Published in final edited form as:

Pharmacol Rep. 2020 February ; 72(1): 179–187. doi:10.1007/s43440-019-00010-3.

Toll-like receptor 9 regulates metabolic profile and contributes to obesity-induced benign prostatic hyperplasia in mice

Fabiano B. Calmasini^{a,b,*}, Cameron G. McCarthy^{a,c}, Camilla F. Wenceslau^{a,c}, Fernanda B.M. Priviero^a, Edson Antunes^b, R. Clinton Webb^a

^aDepartment of Physiology, Augusta University, Augusta, USA

^bDepartment of Pharmacology, Faculty of Medical Science, University of Campinas (UNICAMP), Campinas, Brazil

^cDepartment of Physiology and Pharmacology, College of Medicine and Life Sciences, The University of Toledo, Toledo, USA

Introduction

Obesity is one of the most important risk factors for cardiovascular diseases and diabetes, resulting in about 3 million deaths per year worldwide. Central obesity is associated with insulin resistance and is considered one of the main disorders that contributes to metabolic syndrome [1]. Clinical and experimental studies have demonstrated a direct correlation between metabolic syndrome/obesity and prostatic dysfunction [2, 3]. In fact, benign prostatic hyperplasia (BPH) is commonly associated with obesity [4]. BPH is characterized by increased prostate smooth muscle tone along with augmented prostate size, which may contribute to urethral narrowing and bladder outlet resistance, resulting in lower urinary tract symptoms (LUTS) [5, 6].

Recently, obesity-associated prostatic and systemic inflammation has been shown to play a pivotal role in BPH pathogenesis and progression [7, 8]. Damage-associated molecular patterns (DAMP) are novel, host-derived, pro-inflammatory molecules, released due to cell death or injury. Increased DAMPs are able to elicit an inflammatory response through activation of pattern recognition receptors (e.g., Toll-like receptors) [9]. In obesity, it has been established that DAMPs contribute to a chronic, low-grade inflammatory state, as well as adipose tissue inflammation [10, 11]. Moreover, insulin resistance and metabolic impairments are closely related with increased DAMPs [12]. Specifically, the high mobility group box 1 protein (HMGB1), a nuclear DAMP that modulates chromatin structure, is increased in plasma from obese humans and animals [13]. Additionally, HMGB1 levels have been proposed to be a biomarker of obesity in humans [14, 15].

Terms of use and reuse: academic research for non-commercial purposes, see here for full terms. <https://www.springer.com/aam-terms-v1>

*Corresponding Author: Fabiano B. Calmasini, PhD, 1120 15th street, Augusta, GA, USA, Department of Physiology, Augusta University, Tel: +1 706 7213547, fabiano.b.calmasini@gmail.com.

Publisher's Disclaimer: This Author Accepted Manuscript is a PDF file of an unedited peer-reviewed manuscript that has been accepted for publication but has not been copyedited or corrected. The official version of record that is published in the journal is kept up to date and so may therefore differ from this version.

Toll-like receptors (TLR) are pattern-recognition receptors of the innate immune system that contribute to inflammation in response to DAMPs. Specifically, studies have demonstrated the role of TLR9 activation in the generation and maintenance of chronic inflammation in obesity [16]. The TLR9 signaling involves the recruitment of adaptor protein MyD88, which in turns recruits TNF receptor associated factor (TRAF) and leads to an increase in inflammatory marker expression, through a NF- κ B-dependent pathway in several tissues [17].

Despite all clinical and experimental evidence correlating obesity-induced systemic and prostatic inflammation, nothing is known about the role of TLR9 in generating metabolic impairments and in the genesis of obesity-associated BPH. Using a mouse model of BPH induced by high-fat diet (HFD) and a TLR9 mutant mouse, we hypothesized that obesity leads to metabolic impairments through a TLR9-dependent pathway. Moreover, increased levels of DAMPs in obese animals would further contribute to metabolic dysfunction and consequently BPH. Therefore, the aim of the present study was to evaluate the participation of TLR9 in obesity-induced BPH, focusing on metabolic parameters, HMGB1 levels, prostatic ROS generation and prostatic smooth muscle reactivity.

Materials and methods

Animals

C57BL/6 wild-type (WT) and TLR9 mutant male mice (4-week old) were purchased from Jackson Laboratories (Bar Harbor, ME, USA). All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and ethical standards of Institutional Animal Care and Use Committee of Augusta University. The animals were housed three per cage on a 12 h light–dark cycle, and fed for 12 weeks with either a regular diet (RD: carbohydrate; 70%; protein: 20%; fat; 10%) or a high-fat diet that induces obesity (HFD: carbohydrate; 20%; protein: 20%; fat: 60%; Research Diets, New Brunswick, NJ, USA).

