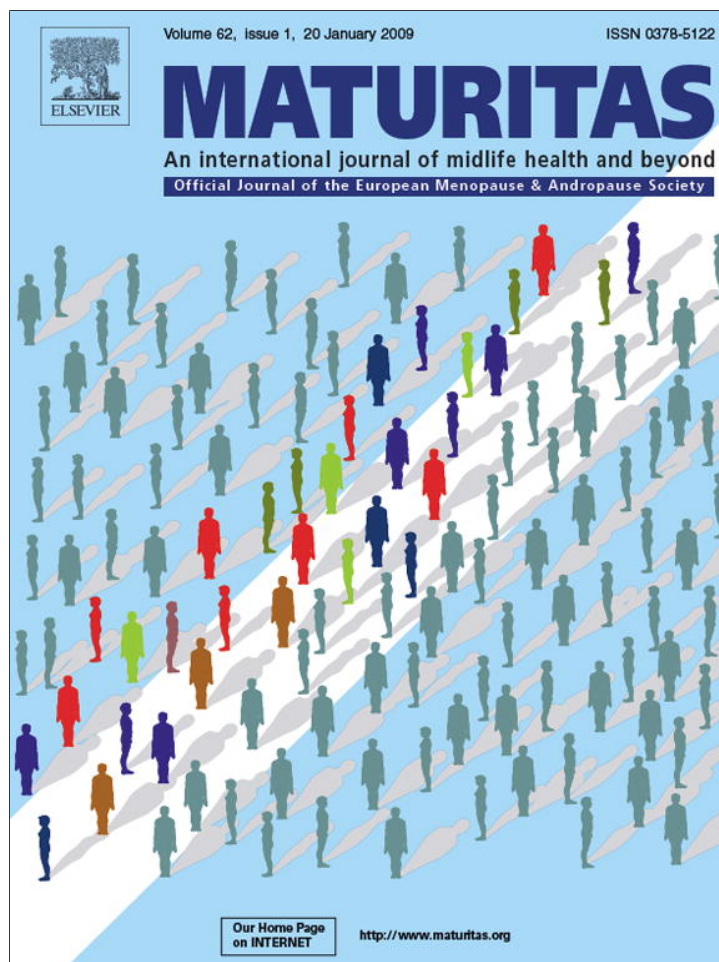


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## Morphological modification of female bladder after prolonged use of soy-based diets<sup>☆</sup>

Tatiane da Silva Faria<sup>a</sup>, Lavínia Leal Soares<sup>b</sup>, Jorge L. Medeiros Jr.<sup>a</sup>, Gilson T. Boaventura<sup>b</sup>, Francisco J.B. Sampaio<sup>a</sup>, Cristiane da Fonte Ramos<sup>a,\*</sup>

<sup>a</sup> Urogenital Research Unit-UERJ, Biomedical Center, State University of Rio de Janeiro, Av. 28 de Setembro, 87-fundos-FCM-terreo, 20551-030 Rio de Janeiro, RJ, Brazil

<sup>b</sup> Laboratory of Experimental Nutrition, Department of Morphology Fluminense, Federal University, Rio de Janeiro, Brazil

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### ABSTRACT

**Objectives:** The aim of this study was to compare the effects of a prolonged use of organic and transgenic soy upon the lipid profile and the collagen/muscle ratio of the detrusor muscle of the bladder.

**Methods:** Wistar rats were fed three different diets from weaning until sacrifice (15 months old): control group (CG) casein-based diet; organic soy group (OSG) organic soy-based diet; genetically modified soy group (GMSG) transgenic soy-based diet.

**Results:** There was no difference in the food consumption or in the diet isoflavone components among the groups. Comparing to CG, both OSG and GMSG groups presented a significant ( $p < 0.05$ ) reduction in the body weight, triglycerides, cholesterol and the smooth muscle of the detrusor and a significant ( $p < 0.05$ ) increase of collagen fibers number of the detrusor muscle.

