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# Research Article
# Aryl Hydrocarbon Receptor Is Expressed in the Prostate Gland of Lean and Obese Rats

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## Abstract

**Background and Objective:** The aryl hydrocarbon receptor (AhR) has been implicated in obesity and related disorders. The tissue distribution of AhR in the prostates of rats suffering from metabolic syndrome were explored in this study. **Materials and Methods:** In animal model, i.e., male Wistar rats' obesity and metabolic syndrome were induced in them. The rats were divided into lean (normal) and obese groups, both groups received standard rat food and drinking water but the obese group also received a sustained supply of 20% w/v of fructose. Morphometric, biochemical, histological and immunohistochemical methods were used. **Results:** AhR was expressed in both the lean and obese rats but it showed higher regulation in the obese group, which had diffused cytoplasmic and nuclear staining in the prostatic epithelium, in lean rats, the expression was only nuclear. This higher expression pattern led to the obese rats having a shorter prostatic epithelium than the lean rats had. AhR was also expressed in the mast cells of both groups. **Conclusion:** Higher regulation of AhR in the prostatic epithelium during the induction of metabolic syndrome points to AhR's direct involvement in prostate pathology. It thus may be possible to use AhR as a therapeutic target to control metabolic syndrome-induced prostate problems.

Key words: Aryl hydrocarbon receptor, metabolic syndrome, prostatic epithelium, diffuse cytoplasmic and nuclear staining, mast cells

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The aryl hydrocarbon receptor (AhR) is an intracellular receptor that is found in the cytoplasm as a part of a protein complex[1,2], upon ligand binding, AhR becomes active and translocates into the nucleus, where it forms a heterodimer with the aryl hydrocarbon nuclear translocator (Arnt). This heterodimer then binds to a specific DNA sequence[3,4] to induce the transcription of a variety of genes called the AhR gene battery[5,6], which includes the cytochromes P450[7,8]. Formerly, AhR was known as the dioxin receptor, it gained notoriety as the receptor underlying dioxin toxicity. However, recent studies have confirmed that AhR is also involved in normal cellular physiology as it induces the transcription of dioxin-independent genes[9]. Furthermore, AhR is involved in development[10], apoptosis[11] and cell proliferation[12]. Researchers have also implicated this receptor in the development of adipocytes[13] and hepatocytes[14]. Although AhR has many known exogenous ligands, its specific endogenous ligand is still unknown.

Diets that are high in carbohydrates induce fatty-acid synthesis and result in obesity[15,16]. Several studies have indicated that central obesity (visceral fat) is a primary cause of metabolic syndrome, insulin resistance[17], inflammatory diseases[18], type 2 diabetes[19-21] and other obesity-related pathologies[22]. In obese individuals, macrophages infiltrate the adipose tissue and release cytokines that lead to low-grade inflammation. Specifically, tumor necrosis factor alpha results in insulin resistance[23] in adipocytes[24].

Recently, scholars have shown an increased interest in AhR as a novel therapeutic target for battling obesity as some studies have shown that AhR is involved in fat metabolism and obesity[25]. For instance, AhR was shown to be rapidly activated in aortic endothelial cells in response to a high glucose dosages and to form complexes with transcription factors other than Arnt[26]. AhR's role in glucose metabolism still requires further study, however. One recent study investigated insulin resistance, the expression of peroxisome proliferator-activated receptor alpha (PPAR-α) and the genes involved in glucose metabolism, using both wild-type and AhR-knockout mice[27]. In that study, AhR-knockout mice (unlike wild-type mice) showed improved glucose tolerance, better insulin sensitivity and decreased PPAR-α, the scholars hypothesized that this effect is mediated by AhR, which causes insulin resistance in wild-type mice. Another recent study on AhR-knockout mice revealed that these mice, when fed a high-fat diet, were protected against obesity, hepatic steatosis, insulin resistance and inflammation due to the absence of AhR, the authors suggested that the AhR-knockout mice had a higher energy expenditure than did the wild-type mice[28].

