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Article

Aging-associated prostate smooth muscle hypercontractility in rats

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Benign prostatic hyperplasia (BPH) is a multifactorial disease, highly associated with aging and characterized by increased prostate smooth muscle (PSM) contractility. Animal models have been employed to explore the aging-associated PSM hypercontractility; however, studies have focused in old animals, neglecting the initial alterations in early ages. The determination of prostatic dysfunctions onset is crucial to understand the BPH pathophysiology and to propose new BPH treatments. Considering that PSM contractility in 10-month-old rats has already been explored, the aim of the present study was to characterize the PSM contractility in younger rats. Male Wistar control (3.5-month-old), 6- and 8-month-old rats were used. Concentration-response curves to phenylephrine and electrical-field stimulation (EFS) were conducted in prostate from all groups. For the first time, we showed that 6- and 8-month-old rats exhibit PSM hypercontractility. The increased prostate contractility to phenylephrine starts around at 6-month-old, worsening during the aging. The 8-month-old rats exhibited hypercontractility to phenylephrine and EFS compared to the control and 6-month-old groups. Reduced phenylephrine potency was observed in 8-month-old rats, indicating an increased age-dependent prostate sensibility to this agonist. Collectively, our findings support the use of 6- and 8-month-old aged rats as new models to explore prostate hypercontractility in BPH.

Keywords: Benign prostatic hyperplasia. Lower urinary tract symptoms. Phenylephrine. Electrical-field stimulation. Smooth muscle.

INTRODUCTION

Benign prostatic hyperplasia (BPH) and prostate cancer are the most prevalent prostatic diseases affecting men (Wah *et al*., 2021; Xiong*et al*., 2020). As a result, several treatments have been investigated in animal models and cell culture in an attempt to prevent or cure these conditions (Lamas *et al*., 2020; Fattahi *et al*., 2018). The epidemiological data indicate an age-dependent incidence of BPH around 50% in middle-aged men (50 to 60 years), increasing to 90% in men aged 90 or older (Calogero *et al*., 2019). BPH is characterized by intense cell proliferation and increased prostate smooth muscle tonus, which are considered hallmarks of this disease. Despite being considered a non-deadly condition, the alterations induced by BPH are closely related to the development of lower urinary tract symptoms (LUTS) (Launer *et al*., 2020). The urethral lumen narrowing and bladder outlet resistance, produced by the increased prostate size and contractility, are key factors in the pathophysiology of LUTS (Gupta, Gange, McVary, 2019). LUTS involve storage, voiding and post-micturition symptoms, negatively impacting the patient's quality of life (Sarma, Wei, 2012).

Prostate smooth muscle is highly innervated by excitatory and inhibitory autonomic neurons, which play a key role in the organ physiology (Sievert *et al*., 2019). The adrenergic nerves are the main pathway in several species and are responsible for the prostate smooth muscle contraction (Calmasini *et al*., 2015; Lau, Ventura,

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Pennefather, 1998). Dysregulations of adrenergic pathway-induced prostate smooth muscle contraction have been associated with BPH genesis and progression in humans and animals (Lee *et al*., 2017; Calmasini *et al*., 2016).

The majority of the published studies have employed aged animals to address aging-related prostate dysfunctions, neglecting the initial alterations that may occur during adulthood and middle-age. Understanding the pathophysiology of BPH, especially in the period before its onset, is crucial for BPH prevention/treatment. Moreover, defining the life period in which rats exhibit initial prostatic alterations may be of interest for those who aim to work with aging-associated prostate smooth muscle dysfunction. Therefore, the aim of the present study was to evaluate the prostate smooth muscle contractility in 6- and 8-month-old rats using functional assays.

