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Plant sterols and casein-derived tripeptides attenuate blood pressure increase in spontaneously hypertensive rats

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ABSTRACT

In this study, we investigated the synergistic effects of plant sterols (PS) and casein-derived tripeptides on arterial tone and blood pressure in experimental hypertension. We hypothesized that PS and tripeptides could have positive, synergistic effects on the development of hypertension and endothelial dysfunction in young spontaneously hypertensive rats (SHR). Six-week-old male SHR were divided into 3 groups to receive milk products containing PS, or PS with tripeptides, or a control containing no active components for 8 weeks. Systolic blood pressure (SBP) was measured weekly, and vascular reactivity measurements with isolated mesenteric arteries were performed at the end of the study. Biochemical measurements for several parameters were performed by enzyme-linked immunosorbent assay using plasma samples. Levels of angiotensin-converting enzyme 1, cyclooxygenase-2, endothelial nitric oxide synthase, and P-selectin messenger RNA expressions were determined from aortic tissue by real-time polymerase chain reaction. The study showed that long-term treatment with PS + tripeptides attenuated the development of hypertension in SHR (SBP, 187 ± 5 mm Hg vs 169 ± 4 mm Hg in control group; $P < .01$). Plant sterols alone did not affect SBP significantly. Endothelial dysfunction was observed in all SHR; however, treatment with PS resulted in poorer endothelium-dependent and nitric oxide–mediated relaxation compared with other groups. Aortic cyclooxygenase-2 and P-selectin were significantly down-regulated in PS and PS + tripeptides groups when compared with the control group. The expression of endothelial nitric oxide synthase was significantly lower in PS than in PS + tripeptides group. In conclusion, long-term treatment with PS has a slight but not significant antihypertensive effect. Plant sterols do not provide any beneficial effects on endothelial function in hypertensive rats; however, treatment with both PS and tripeptides showed mild anti-inflammatory effects.

Abbreviations: ACE1, angiotensin-converting enzyme 1; ACh, acetylcholine; ADMA, asymmetric dimethylarginine; Ang I, angiotensin I; Ang II, angiotensin II; COX-2, cyclooxygenase-2; DBP, diastolic blood pressure; eNOS, endothelial nitric oxide synthase; hs-CRP, high-sensitive C-reactive protein; Ile-Pro-Pro, isoleucine-proline-proline; KCl, potassium chloride; LDL, low-density lipoprotein; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide; NOx, nitrite/nitrate; PE, phenylephrine; PS, plant sterols; PS + T, plant sterols with tripeptides; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; SNP, sodium nitroprusside; TRAM-34, 1-[(2-chlorophenyl)diphenylmethyl]-1*H*-pyrazole; Val-Pro-Pro, valine-proline-proline.

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1. Introduction

Increased blood pressure is one major risk factor for cardiovascular disease, and it often coexists with other risk factors including hypercholesterolemia, insulin resistance, metabolic syndrome, and arterial stiffness. Besides pharmacologic therapy, lifestyle and nutritional factors play a significant role in the prevention and treatment of hypertension and related disorders. Dietary efforts to decrease saturated fat and sodium and increase potassium, calcium, and soluble fiber intake positively affect blood pressure [1]. In addition, both epidemiologic and intervention studies suggest that consumption of low-fat dairy products is inversely related to the risk of hypertension [2-5]. At present, milk proteins are considered the most important source of bioactive peptides [6]. Milk casein–derived tripeptides isoleucine-proline-proline (Ile-Pro-Pro) and valine-proline-proline (Val-Pro-Pro) have been found in milk products fermented with *Lactobacillus helveticus*. They have been shown to possess antihypertensive effects both in humans [7,8] and in experimental animals [9–11]. In addition, Ile-Pro-Pro and Val-Pro-Pro have been shown to have vasoprotective effects in different animal models [10,12], reduce arterial stiffness [13,14], and improve vascular function in humans [15].

