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Chronic treatment with resveratrol improves overactive bladder in obese mice via antioxidant activity

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ABSTRACT

The objective of the present work was to evaluate whether oral intake with resveratrol ameliorates overactive bladder in high-fat fed mice. Male C57BL6 mice fed with standard chow or high-fat diet to induce obesity received a two-week therapy with resveratrol (100 mg/kg, given as a daily gavage). Weight and metabolic profile, together with cystometry and in vitro bladder contractions were evaluated. Measurements of gp91phox and SOD1 mRNA expressions and reactive-oxygen species (ROS) in bladder tissues, and serum TBARS were performed. Obese mice exhibited increases in body weight and epididymal fat mass, which were significantly reduced by oral treatment with resveratrol. Cystometric study in obese mice showed increases in non-voiding contractions, post-voiding pressure and voiding frequency that were reversed by resveratrol treatment. Likewise, the in vitro bladder overactivity in response to electrical-field stimulation (80 V, 1–32 Hz) or carbachol (1 nM to 10 mM) were normalized by resveratrol. The gp91phox and SOD1 mRNA expressions in bladder tissues were markedly higher in obese mice compared with lean group. In addition, ROS levels in bladder tissues and serum lipid peroxidation (TBARS assay) were markedly higher in obese compared with lean mice, all of which were reduced by resveratrol treatment. In lean group, resveratrol had no effect in any parameter evaluated. Our results show that two-week therapy of obese mice with resveratrol reduces the systemic and bladder oxidative stress, and greatly ameliorated the cystometry alterations and in vitro bladder overactivity. Resveratrol treatment could be an option to prevent obesity-associated overactive bladder.

1. Introduction

Obesity has been described as a low-grade chronic inflammation associated with cardiovascular diseases and diabetes along with premature mortality (Apostolopoulos et al., 2016; Wang et al., 2012). Clinical studies have also implicated obesity as a major contributing factor for the lower urinary tract symptoms (Steers, 2009), which comprises storage, voiding or post micturition symptoms (Irwin et al., 2011). Overactive bladder is a subset of storage lower urinary tract symptoms currently defined as urgency, with or without urge incontinence, usually accompanied by frequency and nocturia (Abrams et al., 2002). Overactive bladder is often associated with detrusor overactivity, an urodynamic observation characterized by involuntary detrusor contractions during the filling phase, which may be spontaneous or provoked (Irwin et al., 2006). Obese animals exhibit cystometric alterations such as increases in the frequency of voiding and non-voiding contractions, along with in vitro detrusor overactivity (Leiria et al., 2014; Rahman et al., 2007; Wang et al., 2012). Urethral smooth muscle dysfunction due to impairment of the nitric oxide signal transduction also occurs in conditions of obesity (Alexandre et al., 2014).

Oxidative stress has been defined as an imbalance between the production of reactive-oxygen species and antioxidant defenses (Fatehi-Hassanabad et al., 2010; Frey et al., 2009). Reactive-oxygen species include free radicals such as superoxide anion and hydroxyl radicals, as well as non-radicals such as hydrogen peroxide (Frey et al., 2009). Reactive-oxygen species are generated through different systems including NADPH oxidase, xanthine oxidase, uncoupled nitric oxide synthase, cyclooxygenases and mitochondrial respiratory chain (Fatehi-Hassanabad et al., 2010; Chan,

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2001). It is well established that increased oxidative stress takes place in obesity as a causal factor to the high metabolic load of carbohydrates and/or fats (Santilli et al., 2015). Obesity-associated oxidative stress can be suppressed by caloric restriction (Walsh et al., 2014) or by drugs that mimic the biochemical and functional effects of caloric restriction (Madeo et al., 2014).

Resveratrol is a polyphenol present in many plant-based foods, such as grapes, peanuts and berries (Baur et al., 2006; Smoliga et al., 2011; Szkudelska and Szkudelski, 2010). This polyphenol increases lipolysis and reduces lipogenesis in adipocytes, improving resistance to weight gain caused by high-fat diet, being a therapeutic alternative to obesity-related diseases (Baile et al., 2011; Szkudelska and Szkudelski, 2010). Supplementation with resveratrol for 30 days in obese men was shown to ameliorate the metabolism and metabolic profile mimicking the effects of calorie restriction (Timmers et al., 2011). However, no studies attempted to investigate the actions of resveratrol in obesity-associated voiding dysfunction. Because overactive bladder accompanying obesity may be associated with oxidative stress and that polyphenols can protect bladder cells from an oxidative state, we thought that an antioxidant like resveratrol could provide an auxiliary strategy to treat this disorder (Coyle et al., 2008; Nomiya et al., 2015). This study aimed therefore to evaluate whether oral intake with resveratrol ameliorates overactive bladder in high-fat fed mice, focusing on its antioxidant and anti-obesity properties.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the local Ethics Committee for the Use of Experimental Animals (CEUA #3514-1), and are in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). Male C57BL6/JUnib mice (4 weeks old) were provided by the Central Animal House Services of State University of Campinas (UNICAMP). The animals were housed two per cage on a 12 h light–dark cycle, and fed for 12 weeks with either a standard chow diet (carbohydrate: 70%; protein: 20%; fat: 10%) or a high-fat diet to induce obesity (carbohydrate: 29%; protein: 16%; fat: 55%) (Alexandre et al., 2014).