**Conclusions:** These findings call into question that, the prolonged use of soy-based diets can be deleterious to the bladder by altering the collagen/muscle ratio what can cause bladder dysfunctions similar with that occurring during menopause.

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### 1. Introduction

It has been established that the alterations in circulating estrogen levels that occur during menstruation, pregnancy, and menopause can have marked effects on urogenital organ function [1,2]. Estrogen has been used clinically for the treatment of bladder dysfunctions in postmenopausal women, including stress urinary incontinence, urgency, frequency, unstable bladder contractions, underactive detrusor and recurrent infection [1,3–6].

Some of the bladder dysfunctions are related to the connective tissue and collagen whose fibers are found distributed between the layers of the bladder wall forming an organized pattern. The connective tissue has a predominantly mechanical function, which is the capacity to resist great forces of tension and compression as well as recover shape and structure when the action of these forces terminates [7].

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\* Corresponding author. Tel.: +55 21 2587 6499; fax: +55 21 2587 6121.  
E-mail address: [cristiane@pesquisador.cnpq.br](mailto:cristiane@pesquisador.cnpq.br) (C. da Fonte Ramos).

Several studies have suggested that hormonal deprivation and/or hormonal manipulation can lead to structural changes in the composition of smooth muscle and collagen content of the bladder [8–10]. In addition, several investigators have reported that ovariectomy results in significantly decreased smooth muscle (SM) density and increased connective tissue within the detrusor. Estrogen administration not only reversed these effects, but also increased bladder mass and SM density [8,11,12].

Soyfood, because its high phytoestrogenic isoflavone content has been used to improve cardiovascular disease risk factors [13–15] and in the relief of menopause symptoms [16].

Because of the increased soy consumption, transgenic soy has been created by genetic engineering to try to increase the production and reduce costs. Transgenic soy is a genetically modified organism to which three foreign genes are added, one of them from a virus and the others from a bacterium found in soil. The advantage of this modification is that the plant becomes resistant to glyphosate herbicides used to destroy weeds, which end up by being harmful to the crop itself. With this genetic modification, this problem does not occur, with consequent increased production and reduced costs [17]. In the other hand, organic soy is grown in an ecological manner without chemical products, not polluting the soil, contaminating the producer or modifying the product. This process however, implies a significant loss of productivity and profit [18].

Based on the concern about the use of genetically modified food on health and the loss of data about the effects of soy upon the connective tissue and collagen fibers of urinary bladder, the goal of this paper was to compare the effects of a prolonged use of organic and transgenic soy upon the lipid profile and the collagen/muscle ratio of the detrusor muscle of the bladder.

## 2. Materials and methods

### 2.1. Animals and groups

The biological assay was conducted on 24 female Wistar rats from the Laboratory of Experimental Nutrition (LABNE) of the Department of Nutrition and Dietetics, Nutrition College, Fluminense Federal University. The rats were divided into three groups of eight animals each, which received the experimental diets, as follows: control group (CG) fed a casein-based diet, organic soy group (OSG) fed an organic soy-based diet supplemented with 0.3 g cystine, and a genetically modified soy group (GMSG) receiving a transgenic soy-based diet.

During all the time the rats were kept in polypropylene cages, in an environment with controlled temperature at 22 °C and a 12-h light/dark period. Water and diets were offered ad libitum. Food consumption and animal weight were recorded daily. The research was approved by the Committee of Ethics from Fluminense Federal University.

All the 24 animals used in this study were the offspring of parents (preceding generation) who also received the same diet during all their lives. The animals were fed the above diets exclusively, from weaning until they were 1 year and 3 months old. At the end of this period, the animals were slaughtered under thiopental anesthesia (0.10 mL/100 g body weight), blood collection was made through cardiac puncture and serum stored at –20 °C in order to determine 17 $\beta$ -estradiol, cholesterol and triglycerides serum levels. Bladders were carefully removed and processed by routine methods.

