Increased Cell Proliferation and Contractility of Prostate in Insulin Resistant Rats: Linking Hyperinsulinemia With Benign Prostate Hyperplasia

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BACKGROUND. Obesity, dyslipidemia, Hyperinsulinemia, and insulin resistance (IR) are key features of metabolic syndrome and are considered as risk factors for benign prostatic hyperplasia (BPH) as well as type 2 diabetes. The present study was aimed to determine whether or not IR associated hyperinsulinemia contributes to the BPH.

METHODS. Sprague–Dawley rats (9 weeks) were used in the study. Rats were kept on high fat diet (HFD) for the induction of hyperinsulinemia while hypoinsulinemia was induced by streptozotocin. Effect of HFD feeding on the testosterone-induced prostatic growth was evaluated. Pioglitazone (PG, 20 mg/kg) was used for the reversal of compensatory hyperinsulinemia and to examine the subsequent effect on the prostatic growth.

RESULTS. Prostatic enlargement was observed in the HFD-fed rats. Significant increase in the cell proliferation markers confirmed the occurrence of cellular hyperplasia in the prostate of hyperinsulinemic rat. Enhanced $\alpha$-adrenoceptor mediated contraction in the prostate of HFD-fed rats indicates augmented contractility of the gland. Higher level of phosphorylated-ERK suggests enhanced MEK/ERK signaling. HFD feeding has not led to change in the plasma testosterone level. However, testosterone treatment further augmented the prostatic growth in HFD-fed rats. PG treatment led to improved insulin sensitivity, decreased plasma insulin level and prostate weight, indicating the role of compensatory hyperinsulinemia in the prostate growth.

CONCLUSIONS. The present investigation reports that HFD-feeding induced hyperinsulinemic condition leads to increased cellular proliferation, enhanced $\alpha$-adrenoceptor mediated contraction, and enlargement of the prostate in rats. Prostate 70: 79–89, 2010.

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KEY WORDS: high fat diet; hyperinsulinemia; prostate; insulin resistance; streptozotocin; pioglitazone

INTRODUCTION

Prostatic hyperplasia is a highly prevalent condition of the prostate in aging men, characterized by augmented cell proliferation [1]. The major factors involved in prostatic hyperplasia is an increase in the smooth muscle tone and enlargement of the gland [2]. Prostatic hyperplasia is a highly prevalent disease in men and a number of reports indicate the incidence of 90% in the ninth decade of life [3,4]. The etiology of prostatic hyperplasia is multi-factorial, and its development and differentiation is affected by genetic [5],

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nutritional [6], and hormonal factors [7]. Several etiologic factors have been proposed for the development of prostatic hyperplasia and especially steroidal hormones are considered to be associated with the pathogenesis of prostatic hyperplasia. However, the precise mechanism of its pathophysiology is far from complete understanding. Obesity, insulin resistance (IR), dyslipidemia, and hyperinsulinemia are the risk factors for prostatic hyperplasia [8] as well as type 2 diabetes [9]. Several epidemiological reports indicate parallel increase in the incidence of type 2 diabetes and benign prostatic hyperplasia (BPH) [3,10,11]. However, the possibility of IR associated hyperinsulinemia as a link between these two pathological conditions has not been much explored. Recently Hammarsten et al. [12], has reported insulin and estradiol as an independent risk factor for BPH. Further, Escobar et al. [13], has reported that dietary fatty acids affect the prostatic growth. In experimental studies, streptozotocin (STZ)-treated hypoinsulinemic rats demonstrated regression of the prostate gland and other organs of the genitourinary tract [14]. However, genetically diabetic rats with similar degree of hyperglycemia have little effect on the size of prostate gland [15]. IR associated hyperinsulinemic condition is known to stimulate sympathetic nervous system [16,17] and can affect the dynamic component of the prostate gland. Using these information’s we postulated that hyperinsulinemic condition can promote growth as well as contractility of the prostate gland. Keeping the above facts in mind, we undertook the present investigation to understand the involvement of diet-induced hyperinsulinemia in the pathogenesis of prostatic hyperplasia. Here we report that HFD-feeding induced hyperinsulinemic condition leads to cellular hyperplasia, increased cell proliferation, enhanced α-adrenoceptor mediated contractility, up-regulation of MEK/ERK signaling, and enlargement of the prostate gland.

