ORIGINAL ARTICLE

Effect of diet and exercise intervention on the growth of prostate epithelial cells

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Epidemiological studies suggest a positive association between nutrient intake, hyperinsulinemia and risk of Benign prostatic hyperplasis (BPH). This study tests the hypothesis that a low-fat, high-fiber diet and daily exercise would lower serum insulin and reduce the growth of serum-stimulated primary prostate epithelial cells in culture. Serum samples were obtained from eight overweight men before and after the Pritikin residential, 2-week diet and exercise intervention and from seven men who were long-term followers of the low-fat, high-fiber diet and regular exercise lifestyle. The serum was used to stimulate primary prostate epithelial cells in culture. Growth was measured after 48 and 96 h and apoptosis after 96 h. At 48 h there was no significant difference in growth within the Pre, 2-week or Long-Term groups. At 96 h growth was significantly reduced in the 2-week (13%) and in the Long-Term (14%) groups compared to the Pre data. At 96 h, apoptosis was not significantly different among the three groups. Fasting insulin was reduced by 30% in the 2-week group and by 52% in the Long-Term group compared to the Pre data. Testosterone was unchanged in the 2-week group. The results of this study indicate that a low-fat, high-fiber diet and daily exercise lowers insulin and reduces growth of prostate primary epithelial cells and suggests that lifestyle may be an important factor in the development or progression of BPH. Future prospective trials should address the effects of this lifestyle modification on BPH symptomatology and progression.
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Introduction

Benign prostatic hyperplasis (BPH) is extremely common in middle-aged and older US men. According to Blom and Schroder[1] histological changes consistent with BPH are found in only 10% of Caucasian men below the age of 30 year. However, this increases to 42% for men in their 60s and to 80% for men in their 80s. BPH is characterized by the appearance of hyperplastic nodules in the periurethral region and transition zone of the prostate. As the prostate enlarges it impinges on the urethra causing lower urinary tract symptoms. Since BPH and prostate cancer (PCa) are both common in older US men, it is important to delineate any connection between the two. Studies by Phipps *et al*.[2] have demonstrated fundamental morphological differences between BPH and PCa. Using reverse transcription-PCR to investigate inflammatory and cell biological cascades, Konig *et al*.[3] found distinct differences in gene expression between

prostate tissue obtained from patients with BPH vs PCa. However, they concluded that inflammation appeared to be involved in the development of both prostate problems. Others[4,5] have also suggested that inflammation is involved in the development of BPH. The exact cause of BPH remains unknown, and according to Untergasser *et al*.[6] the only two well-established factors associated with BPH are androgens and age. Epidemiological studies report increased risk for BPH associated with high intake of calories, protein and long-chain polyunsaturated fatty acids.[7,8] Inverse associations have been reported for consumption of fruits and vegetables.[7,9]

Recently it has been suggested that hyperinsulinemia may be involved in the development of BPH and that it is an aspect of the metabolic syndrome, along with other common health problems including PCa.[10,11] We previously reported that a low-fat, high-fiber diet and daily exercise intervention reduced serum insulin, slowed the growth of serum-stimulated, androgen-dependent LNCaP, PCa cells while inducing apoptosis in the LNCaP cells.[12,13] The purpose of the present study was to test the effects of the low-fat, high-fiber diet and exercise intervention on serum-stimulated growth of primary prostate epithelial cells in culture. The results show a reduction in the growth with no change in

apoptosis of the serum-stimulated epithelial cells. These results suggest that lifestyle may play an important role in the development and progression of BPH.

Materials and methods

Subjects and intervention

This study was approved by the UCLA Committee for Protection of Research Subjects and all subjects signed consent forms to participate. The subjects of this study were eight overweight men (61.5 ± 3.2 years old) who attended the Pritikin Longevity Center 2-week Residential Program in Aventura, FL and seven men (59.1 ± 3.7 years old) who were employees of the Center and who were long-term (1–28 year) compliers to the low-fat, high-fiber diet and regular exercise lifestyle. None of the subjects had a diagnosis of PCa, all had prostate-specific antigen values ≤ 4.5 ng ml^{-1}. Information on subject BPH and flow rates was not available. During their stay at the Center, the 2-week subjects were given prepared meals that contained 10–15% of calories from fat, 15–20% of calories from protein and 65–75% of calories from carbohydrates in the form of whole grains, fruits and vegetables. Dietary fiber was approximately 40 g per 1000 kcal per day. Food was provided *ad libitum* except for animal protein that was restricted to 3.5 oz portions, mainly cold-water fish or fowl, served 3 days per week.

