Exercise Does Not Counteract the Effects of a Westernized Diet on Prostate Cancer Xenografts

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BACKGROUND. The relationships between diet, exercise, and prostate cancer (PCa) remain unclear. We have previously reported that a "Western" diet promotes PCa tumor growth in vivo. Presently, we report the effects of sustained aerobic exercise on PCa progression in animals fed a high-fat diet versus a standard diet.

METHODS. Athymic mice (n = 43) were inoculated subcutaneously with human PCa (LNCaP) cells, fed ad libitum with either a high-fat or a standard diet, and randomized into forced exercising and non-exercising groups. Body weight, tumor volume, and food consumption were recorded tri-weekly. Terminal serum samples and tumor biopsies were obtained for analysis.

RESULTS. Body weight differences were not observed between the groups over time. The high-fat-diet with exercise (HF-Ex) group showed significantly increased tumor growth rate compared to all other groups ($P < 0.0007$). Tumor growth rate of the standard diet with exercise (Std-Ex) group was reduced significantly compared to the high-fat-diet without exercise (HF-No Ex) group ($P = 0.0008$). Significant differences ($P \leq 0.012$) were observed in energy consumption (kcal) between the groups over time. Exercising mice consumed significantly more kcal than non-exercising mice, and the HF-Ex group consumed significantly more than each of the other three groups ($P < 0.0007$). The expression levels of p27 and p21 were increased in exercising animals, while AR expression was elevated in the HF-Ex group versus the Std-Ex and HF-No Ex groups.

CONCLUSIONS. Sustained aerobic exercise did not counteract the tumor-promotional effect of increased consumption of a high-fat diet, suggesting that diet is more influential in PCa progression than exercise. Combining exercise with a healthy diet reduced the rate of PCa progression in this model. This study may have implications for PCa risk reduction in humans.

KEY WORDS: **prostate cancer; exercise; high-fat diet; xenograft**

INTRODUCTION

Prostate cancer is a major public health problem [1]. Despite its high prevalence, death rates remain quite low and are decreasing. A recent retrospective study of non-metastatic prostate cancer patients reported a 3% mortality rate [2]. Nevertheless, aggressive localized prostate cancer treatments are associated with numerous side-effects and have significant impacts on quality of life [3–6]. Risk reduction using effective dietary and lifestyle interventions would be a significant achievement.

Animal cancer models provide valuable opportunities to analyze carcinogenesis and the interaction between exercise, diet, and tumor growth [7]. Studies demonstrate that exercise delays or diminishes prostate tumor growth in the SCID mouse xenograft model and transgenic mice [8–10]. Dietary factors are also important in prostate cancer progression. Previously, our group reported that diets high in refined carbohydrates are associated with increased tumor growth in murine LNCaP xenograft models of prostate cancer [11]. A recent study using a similar prostate cancer animal model reported an enhanced proliferative effect of high-fat compared to high-carbohydrate diets [12]. Exercise's inhibitory effect and a poor diet's tumor-promotional effect have also been shown in a variety of other cancers, including breast and colon [13–22].

The exercise–diet–prostate cancer relationship is largely undefined. Improved body composition (such as an increase in fat-free mass and a decrease in fat mass), alterations to the insulin axis, reduced oxidative stress, and enhanced anti-tumor immunity and reduced inflammation have been proposed to explain the widely observed benefits of exercise and diet [23–26]. We hypothesize that aerobic exercise would counteract a high-fat diet's tumor-promotional effects. This study examines the effect of sustained aerobic exercise on prostate tumor growth in mice placed on "Westernized" (high-fat) versus standard diets.

MATERIALS AND METHODS

LNCaP Xenograft Model of Prostate Cancer

The University of Toronto Animal Research Ethics Board and the Sunnybrook Research Institute provided ethical approval for this study. Forty-six male nu/nu athymic nude mice (6 weeks old; Harlan Laboratories, Canada) were maintained in a sterile, pathogen-free facility, according to institutional and Canadian Council on Animal Care guidelines. LNCaP cells (American Type Culture Collection, Rockville, USA) were cultured at 37°C in a 5% CO_2 incubator in RPMI 1640 medium (Invitrogen, Canada) supplemented with 10% fetal bovine serum (Sigma, USA), 0.3 mg/ml L-glutamine and 100 IU/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Canada). Confluent cells were trypsinized and 1.5×10^6 cells resuspended in 100 µl matrigel solution (BD Biosciences) were inoculated subcutaneously unilaterally into each mouse's flank, under inhalational (isofluorane) general anesthesia. Two weeks post-injection, 43 mice had palpable tumors. These were randomly assigned to a high-fat diet with exercise (HF-Ex; n = 10), standard diet with exercise (Std-Ex; n = 10), high-fat diet without exercise (HF-No Ex; n = 11), or standard diet without exercise (Std-No Ex; n = 12) group. Three mice lacked palpable tumors and were excluded.