**MATERIALS AND METHODS**

**Animals**

All the animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) and animals were used according to the CPCSEA (Committee for the Purpose of Control and Supervision of Experimentation on Animals) guidelines. Experiments were performed on male Sprague–Dawley (SD) rats (9 weeks, 190 ± 10 g). Animals were procured from Institute’s Central Animal Facility (CAF) and kept at controlled environmental conditions with room temperature (22 ± 2°C), humidity (50 ± 10%), and light (0600–1800 hr). Rats were allowed to access the food and water ad libitum and acclimatized for 1 week in the experimental conditions prior to the start of experiment. In total 120 SD rats were used in the study. All the animals were killed by cervical dislocation.

**Chemicals and Dose Administration**

Streptozotocin (STZ), alloxan (ALX), insulin (bovine), phenylephrine (PE), clonidine (CL), and D-glucose were procured from Sigma–Aldrich (USA). Pioglitazone was kindly gifted by Ranbaxy Research Laboratories, India. All the primary and secondary antibodies were procured from Santa Cruz Biotechnology, Inc. (USA) until unless mentioned. Ki67 antibody was procured from Genexxbio (India). Commercially available human insulin (Human Mixtard, 30% soluble insulin and 70% isophane insulin, r-DNA origin, t1/2 ≥ 5 hr, Torrent Pharmaceutical, Ltd) was procured from the local medicine dealer and used in the study. Cholesterol and DL-methionine was procured from HiMedia (Mumbai, India). STZ (50 mg/kg) was dissolved in freshly prepared sodium citrate buffer (pH 4.4), and administered immediately after preparation. Concurrent controls received normal saline. For IPGTT and ITT, D-glucose and bovine insulin was dissolved in distilled water to obtain desired concentration and administered immediately after preparation.

**Experimental Design**

Rats were randomly selected and assigned to NPD, HFD, or STZ group. IR and hyperinsulinemia was induced by HFD-feeding while hypoinsulinemia was induced by STZ treatment. Small portion of all the tissues were fixed in formalin for immunohistochemical and histological examination while a fraction was kept for tissue macromolecules content analysis and remaining tissues were stored at −80°C. Phenylephrine and clonidine mediated contraction of the ventral lobe of the prostate gland of NPD/HFD-fed rats were recorded immediately after isolation of tissue. Few animals were kept on HFD for 36 weeks to confirm the prostatic enlargement. To check the relative influence of insulin and testosterone on the prostatic growth, testosterone (1, 3, and 10 mg/kg) was administered to the NPD/HFD-fed rats for 1 week. Further, to substantiate the role of insulin in the prostatic growth, a separate study has been performed with insulin sensitizer PG. The study was aimed to check whether or not reversal of hyperinsulinemia by PG results in the reversal of prostatic hyperplasia. PG was administered to NPD/HFD-fed rats from 9th to 12th week at the dose of 20 mg/kg per orally.

**Diet for the Development of Experimental Insulin Resistant Model**

IR was induced in rats by feeding HFD (5.3 kcal g⁻¹, carbohydrate 17%, protein 25%, fat 58% kcal), while the
control rats were fed with NPD (3.8 kcal g⁻¹, carbohydrate 67%, protein 21%, fat 12% kcal) for a period of 3–12 weeks. The NPD used to feed the animal was standard rodent chow (Pranaw Agro Industries, New Delhi, India). The detailed methodology for the HFD preparation has already been described by Srinivasan et al. [18]. In brief, the content of HFD diet includes NPD-powder (36.5%), lard (31%), casein (25%), vitamin–mineral mix powder (6%), cholesterol (1%), DL-methionine (0.3%), Yee-sac powder (0.1%), and sodium chloride (0.1%).

**Biochemical Parameters**

The blood samples (approximately 0.8 ml) were collected from orbital plexus of rats under light ether anesthesia in heparinized microcentrifuge tubes. The plasma was separated by centrifugation (5,000 rpm, 5 min) and analyzed for glucose (GOD-POD), triglycerides (GPO-POD), total cholesterol (CHOD-POD), and HDL-cholesterol (CHOD-POD) using commercially available spectrophotometric kits (ACCUREX Biomedical Pvt Ltd). The remaining plasma samples were then stored at -20°C till the insulin and testosterone estimation was done. Plasma insulin was estimated by rat/mouse insulin ELISA kit (rat/mouse insulin ELISA kit, Linco Research, USA) as per the manufacturer's instruction. Testosterone level was estimated using a commercially available kit (DIAME-TRA, Testosterone, DK0002, 06034 Foligno (PG), Italy) as per the manufacturer’s instruction.