Blood collection and metabolic profile

After 12 h fasting, the animals were anesthetized with isoflurane (5%) and tail blood samples were collected for glucose measurements using a glucometer (FreeStyle Lite, Abbott, Alameda, CA, USA). Arterial blood was collected from the abdominal aorta in tubes containing heparin. Blood was immediately centrifuged at 1500 g for 15 min at 4° C and then the plasma was collected and stored at –80° C until lipid profile and Western blotting analysis. Lipid profile was analyzed using a lipid monitoring system (CardioChek P.A, Polymer Technology, Indianapolis, IN, USA).

Functional protocols in prostate from WT and TLR9 mutant mouse

After 12 weeks of RD or HFD, mice were euthanized by thoracotomy and exsanguination via cardiac puncture under isoflurane anesthesia (5%). The prostate was dissected and two strips were mounted in a 4-mL strip myograph (Danish Myograph Technology, Aarhus, Denmark) coupled to a PowerLab 8/SP™ data acquisition system (software Chart 5.0, ADInstruments, Colorado Springs, CO, USA). The chambers were filled with a

physiological salt solution (PSS) of the following composition (mmol/L): NaCl (130), NaHCO₃ (14.9), KCl (4.7), KH₂PO₄ (1.18), MgSO₄·7H₂O (1.18), CaCl₂·2H₂O (1.56), EDTA (0.026), and glucose (5.5). The bathing solution was maintained at 37° C and continuously aerated with 95% O₂ and 5% CO₂. Tension of 5 mN was used and the equilibration period was 45 min.

Cumulative concentration-response curves to phenylephrine (PE, α 1-adrenoceptor agonist; 10 nM – 30 μ M) and potassium chloride (KCl, depolarizing agent; 1 mM – 300 mM) were performed. In separate prostate preparations, relaxing responses to isoproterenol (ISO, non-selective β -adrenoceptor agonist; 1 pM – 1 μ M) were also performed in pre-contracted preparations.

Relaxing responses were calculated as percentages of the maximal changes from the steady-state contraction in each tissue. The maximal response ($E_{\max}$) and potency (pEC_{50}) were determined. The $E_{\max}$ values were calculated as mN (contraction protocols) or percentage (relaxation protocols).

Western blotting

Prostate tissues were collected, snap frozen in liquid nitrogen, and then homogenized in ice-cold tissue protein extraction reagent (T-PER) (Thermo Fisher Scientific, Waltham, MA, USA), with protease inhibitors (sodium orthovanadate, PMSF, protease inhibitor cocktail) and phosphatase inhibitors (sodium fluoride and sodium pyrophosphate) (all Sigma-Aldrich, St. Louis, MO, USA). Equal amounts of protein (40 μ g) were loaded into polyacrylamide gels (10-12%). Polyvinylidene difluoride (PVDF) membranes were probed for the expression of TLR9 (1:1000) and MyD88 (1:1000). β -actin (1:50,000) was used as loading control. For plasmatic protein, the samples were prepared according as previously described [18]. Briefly, plasma were diluted 10x in distilled water. Then, 10 μ L of laemilli solution was added in 40 μ L of 10x-diluted samples. Polyacrylamide gels (12%) were loaded with the final volume of 50 μ L for all groups. PVDF membranes were probed for the expression of HMGB1 (1:1000). Densitometry was performed using ImageJ software.

ROS production

The oxidative fluorescent dye hydroethidine (dihydroethidium, DHE; Sigma-Aldrich, St. Louis, MO, USA) was used to evaluate the prostatic ROS generation. Prostate sections were embedded in a freezing medium (OCT compound, Sakura Finetek, Torrance, CA, USA) and transverse sections (10 μ m) of frozen tissue were cut on a cryostat and collected on glass slides. The sections were equilibrated for 10 min in phosphate buffer with 100 μ M of diethylenetriaminepent acetic acid (DTPA, Sigma-Aldrich, St. Louis, MO, USA) in a light-protected humidified chamber at 37° C. Fresh buffer containing DHE (3 μ M) was applied to each tissue section and the slices were incubated in a light-protected humidified chamber at 37° C for 30 min. Images were obtained with an optical microscope equipped with a filter for rhodamine. Three different acini of each slide were captured using a 20x fluorescent objective. The number of nuclei labeled along the prostate was automatically counted using ImageJ Software (National Institutes of Health, Bethesda, MD, USA).

Histological analysis

Prostate was collected, embedded in tissue medium frozen, cross-sectioned in a cryostat (10 μ M section), fixed and stained with hematoxylin-eosin at the Augusta University Imaging Core. Photographs were taken using a Zeiss Axioplan2 microscope.

