Nutrition 31 (2015) 1148–1154

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Dietary silicon-enriched spirulina improves early atherosclerosis markers in hamsters on a high-fat diet

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ARTICLE INFO

Article history:
Received 23 December 2014
Accepted 25 March 2015

Keywords:
Hamsters
Atherosclerosis
Oxidative stress
Liver inflammation
Silicon-enriched spirulina

ABSTRACT

Objective: The aim of this study was to investigate the effects of dietary silicon-enriched spirulina (SES) on atherosclerosis.
Methods: Hamsters (six per group) on a high-fat (HF) diet received SES or non-enriched spirulina (both at 57 mg/kg body weight) daily. This corresponded to 0.57 mg silicon/kg body weight daily.
Results: The HF diet induced dyslipidemia, insulin resistance, oxidative stress, and vascular dysfunction. Compared with the HF group, SES attenuated increases of lipemia and prevented insulin resistance (IR) ($P = 0.001$). SES protected against oxidative stress through a reduction of heart ($P = 0.006$) and liver ($P < 0.0001$) nicotinamide adenine dinucleotide phosphate-oxidase activity and by sparing the activity of superoxide dismutase ($P = 0.0017$) and glutathione peroxidase ($P = 0.01861$). SES decreased inflammation, lowering tumor necrosis factor-α ($P = 0.0006$) and interleukin-6 levels ($P = 0.0112$), decreasing polymorphonuclear cells and preventing nuclear factor-κB activity ($P = 0.0259$). SES corrected plasma level of monocyte chemoattractant protein-1 ($P = 0.0380$), which was increased by the HF diet. Finally, SES supplementation prevented vascular and endothelial functions assessed respectively by the contractile response to the agonist phenylephrine and the relaxation induced by acetylcholine.
Conclusion: SES protects against metabolic imbalance, inflammation, oxidative stress, and vascular dysfunction induced by an HF diet, and could prevent the atherogenic processes. Synergistic effects between spirulina and silicon were observed.

Introduction

The cyanobacteria *Spirulina platensis* is commercially available for human consumption. It represents one of the richest protein sources of plant origin (60–70%), lipids (7%), and carbohydrates (20%) and is a good source of vitamins and minerals such as calcium, magnesium, phosphorus, potassium, sodium, and zinc [1]. This microalga is one of the most potent nutrient sources and is used as a nutraceutical food supplement [2] with no toxic side effects [3], although there is insufficient scientific evidence to recommend supplementation in humans. Spirulina are particularly suitable for the production of specific bioactive compounds and nutritional elements that they are able to accumulate in an organically biotransformed form. Several reports have described successful enrichment of spirulina biomass in selenium [4], iron, or chromium. Thus, a new type of food supplement has been developed and could serve as a rich source of trace elements [5]. As previously reported, ample evidence

J.V. was supported by a CIFRE grant (Convention Industrielle de Formation par la REcherche, no. 0084/2012) from Phyco-Biotech (Montpellier, France) and the French Association Nationale de la Recherche et de la Technologie. N.J. is an employee at Phyco-Biotech R&D. J.V., A.V., C.R., N.J., J.R., S.G., and J-M.R. contributed to the generation, collection, assembly, analysis, and interpretation of data. J.V., A.V., J-P.C., S.R., S.G., and J-M.R. contributed to the drafting and revision of the manuscript. All authors approved the final version of the manuscript. S.G. and J-M.R. contributed equally to this work.
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http://dx.doi.org/10.1016/j.nut.2015.03.014
0899-9007/

exists to indicate that silicon may be an essential nutrient for higher animals, including humans [6].

Silicon is naturally found in insoluble but not bioavailable forms. The main soluble form is orthosilicic acid $Si(OH)_4$, which tends to polymerize at high concentration (>2 mM Si), corresponding to the form absorbed by humans either in drinking water and food or appearing after nutrient hydrolysis in the intestine [7]. Although real dietary deficiencies are unknown, a lack of food diversification and low consumption of fruits and vegetables could result in insufficient coverage of silicon needs. Generally, the main food sources of silicon are bananas, cereal, and beer [8,9]. In humans, the daily intake is about 20 to 50 mg, and the body needs are 9 to 14 mg/d [10,11].

