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Effects of comfort food diet on the penile morphology of stressed rats

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ABSTRACT

Purpose: To investigate the effects of chronic stress, associated or not with comfort food, on the morphology of the penis.
Materials & methods: Thirty-two adult Wistar rats were divided into four experimental groups: Control group (C), receiving standard rat chow, and under normal conditions; Stressed group (S), receiving standard chow, and submitted to stressful situations; Control + comfort food group (C + CF), receiving standard chow and comfort food, and under normal conditions; and Stressed + comfort food group (S + CF), receiving standard chow and comfort food, and submitted to stressful situations. At 10 weeks of age, food supply and stress were initiated. All groups had *ad libitum* access to standard chow and water, and groups receiving comfort food also had access to Froot Loops®. Chronic stress was induced by restriction, animals were contained daily in polypropylene tubes for 2 h, for eight weeks. After eight weeks all animals were killed; penises were removed for histomorphometric analysis.
Results: Body mass was similar among the groups. Food intake in S + CF group was lower than in other groups. Concerning food preference, groups C + CF and S + CF preferred comfort food over the standard chow, with this preference being higher in S + CF than in C + CF. The area of the corpora cavernosa without tunica albuginea was lower in group S + CF than in group C. Most interestingly, the surface density of connective tissue in the corpora cavernosa was higher in groups S and S + CF compared to group C. In contrast, smooth muscle surface density was markedly lower in S + CF compared to groups C and C + CF, while group S also had reduced smooth muscle in comparison to group C.
Conclusion: Chronic stress caused a morphological alteration on penile histomorphometry. Also, stress increased the preference for comfort foods which caused more deleterious effects in some parameters.

1. Introduction

Sexual health is characterized by a multifactorial process coordinated by the neuroendocrine and vascular systems [1]. Sexual dysfunction represents an important medical and physiological problem that adversely affects not only physical health but also

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emotional well-being, seriously compromising the individual's self-esteem, body image, interpersonal relationships, and physical health [2]. Several studies have shown the impact of different (lifestyle-associated) conditions on sexual function, such as obesity [3], sedentarism [4], smoking [2] and chronic stress [5].

Exposure to stressors has become quite common in developed and underdeveloped countries, increasing the risk of erectile dysfunction [6]. Although stress is strongly linked to sexual dysfunction, there are few studies directly correlating the effect of chronic stress on sexual function, quality of life and food preference [7,8]. What is known is that a stressor stimulus hyperactivates the hypothalamic-pituitary-adrenal (HPA) axis, increasing the circulation of glucocorticoids (GCs) in the bloodstream. The elevation of GCs increases the excitation of neuronal receptors for pleasurable activities, such as the ingestion of foods rich in simple carbohydrates, drugs, and physical activities [9].

Regarding food intake, studies in animal models point to a direct relationship between chronic stress and appetite suppression (hypophagia) [10], while in humans it can increase or decrease food consumption [11,12]. In fact, exposure of rats to chronic stress modifies not only food intake but also the dietary pattern, particularly with respect to the intake of highly palatable foods (comfort food), which are rich in simple carbohydrates and lipids [9,13].

Previous studies carried out by our group showed that both isolated macronutrients and chronic stress modified the structure of certain organs of the urogenital system. Campos-Silva and collaborators found that the rats ingesting hypercaloric diets, (either the mother or the offspring) had decreased prostate secretory activity and spermatogenesis [14–16]. On the other hand, rats submitted to chronic stress had harmful effects on the morphology of the penis [17,18], spermatogenic cells [19] and kidneys [20]. However, the effects of chronic stress on food preference and the impact of comfort foods on stressed rats' penile morphology have not yet been investigated.

Thus, the objective of the present study is to investigate the effects of chronic stress, associated or not with comfort food, on the morphology of the penis. Our hypothesis is that the stress stimuli can alter the eating pattern of Wistar rats and the access to comfort food could interfere with the effects on the structure of the penis caused by stress.

