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Beneficial effects of lipidic extracts of saladette tomato pomace and *Serenoa repens* on prostate and bladder health in obese male Wistar rats

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Abstract

BACKGROUND: Obesity is associated with increased risk of a number of serious medical conditions, including urological disorders. This study investigated the effect of lipidic extracts of saladette tomato pomace (STP) and *Serenoa repens* (SR) on the prostate and bladder in a rat obese model induced by high-carbohydrate diet.

RESULTS: High-sucrose-fed rats showed higher prostate weight as well as increased contractility and stromal and epithelial hyperplasia in the prostate. Treatment with STP and SR improved contractility and diminished hyperplasia and hypertrophy in the prostate. Obese animals also showed impaired bladder contractility, but neither extract reversed this deterioration. In the histological study, a disarray in the process of smooth muscle cell proliferation with non-parallel fibers was observed; interestingly, treatment with STP and SR led to improvement in this derangement.

CONCLUSION: These findings indicated impaired contractility and hyperplasia in the prostate and bladder of obese rats induced by high sucrose. STP and SR could enhance prostate function by reducing contractility and hyperplasia and improve smooth muscle fiber structure and decrease cell proliferation in the bladder, suggesting their possible health-beneficial effects on lower urinary tract symptoms.

Keywords: obesity; rats; *Serenoa repens*; lycopene; prostate; bladder

INTRODUCTION

Obesity has reached world epidemic proportions in recent decades and has become the main concern in public health. It has been associated with chronic diseases such as cardiovascular disorders, type II diabetes and several types of cancer and with abnormal growth of the prostate gland in men.[1–3]

Obesity has been identified as one of the factors involved in the onset of prostate cancer and benign prostatic hyperplasia (BPH), which has been considered as a new metabolic disease.[4–7] Several mechanisms have been postulated to explain the relationship, including insulin resistance, hormonal imbalance (androgen/estrogen ratio) and increased activity of the autonomous nervous system.[8–10] Previous studies show that obesity and hyperinsulinemia are strongly associated with increased prostate volume and prostatic hyperplasia.[9] Furthermore, Ribeiro *et al.*[11] demonstrated that diet-induced obesity causes increased cell proliferation and modifies signaling pathways such as PI3K and the estrogen receptor in the rat prostate.

Prostate enlargement contributes to the appearance of lower urinary tract symptoms (LUTS) caused by an increase in smooth muscle tone.[12,13] Current treatments for prostatic hyperplasia include anti-androgens such as finasteride which have several side effects that can cause severe inconvenience to patients and ultimately lead to abandoning treatment. Natural alternatives have been proposed for treatment of this disease, such as lipidosterolic extracts of *Serenoa repens* (SR)[14] and lycopene, which can be extracted from saladette tomato pomace (STP), finding positive results in a decrease in prostate gland size.[15,16] SR powder is a complex mixture of free and esterified long-chain fatty acids, polyprenes and phytosterols that is widely used in the treatment of patients with BPH.[17] This composition of divers ingredients confers to the drug an ability to exhibit several pharmacodynamic properties, which in turn lead to a wide range of mechanisms of action. On the other hand, tomato, containing phytonutrients such as natural tocopherols, phytoene, phytofluene, β-carotene and, mainly, lycopene, which is considered a potent antioxidant among the

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carotenoids, may provide a useful approach for reducing the risk of inflammatory diseases such as BPH/LUTS.[18,19]

The main objective of this study was to investigate alterations in size and smooth muscle tone of the prostate and bladder produced as a consequence of carbohydrate-rich diet-induced obesity, as well as to evaluate the effect of lipidic extracts of STP and SR.

EXPRIMENTAL

Methods

All procedures and protocols of the present study were approved by the ethics committee of our institution (CICUAL-CINVESTAV-IPN) and followed the recommendations of official guidelines (NOM-062-ZOO-1999) establishing the technical specifications for production, care and use of laboratory animals.

Animal model

Fifty-five male Wistar rats obtained from our animal facility (CINVESTAV-Sede Sur) were kept in a controlled temperature room with 12/12 h light/darkness cycle. When the animals reached 10 weeks of age, they were divided into two groups: the first group ($n = 27$) was subjected to a normal diet with standard laboratory chow LabDiet 5008 (LabDiet, St. Louis, MO, USA) and water *ad libitum* (control group); the second group ($n = 28$) was subjected to a hypercaloric diet consisting of standard laboratory chow and 300 g L^{-1} sucrose solution *ad libitum* for 40 weeks. Then the animals were randomized and housed individually. After this, the two groups were each divided into three subgroups to receive orally either vehicle (corn oil), SR at 25 mg kg^{-1} day^{-1} or dehydrated STP at a dose of 5 mg lycopene kg^{-1} day^{-1}. The treatments were administered for 4 weeks; during this period, body weight and food and liquid consumption were evaluated daily. After 4 weeks of treatment, the animals were sacrificed by decapitation.