The biological importance of silicon has to be considered in the context of its body distribution. The highest concentrations are found in bone and connective tissues, such as the aorta, trachea, tendons, and skin, where silicon appears to act on extracellular matrix turnover via collagen and elastin synthesis [6]. Silicon supplementation has been reported to have beneficial effects on these tissues, especially bones [12] and skin [13]. In contrast, silicon deficiency has been associated with detrimental effects on bone mineralization and growth [14] and skin elasticity and healing [6]. The importance of silicon also has been demonstrated in cardiovascular pathophysiology, especially in the prevention of atherosclerosis [15]. Studies have shown an inverse relationship between the ingestion of silicon and the development of atherosclerosis [16,17]. Furthermore, silicon supplementation reduces hypertension and increases antihypertensive and antiatherogenic gene expression in the aorta of spontaneously hypertensive rats [18].

Incorporating silicon into spirulina could be a way to produce a bioavailable food supplement. Thus, in line with the beneficial effects of silicon and the antioxidant, hypolipidemic, and anti-inflammatory properties of spirulina [19], we focused on a model of early atherosclerosis to evaluate the effects of supplementation with silicon-enriched spirulina (SES). We assessed the potential beneficial preventive effects of SES on some major disorders and dysfunctions induced by a high-fat (HF) diet in the Syrian hamster model.

Materials and methods

Animals, diets, and experimental design

Production of the materials

To produce SES, spirulina (PhycoBiotech, Lunel, France) was grown in a 130 L photobioreactor under continuous lighting on Zarouk's medium at 22°C and pH 10.5 in the presence of 1 g/L sodium metasilicate (Na_2O_3Si). This medium contained $NaHCO_3$, 16.8 g/L; K_2HPO_4, 0.5 g/L; $NaNO_3$, 2.5 g/L; K_2SO_4, 1.0 g/L; NaCl, 1.0 g/L; $MgSO_4.7H_20$, 0.2 g/L; $CaCl_3$, 0.04 g/L; $FeSO_4.7H_20$, 0.01 g/L; EDTA, 0.08 g/L; H_3BO_3, 2.86 mg/L; $MnCl_2.4H_2O$, 220 mg/L; $CuSO_4.5H_2O$, 79 mg/L; MoO_3, 15 mg/L; and Na_2MoO_4, 21 mg/L, and was supplied with light aeration (30 L/min) and the addition of 0.03% CO_2. At the end of the growth, the biomass was recovered and filtered through a 20-mm membrane, thoroughly washed with distilled water, frozen, and lyophilized. Resultant SES contained 1% silicon, whereas regular spirulina contained <0.023% silicon (lower detection limit), as indicated by the manufacturer.

Animal husbandry/maintenance

Twenty-four male weanling Golden Syrian hamsters (Janvier-Labs, Le Genest-St-Isle, France), weighing ~90 g each, were randomly divided into four groups of six animals (six animals per plastic cage). They were housed at 23 ± 1°C, subjected to a 12-h light/dark cycle and handled in compliance with European Union rules and according to the guidelines of the National Institutes of Health [20] and the Committee for Animal Care at the University of Montpellier (France) (permission number C 34 249).

Animal protocol design

Three groups were fed an HF atherogenic diet (HFD), consisting of 200 g/kg casein, 3 g/kg L-methionine, 393 g/kg corn starch, 154 g/kg sucrose, 50 g/kg cellulose, 100 g/kg hydrogenated coconut oil, 2 g/kg cholesterol, 35 g/kg mineral mix, and 10 mg/kg vitamin mix, for 12 wk. For reference, a fourth group received a standard diet (STD) consisting of 236 g/kg casein, 3.5 g/kg L-methionine, 300 g/kg corn starch, 30 g/kg maltodextrin 10, 290.5 g/kg sucrose, 50 g/kg cellulose, 45 g/kg vegetable oil, 35 g/kg mineral mix, and 10 g/kg vitamin mix. In both diets, mineral and vitamin mixes were formulated according to American Institute of Nutrition-93 guidelines [21]. The five groups had free access to both food and tap water, which contained <0.023% silicon (lower detection limit).