MATERIAL AND METHODS

Animals

Male Wistar young adult (3.5-month old; body weight 468 ± 14.9 g), 6- and 8-month-old rats were used (body weight 527 ± 16.7 and 581 ± 22.3 g, respectively). The animals were provided by Central Animal House Services of University of Campinas. The rats were kept in temperature-controlled facilities on a 12-h light/dark cycle with *ad libitum* food and water access. All the procedures were conducted in accordance with Institutional guidelines, approved by Ethical Principles in Animal Research by College for Animal Experimentation (COBEA) and local Ethics Committee for the Use of Experimental Animals.

In vitro prostate preparation and concentration-response curves

Rats were killed in CO_2 chamber, the ventral prostate was removed and dissected from fat tissue. Ventral prostatic strips were mounted under resting tension of 5 mN in 4-ml myograph filled with Krebs solution (117 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose and 2.5 mM $CaCl_2$), pH 7.4, 37º C bubbled with carbogenic mixture (95% O_2 and 5% CO_2). PowerLab 400TM Data Acquisition System (Software Chart, version 6.0, AD Instruments, Milford, MA) was used to record the isometric force produced by the prostate smooth muscle. Strips were equilibrated for 1 hour and then concentration-response curves were performed by using one-half log unit concentration rise. Cumulative concentration-response curves to phenylephrine, an α1-adrenoceptor agonist (PE; 1 nM –100μM) were performed. The maximal response (E_{max}) and potency (pEC_{50}) were determined.

Electrical-field stimulation

Electrical-field stimulation (EFS) was performed in prostate strips by placing two platinum electrodes between the tissues, connected to a stimulator (Grass S88, Astro-Med Industrial Park, Warwick, RI). Crescent frequencies (1-32 Hz, 10 sec, pulse of 1msec width at 50 V) were used to construct frequency-response curves, with 2 min interval between stimulations. The contractile responses were exhibited as mN.

Statistical analysis

All data are expressed as means ± S.E.M. The GraphPad Prism Program (GraphPad Software Inc) was used for statistical analysis. Student's t-test was used to assess the results. $p<0.05$ was accepted as significant.

RESULTS

Prostate smooth muscle contractility in 6-month-old rats

Cumulative addition of the α1-adrenoceptor agonist phenylephrine (PE; 1 nM – 100 μM) elicited a concentration-dependent prostate smooth muscle contraction in the control and 6-month-old groups (Figure 1A). The maximal response to phenylephrine in the prostate from 6-month-old rats was higher compared with the control group (Figure 1B); however no differences were seen in the potency for this agonist (Figure 1C). Likewise, EFS (1-32 Hz) produced frequency-dependent prostate smooth muscle contractions that were similar between the control and 6-month-oldgroups (Figure 1D).