Upon entry into the Center the men were given a complete medical examination including a graded treadmill stress test using a modified Bruce protocol. On the basis of the test, the men were assigned a training heart rate and placed in an exercise class that met daily and consisted of warm-up and flexibility activities and 45–60 min of treadmill walking.

Twelve-hour fasting blood samples were drawn from the subjects for standard laboratory tests, including lipids, glucose and insulin that were analyzed at Quest Diagnostics. An additional blood sample was drawn into a Vacutainer containing serum separator tube clot-activating gel, separated by centrifugation and the serum frozen on dry ice and shipped to Los Angeles for the prostate studies.

Cell culture assay

Clonetics primary prostate epithelial cells were purchased from Lonza (Alendale, NJ, USA) and grown in PrEGM growth medium in 75 cm^2 flasks at 37 °C, 5% CO_2. The cells were passaged routinely at a confluence of 80% by trypsinization and the medium was changed every 2 days. For the cell culture assay, 5×10^3 cells per well were plated into 96-well plates and allowed to attach over 24-h in PrEGM medium. The following day the medium was removed and replaced with Basal Medium containing 10% fetal bovine serum (FBS) or 10% subject serum. Each subject serum sample was run in duplicate along with five FBS control samples and placed in the incubator (37 °C, 5% CO_2) for 48 or 96 h. At both 48 and 96 h, cell growth was assessed by the CellTiter 96AQ, MTS assay (Promega, Madison, WI, USA). Due to the slow growth of the cells, apoptosis was determined only after 96 h of growth. For the apoptosis assay, 10×10^3 cells per well were plated in 96-well plates in duplicate

and apoptosis was determined by Cell Death Detection ELISA Plus (Roche Applied Science, Inc., Indianapolis, IN, USA).

Serum values and insulin resistance

Serum lipids, glucose and insulin values were obtained from the subjects' medical charts. Using the fasting glucose and insulin values, insulin resistance was determined by the homeostasis model (HOMA$_{IR}$, homeostasis model assessment of insulin resistance) as (insulin (μU ml^{-1}) × glucose (mmol l^{-1})/22.5). HOMA$_{IR}$ has been shown to correlate with insulin sensitivity determined by the hyperinsulinemic-euglycemic clamp test. Serum testosterone was measured only in the Pre and 2-week samples with an ELISA from Calbiochem (Spring Valley, CA, USA).

Data analysis

The means, standard errors and statistical analyses were determined using Prism 3.0 software. The Pre and 2-week data obtained from the same subjects were analyzed with a paired *t*-test, while the Pre and Long-Term data were analyzed using an unpaired *t*-test.

Results

Serum values and insulin resistance

Table 1 gives the serum values for lipids, glucose and insulin as well as the calculated insulin resistance (HOMA$_{IR}$). Serum insulin was reduced in the 2-week postintervention samples compared to the preintervention samples. Insulin was also significantly lower in the Long-Term samples compared to the preintervention samples. Due to the fact that both serum glucose and insulin were reduced in the 2-week and Long-Term groups, the calculated insulin resistance, HOMA$_{IR}$, was also significantly lower. Both the 2-week postintervention and Long-Term body mass index data were significantly lower compared to the Pre data. All lipid values were significantly reduced in the postintervention 2-week group. Despite the fact that high-density lipoprotein (HDL) cholesterol fell with the intervention after

Table 1 Effect of diet and exercise intervention on serum factors

	Pre	2-Week	Long-Term
BMI (kg m^{-2})	33.4 ± 1.47	32.2 ± 1.49	26.2 ± 1.1
Testosterone (ng ml^{-1})	1.20 ± 0.34	1.94 ± 0.38	—
Total cholesterol (mg dl^{-1})	195.8 ± 12.9	149.3 ± 13.5	180.5 ± 15.1
LDL-cholesterol (mg dl^{-1})	111.9 ± 9.1	83.8 ± 8.7	113.3 ± 13.5
HDL-cholesterol (mg dl^{-1})	46.8 ± 1.9	40.6 ± 0.9	46.6 ± 2.0
Triglycerides (mg dl^{-1})	186.0 ± 15.1	124.4 ± 11.4	102.9 ± 20.3
Glucose (mg dl^{-1})	114.0 ± 13.7	98.4 ± 5.2	90.8 ± 4.5
Insulin (μU ml^{-1})	14.9 ± 2.0	10.4 ± 1.3	7.1 ± 1.0
HOMA$_{IR}$	4.28 ± 0.8	2.55 ± 0.37	1.62 ± 0.27

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA$_{IR}$, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.
All 2-Week values were significantly ($P < 0.05$) lower than Pre values except for testosterone.
All Long-Term values were significantly lower that Pre values except total, LDL and HDL cholesterol.