2. Methods

2.1. Animals

Thirty-two male Wistar rats were used in this study. All animals were bred in the Urogenital Research Unit animal facilities and were kept in a room with controlled temperature (22 °C ± 1 °C) and artificial dark-light cycles (lights on from 7:00 a.m. to 7:00 p.m.) and had free access to standard rat chow and water. This project was formally approved by the local ethics committee under protocol number CEUA-004/2019 and followed national and international regulations on animal experimental use.

2.2. Experimental design and interventions

When animals completed ten weeks of age they were included in the experiment. Four experimental groups, each one consisting of eight rats, were randomly assigned as follows: Control group (C); Stressed group (S); Control + comfort food group (C + CF); and Stressed + comfort food group (S + CF). Fig. 1 illustrates the experimental design.

Animals in groups C and S received only standard rat chow (Nuvilab CR-1, Quimtia, Colombo, Brazil) while groups C + CF and S + CF received Froot Loops (Kellogg Brazil, São Paulo, Brazil) in addition to the standard rat chow. Froot loops was used as comfort food due to its nutritional components which are listed in Table 1. The food intake was measured per cage (with 2 or 3 rats of the same group in each cage). For all groups the standard chow present in each cage was weighted daily and completed for 50 g per animal. For groups C + CF and S + CF, in addition to the standard chow, 30 g of Froot Loops per rat were offered, and its consumption was also weighted daily. Food consumption (in grams and Kilocalories per body mass) as well as food preference (in percentage of consumption in grams; for groups C + CF and S + CF) was calculated and compared among groups. Capillary blood glucose was measured (after a 12-h fast) at the beginning (10 weeks of age) and at the end of the experiment (18 weeks of age) with a portable glucose monitor (Accu-Chek,

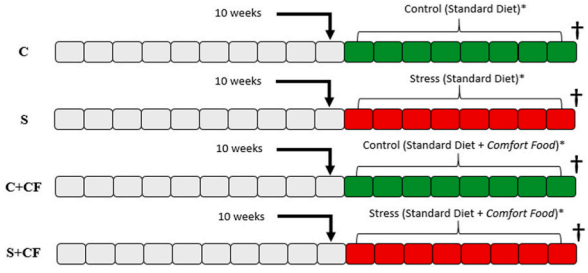

Fig. 1. Illustrative image of the experimental design and group divisions. C: Control group; S: Stressed group; C + CF: Control + comfort food group; S + CF: Stressed + comfort food group. †: Euthanasia and tissue analysis. *: Groups S and S + CF during the stress induction protocol were deprived of water and food. Groups C and C + CF were also deprived of water and food in the same period. All groups started the experiment when they reached ten weeks of age.

Table 1
Experimental diet composition.

	Standard chow (Nuvilab CR-1)	Comfort food (Froot Loops)
Energy (Kcal/100g)	336	376
Carbohydrate (g/100g)	53.0	82.5
Protein (g/100g)	22.0	5.6
Total Fat (g/100g)	4.0	2.7
Dietary Fiber (g/100g)	7.0	3.0

Data provided by the manufacturers.

Roche, São Paulo, Brazil).

Animals in groups S and S + CF were submitted to a chronic stress protocol by the immobilization method [18,21]. Each animal was maintained in a rigid opaque plastic tube to restrain its movements, 2 h daily, during eight weeks. Tubes with different diameters and lengths were adjusted weekly depending on the animal's size. Meanwhile, the control groups (C and C + CF) were kept under normal conditions and not submitted to any stress procedure, but during the same period food was removed from stressed groups (2 h daily) to avoid any bias in food intake measurements. All animals were killed on the day after the last stress stimuli, when the animals were 18 weeks old.

2.3. Sample collection and analyses

After eight weeks of experiments, the animals were weighted and submitted to euthanasia by isoflurane (Isofluorano, BioChimico, Itatiaia, Brazil) inhalation in an induction chamber [22]. The penises were collected and their skin-denuded middle shafts were fixed in a 4% buffered formaldehyde solution. Samples were routinely processed for paraffin embedding and 5 μm-thick sections were used for histomorphometric evaluations [18].

