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Lentil consumption reduces resistance artery remodeling and restores arterial compliance in the spontaneously hypertensive rats☆

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Abstract

We previously established that lentils were able to significantly attenuate the development of hypertension in spontaneously hypertensive rats (SHRs), but the mechanism was not investigated. The current study was therefore designed to examine the effect of lentils on arterial function in relation to arterial stiffness, lipid biochemistry and activation of select aortic proteins. Seventeen-week-old male SHRs were randomly assigned to groups (n=10/group) fed (a) 30% w/w green lentils, (b) 30% red lentils, (c) 30% mixed lentils (red and green) or (d) no lentils for 8 weeks. Normotensive Wistar Kyoto (WKY) groups (n=10/group) received either the mixed lentil or no lentil diet. Blood pressure, pulse wave velocity and serum lipids were measured at baseline and 8 weeks, while pressure myography, arterial morphology and aortic proteins were measured after termination. There were no dietary-related changes in pulse wave velocity or blood pressure for any SHR or WKY group. Low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were significantly lower in only SHR red lentil and WKY mixed lentil groups compared to their controls. The lentil diets reduced the media:lumen ratio of SHRs relative to control-fed SHRs but had no effect on WKYs. Both red and green lentils reduced arterial stiffness of SHRs but not WKYs. SHR lentil groups showed lower aortic p38 mitogen-activated protein kinase (p38MAPK) phosphorylation, thus implying that p38MAPK activation is suppressed with lentil feeding. Lentil-based diets suppress pathological vascular remodeling in SHRs, while green lentils maintain the vascular function of SHRs similar to normotensive WKYs despite the presence of high blood pressure.

Keywords: Hypertension; Lentils; Arterial remodeling; Spontaneously hypertensive rats; p38MAPK

1. Introduction

Hypertension affects more than 1 in 5 adults in North America [1], and its prevalence is increasing as a result of the obesity epidemic and the aging population [2,3]. Over time, hypertension damages organs such as the brain, eyes, heart and kidneys, with resistance arteries considered to be the first organ affected [4,5]. Resistance arteries, those arteries smaller than 350 μm, are important in regulating blood flow and preventing a fluctuating pressure environment in the organs distal to the arterial beds [6]. Damage to the resistance arteries comes in the form of remodeling and is caused by increased shear and tensile stresses resulting in decreased arterial dispensability and compliance, a process termed arterial stiffening [3,7].

Like hypertension, arterial remodeling and stiffening are largely asymptomatic until the late stages of the disease are reached, at which point they affect organ function and ambulation [8]. Typically, arterial remodeling takes the form of eutrophic inward remodeling [9]. Eutrophic inward remodeling decreases the lumen and external diameters, without changing the *tunica media* cross-sectional area, and results in a higher media:lumen ratio, a measurement used to determine the degree of remodeling.

With 90% of hypertension diagnoses being idiopathic and therefore categorized as essential hypertension [10], treatment requires polypharmacy to manage the pressure and associated complications [11]. However, there is an increasing emphasis being placed on diet and exercise to help manage hypertensive patients. Within this context, research on pulse crops has revealed that consumption of dried beans, peas, lentils and chickpeas can provide benefits with respect to cardiovascular health [12–16]. Rimm et al. [12] showed that a daily serving of peas reduced the relative risk of heart attack to 0.52 (95% confidence interval, 0.31–0.88), while Bazzano et al. [13] indicated that eating four servings of pulses a week, compared to one or fewer, reduced the risk of coronary heart disease and cardiovascular disease

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by 22% and 11%, respectively. These observations are supported by a recent meta-analysis that indicated consumption of pulses can lead to a significant reduction in systolic blood pressure (BP) [17]. Additionally, there is evidence that pulses and pulse extracts may decrease vascular remodeling [18,19] in response to AngII [15], even inhibiting ACE directly [14,20].

The spontaneously hypertensive rat (SHR) is a well-established and widely used model of hypertension [21], experiencing marked increases in both BP and arterial stiffness [22]. Additionally, the SHR exhibits increased expression of hypertrophic mediators such as profilin-1 and ACE [23,24], and activation of p38 mitogen-activated protein kinase (p38MAPK) and extracellular signal-regulated kinase 1/2 (ERK1/2) in response to the chronic hypertensive state [25,26]. We have previously shown that a lentil-based diet containing a mixture of red and green lentils was able to attenuate the age-related rise in BP as well as alter aortic structure in the SHR [19], but the effects of the mixed lentil diet on functional parameters as well as mechanism of action were not examined. While red and green lentils have a similar nutritional composition, they differ in their profile of phenolic compounds with potential bioactivity [27,28] and thus could have distinct effects on vascular parameters. Given the positive results we obtained with a mixture of red and green lentils [19], we extended our previous work by comparing the effects of red and green lentils individually on BP, serum lipids and vascular morphology, as well as vascular function, by both pulse wave velocity (PWV) and morphometry. Additionally, changes in aortic p38MAPK, Giα, ERK1/2 and profilin were examined to address mechanism since these proteins have been associated with the vascular changes that can occur in hypertension [25,26,29,30].

