Free Radical Biology and Medicine 72 (2014) 232–237

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Original Contribution

Lowering of oxidative stress improves endothelial function in healthy subjects with habitually low intake of fruit and vegetables: A randomized controlled trial of antioxidant- and polyphenol-rich blackcurrant juice

Faisel Khan [a,*], Sumantra Ray [a,1], Angela M. Craigie [b], Gwen Kennedy [a], Alexander Hill [a], Karen L. Barton [b], Jane Broughton [c], Jill J.F. Belch [a]

[a] Vascular and Inflammatory Diseases Research Unit and, Dundee DD1 9SY, UK
[b] Centre for Public Health Nutrition Research, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK
[c] GlaxoSmithKline, Brentford, Middlesex TW8 9GS, UK

ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form
1 April 2014
Accepted 5 April 2014
Available online 15 April 2014

Keywords:
Antioxidant
Vitamin C
Oxidative stress
Endothelial function
Blackcurrant juice
Free radicals

ABSTRACT

Inadequate intake of the recommended five-a-day fruit and vegetable portions might contribute to increased cardiovascular disease risk. We assessed the effects of dietary intake of a blackcurrant juice drink, rich in vitamin C and polyphenols, on oxidative stress and vascular function. This was a double-blind, placebo-controlled, parallel group study of 66 healthy adults who habitually consume <2 portions of fruit and vegetables per day. Participants were randomly allocated to consume 250 ml of placebo (flavored water) or low or high blackcurrant juice drink four times a day for 6 weeks. Flow-mediated dilation (FMD) and plasma concentrations of F_2-isoprostanes and vitamin C were measured. In the high blackcurrant juice drink group FMD increased significantly (5.8 ± 3.1 to $6.9 \pm 3.1\%$, $P=0.022$) compared with the placebo group (6.0 ± 2.2 to $5.1 \pm 2.4\%$). Plasma vitamin C concentration increased significantly in the low (38.6 ± 17.6 to 49.4 ± 21.0 μmol/L, $P<0.001$) and high (34.6 ± 20.4 to 73.8 ± 23.3 μmol/L, $P<0.001$) blackcurrant juice drink groups compared with the placebo group (38.1 ± 21.0 to 29.0 ± 17.6 μmol/L). F_2-isoprostane concentrations were significantly lower in the high blackcurrant juice drink group (225 ± 64 pg/ml) compared with the low blackcurrant juice drink (257 ± 69 pg/ml, $P=0.002$) and placebo group (254 ± 59 pg/ml, $P=0.003$). At follow-up, changes in plasma vitamin C correlated significantly with changes in FMD ($r=0.308$, $P=0.044$). Consumption of blackcurrant juice drink high in vitamin C and polyphenols can decrease oxidative stress and improve vascular health in individuals with habitually low dietary fruit and vegetable intake.

Generation of reactive oxygen species and oxidative stress are among the many contributory factors involved in the development and progression of cardiovascular disease (CVD) [1]. Inadequate dietary intake of antioxidant-rich foods and beverages can contribute to CVD and thus supplementation of dietary antioxidants has the potential to reduce oxidative stress and CVD risk [2,3]. Oxidative stress can increase CVD risk by causing endothelial dysfunction, which can occur well before the presentation of symptomatic CVD [4]. An established technique for measuring endothelial function in humans is flow-mediated dilation (FMD) in the brachial artery, which has been shown to a validated predictor of CVD risk and outcome [5].

Most studies to date that have explored the association between habitual antioxidant intake and CVD risk have been in individuals with established or increased risk of CVD [3,6,7] and point to potential benefits for secondary prevention. Although studies have reported beneficial effects of acute or short-term consumption (single one-off dose or days) of antioxidant-containing compounds on CV function in healthy subjects [8–10], information is lacking with regard to possible beneficial effects of longer-term (weeks) dietary consumption of antioxidants in healthy individuals. Such information could have implications for primary prevention, especially in individuals in whom antioxidant status might be low because of inadequate intake of the recommended five-a-day fruit and vegetable portions. The purpose of

Abbreviations: CVD, cardiovascular disease; FMD, flow-mediated dilation; GTN, glyceryl trinitrate.