Dietary and Exercise Methodology

All mice had access to food and water ad libitum. Purina Mills Test Diets (Richmond) manufactured the diets. Standard diets were 50.0% carbohydrate, 18.8% protein, 6.0% fat, and 3.8% fiber (caloric density: 3.30 kcal/g), while high-fat diets were 47.5% carbohydrate, 17.6% protein, 23.8% fat, and 4.8% fiber (caloric density: 4.73 kcal/g; Table I).

Mice were exercised 3 times/week, 45 min/day, over 8 weeks using the Forced Exercise/Walking Wheel Bed (Lafayette Instruments). Exercise intensity was gradually increased from 2.0–7.0 m/min to account for training effects. Body weight, tumor volume, and food consumption were measured tri-weekly. Tumor size (measured with Vernier callipers) was converted to tumor volume using the equation $\pi/6 \times (\text{tumor width})^2 \times \text{tumor length}$. Food consumption data were converted into energy consumption using established caloric density (kcal/g) values for the respective diets. Mice were sacrificed following the 8-week treatment period or once tumors reached 17 mm, the Canadian Council on Animal Care and Cancer Endpoint Guidelines maximum permissible tumor diameter.

TABLE I. Comparison of the Macronutrient Composition of the High-Fat and Standard Diets*

Dietary parameters	High-fat diet	Standard diet
Composition of diet, % weight		
Carbohydrates	47.5	50.0
Protein	17.6	18.8
Fat	23.8	6.0
Fiber	4.8	3.8
Energy contribution, %		
Carbohydrates	40.1	60
Protein	14.8	23
Fat	45.1	17

*These data were provided in the specification sheets of the manufacturer.

Blood and Tissue Samples

At sacrifice, terminal blood samples were collected by direct cardiac puncture. Serum was separated, aliquot, and stored at −80°C. Each group's samples were pooled for the mitogenicity and oxidative stress assays (described below). Tumors were removed, weighed, and a portion was snap frozen in liquid nitrogen. The remaining portions of the tumors were fixed in 10% v/v buffered formalin, mounted on slides, and stained with hematoxylin and eosin. Stained tumor sections were analyzed by a blinded pathologist to confirm the presence of prostate cancer.

Serum Mitogenicity Assay

LNCaP cells were plated in 96-well plates (5×10^3 cells per well) and left to adhere. After 24 h of incubation, cells were washed with PBS and treated with serum-free media for an additional 24 h to synchronize cell cycling. Cells were then treated for 72 h with appropriate media supplemented with 10% pooled serum (described above) or fetal bovine serum (control). The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) method was used to measure cell proliferation as described previously [27].

Oxidative Stress Measurement

The Quantitative Assay for 8-Hydroxy-2′ -Deoxyguanosine (8-OHdG) (Oxford Biomedical Research) was used according to the manufacturer's specifications to measure oxidative stress in the pooled serum of each group. Using a 96-well plate that was precoated with 8-OHdG, 50 µl of sample or standard (0.5, 2.0, 8.0, 20.0, 80.0, or 200.0 ng 8-OHdg/ml serum) was added to each well (except blanks), followed by 50 µl of reconstituted primary antibody. After mixing, incubation, and washing, 100 µl of reconstituted secondary antibody was added to each well, followed by the addition of 100 µl of diluted Chromogen. Stop solution (100 µl) was added after further incubation, and absorbance was read at 450 nm. Six replicates were conducted for each pooled sample group.

Analysis of Serum Insulin

The C-peptide Mouse ELISA (ALPCO Immunoassays, USA) was used according to the manufacturer's specifications. C-peptide is a commonly used surrogate marker that indirectly measures serum insulin levels. Serum C-peptide was analyzed in each individual mouse in duplicate and expressed in picomolar units (pM). Ten microliters of standards (0, 58, 220, 656, 1,337, and 2,992 pM), high and low controls, and serum samples were pipetted into a 96-well microplate pre-coated with a specific antibody for C-peptide. Working strength conjugate (100 µl) was added to each well. Following incubation and washing, 100 µl of TMB substrate was added. 100 µl of Stop Solution was added after further incubation, and absorbance was read at 450 nm.