Statistical Analysis

All analyses were performed using data analysis software GraphPad Prism 5.0. Unpaired Student's t-tests and one-way ANOVA were used. Data are expressed as the mean $\pm$ S.E.M. of N experiments. To follow the principles of the 3Rs (Replacement, Reduction and Refinement) we have used a minimal amount of sample number, enough to the differences between groups become clear. P-values from groups smaller than $n=5$ are considered a pilot study, since p-values calculation requires group sizes of 5 or more.

Results

Body composition and metabolic profile in TLR9 mutant obese mice

WT obese mice exhibited increases in body (by 70%), absolute and relative prostate (by 59% and 56%) and epididymal fat (6-fold) weights compared with WT lean group ($p < 0.001$; Table 1). Fasting glucose and triglycerides levels were also higher in WT obese group compared with WT lean mice (by 161% and 28%, respectively, $p < 0.001$). In contrast, TLR9 mutant obese mice exhibited reductions in fasting glucose (by 21%) and triglycerides levels (14.4%, $p < 0.05$) along with reduction in epididymal fat mass (22.6%, $p < 0.001$) compared with WT obese mice. No differences were seen in body and prostate weights between WT obese and TLR9 obese animals (Table 1). Curiously, TLR9 lean group exhibited higher levels of fasting glucose in comparison with WT lean group ($p < 0.05$).

Obesity-induced prostatic hypercontractility is reduced in TLR9 mutant obese mice

Phenylephrine (PE, 10 nM – 30 μ M; Fig. 1A) and potassium chloride (KCl, 1 – 300 mM; Fig. 1C) produced a concentration-dependent prostatic smooth muscle contraction in all groups, however, the maximal response were greater for PE (Fig. 1B, $p < 0.05$) and KCl (Fig. 1D, $p < 0.05$) in prostate from WT obese group compared with WT lean mice. On the other hand, the enhanced contractile responses seen for PE and KCl under obesity conditions in WT group were reduced in TLR9 mutant obese mice (Fig. 1B and D, $p < 0.05$). No significant changes in the contractile responses elicited by PE and KCl in prostate of TLR9 mutant lean mice compared with WT lean group were observed (Fig. 1A and C).

The non-selective β -adrenoceptor agonist isoproterenol (ISO, 1 pM – 1 μ M; Fig. 1E) produced concentration-dependent prostatic relaxation in all groups. The potency for isoproterenol was lower in WT obese and TLR9 mutant lean and obese groups in comparison with WT lean mice (fig. 1F, $p < 0.05$).

ROS generation is attenuated in prostate from TLR9 mutant obese mice

Reactive-oxygen species in the WT obese group was increased by 56% compared with WT lean mice (from 17.2 ± 3.1 to 39 ± 2.4 nuclei/ mm^2 , $p < 0.05$; Fig. 2). On the other hand, the fluorescence produced in prostate of TLR9 mutant obese mice was reduced by 21%

compared with WT obese group (from 39 ± 2.4 to 30.9 ± 1.5 nuclei/mm², $p < 0.05$; Fig. 2). Interestingly, TLR9 mutant lean animals exhibited 39% of increasing in ROS generation compared with WT lean group (from 17.2 ± 3.1 to 28.3 ± 2 nuclei/mm², $p < 0.05$).

Protein expression of TLR9 and MyD88 in prostate

The protein expression for TLR9 was increased by 41% in prostate of WT obese mice compared with WT lean group ($p < 0.05$; Fig. 3A). However, the prostatic protein expression of MyD88 changed neither in WT or TLR9 mutant groups (Fig. 3B).

Damage-associated molecular patterns levels

HMGB1 protein expression was significantly higher (by 31%) in plasma from WT obese group compared with WT lean mice (Fig. 4; $p < 0.05$). Conversely, TLR9 mutant obese mice exhibited reduced levels of HMGB1 (34% of reduction) as evidenced in Fig. 4 ($p < 0.05$).

Histological analysis

In WT and TLR9 mutant lean groups, histological analysis of the prostate revealed a regular morphological structure characterized by a simple columnar epithelium (Fig. 5 top panels). In contrast, prostate from WT and TLR9 mutant obese mice exhibited epithelial hyperplasia and polyps formation in the epithelial layer (Fig. 5 bottom panels).

Discussion

In the present study, we observed that obesity-induced BPH-related hypercontractility is attenuated in TLR9 mutant mice. This protective effect may be associated with reduced levels of prostatic ROS seen on this group. Moreover, reduction in epididymal fat accumulation and improvements of glucose, triglyceride and DAMP levels in TLR9 mutant obese mice reveals an important role of TLR9 signaling pathway in generating metabolic impairments and consequently BPH-related prostatic smooth muscle hypercontractility.

