Effect of Isocaloric Low-fat Diet on Human LAPC-4 Prostate Cancer Xenografts in Severe Combined Immunodeficient Mice and the Insulin-like Growth Factor Axis

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ABSTRACT

Over-consumption of dietary fat has been suggested to promote the development and progression of prostate cancer in men. The present study was conducted to answer the following questions: (a) Can dietary fat reduction decrease tumor growth rates of Los Angeles prostate cancer (LAPC)-4 xenografts in severe combined immunodeficient (SCID) mice independent of total caloric intake? and (b) Is the insulin-like growth factor (IGF) axis involved in the effects of dietary fat on LAPC-4 tumor growth in SCID mice? Twenty-eight male CB17 beige SCID mice (8 weeks old) were individually caged, randomized, and fed an isocaloric high-fat (HF, 42% kcal) or low-fat (LF, 12% kcal) diet. Each mouse was s.c. injected with 1 x 10^5 LAPC-4 cells, and tumor volumes were measured weekly. At week 16, all animals were sacrificed, and serum and tumors were obtained for analysis. Although caloric intakes and mouse weights were equal between groups, the LF mice had significantly slower tumor growth rates and lower serum prostate-specific antigen levels compared with the HF mice. LF mice had significantly lower levels of serum insulin, tumor IGF-1 mRNA expression, and tumor IGFBP-2 immunostaining and higher levels of serum IGFBP-1 (by Western ligand blot) relative to the HF mice. There were no differences in the serum levels of IGFBP-3 and IGFBP-4 between the groups. LAPC-4 cells cultured in vitro with media containing serum from LF mice demonstrated slower growth than LAPC-4 cells cultured in media containing HF mice serum. These results demonstrate that intake of an LF diet was associated with slower LAPC-4 prostate tumor growth relative to mice fed an HF diet, independent of total caloric intake, and this effect may be mediated through modulation of the insulin/IGF axis.

INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer in United States men and the second leading cause of cancer death (1). Although men in Asian countries and the United States have similar rates of autopsy-detected latent prostate cancer (Refs. 2 and 3; ~30% of men older than age 50 years), the incidence of clinically detected prostate cancer is 15-fold higher among men in the United States (4–7). Furthermore, Chinese and Japanese men who immigrate to this country have an increased incidence and mortality from prostate cancer compared with Chinese and Japanese men in their native country (8–11). These epidemiological studies suggest that environmental factors associated with Western culture may promote the development of clinical prostate cancer. One such factor that has been implicated is dietary fat. A number of case controlled and cohort studies found that increased intake of dietary fat was associated with a higher risk of developing and dying from prostate cancer (11–14).

Animal and in vitro studies have also demonstrated a relationship between dietary fat and prostate cancer. Pollard and Luckert (15) found that a 20% corn oil ad libitum diet induced more frequent and earlier prostate tumor development in testosterone-treated Lobund-Wistar rats, as compared with the same rats fed an ad libitum vegetarian diet containing <5% fat. Kondo et al. (16) showed that ad libitum feeding ACI/Seg rats (a strain that can spontaneously develop prostate cancer) a HF3 diet during pregnancy promoted prostate carcinogenesis in male offspring. In addition, Wang et al. (17) found that lowering dietary fat in nude mice resulted in reduced growth of human prostate LNCaP tumors. However, none of these feeding experiments housed the animals one mouse per cage or monitored the caloric intake of each mouse to ensure equal caloric intake...
between the groups. Caloric excess therefore may have played a role in these experiments in the increased development and growth of the prostate tumors (18–21). In vitro studies also indicate an association between dietary fat and prostate cancer. Adding ω-6 fatty acids to cultured prostate cancer cells yielded a dose-dependent promotional effect on six different cell lines, including androgen-responsive (LNCaP) and androgen-independent (PC-3) cell lines (22).

Numerous studies suggest that the IGF axis may play an important role in the development and progression of prostate cancer (23–25). To date, however, no clear link has been established between dietary intake of fat, the IGF axis, and the development and progression of prostate cancer. Although caloric restriction has been shown to decrease serum IGF-1, and result in slower xenograft tumor growth (26), no animal studies have specifically addressed whether altering dietary fat without caloric restriction has similar effects on the IGF axis and tumor growth. We sought to further study the relationship between the IGF axis and dietary fat in an in vivo xenograft model. Specifically, we addressed the following two questions: (a) Can dietary fat reduction decrease tumor growth rates of LAPC-4 prostate cancer xenografts in SCID mice independent of total caloric intake? and (b) Is the IGF axis involved in the effects of dietary fat on LAPC-4 tumor growth in SCID mice?