Western Blotting

Frozen tumor tissues from each individual mouse were cut into approximately 1-mm pieces using a sterile razor blade and homogenized separately in ice-cold radio-immuno precipitation assay (RIPA) buffer with added protease and phosphatase inhibitors as previously published [11]. Protein quantification by the Bradford method was completed prior to SDS–PAGE (sodium dodecyl sulphate–poly acrylamide) gel electrophoresis. After overnight transfer, membranes were probed to investigate protein expression relating to: (1) cell cycle regulation (p21 and p27), (2) insulin axis alterations (IR and IGF1R-β), and (3) receptor expression levels (AR). All antibodies were purchased from Cell Signaling (Danvers), except for AR (Santa Cruz; Santa Cruz). Protein expression levels, relative to β-actin, were determined using image quantification software (ImageJ, US National Institute of Health). Western blot experiments were performed on each individual mouse in duplicate. Average protein expression levels were calculated for each group.

Statistical Analysis

Group by time interactions for body weight, tumor volume, and energy consumption were analyzed using repeated measures ANOVA techniques. When significant differences occurred between groups over time, pair-wise analysis of individual groups was performed. For pair-wise comparisons, a Bonferroni adjustment was applied to account for multiple testing, such that statistical significance was denoted by a P-value < 0.0125 (since comparisons were made between four groups, the standard P-value of 0.05 was divided by 4). The two-tailed Student's t-test was used to analyze C-peptide, mitogenicity, and oxidative stress levels from serum, and protein expression levels from Western blotting.

RESULTS

Significant Alterations in Body Weight Were Not Observed With Diet and Exercise

Mice bearing palpable tumors were randomly assigned to high-fat and standard diet groups, with or without exercise. Both diets were well tolerated as was adaptation to the running wheels. Some mice experienced mild tail lesions due to trauma. This did not affect their ability to perform the exercise regimen.

Although each group's overall body weight decreased during the study, repeat measures ANOVA showed no significant differences between groups over time ($P = 0.077$). Differences in terminal body weight were minor and not significant ($P > 0.07$; Fig. 1).

High-Fat Diet With Exercise Significantly Increased Tumor Volume Over Time

Tumors were measured tri-weekly. Significant tumor volume differences were observed between the four groups over time ($P < 0.001$; Fig. 2). Tumor growth rates were compared in a pair-wise manner. A significant increase in tumor volume over time was seen in the HF-Ex versus the other three groups ($P < 0.0007$). Tumor growth rate was also significantly greater in the HF-No Ex group versus the Std-Ex group ($P = 0.0008$). Although the HF-No Ex group's tumor growth rate was greater than that of the Std-No Ex group, when accounting for the Bonferroni adjustment, this difference was not significant ($P = 0.013$), nor was that between the Std-No Ex and Std-Ex groups ($P = 0.016$). At sacrifice, tumor weights and volumes were measured. The mean tumor volume was significantly greater in the HF-Ex group compared to the Std-Ex group ($P = 0.03$). Pathological analysis confirmed the presence of adenocarcinoma in all tumors. The amount of fatty deposits and signs of necrosis within the tumors did not differ between groups (data not shown). Tumor angiogenesis was not measured.

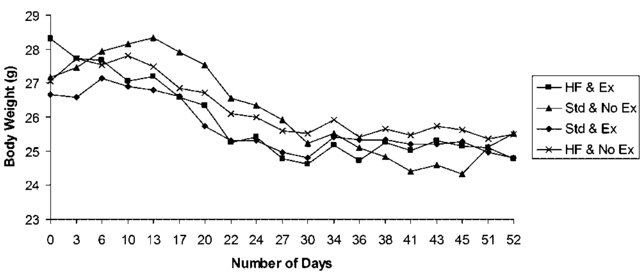

Fig. 1. Mean body weights of the four groups over time. There was an overall decrease in the body weights of the mice, but statistically significant differences were not observed either over time or at the time of sacrifice.

Exercise Significantly Increased Consumption of Both Diets

The average food consumption of each mouse was measured tri-weekly. This was converted to energy consumption per day (kcal): [grams of food $\times$ 4.73 kcal/g or 3.30 kcal/g (for high-fat and standard diets, respectively)/number of days between measurements].