Preparation of extracts

The SR extract was prepared in our laboratory following the patent for Permixon®[20] by mixing dry *S. repens* berry powder (GNC, Syracuse, NY, USA) with corn oil. The dehydrated SR powder contains phytosterols (campesterol, cycloartenol, lupenone, lupeol, β-sistosterol and stigmasterol).[21] The content of several capsules (14.5 g) was mixed with 100 mL of corn oil, stirred at 60 °C for 1 h and filtered to remove solid particles. The extract was administered orally for 4 weeks to its respective groups at a dose of 25 mg kg^{-1} day^{-1}; vehicle groups only received corn oil. For the STP extract, dehydrated saladette tomato variety (Labizet, Mexico City, Mexico) was used following the patent for Lyc-O-Mato® 15%.[22] The dehydrated STP containing lycopene as well as other phytonutrients (natural tocopherols, phytoene, phytofluene and β-carotene)[18] was prepared by mixing sun-dried tomato powder with corn oil (25:75 w/v). The mixture was subjected to mechanical stirring and sonication at 40 kHz for 1.5 h and filtered to remove solid particles. Lycopene content was evaluated spectrophotometrically at 503 nm. The extract was administered orally for 4 weeks using corn oil as vehicle.

Hemodynamic parameters

After 40 weeks on the hypercaloric diet, blood pressure and heart rate were measured using a plethysmograph (LE5002 storage pressure meter, Barcelona, Spain) by the tail-cuff technique. The animals were previously trained to prepare them for testing by this method. Four measurements were performed on each animal and the average value was used for analysis.

Biochemical parameters

The animals were subjected to a 12 h fasting period before sacrifice; trunk blood was taken and serum was obtained by centrifugation. Glucose, triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDLc) and low-density lipoprotein cholesterol (LDLc) assays were conducted using enzymatic/colorimetric assay kits (Spinreact, Girona, Spain), and spectrophotometry was performed with a Microlab 100 (Merck, Dieren, The Netherlands).

Histopathology

Bladders and prostates were excised from the sacrificed animals. Excess adipose and connective tissues were removed from the organs and fixed in 100 mL L^{-1} formalin/phosphate-buffered saline solution. The organs were then dehydrated with a histokinette (Was Bath® E7606, Biopur Diagnostics, Rosario, Argentina) for inclusion in paraffin. The organs were sliced at 6 µm with a microtome and stained with hematoxylin and eosin (H&E) for microscopic analysis.

Smooth muscle reactivity

The ventral lobes of prostates and slices (1.5 mm × 3.5 mm) of bladders extracted from the sacrificed rats were used to test the smooth muscle reactivity *in vitro*. For this purpose, after excision and slicing, the tissues were immediately put into Krebs solution (112 mmol NaCl, 5 mmol KCl, 25 mmol $NaHCO_3$, 1.2 mmol $MgSO_4$, 11.5 mmol glucose, 1.2 mmol KH_2PO_4 and 1.25 mmol $CaCl_2$ L^{-1}) at 37 °C and placed in isolated tissue chambers while bubbling 95% O_2/5% CO_2. The tissues were stabilized for 60 min at 1 g resting tension. After this, maximal contraction using a depolarizing solution of KCl (17 mmol NaCl, 100 mmol KCl, 25 mmol $NaHCO_3$, 1.2 mmol $MgSO_4$, 115 mmol glucose, 1.2 mmol KH_2PO_4 and 1.25 mmol $CaCl_2$ L^{-1}) was performed. After 1 h of stabilization, concentration–response curves to phenylephrine (PE) and isoproterenol (ISO) of prostate tissue and to PE and acetylcholine (ACh) of bladder tissue were constructed. Tension was recorded with force transducers (Grass FT03®, Adinstruments, San Diego, CA, USA) coupled to a computer with registering software.

Statistical analysis

Data are shown as mean ± standard error of mean (SEM). The data were subjected to the Shapiro–Wilk test to verify normality, the Student *t* test to compare two groups, and analysis of variance (ANOVA) for multiple comparisons. The results were considered significant at $P < 0.05$.