MATERIALS AND METHODS

Animal Husbandry and Feeding Protocol. Twenty-eight male CB17 beige SCID mice (8 weeks old) were obtained from the UCLA Department of Laboratory Animal Medicine facility, which is accredited by the American Association for Accreditation of Laboratory Animal Care. The mice were housed one mouse per cage to allow for the maintenance of isocaloric intake between the diet groups. The cages were kept in a sterile and pathogen-free facility on campus. Cages, bedding, and water were autoclaved before use. The feeding receptacles were on the top of the cages so food intake could be monitored and new feedings given without opening the cage. Sterile techniques were used whenever handling the cages, mice, and food. The experiments were approved by the UCLA Chancellor’s Animal Research Committee, and animals were cared for in accordance with institutional guidelines.

The diets were prepared and sterilized (irradiated) by DYETS, Inc. (Bethlehem, PA). The HF diet contained 42% calories from fat; the LF diet contained 12% calories from fat (Table 1). Initially, a palatability study (without tumor injection) comparing ad libitum intake of the LF and HF diets was performed, and the LF group consumed slightly fewer calories than the HF group. Thus, during the xenograft experiment, the LF mice were fed ad libitum, and the average caloric intake of the LF group was measured three times per week (Monday, Wednesday, and Friday). Each feeding period (Monday, Wednesday, and Friday), the HF group was given HF food to match the average caloric intake of the LF group from the previous feeding period. This modified pair feeding technique has provided equal caloric intake in previous isocaloric feeding studies (27).

LAPC-4 Xenografts. The LAPC-4 cell line (a generous gift from Drs. Robert Reiter and Charles Sawyers) was developed in paraffin were stained with H&E. Immunohistochemistry

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* Two experimental diets were formulated containing various amounts of corn oil, a source of fat.
* Depolymerized cornstarch used to pelletize diets.
* See Ref. 66.

Table 1 Ingredients of experimental diets

After LAPC-4 xenografts were established at UCLA by direct transfer of cancer cells from a patient with advanced adenocarcinoma of the prostate into the s.c. tissue of SCID mice (28). LAPC-4 produces PSA, has a wild-type androgen receptor, and shows features of hormone-dependent growth and metastasis (28). Four weeks after starting their respective diets, the mice were injected s.c. in the lateral flank with $1 \times 10^7$ LAPC-4 tumor cells in 0.1 ml of Matrigel (Collaborative Biomedical Products, Bedford, MA). The LAPC-4 tumor cells used for injection were harvested from xenograft tumors propagated in SCID mice as described previously (29). Throughout the experiment, mice were weighed, and tumors were examined weekly. When tumors became palpable, the tumor dimensions were measured using a caliper. Tumor volumes were calculated using the formula: length $\times$ width $\times$ height $\times 0.5236$ (30).

Serum and Tumor Collection. Sixteen weeks after the LAPC-4 cells were implanted, the mice in each group were euthanized, serum was collected, and tumor tissue was obtained and weighed. Serum was stored at $-70^\circ$C. Tumor tissue was rinsed with saline to remove any traces of blood to avoid confounding mRNA and protein tissue measurements. Half of the tumor was snap frozen in liquid nitrogen, and the other half was fixed for 4–8 h in 10% neutral buffered formalin and then embedded in paraffin blocks for histological sections.

Serum Studies. Human serum PSA and mouse serum IGF-I were measured by ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX). Mouse serum insulin was measured by ELISA (Crystal Chem Inc., Chicago, IL), and mouse serum IGFBPs were detected by Western ligand blot. Western ligand blotting for the detection of IGFBPs was performed using electrohoresis of 25 μg of protein on 12% SDS-polyacrylamide gels overnight at constant voltage, electroblotting onto nitrocelulose, sequential washing with NP40, 1% BSA, and Tween 20, followed by incubation with two million cpm of $^{125I}$-IGF-I (Amersham, Piscataway, NJ) for 12 h, and exposure to film, as described previously (31).