Significant differences in energy consumption existed between the groups over time ($P < 0.0001$; Fig. 3). Pair-wise comparison of groups demonstrated that exercise stimulated a significant increase in energy

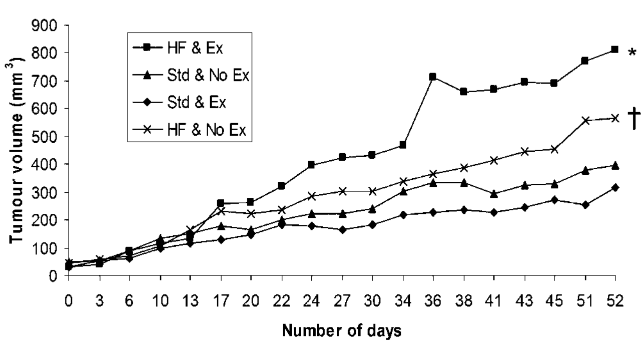

Fig. 2. Mean tumor volumes of the four groups over time. A statistically significant difference in tumor volume was observed between the groups over time ($P < 0.001$). When comparing each group individually, it was determined that a significantly greater tumor volume increase over time occurred in the HF-Ex group compared to each of the three other groups ($^{*}P < 0.0007$). A significant increase in tumor volume was also observed in the HF-No Ex group when compared to the Std-Ex group ($^{\dagger}P = 0.0008$). Significant differences in tumor volume over time were not observed between the HF-No Ex and Std-No Ex groups ($P = 0.013$) or between the Std-No Ex and Std-Ex groups ($P = 0.016$).

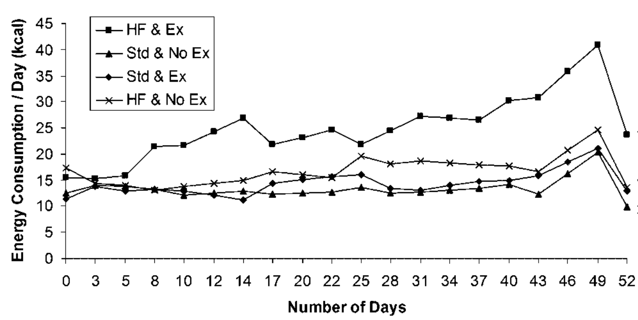

Fig. 3. Mean energy consumption per day of the four groups. Statistically significant differences in energy consumption were observed between the groups over time ($P < 0.0001$). The HF-Ex group consumed significantly more energy than each of the other three groups ($^{*}P < 0.0007$), while the Std-Ex group consumed significantly more energy than the Std-No Ex group ($^{\dagger}P = 0.0019$). This result indicates that exercise stimulated an increase in food consumption in both diet groups. The HF-No Ex group consumed significantly more energy than did the Std-No Ex group ($^{\ddagger}P = 0.012$), which follows the increased caloric density of the high-fat diet versus the standard diet. Energy consumption between the Std-Ex group and the HF-No Ex group were not significantly different.

consumption irrespective of diet. The HF-Ex group consumed significantly more energy than the other groups ($P < 0.0007$). Energy consumption was significantly greater in the Std-Ex group versus the Std-No Ex group ($P = 0.0019$), and in the HF-No Ex group versus the Std-No Ex group ($P = 0.012$), consistent with the increased caloric density of the high-fat versus the standard diet. Energy consumption of the Std-Ex and HF-No Ex groups were not significantly different.

The Influence of Diet and Exercise on the Insulin Axis

To investigate whether alterations in the insulin axis contributed to observed differences in tumor growth rate between groups, serum C-peptide levels obtained at sacrifice were analyzed. Serum C-peptide levels (pM) were greatest in the HF-Ex group (470.94 ± 130.24), followed by the Std-Ex (343.34 ± 169.28), HF-No Ex (306.93 ± 109.18), and Std-No Ex groups (208.82 ± 77.12; Fig. 4). C-peptide levels of the HF-Ex group were significantly higher than in the HF-No Ex ($P = 0.00844$) and Std-No Ex groups ($P = 0.00016$), but not the Std-Ex group ($P = 0.082$). Serum C-peptide levels were significantly higher in the HF-No Ex ($P = 0.024$) and Std-Ex groups ($P = 0.0386$) than the Std-No Ex group. C-peptide levels corresponded with the differences in the energy consumption of the groups over time, but not with the observed tumor growth rate differences.