2. Materials and methods

2.1. Animals and experimental diets

Seventeen-week-old male SHR and Wistar Kyoto (WKY) rats (Charles River Laboratories, Saint-Constant, QC, Canada) were housed individually for the 8-week dietary intervention period. There were six study groups (n=10/group): SHR control (SHR-Ctrl), SHR mixed lentil (SHR-ML), SHR green lentil (SHR-GL), SHR red lentil (SHR-RL), WKY control (WKY-Ctrl) and WKY mixed lentil (WKY-ML). Diets were formulated and prepared as previously described [19], and ingredients and proximate analysis data are provided in Supplemental Table 1. Whole lentils were cooked, freeze-dried and powdered before addition to the diets to inactivate antinutritional factors and mimic preparation for human consumption. Based on our previous study [19] and the work of others [31], the dose (30% w/w lentils) provides approximately 26% of total energy based on feed consumption. These experiments were carried out in accordance to proper animal care and experimentation as outlined by the Canadian Council on Animal Care and a protocol approved by the University of Manitoba Animal Care Committee.

2.2. BP and PWV

BP was measured at baseline and week 8 by tail-cuff plethysmography (CODA system; Kent Scientific, Torrington, CT, USA). At least five values were obtained for each animal at each time point for calculations of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) averages. PWV in the femoral artery was measured in anesthetized rats with a 10-MHz electrocardiogram-triggered Doppler probe (Indus Instruments, Houston, TX, USA). The PWV analysis was done in a blinded manner using the Doppler Signal Processing Workstation program (DSPW Version 1.624; Indus Instruments, Houston, TX, USA) as previously described [19]. Briefly, the software was used to locate the baseline of the PWV trace as well as determine the pulse waveforms on the electrocardiogram trace. Afterward, the peak velocity, the mean flow velocity, the minimum flow velocity, the pulsatile index and the resistivity index were manually identified on the Doppler trace. Approximately 17 peaks were analyzed per trace, with 3 traces per animal per time point.

2.3. Body composition

Body composition (fat mass, lean body mass, total and free water) was assessed *in vivo* through use of an EchoMRI-700 whole body quantitative magnetic resonance instrument (EchoMRI, Houston, TX, USA) at baseline, week 4 and week 7.

2.4. Serum biochemistry

Fasting serum samples obtained at baseline (week 0), week 4 and week 7 from the saphenous vein were analyzed with a Cobas C111 auto analyzer (Roche Diagnostics, Indianapolis, IN, USA) for total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, glucose, creatinine and urea.

2.5. Tissue collection

Rats were euthanized by injecting an overdose of pentobarbital. The heart, liver, perirenal and epididymal adipose tissues were excised and weighed to determine organ to body weight ratios. The left ventricle was isolated from the rest of the heart and weighed. The aorta was excised, and a portion of descending aorta was embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA, USA) and frozen in a dry ice-ethanol bath, while the remainder of the aorta was snap frozen in liquid nitrogen and stored at −80°C.

2.6. Pressure myography

A third order vessel isolated from the first 10 cm of mesenteric fat was mounted on a pressure myograph (Living Systems Instrumentation, Burlington, VT, USA), pressurized to 45 mmHg, allowed to equilibrate for 1 h in Krebs–Henseleit (KH) buffer (25 mM $NaHCO_3$, 5.5 mM glucose, 2.7 μM NaEDTA, 120 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, pH 7.4) at 37°C, then challenged with 125 mM KCl solution (in KH buffer) to constrict and check artery viability. After the 30 min in Ca^{2+}-free KH buffer containing 10 mM EGTA, triplicate measurements of lumen diameter and left and right wall thickness were made at 3, 10, 20, 30, 40, 60, 80, 100, 120 and 140 mmHg. Calculations of stiffness of the artery media stress, media strain, elastic modulus and media cross-sectional area were done as described previously [4,32].

2.7. Aorta histology

Sections were prepared and fixed as described previously [19]. Elastin and collagen were differentially stained with an Elastin Stain Kit (Sigma-Aldrich, St. Louis, MO, USA), and digital images were analyzed with ImagePro Plus (Media Cybernetics, Rockville, MD, USA) to obtain lumen diameter, media thickness, media:lumen ratio, media cross-sectional area and external diameter [19]. The sections were also analyzed with ImageJ software [33] to determine the relative elastin (black), collagen (red) and cellular (yellow) components of the vessels. Sections stained with Lee's methylene blue [34] were used to quantify cell number per unit area.

2.8. Western blotting

Aorta lysates were prepared as previously described [35], and the proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis prior to transfer to polyvinylidene fluoride membranes. The blots were stained with Ponceau S to assess protein loading. All primary antibodies (Giα, p38MAPK, phospho-p38MAPK Thr-180/Tyr-182, profilin1, ERK1/2, phospho-ERK1/2) were from Cell Signaling (Danvers, MA, USA). Bands were visualized with Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare, Little Chalfont, UK), imaged with a FluorChem Q Western Blot imager and quantified using AlphaView SA software (Protein Simple, Santa Clara, CA, USA).

2.9. Statistical analysis

Measurements over time and end point data were analyzed by repeated-measures analysis of variance (ANOVA) and one-way ANOVA, respectively, using Statistical Analysis Software (SAS) (version 9.2; SAS, Cary, NC, USA). Outliers were identified as those values outside the mean±2.5 standard deviations. Duncan's multiple-range test was used for *post hoc* means testing. When required, data were log-transformed to achieve normality and homogeneity, or nonparametric statistics were used. Pressure myography data were analyzed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). Linear regression one-way ANOVA was calculated, and a *post hoc* Newman–Keuls multiple-comparison test was performed to determine significance between groups. Log-transformed stress–strain relationships were calculated using a one-way ANOVA in SAS. Data are expressed as mean±standard error (S.E.) unless otherwise indicated. A P value ≤.05 was considered to be significant.

3. Results

3.1. Tissue weights and body composition

Differences between SHR-Ctrls and WKY-Ctrls were observed for body and organ weights, and body composition, but no changes due to the lentil diets were seen (Tables 1 and 2).