Life Sciences 210 (2018) 192–200

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Dietary macronutrient content affects inflammatory and fibrotic factors in normal and obstructed bladders

Temitope Gabriel Adedeji[a,c,*], Emiola Oluwabunmi Olapade-Olaopa[b,c]

[a] Department of Physiology, School of Health and Health Technology, Federal University of Technology, Akure, Nigeria
[b] Urology Division, Department of Surgery, College of Medicine, University of Ibadan, Nigeria
[c] PIUTA Ibadan Centre, Department of Surgery, University of Ibadan, University College Hospital, Ibadan, Nigeria

ARTICLE INFO

Keywords:
Diet
C-reactive protein
CXCL12
NGF
CTGF
HIF-1α
TGF-β
Collagen

ABSTRACT

Aims: To investigate the effects of diets on factors and markers of inflammation and fibrosis in unobstructed and obstructed bladders of male Wistar rats.
Materials and methods: Partial BOO was surgically induced in twelve-week old rats after feeding on different diets for eight (8) weeks. Feeding continued for 4 weeks after surgery. Rats were divided into sham-operated and BOO groups as follow: control, high-carbohydrate (HCD), high-fat (HFD) and high-protein (HPD). After the feeding period, bladder weight, CRP, nerve growth factor (NGF), tissue growth factor-β (TGF-β), connective tissue growth factor (CTGF), hypoxia inducible factor-1α (HIF-1 α), platelet-derived growth factor-A (PDGF-A) and CXCL12 were all determined.
Key findings: In both unobstructed and obstructed bladders, CRP was increased in animals fed on the HFD ($P < 0.05$). NGF was increased in animals fed on HFD and HPD but decreased only in HCD-BOO. CXCL12 was increased in animals fed on HFD and HPD ($P < 0.05$) and decreased in HCD. The HCD-BOO group exhibited a decrease in CXCL12, while CXCL12 increased in HFD-BOO. TGF-β was elevated in HFD and all the dietary-BOO groups, but animals with obstructed bladders fed on the HPD and HCD had significant reduction in TGF-β expression. CTGF was increased in HFD- and HPD-fed animals. HIF-1α, PDGF-A and collagen were increased in both HFD dietary groups and HPD-BOO.
Significance: Feeding on a high fat diet results in increased activity of factors and mediators of inflammation and fibrosis in both unobstructed and obstructed rat bladders. This might increase predisposition to or further worsen symptoms in BOO.

1. Introduction

Diet affects human health, and nutritionally-poor diets play a crucial role in the mechanisms involved in many diseases [1]. Obesity and metabolic syndrome result from consumption of high-calorie diets. Diets high in fats and carbohydrates, which are commonly consumed because of their palatability, directly result in these conditions with high intake [1]. Metabolic syndrome is a major factor in the aetiology of lower urinary tract symptoms (LUTS) in the bladder, increasing the incidence and also aggressiveness of the symptoms [2]. Documented evidence show that diet and nutr*ition could have an effect on the aetiology of LUTS, even though there is a dearth of literature on this [3,4]. Also, the composition and amount of macronutrients consumed in diet have been described as factors in lower urinary tract functions [5,6].

A common cause of LUTS is bladder outlet obstruction (BOO), a major cause of morbidity in urology, especially in adult males [7]. In spite of its high morbidity and negative impact on the quality of life (QoL) of affected individuals, the pathophysiological mechanisms involved in this condition are still poorly understood [8]. However, it has been shown that progression of this condition to an end-stage bladder is a complex biochemical pathway involving inflammation and fibrosis [9].

Inflammation has been implicated in various disease conditions, [10,11,12], and there are many mediators of inflammation in the bladder, but of particular note are chemokines and growth factor activity [13,14]. Serum levels of C-reactive protein increase when chronic inflammation occurs in the body, and show a correlation with LUTS [15]. Chemokines, such as CXCL12, are mediators of immune responses and inflammatory processes. The influence these chemokines exert on

* Corresponding author at: Department of Physiology, Federal University of Technology, Akure, Nigeria.
E-mail address: tgadedeji@futa.edu.ng (T.G. Adedeji).

https://doi.org/10.1016/j.lfs.2018.08.069
Received 21 June 2018; Received in revised form 23 August 2018; Accepted 30 August 2018
Available online 04 September 2018
0024-3205/ © 2018 Elsevier Inc. All rights reserved.

inflammation in the bladder has been reported [13,15]. When administered exogenously, chemokines induce thermal hyperalgesia and mechanical allodynia [16,17]. Another mediator of inflammation, Nerve Growth Factor (NGF), is responsible for proper growth and maintenance of sensory neurons. Increased levels of this factor have been reported in patients with idiopathic detrusor overactivity, neurogenic bladder and chronic bladder inflammation [18]. It is believed to play a major role in neuro-immune interactions, tissue inflammation, and also in neuroplasticity. Increased levels in the bladder usually result in increased bladder sensation and hyperactivity [19].

In obstructed bladders, increased pressure leads to various pathophysiological changes, including hyperplasia and hypertrophy of smooth muscle, increase in extracellular matrix storage, degradation of the collagen structure, increased activity of different growth factors etc. [20,21]. In murine models of BOO, transforming growth factor-beta (TGF-β) and connective tissue growth factor (CTGF) have been reported to be increased [22]. These changes eventually cause detrusor hypertrophy, elevated voiding pressure and detrusor instability, resulting in a pathologically decompensated detrusor [22]. Bladder outlet obstruction has also been reported to cause ischaemic and hypoxic changes in the bladder, which increase concentrations of Hypoxia Inducible Factor-1α and Platelet-Derived Growth Factor-A (PDGF-A) [23].

