have more bioactivity (Sinha 1992, Walker 1994). Indeed, the importance of phosporylation has been demonstrated in target tissues, in which the monophosphorylated variant seems to act as an antagonist to the unmodified hormone (Sinha 1992, Walker 1994). A further understanding of how diet may modulate the forms of prolactin produced and their pleotropic biological effects is clearly necessary.

Dietary modulation of prolactin receptors in the prostate. The biological effects of prolactin in the prostate are initiated through membrane receptors. We observed that the binding capacity of isolated prostatic membranes for prolactin was reduced in rats fed diets low in protein and energy. Based on the known role of prolactin in regulating prostatic growth and function (Costello and Franklin 1994), particularly in immature animals (Negro-Vilar et al. 1977), this observation may have importance in explaining the interrelationships of diet and hormones to the progression of prostate cancer. This

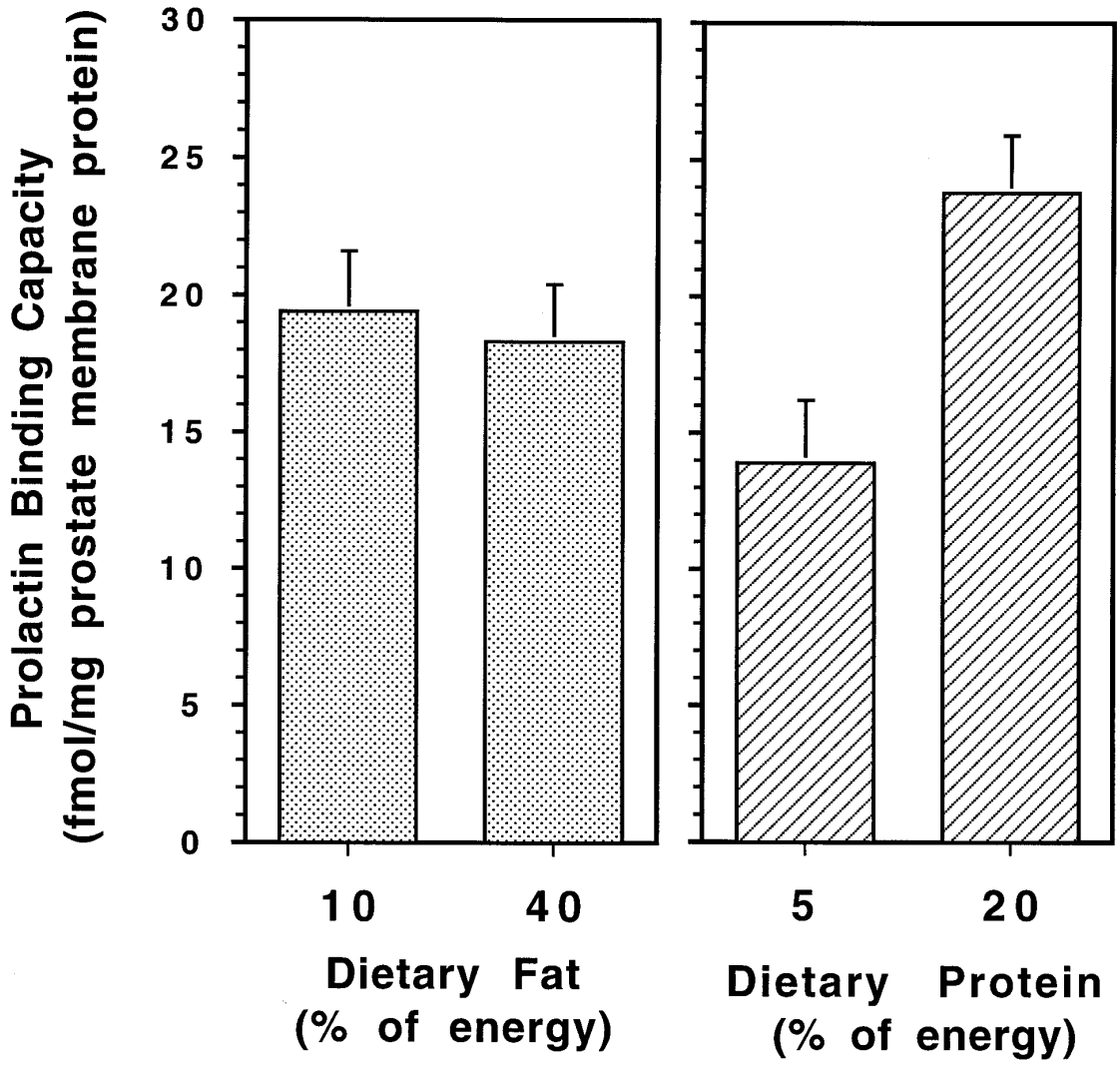

FIGURE 8 The main effects of dietary protein (5 or 20% of energy) and fat (10 or 40% of energy) on the prolactin binding capacity of prostatic tissue from male Sprague-Dawley rats after 16 wk of feeding. Prostatic membranes from rats fed the protein-restricted diet had only 58% of the total prolactin binding capacity of prostate membranes isolated from rats fed normal protein for 16 wk ($P < 0.01$). The binding of prolactin to isolated prostate membrane fractions was not influenced by dietary fat intake and there were no interactions with dietary protein (data not shown). Values represent the mean ± SEM ($n = 32$ for main effect of each level of dietary fat or protein).

