Clinical Nutrition 34 (2015) 415–421

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized control trials

Acute effect of sorghum flour-containing pasta on plasma total polyphenols, antioxidant capacity and oxidative stress markers in healthy subjects: A randomised controlled trial

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ARTICLE INFO

Article history:
Received 25 February 2014
Accepted 4 August 2014

Keywords:
Sorghum
Polyphenols
Antioxidant capacity
Oxidative stress
Pasta
Durum

SUMMARY

Background & aims: It has been previously reported that pasta containing wholegrain sorghum flour exhibits high content of polyphenols and antioxidant capacity and hence might enhance antioxidant status and reduce markers of oxidative stress *in vivo*; however no clinical studies have yet been reported. Therefore, the present study assessed the effect of pasta containing red or white wholegrain sorghum flour on plasma total polyphenols, antioxidant capacity and oxidative stress markers in humans. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN: 12612000324819).
Methods: In a randomised crossover design, healthy subjects ($n = 20$) consumed three test meals of control pasta (CP), 30% red sorghum pasta (RSP) or 30% white sorghum pasta (WSP), 1–2 wk apart. The test meals were consumed as breakfast after an overnight fast. Blood samples were obtained at fasting and 2 h after consumption and analysed for total polyphenols, antioxidant capacity, superoxide dismutase (SOD) activity, protein carbonyl and 8-isoprostanes.
Results: Compared to baseline, the 2 h post-prandial levels following the RSP meal of plasma polyphenols, antioxidant capacity and SOD activity were significantly ($P < 0.001$) higher while the protein carbonyl level was significantly lower ($P = 0.035$). Furthermore, net changes in polyphenols, antioxidant capacity and SOD activity were significantly ($P < 0.001$) higher while protein carbonyl were significantly ($P = 0.035$) lower following consumption of the RSP meal than the CP meal.
Conclusion: The results demonstrated that pasta containing red wholegrain sorghum flour enhanced antioxidant status and improved markers of oxidative stress in healthy subjects.

1. Introduction

Oxidative stress, an imbalance between the generation of free radical such as reactive oxygen/nitrogen species and the antioxidant defences, may play a role in the development and progression of many chronic diseases such as CVD [1] and diabetes [2]. The antioxidant defence system consists of endogenous antioxidants glutathione, catalase and superoxide dismutase (SOD) and exogenous antioxidants obtained by dietary intake, such as polyphenols [3]. Dietary polyphenols have been identified as both powerful chemical antioxidants and have also been shown to up-regulate the synthesis of endogenous antioxidants [4].

Epidemiological studies have consistently shown an inverse association between the consumption of polyphenolic-rich foods and the risk of chronic diseases associated with oxidative stress [5]. This is supported by data from cross-sectional studies, which show that markers of oxidative stress are inversely related to intake of polyphenolic-rich foods [6,7]. Furthermore, polyphenolic-rich foods such as apple juice [8], various nuts such as walnut, almond and pecan [9–12], red wine [13] and wholegrain foods such as wheat aleurone-rich bread and extruded cereals [14] have been demonstrated to enhance antioxidant status and improve markers of oxidative stress in clinical studies.

Consumption of foods containing wholegrain sorghum flour as an ingredient has the potential to enhance antioxidant status and

Abbreviations: B_0, maximum binding; CP, control pasta; DNPH, 2,4-dinitrophenylhydrazine; GAE, gallic acid equivalents; H_2O_2, hydrogen peroxide; NSB, non-specific binding; pNpp, p-nitrophenyl phosphate; RSP, red sorghum pasta; SOD, superoxide dismutase; TA, total activity; WSP, white sorghum pasta.

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http://dx.doi.org/10.1016/j.clnu.2014.08.005
0261-5614/

beneficially modulate markers of oxidative stress. Among cereal grains, some varieties of sorghum have the highest polyphenolic content [15]. These sorghum polyphenols are known to function as strong antioxidants, at least *in vitro* [15]. *In vivo* studies on the oxidative stress related properties of sorghum foods are limited to just one study conducted on Wistar rats which demonstrated that a diet containing sorghum flour reduced markers of oxidative stress and inflammation [16].

Studies on the effect of foods containing sorghum flour on antioxidant status and markers of oxidative stress in humans are lacking and the bioavailability of polyphenols from such foods has not yet determined. Therefore, the present study aimed to investigate the acute effect of consumption of pasta containing red or white wholegrain sorghum flour on plasma total polyphenols, antioxidant capacity and markers of oxidative stress in healthy human subjects. It was hypothesised that pasta containing the red or white sorghum flour would enhance plasma polyphenols and antioxidant status (enzymatic and/or non-enzymatic) and would supress markers of oxidative stress compared to pasta made from durum wheat semolina only.