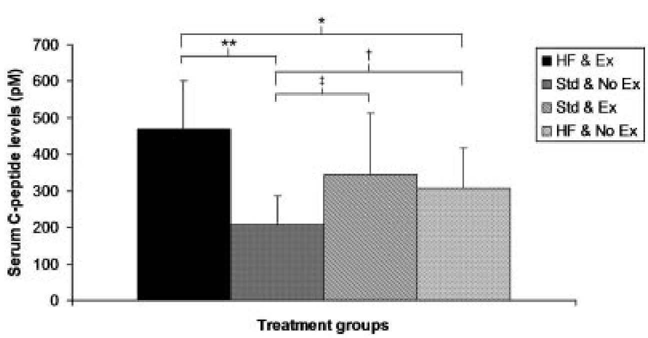

Fig. 4. Mean C-peptide levels in the serum of the four groups at the time of sacrifice. Levels in the HF-Ex group were significantly higher than in the HF-No Ex group ($^{*}P = 0.00844$) and the Std-No Ex group ($^{**}P = 0.00016$), but not the Std-Ex group ($P = 0.082$). C-peptide levels of the HF-No Ex group ($^{\dagger}P = 0.024$) and the Std-Ex group ($^{\ddagger}P = 0.0386$) were significant increased compared to the Std-No Ex group. A significant difference was not observed between the HF-No Ex and the Std-Ex groups. Serum C-peptide levels were consistent with variations in the energy consumption of the groups, but did not correspond to differences in tumor growth. Error bars represent the standard deviation within each group.

The effect on prostate cancer cell growth in vitro was assessed by the MTS cell proliferation assay. LNCaP cell proliferation did not significantly differ between groups (data not shown).

IGF1R-β and IR expression in tumor samples were also measured in duplicate by Western blot. Mean expression levels were quantified and normalized to β-actin. IGF1R-β expression levels did not differ between groups. IR expression was significantly greater in the Std-Ex versus the HF-No Ex groups ($P = 0.036$; Fig. 5a–c).

The Influence of Diet and Exercise on Oxidative Stress

To determine whether reductions in oxidative stress contributed to observed differences in tumor growth,

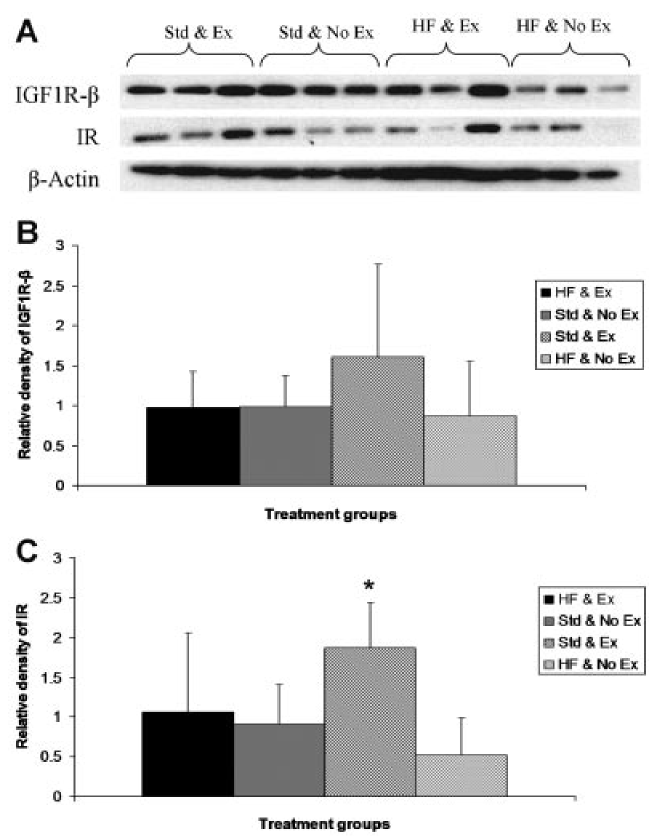

Fig. 5. **A**: A representative comparison of expression levels of IGFIR-β and IR in the tumor lysates of the four groups. β-actin was used as an internal control. **B**: Quantification of IGFIR-β expression between the groups. Significant differences were not observed, possibly due to a large amount of individual variation within each group. **C**: Quantification of IR expression between the groups. A significant increase was observed in the Std-Ex group compared to the HF-No Ex group ($^{*}P = 0.036$). The differences between the other groups were not significant. Error bars represent the standard deviation within each group.

pooled serum from each group was analyzed for 8-Hydroxy-2′ -Deoxyguanosine (8-OHdG). Six replicates of each sample were measured and mean concentration levels were calculated. Average 8-OHdG concentration in the groups ranged from 4.0 to 4.6 ng/ml. Significant differences were not observed between groups (data not shown).