The cross-sectional penile area, the area of CC (including its tunica albuginea), and the area of CC without the tunica albuginea were evaluated in Sirius red stained sections. For this purpose, images were captured under 20 × magnification by a digital camera (Axiocam 506 color, Carl Zeiss, Jena, Germany) coupled to a stereomicroscope (Discovery V.8, Carl Zeiss). These areas were measured with the "Polygons" tool of the Image J software (version 1.45s, National Institutes of Health, Bethesda, USA), and expressed in mm^2. The area of the tunica albuginea was calculated as the difference of CC area with and without its tunica albuginea [23,24].

The surface density (Sv) of corpus cavernosum connective tissue, sinusoidal space, and smooth muscle fibers was evaluated in Masson's trichrome stained sections. These sections were captured under 400× magnification by a digital camera (DP70, Olympus, Tokyo, Japan) coupled to a microscope (BX51, Olympus). The Sv was calculated (for each structure) by the point counting method [25]. Briefly, a 100-point grid was superimposed over the images using the Image J software, and each structure "touched" by a point was counted. The result, expressed as a percentage, was calculated after measuring 25 images from different randomly captured fields for each animal [23,25].

2.4. Statistical analyses

All data was tested for normal distribution by the Kolmogorov-Smirnov test. Comparisons among all groups were performed by one-way ANOVA and Tukey's post-test. Food preference was analyzed by Student-t-test. Statistically significant differences were considered at $p < 0.05$. All results were presented as mean ± standard deviation. All statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, USA).

3. Results

3.1. Biometric and dietary analyses

Body mass and capillary blood glucose were similar among all groups at the beginning and at the end of the study. The food intake of S + CF was 14.2% lower than group C, 14.4% lower than group S, and 10.2% lower than group C + CF. Despite this lower food consumption, when energy intake was studied, it was found that group S + CF had similar values to groups C and S. Group C + CF (which was not submitted to stress but had access to comfort food) showed a higher energy intake in comparison to group C (12.5% higher) and group S + CF (8.0% higher).

For both groups that had access to comfort food (C + CF and S + CF), Froot Loops was preferred over standard chow. Animals in group C + CF consumed 81% more of the comfort food than standard chow, while in group S + CF, Froot Loops was consumed 142% more than standard chow. When comparing these results, a statistical difference of food preference was observed, with stressed animals consuming more comfort food than non-stressed animals.

3.2. Penile cross-sectional areas

Regarding the penile cross-sectional area, it was found that group C + CF had an 8.1% increase in comparison to group S. Also,

group C + CF had a 7.5% increase of the corpus cavernosum area (with tunica albuginea) in comparison to group S. Meanwhile, group S + CF showed a 15.5% reduction of the corpus cavernosum area (without tunica albuginea), in comparison to group C. No statistical difference was observed regarding the tunica albuginea area. Fig. 2 [A – G]illustrates these findings.

3.3. Cavernosal tissue analyses

The cavernosal smooth muscle surface density was reduced by 39.2% in group S in comparison to group C. A more drastic reduction was noted in group S + CF, with 45.7% and 35.5% lower values when compared to groups C and C + CF, respectively. The connective tissue surface density was augmented by 13.6% in group S in comparison to group C, and by 9.2%, in comparison to group C + CF. Group S + CF showed 11.6% higher values of this parameter in comparison to group C. The sinusoidal space surface density of group S showed a 27.3% reduction in comparison to group C. Fig. 3 [A – G] illustrates these findings. All numerical data are presented in Table 2. Table 3 presents the initial and final body mass of the animals.