2.2. Resveratrol treatment

Lean and obese mice were orally treated with resveratrol (Sigma-Aldrich, St. Louis, MO, USA) at the dose of 100 mg/kg/day (Rieder et al., 2012), given as a daily gavage from weeks 10–12 of the diet. Controls received daily gavage of vehicle (Water). Our protocols consisted of four experimental groups, namely lean+vehicle, lean+resveratrol, obese+vehicle and obese+resveratrol. A selection of animals was made before treatment to ensure similar body weights between groups.

2.3. Weight and metabolic profile

Weight and metabolic profile were assessed by measuring body weight, epididymal fat mass, total cholesterol, LDL and HDL. Serum cholesterol, LDL and HDL were determined by a commercial kit assay (Katal, São Paulo, SP, Brazil).

2.4. Cystometry

Mice were anesthetized by intraperitoneal injection of urethane (1.8 gm/kg). A 1 cm abdominal incision was made. The bladder was exposed and a 25 gauge butterfly cannula was inserted in the bladder dome. The cannula was connected to a 3-way tap, of which 1 port was connected to a pressure transducer and the other was connected to the infusion pump through a polyethylene-50 catheter. Before starting cystometry the bladder was emptied via the third port. Continuous cystometry was performed by infusing saline in the bladder at 0.6 ml per hour for 30 min after the end of the first micturition cycle. The parameters assessed were non-voiding contractions, capacity, threshold pressure, compliance, maximum voiding pressure, post-voiding pressure and frequency of voiding. The bladders of mice used for cystometry were not used in the other experiments.

Table 1
Primers sequence to β-actin, gp91phox and SOD1.

Gene	Primer	Optimal primer concentration
β-actin – F	5′-ACTGCCGCATCCTCTTCCT-3′	150 nM
β-actin – R	5′-GAACCGCTCGTTGCCAATA-3′	
gp91phox – F	5′-TTGGGTCAGCACTGGCTCTG-3′	70 nM
gp91phox – R	5′-TGGCGGTGTGCAGTGCTATC-3′	
SOD1 – F	5′-CAGCATGGGTTCCACGTCCA-3′	70 nM
SOD1 – R	5′-CACATTGGCCACACCGTCCT-3	

2.5. In vitro functional studies

Mice were killed in a CO_2 chamber. Longitudinal detrusor smooth muscle strips were obtained and mounted in 4 ml organ baths containing Krebs-Henseleit solution composed of 117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 11 mM glucose continuously bubbled with a mixture of 95% O_2 and 5% CO_2 (pH 7.4) at 37 °C. Changes in isometric force were recorded using a PowerLab, version 7.2 system (ADInstruments). Resting tension was adjusted to 5 mN at the beginning of the experiments. The equilibration period was 45 min and the bathing medium was changed every 15 min. Cumulative concentration-response curves to the muscarinic receptor agonist carbachol (1 nM to 10 mM; Sigma-Aldrich, St. Louis, MO, USA) and electrical-field stimulation (80 V, 1–32 Hz, 10 s of stimulation) were performed in detrusor smooth muscle strips from vehicle and resveratrol treated mice. Contractile response data were normalized to the wet weight of the respective bladder strips. Nonlinear regression analysis to determine the pEC_{50} was carried out using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) with the constraint that F=0. The concentration–response data were evaluated for a fit to a logistics function in the form: $E = Emax/([1+(10c/10x)n]+F)$, where E is the maximum response produced by agonists; c is the logarithm of the EC_{50}, the concentration of drug that produces a half-maximal response; x is the logarithm of the concentration of the drug; the exponential term, *n*, is a curve-fitting parameter that defines the slope of the concentration–response line, and F is the response observed in the absence of added drug. Data are expressed as the mean values ± S.E.M. EC_{50} values are presented as the negative logarithm (pEC_{50}), and calculated by a fitting concentration–response relationship to a sigmoidal model of the form log-concentrations vs response using the GraphPad Software.

2.6. Local and systemic oxidative stress measurements

The oxidative fluorescent dye dihydroethidium (Sigma-Aldrich, St. Louis, MO, USA) was used to evaluate in situ reactive-oxygen species generation. The bladder was embedded in freezing medium. Transverse sections (12 μm) of frozen tissue were obtained using a cryostat, collected on glass slides and equilibrated for 10 min in Hanks solution composed of 1.6 mM $CaCl_2$, 1.0 mM $MgSO_4$, 145.0 mM NaCl, 5.0 mM KCl, 0.5 mM NaH_2PO_4, 10.0 mM