RESULTS

Weight and food/liquid consumption

The general characteristics are shown in Table 1. Compared with the control groups, solid food consumption in the obese groups was lower (control groups: vehicle, 19.9 ± 0.2 g; SR, 19.5 ± 0.3 g; STP, 19.8 ± 0.3 g; obese groups: vehicle: 7.64 ± 0.15 g; SR, 7.7 ± 0.2 g; STP, 8.48 ± 0.2 g).

There were no significant differences among the studied groups regarding liquid consumption. By the end of week 40 of the hypercaloric diet, rat weights in the obese groups (634 ± 25.8 g) were

Table 1. Arterial blood pressure (MAP), heart rate (HR) and plasma glucose, triglycerides, total cholesterol, LDLc and HDLc from all experimental groups

	Pre-treatment		Post-treatment					
			Control			Obese		
Item	Control	Obese	Vehicle	SR	STP	Vehicle	SR	STP
MAP (mmHg)	100 ± 2	112 ± 2**	99 ± 1	98 ± 1	101 ± 2	117 ± 6**	110 ± 2**	99 ± 2††
HR (beats min^{-1})	368 ± 7	370 ± 7	386 ± 14	380 ± 12	368 ± 7	398 ± 21	382 ± 10	396 ± 10
Glucose (mg dL^{-1})			88 ± 6	94 ± 5	94 ± 6	120 ± 6*	121 ± 5*	119 ± 8*
Triglycerides (mg dL^{-1})			63 ± 14	73 ± 13	58 ± 5	148 ± 15**	135 ± 21**	90 ± 13†
Total cholesterol (mg dL^{-1})			49 ± 8	51 ± 7	50 ± 4	57 ± 3	57 ± 4	51 ± 4
LDLc (mg dL^{-1})			26 ± 2	27 ± 2	23 ± 2	26 ± 1	27 ± 2	24 ± 1.6
HDLc (mg dL^{-1})			29 ± 2	27 ± 2	26 ± 2	25 ± 2	28 ± 2	27 ± 1.6

Data are mean ± SEM. *$P < 0.05$, **$P < 0.01$ *vs* vehicle control; †$P < 0.05$, ††$P < 0.01$ *vs* vehicle obese.