Obesity is clinically associated with LUTS and BPH [19]. The association between BPH and LUTS is one of the most costly conditions among the ten most prevalent diseases in aging men [20]. Animals model of obesity have been used in the literature to mimic the metabolic and non-metabolic impairments seen in obese patients [21] and have reinforced the relationship between obesity and prostatic dysfunction. In insulin resistant obese mice and rats, *ex vivo* functional experiments showed increases in phenylephrine- and clonidine-induced prostatic smooth muscle contraction [22, 23]. Increases in prostate weight and epithelial hyperplasia were also reported in HFD fed obese mice and rats [22, 24, 25].

Recently, inflammatory processes have been shown to play a pivotal role in BPH pathogenesis and progression [26, 27]. Accordingly, prostate from obese mice exhibits increased NF- κ B protein expression along with increased intraprostatic levels of IL-6, all of which contributing to prostatic inflammation [28, 29]. Moreover, rabbits fed with HFD exhibited prostatic inflammation characterized by increased levels of proinflammatory cytokines (e.g. TNF- α) and intense macrophage infiltration, suggesting the role of the innate immune system on HFD-induced prostatic dysfunction [30]. Based on this data, we

employed an established model of BPH induced by HFD and a TLR9 mutant mouse to address the role of TLR9 in generating systemic and prostatic dysfunctions, which may contribute to BPH process.

Systemically, we demonstrated that TLR9 mutant mice exhibit reduced epididymal fat mass and improvements in metabolic profile, characterized by lower fasting glucose and triglycerides levels compared with WT obese animals. In humans and rodents, obesity was shown to increase DAMPs release, probably due to necrotic or apoptotic cell death [31]. Increased serum glucose levels in type 2 diabetic patients have been positively correlated with high circulating DAMPs [32], which contributes to insulin resistance, glucose imbalance and chronic adipose tissue inflammation related to obesity [10, 33]. In agreement with previous publications, we report here increased circulating levels of HMGB1 in WT obese group [13, 34]. However, we have shown for the first time that HMGB1 serum levels are completely restored in TLR9 mutant obese mice and might be related to metabolic improvements seen on this group. A previous study has shown improvement of obesity-associated inflammatory state, secondary to restored glucose homeostasis, resulting in amelioration of steatohepatitis in TLR9 mutant obese mice [35].

Prostate overgrowth and increased prostate smooth muscle contractility are common features of BPH, also called static and dynamic components, respectively. The combination of both components generally result in urethral narrowing and LUTS [36]. Increased oxidative stress have been proposed to be an important condition in BPH genesis and progression. It was demonstrated that obese animals exhibit increased prostatic ROS generation associated with increased gene and protein expression for NADPH oxidase, contributing for prostate enlargement and prostate smooth muscle hypercontractility [22, 27]. Moreover, the complete restoration of the prostatic ROS levels led to amelioration of static and dynamic components in obesity-induced BPH in mice [22]. In the present study and in accordance with the literature, we demonstrated that obesity led to increased prostatic ROS generation and prostate hypercontractility in WT obese animals compared with WT lean animals. Conversely, we have shown for the first time that TLR9 mutant obese mice exhibit reduced prostatic ROS levels and improved prostate smooth muscle reactivity. However, TLR9 mutant obese mice were not protected from obesity-associated prostatic overweight and histological abnormalities. Considering that TLR9 mutant group was only partially protected from obesity-induced prostatic ROS generation, our data suggest that a completely restoration of oxidative status is needed for complete reversion of BPH.

Hyperglycemia and dyslipidemia have been closely related to increased oxidative state and prostate hypercontractility [28]. The amelioration in metabolic profile observed in TLR9 mutant obese mice may be an explanation for the reduced oxidative status and consequent functional improvement seen on this group. Despite an increase in TLR9 expression in prostate of WT obese group, no differences were seen in prostatic expression for MyD88 between the groups, which reinforce the idea that amelioration in metabolic profile might be implicated in oxidative and functional improvements observed in prostate from TLR9 mutant obese mice.