Tumor Studies. Formalin-fixed tumor sections embedded in paraffin were stained with H&E. Immunohistochemistry
of representative tumor sections was performed for Ki-67 
(DAKO, Carpinteria, CA) and human IGFBP-2 and IGFBP-3 
(UBI, Lake Placid, NY) using the following protocol. Serial, 
4-μm sections were cut from each archived paraffin-embedded 
block and deparaffinized with xylene, rehydrated through 
graded alcohol rinses, and incubated with 3% hydrogen pero- 
oxide to quench endogenous horseradish peroxidase for 5 min. 
Antigen retrieval was performed by placing the slides in a 
vegetable steamer for 30 min with sodium citrate buffer (10 
Mm, pH 6.0). Subsequently, the slides were treated with normal 
horse serum (dilution 1:20) in 0.1 M Tris-buffered saline at pH 
6.0 and incubated for 5 min. Next, the slides were incubated for 
30 min with the primary antibodies IGFBP-2, IGFBP-3, and 
Ki-67 (dilution 1:100). Slides were then treated with biotin- 
labeled antimouse IgG and incubated for 20 min. Finally, the 
slides were incubated with the preformed avidin biotin peroxi- 
dase complex for 20 min. Metal-enhanced diaminobenzidine 
substrate was then added in the presence of horseradish pero- 
oxidase, and sections were counterstained with hematoxylin, de- 
hydrated, and mounted. Positive controls were used for each 
immunostain studied. For the IGFBP-2 and IGFBP-3 immuno- 
stains, the slides were examined by a group of pathologists to 
determine the criteria for positive or negative staining. All slides 
were then read in a blinded fashion by a single pathologist 
(Yahiya I. Elshimaili). Two-hundred cells were counted per slide. 

**Quantitative RT-PCR.** Total RNA was extracted from 
LAPC-4 tumors using TRIzol reagent (Life Technologies, Inc., 
Grand Island, NY). One-hundred mg of LAPC-4 tissue were 
homogenized in 1 ml of TRIzol reagent and incubated for 5 min 
at 25°C. Chloroform (0.2 ml) was added to the homogenized 
sample and incubated for 3 min at 25°C. The mixture was 
centrífuged (11,000 rpm, 4°C, 10 min). RNA was precipitated 
from the aqueous phase by the addition of isopropanol (0.5 ml) 
for 10 min and centrifugation at 11,000 rpm for 10 min at 4°C. 
The RNA pellet was washed with 1 ml of 75% ethanol and air 
dried for 10 min. The RNA was then dissolved in RNase-free 
water and determined to have an A260/A280 ratio of <1.6. Reverse 
transcription of mRNA into cDNA was carried out by incubat- 
ing tritiated RNA with avian myeloblastosis virus reverse tran-
scriptiona prim, oligo (dT), deoxynucleoside triphosphate, and 
RNase inhibitor at 42°C for 1 h. One µl of each cDNA sample 
was amplified using PCR in a total volume of 25 µl (30 ng of 
[32P]-5′-oligonucleotide primer, 100 ng of 3′-oligonucleotide 
primer, 2.5 µl of modified 10 × PCR buffer, 1.25 units of Taq pol-

derase, and autoclaved double distilled water to a volume of 25 
µl). The PCR mixture was amplified for 25 cycles in a DNA 
Thermocycler (Perkin-Elmer, Norwalk, CT). Each cycle con-
sisted of denaturation at 94°C for 1 min and annealing/extension 
at 65°C for 2 min. The [32P]-labeled PCR products were visual- 
ized directly by acrylamide gel electrophoresis and autoradiog-
raphy. The signal power of each product was calibrated to its 
matching β-actin mRNA expression. The following oligonu- 
ucleotide primer pair sequences were used: 

β-actin (184 bp): 5′ CAACCTCCATCATGAGTGTGAC and 3′CTCGGCTTACGAGCAACAC; and IGF-I (1175 
bp): 5′ AAGTCAGTCTGCTGTCCG and 3′ CTGTTACCT- 
CCCTCAGCTTCT.

