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Daily non-soy legume consumption reverses vascular impairment due to peripheral artery disease

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ABSTRACT

Objective: Peripheral artery disease (PAD) results from a decrease in blood flow to the limbs due to the presence of atherosclerotic plaque. It has been reported that isoflavones isolated from soybeans reduce arterial stiffness, a component of atherosclerotic disease. This study examined the effect of consuming whole legumes (non-soy) on arterial function in humans with PAD.
Methods: Twenty-six individuals with PAD consumed ½ cup/day cooked legumes (beans, peas, lentils, chickpeas) daily for 8 weeks. Measurements of circulating factors and vascular function at baseline and study conclusion were compared.
Results: No changes in were detected relative to baseline values for most parameters. Total and LDL-cholesterol were reduced by 5.0% and 8.7%, respectively. The ankle-brachial index (ABI) showed a 5.5% increase. Changes in ABI and LDL-cholesterol did not correlate. Serum markers of endothelial dysfunction and inflammation were unchanged, but short-chain acylcarnitine concentrations were significantly decreased.
Conclusions: A legume-rich diet can elicit major improvements in arterial function and serum cholesterol in the absence of changes in either body mass or blood pressure, although the improvements in vascular function and serum lipids were unrelated. Although the positive results obtained with this dietary intervention were not explained by biomarkers of endothelial function and inflammation, altered acylcarnitine levels indicate an improvement in skeletal muscle metabolism due to enhanced tissue perfusion.

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1. Introduction

Peripheral artery disease (PAD) represents one manifestation of systemic atherosclerosis, affecting up to 20% of persons over 60 years of age [1]. PAD diagnosis and progression are typically based on the ankle-brachial index (ABI), which is a ratio of systolic pressure at the ankle (posterior tibial or dorsalis pedis artery) and the arm (brachial artery). An ABI value below 0.9 indicates a reduction in the ankle blood pressure, due to the presence of arterial stenosis which reduces blood flow to the legs [2]. As a result, PAD is associated with a decrease in functional performance that diminishes quality of life [3] and is an independent predictor of a high risk of death due to coronary artery and cerebrovascular disease [4].

A consequence of PAD is increased arterial dysfunction, which can be detected as decreased arterial compliance [3], and reliably detected by pulse wave analysis (PWA) measurements [5]. To date, pharmacological interventions have had equivocal success in improving arterial compliance [6], however, both epidemiological evidence and interventional studies have shown arterial stiffness may be improved with dietary manipulations [7,8].

Legumes are grown primarily for the production of oil (e.g. soybeans) or their protein and fiber-rich seed (e.g. beans, peas, lentils, chickpeas). Epidemiological studies have reported an association between a decreased incidence of atherosclerosis and higher consumption of legumes (soy and non-soy) [9,10], presumably through a reduction in serum lipids due to the high fiber content and low glycemic index of these seeds [11]. At the

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same time, there is strong evidence that changes to atherosclerosis as characterized by arterial stiffness may result directly through the action of the isoflavones present in soybeans [12]. These data are supported by the fact that intervention trials employing isoflavones extracted from soybeans have produced beneficial effects on atherosclerosis [13] and have been shown to reduce arterial stiffness [14]. Interestingly, no study has yet examined whether consumption of whole legumes, either soy or non-soy, is capable of modulating arterial stiffness. We therefore initiated a clinical study to determine if daily consumption of mixed non-soy legumes (beans, peas, lentils, chickpeas) for 8 weeks would positively affect arterial dysfunction in a cohort with PAD.

2. Materials and methods

2.1. Study design

Twenty-six individuals, thirteen men and thirteen women, with PAD as defined by an ankle-brachial index $\leq$0.90 or asymptomatic carotid stenosis lesions of >50%, were recruited from the Vascular Surgery Clinic at St. Boniface Hospital, Winnipeg, Canada. The participants completed an eight-week trial that required the daily consumption of foods containing ¼ cup in week 1 and ½ cup in weeks 2–8 of cooked mixed non-soy legumes (beans, peas, lentils, chickpeas). Each participant was expected to incorporate one of fourteen distinct food items (soups, side dishes, entrees; each consisting of ½ cup cooked legume) each day into their usual meal planning. Approximately equal amounts of beans (mixture of pinto, kidney, black and navy), peas (green and yellow), lentils (green and red) and chickpeas were consumed. Inclusion criteria were age >40 and a stable medication profile with no changes anticipated for the duration of the study. Exclusion criteria included renal failure requiring dialysis, hormone replacement therapy and currently smoking, as well as a history of gastrointestinal reactions or allergies to legumes.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the University of Manitoba Research Ethics Board and the St. Boniface General Hospital Research Review Committee. The study protocol was registered with an ICMJE approved registry (www.Clinicaltrials.gov NCT01382056). All participants provided written informed consent consistent with guidelines for the protection of human research participants.

