Journal of Functional Foods 18 (2015) 275–288

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/jff

Oral and faecal microbiota in volunteers with hypertension in a double blind, randomised placebo controlled trial with probiotics and fermented bilberries

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ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form 4 July 2015
Accepted 7 July 2015
Available online

Keywords:
Microbiota
Blood pressure
Lactobacillus plantarum DSM 15313
Bilberry

ABSTRACT

Two probiotic products containing either live *Lactobacillus plantarum* strain DSM 15313 or bilberries fermented by the same bacterial strain were tested for their antihypertensive potentials and their impact on the oral and the faecal microbiota in hypertensive adults. The results showed that consumption of the tested products for three months did not reduce the blood pressure in adults with hypertension. Neither the diversity nor the composition of the oral and faecal microbiota was significantly affected by the tested probiotic products. Moreover, the oral and the faecal microbiota remained generally stable over the intervention period of 3 months.

1. Introduction

In recent years, probiotics have gained growing interest from researchers for their potential to convey health benefits. They have been studied for their efficacy against gastrointestinal disorders, inflammatory diseases, respiratory tract infections, and other health-related conditions (Amara & Shibl, 2015; Mortaz et al., 2013). Hypertension as a major risk factor for cardiovascular disease affects one billion adults globally and leads to nine million deaths every year according to estimates by the World Health Organization (WHO, 2013). Unhealthy diet is one of many factors contributing to the development of hypertension and thus modifications in dietary patterns are

Trial registration: ClinicalTrials.gov: NCT01989702.

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http://dx.doi.org/10.1016/j.jff.2015.07.005
1756-4646/

recommended for the prevention and management of high blood pressure (Chobanian et al., 2003; Whitworth & WHO, 2003; WHO, 2013). Consumption of probiotic food products has for example been reported to lower blood pressure through postulated mechanisms linked to anti-inflammatory effects, the generation of nitric oxide or the production of peptides inhibiting the activity of angiotensin-I-converting enzyme (Aihara, Kajimoto, Hirata, Takahashi, & Nakamura, 2005; Hutt et al., 2015; Naruszewicz, Johansson, Zapolska-Downar, & Bukowska, 2002). Although the exact mechanisms behind the blood pressure lowering activity of various probiotic strains remain to be unraveled, the observed antihypertensive effects have often been linked to bacterial metabolic products. Furthermore, an altered microbiota composition has been associated with conditions of the metabolic syndrome such as obesity and type 2 diabetes (Le Chatelier et al, 2013; Ley, Turnbaugh, Klein, & Gordon, 2006; Qin et al., 2012). Thus, it seems reasonable to investigate the potential relationship between the structure of the gut microbiota and hypertension which is another common component of the metabolic syndrome. A beneficial shift in the composition of the indigenous microbiota could be one of the possible mechanisms of action of the administered probiotics (Mekkes, Weenen, Brummer, & Claassen, 2014; Sherman, Ossa, & Johnson-Henry, 2009; Wang et al., 2015).

In a previous study using a rat model (Xu et al., 2013), we observed that N^{ω}-nitro-L-arginine methyl ester (L-NAME)-induced hypertension was accompanied by a shift in caecal microbiota. Although the administration of the study product containing *Lactobacillus plantarum* DSM 15313 and bilberries (European blueberry) fermented by the same bacterial strain did not significantly prevent the increase in blood pressure, it altered the composition of the caecal microbiota. In another study involving healthy rats and a product similar to the aforementioned one but with a higher content of certain fermentation products, we observed both an antihypertensive effect and a shift in the microbiota composition (Ahren et al., 2015). Even though we did not identify specific bacterial members that were directly associated with the obtained antihypertensive effects, the observed shifts in the gut microbiota support the need for further investigation.

In the present human study, we primarily aimed to test the antihypertensive effect of live *L. plantarum* DSM 15313 or bilberries fermented by the same strain in hypertensive adults. It has previously been reported in human studies that consumption of antioxidant-rich blueberries could lower blood pressure (Basu et al., 2010; Cassidy et al., 2011; Rodriguez-Mateos et al., 2013). Furthermore, as described in the animal study by Xu et al. (2013) the fermentation of bilberries by *L. plantarum* DSM 15313 resulted in the production of new phenolic compounds (i.e. not present in the non-fermented berries) with possible, based on the literature, anti-inflammatory activities. These phenolic compounds were shown in the same animal study to possess a blood-pressure lowering activity after administration to the animals for 2 weeks. In the present human study the possible blood-pressure lowering effect of the fermented bilberry product was compared to that of a placebo or *L. plantarum* DSM 15313. We hypothesised that the combination of the tannase-rich *L. plantarum* DSM 15313 with the tannin-rich bilberries would be able to either reduce a high blood pressure or prevent a normal-high blood pressure from getting even higher through an anti-inflammatory mechanism. At the same time we wanted to take the opportunity to study the microbiota in order to see whether a possible antihypertensive effect could also be linked to changes in the microbiota.

2. Materials and methods

2.1. *Participants and study design*

Participants were recruited through advertisements in the local newspapers and were enrolled at the Clinical Research Department at the University Hospital in Malmö, Sweden. The study participants and the nurse who met the study subjects were blind to the intervention. In total, 142 participants were included in this double-blind placebo controlled study after providing written informed consent, according to the following inclusion criteria: (i) healthy individuals at the age of 40–75 years, (ii) systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) > 90 mmHg, (iii) triglycerides >1.7 mmol/L and/or HDL <1 mmol/L (men)/1.29 mmol/L (women), (iv) fasting plasma glucose levels >5.6 mmol/L and/or waist circumference >102 cm (men)/88 cm (women). Ongoing medication for high levels of blood lipids excluded people from participating in the study.

The study consisted of a 2-week "run-in" period and a 12-week intervention period during which the subjects were taking the probiotic study products or placebo once daily. All study participants were asked to refrain from consumption of other products containing probiotic bacteria during their participation in the study. Exclusion criteria at the time of enrolment were: medically treated allergy or allergy to any of the ingredients of the study product, medication for high levels of blood lipids, presence of metabolic disease, such as type one diabetes, confirmed disease of the heart, liver or kidneys, chronic inflammatory disease requiring medication, pregnant or nursing, regular intake of a probiotic product (5–7 days per week) during the last three months before inclusion into the study. Antihypertensive medication during the time of the study was allowed only if the dosage for the medication had been stable for at least three months before the start of intervention. Any changes in this type of medication, either in dosage or pharmaceutical product, excluded participants from further participation in the study.

The study was conducted in accordance with the Declaration of Helsinki and ethical approval was given by the Ethics Committee at Lund University, Sweden (Dnr 2009/66).

2.2. *Intervention*

Participants were randomly allocated to receive either one of the two probiotic products or placebo, sequentially labelled according to a randomisation list consisting of blocks of six. The randomisation list was produced by a statistician not involved in the conduct of the study. The study product was a combination of a fruit drink with or without added fermented bilberries and a powder consisting of maltodextrin with or without the probiotic bacteria *L. plantarum* DSM 15313 (strain