**In Vitro Bioassay.** Androgen-sensitive LAPC-4 prostate 
tumor cells were grown in 75-cm² flasks (Falcon) in Iscove's 
modified Dulbecco’s medium without phenol red, supplemented 
with 10% FBS, 200 IU penicillin, 200 mg/ml streptomycin, and 
4 μM t-glutamine (Omega Scientific, Inc., CA). The cultures 
were maintained at 37°C and supplemented with 5% CO₂ in 
a humidified incubator. Cells were passaged routinely at 80% 
confluence, and fresh medium was replaced every 3rd day. Cells 
used in experiments were not passaged >10 times. Cells were 
detached with 0.25% trypsin-EDTA solution (Sigma Chemical 
Co., St. Louis, MO), centrifuged at 1250 rpm, and resuspended 
in fresh medium. Cell viability was assessed via trypan blue 
exclusion. Cells were plated at 5 × 10⁴ cells/well in 96-well 
plates. After 24 h, fresh media (Iscove’s modified Dulbecco’s 
medium, 200 IU penicillin, 200 mg/ml streptomycin, and 4 µl 
t-glutamine) with 10% FBS or 10% mouse serum (obtained 
from individual mice on sacrifice, i.e., nonpooled sera) were 
added to the wells, and the plates were incubated (37°C, 5% 
CO₂) for 48 h. LAPC-4 cell growth in media containing mouse 
serum was expressed as a percentage of LAPC-4 growth in 
media containing FBS. Cell growth was determined by CellTiter 
96AQ Assay (Promega Corp., Madison, WI). The assay con-
tained a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5- 
(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] or 
3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)- 
2-(4-sulfonyl)-2H-tetrazolium, which was bioreduced by viable 
cells via dehydrogenase enzymes into a colored formazan pro-
duct that was soluble in tissue culture medium. Twenty µl of 
this solution were added to each well of the 96-well plate containing 
100 µl of culture medium and incubated for 2 h (37°C, 5% 
CO₂). The quantity of formazan product, as measured by a 
microplate reader (Molecular Devices) at 490 and 655 nm, was 
directly proportional to the number of living cells in each well. 
This method has been shown to correlate (<5% difference with 
[3H] thymidine incorporation and other proliferation assays, 
such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium 
brmide and 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-
tetrazolium-5-carboxanilide inner salt.

**Statistical Analysis.** Statistical analyses (InStat Statistical 
Software; GraphPad, San Diego, CA and STATA 7.0; Stata 
Corp., College Station, TX) were performed by Student’s t test 
or ANOVA followed by Newman-Keuls post hoc analyses. 
Correlations between outcome variables were computed using 
the Spearman correlation coefficient. Multivariate analysis of 
the predictors’ serum-stimulated LAPC-4 tumor growth was 
calculated using a forward stepwise linear regression model 
with a P < 0.05 used for evaluating which variables should be 
included in the model at each step. Power calculation was also 
done on negative findings using the InStat software. P < 0.05 
was considered significant. Data are expressed as mean ± SE. 
The coefficient of variation was calculated by dividing the SD 
by the mean (expressed as a percentage).

**RESULTS**

**Reduced LAPC-4 Tumor Growth and Ki-67 Labeling** 
in LF Diet Group. The mice in the LF and HF groups 
maintained equal caloric intake throughout the experiment with 
each mouse consuming an average of 12 kcal/mouse/day (Fig. 
1A). Mouse weights were also equal between the two groups 
throughout the study (Fig. 1B). All mice from both groups
developed tumors, and there were no differences in time to development of palpable tumor between the groups. The tumors in the LF mice had significantly slower growth rates when compared with the tumors in the HF group (\( P < 0.01 \)). At the time of sacrifice, mice fed the LF diet had significantly smaller tumor volumes (Fig. 2A) and lower tumor weights (Fig. 2B) than mice fed an HF diet. Mean serum PSA was 34% lower in the LF group relative to the HF group (69.5 ± 6.1 versus 106 ± 13 ng/ml, \( P = 0.02 \); Fig. 2C).

The LAPC-4 tumors in the LF and HF groups appeared histologically similar and revealed polygonal cells with pleomorphic vesicular nuclei and prominent nucleoli. There were large areas of necrosis and prominent apoptosis with no evidence of tubule formation consistent with a poorly differentiated adenocarcinoma of the prostate. The mean Ki-67 labeling index of the LF tumors was 14% lower than the labeling index in the HF tumors (62.1 ± 3.4 versus 72.2 ± 2.2%, \( P = 0.02 \); Fig. 3A).

