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Lingonberry juice negates the effects of a high salt diet on vascular function and low-grade inflammation

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ABSTRACT

High salt in diet elevates blood pressure in man. Berries and fruits are regarded beneficial in antagonizing hypertension and related vascular complications. We aimed to test whether lingonberry juice shows beneficial and anti-inflammatory effects in salt induced hypertension rat model. Male Wistar–Kyoto rats were fed with 8% sodium chloride enriched pellets for 10 weeks having tap water or diluted lingonberry juice as drinking fluid. Blood pressure was recorded weekly and mesenteric artery functions tested ex vivo. Urine was collected in metabolic caging. Salt loading had only minor effects on blood pressure or endothelial function. Increased urine excretion of 8-isoprostane, cGMP and albumin reflected general or renal inflammation. Elevated expression (mRNA) of proinflammatory COX-2 by salt was normalized by lingonberry. Salt increased kidney, heart and left ventricle masses and changed serum chloride, alkaline phosphatase, albumin and lipid concentrations. Taken together the results show that l0 weeks' high-salt diet impaired kidney function of young rats without clear effect on blood pressure or vascular function. Lingonberry juice moderately reduced biomarkers of low-grade inflammation probably due to its high polyphenol concentrations.

1. Introduction

Excess salt intake and poor dietary habits are a worldwide problem. Humans are physiologically and genetically adapted to much lower salt intake, and high salt concentration in diet force kidneys to function at their extreme limit. High dietary salt intake increases the risk for cardiovascular disease and urinary sodium excretion correlates to blood pressure (Feng & MacGregor, 2012; Intersalt Cooperative Research Group, 1988; Tuomilehto et al., 2001). It has been shown that reduction of salt intake from current 10–12 g/day to the recommended level of 5–6 g/day lowers blood pressure of hypertensive and normotensive subjects, thus decreasing the risk of cardiovascular problems (Feng & MacGregor, 2012). In Finland, 30% decrease in salt intake during past 30 years has lowered the systolic and diastolic blood pressure over 10 mmHg, and also a decrease in stroke and coronary heart disease mortality has been accomplished (Karppanen & Mervaala, 2006). Because salt plays such a marked part in the development of hypertension in man, hypertensive rat models have been developed to test the effects of new drugs in excess salt intake related human hypertension. However,

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in these models much higher salt loading up to 8% in diet should be used (Gordon, Drury, & Schapiro, 1953; Zicha et al., 2012) unless a salt sensitive "Dahl's rat" strain is considered (Dahl, 1961).

Sodium is an important regulator of normal body physiology. The ability of the kidneys to excrete excessive sodium to urine inhibits plasma sodium elevation caused by diet (Haddy & Pamnani, 1995). In some cases, kidney failure causes inability to excrete sodium leading to increase in blood pressure and disturbing compensatory mechanisms (Guyton, 1991).

Salt itself and other hypertensive agents, like angiotensin II cause vascular inflammation suggested to be related mainly but not only to elevated blood pressure (Harrison et al., 2011). Aldosterone, an important regulator of blood pressure and salt excretion, stimulates inflammation regulator of blood pressure and salt excretion, stimulates inflammation by inducing oxidative stress which leads to activation of proinflammatory transcription factors, such as nuclear factor (NF)-κB (Gilbert & Brown, 2010). Inflammatory molecules in vasculature are also released by dysfunctional endothelium causing e.g. platelet activation and adhesion to the vascular wall (Libby, 2008).

Polyphenols have positive effects on blood pressure and cardiovascular health (Stoclet et al., 2004). We have found that lingonberry (*Vaccinium vitis-idaea*), a northern berry rich in polyphenols, improves vascular endothelium-dependent relaxation and decreases blood pressure of spontaneously hypertensive rats (SHR) (Kivimäki, Ehlers, Turpeinen, Vapaatalo, & Korpela, 2011; Kivimäki et al., 2012). Anti-inflammatory effects of polyphenols are proposed to explain positive cardiovascular effects (González et al., 2011; Tangney & Rasmussen, 2013). Accordingly, we found that lingonberry and cranberry juices given at high concentrations in drinking fluid inhibited mRNA expressions of cyclooxygenase 2 (COX 2), monocyte chemoattractant protein 1 (MCP1) and P-selectin in spontaneously hypertensive rats (SHR) (Kivimäki et al., 2012).