**TUNEL Assay**

The in situ cell death was detected by TUNEL assay kit (Calbiochem, USA) according to the manufacturer’s instruction.

**Glucose and Insulin Tolerance Tests**

Animals were kept on 6 hr fasting for glucose and insulin tolerance test (ITT) and a basal sample was taken, followed by intraperitoneal injection of d-glucose (1,000 mg/kg) or bovine insulin (0.5 IU/kg). Blood sample were collected at 5, 15, 30, 60, 90, and 120 min and plasma glucose concentration was determined to assess impairment in the glucose tolerance and glucose disappearance with time.

**In Vitro Isometric Tension Experiments**

Rats were sacrificed by cervical dislocation under ether anesthesia and prostate gland was quickly isolated and kept in the ice-cold Krebs–Henseleit buffer (KHB). Glandular prostatic tissue is then cut into two parts and tissue was suspended in the water-jacketed organ bath, filled with 10 ml of oxygenated KHB (37°C). A resting tension of 1 g was maintained to the prostate tissue and allowed to equilibrate for 60 min. Isometric tension responses were measured on a physiograph chart recorder (BioDevices, Ambala, India) using an isometric force transducer (T-305, BioDevices). A cumulative concentration response curve of phenylephrine and clonidine was recorded. Data are expressed as percent control response of phenylephrine (10⁻⁵ M).

**Immunoblotting and Immunoprecipitation**

Protein samples were resolved on 10–12% SDS–PAGE, transferred to PVDF membrane, and analyzed with antibodies against insulin receptor (α-subunit), PCNA, MEK, ERK, p-ERK, and β-actin. The antigen–primary antibody complexes were incubated with HRP conjugated secondary antibodies and visualized by Western blot luminal reagent (Santa Cruz Biotechnology, Inc.). Image was captured by ImageQuant-350 (Ver. 1.0.2). The protein quantification was done with ImageQuant TL (GE Healthcare, UK) software and intensity values were normalized to actin. Tissue lysate was precleaned by incubating with irrelevant primary antibody and Protein A/G plus-Agarose for 90 min. The pellet obtained after centrifugation is discarded and supernatant was used for the further processing. Precleaned tissue lysates (500 μg) were incubated with anti-insulin receptor (α-subunit) antibody for 16 hr at 4°C. Subsequently, the immune-complex was precipitated with Protein A/G plus-Agarose (Santa Cruz Biotechnology, Inc.) for 6 hr at 4°C. The immunoprecipitate was washed three times with lysis buffer. The protein sample was resolved on SDS–PAGE and immunoblotted as described above.

**Immunohistochemistry**

Prostatic sections were deparaffinised with xylene, followed by antigen retrieval by heating in citrate buffer (10 mM). The following rabbit polyclonal primary antibodies were used: PCNA, 1:125; Ki67, 1:100; insulin receptor (α-subunit), 1:100; MEK, 1:100; ERK, 1:100; p-ERK, 1:50. Polyclonal biotinylated goat anti-rabbit secondary antibody and streptavidin peroxidase (STV-HRP) system was used to amplify the signals, followed by detection with dianinobenzidine (DAB) as a chromogen. Slides were counterstained with hematoxylin, dehydrated with alcohols and xylene, and mounted in DPX.
Statistical Analysis

Statistical analysis was performed using Jandel SigmaStat statistical software. Significance of difference between two groups was evaluated using Student’s t-test. For multiple comparisons, ANOVA was used and post hoc analysis was performed with Tukey’s test. Results were considered significant if $P$ values were $\leq 0.05$.

RESULTS

HFD-Feeding Induced Hyperinsulinemia in Rats

HFD feeding has led to the induction of mild hyperglycemia and hyperinsulinemia in HFD-fed rats as compared to NPD-fed rats (Fig. 1A,B). Further, development of IR was determined by intraperitoneal glucose tolerance test (IPGTT) and ITT (Fig. 1C–F). Dietary manipulation has not led to change in the plasma testosterone level (Fig. 1G). As expected, significant increase in the adiposity index of HFD-fed rats was observed as compared to NPD-fed rats (Fig. 1H). The increased adiposity of HFD-fed rats was further confirmed by histological examination (Fig. 1I).