In summary, AhR, in addition to its development as a receptor of xenobiotics, has been implicated in many physiological processes and pathological syndromes. Indeed, recent studies have revealed that AhR is implicated in obesity and related metabolic pathologies. The current study was the first to evaluate AhR expression in the prostates of lean and obese rats, an area which had not been previously explored. This will provide an idea of AhR distribution in the prostate and will shed a light on AhR's therapeutic potential as a novel drug target for managing obesity's complications.

## MATERIALS AND METHODS

This study's research methodologies were in accordance with the Regulations of Research Bioethics on Living Creatures created by the National Committee of Bio. and Med. Ethics, Kingdom of Saudi Arabia (Ethical approval no. 1438-111).The study's duration was 12 weeks.

**Animals:** This study used 20 male, 6-weeks-old Wistar rats (King Fahad Medical Research Center, King Abdulaziz University, KSA), weighing roughly 170g each. They were maintained in a cycle of 12 h of light and 12 h of dark during the experiment and were kept for 7 days of acclimatization before the experiment was performed. The rats were fed commercial rodent food and had access to water ad libitum.

**Study protocol:** The rats were randomly divided into two groups (10 per group). Both groups received standard rodent food and had free access to water for 12 weeks. The obese group also had free access to 20% w/v fructose solution throughout this period, this was intended to induce obesity and metabolic syndrome.

**Tissue sampling:** At the end of the experiment, the rats were weighed. Their fasting blood sugar was measured in blood samples from the tail vein using an Accu-Check glucometer (Roche). The rats were first anaesthetized with an intraperitoneal injection of urethane (1 g $kg^{-1}$). Blood was then collected from the retro-orbital plexus and centrifuged for 20 min at 4000 g and 4°C. The resulting serum was analyzed for insulin level and assayed with an enzyme-linked immunosorbent assay kit (Millipore, Billerica, MA, USA)using a plate coated with monoclonal anti-rat insulin antibodies.

The ventral prostate glands were then collected and weighed. A portion of the ventral prostate from each rat was frozen in liquid nitrogen and stored at -80°C for western blot analysis. Another portion of the ventral prostate gland from each rat was fixed in 10% formalin solution for 24 h for use in routine histology and immunohistochemistry.

**Western blot analysis:** To establish the specificity of the primary antibody (polyclonal rabbit anti-AhR, ab84833), western blot analysis was performed as described below.

**Protein extraction:** For each rat, a 100 mg prostate-gland sample was minced, homogenized and lysed in a lysis buffer (0.5 mL sodium dodecyl sulfate (SDS)). The samples were heated for 5 min in a 90°C water bath and then cooled. The protein concentration (μg $mL^{-1}$) in each sample was then evaluated using a Bio-Rad protein assay[29]. The protein samples were divided into 30 μg probes and frozen at -80°C until use.

**SDS with polyacrylamide gel electrophoresis:** Two concentrations of polyacrylamide gel were prepared (a 5% stacking gel and an 8% slab gel). The samples were denatured and mixed with a loading buffer and then loaded on the gel. Using a gel slab apparatus, the proteins were separated into bands, their molecular weights were then evaluated with a high-range protein marker. The bands of separated proteins were then blotted onto a nitrocellulose membrane using a gel blotter (for 45 min at 16°C and 20 mA).

**Immunoreaction:** The nitrocellulose membranes that carried the protein bands were incubated overnight in 5% dried skimmed milk in a phosphate-buffered solution with tween 20 (PBS-T) at 4°C, then, for 1 h at room temperature, the membranes were incubated with the primary antibody (polyclonal rabbit anti-AhR ab84833) at a 1:1000 dilution in PBS-T with 3% BSA (bovine serum albumin). The membranes were then washed 3×15 min in PBS-T with shaking. The secondary antibody (goat anti-rabbit ab205718) was applied at a 1:10000 dilution in PBS-T with 3% BSA for 1 h. The membranes were then washed 3×15 min in PBS-T and an additional time in PBS for 5 min. Using an enhanced chemiluminescence kit, specific reactions were visualized and permanent pictures were obtained using x-ray film.