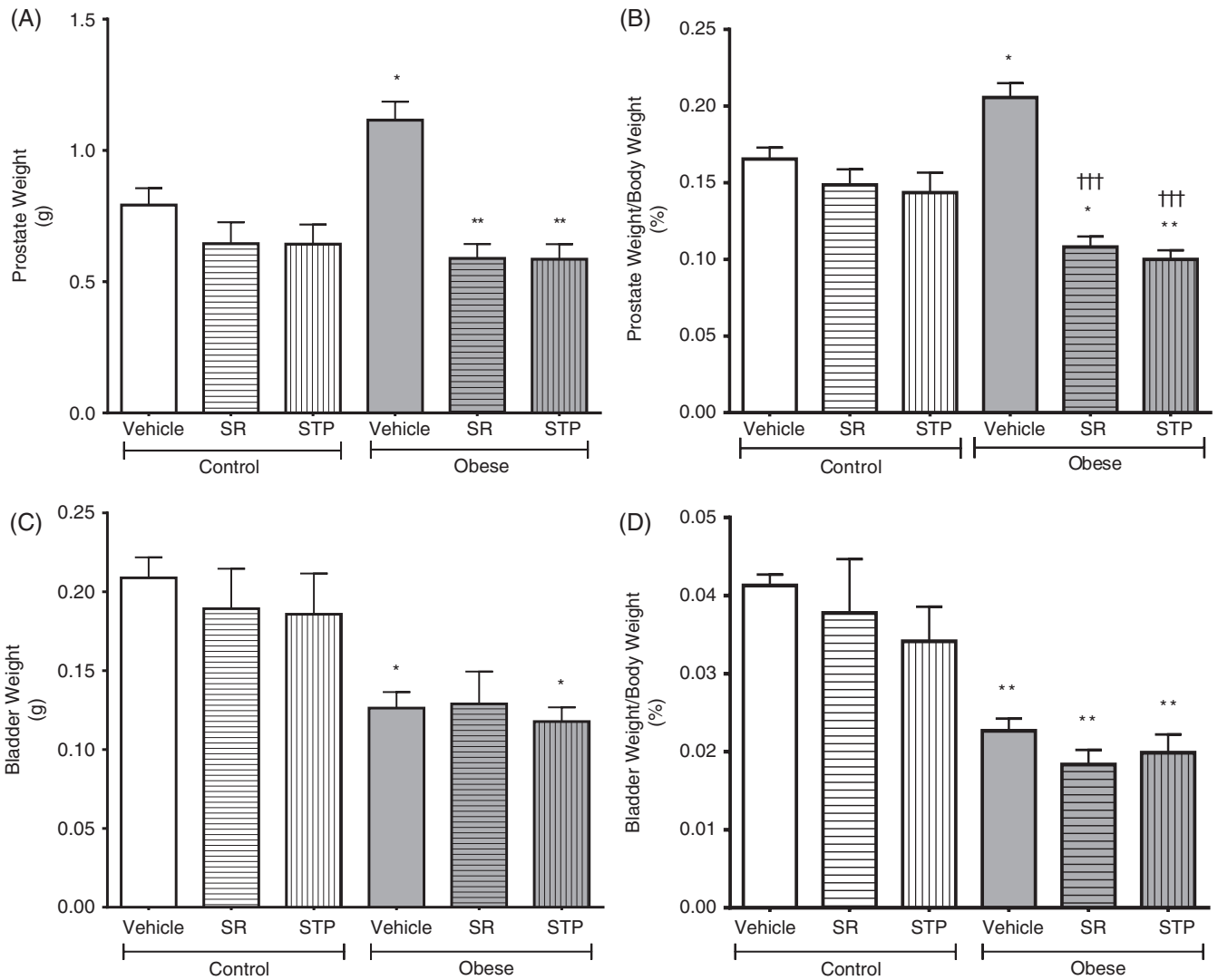

Figure 1. (A) Absolute and (B) relative weights of prostate gland and (C) absolute and (D) relative weights of bladder. Control: vehicle ($n = 8$), SR ($n = 9$), STP ($n = 10$). Obese: vehicle ($n = 10$), SR ($n = 9$), STP ($n = 9$). Data are mean ± SEM. *$P < 0.05$, **$P < 0.01$ *vs* vehicle control; †††$P < 0.001$ *vs* vehicle obese.

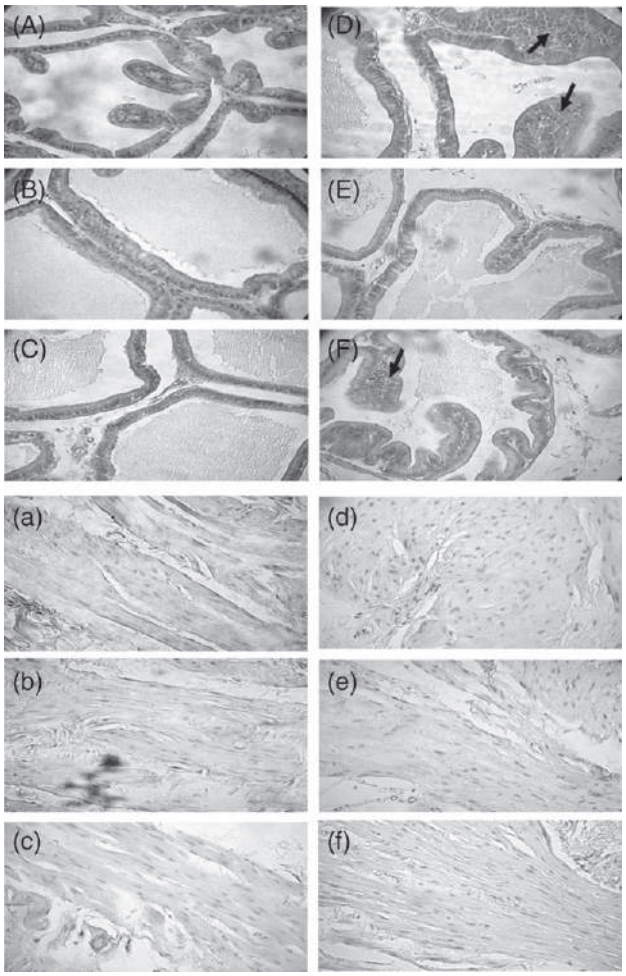

Figure 2. Histological analysis of (A–F) prostate and (a–f) bladder. Control group: vehicle (A), SR (B), STP (C). Obese group: vehicle (D), SR (E), STP (F). Arrows indicate cell proliferation. Magnification × 100. Control group: vehicle (a), SR (b), STP (c). Obese group: vehicle (d), SR (c), STP (f). Staining was performed with H&E. Magnification × 40.

significantly higher ($P < 0.01$) than those in the control groups (489.4 ± 16.7 g), and no change was observed over the 4 weeks of treatment.