### 2.2. Diets

Transgenic soy was supplied by Jasmine Integral Foods (Curitiba, PR, Brazil) and organic soy was supplied by Bunge Foods (Porto Alegre, RS, Brazil). The suppliers of the other components of the diets were: Maizena starch by Refinements of Maize Ltda (Granhuns, Recife, PE, Brazil), refined sugar by União (Rio de Janeiro, RJ, Brazil), Liza soy oil by Cargill Agricultural Ltda (Mairinque, SP, Brazil), Microcell cellulose by Blanver Ltda (Cotia, SP, Brazil) and cysteine, choline bitartrate, casein and mixtures of vitamins and minerals by Rhoster Commerce and Industry (Vargem Grande Paulista, SP, Brazil).

The soybeans were processed as described by Soares et al. [19] to minimize the antinutritional factors, and then the beans were used as the protein source for diet preparation. All diets were prepared in the LABNE and contained 10% protein (1.75% nitrogen) and 363.95 kcal per 100 g, added to the mixtures of vitamins and minerals according to the rules of the Committee on Laboratory Animal Diets, 1979, modified according to the recommendations of the American Institute of Nutrition-93 [20]. The ingredients of the diets were homogenized in an industrial mixer with boiling water. The mass obtained was transformed into tablets, which were dried in a ventilated oven at 60 °C for 24 h, properly identified and stored refrigeration until the time for use.

The isoflavone content was determined as described by Klump et al. [21]. Briefly, samples of organic and transgenic soy were extracted at 65 °C with methanol–water (80 + 20), saponified with dilute sodium hydroxide solution, and analyzed by reversed-phase

liquid chromatography with UV detection at 260 nm. The data were analyzed for individual isoflavone components, subtotals of daidzin, daidzein, glycitin, genistin and genistein.

### 2.3. Histological studies

The material obtained was fixed in formalin (pH 7.2) and processed following the routine histological procedures for the inclusion in paraffin. Section of 5  $\mu$ m of thickness was stained by the following methods: Hematoxylin and Eosin for the analysis of the integrity of the specimens and exclusion of the samples with artifacts, Picro-Sirius-Red in polarizations microscope to show the different possible types of collagen in the samples, Van Gieson for the quantifying of collagen and smooth muscle. This technique brings about a marked color difference between these two components.

### 2.4. Image acquisition and analysis

Five different sections were selected from five fragments. Then, five random fields were evaluated from each section. Therefore, there were 25 test areas from each bladder. Images were digitised using a Olympus DP70 (12.5 megapixels) video camera coupled to a BX51 Olympus light microscope, which transferred all images captured to a microcomputer.

#### 2.4.1. Morphometric quantification of detrusor

The quantitative analysis was performed using paraffin sections. After image digitalization, all procedures: areas selection, apposition test-system, linear measure was performed using software ImageJ (National Institutes of Health, USA), with previous calibrations. Quantification was performed at a final magnification of 400 $\times$  using software ImageJ.

### 2.5. Biochemical analysis

The cholesterol and triglycerides were determined by a colorimetric method (Bioclin, Belo Horizonte, MG, Brazil). 17 $\beta$ -Estradiol serum concentration was determined by radioimmunoassay, using a commercial kit (Solid Phase Component System, INC Pharmaceuticals, USA). The sensitiveness of the kit was 0.13 pg/dl and the intra- and inter-assay variation coefficient were of 5.5% and 5.3% respectively.

#### 2.5.1. Statistical analysis

Statistical significance of experimental observations was determined by the one-way analysis of variance followed by Newman Keuls test and by the Kruskal–Wallis non-parametric test. All results are presented as the mean  $\pm$  S.D. with statistical significance considered at  $p < 0.05$ .

## 3. Results

The data related to isoflavone consumption per 100 g of body weight and consumption of individual isoflavone components as daidzein, genistein, daidzin, glycitin and genistin are showed in Table 1. There was no significant difference in the food consumption per 100 g of body weight among the groups or in none of the individual isoflavone components between transgenic and organic soy.