**Routine histology and immunohistochemistry:** The specimens were fixed in formalin and processed according to the technique described by Bancroft and Stevens[30], after which, 5 μm sections were sectioned by microtome and stored for use in the routine histology and immunohistochemistry.

**Routine histology:** The tissue sections were stained with hematoxylin and eosin (H and E) to examine the normal architecture of the prostatic tissues and to make cellular measurements[30]. The heights of the glandular epithelial cells were measured using image analyzer software (Leica Application Suite X).

**Immunohistochemistry protocol:** The sections were rehydrated using descending dilutions of alcohol. To avoid the loss of reagents, the sections were surrounded with dissolved wax using a barrier pen. An indirect immunohistochemical method[12] was applied to localize and visualize the AhR. Endogenous peroxidase was blocked using 3% $H_2O_2$ in methanol for 25 min and unspecific reactions were blocked using10% normal goat serum (ab7481) for 30 min. Rabbit anti-AhR antibody (ab84833) was then applied as a primary antibody in concentration of 1:100 in 3% BSA and kept overnight at 4°C. Next, the sections were washed 3×15 min in PBS-T. A secondary antibody (goat anti-rabbit, ab205718) was applied for 1 h at a concentration of 1:500 in 3% BSA. The sections were again washed 3×15 min in PBS-T. Positive immunostaining was visualized using diaminobenzidine chromogen at a 1:10 dilution in stable $H_2O_2$. Brown color and/or precipitate appeared at sites of positive immunoreactions. The slides were mounted in distyrene plasticizer xylene (DPX) and examined with a light microscope. Without the application of a primary antibody, the controls were always negative.

**Statistical analysis:** The obtained data were represented as Mean±Standard deviation. A comparison between the two groups was performed using Student's t-test. The difference was statistically significant ($p<0.05$). The data were analyzed using Statistical Package of Social Sciences (version 16).

## RESULTS

After 2 months of pretreatment, the body weights averaged 281±23 g for lean rats and 326±39 g for obese rats, indicating the occurrence of obesity (Table 1). The prostate

Table 1: Mean values of body weight, prostatic weight and epithelial height in lean and obese rats

| Measured parameters | Lean rats | Obese rats |
|---|---|---|
| Body weight (g) | 281.000±23.00 | 326.000±39.00* |
| Prostatic weight (g) | 0.147±0.023 | 0.218±0.049* |
| Epithelial length (μm) | 11.460±1.510 | 5.410±1.220* |

Data are expressed as Mean±SD, SD: Standard deviation,*$p<0.05$ in comparison to lean

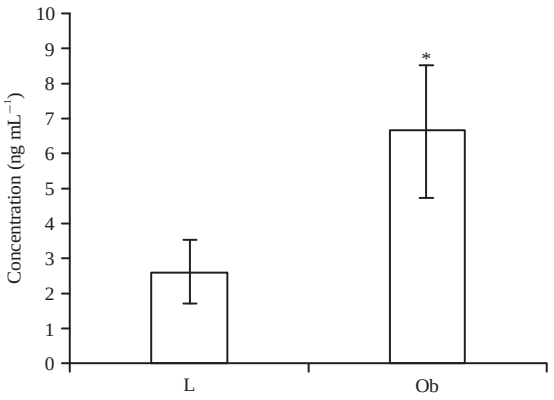


Fig. 1: Measurements of serum insulin levels (ng $mL^{-1}$) in lean (L) and obese (Ob) rats. Data are expressed as Mean±SD, SD: Standard deviation, $p<0.05$ in comparison to lean

weights were 0.147±0.023 g in lean rats and 0.218±0.049 g in obese rats (Table 1). The fasting blood-glucose levels were 95.25±3.09 mg $dL^{-1}$ in lean rats and 109±3.11 mg $dL^{-1}$ in obese rats (data not presented). As shown in Fig. 1, serum insulin levels were higher in obese rats (6.70±1.91ng $mL^{-1}$) than in lean rats (2.65±0.93 ng $mL^{-1}$), indicating insulin resistance and metabolic syndrome.