Effect of Diet Induced Hyperinsulinemia on the Prostate Gland

Significant increase or decrease in the weight of prostate gland of HFD-fed (hyperinsulinemic) or STZ-treated (hypoinsulinemic) rats was observed as compared to NPD-fed rats (Fig. 2D). Further, to confirm the results observed in 12-week study, effect of long-term (36 weeks) dietary manipulation on the prostate gland was evaluated and as expected, significant increase in the weight of ventral prostate was observed in HFD-fed rats as compared to NPD-fed rats (Fig. 2E). Positive relationship has been observed between plasma insulin level and prostatic growth in rats (Fig. 2F). Further, to investigate the per se effect of insulin, exogenous long-acting insulin was administered to the NPD-fed rats and marginal increase in the absolute prostate weight was observed. However, the regression of prostate gland in STZ-treated hypoinsulinemic rats

Fig. 1. Insulin resistance and compensatory hyperinsulinemia in HFD-fed rats. A, B: HFD feeding leads to increase in the plasma glucose (A) and insulin (B) level ($n = 6–12$). C: HFD induces glucose intolerance in Sprague–Dawley (SD) rat as determined by IPGTT ($n = 5$). D: HFD feeding leads to significant increase in the area under curve (AUC) of the IPGTT in (C). E: HFD feeding led to impaired insulin sensitivity in SD rat as determined by ITT ($n = 5$). F: HFD feeding leads to significant increase in AUC of the ITT in (E). G: HFD feeding does not alter the plasma testosterone level. H: HFD feeding leads to significant increase in the adiposity index ($n = 5$). I: Representative photomicrograph showing fat deposition in the liver and increased size of adipocytes in brown adipose tissue (BAT) and white adipose tissue (WAT). Bar length indicates the magnification. All the values are mean $\pm$ SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus NPD. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
was significantly restored by chronic administration of long-acting exogenous insulin (Fig. 2G).

**Effect of Diet Induced Hyperinsulinemia on the α-Adrenoceptor Mediated Contraction of the Prostate Tissue**

Effect of persistent hyperinsulinemia was evaluated on the contractility of the prostate gland. α-Adrenoceptor agonist phenylephrine and clonidine mediated contractile responses were recorded in a concentration-dependent manner and significant increase in the contraction ($E_{\text{max}}$) without appreciable change in pD$_2$ value was observed in the prostate of HFD-fed rats as compared to NPD-fed rats (Fig. 2H,I).

**Cellular Hyperplasia and Enhanced Cell Proliferation in the Hyperinsulinemic Rats**

Nucleic acid and protein content analysis suggests the occurrence of cellular hyperplasia in the prostate gland (Fig. 3A,B). Significant increase in the proliferating cell nuclear antigen (PCNA) and Ki67 level in the prostate of HFD-fed hyperinsulinemic rat confirms enhanced proliferation of prostatic cells (Fig. 3D–F). Slight decrease in the TUNEL positive cells was observed in the prostate of HFD-fed rats as compared to NPD-fed rats. However, as expected, significant increase in the frequency of TUNEL positive cells was observed in the STZ-treated hypoinsulinemic rats (Fig. 3C). Histological examination of the prostate...
isolated from HFD-fed rats revealed higher infolding of the epithelial layer in prostatic follicles, however, no appreciable difference in the cellular height and morphology was observed between luminal secretory cells (LSCs) of NPD- and HFD-fed rats (Fig. 3F).

**ERK Signaling in the Prostate of Hyperinsulinemic Rats**

Significant increase in the phosphorylated-ERK was observed in the prostate of hyperinsulinemic rats, indicating the upregulation of MEK/ERK signaling (Fig. 3G,H).

**Effect of Dietary Manipulation on the Testosterone Induced Prostatic Growth**

Testosterone treatment augmented prostatic growth in both NPD- and HFD-fed rats in a dose-dependent manner. In HFD-fed rats, leftward shift in the testosterone-prostatic growth curve was observed as compared to the NPD-fed group, indicating the per se effect of
dietary manipulation on the prostate (Fig. 4A). The higher percentage of Ki67 positive LSCs in the prostate of testosterone-treated rats as compared to their respective diet (HFD/NPD) matched control further confirms the above observations (Fig. 4B). Higher infolding of prostate epithelial layer was observed in the hyperinsulinemic rats and the degree of infolding was further increased with testosterone treatment (Fig. 4C).

