Morphological and functional changes in urinary bladder of multiparous female rats submitted to resistance exercise

Alterações morfológicas e funcionais da bexiga urinária de ratas múltiparas submetidas ao exercício resistido

Monise Moreno,1,2 Fernanda Milani Magaldi,1,2 Cristiane Milani Magaldi,1,2 Fernando Luiz Affonso Fonseca,2,3 Eduardo Mazuco Cafarchio,4 Ricardo Aparecido Baptista Nucci,1,5 Monica Akemi Sato,4 Laura Beatriz Mesiano Maifrino2

ABSTRACT

Objective: investigate whether resistance exercise can induce morphological and functional alterations in urinary bladder (UB) in multiparous rats. Methods: we used 40 adults female Wistar rats of which 20 were nulliparous and 20 were multiparous submitted to different volumes of moderate resistance exercise (one, three or ten weeks). Animals were submitted to the functional evaluation of UB. At the end of the protocol, the UB was removed, weighed and stained with hematoxylin and eosin for structural evaluation, and picrosirius red for collagen fibers. Results: we observed that multiparity promoted increase in body mass, reduction in UB layers, decrease in volume densities of collagen fibers I and III. However, 10-weeks of training was able to reverse the negative effects of multiparity. Conclusion: the intervention of physical exercise in 10 weeks seems to cause greater benefit in UB of multiparous animals by preventing morpho-functional changes that trigger lower urinary tract symptoms, such as urinary loss. Keywords: urinary bladder; parity; exercise; pregnancy; incontinence urinary; rats.

RESUMO

Objetivo: investigar se o exercício resistido pode induzir alterações morfológicas e funcionais na bexiga urinária (BU) em ratas múltiparas. Métodos: foram utilizadas 40 ratas Wistar adultas, sendo 20 nulíparas e 20 múltiparas, submetidas a diferentes volumes de exercício resistido moderado (uma, três ou dez semanas). Os animais foram submetidos à avaliação funcional da BU. Ao final do protocolo, a BU foi retirada, pesada e corada com hematoxilina e eosina para avaliação estrutural, e picrosirius red para fibras colágenas. Resultados: observamos que a multiparidade promoveu aumento da massa corporal, redução das camadas da BU, diminuição das densidades de volume das fibras colágenas I e III. No entanto, 10 semanas de treinamento foram capazes de reverter os efeitos negativos da multiparidade. Conclusão: a intervenção do exercício resistido por 10 semanas parece trazer maior benefício na BU de múltiparas por prevenir alterações morfofuncionais que desencadeiam sintomas do trato urinário inferior, como a perda urinária. Palavras-chave: bexiga urinária; paridade; exercício físico; incontinência urinária; gravidez; ratos.

1 São Judas Tadeu University. Laboratory of Morphological and Immunohistochemical Studies – São Paulo (SP), Brazil.
2 Faculty of Medicine of the ABC District. Laboratory of Clinical Analysis – Santo André (SP), Brazil.
3 Federal University of São Paulo (UNIFESP). Department of Pharmaceutical Sciences – São Paulo (SP), Brazil.
4 Faculty of Medicine of the ABC District. Department of Morphology and Physiology – Santo André (SP), Brazil.
5 University of São Paulo. Faculty of Medicine. Department of Pathology – São Paulo (SP), Brazil.
6 Dante Pazzanese Institute of Cardiology – São Paulo (SP), Brazil.

Corresponding author: Ricardo Aparecido Baptista Nucci
Department of Pathology, Faculty of Medicine of the University of São Paulo, Av.: Dr. Arnaldo, 455, Postal Code: 01246903 – São Paulo (SP), Brazil
E-mail: nucci.ricardo.ab@gmail.com
Received in 10/02/2023 - Accepted for publication in 14/06/2023.
INTRODUCTION

Pregnancy and the puerperium are characterized by marked changes in the woman's body structure, however, little is known about the changes undergone by the urinary bladder (UB) throughout the gestational period and in the postpartum period. Additionally, symptoms related to the lower urinary tract, such as stress urinary incontinence (UI), are increasingly frequent in women in this period of life, especially in multiparous women. The hormonal action and the mechanical impact caused by the increase in body mass and childbirth suggest that the UB may be subject to structural changes and, as a result, impact the appearance of these symptoms. This condition has several implications and complications negatively affecting the quality of life of many women since their social, family, professional and sexual activities are impaired, mainly by the appearance of UI symptoms which leads to a Public Health problem.