**Histological profile:** The epithelium in the prostatic ductules was much shorter in obese rats than in lean rats (Table 1). In obese rats (Fig. 2b,d), the main ductules (the bulk of the gland) had relatively thin, undulated walls when compared to those of lean rats (Fig. 2a,c). Wide interductal spaces were observed between the prostatic ductules of obese rats (Fig. 2d).

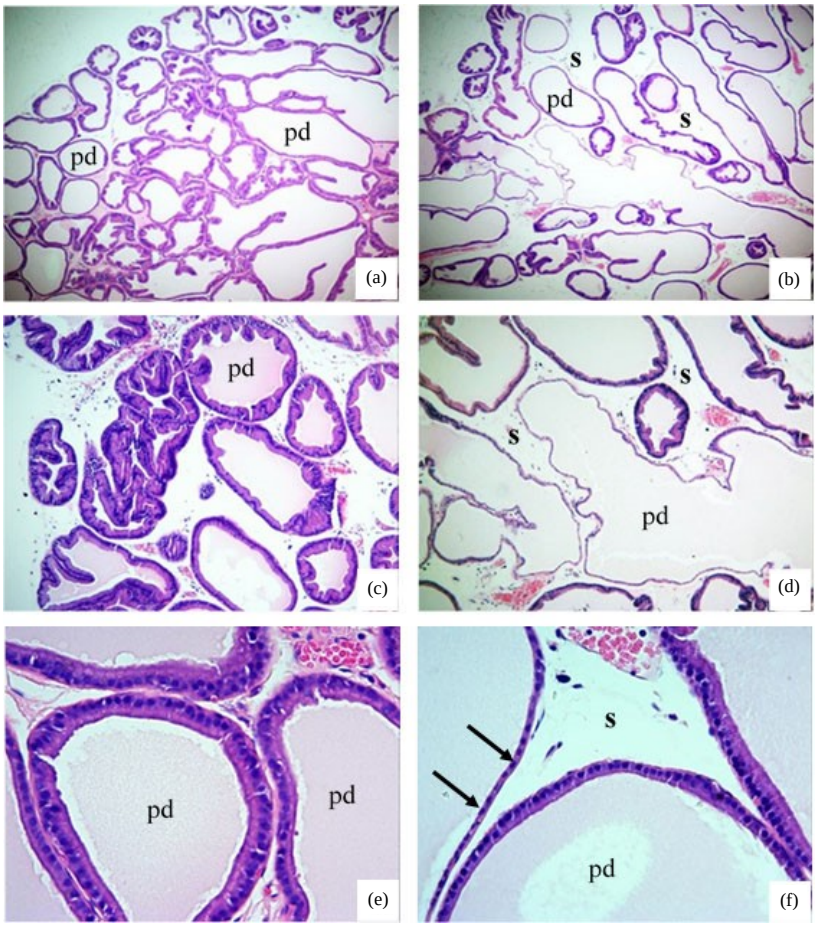


Fig.2(a-f): Photomicrographs showing different magnifications of prostatic ductules (pd) in lean (a,c,e) and obese (b,d,f) rats. In lean (normal) rats, many prostatic ductules were adhered together with narrow interductal spaces (a) while in obese rats wider interductal spaces (s) were observed (b). Walls of prostatic ductules in lean rats were thick and regular in outline (c) while in obese rats, they were relatively thin and undulant (d). Prostatic epithelium was formed of tall columnar and cuboidal cells in lean rats (e) while in obese rats it was relatively short with flattened cells, arrows (f) Magnifications (A,B X40-C,D X100-E,F X400). Stain, H and E