Before treatment with the extracts, the obese groups had significantly higher mean arterial pressure. By the end of treatment, the obese animals treated with STP showed a significant decrease in this parameter. There were no significant differences in heart rate after treatment. In regard to biochemical parameters, there were significantly higher levels of glucose and triglycerides in obese *versus* control animals. Treatment with STP significantly decreased triglyceride levels, while no changes were observed in total cholesterol, LDLc and HDLc (Table 1).

Prostate and bladder morphology and histology

There was a significant increase in both absolute and relative weights of the prostate in obese animals (Figs 1A and 1B). Bladder weights showed the opposite trend, i.e. lower absolute and relative weights of bladders from obese animals ($P < 0.05$) (Figs 1C and 1D). Histological alterations observed in prostate tissue are shown in Fig. 2. The tissues from control animals treated with vehicle and the extracts showed no alterations in their structure (Figs 2A–2C); however, in obese animals treated with vehicle, epithelial hyperplasia as well as cell hypertrophy was observed (Fig. 2D). This alteration was reversed by treatment with SR (Fig. 2E). Treatment with STP led to partial regeneration of the microarchitecture, with some hyperplastic processes being observed (Fig. 2F).

No alteration in bladder structure was observed in the three subgroups of control rats (Figs 2a–2c). In the micrographs of bladder tissue from obese animals treated with vehicle, a disarray in the process of smooth muscle cell proliferation with non-parallel fibers was seen (Fig. 2d). Treatment with SR and STP resulted in a decrease in cell proliferation and parallel smooth muscle fibers (Figs 2e and 2f).

In vitro contractility

Prostate contractility in obese animals with vehicle was significantly higher than in control rats (Fig. 3A). There was no significant difference in maximum response to PE among control rats regardless of treatment (Fig. 3B), but obese animals treated with STP and SR had significantly lower prostate contractility compared with vehicle-treated rats (Fig. 3C). Moreover, there was a higher relaxation in response to ISO in prostates from obese animals treated with vehicle *versus* control rats (Fig. 4A). The extracts did not show any significant difference in relaxation to ISO in either control or obese animals (Figs 4B and 4C).

In bladder tissues, there was a lower contractility response to PE in obese animals (Fig. 5A), but no significant differences were observed with any of the treatments (Figs 5B and 5C). There were no significant differences in bladder contractility to ACh among all groups (Fig. 6).

DISCUSSION

To the best of our knowledge, this study is the first report describing alterations in prostate and bladder contractility and hyperplasia in obese rats induced by high-carbohydrate diet, as well as the beneficial effect of SR and STP extracts. The positive effects observed in these data were probably due to the mixture of components of each extract, namely phytosterols in the case of SR and a mixture of phytonutrients in the case of STP.

We demonstrated that obesity leads to prostate enlargement, consistent with previous studies in which obese men had a significantly larger prostate size compared with non-obese men.[23–25] Although the mechanism linking prostate enlargement and obesity remains unclear, pro-inflammation, insulin resistance and adipokine abnormality play plausible roles in prostate enlargement. For example, a previous study has demonstrated that increased IL-6 levels are associated with BPH.[26] Such changes, resulting from systemic insult, may increase the pro-inflammatory environment systemically, thus supporting pro-inflammatory signaling within prostate tissue. Leptin may also play an important role in such association owing to its mitotic and antiapoptotic effect. It has been demonstrated that leptin promotes prostatic cell proliferation and, as a consequence, gland weight.[27] Moreover, insulin resistance promotes an increase in cell proliferation of the prostatic epithelium.[9] Oxidative stress can cause atherosclerosis and then fibrosis in smooth muscle of prostatic arterioles, increasing the weight of the gland.[28] Prostate growth has also been correlated with biochemical parameters and hypertension, as well as alterations of contractility by over-activation of parasympathetic neural pathways.[29] Therefore it is rational to postulate that at least some of these above-mentioned factors may be involved in explaining the relationship between prostate enlargement and obesity in our model.