The present study aimed to investigate whether lingonberry juice could abolish the development of hypertension and impaired vascular function in a widely used experimental model, salt induced hypertension.

2. Materials and methods

2.1. Animals

Thirty-two male Wistar Kyoto rats (WKY) six weeks of age were purchased from Charles River Laboratories (Sulzfeld, Germany). The rats were randomized by weight and systolic blood pressure and housed four to a cage in a standard animal laboratory to four treatment groups. Two high-salt groups received standard rodent diet (Harlan, Venray, The Netherlans) containing 8% NaCl which is a concentration most often used to induction of hypertension since the pioneering works of Gordon and Dahl (Dahl, 1961; Gordon et al., 1953). Lower concentrations have not consistently elevated blood pressure in rat unless unilaterally nephrectomised (Vapaatalo, Lahovaara, & Hackman, 1970) or mineralocorticoid treated (Vapaatalo, Lahovaara, Torsti, & Paasonen, 1969). This high salt concentration is not possible to use in the clinical studies, but is needed when fast increase (in weeks) of blood pressure and development of its complications are of interest in otherwise healthy animals. The control groups received standard rodent diet (Harlan) containing 0.2% Na. The study groups were as follows: (1) normal diet and tap water, (2) normal diet and cold-compressed lingonberry juice with 1% of sucrose, (3) high-salt diet and tap water, (4) high-salt diet and lingonberry juice with 1% of sucrose. One percent of sucrose-containing tap water was given to the water groups to reach about similar energy intake as juice-drinking groups and to exclude possible sucrose effect. The amount of given sucrose-containing tap water was calculated from the amount of daily intake of lingonberry juice. In addition, all groups received normal tap water *ad libitum*. The lingonberry puree was purchased from Bandedosa PLC (Ilmajoki, Finland), compressed without heating to lingonberry concentrate and diluted to one part of lingonberry concentrate and four parts of water. The dilution was selected based on the polyphenol concentration being now about half of that in our previous studies (Kivimäki et al., 2011, 2012). This was aimed to indicate dose–response dependency. The consumption of drinking fluids was recorded daily and consumption of feed was recorded weekly. The protocol was approved by National Animal Experimentation Committee of Finland according to EC Directive 86/609/ECC and Finnish Experimental Animal Act 62/2006 (ESLH-2008-05775/Ym-23).

Once a week, systolic blood pressure (SBP) and heart rate were measured with non-invasive blood pressure measurement system (tail-cuff method) CODA (Kent Scientific Corporation, Torrington, CT, USA). The rats were pre-warmed for 10–15 min at 32 °C to intensify the pulsation of the tail artery. Fifteen measurements (automatically) of each rat were made before noon by the same researcher. SBP, DBP and heart rate were calculated as the mean of the measurements accepted by the CODA program.

Between nine and ten weeks of treatment, rats were housed individually in metabolic cages for 24 h. They received same feed and drinking fluid as during the experiment *ad libitum*. Urine rats was collected during 24 h and stored at −80 °C until further analysis within two weeks.

After ten weeks' treatment the rats were rendered unconscious with CO_2/O_2 (30%/70%, AGA, Riihimäki, Finland) and decapitated.

2.2. Collection of samples

Blood was collected after decapitation with or without ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, and centrifuged 3000*g* for 10 min at +4 °C. Aliquots were stored in −80 °C until analysis. Superior mesenteric arteries were excised and placed in ice-cold oxygenated Krebs buffer (pH 7.4–7.6 composition in mM: NaCl 119.0, $NaHCO_3$ 25.0, glucose 11.1, KCl 4.7, $CaCl_2$ 1.6, KH_2PO_4 1.2, $MgSO_4$ 1.2) for vascular reactivity studies. The hearts, left kidneys, left ventricles and adrenal glands were excised and weighted. The aortas were cleaned of adherent connective tissue in sterile saline solution in petri dish and cutted in three parts and stored in All-protect Tissue Reagent (Qiagen GmbH, Hilden, Germany).

Adherent connective tissue was carefully cleaned from mesenteric artery and 4 mm section from proximal end of mesenteric-artery junction was cut off and the following 4 mm section was used for vascular reactivity measurements. The artery rings were placed in oxygenated (O_2/CO_2; 95:5,