2. Materials and methods

2.1. Subjects

A total of twenty-two healthy subjects (both male and female) were recruited through posted flyers, newspaper advertisements and direct personal communication in Melbourne, Australia. After providing written informed consent, subjects were screened for suitability using a health questionnaire. Exclusion criteria included: history of cardiovascular disease; diabetes, asthma, hypertension or gastrointestinal disease; pregnancy; use of medications (oral contraceptives were allowed); allergy to gluten; smoking; excessive alcohol intake; and breakfast skipping. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Deakin University Human Research Ethics Committee. This trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN: 12612000324819).

2.2. Study design and protocol

A randomised, controlled, crossover design with at least 1 week wash-out period between treatments was used. The study was conducted at Deakin University, Burwood campus, Melbourne, Australia and was initiated on June 20, 2012. Each subject attended three testing sessions 1–2 wk apart and at each session, consumed one of the three test meals of control pasta (CP), 30% red sorghum pasta (RSP) or 30% white sorghum pasta (WSP). The test meals were administered in a random order assigned by computer-generated random numbers concealed in opaque envelopes that were opened sequentially by the chief investigator. Subjects were instructed to maintain their usual diet during the study period, but to abstain from alcohol and vigorous physical activity the day before each study session. Subjects consumed their evening meal no later than 9:00 pm, after which they fasted (consumption of 500 ml of water was allowed between 9 pm and the next morning).

On the test day, subjects arrived to the laboratory after the 10–12 h overnight fast. Baseline measurements including weight, height, waist circumference, blood pressure and heart rate were taken. After 5 min rest in the supine position, a baseline venous blood sample was drawn into EDTA vacutainers by venepuncture by a qualified phlebotomist. Then, according to the randomisation schedule, subjects were provided with one of the test meals for breakfast, which was consumed within 10–12 min. A second venous blood sample was collected at exactly 2 h after starting the breakfast. This time point was chosen to correspond to the maximal post-prandial antioxidant capacity and polyphenol concentration in blood reported in the literature [9,12]. The subjects were not allowed to eat or drink anything during the 2 h period, but were allowed to read, watch TV or use their computer.

2.3. Test meals

The test meals were three experimental pasta samples (one control and two sorghum-containing pastas). The control pasta was made from 100% semolina. For the sorghum-containing pastas 30% of the semolina was replaced with either red or white wholegrain sorghum flour. Tannins may be present in sorghum grain varieties with pigmented testa, however the red sorghum grain had been previously confirmed as tannin free [17] as determined by the standard bleach test [18]. The sorghum level used was based on earlier consumer sensory studies conducted in our laboratory which indicated that a palatable product could be made at this incorporation level [19]. Pasta was prepared as previously reported [20]. In terms of nutritional composition, the sorghum-containing pastas had slightly lower protein and slightly higher fat and total dietary fibre content than the control [20]. All pasta samples were cooked at the optimal time [20] and served warm with 50 g tomato sauce and 200 ml water. The composition of test meals were calculated using FoodWorks version 7 (Xyris Software, Kenmore Hills, Qld, Australia) using AusNut database (All Foods, Rev. 14, Food Standards Australia New Zealand, Canberra, Australia). The database was supplemented with direct analysis of the experimental foods and manufacturers' information for foods not found on the database. The ingredients used in all the test meals and their compositional profiles are given in Table 1. All test meals contained similar amounts of macronutrients, except dietary fibre that differed somewhat between the meals. All the test meals were adjusted to an equal weight with drinking water.

2.4. Baseline measurements

Height was recorded to the nearest centimetre using a stadiometer (Seca Limited, Birmingham, UK), and weight was measured with a digital medical scale (model S-YB; Wedderburn, Shanghai, China), with the participant wearing light clothing and no shoes. Height and weight were used to calculate BMI. Waist circumference was measured midway between the lowest rib margin and iliac crest and recorded to the nearest 0.1 cm. Systolic and diastolic blood pressures and heart rate were measured in the supine position with an automatic digital blood pressure monitor (UA-767, A & D Company Limited, Tokyo, Japan).

Table 1
Ingredients and energy/nutrient composition of test meals.

	CP	WSP	RSP
Ingredients			
Uncooked pasta (g)	67	65	66
Tomato sauce (g)	50	50	50
Water (g)	196	200	198
Total weight (g)	400	400	400
Energy (kJ)	1119	1112	1113
Available carbohydrate (g)	50	50	50
Protein (g)	9.2	8.6	8.5
Fat (g)	2.6	2.7	2.8
Total dietary fibre (g)	3.4	3.8	4.2
Total phenolics (mg GAE)[a]	31.5	63.0	123.4

CP: control pasta; WSP: 30% white sorghum pasta; RSP: 30% red sorghum pasta.
[a] GAE: gallic acid equivalents (Folin Ciocalteu method).