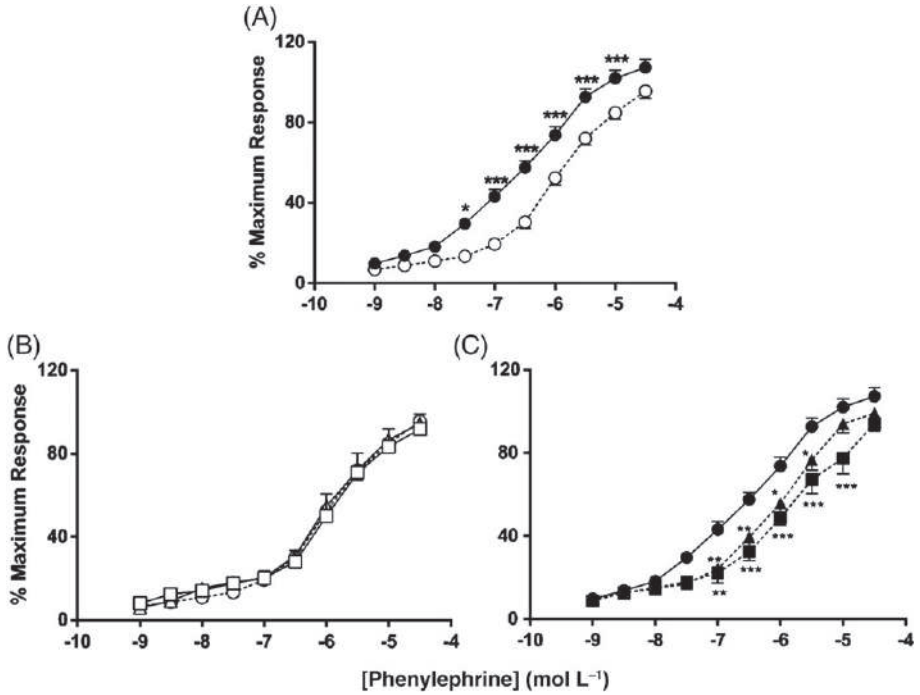

Figure 3. (A) Comparison of concentration–response curve to phenylephrine of prostate tissue obtained from control (○, $n = 8$) or obese (●, $n = 10$) rats, (B) control group: vehicle (○, $n = 8$), SR (△, $n = 9$), STP (□, $n = 10$) and (C) obese group: vehicle (●, $n = 10$), SR (▲, $n = 9$), STP (■, $n = 9$). Data are mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* obese group.

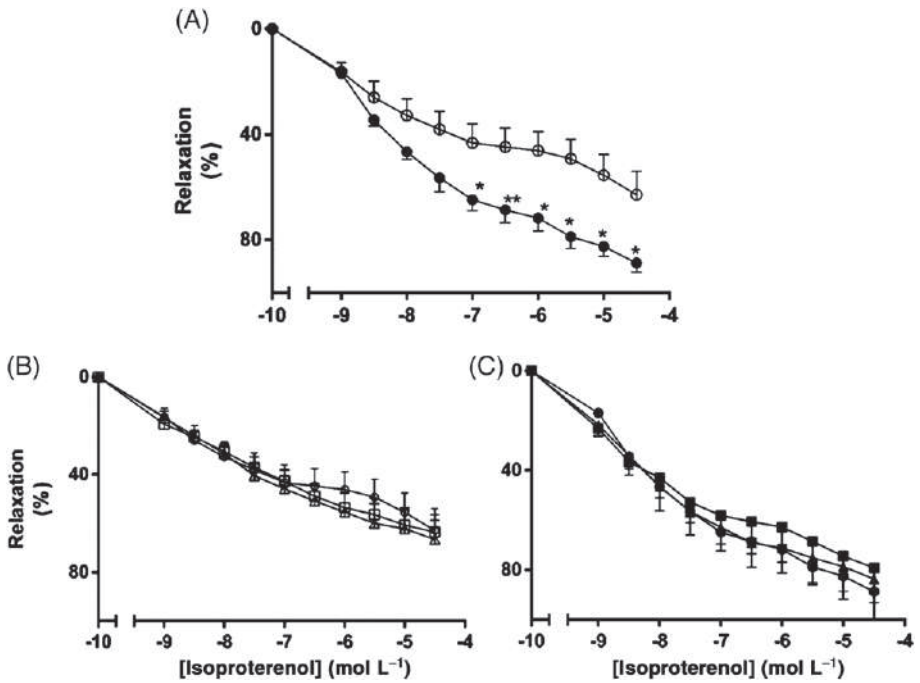

Figure 4. (A) Comparison of concentration–response curve to isoproterenol of prostate tissue obtained from control (○, $n = 8$) or obese (●, $n = 10$) rats, (B) control group: vehicle (○, $n = 8$), SR (△, $n = 9$), STP (□, $n = 10$) and (C) obese group: vehicle (●, $n = 10$), SR (▲, $n = 9$), STP (■, $n = 9$). Data are mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* control group.