Reduced Serum Insulin and Increased Serum IGFBP-1/-2 in LF Diet Group. To examine the role of the IGF axis in tumor growth in our model, Western ligand blots were performed to detect serum levels of IGFBPs using \(^{125}\text{I}-\text{IGF-I}\). Three well-characterized bands (32) were found (Fig. 4A) with molecular weights corresponding to IGFBP-3 (at the \( M_r 40–44,000 \) range), IGFBP-1/-2 (at \( M_r \sim 29,000 \)), and IGFBP-4 (at the \( M_r 24–28,000 \) range) from top to bottom. The only marked difference between the LF and HF groups was increased band intensity in the middle bands (IGFBP-1/-2) in the LF group (111.2 ± 4.4 AU) relative to the HF group (72.1 ± 5.5 AU, \( P < 0.01 \); Fig. 4B). Mean relative optical densities for IGFBP-3 and IGFBP-4 bands were not significantly different between the

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**Fig. 1** SCID mouse energy intake, weights, and LAPC-4 tumor growth. Twenty-eight 8-week-old male SCID mice were fed either an HF or LF diet for 4 weeks and then injected s.c. in the lateral flank with \( 1 \times 10^5 \) LAPC-4 tumor cells in 0.1 ml of Matrigel. In A, energy intake was measured for each mouse three times per week by subtracting the weight of uneaten food from the weight of the food placed into the feeding receptacles at the beginning of each feeding period. In B, mice were weighed weekly from the start of the experiment. In C, once the tumors became palpable, tumor volume was measured weekly. Values are expressed as mean ± SE. **, \( P < 0.01 \); * \( P < 0.05 \).
groups. Mean serum insulin concentrations were significantly lower in the LF mice (309 ± 105 pg/ml) relative to the HF mice (908 ± 152, P < 0.01; Fig. 4C). Although mean serum IGF-I levels were slightly lower among the LF mice (350 ± 380 ng/ml), the difference was not statistically significant (P = 0.46).

Reduced Tumor IGFBP-2 in LF Diet Group. The mean percentage of tumor cells staining positive for IGFBP-2 was significantly lower in the LF mice relative to the HF mice (2.29 ± 0.4 versus 3.82 ± 0.39, P = 0.01; Fig. 5). There was no difference in the percentage of tumor cells staining for IGFBP-3 between the groups (1.73 ± 0.36 versus 1.57 ± 0.32, P = 0.75). RT-PCR for IGF-I mRNA expression in the tumors showed a trend for less intense bands for LF IGF-I mRNA, compared with HF (P = 0.06; Fig. 6).

Increased LAPC-4 Growth in Vitro in Media Containing HF Mouse Serum. In vitro 48-h incubation of LAPC-4 cells with media containing 10% HF mouse serum resulted in more LAPC-4 cell growth (110% of FBS control, 95% confidence interval = 94–127%), compared with LAPC-4 cells grown in media containing 10% LF mouse serum (72% of FBS control, 95% confidence interval = 58–85%, P < 0.01). Using multivariate analysis, we compared the variables of serum insulin, IGFBP-1, and IGF-1 levels, final tumor volume, and diet group for their ability to predict serum-stimulated LAPC-4 cell growth. Serum insulin (P = 0.001) and IGF-1 (P = 0.034) were both significant independent predictors of serum-stimulated LAPC-4 cell growth.

Analysis of the serum and tumor measurements produced several significant correlations (Table 2). Human serum PSA was positively correlated with final tumor weight and volume and serum insulin (P ≤ 0.02). Serum insulin positively correlated with serum PSA, tumor weight and volumes, serum-stimulated LAPC-4 growth in vitro, and IGFBP-2 immunostaining (P < 0.03). Serum insulin was negatively correlated with serum IGFBP-1/2 (P < 0.01). Serum IGF-I was positively correlated with serum-stimulated LAPC-4 growth in vitro (P ≤ 0.05). In addition, IGFBP-2 tumor immunostaining was positively correlated with tumor weight (P < 0.01), final tumor...
volume, and serum-stimulated LAPC-4 growth in vitro (P < 0.05).