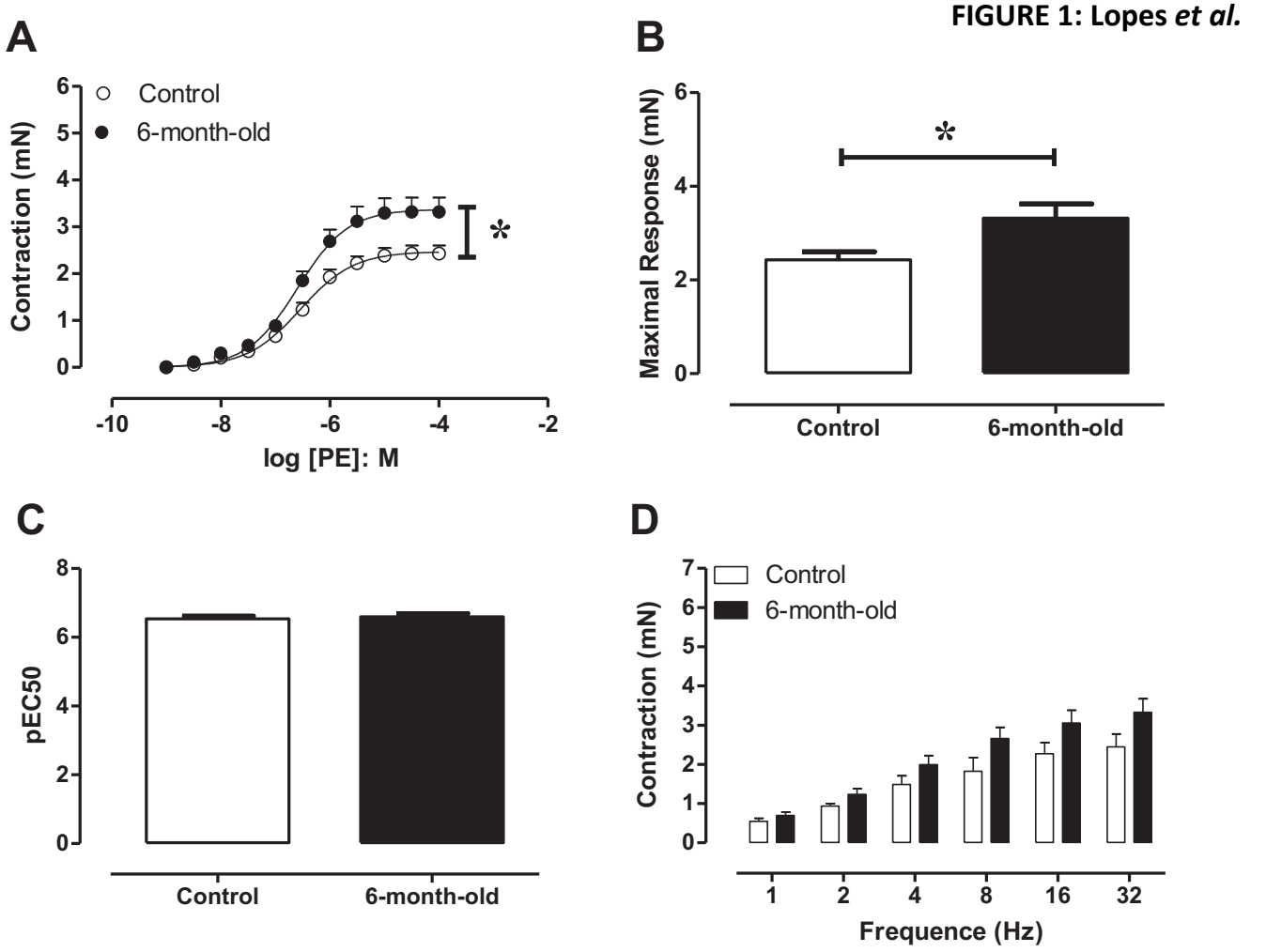

FIGURE 1 - Prostate smooth muscle contraction induced by electrical-field stimulation (EFS 1-32 Hz; A), concentration-response curve to phenylephrine (PE; 1 nM – 100 μM; B), maximal response (C) and potency (D) in prostate from control and 6-month-old rats (6 months). Data represent the mean ± S.E.M. (n=5-11). *p<0.05 compared to control group.

Prostate smooth muscle contractility in 8-month-old rats

The prostate smooth muscle contraction elicited by EFS was higher at almost all frequencies tested (2-32 Hz, Figure 2A) in the 8-month-old group compared with control rats. The biggest differences were observed at higher frequencies (8-32 Hz, Figure 2A). Similarly, phenylephrine produced concentration-dependent prostate smooth muscle contractions that were higher in the 8-month-old group compared with control rats (Figure 2B and C). Reduced potency values (leftward shift of 2.2) for phenylephrine were also found in prostates from 8-month-old rats (Figure 2D).