Pregnancy itself is related to the risk of urinary symptoms, which is increased when associated with vaginal delivery and multiparity. Although both clinical features and prognosis seems to be more severe with the advancing age, the presence of urinary symptoms in young adult women cannot be excluded. During pregnancy, the pelvic floor undergoes changes through hormonal influences and mechanical effects, to progressively adapt to the increasing size of the uterus and the need to distend during childbirth. The most frequent symptoms during pregnancy, whose peak prevalence is in the third trimester, are increased frequency and nocturia, together with incontinence.

Several authors agree that the practice of physical exercise, especially resistance training, can be a great ally in the strengthening and hypertrophy of large muscle groups such as the pelvic muscles. On the other hand, studies showed that strenuous physical activity would lead to increased abdominal pressure, combined with overload, stretching and weakening of the pelvic floor, resulting in UI.

In the current scenario, the hypotheses about the development of UI induced by strenuous physical exercise or heavy work are based on the fact that there may be stretching of the pelvic floor muscles or their weakening, due to the overload promoted by physical activity. However, more studies are needed to understand the effect of a routine of exercise in multiparous women and its relation with UI. Thus, we aimed to analyze the morphological and functional changes in urinary bladder of multiparous female rats submitted to resistance training.

MATERIALS AND METHODS

Division of Animals

The study was approved by the Ethics Committee in Research of the São Judas Tadeu University, São Paulo, under protocol number 024/16. It was used 40 female rats, Wistar strain, adults (250-300g, 8-9 months of age) divided in two major groups (n=20/per group): nulliparous and multiparous.

Animals were kept in cages in a room with controlled temperature (22–24 °C) and a light/dark cycle of 12/12 h. All mice were fed standard chow and ‘ad libitum’ water. At 6 months of age, the 20 females from the multiparous group were mated using two matrices and one male per cage, being kept together until the end of weaning. Then, to investigate the effects of exercise, each major group was divided in four groups (n=5/per group): (1) sedentary nulliparous (NS); (2) nulliparous trained for one week (N1); (3) nulliparous trained for three weeks (N3); (4) nulliparous trained for ten weeks (N10); (5) sedentary multiparous (MS); (6) multiparous trained for one week (M1); (7) multiparous trained for three weeks (M3); (8) multiparous trained for ten weeks (M10). Animals were weight during the study protocol.

Resistance Training Protocol

We used the ladder climbing model for the strength training, as this model show effect in a variety of biological systems. The training protocol was progressive with the load being adjusted every week. The load was composed of lead weights that were attached to their tails with tape. The animals were supposed to climb the ladder to reach the resting area at the top that was considered one repetition. The adaptation process underwent three alternate days with four repetitions every day.

Animals were trained once a day throughout three days per week for 1, 3 or 10 weeks with a rest interval of 60 seconds between repetitions. Each training session consisted of six climbs. The amount of weight carried by each rat was equivalent to 75% of its body weight (BW). The BW was measured at the beginning of each week of the experiment and the new weight to be carried by the animals during that week was adjusted according to their BW. No external stimulus was necessary so that the animals conduct the training.

Functional Analysis

At the end of the experimental protocol, animals were weighted, anesthetized and submitted to femoral artery and vein cannulation, urinary bladder cannulation after medial incision, and subsequent “in situ” administration of acetylcholine (0.1, 1.0 and 2.0 μg/mL), noradrenaline (0.5, 1.0 and 2.0 μg/mL), our saline, dripping (0.1 mL) into the urinary bladder. Pulsatile arterial pressure (PAP), mean arterial pressure (MAP), heart rate (HR), and intravesical pressure (IP) values were monitored and recorded in a data acquisition system (PowerLab 16 SP, AD Instruments, Melbourne, AU). A baseline IP value was established at 5 mmHg by saline infusion or urine withdrawal through the catheter inserted into the UB. At the end of the experiments, the animals received an overdose of sodium thiopental 170 mg/kg intravenously, for subsequent extraction of the urinary bladder and organ weighing.

Histological Techniques

Bladder samples were fixed in 10% buffered formaldehyde solution for a period of 24 hours.
Afterwards, they were dehydrated in ethyl alcohol, cleared in xylene and embedded in paraffin. Two non-serial histological sections of 5 μm thickness were collected on glass slides, stained with Eosin and Hematoxylin for the layers thickness analysis; and Picrosirius Red technique for the collagen fibers I and III analysis under a polarized light microscope.