SR could decrease prostate gland weight in obese animals likely via its antiproliferative and anti-inflammatory effects.[17,30] A previous study demonstrated that SR is an antagonist of α-adrenergic receptors,[31] and could inhibit 5α-reductase, which may also be a possible mechanism involved. These results suggest the possibility of beneficial effects in patients suffering from LUTS.

Treatment with STP in obese animals also showed an effect in reversing the alterations in the prostate associated with obesity. We also showed a decrease in prostate weight, improvements in cell microarchitecture and improvements in tissue function in contractility testing. These effects are probably due to the mixture of phytonutrients contained in our extract and not to

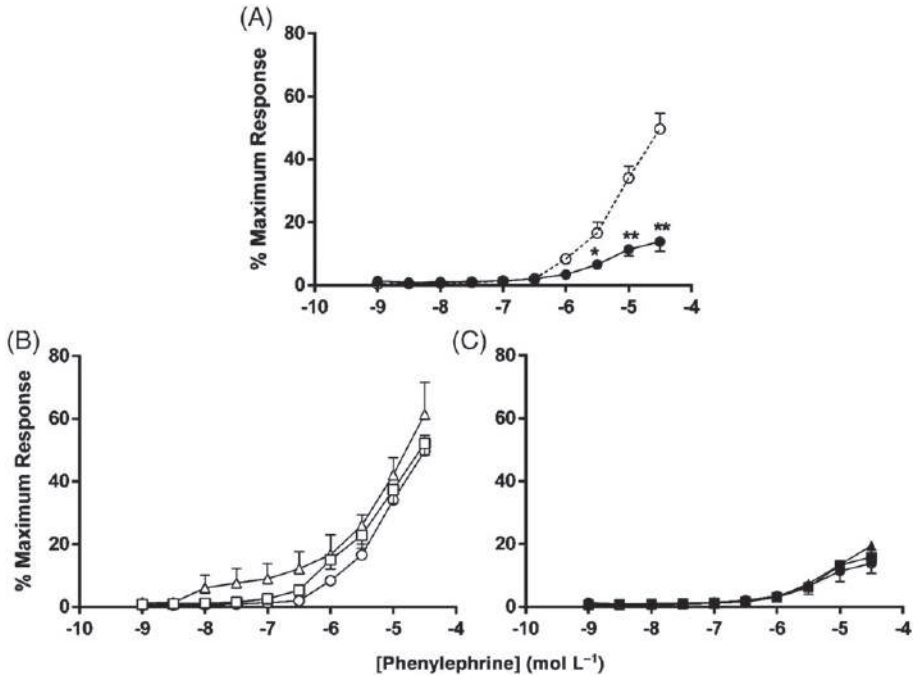

Figure 5. (A) Comparison of concentration–response curve to phenylephrine of bladder tissue obtained from control (○, $n = 8$) or obese (●, $n = 10$) rats, (B) control group: vehicle (○, $n = 8$), SR (△, $n = 9$), STP (□, $n = 10$) and (C) obese group: vehicle (●, $n = 10$), SR (▲, $n = 9$), STP (■, $n = 9$). Data are mean ± SEM. $^{*}P < 0.01$, $^{**}P < 0.001$ *vs* control group.