The reduction in prostatic smooth muscle contraction is one of the main clinical therapies to treat BPH-induced LUTS under obese and non-obese conditions. The β -adrenoceptor activation counteracts the contractile pathways, contributes physiologically to maintain the prostatic tonus in several species and has been proposed as a new pharmacological therapeutic indication for BPH [37, 38]. Studies in the literature have shown that impaired β -adrenergic signaling plays a role in prostatic dysfunction in animal models of aging and hypertension [39, 40]. However, to the best of our knowledge, no previous study evaluated this signaling pathway in prostatic tissue under obesity conditions. In the present study, we have demonstrated for the first time that obesity led to impaired β -adrenergic-induced prostatic smooth muscle relaxation, characterized by reduced potency of β -adrenoceptor agonist isoproterenol in WT obese group. However, the TLR9 mutant obese mice were not protected from prostatic β -adrenergic impairments, suggesting that TLR9 is not implicated in reduced β -adrenoceptor sensitivity in obese mice. It is important to note that the sigmoidal shape of the concentration-response curves in the obese groups is lacking. Studies have indicated the presence of three different subtypes of β -adrenoceptor in prostatic tissue of humans and rodents [41, 42]. Considering that isoproterenol is a non-selective β -adrenoceptor agonist, it is possible that changes in β -adrenoceptor density under obesity conditions, especially in the receptors activated with high concentrations of isoproterenol, may be implicated on this non-sigmoidal shape.

Finally, it is important to note that under physiological condition, TLR9 mutant lean mice exhibited higher levels of fasting glucose and increased prostatic ROS generation compared with WT lean mice. Moreover, functional impairment characterized by a rightward shift to isoproterenol-induced prostate smooth muscle relaxation was also observed. Studies have demonstrated an important role for TLR in the physiological process in mammals. For instance, TLR9 signaling is important for cardiac vagal tone and baroreflex control of arterial pressure in mice [43]. Whether TLR9 also contributes for prostate functioning under physiological condition is still unknown. However, the data presented here are suggestive of an important role of TLR9 signaling pathway in the mice prostate and may be a matter of future investigations.

Overall, our data have shown that TLR9 mutant obese mice exhibited improvements in obesity-induced prostate hypercontractility, which may be associated with reduced prostatic ROS levels, secondary to improvements in metabolic profile and DAMPs generation. Thus, TLR9 and its signaling pathway could be a valuable target to improve obesity-associated metabolic disorders and consequently BPH-related prostatic smooth muscle hypercontractility.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; 2016/20592-8), National Institute of Health (NIH HL-134604 and NIH GM - 118885).

References

- [1]. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference

on scientific issues related to definition. *Circulation*, 2004; 109(3) 433–438. [PubMed: 14744958]