The Influence of Diet and Exercise on Cell Cycle Regulation

Expression of p27 and p21 was measured in tumor lysates via Western blotting to investigate whether alterations in cell cycle regulation contributed to observed differences in tumor growth over time. Significant increases in p27 ($P = 0.0043$) and p21 ($P = 0.00164$) were seen in the HF-Ex versus the HF-No Ex groups. p21 expression was also significantly greater in the Std-Ex than the HF-No Ex groups ($P = 0.048$), and was significantly increased in the HF-Ex versus the Std-No Ex groups ($P = 0.0064$). Comparisons between other groups were not significant (Fig. 6a–c).

AR Expression in Tumor Lysates

Androgen receptor (AR) expression was also measured in tumor lysates via western blotting. AR expression was significantly greater in the HF-Ex versus the Std-Ex ($P = 0.019$) and HF-No Ex ($P = 0.016$) groups. Additional comparisons were not significant (Fig. 7a and b).

DISCUSSION

We examined the effects of diet and exercise on prostate tumor growth using a LNCaP xenograft model, focusing on whether an aerobic exercise program would counteract the effects of a high-fat diet known to be tumor-promotional [11]. A summary of our key findings are listed in Table II. Despite differences in the diets' caloric densities, significant differences in body weight were not observed over time or at sacrifice. While some studies using similar animal models have reported significant increases in body weight in mice placed on a high-fat diet compared to those fed a standard diet, others have not [11,12]. The conflicting observations between different studies may be due to differences in dietary composition and type of dietary fat used [12]. As tumors progressed, body weight declined gradually, possibly from cachexia (characterized by weight loss, anemia, and fat and muscle depletion). One study using prostate cancer xenografts found weight loss to be related to cachexia in 8 of 11 PC-3M and 5 of 6 LuCaP 35 mice [28].

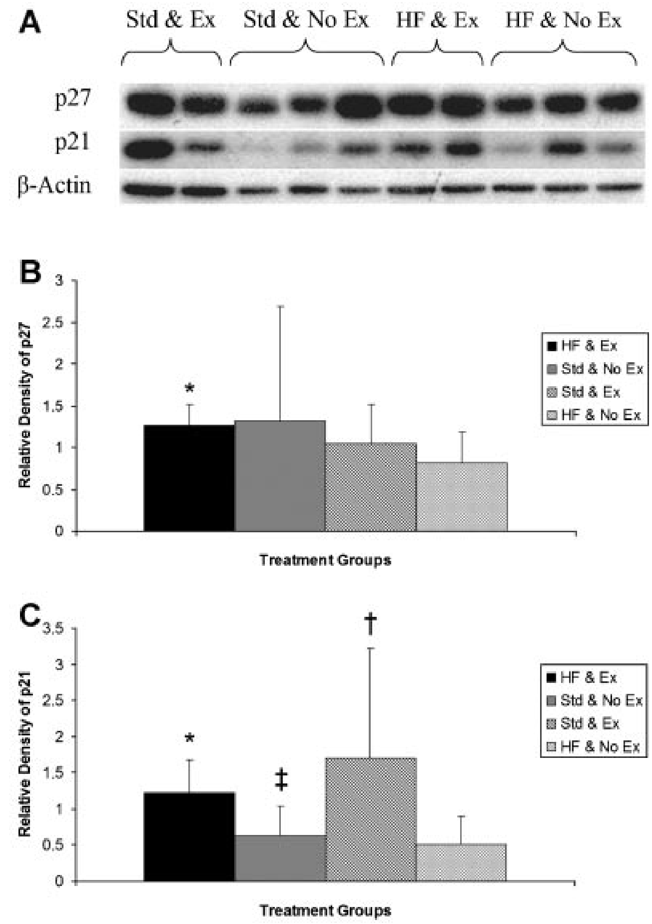

Fig. 6. **A**: A representative comparison of p27 and p21 expression in the tumor lysates of the four groups. β-actin was used as an internal control. **B**: Quantification of average p27 expression levels in the four groups. A significant increase was observed in the HF-Ex group versus the HF-No Ex group ($^*P = 0.0043$), but other comparisons were not significant. **C**: Quantification of average p21 expression levels in the four groups. p21 expression in the HF-Ex group was significantly increased compared to the HF-No Ex group ($^*P = 0.00164$), while p21 expression in the Std-Ex group was significantly greater than the HF-No Ex group ($^\dagger P = 0.048$). A significant increase in p21 expression was observed in the HF-Ex group versus the Std-No Ex group as well ($^\ddagger P = 0.0064$). Error bars represent standard deviation within each group.