2 weeks, the ratio of total cholesterol/HDL cholesterol fell from 4.18 to 3.68. In the Long-Term group total low-density lipoprotein and HDL cholesterol were not significantly different from the Pre data; however the ratio of total cholesterol/HDL cholesterol (3.87) was lower than the Pre group. Testosterone was measured in only the Pre and 2-week samples and was unchanged by the intervention.

Epithelial cell growth and apoptosis

Primary epithelial cell growth studies were performed at both 48 and 96 h after introducing the human serum. At 48 h there was slower growth in the 2-week postintervention and Long-Term groups but the differences were not significant (Pre $103.8 \pm 3.2\%$ FBS, 2-week $95.3 \pm 5.2\%$ FBS, Long-Term $96.6 \pm 8.1\%$ FBS). However, after 96 h of growth statistically significant reductions were noted in both the 2-week postintervention and Long-Term groups (Figure 1). In the 2-week group all 8 subjects showed reductions in epithelial cell growth after the intervention.

Apoptosis studied after 96 h of growth was slightly higher in the 2-week postintervention and Long-Term groups, but the differences compared to the Pre group were not statistically significant. Optical density units of apoptosis were 0.405 ± 0.073, 0.488 ± 0.069 and 0.553 ± 0.114 for the Pre, 2-week and Long-Term groups, respectively.

Discussion

BPH is found in the majority of older men in the US and other westernized societies and often results in urinary problems. Despite the common occurrence of BPH, the underlying cause remains unknown.

According to Untergasser et al.,[6] the only two factors clearly associated with BPH are age and androgens. Age itself cannot be the mechanism and as men age androgens decline significantly, thus questioning the involvement of these as possible risk factors in the etiology of BPH. The results of the present study indicate that lifestyle that is, diet and exercise, is an important factor that supports the epidemiological data reporting that BPH is related to the intake of calories, protein and polyunsaturated fatty acids and inversely to the intake of fruits and vegetables.[7–9]

In 1998, Hammarsten et al.[10] reported that BPH patients share the same metabolic abnormalities of defective insulin-mediated glucose uptake and secondary hyperinsulinemia as patients with the metabolic syndrome, and suggested that BPH is another facet of the metabolic syndrome. Despite the fact that their patients with BPH had many of the metabolic abnormalities of the metabolic syndrome, they suggested that hyperinsulinemia might be a causal factor in the development of BPH. In a follow-up study they confirmed that hyperinsulinemia is in deed a risk factor for BPH.[14] In a case–control study, Nandeesha et al.[15] also reported that BPH was associated with metabolic syndrome factors. They too emphasized the fact that hyperinsulinemia and insulin resistance were independent risk factors for BPH despite the presence of other metabolic abnormalities. In an epidemiological study, Dahle et al.[16] reported
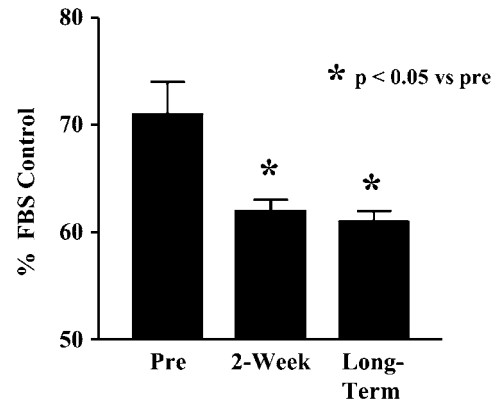

Figure 1 Effect of diet and exercise intervention on serum-stimulated growth (96 h) of primary prostate epithelial cells. Growth was significantly reduced in both the 2-week and Long-Term samples.

that a higher waist-to-hip ratio and higher serum insulin increased the risk for BPH. Ozden et al.[17] studied 78 patients with BPH and found that those with the metabolic syndrome vs those without the syndrome had a significantly greater growth rate for both the whole prostate and especially the transitional zone. These data collectively suggest that hyperinsulinemia is an important factor in the development of BPH. However, to our knowledge no one has demonstrated a direct effect of insulin on normal prostate tissue. A previous study from our laboratory[18] reported that insulin does stimulate the growth of the human LNCaP PCa cell line, and Polychronakos et al.[19] reported that insulin stimulated the growth of rat PA-III adenocarcinoma cells.