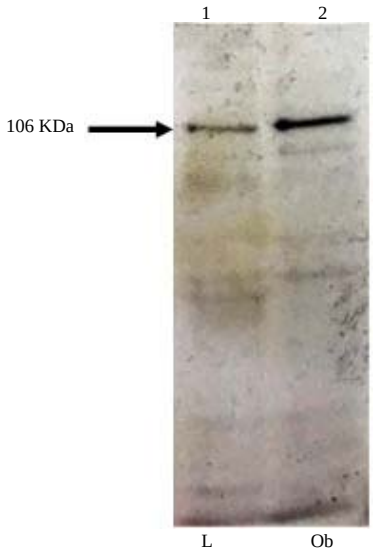


Fig. 3: Western blot analysis of ventral prostatic tissues from lean (L-lane 1) and obese (Ob-lane 2) rats. A single protein band of expected molecular weight (about 106 KDa) was identified. Observe, the protein band in lane 2 is relatively thicker and darker than that of lane 1

The majority of the luminal epithelial cells in the prostates of the lean rats were high columnar and cuboidal cells (Fig. 2e) but in obese rats, these cells were low columnar, cuboidal or even flattened (Fig. 2f).

There were no signs of inflammation or necrosis in any of the examined specimens.

**Western blot analysis:** The primary antibody identified a single band (about 106 kDa) in both lean and obese rats (Fig. 3). This confirmed the specificity of the antibody.

**Immunohistochemical study:** AhR was expressed in the prostatic tissues of both lean (Fig. 4a) and obese (Fig. 4b) rats, although in varying degrees in each prostatic tissue.

AhR had a very selective expression pattern, it was weakly expressed in the main ductules in lean rats (Fig. 4a) but was strongly expressed in the same ductules in obese rats (Fig. 4b,d). When expressed in the main ductules of lean rats, the reaction was nuclear (Fig. 4c) but in the main ductules of obese rats, the reaction was nuclear with diffuse cytoplasmic staining (Fig. 4d), even in flattened cells (Fig. 4f).

In the terminal prostatic ductules, AhR showed nuclear staining in lean rats (Fig. 4e) but nuclear and diffuse cytoplasmic staining in obese rats (Fig. 4g). AhR was always present in the epithelia of the secretory prostatic ductules (near the prostatic urethra)in both lean rats (Fig. 4a, 5a,c) and obese rats (Fig. 5b,d). This reaction was in the form of nuclear and diffuse cytoplasmic staining (Fig. 5c,d). In the prostatic stroma, AhR was expressed in the mast cells, which were either solitary or in groups for both lean (Fig. 5e) and obese (Fig. 5f) rats.

## DISCUSSION

In the current study, a concentrated fructose solution was given as a drinking fluid to induce obesity and metabolic syndrome, in this model, the occurrence of type 2 diabetes was confirmed by testing insulin resistance rather than blood-glucose level (which averaged 109 mg $dL^{-1}$). Researchers who used streptozotocin to induce type 2 diabetes after a single intraperitoneal injection recorded blood-glucose levels of up to 385 mg $dL^{-1}$ [31]. Streptozotocin-induced diabetes has also been shown to cause a decrease in both body weight and prostate weight[31,32]. However, the current study indicated a marked increase in both body weight and prostate weight. This may be due to the accumulation of body fat and the precipitation of fat in the prostatic stroma rather than to a proliferated ductal epithelium.

In general, the prostate gland is sensitive to metabolic disorders, a marked reduction in the length of the ductal epithelial cells was recorded in this study (Table 1). Similar results have been recorded in the prostate glands of streptozotocin-induced diabetic rats[31,32], this indicates decreased prostate activity in diabetes, regardless of the model used.