- [2]. De Nunzio C, Aronson W, Freedland SJ, Giovannucci E, Parsons JK. The correlation between metabolic syndrome and prostatic diseases. *Eur Urol* 2012; 61(3) 560–570. [PubMed: 22119157]
- [3]. Gacci M, Corona G, Vignozzi L, Salvi M, Semi S, De Nunzio C, Tubaro A, Oelke M, Carini M, Maggi M. Metabolic syndrome and benign prostatic enlargement: a systematic review and meta-analysis. *BJU Int*, 2015; 115(1) 24–31.
- [4]. Vignozzi L, Gacci M, Maggi M. Lower urinary tract symptoms, benign prostatic hyperplasia and metabolic syndrome. *Nat Rev Urol*, 2016; 13(2) 108–19. [PubMed: 26754190]
- [5]. Liu RT, Chung MS, Lee WC, Chang SW, Huang ST, Yang KD, Chancellor MB, Chuang YC. Prevalence of overactive bladder and associated risk factors in 1359 patients with type 2 diabetes. *Urology*, 2011; 78(5) 1040–1045. [PubMed: 21958505]
- [6]. Steers WD. Food for thought: obesity as the major contributing factor for most urological disorders. *J Urol*, 2009; 181(5) 1983–1984. [PubMed: 19286212]
- [7]. Bushman WA, Jerde TJ. The role of prostate inflammation and fibrosis in lower urinary tract symptoms. *Am J Physiol Renal Physiol*, 2016; 311(4) F817–F821. [PubMed: 27440781]
- [8]. Fowke JH, Koyama T, Fadare O, Clark PE. Does Inflammation Mediate the Obesity and BPH Relationship? An Epidemiologic Analysis of Body Composition and Inflammatory Markers in Blood, Urine, and Prostate Tissue, and the Relationship with Prostate Enlargement and Lower Urinary Tract Symptoms. *PloS one*, 2016; 11(6) e0156918. [PubMed: 27336586]
- [9]. McCarthy CG, Gouloupoulou S, Wenceslau CF, Spitler K, Matsumoto T, Webb RC. Toll-like receptors and damage-associated molecular patterns: novel links between inflammation and hypertension. *Am J Physiol Heart Circ Physiol*, 2014; 306(2) H184–196. [PubMed: 24163075]
- [10]. Haghiac M, Vora NL, Basu S, Johnson KL, Presley L, Bianchi DW, Hauguel-de Mouzon S. Increased death of adipose cells, a path to release cell-free DNA into systemic circulation of obese women. *Obesity (Silver Spring)*, 2012; 20(11) 2213–2219. [PubMed: 22836687]
- [11]. Miura K, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, Olefsky JM, Brenner DA, Seki E. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1 β in mice. *Gastroenterol*, 2010; 139(1) 323–334 e327.
- [12]. Shin JJ, Lee EK, Park TJ, Kim W. Damage-associated molecular patterns and their pathological relevance in diabetes mellitus. *Ageing Res Rev*, 2015; 24(Pt A) 66–76. [PubMed: 26197086]
- [13]. Ganz M, Bukong TN, Csak T, Saha B, Park JK, Ambade A, Kodys K, Szabo G. Progression of non-alcoholic steatosis to steatohepatitis and fibrosis parallels cumulative accumulation of danger signals that promote inflammation and liver tumors in a high fat-cholesterol-sugar diet model in mice. *J Transl Med*, 2015; 13 193. [PubMed: 26077675]
- [14]. Giacobbe A, Grasso R, Imbesi G, Salpietro CD, Grasso L, Lagana AS, Triolo O, Di Benedetto A. High mobility group protein B1: a new biomarker of obesity in pregnant women? *Gynecol Endocrinol*, 2015; 31(2) 113–115. [PubMed: 25356847]
- [15]. Arrigo T, Chirico V, Salpietro V, Munafo C, Ferrau V, Gitto E, Lacquaniti A, Salpietro C. High-mobility group protein B1: a new biomarker of metabolic syndrome in obese children. *Eur J Endocrinol*, 2013; 168(4) 631–638. [PubMed: 23384711]
- [16]. Kim SJ, Choi Y, Choi YH, Park T. Obesity activates toll-like receptor-mediated proinflammatory signaling cascades in the adipose tissue of mice. *J Nutr Biochem*, 2012; 23(2) 113–122. [PubMed: 21414767]
- [17]. Mann DL. The emerging role of innate immunity in the heart and vascular system: from whom the cell tolls. *Circ Res*, 2011; 108(9) 1133–1145. [PubMed: 21527743]
- [18]. Szasz T, Wenceslau CF, Burgess B, Nunes KP, Webb RC. Toll-Like Receptor 4 Activation Contributes to Diabetic Bladder Dysfunction in a Murine Model of Type 1 Diabetes. *Diabetes*, 2016; 65(12) 3754–3764. [PubMed: 27650857]
- [19]. Daneshgari F, Liu G, Hanna-Mitchell AT. Path of translational discovery of urological complications of obesity and diabetes. *Am J Physiol Renal Physiol*, 2016; 312(5) F887–F896.
- [20]. Fenter TC, Naslund MJ, Shah MB, Eaddy MT, Black L. The cost of treating the 10 most prevalent diseases in men 50 years of age or older. *Am J Manag Care*, 2006; 12(4 Suppl) S90–98. [PubMed: 16551207]