Significant tumor volume differences were observed between groups over time. In standard diet-fed mice, tumor growth did not differ significantly between exercise groups. Moreover, in non-exercising mice, the high-fat diet did not significantly increase tumor growth rate compared to standard-diet fed mice. Although this comparison was not significant ($P = 0.013$; significance indicated by $P = 0.0125$), statistical analysis (applying a Bonferroni adjustment to account for multiple testing) suggests that short treatment duration and modest group sizes may have

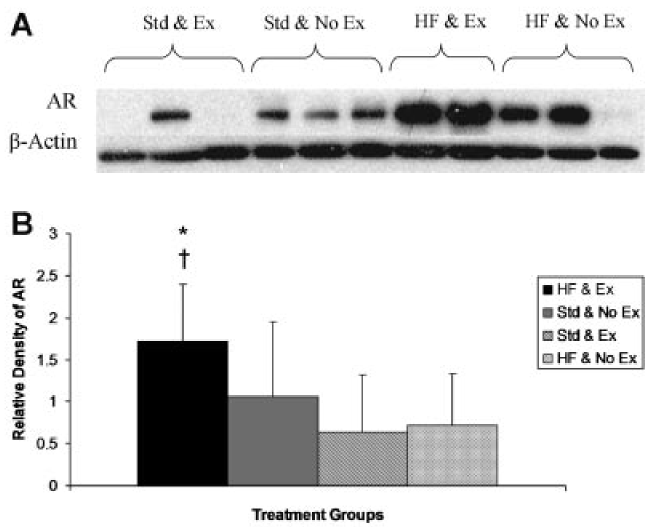

Fig. 7. **A**: A representative comparison of AR expression in the tumor lysates of the four groups. β-actin was used as an internal control. **B**: Quantification of the average AR expression in the four groups. The HF-Ex group showed a significant increase in AR expression compared to the Std-Ex group ($^{*}P = 0.019$) and compared to the HF-No Ex group ($^{\dagger}P = 0.016$). Significant differences were not observed between the other groups. Error bars represent standard deviation within each group.

been factors. The significant increase in tumor growth rate in the HF-Ex group versus the other groups is consistent with several other studies. Studies of other cancers using animal models have reported greater tumor incidence when combining exercise with high-fat diets [29–31].

Energy consumption data provided an explanation for this result. Exercise significantly increased food consumption. Furthermore, despite the high-fat diet's increased caloric density, energy consumption did not significantly differ between the HF-No Ex and Std-Ex groups. The exercise-stimulated increase in energy consumption only corresponded to increased tumor growth in high-fat diet-fed mice, suggesting that diet may have a greater impact on prostate cancer progression than exercise. Although benefits from exercise alone were unable to counteract tumor-promotional effects of the high-fat diet or cause significant tumor growth reduction in standard diet-fed mice, the significant reduction in tumor growth in the Std-Ex versus HF-No Ex groups provides further evidence that the combination of exercise with a healthy diet may slow prostate cancer progression.

At sacrifice, serum samples were obtained and tumors were excised for mechanistic analyses. We examined the effect of diet and exercise on C-peptide, markers of proliferation, IR and IGF1R-β, oxidative stress assessed by 8-Hydroxy-2′ -Deoxyguanosine (8-OHdG) levels, cell regulatory markers p27 and p21, and AR expression.

C-peptide is an independent marker of insulin biosynthesis and secretion [32]. Insulin axis modifications are thought to contribute to diet and exercise's prostate cancer benefits, while increases in IGF-1 are associated with increased cancer risk [33]. Combining low-fat diets with regular exercise has been shown to reduce insulin and IGF-1 levels while increasing IGFBP-1 and IGFBP-3 levels [23,24,34–37]. LNCaP cells treated with serum from men on a diet and exercise intervention displayed reduced growth correlating with alterations in the IGF-1 axis [24,36,37]. Lifestyle interventions have also been effective for breast and colorectal cancers [24,34]. Previously, our group reported significant increases in serum insulin and IGF-1 in mice on the high-fat diet [11], while saturated fat consumption has been associated with greater risk of advanced prostate cancer, possibly due to an IGF-1 related mechanism [25]. In the present study, serum C-peptide levels were significantly greater in the HF-Ex versus HF-No Ex and Std-No Ex groups, but not the Std-Ex group. Serum C-peptide levels were also significantly elevated in the HF-No Ex versus Std-No Ex groups, as well as in the Std-Ex versus Std-No Ex groups. These results reflect the observed energy consumption differences and highlight the importance of diet in prostate cancer progression.