**Morphological analyzes**

The images of the muscular layers, mucosa and the transitional epithelium (urothelium) of the UB were captured by a Sony video micro-camera attached to the Zeiss Microscope. The thickness (μm) of each layer (muscular, mucosa and epithelial) was measured using Axio Vision version 4.8 software in 4 fields/slice (0º, 30º, 60º and 90º), with 2 cuts/blade, performing 3 measurements/layer, totaling 24 measurements per layer/animal.

For the stereological analysis, 16 fields were analyzed in each cut, being 2 cuts/slide, making a total of 16 fields per animal, totaling 80 fields for each group. Image J software (NIH, Bathesda, USA) was used to quantify the volume density of type I (red) and III (green) collagen fibers, with the aid of a grid containing 196 points.

**Statistical Analysis**

For statistical analysis, data were presented as mean and standard error (mean ± SEM). ANOVA one-way (post hoc Tukey) was applied for data comparison between groups, with statistical differences set at p ≤ 0.05. For data management, GraphPad Prism 5.0 software was used (GraphPad Prism, Inc., San Diego, CA).

**RESULTS**

We observed that the body mass of the animals in the study increased throughout their growth, and that multiparity promoted a significant increase in the body mass of this group (Fig 1A). However, we observed in the adaptation week and in the 1-week training animals of both the nulliparous and multiparous groups had a decrease in body mass, which was recovered throughout the protocol of training at 3 and 10 weeks (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N1</th>
<th>N3</th>
<th>N10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous Adaptation 1-week Adaptation 1-week 3-week Adaptation 1-week 3-week 7-week 10-week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (g)</td>
<td>234±11</td>
<td>194±9a</td>
<td>248±13b</td>
</tr>
<tr>
<td>Multiparous Adaptation 1-week Adaptation 1-week 3-week Adaptation 1-week 3-week 7-week 10-week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (g)</td>
<td>260±15</td>
<td>245±15</td>
<td>270±15</td>
</tr>
</tbody>
</table>

*a p<0.05 vs N1 adapt; b p<0.05 vs N1 1-week; c p<0.05 vs N3 adapt; d p<0.05 vs N3 1-week; e p<0.05 vs N3 3-week; f p<0.05 vs N3 7-week; g p<0.05 vs N10 adapt; h p<0.05 vs N10 1-week
Figure 1B showed that multiparity did not promote a significant change in the percentage change in urinary bladder weight (Δ%UB) when compared with nulliparous groups (MS versus NS), however, physical exercise tended to decrease in all training times (N1, N3 and N10). On the other hand, in the multiparous groups, this decrease was only observed at 3 and 10 weeks (M3 and M10), however the M1 group showed a significant increase when compared to nulliparous groups submitted to resistance training. Our functional analysis showed that multiparity alone did not influence acetylcholine (Fig 1C) and noradrenaline (Fig 1D) reactivity in the urinary bladder in both groups (NS and MS). However, our data suggest a decrease in intravesical pressure (%ΔIP) of the animals due to a short-period of training with a significant increase in 10 weeks of training.

Our morphometrical analysis showed that multiparity promoted a significant reduction in the thickness of the muscular (16.4%) and epithelial (25.4%) layers, in addition to tend a decrease in the mucous layer (9.1%) in the animals of the MS multiparous group, when compared to the NS group (Table 2). Additionally, we found that the 3-week training (M3) causes a significant increase in the muscular (31.4%), mucosa (30.1%) and epithelial (22.9%) layers when compared to the MS group. When analyzing the M10 group, we observed that the thickness of the muscular layers reduced when compared to the M3 group and returned to the values of the N10 group; the mucous layer showed a significant increase in relation to all groups studied and the epithelial layer (M3) increased when compared to the MS group and the M10 returned to the values of the N10 group.
Regarding the collagen density, our results showed that the volume densities of collagen fibers in the muscular and mucosal layers of the UB, we found that multiparity promoted a significant decrease in collagen fibers I and III, when compared to nulliparous groups (Figure 2). Additionally, we observed that the increase in training period (M1, M3 and M10) reversed the process, when comparing to the MS group, similar data were observed in the control group. In the M10 group, collagen fibers I approached the values of N10, and collagen fibers III showed a significant increase when compared to its control (N10).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NS</th>
<th>N1</th>
<th>N3</th>
<th>N10</th>
<th>MS</th>
<th>M1</th>
<th>M3</th>
<th>M10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle layer</td>
<td>466.9±17.4</td>
<td>487.9±24.3</td>
<td>401.5±19.6</td>
<td>508.2±19.3</td>
<td>390.5±23.1</td>
<td>390.6±26.2</td>
<td>513.1±25.8</td>
<td>453.8±18.7</td>
</tr>
<tr>
<td>Mucosal layer</td>
<td>437.3±18.6</td>
<td>481.2±27.9</td>
<td>370.5±31.1</td>
<td>460.2±17.2</td>
<td>396.0±30.8</td>
<td>397.3±27.1</td>
<td>508.2±25.1</td>
<td>529.2±27.6</td>
</tr>
<tr>
<td>Epithelial layer</td>
<td>39.8±2.4</td>
<td>50.6±3.7</td>
<td>22.7±1.1</td>
<td>26.2±1.1</td>
<td>29.7±1.9</td>
<td>24.9±2.3</td>
<td>36.5±2.1</td>
<td>26.8±1.4</td>
</tr>
</tbody>
</table>