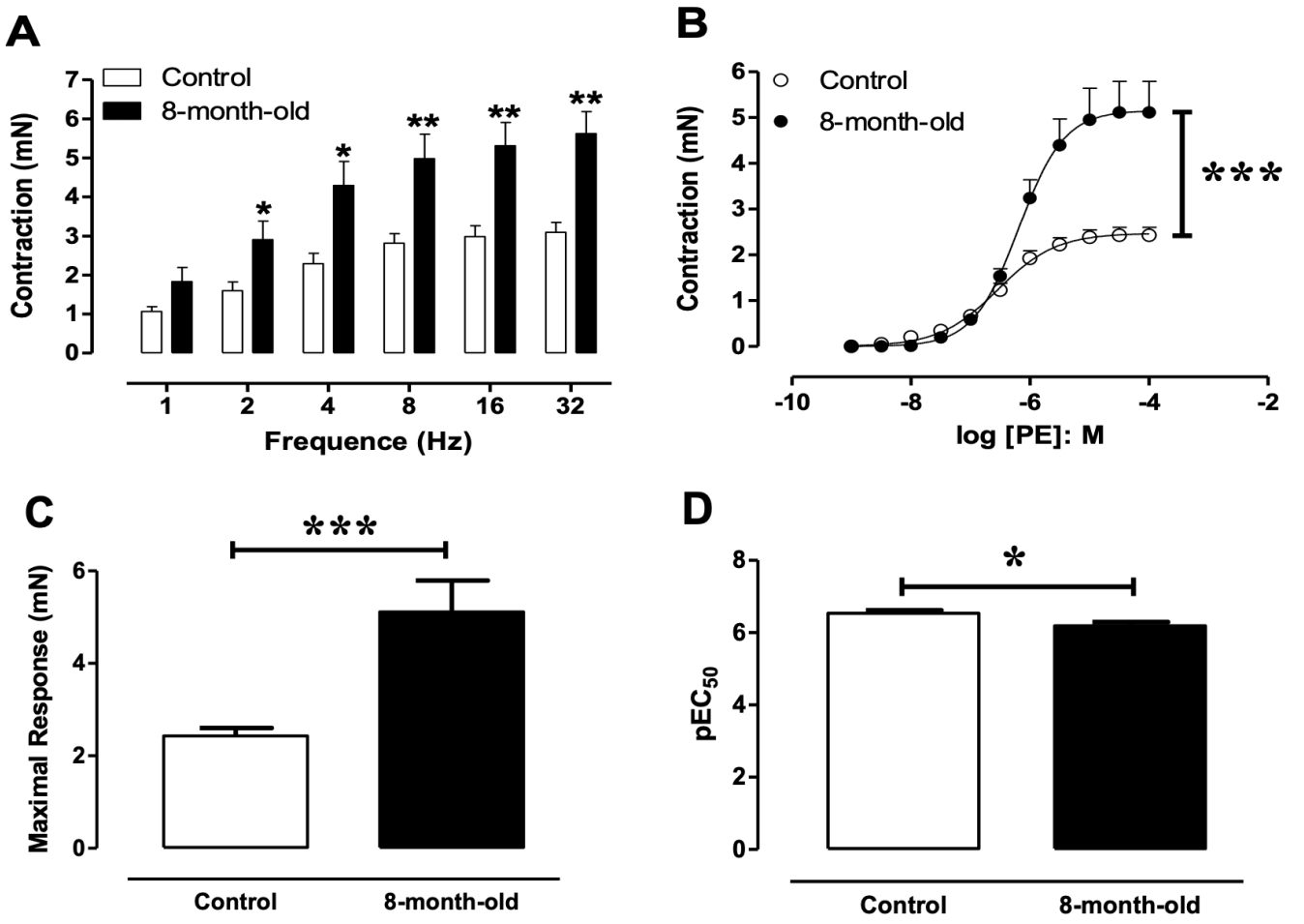

FIGURE 2 - Prostate smooth muscle contraction induced by electrical-field stimulation (EFS 1-32 Hz; A), concentration-response curve to phenylephrine (PE; 1 nM – 100 μM; B), maximal response (C) and potency (D) in prostate from control and 8-month-old rats (8 months). Data represent the mean ± S.E.M. (n=6-11). $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ compared to control group.