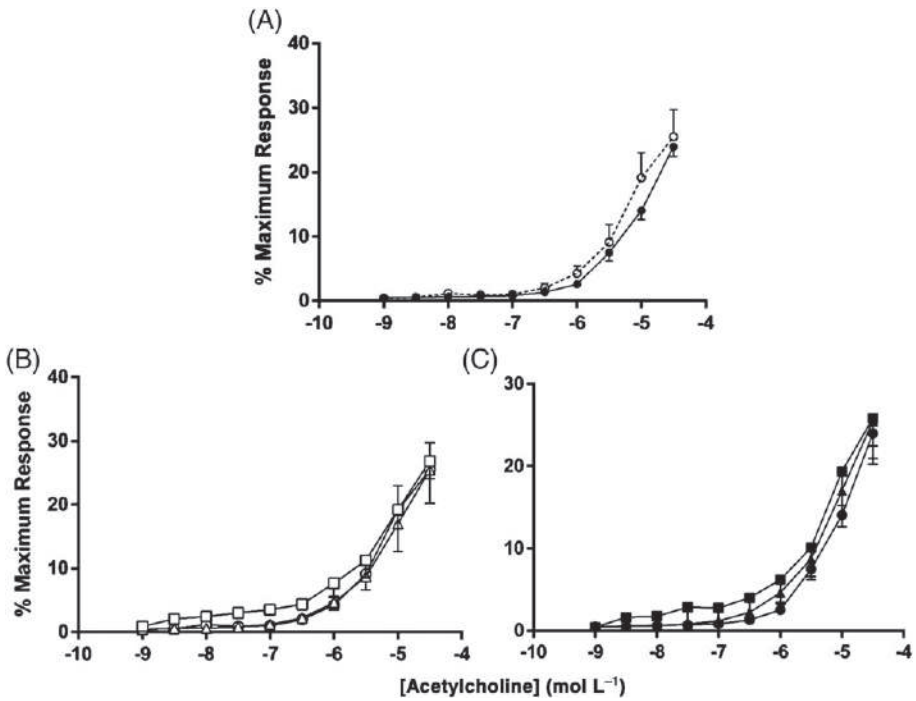

Figure 6. (A) Comparison of concentration–response curve to acetylcholine of bladder tissue obtained from control (○, $n = 8$) or obese (●, $n = 10$) rats, (B) control group: vehicle (○, $n = 8$), SR (△, $n = 9$), STP (□, $n = 10$) and (C) obese group: vehicle (●, $n = 10$), SR (▲, $n = 9$), STP (■, $n = 9$).

a particular element. STP could prevent the damage caused by reactive oxygen species, which are increased in obesity.[32,33] Other proposed mechanisms include inhibition of cell proliferation, apoptosis induction, inhibition of IGF-1 signal transduction and inhibition of androgen-dependent signaling.[15,34,35] Such mechanisms can be complementary and can overlap at the same time. Indeed, a combination of all these mechanisms may be responsible for the improvements we observed in this study.

Obesity altered bladder weight; however, contrary to what we expected, there was a decrease in the weight of this organ. There are very few reports in the literature focusing on this effect. It has been suggested that increased adipose tissue alters bladder consistency through a reduction of bladder volume, leading

to reduced bladder thickness;[36] oxidative stress might also be involved, because it leads to fibrosis, alteration in the microarchitecture, reduction in wall thickness and contractility alteration.[37] Histological analysis showed that obesity causes a disarray of the normal distribution of smooth muscle cells in the bladder, as well as increased cell proliferation in the inferior part of the bladder. Our results show that these alterations were improved by extracts that contain a mixture of phytonutrients or phytosterols.

All these changes in prostate and bladder sizes are known as 'static and dynamic components' to the development of bladder outlet obstruction from LUTS/BPH.[38] We suggest that our data presented both components. The static component is mediated by the volume effect of BPH. It is characterized by an increase in epithelial and stromal cell numbers (hyperplasia) in the periurethral area of the prostate, while the dynamic component is characterized by a modification of muscle tone of the bladder neck and prostate.

The alterations observed showed increased sensitivity to PE in the prostate and bladder and to ISO in the prostate. These changes reflect that there are alterations in the adrenergic system, in accordance with a previous report that demonstrated prostate growth associated with adrenergic sensitivity.[39] The increased sensitivity to adrenergic agonists could suggest a process of up-regulation of adrenergic receptors. Contrary to these results, it has been reported that BPH is associated with a decrease in β-adrenergic receptor density, causing impaired relaxation.[40] This discrepancy requires future research regarding the activity of adrenergic receptors in these conditions, as well as possible alternative explanations such as catecholamine breakdown or a possible increase in reuptake.

The present data demonstrated that contractility to PE in the bladder decreased in obese tissues. The density of adrenergic receptors in the bladder, which are the main mediators of the filling process, preventing urine flow by closing the passage to the urethra probably, is higher in the inferior part of the bladder. The decreased contractile activity contributes to urinary symptoms such as urinary incontinence.[12,13] According to our histological observations, the decreased contractility may be explained by the disarray of smooth muscle fibers.

CONCLUSIONS

The present findings showed the alterations in the prostate and bladder in a rat obese model induced by chronic sucrose ingestion. Moreover, SR and STP extracts could be a potential supplement with a role in managing obesity in relation to the appearance of LUTS.

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