study illustrates that in situations in which serum concentrations of hormones may be normal, diet may cause a reduction in target tissue hormone response by altering hormone receptor number or affinity. The recent cloning and ongoing characterization of the prolactin receptor indicates that it is a member of a cytokine-growth factor-hormone superfamily based upon conserved sequences in their extracellular domains (Kelly et al. 1992). The primary structure of two forms of the prolactin receptor that vary in length has been defined (Kelly et al. 1992). The receptors contain ~300 and 600 amino acids and share similar extracellular, transmembrane and proximal cytoplasmic domains (Kelly et al. 1992). The two forms show tissue-specific patterns of expression (Kelly et al. 1992). Efforts to define how diet regulates the expression of the prolactin receptors and identify biomarkers of prolactin activity in the prostate are necessary. We observed no effect of dietary fat between 10 and 40% of energy on prolactin receptors in the prostate which is supported by other studies showing no effect of lipid over this range of intake on prolactin receptors in liver and breast tumors (Cave and Jurkowski 1984, Wetsel and Rogers 1984).

Dietary modulation of serum growth hormone. In contrast to prolactin and particularly androgens, very little is known about the role of growth hormone in prostate carcinogenesis and its direct or indirect effects via modulation of other hormones such as insulin-like growth factor I or II. A provocative study by Hursting et al. (1993) suggests that the profound effects of energy restriction on tumor growth in experimental models may be mediated by reduced growth hormone and insulin-like growth factor-I levels. In their study, diet restriction reduced serum concentrations of growth hormone and insulin-like growth factor-I and inhibited the replication of transplanted leukemia cells. Infusion of growth hormone or insulin-like growth factor-I restored tumor growth in restricted rats to that observed in freely fed controls. The possibility that the growth hormone/insulin- like growth factor-I axis plays a critical role in the prostate tumor growth by dietary protein and energy warrants additional investigation.

Dietary modulation of serum testosterone. Our study shows that insufficient protein and energy have a significant effect on serum testosterone in weanling rats, but that serum concentrations normalize over a period of weeks despite con-

tinued consumption of an inadequate diet as the rats mature. In studies which focus primarily on acute and severe dietary restriction or fasting, others have documented suppression of the hypothalamus-pituitary-testicular axis (Bergendahl and Veldhuis 1995). It is probable that the effects we observed are also mediated by changes in the synthesis and secretion of hypothalamic gonadotropin-releasing hormone and gonadotropins, although they were not measured in our study. The importance of testosterone in normal and malignant prostate biology is well established (Huggins and Clark 1940, Huggins and Hodges 1941). A number of studies evaluating the relationship between affluent dietary patterns and circulating hormones in men have reported increases in serum concentrations of testosterone and prolactin (Hill et al. 1979). We propose that a lower serum testosterone as a result of dietary variables, particularly during adolescence when prostate growth is greatest, may have long-lasting effects on the risk of prostate carcinogenesis.

Summary. Our studies show a profound effect of dietary protein and energy restriction on serum prolactin, growth hormone and testosterone during early growth and development. Although hormone levels normalized with prolonged dietary restriction despite significant stunting in growth, a lasting effect on prostate gland hormone receptor status was observed. It is our hypothesis that etiologic factors responsible for altering human prostate carcinogenesis begin early in life and gradually alter risk over decades. Many populations exhibiting lower rates of prostate cancer mortality consume diets lower in energy and protein content or protein sources of lower biological value which have a greater effect on children and adolescents who have greater protein requirements. The diet-induced endocrine changes suggest mechanisms whereby small, low grade prostate carcinomas, which occur at a more homogeneous frequency though the world (Yatani et al. 1989), may progress to clinically significant disease in Western cultures consuming diets rich in energy, fat and protein, and low in fiber and foods of plant origin. These studies do not imply that protein restriction is an appropriate therapy for established prostate cancer.

ACKNOWLEDGMENT

The authors are grateful to the Papanicolaou Cancer Research Institute at Miami, FL for providing the Dunning R3327-H tumor and the Fisher × Copenhagen male F_1 rats.

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