Prostate smooth muscle contractility worsens during aging process

Figure 3 compares the prostate smooth muscle contractility between 6- and 8-month-old rats. As indicated in the panel A, EFS-induced prostate smooth muscle contractions were higher in all frequencies tested in 8-month-old rats compared with the 6-month-old group. Similarly, prostate smooth muscle contractions induced by phenylephrine were increased by approximately 55% in the 8-month-old group compared with 6-month-old rats (Figure 3B and C). The prostates from 8-month-old rats also exhibited a leftward shift in potency (shift of 2.6) compared with 6-month-old rats, indicating an increase in tissue sensitivity to phenylephrine (Figure 3D).

FIGURE 3: Lopes *et al.*

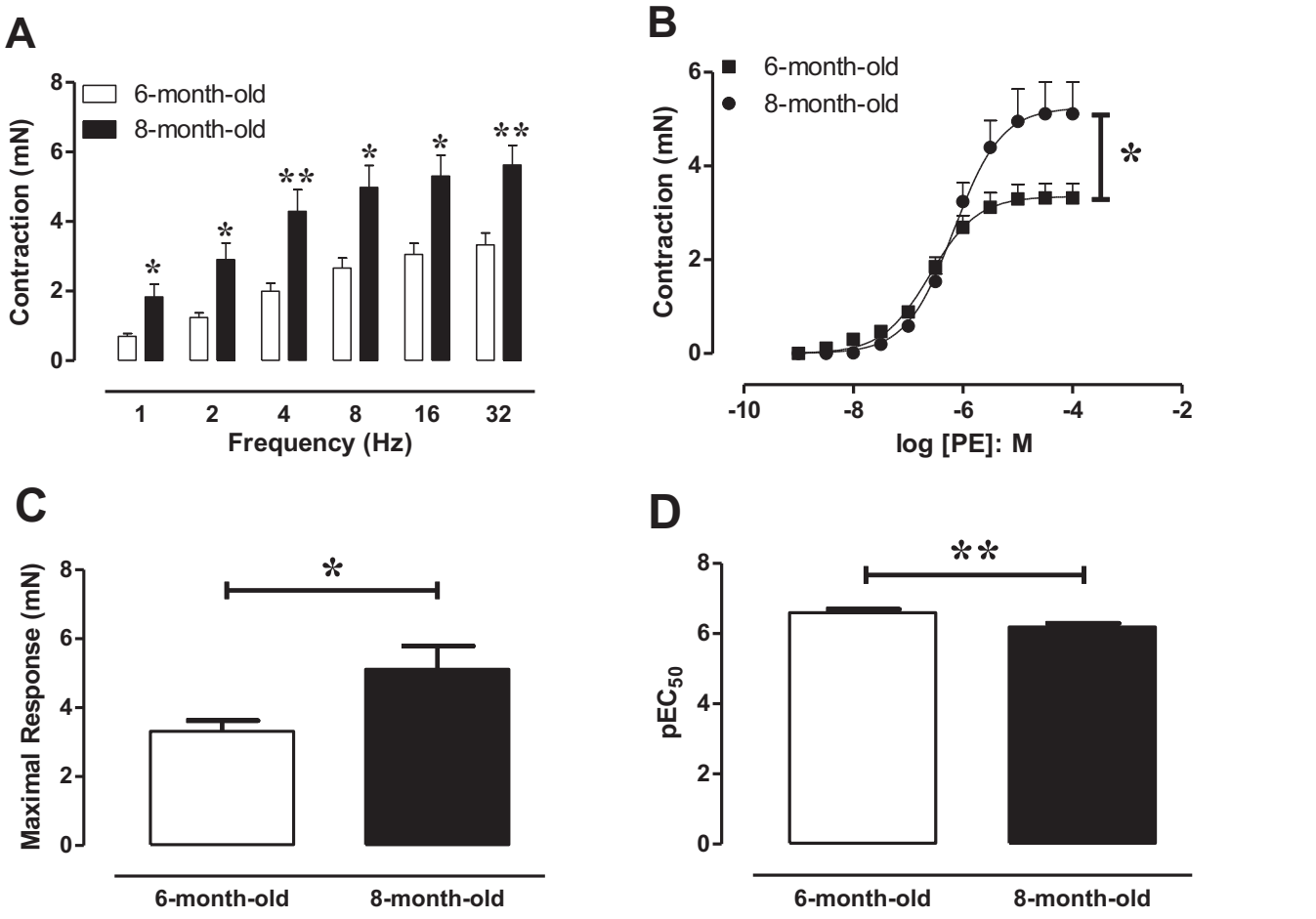

FIGURE 3 - Prostate smooth muscle contraction induced by electrical-field stimulation (EFS 1-32 Hz; A), concentration-response curve to phenylephrine (PE; 1 nM – 100 μM; B), maximal response (C) and potency (D) in prostate from 6- and 8-month-old. Data represent the mean ± S.E.M. (n=8). $^{*}p<0.05$ and $^{**}p<0.01$ compared to 6-month-old group.

DISCUSSION

In the present study, we have demonstrated for the first time that 6- and 8-month-old rats exhibit prostatic dysfunction characterized by prostate smooth muscle hypercontractility. The increased prostate smooth muscle contractions start at around 5 to 6-months-old, and worse during the aging process. Specifically, 8-month-old rats exhibited an increased maximal response to the adrenergic agonist phenylephrine and to EFS compared to control and 6-month-old rats. In addition, the potency for phenylephrine was reduced in 8-month-old rats, indicating an increased age-dependent prostate sensitivity to this agonist.

Aging has been strongly associated with BPH in humans and animal models (Liu *et al*., 2019; Mazur, Helfand, McVary, 2012). The exact BPH pathophysiology remains unclear; however, some aging-associated conditions, such as increased oxidative stress, low-grade inflammatory state and autonomic dysregulation have been shown to play a key role in the