DISCUSSION

The present study found that mice fed an isocaloric LF diet demonstrated slower LAPC-4 xenograft tumor growth and lower serum PSA levels relative to mice fed an HF diet. Serum from mice consuming an LF diet also stimulated less LAPC-4 cell growth in vitro, compared with serum from mice consuming an HF diet. Consuming an LF diet was also associated with a significant decrease in serum insulin levels, lower IGFBP-2 tumor immunostaining, lower IGF-I mRNA tumor expression, and an increase in serum IGFBP-1/2. Serum IGFBP-3 and -4 and tumor IGFBP-3 tumor expression were not significantly associated with dietary fat content. This suggests that altering dietary fat intake may affect tumor growth and that the IGF axis may be involved in this process.

It has been suggested that an HF diet may promote the development and progression of prostate cancer through modulation of the IGF axis (33–35). An HF diet is known to result in elevated serum insulin levels, and several recent studies have found a positive association between serum insulin levels and the development of prostate cancer and advanced prostate cancer (36, 37). Insulin is known to stimulate hepatic production of IGF-I (38, 39), while suppressing hepatic IGFBP-1 (40). As well, LF diet and exercise intervention program in men have been shown previously to reduce serum insulin and IGF-I levels and increase serum IGFBP-1 levels (33). LNCaP cells cultured in media containing 10% postintervention serum from men in this LF diet and exercise study had reduced growth in vitro and increased apoptosis relative to LNCaP cells cultured in media containing 10% preintervention subject serum, and these effects appeared to be mediated through changes in serum levels of IGF-1 and IGFBP-1 (33). In the present experiment, LAPC-4 cells cultured in media containing LF mice serum had reduced growth relative to LAPC-4 cells cultured in media containing HF mice serum. On multivariate analysis, we found that this effect was significantly related to changes in serum insulin and IGF-1 levels and was independent of tumor volume. Of interest, LeRoith’s group (41, 42) developed a liver-specific, IGF-I-deficient mouse model with a substantial (~75%) reduction in serum IGF-I. These animals have been found to be less prone to development and metastasis of colon cancer (43). These animals may prove to be extremely useful for further evaluating the role of dietary fat and IGF-1 in the development and progression of prostate cancer.

In the present experiment, Western ligand blotting showed an up-regulation of serum levels of the band at M_r 29,000 in the LF mice group compared with the HF group. Although IGFBP-1, -2, -5, and -6 all have molecular weights M_r 29,000, it is most likely that the observed band is IGFBP-1. Because the Western ligand blot was carried out using 125I-IGF-I as the probe, IGFBP-6 would be less likely because of its lower affinity for IGF-I and high affinity for IGF-II (44, 45). Furthermore, most of the IGFBP-5 in serum is cleaved into M_r 23,000 and 16,000 fragments by a serine protease, making it extremely unstable (44). IGFBP-2 is not a likely candidate either, because elevated serum IGFBP-2 is associated with a reduction in mouse body weight (46), and the mice did not lose weight in the present experiment. Close inspection of the band at M_r 29,000 in Fig. 4A reveals that it appears as a doublet with molecular weights M_r 29,000. IGFBP-1, -2, -5, and -6 all have molecular weights M_r in that range (47). The finding of increased serum IGFBP-1 in the LF group would be expected given that the LF group had lower serum insulin levels, and insulin is known to suppress hepatic production of IGFBP-1 through an insulin-response element in the IGFBP-1 gene (48–50). Therefore, the doublet band in Fig. 4 with molecular weights M_r 29,000 likely represents IGFBP-1 and not IGFBP-2.

In the present study, serum IGF-I levels were 8% lower in the LF group relative to the HF group, which did not reach statistical significance. Interestingly, Chan et al. (34) conducted a prostate cancer case control study in which they found plasma IGF-I was 7% lower among controls compared with cases, which was statistically significant. These investigators found a significant positive correlation between plasma IGF-I levels and prostate cancer risk. The results of these
investigators and others (35, 51, 52) suggest that a small decrease in plasma IGF-I could possibly lower the risk of developing prostate cancer. Repeating our experiment with a larger sample size may allow the small reduction in serum IGF-1 in the LF group to reach statistical significance.