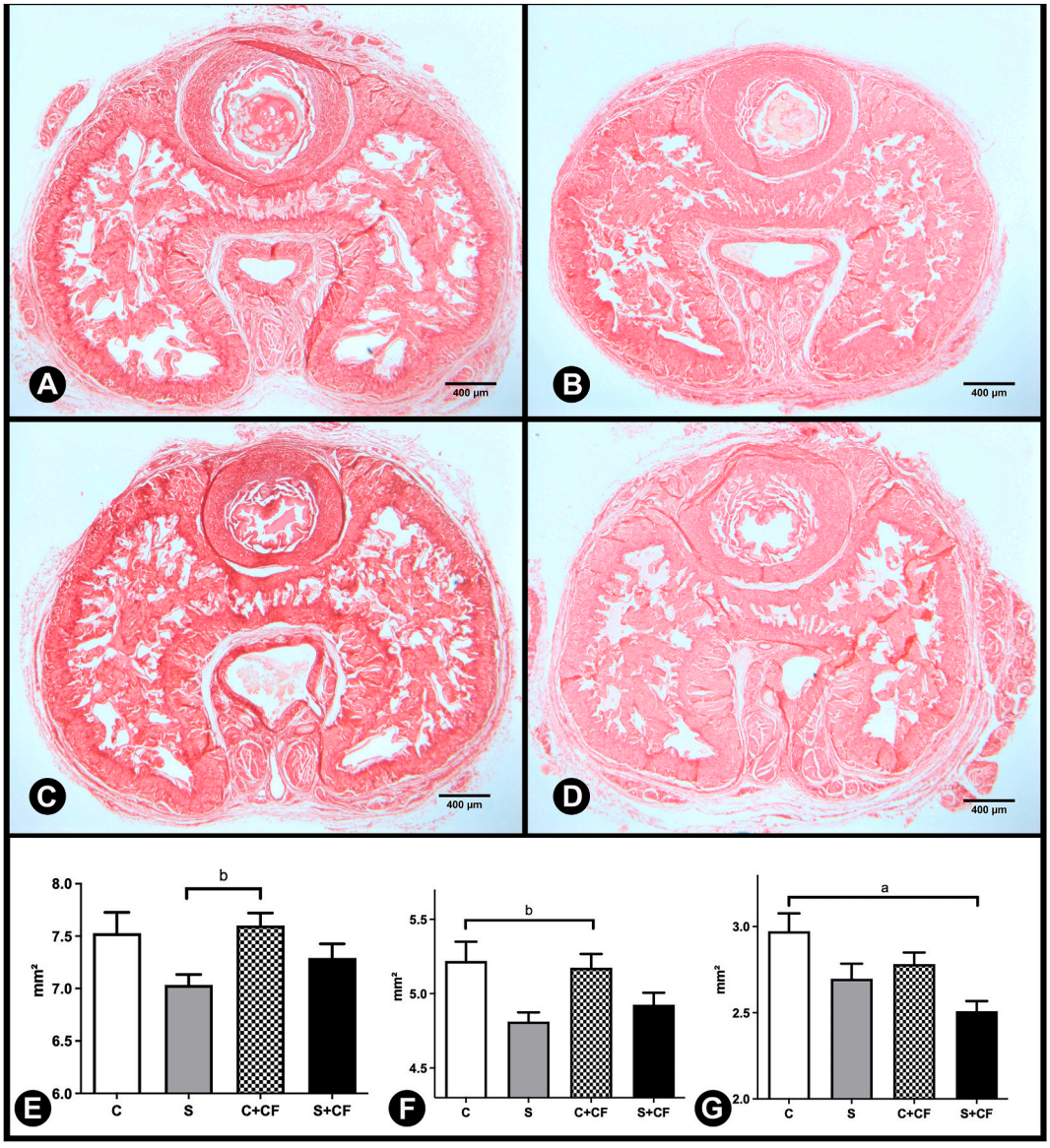

Fig. 2. Photomicrographs and graphics representing the penile cross-sectional areas. Cross sectional images of penis from Control group (A), Stressed group (B), Control + comfort food group (C), and Stressed + comfort food group (D). Sections were stained by Sirius red and captured under 20 × magnification. The graphics below presents the results of cross-sectional penile area (E), corpus cavernosum area with tunica albuginea (F), and corpus cavernosum area without tunica albuginea (G).

4. Discussion

The results of the present study showed that highly palatable foods were preferentially consumed in both groups which had access to it. Nevertheless, the S + CF group had a higher preference for comfort foods than the C + CF group. This supports the hypothesis that stress stimuli can alter the eating pattern of rats, with stressed animals preferring comfort foods more than non-stressed animals. Some previous studies support these findings and suggest that stress conditions can lead to two profiles: the anxiogenic and the anorexigenic [9,26]. Although behavioral tests were not used in the present study, it was possible to observe that group S + CF had lower food consumption than group C + CF (both in grams and in kcal/kg of body mass). Thus, it is possible to state that in the current study stressed animals reduced food consumption while preferring comfort foods. It has been shown that exposure to highly palatable foods reduces anxiety-related behaviors in rodents, regardless of the stressor stimulus [27–29]. It is likely that the consumption of comfort foods may be an unconscious strategy used by animals to alleviate or ameliorate a provoked stress [30].

Regarding the histomorphometric parameters of the penis, group S + CF had a decrease in the area of the corpora cavernosa without tunica albuginea (when compared to C). A similar fact occurred in a study by Ribeiro et al. (2019) where a reduction of this parameter in the penis of stressed rats was found [17]. Interestingly, the control animals with access to comfort foods had augmented areas of the penis and of the corpora cavernosa with tunica albuginea. It is possible to suppose that in these animals the (non-statistically significant) rise in body mass could have led to these raised penile areas.

When the cavernosal tissue was analyzed, it was found that stress caused a marked reduction in smooth muscle content, with a more drastic reduction in stressed animals with access to comfort food. Meanwhile, connective tissue was augmented by stress. Penile

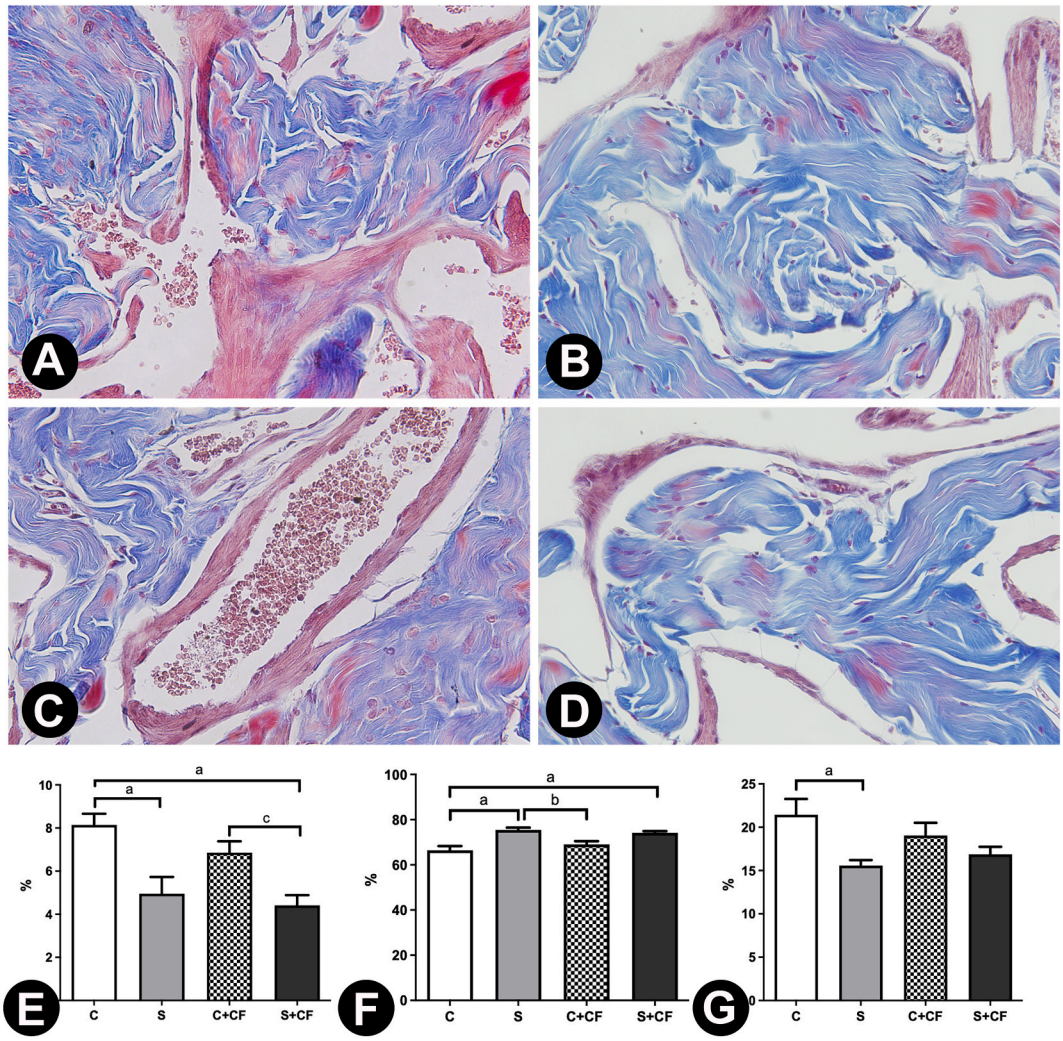

Fig. 3. Photomicrographs and graphics representing the Surface densities of corpus cavernosum structures. Images from the corpus cavernosum of Control group (A), Stressed group (B), Control + comfort food group (C), and Stressed + comfort food group (D). Sections were stained by Masson's trichrome and captured under 400 × magnification. The graphics below presents the results of smooth muscle surface density (E), connective tissue surface density (F), and sinusoidal space surface density (G).

Table 2
Biometric data and penile morphometric analysis of experimental groups.

	C	S	C + CF		S + CF		*p* value
Blood glucose - initial (mg/dL)	89.25 ± 9.19	98.63 ± 13.21	95.38 ± 14.69		89.29 ± 11.88		0.3701
Blood glucose - final (mg/dL)	89.19 ± 7.95	96.13 ± 13.67	102.90 ± 15.30		87.88 ± 11.22		0.0786
Food intake (g)	27.77 ± 1.69	27.81 ± 1.95	26.51 ± 2.04		23.81 ± 1.67 [a,b,c]		**0.0004**
Food intake (kcal/kg BM)	0.24 ± 0.01	0.24 ± 0.02	0.27 ± 0.03 [a]		0.25 ± 0.03 [c]		**0.0098**
Food preference (%)	–	–	**SC**	**CF**	**SC**	**CF**	**0.0110**
			35.57 ± 2.82	64.43 ± 2.82	29.20 ± 5.30 [c]	70.80 ± 5.30 [c]	
Cross-sectional penile area (mm^2)	7.53 ± 1.26	7.03 ± 0.62	7.60 ± 0.75 [b]		7.29 ± 0.79		**0.0183**
CC area with TA (mm^2)	5.22 ± 0.82	4.81 ± 0.39	5.17 ± 0.59 [b]		4.93 ± 0.48		**0.0084**
CC area without TA (mm^2)	2.97 ± 0.65	2.70 ± 0.55	2.78 ± 0.42		2.51 ± 0.34 [a]		**0.0064**
Tunica albuginea area (mm^2)	2.36 ± 0.27	2.17 ± 0.51	2.36 ± 0.33		2.42 ± 0.39		0.2725
Sv [smooth muscle] (%)	8.14 ± 1.48	4.95 ± 2.20[a]	6.86 ± 1.48		4.42 ± 1.24 [a,c]		**0.0004**
Sv [connective tissue] (%)	66.43 ± 5.40	75.47 ± 2.86 [a]	69.09 ± 3.70 [b]		74.18 ± 1.92 [a]		**0.0001**
Sv [sinusoidal space] (%)	21.44 ± 5.16	15.58 ± 1.78 [a]	19.04 ± 4.16		16.87 ± 2.31		**0.0197**