If hyperinsulinemia is indeed an important factor in the development of BPH, lifestyle is an important underlying factor as we have previously reported that a low-fat, high-fiber diet combined with daily exercise lowers serum insulin by 30–40%.[13,20] The importance of daily exercise should be emphasized as exercise has an insulin-like effect to increase glucose transport and reduce serum insulin. In the present study just 2 weeks of diet and exercise intervention improved insulin resistance, lowered fasting insulin by 30% and reduced the growth of cultured prostate epithelial cells by 13% over 4 days of growth. This reduction was sustained in the Long-Term diet and exercised group. Is the effect of insulin on prostate tissue a direct or indirect effect? Insulin is a well-known anabolic hormone and may have a direct effect or may be acting through multiple indirect effects. In our studies with PCa cell lines we found that insulin had a direct growth-stimulating effect, but other factors related to the reduction in insulin in response to diet and exercise appeared to be more important[13,18,21]

As testosterone is a recognized factor associated with BPH, we measured serum levels and found them to be low but unchanged in the 2-week samples. However, in men with hyperinsulinemia, free testosterone may be higher due to suppression of sex hormone-binding globulin (SHBG) production by the liver.[22] Reducing insulin by diet and exercise increases SHBG and lowers free testosterone even while total testosterone remains unchanged.[12,20] In addition to suppressing SHBG

production by the liver, insulin also increases production of insulin-like growth factor-I (IGF-I) while suppressing the production of IGF-binding protein-1 (IGFBP-1) to increase free IGF-I.[23] IGF-I is a peptide growth factor and is known to play a pivotal role in regulating cell growth, differentiation and apoptosis.[24] IGF-I has been shown to stimulate the growth of human primary cultures of prostate epithelial and stromal cells.[25,26] IGF-I stimulates the growth of LNCaP PCa cells and inhibits apoptosis.[21] The same diet and exercise intervention used in this study reduced serum IGF-I and increased IGFBP-1; these changes reduced growth and induced apoptosis in serum-stimulated LNCaP cells.[12,13]

Another serum factor that might be important in the development of BPH and the response to diet and exercise is estrogen. In a review, Thomas and Keenen[27] concluded that estrogens exert a synergistic effect with androgens on the growth of both epithelial and stromal cells of the prostate. We previously reported that the low-fat, high-fiber diet and exercise intervention reduced estradiol in men by 50%.[28]

In conclusion, the results from the present study suggest that lifestyle that is, diet and exercise may potentially impact on the development or progression of BPH by reducing the growth of prostate epithelial cells. The low-fat, high-fiber diet combined with daily exercise reduced the growth of serum-stimulated prostate epithelial cells while apoptosis was unchanged. While BPH and PCa are common in older men, especially in the westernized countries, they generally develop in different areas of the prostate and the underlying etiologies are still unknown.[29] Insulin has been suggested to be an important factor in both BPH and PCa and both have been suggested to be additional aspects of the metabolic syndrome.[10,11] Future prospective trials should address the effects of this lifestyle modification on BPH symptomatology and progression.

