

## PHENOTYPIC MODULATION ON TYPES I AND III COLLAGEN AND IN CELL ADHESION PROTEIN AS CATENIN INDUCED BY HYPERCHOLESTEROLEMIC DIET IN NORMAL AND BLADDER OBSTRUCTED RATS

### Hypothesis / aims of study

Partial bladder outlet obstruction (PBOO) induces an increase in bladder mass, smooth muscle content and collagen deposition, sometimes generating bladder dysfunction represented by impaired contractility and compliance. We conduct this study aiming to compare this representative model of outlet obstruction with the morphological and histological changes of the bladder wall after a hypercholesterolemic diet (HCD).

### Study design, materials and methods

Twenty-nine four weeks old female Wistar rats were divided into three groups: 1. control (ten rats); 2. six-week obstructed (twelve rats) and 3. hypercholesterolemic diet (seven rats). The control and obstructed groups were fed with normal diet (0.2% cholesterol), while the group 3 was fed with HCD (1,25% cholesterol) for 14 weeks. At the 8<sup>th</sup> week, groups 1 and 3 initially underwent to a sham operation, while group 2 underwent to a partial outlet obstruction surgery under general anesthesia. After the urethra was dissected a 5-zero nylon suture was passed and tied loosely around the urethra with a 22G needle besides it. Six weeks later all animals were sacrificed and the bladders removed. All bladders were fixed in 10% formaldehyde. Serial sections of 5 µm were obtained from paraffin embedded material and stained with Picosirius red. Immunohistochemistry to types I and III collagen and analysis of cadherin complex (E-cadherin, α-, β- and γ-catenin) were also performed. Weight gain, total cholesterol and LDL-cholesterol were measured initially, at the moment of surgical procedure and at sacrifice.

### Results

Picosirius analysis showed that mature dense fibers, suggestive of type I collagen, did not altered significantly among all groups ( $p = 0.29$ ), while immature smooth fibers, suggestive of type III collagen, increased significantly in obstructed as well as in HCD group ( $p = 0.001$ ), but was similar between these two groups ( $p = 0.09$ ). In accordance with these findings, immunochemistry presented no significant increase in type I ( $p = 0.157$ ) and a significant increase in type III collagen ( $p = 0.046$ ) in groups 2 and 3 comparing with group 1 and this increase was statistically greater in obstructed group ( $p < 0.01$ ). Catenin analysis presented a similar alteration pattern in β- and γ-catenin expressions in groups 2 and 3, comparing to control group ( $p = 0.02$  and  $p = 0.04$ , respectively). While total serum cholesterol did not alter significantly between the groups ( $p = 0.75$ ), LDL-cholesterol and weight were statistically higher in the group 3 at the sacrifice moment ( $p = 0.003$  and  $p < 0.001$ , respectively).

### Interpretation of results

The results suggest that hypercholesterolic-based diet alone can induce alterations in the detrusor muscle cells (DMC) matrix, regarding an increase in immature type III collagen deposition and changes in cell adhesion protein pattern as catenin, resembling those occurred in the obstructed group.

### Concluding message

It is possible that weight gain and LDL-cholesterol increase, both induced by HCD, could lead or at least contribute to these alterations in DMC matrix.

		GROUPS			p
		Control	Obstructed	HCD	
E - cadherin	Normal (%)	70	41.7	71.4	0.393
	Reduced (%)	30	58.3	28.6	
β-catenin	Normal (%)	20	66.7	71.4	0.021
	Reduced (%)	80	33.3	28.6	
α-catenin	Normal (%)	40	58.3	42.9	0.717
	Reduced (%)	60	41.7	57.1	
γ-catenin	Normal (%)	10	50	57.1	0.044
	Reduced (%)	90	50	42.9	

Specify source of funding or grant	None
Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	Yes
Name of ethics committee	Ethics Committee for Analysis of Research Projects - CAPPesq of University of Sao Paulo