* p < 0.05 versus NS; *b* p < 0.05 versus N1; *c* p < 0.05 versus N3; *d* p < 0.05 versus N10; *e* p < 0.05 versus MS; *f* p < 0.05 versus M1; *g* p < 0.05 versus M3.
We observed that resistance exercise, at different frequencies, increased the amount of collagen and elastin and collagen, reinforcing the hypothesis of possible changes in the bladder structure in stressful situations, which may favor the prevalence of urinary incontinence symptoms also associated with obesity. In fact, the animals in the study were not weighed during pregnancy or shortly after the birth of the pups, or during breastfeeding, in order to avoid stress in these periods, both for the mother and the calf, which leads to a limitation in the study.

After the resistance exercise intervention, we found that all animals in the study suffered a decrease in body mass during the period of hormonal reorganization and structural adaptation, as previously described. Some studies also suggest that the increase in body mass along the growth/aging of the animals is expected and can be verified in our study. In addition to the effect of aging on this parameter, we also verified that the increase in body mass was more evident in the groups of multiparous animals, thus suggesting the influence of pregnancy and postpartum on this variable as previously described. Some studies also suggest that the increase in body mass, from the beginning of pregnancy to six months after delivery, may favor the prevalence of urinary incontinence symptoms also associated with obesity. In fact, the animals in the study were not weighed during pregnancy or shortly after the birth of the pups, or during breastfeeding, in order to avoid stress in these periods, both for the mother and the calf, which leads to a limitation in the study.

We also evidenced that multiparity alone or associated with physical exercise led to a reduction in bladder mass in all groups when compared to nulliparous groups seen as a reduction in the thickness of the UB layers, where there is possibly an adaptation related to the abdominal pressure exerted during resistance training, pregnancy and delivery. However, the group M10 was an exception as it remained with an increase in the thickness of the UB layers, which may cause urinary loss in pregnant and postpartum women.

We also observed that multiparity caused a decrease in collagen fibers type I and III in both the muscular and mucosal layers. However, a previous study showed that birth-induced trauma in adult rats increased the amount of collagen and elastic fibers in the bladder wall. The hypothesis that only multiparity can influence the reduction of these fibers can also be considered, mainly because it is a period of hormonal reorganization and structural adaptation, as type III collagen undergoes conformational changes to accommodate intravesical volume, and is also the first type of collagen to be synthesized in repair processes.

We observed that resistance exercise, at different frequencies, added to pregnancy and childbirth, is considered a bladder stressor, as we observed an increase in the volume density of collagen fibers type I and III in the bladder wall of the multiparous group (M), mainly in the muscular layer. Experimental studies showed an increase in collagen in the musculature with advancing age. In addition, studies have shown structural abnormalities in the detrusor muscle from overactive bladders, including increased elastin and collagen, reinforcing the hypothesis of possible changes in the bladder structure in stressful situations, which may trigger neurological abnormalities.

The loss of muscle strength in the pelvic region may be explained by the influence of pregnancy hormones, especially progesterone, which causes the bladder muscles to relax, allowing involuntary elimination of urine.
CONCLUSION

In summary, resistance physical exercise at different periods caused changes in the structure and function of the UB of multiparous animals. Thus, the intervention of a longer period of physical exercise (10 weeks) seems to cause greater benefit in the UB of multiparous animals. Being more interesting in the sense of preventing morpho-functional changes that trigger lower urinary tract symptoms, such as urinary loss.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

Funding Sources

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

REFERENCES


Como citar este artigo: