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Diet-induced metabolic syndrome altered bladder urothelium in adult female rats

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Abstract: In recent years, the prevalence of chronic non-communicable diseases has increased. In females, there is a close relationship in the development of these diseases after menopause, related to the estrogenic signaling occurring in various tissues; such is the case of the bladder, compromising its physiology in females. We sought to analyze the effect of diet-induced metabolic syndrome on the bladder epithelium. Eighteen 12-week-old Wistar rats were divided into an intact control group (C, n=6), a cafeteria diet SMet group (CAF, n=6), and a high-fat/high-sugar diet SMet group (HF/HS, n=6). Atrophy and hyperplasia in bladder epithelium were observed in the case of the CAF diet, while the other scheme was only inflammation.

Keywords: Bladder endothelium; metabolic syndrome; urinary incontinence

1. Introduction

In recent years, there has been a constant growth in the prevalence of chronic non-communicable diseases, which even appear increasingly at an earlier age [1]. However, there is a close relationship in the development of these diseases in females, the role of oestrogen fluctuation in reproductive stages such as gestation and menopause has been widely discussed [2, 3], compromising its physiology in females. Urinary incontinence (UI) is a common experience throughout a woman's life and has a significant impact on well-being and quality of life [4]. The prevalence of urinary incontinence worldwide is reported to be 8.5%; it is the most common urinary tract disease affecting adult women. The main risk factors associated with stress UI include age, pregnancy, and parity history of hysterectomy, obesity, and pelvic radiation [5]. Most of the related studies focus on analyzing the morphophysiology of the pelvic floor, and bladder musculature, neglecting the effects on the urothelium [6, 7]. Metabolic syndrome (MetS) is a set of disorders characterized by low-grade inflammation that alters various systems and is associated with type 2 diabetes mellitus and cardiovascular disease [8]. Diet plays a crucial role in the development of MetS, with the combination of high caloric intake, poor nutrient quality, and unhealthy food choices contributing to its negative impact on overall health [9]. Considering that women are more affected by bladder diseases such as UI, it is of relevance to analyze the effect of metabolic syndrome (MS) models of diet through cafeteria diet (CAF) or high fat/high sugar diet (HF/HS) on the bladder urothelial of female rats.

2. Materials and Methods

Eighteen 12-week-old Wistar rats were divided into an intact control group (C, n=6), a cafeteria diet SMet group (CAF, n=6), and a high-fat/high-sugar diet SMet group (HF/HS, n=6). Were housed with temperature and controlled artificial illumination (20 ±

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2 C; light:dark 7 AM to 9 PM). This light condition was used. All animal procedures followed the Guidelines of Mexican Law of Production, Care, and Use of Laboratory Animals (NOM-062-ZOO-1999), and were approved by the research committee of the division de Ciencias de la Salud from Universidad Cristóbal Colón (Registration code: COVID-100).

Control group had access to water and feed (23% protein, 50% carbohydrate, and 27% lipid) ad libitum; the cafeteria group had a designed diet (approximately 11% protein, 60% carbohydrate, and 29% lipid) in which ultra-processed feeds were used, the high-fat/high-sugar diet (HF/HS) was designed and pellets were prepared with a composition of (18% protein, 55% carbohydrate, and 27% lipid). In the case of the CAF group, the animals had access to chow and water, along with the diet, which consisted of bread rolls, French fries, soft drinks, sausages, among others. The duration of the treatments was 10 weeks. All rats used in the experimental procedures were euthanized with an overdose of sodium pentobarbital (60 mg/kg). Blood was obtained by cardiac puncture for biochemical measures.

Bladder were fixed in formaline and histologically processed, then were embedded in Paraplast X-tra (Sigma-Aldrich) was transversally cut at a thickness of 5 μ using a microtome (Thermo Scientific, Model 325). Tissue sections were mounted on gelatin-coated slides.Each tissue was stained with Masson's trichrome and PAS, and photographs were taken at 10x, 40x, and 100x. Data were analyzed statistically and differences were considered when $P< 0.05$, using graph Pad v.6 statistical packages.

3. Results

The cafeteria diet was effective in generating metabolic syndrome, with the presence of hyperglycemia, elevated cholesterol, and triglycerides, as well as higher body weight gain (table 1), while the HF/HS diet generated increased body weight and hypercholesterolemia.

Table 1. Parameters measure to identify metabolic syndrome.