* Corresponding author. Fax: +44 1382 632333.
E-mail address: f.khan@dundee.ac.uk (F. Khan).
[1] *Current address:* Medical Research Council Human Nutrition Research Unit, Elsie Widdowson Laboratory, Cambridge, UK.

http://dx.doi.org/10.1016/j.freeradbiomed.2014.04.006
0891-5849/

this study therefore was to carry out a randomized controlled trial to assess the effects of 6 weeks' dietary intake of a blackcurrant juice drink, rich in antioxidant vitamin C as well as polyphenols, on FMD in healthy volunteers who habitually have low consumption of fruit and vegetables. Additionally, we assessed whether consumption of a high (20%) blackcurrant juice drink produced significant changes in blood markers of oxidative stress.

Materials and methods

Fig. 1 shows the pathway for subjects from recruitment to follow-up. We used a double-blind, placebo-controlled, parallel group study design. Ethical approval was obtained from the Tayside research ethics committee and all participants gave written, informed consent to the protocol before starting. Screening and baseline assessments took place between January 2006 and July 2007, and follow-up visits between March 2006 and May 2008. We recruited volunteers from the general population in Tayside, Scotland, via advertisements using posters, newspapers, and lay press. Initial screening of participants took place via telephone conversation to determine intake of fruits and vegetables, and those potentially eligible (i.e., healthy and consuming <2 portions per day) were invited to attend Ninewells Hospital and Medical School, Dundee, for a face-to-face screening visit. Before attending this visit, participants were requested to complete a 3-day record of their dietary intake using the FACET tool [11] to obtain an estimation of their daily fruit and vegetable consumption. At this visit, assessment of dietary records was reviewed further to assess intake of fruit and vegetables, and in addition a general medical assessment was conducted.

After inclusion at screening, participants returned to the hospital on a further occasion for baseline assessments of vascular function (described below), after which they were block-randomized (in blocks of six, sequentially numbered, cartons) to one of three study groups, each consisting of 22 subjects. The groups were placebo (flavored water), low blackcurrant juice drink (6.4% juice; final diluted concentration 1.1 mg vitamin C/100 ml, 27.3 mg/100 ml total polyphenols, 4 mg/100 ml anthocyanins), and high blackcurrant juice drink (20% juice; 10.2 mg vitamin C/100 ml, 81.5 mg/100 ml total polyphenols, 14.3 mg/100 ml anthocyanins) provided in ready-to-drink cartons provided by GlaxoSmithKline. Participants were requested to consume 250 ml of juice drink four times a day for 6 weeks. The composition of the low and high blackcurrant juice drinks per 250 ml was 1.7 g carbohydrate, 1.2 g sugars, 0.1 g protein and they provided 10.4 kcal. Participants were instructed to maintain their usual dietary intake and exercise habits throughout the 6 weeks, with only the addition of the allocated juice to their intake. Baseline measurements were repeated at the 6-week follow-up visit. Compliance was assessed by monitoring fruit and vegetable intake at weeks 3 and 5 using the FACET tool [11] and using juice record cards of daily juice consumption throughout. Additionally, assessment of plasma concentrations of vitamin C served as a further marker of compliance.

Flow-mediated dilation

Measurements were conducted in a temperature-controlled room ($24 \pm 1\ ^{\circ}C$). Subjects attended fasted, having not consumed food or beverages (other than water) since 1200 hours the night before. Resting blood pressure was measured in triplicate using a semiautomated oscillometric device (Omron 705 CPII). The mean of the last two measurements was used for analysis.