The hamsters of each group received daily by gavage 1 mL of either tap water (STD and HFD groups), or crude spirulina (Sp) suspended in tap water at 57 mg/kg body weight (HF-Sp group), or SES suspended in tap water at 57 mg/kg body weight (HF-SES group). Spirulina concentration was determined according to a previous study [22] and its silicon content (1%), and corresponded to a daily intake of 40 mg for a 70 kg-human [23].

Analytical procedures

Plasma analysis

At the end of the experimental period, fasting blood samples were collected by cardiac puncture. Plasma was prepared by centrifugation at 2000*g* for 10 min. Total cholesterol and high-density lipoprotein cholesterol (HDL-C) were determined in plasma (10 μL) using enzymatic kits (Randox Laboratories Ltd, Crumlin, UK) according to the manufacturer; HDL-C was measured after precipitation of very low- and low-density lipoprotein cholesterol (LDL-C) using phosphotungstate reagent. Triacylglycerols and glucose levels were measured in plasma (10 μL) using a Randox enzymatic kit and reagents from the Thermo Electron Corporation (Cergy Pontoise, France), respectively. Paraoxonase activity (PON) was determined using paraoxon as a substrate and measured by the increase in absorbance at 412 nm, as previously described [24]. Insulinemia (10 μL plasma) was determined using enzyme-linked immunosorbent assay (ELISA) kits (Mercodia AB, Uppsala, Sweden). Plasma monocyte chemoattractant protein (MCP)-1 concentration was measured using specific ELISA kit according to the manufacturer (R&D Systems, Europe, Lille, France) and 100-μL plasma.

The homeostatic model assessment for insulin resistance (HOMA-IR) was determined from fasting insulin and glucose values as previously described [25] and according to:

$$\text{HOMA} - \text{IR} = \frac{\left[\text{fasting glucose}\left(\text{mmol}/\text{L}\right) \times \text{fasting insulin}\left(\text{mU}/\text{L}\right)\right]}{22.5}$$

Liver anatomo-pathology

The liver was excised; some samples were removed for histology, whereas others were stored at −80°C until further use. For pathologic analysis, liver samples were fixed in 10% neutral-buffered formaldehyde and paraffin embedded, and 3 μm-thick serial sections prepared. Sections were deparaffinized and stained with hematoxylin and eosin.

Liver antioxidant enzymes activity

Unfixed liver samples were homogenized (5%, w/v) in ice-cold 0.1 mol/L potassium phosphate buffer (pH 7.4) and the homogenate was spun at 13 000*g* for 15 min at 4°C. The supernatant was then stored at −80°C for subsequent assay of superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity using commercial kits (Randox Laboratories LTD, Crumlin, UK), and to quantify liver tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels using specific ELISA kits according to the manufacturer's instructions (R&D Systems Europe, Lille, France).

Liver inflammation

To assay nuclear factor (NF)-κB activity, liver tissue was homogenized (7.5% w/v) in a hypotonic buffer (pH 7.9) containing 20 mM HEPES, 10 mM EDTA, 10 mM KCl, 1% protease inhibitor cocktail, 0.1% dithiothreitol, and 0.1% Igepal. Nuclear extracts were then obtained by homogenizing the pellet in lysis buffer (pH 7.9) containing 20 mM HEPES, 1 mM EDTA, 200 mM NaCl, 10% glycerol, 1 mM dithiothreitol, and 1% protease inhibitor cocktail. NF-κB activity was determined from nuclear extracts using a commercial immunoassay kit (Active Motif, Rixensart, Belgium) and according to manufacturer's instructions.

Liver and cardiac oxidative stress

Hepatic and cardiac superoxide anion ($O2^{\circ-}$) production was evaluated by the intensity of lucigenin-enhanced chemiluminescence (10 μM lucigenin) as previously described [24]. Results were expressed as relative luminescence units/mg of protein.