**Effect of Pioglitazone on Hyperinsulinemia Associated Prostatic Hyperplasia**

Significant increase in the weight of prostate gland of HFD-fed rats was observed as compared to NPD-fed rats, reconfirming the earlier results of dietary manipulation on the prostate. However, in HFD + PG group significant decrease in the prostate weight was observed as compared to HFD-fed rats (Fig. 5A). Smaller prostate in HFD + PG group as compared to the diet-matched control can be attributed to decreased plasma insulin level (Fig. 5B). Lower PCNA level was observed in HFD + PG group as compared to diet-matched control (Fig. 5F).

**DISCUSSION**

Structural and functional development of prostate is a very complex phenomenon and is sensitive to the overall endocrine environment. Prostatic development is primarily governed by androgens, estrogen, and mesenchymal–epithelial interactions [7]. In the present investigation, we report that HFD feeding leads to cellular hyperplasia, enhanced a1-adrenoceptor mediated contraction and overall enlargement of the prostate gland in rats. Recent epidemiological reports indicate positive correlation between dyslipidemia, obesity, and hyperinsulinemia with PH [8,11]. These factors are well-established forerunners of type II diabetes [19,20]. HFD feeding led to the development of IR, compensatory hyperinsulinemia, and enlargement of the prostate gland in experimental animals. Prostatic enlargement in HFD-fed rats was supported by the tissue macromolecules analysis. On the other hand, significant decrease in the weight of prostate gland as well as LSCs size was observed in the STZ-treated (hypoinsulinemic) rats. The enlargement of the
prostate gland in the HFD-fed hyperinsulinemic rats can be attributed to; (i) enhanced mitogenic activity of insulin (hyperinsulinemia); (ii) altered steroidal hormonal activity; (iii) increased sympathetic tone or to the (iv) perturbed endocrine microenvironment of the prostate. However, the regression of prostate gland in the STZ-treated rats can be attributed to decrease in the plasma insulin and testosterone level and increased expression of transforming growth factor-β (TGF-β) family proteins [14]. LSCs require testosterone for their survival, development, and differentiation [21]. Irrespective of the β-cell toxin (STZ or alloxan), decrease in prostatic weight was observed as a consequence of hypoinsulinemia (data not shown). Yono et al. [15] reported the higher prostatic weight in genetically diabetic rats as compared to STZ-treated hypoinsulinemic rats due to difference in the level of insulin. These findings indicate the critical role of insulin in the development and differentiation of prostate gland. Further, enhanced cell proliferation in the prostate of hyperinsulinemic rats was confirmed by PCNA and Ki67 immunostaining. Higher level of PCNA suggests increased cellular proliferation in the prostate of HFD-fed hyperinsulinemic rats.

HFD feeding is known to stimulate sympathetic nervous system [22]. Hyperinsulinemia and mild hyperglycemia is sensed by the ventromedial hypothalamus which ultimately causes stimulation of the sympathetic nervous system [23]. Rahman et al. [24] reported increased immunostaining for α-adrenergic receptor in the prostate of hyperlipidemic rats. Recently Silva et al. [25] emphasized the role of autonomic nervous system in the prostatic growth. HFD feeding led to increased α₁- and α₂-adrenoceptor-mediated contractility of the prostate isolated from HFD-fed rats. These results clearly indicate that HFD feeding leads to increased sensitivity of prostate to the α₁- and α₂-adrenoceptor-mediated contractile responses. To eliminate the influence of different rates of uptake of agonists in the prostatic tissue of NPD- and HFD-fed rats, synthetic compounds were used, which are poor substrate for an uptake mechanism. Golomb et al. [26] reported the occurrence of spontaneous prostatic hyperplasia in the genetically hypertensive rats, which are known to have higher sympathetic tone [27].