2.2. Clinical characteristics

Prior to beginning the study, a medical history was obtained regarding concurrent conditions, medications and food consumption patterns. Anthropometric data (height, weight, age, body-mass index (BMI)), blood pressure, ankle-brachial index (ABI) and fasting blood were obtained at baseline and terminal visits. Lipid profiles and glycated hemoglobin (HbA1c) were measured by the hospital's clinical laboratory.

2.3. Pulse wave analysis

The pulse waveform of the radial artery was recorded by applanation tonometry (SphygmoCor®, AtCor Medical, Sydney, Australia). The central (aortic) augmentation index (AIx), which represents the percentage increase in blood pressure due to pulse wave reflections, was normalized to a standard heart rate of 75 beats/min [15]. All quality control parameters, including an operator index of >85%, were used to define the minimum criteria for an acceptable recording.

2.4. Laboratory analyses

Serum was prepared from fasting blood samples and stored at −80 °C. Circulating levels of biomarkers associated with endothelial dysfunction, inflammatory cytokines and metabolism were determined by ELISA. Commercial kits were obtained from ALPCO Diagnostics (Salem, NH) for adiponectin, insulin and vitamin B12, BioVendor (Candler, NC) for arginase and glucagon-like peptide-1 (GLP-1), Diagnostic Systems Laboratories (Webster, TX) for C-reactive protein, EMD Millipore (Billerica, MA) for E-selectin and intercellular adhesion molecule (ICAM), R&D Systems Inc (Minneapolis, MN) for interleukin-6 (IL-6), interleukin-10 (IL-10) and total nitric oxide (nitrite/nitrate), ALerCHEK Inc (Portland, MA) for Lp(a), Diagnostic Chemicals Ltd (Charlottetown, PEI) for glucose, Biovision Research Products (Milpitas, CA) for uric acid and Abbott Diagnostics Division (Abbott Park, IL) for homocysteine.

2.5. Acylcarnitine profile

Serum acylcarnitine levels were measured at the Metabolomics Innovation Centre, University of Alberta, using an ABI 4000Q Trap ESI-MS/MS for direct flow injection mass spectrometry. The extracted analytes were derivitized prior to selective mass-spectrometric detection using multiple reaction monitoring pairs. Isotope-labeled internal standards were used for metabolite quantification.

2.6. Statistical analysis

The data from all sources were entered into a single database. The data were analyzed with a paired *t*-test to identify significant differences between the baseline and final values using SPSS (PASW Statistics 18; Armonk, NY). Correlation analysis was also completed using SPSS software.

3. Results

3.1. Demographics

The mean age of the participants (n = 26; 13 male, 13 female) was 69.5 ± 8.9 years, with a range of 48–84 years. The participants were on prescribed medication for one or more of hypertension (n = 23), hyperlipidemia (n = 20) or type 2 diabetes mellitus (n = 7). No participants had renal disease. ABI was <0.9 in at least one limb.

3.2. Consumption patterns

A study questionnaire administered at baseline showed that 90% of the participants consumed legumes 1–3 times per month or less. Three day food records at baseline and at 6–7 weeks also showed there were no significant changes in energy intake (1698 ± 81 kcal at baseline vs. 1764 ± 86 kcal at 6–7 weeks), or consumption of carbohydrate (192 ± 11 g vs. 200 ± 12 g), total fat (59.5 ± 3.7 g vs. 58.2 ± 3.8 g) or saturated fat (19.9 ± 1.7 g vs. 20.5 ± 1.9 g) as a result of participating in the study. Similarly, no significant changes in nutrient intake were detected as a result of the intervention except for fiber (17.0 ± 1.2 g vs. 22.0 ± 1.2 g), vitamin C (60.4 ± 8 mg vs. 89.5 ± 9.7 mg) and iron (11.5 ± 0.5 mg vs. 13.3 ± 0.5 mg), suggesting that participants did not significantly alter their background diet.

3.3. Anthropometric parameters

The initial weight and BMI of the subjects ranged from 40.4 to 128.4 kg and 18.6–41.8 kg/m^2, respectively. After 8 weeks on the

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