In this study, the expression of AhR in the prostate gland of lean and obese rats was studied and compared or the first time. AhR was expressed in the prostatic tissues of lean (normal) rats, indicating that this protein is involved in the normal physiology of the prostate gland. Earlier studies have reported that AhR was present in the prostates of fetal and adult rats[33] and in physiologically active normal tissues and organs such as the human endometrium[34], the bovine uterus[35], the rabbit uterus and ovary[36] and the chicken ovary[37]. In the current study, AhR was expressed in the prostates of obese rats, revealing a higher pattern of expression when compared with the prostates of lean rats. This indicates AhR's direct involvement in prostate pathology. Furthermore, recent studies have pointed to the involvement of AhR in lipid metabolism and gluconeogenesis[38-40], in addition, AhR may be activated in rats' aortic endothelial cells in response to high glucose treatment[26].

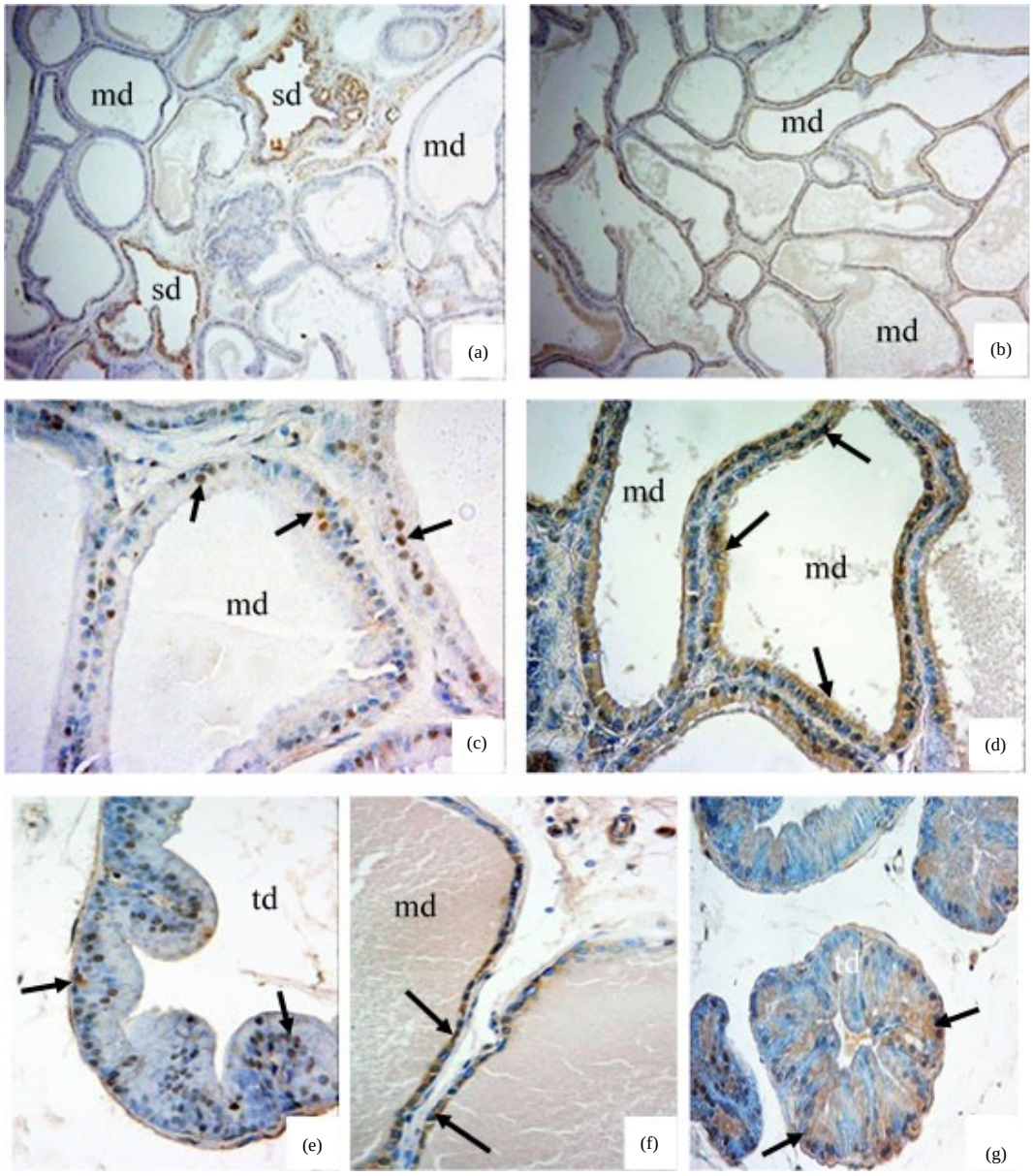


Fig. 4(a-g): AhR was expressed in prostatic tissues of lean (a,c,e) and obese (b,d,f,g) rats. Low magnification revealed large number of immunopositive main prostatic ductules (md) in obese rats (b) if compared with these of lean rats (a). Higher magnification of (a) showed nuclear staining (arrows) in main ductules (md), while higher magnification of (b) showed nuclear and diffuse cytoplasmic staining (arrows) (d). In terminal ductules (td), AhR was nuclear (Arrows) in lean rats (e) while in obese rats (g) it was nuclear with diffuse cytoplasmic (arrows). In obese rats (f), flat epithelial cells showed nuclear and diffuse cytoplasmic staining (arrows). Secretory ductules (sd) were immunopositive (a). Magnifications (A,B X40-C,D,E,F,G X400)

In a recent experimental study on AhR-deficient mice that were fed a high-fat diet, researchers reported that the mice were protected from obesity and its complications due to increased energy loss[28], the researchers suggested that AhR could be a therapeutic target for controlling obesity and its complications. Similarly, the results of the current study suggest that AhR is involved in obesity-induced problems in the rat prostate. This is supported by the nuclear and diffuse cytoplasmic staining in the luminal epithelia of the obese rats as AhR had higher expression in these rats. The exact