- [21]. Sah SP, Sinh B, Choudhary S, Kumar A. Animal models of insulin resistance: A review. *Pharmacol Rep*, 2016; 68(6) 1165–1177. [PubMed: 27639595]
- [22]. Calmasini FB, de Oliveira MG, Alexandre EC, Silva FH, Tavares EBG, Andre DM, Zapparoli A, Antunes E. Obesity-induced mouse benign prostatic hyperplasia (BPH) is improved by treatment with resveratrol: implication of oxidative stress, insulin sensitivity and neuronal growth factor. *J Nutr Biochem*, 2018; 55 53–58. [PubMed: 29413489]
- [23]. Vikram A, Jena GB, Ramarao P. Increased cell proliferation and contractility of prostate in insulin resistant rats: linking hyperinsulinemia with benign prostate hyperplasia. *Prostate*, 2010; 70(1) 79–89. [PubMed: 19790233]
- [24]. Cai X, Haleem R, Oram S, Cyriac J, Jiang F, Grayhack JT, Kozlowski JM, Wang Z. High fat diet increases the weight of rat ventral prostate. *Prostate*, 2001; 49(1) 1–8. [PubMed: 11550205]
- [25]. Silva SA, Gobbo MG, Pinto-Fochi ME, Rafacho A, Taboga SR, Almeida EA, Goes RM, Ribeiro DL. Prostate hyperplasia caused by long-term obesity is characterized by high deposition of extracellular matrix and increased content of MMP-9 and VEGF. *Int J Exp Pathol*, 2015; 96(1) 21–30. [PubMed: 25529509]
- [26]. Gandaglia G, Briganti A, Gontero P, Mondaini N, Novara G, Salonia A, Sciarra A, Montorsi F. The role of chronic prostatic inflammation in the pathogenesis and progression of benign prostatic hyperplasia (BPH). *BJU Int*, 2013; 112(4) 432–441. [PubMed: 23650937]
- [27]. Wang L, Zoetemelk M, Chitteti BR, Ratliff TL, Myers JD, Srour EF, Broxmeyer H, Jerde TJ. Expansion of prostate epithelial progenitor cells after inflammation of the mouse prostate. *Am J Physiol Renal Physiol*, 2015; 308(12) F1421–1430. [PubMed: 25925259]
- [28]. Vykhovanets EV, Shankar E, Vykhovanets OV, Shukla S, Gupta S. High-fat diet increases NF-kappaB signaling in the prostate of reporter mice. *Prostate*, 2011; 71(2) 147–156. [PubMed: 20632379]
- [29]. Shankar E, Vykhovanets EV, Vykhovanets OV, MacLennan GT, Singh R, Bhaskaran N, Shukla S, Gupta S. High-fat diet activates pro-inflammatory response in the prostate through association of Stat-3 and NF-kappaB. *Prostate*, 2012; 72(3) 233–243. [PubMed: 21604287]
- [30]. Vignozzi L, Morelli A, Sarchielli E, Comeglio P, Filippi S, Cellai I, Maneschi E, Serni S, Gacci M, Carini M, Piccinni MP, Saad F, Adorini L, Vannelli GB, Maggi M. Testosterone protects from metabolic syndrome-associated prostate inflammation: an experimental study in rabbit. *J Endocrinol*, 2012; 212(1) 71–84. [PubMed: 22010203]
- [31]. Wang H, Qu H, Deng H. Plasma HMGB-1 Levels in Subjects with Obesity and Type 2 Diabetes: A Cross-Sectional Study in China. *PloS one*, 2015; 10(8) e0136564. [PubMed: 26317615]
- [32]. Alvarado-Vasquez N. Circulating cell-free mitochondrial DNA as the probable inducer of early endothelial dysfunction in the prediabetic patient. *Exp Gerontol*, 2015; 69 70–78. [PubMed: 26026597]
- [33]. Nishimoto S, Fukuda D, Higashikuni Y, Tanaka K, Hirata Y, Murata C, Kim-Kaneyama JR, Sato F, Bando M, Yagi S, Soeki T, Hayashi T, Imoto I, Sakaue H, Shimabukuro M, Sata M. Obesity-induced DNA released from adipocytes stimulates chronic adipose tissue inflammation and insulin resistance. *Sci Adv*, 2016; 2(3) e1501332. [PubMed: 27051864]
- [34]. Garcia-Martinez I, Santoro N, Chen Y, Hoque R, Ouyang X, Caprio S, Shlomchik MJ, Coffman RL, Candia A, Mehal WZ. Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9. *J Clin Invest*, 2016; 126(3) 859–864. [PubMed: 26808498]
- [35]. Revelo XS, Ghazarian M, Chng MH, Luck H, Kim JH, Zeng K, Shi SY, Tsai S, Lei H, Kenkel J, Liu CL, Tangsombatvisit S, Tsui H, Sima C, Xiao C, Shen L, Li X, Jin T, Lewis GF, Woo M, Utz PJ, Glogauer M, Engleman E, Winer S, Winer DA. Nucleic Acid-Targeting Pathways Promote Inflammation in Obesity-Related Insulin Resistance. *Cell Rep*, 2016; 16(3) 717–730. [PubMed: 27373163]
- [36]. Vikram A, Jena G, Ramarao P. Insulin-resistance and benign prostatic hyperplasia: the connection. *Eur J Pharmacol*, 2010; 641(2-3) 75–81. [PubMed: 20553919]
- [37]. Kedia GT, Uckert S, Kedia M, Kuczyk MA. Effects of phosphodiesterase inhibitors on contraction induced by endothelin-1 of isolated human prostatic tissue. *Urology*, 2009; 73(6) 1397–401. [PubMed: 19285714]