In the present study, LAPC-4 tumor IGFBP-3 levels did not change in response to varying dietary fat content. Serum IGFBP-3 levels have been shown previously not to change in response to an LF diet and exercise intervention (33). Of interest, tumor IGFBP-2 levels (by immunostaining) were found to be significantly higher in the HF group compared with the LF group. Previous studies found IGFBP-2 immunostaining intensity and mRNA expression were significantly elevated in prostate intraepithelial neoplasia, compared with normal epithelium, and further elevated in malignant prostate cancer cells (53). In addition, Mita et al. (54) found a positive correlation between IGFBP-2 mRNA expression in prostate cancer tissue and advanced stage and more poorly differentiated tumors in men with prostate cancer. A number of groups, including our own, have also demonstrated that serum IGFBP-2 levels were elevated in prostate cancer patients relative to controls (55, 56) and in TRAMP mice with more advanced tumors (24). We have also recently demonstrated that IGFBP-2 is a potent growth factor for several prostate cancer cell lines in vitro and that this effect occurs independently of IGF-I (57). Thus, the finding that an LF diet reduced tumor IGFBP-2 suggests a possible direct mechanism for reduced tumor growth in this setting.

Although the decreased fat consumption in the LF group may have slowed tumor growth via effects on the IGF axis as described above, other mechanisms may also be responsible. The fat used in the diets in the present study was from corn oil, which is primarily composed of linoleic acid, an ω-6 polyunsaturated fatty acid. Linoleic acid has been found to exert a stimulatory effect on the growth of androgen-responsive (LNCaP) and androgen-independent (PC-3) human prostate cancer cell lines (22, 58). Membrane arachidonic acid (ω-6)
derived from linoleic acid is converted by cyclooxygenase-2 to prostaglandin E2, which has been shown to promote the growth of prostate cancer cells in tissue culture, affect tumor cell invasion, and may play a role in controlling growth and metastasis in prostate cancer (59–61). Arachidonic acid derived from linoleic acid is also metabolized by the lipoxygenase pathway to eicosanoids (leukotrienes and hydroxy derivatives of fatty acids), which have been implicated in the pathogenesis of cancer, and are believed to play important roles in tumor promotion, progression, and metastasis (62, 63). One of the metabolites of lipoxygenase-5, 5-HETE, was found in higher levels in malignant prostate tissue than adjacent benign tissue, and 5-HETE has also been shown to support the growth of androgen-dependent and -independent prostate cancer cell lines (64). Thus, the eicosanoids derived from the cyclooxygenase-2 and lipoxygenase pathways may also play a role in the observed differences in tumor growth in the LF and HF groups. The present study was specifically designed to evaluate the effects of corn oil on tumor growth and the IGF axis, but future studies need to also address the various compositions of fat in the human diet, including ω-3 polyunsaturated fatty acids, monounsaturated fatty acids, and saturated fats, which may also play an important role in the development and progression of prostate cancer.

The feeding method used in the present study successfully maintained isocaloric intake between the two groups as demonstrated by the equivalent mouse weights and equal measured caloric intake throughout the study. This was a critical element, given that previous animal studies have shown that consumption of a calorie-dense diet promotes weight gain and prostate tumor growth, and caloric restriction results in decreased tumor growth (18, 20, 21, 65). This may ultimately have implications for future trials studying fat intake and prostate cancer. Although compliance would likely not be feasible in long-term trials combining reduction of dietary fat, caloric restriction, and weight loss, trials incorporating modification of fat intake without weight loss may be more attainable. In addition, future animal feeding studies evaluating nutrients should also monitor and control caloric intake to ensure isocaloric intake between the different treatment groups.

In summary, SCID mice consuming an isocaloric LF diet had reduced growth of LAPC-4 prostate cancer xenografts, decreased serum insulin levels, and increased serum IGFBP-1 levels when compared with an HF diet. Furthermore, tumor levels of IGFBP-2 and IGF-I decreased in response to an LF diet. These data suggest that the IGF axis may play a role in the LF diet-induced reduction in prostate tumor growth. This study also suggests the potential benefits of an LF diet in the prevention and treatment of men with prostate cancer, although this will require prospective randomized trials. In addition, measurement of serum levels of insulin, IGF-I, and IGFBP-1 and tissue levels of IGF-1 and IGFBP-2 may be useful surrogate biomarkers to monitor the clinical efficacy of dietary prevention and treatment trials for prostate cancer.

**ACKNOWLEDGMENTS**

We thank Colin McClean for his expert assistance in the SCID mouse facility.

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Low-fat Diet, LAPC-4 Growth, and the IGF Axis


Clinical Cancer Research

Effect of Isocaloric Low-fat Diet on Human LAPC-4 Prostate Cancer Xenografts in Severe Combined Immunodeficient Mice and the Insulin-like Growth Factor Axis

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