Plant sterols (PS) and stanols have well-established cholesterol-lowering effects. Consuming 2 g/d of PS or stanols has been shown to lower both total and low-density lipoprotein (LDL) cholesterol concentrations by approximately 10% in humans in different population groups [16,17]. They reduce blood cholesterol levels by competitively inhibiting absorption of both dietary and endogenous cholesterol and also by up-regulating intestinal cholesterol efflux transporters [18]. The effects of PS and stanols on endothelial function in humans seem to be mild; however, consuming sterol ester for 10 weeks increased brachial artery diameter [19], and that for 2 or more years was associated with higher carotid artery compliance [20]. In animals, there are few studies addressing the effects that PS and stanols have on vascular function. The use of PS or stanols, in coordination with pharmacotherapy, to achieve cholesterol-lowering goals is currently recommended by the US National Cholesterol Education Program Adult Treatment Panel III Guidelines [21]. The European Commission has also approved the use of a health claim in context with PS- or stanol-containing products [22].

General interest in functional food products that may be consumed as a part of a normal diet to prevent or non-pharmacologically treat cardiovascular diseases has increased. More data describing the effects of functional food components are needed to either support or prove the opposite. Because the effects of PS on vascular function and blood pressure are largely unknown, the present study aimed at investigating these issues. Based on the known beneficial cardiovascular effects of PS and stanols and previously shown antihypertensive effects of casein-derived tripeptides, we hypothesized that PS and tripeptides could have positive, synergistic effects on the development of hypertension and endothelial dysfunction in young spontaneously hypertensive rats (SHR).Young SHR is a widely used and well-characterized animal model of human essential hypertension, and it has been used in several studies that investigated the effects of nutritional factors on cardiovascular function [9,23,24]. In addition to monitoring the rat blood pressure, vascular reactivity measurements with isolated arteries and biochemical measurements from tissue and blood samples were performed to gain more knowledge of possible changes in the vascular level and clarify the mechanisms behind the observed effects.

2. Methods and materials

2.1. Experimental protocol

The protocol was approved by the National Animal Experimentation Committee according to EC Directive 86/609/EEC and Finnish Experimental Animal Act 62/2006. Twenty-four male SHR were obtained from Charles River Laboratories (Sulzfeld, Germany) at the age of 5 weeks. The rats were housed 4 per cage in a standard experimental animal laboratory (illuminated from 7:00 AM to 7:00 PM; temperature, 22°C ± 2°C; humidity, 55°C ± 15°C). The rats had free access to standard rat pellet (2018 Teklad Global 18% Protein Rodent Diet; Harlan Laboratories, Madison, Wisc) and water.

After 1 week's adaptation, baseline blood pressure measurements were performed. The systolic blood pressure (SBP) of conscious rats was assessed by the tail-cuff method using Apollo 2AB Blood Pressure Analyzer, model 179-2AB (IITC Life Science, Woodland Hills, California). The rats were placed in restrainer tubes and warmed in a heated chamber (32°C-34°C) for 15 to 20 minutes to make the pulsations of the tail artery detectable. After obtaining 3 consecutive and successful recordings without disturbance of the signal, the average of the values was determined to be the SBP. Thereafter, based on the SBP values and body weights, 6-week-old rats were randomized into 3 groups (n = 8) to receive one of the study products for 8 weeks.

2.2. Study products

The study products were drinkable, fermented milk products, which were administered to the rats from standard rat drinking bottles. The contents of the study products were as follows:

PS: contained a PS mixture (Cognis, Monheim, Germany) that was obtained by esterification of free PS with fatty acids obtained from vegetable oil (5.1 g saturated, 7.6 g monounsaturated, and 27.3 g polyunsaturated fatty acids per 100 g) and contained mainly β-sitosterol (69%), campesterol (15%), β-sitostanol (8%), and brassicasterol (3%) and other sterols or stanols in minor amounts.

PS + T: contained the PS mixture (as described previously) and tripeptides Ile-Pro-Pro, Val-Pro-Pro, and Leu-Pro-Pro, which were obtained by fermentation with *L helveticus* and enzymatically by proline-specific endoprotease (DSM, Heerlen, the Netherlands).

Control: a fermented milk product containing neither PS nor tripeptides in detectable amounts.

Study products were provided by Valio Ltd (Helsinki, Finland). All study products were low-fat (<1%) and low-