Recommended guidelines were used to assess endothelial function in the brachial artery using FMD [5]. The brachial artery was imaged in longitudinal section using an Acuson Sequoia C512 ultrasound system (Siemens Medical Solutions USA, Malvern, PA, USA) with a 5- to 8-MHz linear array transducer held in position using an adjustable arm. Images were captured at end diastole of each cardiac cycle by gating image acquisition with the R-wave of a three-lead electrocardiogram trace. Images were recorded for

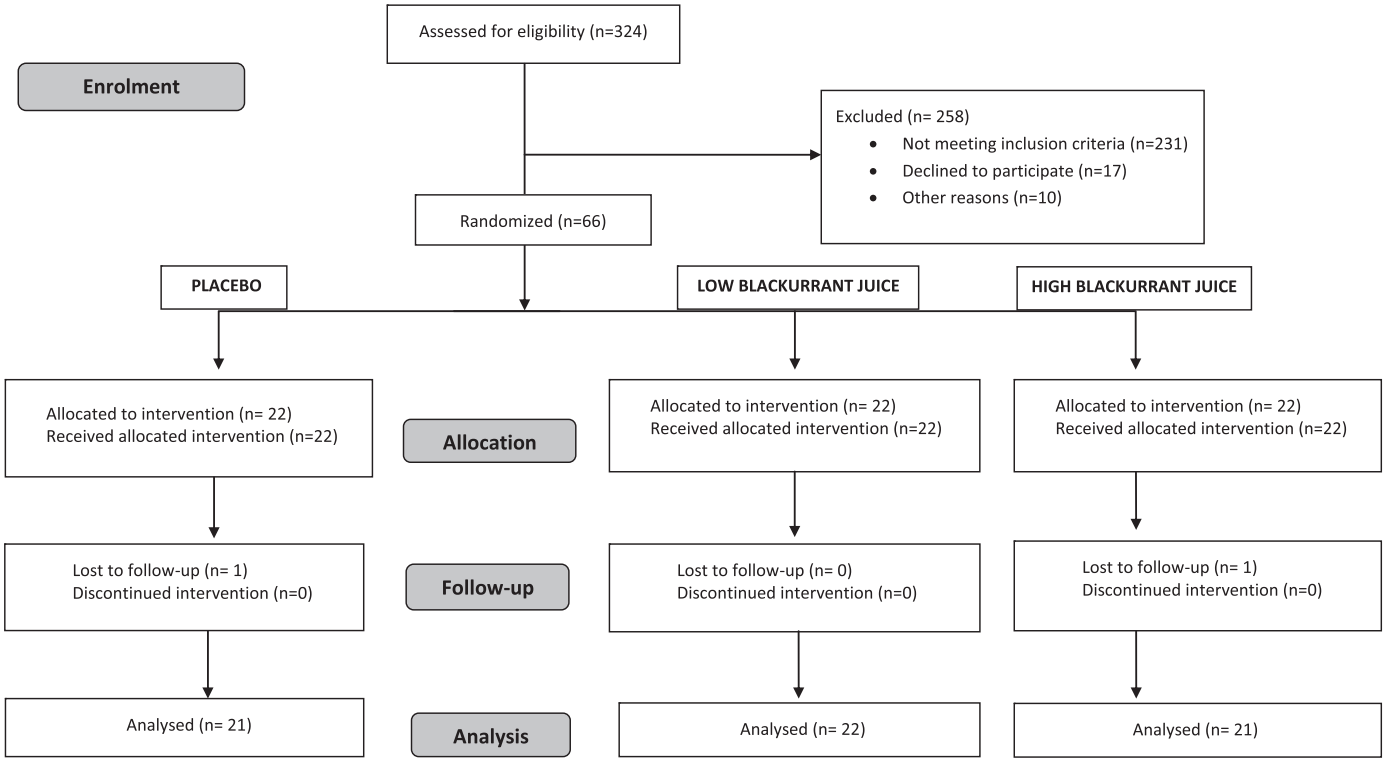

Fig. 1. Recruitment and follow-up of participants.