Table 2 shows the body weight, cholesterol, triglycerides and estradiol serum levels of all groups. Both OSG and GMSG groups had lower body weight when compared to CG, but this reduction was significant only in the GMSG ( $p \leq 0.05$ ). Both OSG and GMSG groups presented low estradiol serum levels ( $p < 0.05$ ) compared to CG. In relation to lipid profile, both OSG and GMSG groups presented



**Table 1**  
Total and individual isoflavone components of diets (mg/g diet).

Groups	Total isoflavone (mg/g)	Daidzein (mg/g)	Genistein (mg/g)	Daidzin (mg/g)	Glicitin (mg/g)	Genistin (mg/g)
OSG	0.384 ± 0.04	0.030 ± 0.004	0.034 ± 0.002	0.067 ± 0.005	0.018 ± 0.001	0.235 ± 0.06
GMSG	0.396 ± 0.03	0.032 ± 0.003	0.038 ± 0.003	0.063 ± 0.002	0.014 ± 0.002	0.249 ± 0.05

OSG = organic soy group, GMSG = genetically modified soy group. Data are reported as mean ± standard deviation of eight animals. The CG (control group) diet presents no isoflavones.

**Table 2**  
Body weight, cholesterol, triglycerides and estradiol serum levels in control group (CG), organic soy group (OSG) and genetically modified soy group (GMSG).

	CG	OSG	GMSG
Body weight (g)	406 ± 23.1	389 ± 23.5	368 ± 17.6*
Cholesterol (mg/dL)	117.9 ± 7.3	95.5 ± 8.0†	83.3 ± 5.7#
Triglycerides (mg/dL)	104.3 ± 13.2	72.3 ± 12.5*	60.3 ± 4.6*
Estradiol (pg/dl)	149.3 ± 1.0	102 ± 6.1†	94.7 ± 15.4*

Values are given as mean ± standard deviation of seven animals.

\*  $p < 0.05$  compared with control group.

#  $p < 0.01$  compared with control group.

**Table 3**  
Collagen and muscle fibers of bladder detrusor muscle in control group (CG), organic soy group (OSG) and genetically modified soy group (GMSG).

Groups	Collagen (%)	Smooth muscle (%)
CG	24.20 ± 2.68	73.40 ± 3.50
OSG	37.20 ± 5.26*	60.40 ± 6.46*
GMSG	38.8 ± 3.03*	59.80 ± 2.28*

Values are given as mean ± standard deviation of five animals.

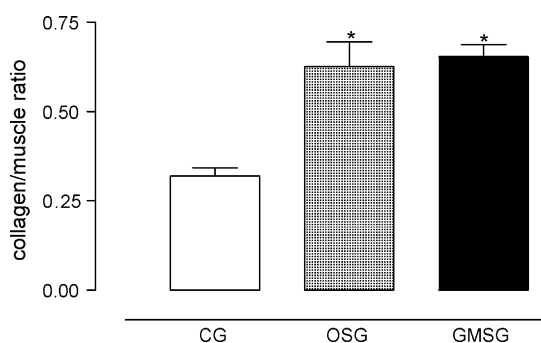
\*  $p < 0.05$  compared with control group.

low triglycerides and cholesterol serum levels ( $p < 0.05$ ) compared to CG.