In light of observed C-peptide level differences, we assessed the proliferative effect of the serum on LNCaP cells in vitro, but found no differences between groups. Due to individual variation within groups, drawing conclusions from IR and IGF1R-β expression levels in the tumors is difficult. The reported data on diet and exercise's impact on the insulin axis suggests that these alterations contribute to prostate cancer progression. Our results may be due to limitations in the study design, particularly the fact that these levels were only assessed at the end of the study.

Oxidative stress is also linked to cancer progression [38]. Despite causing transient increases in reactive oxygen species, sustained exercise has been shown to decrease oxidative stress [39–41]. High-fat diets have also been reported to increase oxidative stress by decreasing antioxidant defenses [42]. 8-Hydroxy-2′ -Deoxyguanosine (8-OHdG) is a widely-used biomarker for oxidative stress that effectively assesses the risk of carcinogenesis [43]. Significant differences in serum 8-OHdG levels were not observed between groups. Possible differences in oxidative stress may have been observed had interim samples been collected.

Cell cycle regulators are crucial for controlling tumor growth. LNCaP growth reductions following treatment with serum from men post-exercise have

TABLE II. Summary of Important Findings Between the Four Groups

Body weight	Significant differences were not observed between the four groups over time
Tumor volume	Significant differences observed between the four groups over time ($P < 0.001$) Pairwise comparisons HF-Ex: significantly greater than all three other groups ($P < 0.0007$) HF-No Ex: significantly greater than Std-Ex group ($P = 0.0008$) HF-No Ex vs. Std-No Ex: not significant ($P = 0.013$) Std-No Ex vs. Std-Ex: not significant ($P = 0.016$)
Energy consumption	Significant differences observed between the four groups over time ($P < 0.0001$) Pairwise comparisons HF-Ex: significantly greater than all three other groups ($P < 0.0007$) Std-Ex: significantly greater than Std-No Ex group ($P = 0.0019$) HF-No Ex: significantly greater than Std-No Ex group ($P = 0.012$) Std-Ex vs. HF-No Ex: not significant
C-peptide	HF-Ex: significantly greater than HF-No Ex group ($P = 0.00844$) and Std-No Ex group ($P = 0.00016$), but not Std-Ex group ($P = 0.082$) HF-No Ex: significantly greater than Std-No Ex group ($P = 0.024$) Std-Ex: significantly greater than Std-No Ex group ($P = 0.0386$) HF-No Ex vs. Std-Ex: not significant
p21	Std-Ex: significantly greater than HF-No Ex group ($P = 0.048$)
AR	HF-Ex: significantly greater than Std-Ex ($P = 0.019$) and HF-No Ex groups ($P = 0.016$)

been attributed to p21 increases [23]. Moreover, animal studies have shown that energy restriction and physical activity result in dose-dependent p27 expression increases [44,45]. In the Std-Ex versus HF-No Ex groups, p21 expression was significantly increased, corresponding to the significant tumor growth reduction observed between these groups. These results may help explain the benefit of combining healthy diets with exercise for slowing prostate cancer progression. A significant p21 increase was also observed in the HF-Ex versus HF-No Ex and Std-No Ex groups, and p27 expression in the HF-Ex versus HF-No Ex groups was significantly increased as well. As the observed increase in the HF-Ex group's cell cycle inhibition did not correlate with decreased tumor growth, these results likely reflect the group's rapid tumor growth at the beginning of the study more than the effect of the exercise program alone.

The androgen receptor (AR) is essential to normal prostate growth and development, allowing androgens to regulate cell growth and differentiation [46]. AR expression increases have been associated with disease progression, as AR promotes cell survival as a downstream target of IGF-1 and IL-6 signaling pathways [47]. A significant increase in AR expression was seen in the HF-Ex versus Std-Ex and HF-No Ex groups, consistent with tumor volume differences. Significant differences between the other groups were not observed. Further investigation into the effect of diet and exercise on AR signaling may be warranted.

While providing all animals with ad libitum access to food allowed for the recording of the exercise-stimulated increase in energy consumption, the effects of the exercise regimen alone (while controlling for diet) could not be observed. The implementation of iso-caloric diets via paired feeding would provide valuable insight into these effects. Future studies should consider this factor as well as the impact of exercise intensity and type of dietary fat.

We have demonstrated that exercise alone cannot counteract the tumor-promotional effects of an increased consumption of a high-fat diet. Importantly, this suggests that diet plays a more influential role in prostate cancer progression than does exercise. Moreover, our findings provide supporting evidence that healthy diet and regular exercise are both implicated in slowing prostate cancer progression. Alterations in cell cycle regulatory proteins and AR signaling may be involved in the relationship between diet, exercise, and prostate cancer progression.

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