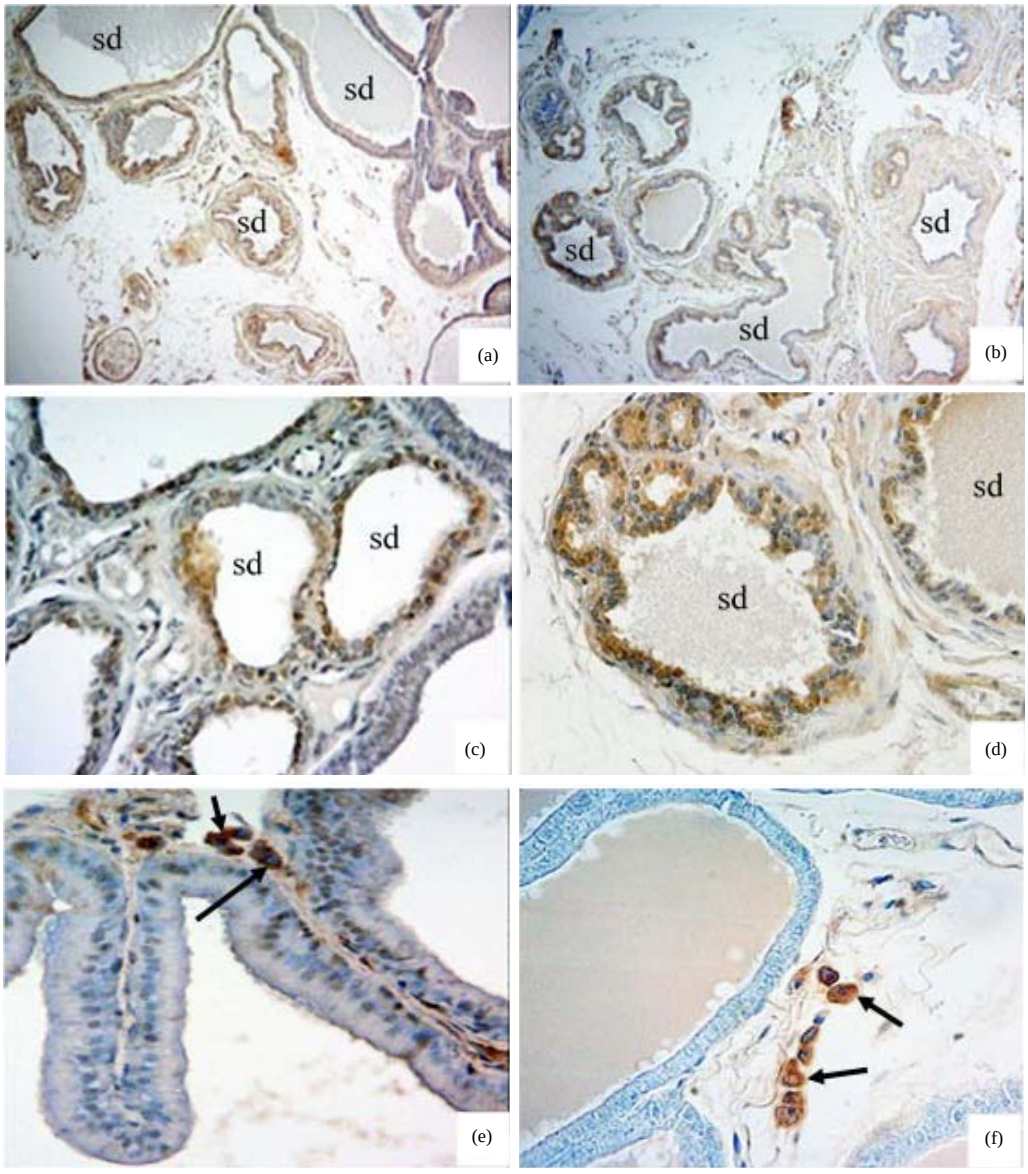


Fig. 5(a-f): AhR was expressed in the epithelium of all secretory prostatic ductules (sd) in lean (a,c) and obese (b,d) rats. The reaction was diffuse cytoplasmic with nuclear staining (c,d). Mast cells in both lean (e) and obese (f) expressed AhR in their cytoplasm (arrows). Magnifications (A,B X100-C,D X200-E, F X400)

mechanism by which AhR mediated the effects in these tissues, however, requires further investigation.

Mast cells are specialized connective cells of myeloid origin. They play basic roles in the allergic and immune responses, homoeostasis maintenance, wound healing and neuronal activity[41], furthermore, they are responsible for the secretion of histamines, heparin, serotonin, prostaglandins, proteases and tryptases as well as a variety of chemokines and cytokines[41]. In this study, AhR was expressed in the mast cells of both the lean and obese rats, indicating its involvement in mast-cell physiological activity. Although mast cells are normal inhabitants of the prostatic stroma[31], many such cells are also recruited in benign prostatic hyperplasia[42], leading to the proliferation of benign hyperplastic cells[43]. One of the predisposing factors that induces this condition is metabolic syndrome[44]. Whether mast cells were involved in the reduction of epithelial length during the induction of obesity and metabolic syndrome as is implied in these experiments, requires further study-as does AhR's exact role in prostatic mast cells.

## CONCLUSION

This study provided two novel findings about AhR expression in the prostate gland and its tissue distribution. First, AhR is involved in prostate activity and is implicated in the adverse effects that metabolic syndrome has in the rat prostate. Second, AhR is expressed in mast cells, however, further studies are necessary to elucidate AhR's exact role in mast-cell physiology. Because of this expression, AhR could serve as a novel therapeutic target for controlling complications in the prostate due to obesity and related metabolic disorders.

## SIGNIFICANCE STATEMENT

Prostate-gland pathologies, which are life-threatening in humans, have shown sensitivity to the effects of type 2 diabetes and metabolic disorders. This study was the first to explore the involvement of AhR in the prostate gland of lean and obese rats suffering from metabolic syndrome and to directly compare the expression between them. This may open new directions for treating prostatic complications of metabolic disorders and hence provide patients suffering from this condition with a better quality of life and provides new therapeutic targets.

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