In addition of maintaining plasma glucose level, insulin has a growth-stimulating effect. IRS/PI3-kinase-dependent downstream signaling of insulin is primarily concerned with glucose uptake and metabolic effects, whereas MEK/ERK-dependent signaling is responsible for its mitogenic action. In IR, IRS/PI3-kinase pathway is impaired, whereas MEK/ERK pathway remains unaffected [28]. Montagnani et al. [29] reported that inhibition of PI3-kinase leads to the enhanced mitogenic effects of insulin in the endothelial cells in vitro. In response to the mitogenic stimuli, such as insulin, tyrosine kinase receptor undergoes autophosphorylation [30,31] and phosphorylates IRS [32]. Grb-2 is a small cytoplasmic protein, acts as an adaptor molecule, binds to IRS-1 and localizes Sos to the plasma membrane [33,34]. Grb-2/Sos complex activates p21ras by exchanging GTP for GDP [32]. However, other mechanism of insulin mediated Ras activation may exist, including insulin-stimulated tyrosine phosphorylation of Shc [35] or direct binding of p21ras to the insulin receptor [36]. Microinjection of Grb-2 and p21ras together is reported to stimulate cell proliferation [37]. Ras directly binds to serine/threonine kinase Raf-1 which in turn activates MEK. It is generally believed that MEKI binds with ERK1/2 and phosphorylates

**Fig. 5.** Effect of pioglitazone treatment on hyperinsulinemia and prostatic growth. **A:** Significant decrease in the absolute prostate weight was observed in the HFD + PG rats as compared to HFD. However, no difference in the prostate size of was observed in NPD + PG group as compared to NPD (n = 6). **B,C:** PG treatment led to significant decrease in the plasma insulin and glucose level in HFD + PG group as compared to HFD. **D,E:** PG treatment partially restores HFD induced glucose intolerance as determined by IPGTT (n = 4). **F:** PG treatment led to decreased PCNA level in HFD + PG as compared to HFD. **G:** Schematic representation of experimental design. All the values are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.
either a tyrosine or threonine residue and dissociates. The monophosphorylated ERK1/2 again binds with activated MEK-1 for dual phosphorylation and complete activation [38]. Activated ERKs phosphorylates mainly p90\textsuperscript{rsk} [39], and also migrates to the nucleus and phosphorylates transcription factor Elk-1 [40]. Controlled regulation of Raf/MEK/ERK signaling cascade is involved in the cell proliferation and differentiation [41,42]. We found significant increase in the level of phosphorylated-ERK in the prostate of hyperinsulinemic rats, indicating the activation of MEK/ERK signaling. Increased plasma insulin level in HFD-fed rats can result in the enhanced growth of prostate gland and hence prostatic hyperplasia (Fig. 6). Increased expression of PCNA and Ki67 labeling in the prostatic LSCs of the hyperinsulinemic rats strengthens the evidence that directly or indirectly insulin is promoting cell proliferation in the prostate gland.

Male sex hormone testosterone and dihydrotestosterone is known to be associated with the pathogenesis of prostatic hyperplasia. To delineate the relative influence of androgen and insulin on the prostatic development, effect of testosterone on the prostate growth was evaluated in both HFD-fed hyperinsulinemic and NPD-fed normoinsulinemic rats. In HFD-fed rats leftward shift in the testosterone-prostatic growth curve indicates the per se effect of dietary manipulation on the prostatic development. Higher percentage of Ki67 positive LSCs in the prostate of testosterone-treated rats as compared to respective diet (HFD/NPD) matched control further confirms the above observations. Enhanced infolding of the epithelial layer in the HFD fed rats, and further increase in the degree of infolding with testosterone treatment indicates that hyperinsulinemia per se promotes the cell proliferation. Further, to substantiate the findings, a separate study has been performed with insulin sensitizer PG. Smaller prostate in HFD + PG group as compared to the diet-matched control can be attributed to decreased plasma insulin level (Fig. 5B). PG is a synthetic ligand of peroxisome proliferative-activated receptor-γ (PPARγ), which increases glucose disposal and improves insulin sensitivity (Fig. 5D,E). Previously we have reported the reversal of glucose intolerance and normalization of figure 6. A schematic model of insulin signaling in the prostate gland under IR associated hyperinsulinemic condition. High fat diet feeding induces insulin resistance and hyperinsulinemia in experimental animals. The IRS/Pi3-kinase downstream signaling is primarily concerned with glucose uptake, while RAF/MEK/ERK-dependent signaling is responsible for the growth-stimulating effects of insulin. Increased plasma insulin level in HFD-fed rats can result in enhanced mitogenic signaling in the prostate gland and hence prostatic hyperplasia. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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plasma insulin level by PG treatment in HFD-fed rats [18]. The above results can be attributed to the insulin sensitizing effect of PG resulting in the decreased plasma glucose. Hyperinsulinemia is observed as a compensatory mechanism and hence decreased insulin level was observed in HFD + PG as compared to HFD. PG per se have anti-proliferative activity [43] which can also affect the prostatic growth. However, PG treatment did not led to any change in the prostate size of NPD-fed rats, suggesting that observed modulation in the prostate size is associated with insulin level. The results of PG study further substantiate the findings that IR associated compensatory hyperinsulinemia is primarily responsible for the enhanced cell proliferation and overall enlargement of the prostate gland.