development of BPH (Calmasini *et al*., 2020, Madersbacher, Sampson, Culig, 2019; Calmasini *et al*., 2018). Regarding autonomic dysregulation, Thiyagarajan and collaborators (2002) have demonstrated a leftward shift in the potency to α-adrenoceptor-induced prostate smooth muscle contractility in a rat model of BPH. Similarly, phenylephrine- and clonidine-induced prostate smooth

muscle contractions were also increased in rats with BPH (Vikram, Jena, Ramarao, 2010). Considering middle-aged rats, Calmasini and collaborators (2016) demonstrated that 10-month-old rats exhibited adrenergic impairments in the prostate, resulting in phenylephrine-induced prostate smooth muscle hypercontractility. In addition, we have demonstrated that 6- and 8-month-old rats exhibited prostate hypercontractility, which started at around 6 months of age. Interestingly, 8-month-old rats also exhibited higher sensitivity to phenylephrine, indicating alterations at the receptor level.

The neurogenic contractions induced by EFS reflect the tissue depolarization and consequent neurotransmitter release from the autonomic fibers. In the prostatic tissue from humans and rodents, noradrenaline is the main neurotransmitter released upon neuronal depolarization (Sievert *et al*., 2019; Pennefather*et al*., 2000). This neurotransmitter elicits smooth muscle contraction through post-junctional α1-adrenoceptor stimulation, Gq protein activation and Ca^{2+}-IP_3 signaling-based downstream (Michel, Vrydag, 2006). In the present study, the contractions induced by EFS were greater in prostates from 8-month-old rats compared with the control group, corroborating the results obtained with direct α1-adrenoceptor stimulation. EFS-induced prostate smooth muscle contractions were higher in 8-month-old rats compared with the 6-month-old group, indicating that prostate dysfunction worsened as the rats aged. It is important to note that chronic α1-adrenergic receptor stimulation led to prostatic hyperplasia in rats, suggesting that the increased EFS could be involved in prostate abnormalities found in aged rats (Golomb*et al*., 1998).