C: Control group; S: Stressed group; C + CF: Control + comfort food group; S + CF: Stressed + comfort food group; CC: Corpus cavernosum; TA: Albuginea tunic. BM: Body mass. SC: Standard chow. CF: Comfort food. Data expressed as mean ± standard deviation. Data were considered different when $p < 0.05$. [a]: Indicates statistical difference with group C; [b]: indicates statistical difference with group S; [c]: Indicates statistical difference with group C + CF.

Table 3
Body mass of animals at the beginning and at the end of the study.

	Initial Body mass (g)	Final Body mass (g)
C	319.1 ± 57.85	388.1 ± 70.45
S	348.0 ± 29.63	378.6 ± 43.53
C + CF	373.0 ± 41.24	423.7 ± 38.93
S + CF	313.4 ± 24.90	364.9 ± 31.75

C: Control group; S: Stressed group; C + CF: Control + comfort food group; S + CF: Stressed + comfort food group. Initial (beginning of the experiment - 10 weeks) and final (end of the experiment - 18 weeks) body mass values. Data expressed as mean ± standard deviation.

erection occurs via smooth muscle relaxation, arterial dilation, and venous occlusion [31]. Therefore, any modification in one of these structures will trigger erectile dysfunction. Stress stimuli affects penile architecture, leading to greater deposition of connective tissue and lower concentration of smooth muscle in the sinusoidal space [18], in addition to decreasing endothelial nitric oxide synthesis in the corpus cavernosum of the penis [32]. Here, we realize that diets rich in simple carbohydrates (comfort food) follow the same line and can be considered a risk factor for the development of erectile dysfunction.

Chronic stress is associated with erectile dysfunction, and the loss of erectile tissue can decrease the functional capacity of the penis [18]. However, the deleterious effects of this condition on penile structures, especially the corpora cavernosa, are still poorly understood. Some conditions associated with erectile dysfunction, such as cardiovascular disease, smoking and alcohol, are positively related to a reduction in testosterone levels [28]. This hormone has a unique importance in erectile function and in the modulation of penile architecture [33], and its production may be compromised by the imbalance in the hypothalamic-pituitary-adrenal axis generated by stress [34]. Previous studies have shown that testosterone levels are reduced as a result of chronic stress [19], thus, a hypothesis that should be further investigated is that the suppressed production of testosterone in stressed animals may trigger these structural changes found in the penis.

According to Cunha et al. (2020), comfort foods seem to decrease the concentration of serum corticosterone, and thus, attenuate the responses to the stressor stimulus. However, in the present study the association with the comfort food diet did not attenuate the effects on the structure of the penis caused by stress. On the contrary, in some parameters, stressed animals with access to comfort foods showed worse results than group S. The mechanism is not yet fully elucidated; however, it represents an imminent risk for triggering binge eating, and consequently obesity and associated metabolic disorders [26].

This work has limitations. As it was performed in animals, transposing the results to humans becomes a great challenge. Furthermore, the stress induction method was also considered a limitation, since the animals were immobilized at the same time of day, under the same stress conditions and at the same time, differently to what commonly occurs in stressed humans. Even so, these results help to understand some aspects of the biology of stress, its effects on penile structure, and its relation to food preference.

5. Conclusion

Chronic stress caused important structural damage to the penis which may be related to erectile dysfunction. Comfort foods are preferable for both groups with access to it, but stressed animals tended to consume even more of this type of diet. The consumption of comfort food aggravated penile damage caused by chronic stress and should be avoided during stressful situations.

Author contribution statement

Roger G. Marchon: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bianca M. Gregório: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Waldemar S. Costa; Marco A. Pereira-Sampaio: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Francisco J. Sampaio: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Diogo B. De Souza: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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