**CONCLUSIONS**

Augmented cell proliferation is the key signature of prostatic hyperplasia in contrast to the normal adult prostate. This is, to our knowledge, the first report to demonstrate that diet-induced hyperinsulinemic condition leads to enlargement as well as enhanced α-adrenoceptor mediated contractility of the prostate gland. We demonstrate, higher proliferation in the prostate of adult insulin-resistant rats, linking the hyperinsulinemia with prostatic hyperplasia. The present report proposes a novel mechanism for the pathogenesis of prostatic hyperplasia, further research in this direction will help to better understand the etiology of the disease as well as facilitate the rationale intervention of different pharmacological agents for therapeutic use. In addition to this, HFD-fed hyperinsulinemic rats could be utilized as an experimental model to study the pathogenesis of prostatic hyperplasia and to screen drugs which are designed to alleviate these conditions.

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Increased Cell Proliferation and Contractility of Prostate in Insulin Resistant Rats: Linking Hyperinsulinemia With Benign Prostate Hyperplasia

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Background: Obesity, dyslipidemia, Hyperinsulinemia, and insulin resistance (IR) are key features of metabolic syndrome and are considered as risk factors for benign prostatic hyperplasia (BPH) as well as type 2 diabetes. The present study was aimed to determine whether or not IR associated hyperinsulinemia contributes to the BPH. Methods: Sprague-Dawley rats (9 weeks) were used in the study. Rats were kept on high fat diet (HFD) for the induction of hyperinsulinemia while hypoinsulinemia was induced by streptozotocin. Effect of HFD feeding on the testosterone-induced prostatic growth was evaluated. Pioglitazone (PG, 20 mg/kg) was used for the reversal of compensatory hyperinsulinemia and to examine the subsequent effect on the prostatic growth. Results: Prostatic enlargement was observed in the HFD-fed rats. Significant increase in the cell proliferation markers confirmed the occurrence of cellular hyperplasia in the prostate of hyperinsulinemic rat. Enhanced alpha-adrenoceptor mediated contraction in the prostate of HFD-fed rats indicates augmented contractility of the gland. Higher level of phosphorylated-ERK suggests enhanced MEK/ERK signaling. HFD feeding has not led to change in the plasma testosterone level. However, testosterone treatment further augmented the prostatic growth in HFD-fed rats. PG treatment led to improved insulin sensitivity, decreased plasma insulin level and prostate weight, indicating the role of compensatory hyperinsulinemia in the prostate growth. Conclusions: The present investigation reports that HFD-feeding induced hyperinsulinemic condition leads to increased cellular proliferation, enhanced alpha-adrenoceptor mediated contraction, and enlargement of the prostate in rats.

Editorial Comment: Obesity is widely regarded as one of the most significant health problems in the Western world. The almost frightening increase in the incidence of diabetes, dyslipidemia and central obesity, and the consequent effects on cardiovascular and metabolic health have been well documented. In our world of urology these factors have been well documented to be associated with worsening pelvic function, ie voiding and sexual health, as well as urological cancers. Our understanding of the causative association of central obesity, diabetes and hyperinsulinemia with prostatic growth is evolving.

In this study the authors demonstrate that in rats fed high fat diets there is subsequent development of insulin resistance and compensatory hyperinsulinemia. Simultaneously there is marked prostatic enlargement, as demonstrated by various cell proliferation markers and increased alpha-adrenoceptor mediated contraction. Interestingly testosterone supplementation serves as a further modulator of prostatic growth. Purported reasons why these associations occur include enhanced mitogenic activity of hyperinsulinemic states, increased sympathetic tone, altered steroid metabolism and changes in the endocrine microenvironment within the prostate. Interestingly central obesity is a major risk factor for overactive bladder symptoms in women. Moreover, the effect of weight loss and control of diabetes on these events remains unknown. Nevertheless, as urologists we need to be attuned to the intimate associations among obesity, metabolic processes and pelvic dysfunction. It is reasonable to support the notion that as we further identify ourselves as leaders in delivering expertise in male health these concepts become part of our residency training and practice.

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