Few studies in the literature have addressed prostate smooth muscle contractility in 6 to 10-month-old rats. According to the published data, 10-month-old rats exhibited EFS- and phenylephrine-induced prostate smooth muscle hypercontractility (Calmasini *et al*., 2016), which are of the same magnitude as in the 8-month-old rats presented here. This is a valuable finding, suggesting that increased prostate smooth muscle contractility achieves a plateau in 8-month-old rats. Based on this finding, it is reasonable to say that 8-month-old rats would be a greater model to test new curative treatments to BPH-related smooth muscle hypercontractility than 10-month old rats, saving time and money related to animal maintenance in the facilities. On the other hand, 6-month-old rats would be a suitable model for BPH prevention studies, since prostate hypercontractility is initiated at around this age.

It is important to note that a number of animal models for BPH are available in the literature. For instance, rats supplemented with testosterone exhibit prostate enlargement and epithelial hyperplasia (Zhang *et al*., 2021). Similarly, mice fed a high-fat diet also develop BPH, characterized by prostate overgrowth and smooth muscle hypercontractility (Calmasini *et al.,* 2018). However, for these models, some aspects should be taken into consideration. For the testosterone-induced BPH, it is worth noting that the maximal response elicited by alpha-adrenoceptor agonists in the prostatic tissue is not altered (Thiyagarajan, Kaul, Ramarao, 2002), which restricts the use of this model to explore pathways and drugs that reduce prostate contractility. Besides, it is well characterized that the aging process leads to a reduction in testosterone levels, which is implicated in BPH genesis and/or progression (Banerjee *et al*., 2018). Therefore, the testosterone-induced BPH model is not in accordance with the real environment when BPH originates in humans. The HFD-induced BPH is another important model employed to better understand the BPH pathophysiology and to test new drugs under obesity conditions, which also involves impaired glucose and lipid homeostasis (Zhang *et al*., 2021). However, BPH in humans also occurs in non-obese and metabolic health individuals. Taking this scenario into consideration, animal models that involve prostate hypercontractility secondary to the aging process are more representative of that which occurs naturally in humans and are valuable tools to better understand BPH.

In the present study we have focused in prostate contractility induced by EFS and alpha-1 adrenoceptor stimulation, which involve smooth muscle activation. Studies have shown that aging is associated with altered smooth muscle phenotype (Schauer, Rowley, 2011). The switch from smooth muscle cells to a synthetic phenotype contributes to prostate cell proliferation and might be involved in prostatic dysfunctions during the aging process (Schauer, Rowley, 2011). However, the synthetic

phenotype exhibits low contractile profile; therefore, considering that 6- and 8-month-old rats exhibit prostate hypercontractility, it is unlikely that altered smooth cells, at least to the synthetic phenotype, take place during this process.

Besides the adrenergic pathway, it is well characterized that human and rodent prostates are also innerved by cholinergic and non-adrenergic non-cholinergic neurons (Michel, Vrydag, 2006), which are altered during the aging process. For instance, 10-month-old rats exhibited increased prostate smooth muscle contractility to the purinergic agonist alpha, beta-methylene ATP and reduced isoproterenol (a beta-adrenoceptor agonist)-induced prostate smooth muscle relaxation(Calmasini *et al*, 2016). In aged mice, increased PSM contraction induced by purinoceptor activation was associated with reduced ATP breakdown in the prostate (White *et al*., 2015). Therefore, it is possible that pathways other than the adrenergic one may also be involved in prostate hypercontractility in 6- and 8-month-old rats. Further investigations are needed to confirm this hypothesis.

In conclusion, our findings confirm that the aging process is related to prostate smooth muscle hypercontractility and reveal 6- and 8-month-old rats as new models to better understand the pathophysiology of BPH.

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