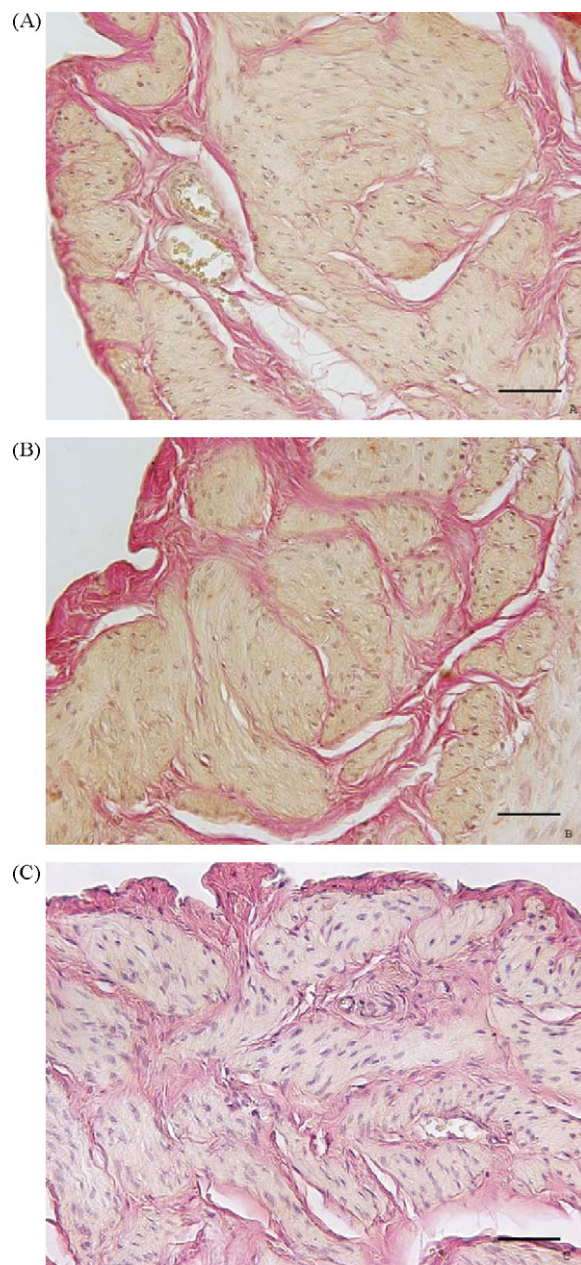
Table 3 shows the mean and standard error of collagen and muscle fibers of bladder detrusor muscle. In relation to collagen fibers there was a significant increase ( $p < 0.05$ ) in the OSG and GMSG groups compared to control (OSG = 54%; GMSG = 60%). Otherwise, both OSG and GMSG groups presented a significant decrease ( $p < 0.05$ ) in the muscle fibers (OSG = 18%; GMSG = 19%). The collagen/muscle fibers ratio is increased in both OSG and GMSG groups and this ratio is shown in Fig. 1.

Fig. 2 shows the histological sections of detrusor bladder muscle stained with Van Gieson that was used to quantify the collagen and smooth muscle fibers.

Fig. 3 shows the histological sections of detrusor bladder muscle stained with Picro-Sirius-Red in polarizations microscope to show the different possible types of collagen in the samples.



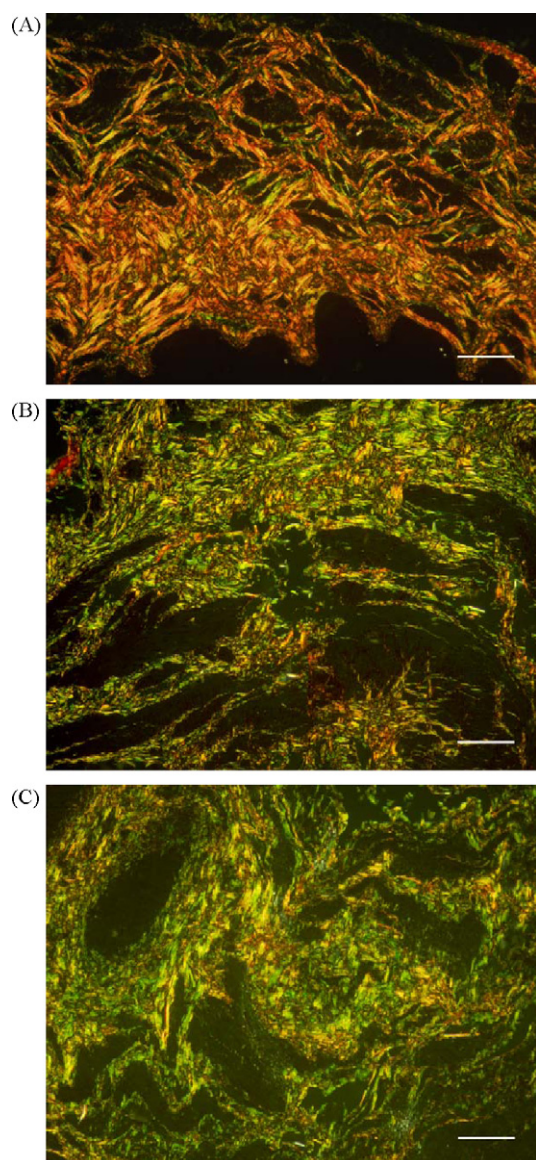
**Fig. 1.** The collagen and muscle ratio of the detrusor. Values are given as mean ± standard deviation of five animals. \* $p < 0.05$  vs CG.