Parameter	C *n=6*	HF/HS *n=6*	CAF *n=6*
Average food consumption (Kcal/día)	60.73 ± 0.74 [a]	63.95 ± 1.12 [a]	109 ± 3.24 [b]
Δ Body weight (from week 0 to week 10) gr	38.47 ± 8.41[a]	52.78 ± 4.96[ab]	77.18 ± 12.02[b]
Glucose (mg/dL)	112.5 ± 6.22[a]	148.4 ± 23.54[b]	195.5 ± 6.36[b]
Triglycerides (mg/dL)	52.70 ± 3.65[a]	69.80 ± 5.65[a]	93.03 ± 2.13[b]
Cholesterol (mg/dL)	32.60 ± 3.24[a]	77.97 ± 6.75[b]	99.12 ± 12.51[b]

Data are mean ± SEM. Different letters indicate significant differences between groups.

With respect to the bladder epithelium in the case of the HF/HS diet, it generates a detachment of the umbrella cells, and areas of desquamation and it can be observed that there are foci of inflammation. In the case of the CAF diet, it generated an important disarrangement of the epithelium, with many pyknotic nuclei, changes in basal cells, as well as blank spaces that do not appear in the other groups (Fig.1).

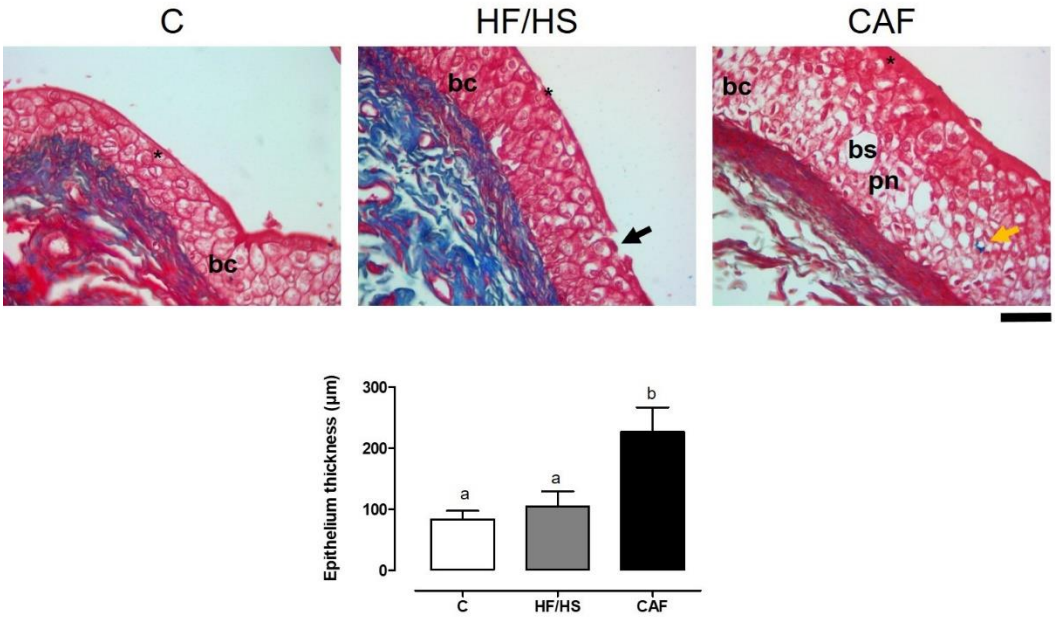

Figure 1. Bladder epithelium from control, HF/HS, and CAF groups. Data are mean ± SEM. Different letters indicate significant differences between groups. Scale: 50 µ. Abbreviation: * umbrella cells; black arrows, areas of desquamation; bc, basal cells, pn, pyknotic nuclei; yellow arrows, fibrosis; bs, blank space.

4. Discussion

Several studies have shown that different diet patterns can emulate the clinical manifestations of MetS. In the case of high fat/high sugar (HF/HS) diets, we find similarities to what has been shown previously, where it appears to be less effective in females [10]. In the case of the cafeteria diet, it was shown to be an excellent model with the classical manifestations of MetS, hyperglycemia, dyslipidemia, and higher adiposity. The treatment time was adjusted to 10 weeks, derived from the fact that, as indicated above, pathogenesis in females is slower due to the protective effect of estrogens **[8]**. **T**he manifestations observed in the bladder, coincide with problems such as cystitis **[9]**, in which there is desquamation and loss of umbrella cells. In the case of the CAF diet, in addition, disarray and many pyknotic nuclei were found, suggesting an increase in cell death. While histology alone does not indicate urinary incontinence, other studies show that the cafeteria diet leads to changes in the electrophysiological profile **[10]**. The results show that the cafeteria diet is a model that could be more useful for analyzing metabolic syndrome in females than other diet-generated models. Further studies are required to analyze the relationship of bladder alterations in females.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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