Acknowledgements

References

1 Blom JH, Schroder FH. Epidemiology and natural course of benign prostatic hyperplasia. *Urologe A* 1992; **31**: 129–134.
2 Phipps S, Yang TH, Habib FK, Reuben RL, McNeill SA. Measurement of tissue mechanical characteristics to distinguish between benign and malignant prostatic disease. *Urology* 2005; **66**: 447–450.
3 Konig JE, Senge T, Allhoff EP, König W. Analysis of the inflammatory network in benign prostate hyperplasia and prostate cancer. *Prostate* 2004; **58**: 121–129.
4 Kramer G, Mitteregger D, Marberger M. Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? *Eur Urol* 2007; **51**: 1202–1216.
5 Sciarra A, Di Silverio F, Salciccia S, Autran Gomez AM, Gentilucci A, Gentile V. Inflammation and chronic prostatic diseases: evidence for a link? *Eur Urol* 2007; **52**: 964–972.
6 Untergasser G, Madersbacher S, Berger P. Benign prostatic hyperplasia: age-related tissue-remodeling. *Exp Gerontol* 2005; **40**: 121–128.
7 Bravi F, Bosetti C, Dal Maso L, Talamini R, Montella M, Negri E et al. Food groups and risk of benign prostatic hyperplasia. *Urology* 2006; **67**: 73–79.
8 Suzuki S, Platz EA, Kawachi I, Willett WC, Giovannucci E. Intakes of energy and macronutrients and the risk of benign prostatic hyperplasia. *Am J Clin Nutr* 2002; **75**: 689–697.
9 Rohrmann S, Giovannucci E, Willett WC, Platz EA. Fruit and vegetable consumption, intake of micronutrients, and benign prostatic hyperplasia in US men. *Am J Clin Nutr* 2007; **85**: 523–529.
10 Hammarsten J, Hogstedt B, Holthuis N, Mellström D. Components of the metabolic syndrome-risk factors for the development of benign prostatic hyperplasia. *Prostate Cancer Prostatic Dis* 1998; **1**: 157–162.
11 Barnard RJ, Aronson WJ, Tymchuk CN, Ngo TH. Prostate cancer: another aspect of the insulin-resistance syndrome? *Obes Rev* 2002; **3**: 303–308.
12 Tymchuk CN, Barnard RJ, Heber D, Aronson WJ. Evidence of an inhibitory effect of diet and exercise on prostate cancer cell growth. *J Urol* 2001; **166**: 1185–1189.
13 Ngo TH, Barnard RJ, Tymchuk CN, Cohen P, Aronson WJ. Effect of diet and exercise on serum insulin, IGF-I, and IGFBP-1 levels and growth of LNCaP cells in vitro (United States). *Cancer Causes Control* 2002; **13**: 929–935.
14 Hammarsten J, Hogstedt B. Hyperinsulinaemia as a risk factor for developing benign prostatic hyperplasia. *Eur Urol* 2001; **39**: 151–158.
15 Nandeesha H, Koner BC, Dorairajan LN, Sen SK. Hyperinsulinemia and dyslipidemia in non-diabetic benign prostatic hyperplasia. *Clin Chim Acta* 2006; **370**: 89–93.
16 Dahle SE, Chokkalingam AP, Gao YT, Deng J, Stanczyk FZ, Hsing AW. Body size and serum levels of insulin and leptin in relation to the risk of benign prostatic hyperplasia. *J Urol* 2002; **168**: 599–604.
17 Ozden C, Ozdal OL, Urgancioglu G, Koyuncu H, Gokkaya S, Memis A. The correlation between metabolic syndrome and prostatic growth in patients with benign prostatic hyperplasia. *Eur Urol* 2007; **51**: 199–203.
18 Tymchuk CN, Barnard RJ, Ngo TH, Aronson WJ. The role of testosterone, estradiol, and insulin in diet and exercise-induced reductions in serum-stimulated prostate cancer cell growth in vitro. *Nutr Cancer* 2002; **42**: 112–116.
19 Polychronakos CJU, Lehoux JG, Koutsilieris M. Mitogenic effects of insulin and insulin-like growth factors on PA0III rat prostate adenocarcinoma cells: characterization of the receptors involved. *Prostate* 1991; **19**: 313–321.
20 Tymchuk CN, Tessler SB, Aronson WJ, Barnard RJ. Effects of diet and exercise on insulin, sex hormone-binding globulin, and prostate-specific antigen. *Nutr Cancer* 1998; **31**: 127–131.
21 Ngo TH, Barnard RJ, Leung PS, Cohen P, Aronson WJ. Insulin-like growth factor I (IGF-I) and IGF binding protein-1 (IGFBP-1) modulates prostate cancer cell growth and apoptosis: a possible mediators for the effects of diet and exercise on cancer cell survival. *Endocrinology* 2003; **144**: 2319–2324.
22 Plymate SR, Matej LA, Jones RE, Friedl KE. Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. *J Clin Endocrinol Metab* 1988; **67**: 460–464.
23 Barnard RJ, Aronson WJ. Preclinical models relevant to diet, exercise, and cancer risk. *Recent Results Cancer Res* 2005; **166**: 47–61.
24 Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. *J Natl Cancer Inst* 2000; **92**: 1472–1489.
25 Cohen P, Peehl DM, Lamson G, Rosenfeld RG. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins in primary cultures of prostate epithelial cells. *J Clin Endocrinol Metab* 1991; **73**: 401–407.

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26 Grant ES, Ross MB, Ballard S, Naylor A, Habib FK. The insulin-like growth factor type I receptor stimulates growth and suppresses apoptosis in prostatic stromal cells. *J Clin Endocrinol Metab* 1998; **83**: 3252–3257.

27 Thomas JA, Keenan EJ. Effects of estrogens on the prostate. *J Androl* 1994; **15**: 97–99.

28 Rosenthal MB, Barnard RJ, Rose DP, Inkeles S, Hall J, Pritikin N. Effects of a high-complex-carbohydrate, low-fat, low-cholesterol diet on levels of serum lipids and estradiol. *Am J Med* 1985; **78**: 23–27.

29 Erbersdobler A, Augustin H, Schlomm T, Henke RP. Prostate cancers in the transition zone: Part 1; pathological aspects. *BJU Int* 2004; **94**: 1221–1225.