We hypothesized that diet could have an effect on the progression of inflammation and fibrosis by altering concentrations of growth factors and other mediators in both the obstructed and unobstructed bladder. We therefore assessed different factors associated with bladder inflammation and fibrosis in animals fed on diets of different macronutrient compositions.

2. Materials and methods

2.1. Animals

A total of eighty (80) twelve-week old male albino rats of the Wistar strain were used in this study. The animals were obtained from the Animal House of the College of Medicine, University of Ibadan, Nigeria. All studies were approved by the University of Ibadan Animal Ethics Committee. The animals were divided into eight (8) dietary and BOO groups of ten animals each and were housed in well-aerated experimental animal cages, maintained under standard lighting conditions. They were acclimatised for 7 days prior to commencement of the grouping. During this period, they were fed on standard rat chow (Ladokun feeds, Nigeria Limited) and had access to clean drinking water.

2.2. Experimental design

Animal feeds were mixed from individual feed constituents in particular compositions for each of the dietary groups. The mixes were then pelletised to ensure even distribution of components and consumption by animals. The control diet was derived from standard rats' feeds commercially-propounded and sold by Ladokun feeds. Each of the experimental diets was formulated by altering the proportion of components supplying each macronutrient in the original standard feed. Adequate nutritional requirements were ascertained and essential amino acids were added to the feeds to prevent under-nutrition. These were then fed to the animals as follows:

– Group 1: Sham-operated rats fed on normal rats' chow (26.5% protein, 40% carbohydrates, 29% fat, and 4.5% crude fibre)
– Group 2: BOO animals fed on normal rats' chow
– Group 3: Sham-operated rats fed on a High Carbohydrate Diet (HCD) (20% protein, 58.5% carbohydrates, 17% fat, and 4.5% crude fibre)
– Group 4: BOO rats fed on a High Carbohydrate Diet (HCD) (20% protein, 58.5% carbohydrates, 17% fat, and 4.5% crude fibre)
– Group 5: Sham-operated rats fed on a High Fat diet (HFD) (22% protein, 13.5% carbohydrates, 60% fat, and 4.5% crude fibre)
– Group 6: BOO animals fed on a High Fat diet (HFD) (22% protein, 13.5% carbohydrates, 60% fat, and 4.5% crude fibre)
– Group 7: Sham-operated rats fed on a High Protein diet (HPD) (55% protein, 25.5% carbohydrates, 15% fat, and 4.5% crude fibre)
– Group 8: BOO animals fed on a High Protein diet (HPD) (55% protein, 25.5% carbohydrates, 15% fat, and 4.5% crude fibre)

The animals were fed for a period of 8 weeks, after which partial bladder outlet obstruction was induced in them surgically. Subsequently, they were fed for 4 weeks after induction.

2.3. Induction of bladder outlet obstruction

Twelve (12) hours prior to surgery, animals were fasted but allowed free access to drinking water. Anaesthesia was induced using ketamine (75 mg/kg ip) and xylazine (15 mg/kg ip). The bladder was approached through a lower midline incision which was used to expose the proximal urethra. A 3-0 Novafil (monofilament polybutester; Davis & Geck, Wayne, NJ) ligature was placed around the urethra and tied while a steel rod was placed in the lumen to create partial obstruction of the outlet. After the knot was tied, the steel rod was removed, the bladder repositioned, and the abdominal wall was closed. Sham-operated rats underwent the same procedure, however, no suture was placed around the urethra.

2.4. Preparation of tissue samples for ELISA

Rats were sacrificed with isoflurane (4%), after which a thoracotomy was performed to remove the bladder, which was weighed and then solubilized in tissue protein extraction reagent (1 g tissue/20 ml; Pierce Biotechnology, Woburn, MA) after which it was treated with protease inhibitor cocktail tablets (Roche, Indianapolis, IN). The tissue was then homogenized and then centrifuged at 10,000 rpm for 10 min. The resulting supernatant was subsequently used for CXCL12 protein quantification. Total protein was determined using the Pierce™ Coomassie Plus (Bradford) Assay Kit (ThermoFisher Scientific, UK). CXCL12 was quantified using standard 96-well ELISA plates (R&D Systems, Minneapolis, MN) according to the manufacturer's recommendations.

2.5. Estimation of plasma C - reactive protein, bladder tissue NGF and CXCL12 concentrations

Serum was separated from blood collected from rats and assayed for serum C-reactive protein (CRP). Urinary bladder was removed under anaesthesia blotted dry weighed and then homogenized for 1 min and centrifuged at 33,000 rpm for 1 h. Bradford assay was performed to determine the total protein in each sample, then the homogenate was concentrated using a protein concentrator (Millipore, Billerica, MA). Commercially-available rat CRP, NGF and CXCL12 ELISA kits (Merck KGaA, Darmstadt, Germany) were procured and used in accordance with manufacturer's protocols and instructions.

2.6. RT-PCR for TGF-β, CTGF, HIF-1α and PDGF-A

Total RNA was extracted from bladder samples and DNAse digestion performed for 60 min to remove any contamination by genomic DNA. First strand cDNA synthesis was carried out using a cDNA synthesis kit (Merck KGaA, Darmstadt, Germany) at 42 °C using 500 ng total RNA extract. Real-time RT-PCR was conducted using Power SYBR® Green PCR Master Mix (ABI, Foster, CA, USA) in a 25-μL tube with a total reaction volume of 25 μL containing 1 μL of a 1:2 dilution of first-strand reaction product, 0.2 μM gene-specific upstream and downstream primers (Table 1). Amplification and analysis of cDNA fragments was

Download English Version:

https://daneshyari.com/en/article/10137627

Download Persian Version:

https://daneshyari.com/article/10137627

Daneshyari.com