**Fig. 2.** Histological sections of bladder stained with Van Gieson in control group (A), organic soy group (B) and genetically modified soy group (C). The final magnification is 400× (bar: 400 μm).

#### 4. Discussion

Soyfood has many beneficial effects improving the lipid profile [22–27], bone metabolism [28,29] and in the relief of menopause symptoms [16]. In this paper we evaluated whether the prolonged use of organic or transgenic soy upon the lipid profile and the collagen/muscle ratio of the detrusor could be advantageous.



**Fig. 3.** Picro-Sirius-Red staining under polarization microscopy of the detrusor demonstrating the amount of collagen in control group (A), organic soy group (B) and genetically modified soy group (C). The final magnification is 400 $\times$  (bar: 400  $\mu$ m).

The isoflavone content of both organic and transgenic soy was evaluated and no significant difference in the individual components or in food consumption was found.

In agreement with the literature, both organic and transgenic soy reduced the body weight [30,31] and estradiol serum levels [32–35] and improved the lipid profile by reducing cholesterol and triglycerides serum levels [22–27].

Our analysis showed an increase in the collagen and a decrease in the muscle fibers of bladder detrusor of both organic and transgenic groups compared to control, resulting in a higher collagen/muscle fibers ratio. This findings are consistent with those in which estradiol serum levels are low [8,12,36,37]. Studies using hormonal therapy in ovariectomized rats show a significant decrease in the bladder collagen/muscle ratio in relation to the castrated group [38–41]. So, despite of reducing body weight and improving the lipid profile, the consumption of soy-based diets was not capable of normalizing the alterations caused by low estradiol levels.

Using the Picro-Sirius-Red stain and observing the cuts in polarizing light microscopy we can suggest that there is a difference in

the type of collagen between the soy-based groups and control. Even though this stain may not be reliable to determine the different types of collagen, it is possible to suppose that a prevalence of one color is indicative of a predominance of one specific collagen type. In this study, there was a prevalence of the green color, suggesting a predominance of a new synthesized collagen, indicating a possible bladder detrusor muscle remodeling. The higher collagen/muscle fibers ratio and the predominance of a new synthesized collagen suggest that these alterations can be involved in the bladder dysfunctions that occurring during menopause.

Although the use of genetically modified food is still questionable, there is no evidence that genetic modification through biotechnology will impose immediate significant risks as food allergen sources beyond that of our daily dietary intake of foods from crop plants [42] or beyond other methodologies widely accepted in the food industry [43]. Also, there is no evidence suggesting that recombinant DNA would be processed in the gut in any manner different from endogenous feed-ingested genetic material [44,45]. The data presented here, with no statistical difference between both soy-based groups, reinforces this concept.

In conclusion, these findings call into question that, the prolonged use of soy-based diets can be deleterious to the bladder by altering the collagen/muscle ratio what can cause bladder dysfunctions similar with that occurring during menopause.

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