- [38]. Calmasini FB, Candido TZ, Alexandre EC, D'Ancona CA, Silva D, de Oliveira MA, De Nucci G, Antunes E, Monica FZ. The beta-3 adrenoceptor agonist, mirabegron relaxes isolated prostate from human and rabbit: new therapeutic indication? *Prostate*, 2015; 75(4) 440–7. [PubMed: 25417911]
- [39]. Calmasini FB, Leiria LO, Alves Junior MJ, Bau FR, Alexandre EC, Silva FH, Monica FZ, Antunes E. Increased Rho-kinase-mediated prostate contractions associated with impairment of beta-adrenergic-cAMP-signaling pathway by chronic nitric oxide deficiency. *Eur J Pharmacol*, 2015; 758, 24–30. [PubMed: 25835610]
- [40]. Calmasini FB, Silva FH, Alexandre EC, Rodrigues RL, Barbosa AP, Ferrucci DL, Carvalho HF, Anhe GF, Pupo AS, Antunes E. Implication of Rho-kinase and soluble guanylyl cyclase enzyme in prostate smooth muscle dysfunction in middle-aged rats. *Neurourol Urodyn*, 2017; 36(3) 589–596. [PubMed: 26999618]
- [41]. Goepel M, Wittmann A, Rubben H, Michel MC. Comparison of adrenoceptor subtype expression in porcine and human bladder and prostate. *Urol Res*, 1997; 25(3) 199–206. [PubMed: 9228673]
- [42]. Haynes JM. Beta(2) and beta(3)-adrenoceptor inhibition of alpha(1)-adrenoceptor-stimulated Ca^{2+} elevation in human cultured prostatic stromal cells. *Eur J Pharmacol*, 2007; 570(1-3) 18–26. [PubMed: 17617401]
- [43]. Rodrigues FL, Silva LE, Hott SC, Bomfim GF, da Silva CA, Fazan JrR, Resstel LB, Tostes RC, Carneiro FS. Toll-like receptor 9 plays a key role in the autonomic cardiac and baroreflex control of arterial pressure. *Am J Physiol Regul Integr Comp Physiol*, 2015; 308(8) R714–723. [PubMed: 25673780]

Figure 1.

Concentration-response curves to phenylephrine (PE; A), potassium chloride (KCl; C) and isoproterenol (E), maximal response to PE (B) and KCl (D), and pEC₅₀ for isoproterenol (F) in prostate from wild-type (WT) and TLR9 mutant (mutant) lean and obese mice. Data represents mean $\pm$ SEM. The sample sizes are inserted within figure (panels B, D and F). * $p < 0.05$ compared with WT lean group, # $p < 0.05$ compared with WT obese group, one way ANOVA, Bonferroni multiple comparison test.

Figure 2. Reactive-oxygen species (ROS) levels through DHE-induced fluorescence (top) and quantification of ethidium-bromide-positive nuclei (bottom) were measured in prostate from wild-type (WT) and TLR9 mutant (mutant) lean and obese mice. Data represents mean $\pm$ SEM. The sample sizes are inserted within figure. * $p < 0.05$ compared with WT lean, # $p < 0.05$ compared with WT obese group, one way ANOVA, Bonferroni multiple comparison test.

Figure 3. Representative western blotting images (top panels) and protein expression values (bottom panels) for TLR9 (A) and MyD88 (B) in prostate homogenates from wild-type (WT) and TLR9 mutant (mutant) lean and obese mice. Data represents mean $\pm$ SEM. The sample sizes are inserted within figure. * $p < 0.05$ compared with WT, student's t-test.

Figure 4.

Circulating HMGB1 levels for wild-type (WT) and TLR9 mutant (mutant) lean and obese mice. Data represents mean $\pm$ SEM. The sample sizes are inserted within figure. * $p < 0.05$ compared with WT lean and # $p < 0.05$ compared with WT obese group, one way ANOVA, Bonferroni multiple comparison test.

Figure 5. Hematoxylin-eosin sections of prostate from wild-type (WT) and TLR9 mutant (mutant) lean and obese mice. Four animals were analyzed per group. Red arrows indicate epithelial hyperplasia; and black arrow polyps formation. St = stroma; ep = epithelium. Scale bar: 100 μ M.

Table 1 -

Body and prostate weight, epididymal fat mass and metabolic parameters

	LEAN		OBESE	
	Wild Type	TLR9 mutant	Wild Type	TLR9 mutant
Body weight (g)	26.33 ± 0.84	26 ± 0.45	45 ± 0.78 ^{***}	45.11 ± 1.18 ^{***}
Prostate (mg)	13.9 ± 0.83	17.8 ± 1.03	22.19 ± 1.38 ^{***}	24.04 ± 1.77 ^{***}
Relative weight (mg/cm)	6.23 ± 0.39	7.59 ± 0.45	9.72 ± 0.71 ^{**}	10.05 ± 0.66 ^{**}
Epididymal fat (g)	0.38 ± 0.03	0.48 ± 0.02	2.74 ± 0.78 ^{***}	2.12 ± 0.13 ^{###}
Fasting glucose (mg/dL)	80.33 ± 6.85	121 ± 8.91 [*]	210 ± 16.9 ^{***}	165.8 ± 11.1 [#]
Triglyceride (mg/dL)	77.20 ± 3.41	73.4 ± 3.16	98.75 ± 2.84 ^{**}	84.5 ± 4.07 [#]

Data represent mean ± SEM (n=5-8).

P* < 0.05, *P* < 0.01 and ****P* < 0.001 compared with Wild type lean group.#*P* < 0.05 and ###*P* < 0.001 compared with wild type obese group.

Relative weight = prostate weight (mg) / tibia length (cm).