



Nutrición Hospitalaria

ISSN: 0212-1611

info@nutriciónhospitalaria.com

Grupo Aula Médica

España

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Nutrición Hospitalaria, vol. 31, núm. 2, febrero, 2015, pp. 559-569

Grupo Aula Médica

Madrid, España

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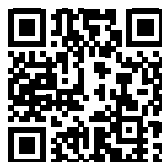
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Revisión

Clinical and molecular evidence of the consumption of broccoli, glucoraphanin and sulforaphane in humans

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Abstract

Introduction: Sulforaphane (SFN) is an isothiocyanate derived from glucoraphanin (GRA), which is found in great amounts especially in broccoli. Its consumption has been reported to be associated with a lower risk of myocardial infarction and cancer development. Additionally, its effects have been studied in neurodegenerative diseases, diabetes, and atherosclerosis, most of the times using animal models and cell cultures.

Objectives: Given the promising results of SFN, this review aimed to investigate evidence documented in human intervention studies with broccoli, GRA and SFN.

Methods: A search was performed on PubMed and Virtual Health Library databases by two independent researchers using the descriptors “broccoli” or “glucoraphanin” or “sulforaphane”, which should appear on the study’s title or abstract. This review included randomized clinical trials performed in humans that were published in English and Portuguese from 2003 to 2013 and that considered clinical and molecular parameters of cell damage as outcomes of interest.

Results: Seventeen studies were selected, and the predominant type of intervention was broccoli sprouts. More consistent results were obtained for the clinical parameters blood glucose and lipid profile and for molecular parameters of oxidative stress, indicating that there was an improvement in these parameters after intervention. Less solid evidence was found with regard to decreased inflammation, *Helicobacter pylori* colonization, and protection against cancer.

Conclusion: Although being relevant, the evidence for the use of broccoli, GRA and SFN in humans are limited;

EVIDENCIAS CLÍNICAS Y MOLECULARES DEL CONSUMO DE BRÓCOLI, GLUCORAFANINA Y SULFORAFANO EN HUMANOS

Resumen

Introducción: El sulforafano (SFN) es un isotiocianato derivado de la glucorafanina (GRA), encontrada en gran cantidad especialmente en el brócoli. Su consumo está asociado a un menor riesgo de infarto del miocardio y de cáncer. Además, sus efectos están siendo estudiados en enfermedades neurodegenerativas, diabetes y aterosclerosis, casi siempre utilizando modelos animales y cultivos celulares.

Objetivos: Debido a los resultados prometedores del compuesto SFN, esta revisión buscó investigar evidencias ya documentadas en intervenciones con brócoli, GRA y SFN en humanos.

Métodos: Se realizó una búsqueda en las bases de datos PubMed y Biblioteca Virtual en Salud, por dos investigadores independientes, utilizando los descriptors “broccoli” o “glucoraphanin” o “sulforaphane”, que debían constar en el título o resumen del trabajo. Se incluyeron ensayos clínicos randomizados realizados en humanos, publicados en inglés y portugués entre 2003 y 2013, y que consideraron como desenlaces de interés parámetros clínicos y moleculares de daño celular.

Resultados: Se seleccionaron 17 estudios y el tipo de intervención predominante fueron brotes de brócoli. Los resultados más consistentes fueron obtenidos con los parámetros clínicos glicemia y perfil lipídico y los parámetros moleculares de estrés oxidativo, que presentaron mejora después de la intervención. Se encontraron evidencias menos sólidas respecto a la disminución de la inflamación, de la colonización por *Helicobacter pylori* y protección contra cáncer.

Conclusión: Aunque relevantes, las evidencias del uso de brócoli, GRA y SFN en humanos son limitadas, siendo

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Recibido: 14-VI-2014.

Aceptado: 1-IX-2014.

thus, further intervention studies are needed to evaluate outcomes more consistently and reach better grounded conclusions.

(*Nutr Hosp.* 2015;31:559-569)

DOI:10.3305/nh.2015.31.2.7685

Key words: *Broccoli. Sulforaphane. Glucoraphanin. Isothiocyanates.*

Abbreviations

GSL = Glucosinolate.
GRA = Glucoraphanin.
SFN = Sulforaphane.
Nrf2 = Nuclear factor erythroid 2-related factor 2.
Keap1 = Kelch-like ECH-associated protein 1.
ARE = Antioxidant response element.
DNA = Deoxyribonucleic acid.
NQO1 = NADPH quinone oxidoreductase 1.
HO-1 = Heme oxygenase-1.
GST = Glutathione S-transferase.
PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses.
RCT = Randomized clinical trial.
VHL = Virtual Health Library.
H. Pylori = *Helicobacter pylori*.
DM2 = Diabetes mellitus type 2.
HOMA IR = Homeostatic Model Assessment for Insulin Resistance.
TC = Total cholesterol.
LDL = Low-density lipoprotein.
HDL = High-density lipoprotein.
TG = Triglycerides.
IL-6 = Interleukin-6.
CRP = C-reactive protein.
TNF-α = Tumor necrosis factor α.
CVD = Cardiovascular disease.
GSTM1 = Glutathione S-transferase M1.
GSTP1 = Glutathione S-transferase P1.
PSA = Prostate-specific antigen.
IGF-1 = Insulin-like growth factor-1.
HpSA = *H. pylori* stool antigen.
UBT = Urea breath test.
PGI = Pepsinogen I.
PGII = Pepsinogen II.
NF-κB = Nuclear factor kappa-B.

Introduction

Epidemiological evidence suggests that the consumption of cruciferous vegetables is related to beneficial health effects, such as lower risk for myocardial infarction and for the development of cancers¹⁻⁵. Additionally, compounds extracted from these vegetables have been studied for the treatment of neurodegenerative diseases, diabetes, and atherosclerosis, showing promising results⁶⁻⁸.

necesarios más estudios de intervención para avaluar los desenlaces de forma más consistente y producir conclusiones mejor fundamentadas.

(*Nutr Hosp.* 2015;31:559-569)

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Palabras clave: *Brócoli. Sulforafano. Glucorafanina. Iso-tiocianato.*

Cruciferous species belong to the genus *Brassica* (families *Brassicaceae* and *Cruciferae*) and include vegetables such as broccoli, cauliflower, kale, brussels sprout, cress, radish, cabbage, and mustard⁹. These vegetables represent a good source of phytochemicals, including phenolic compounds, sulphur glycosides, and carotenoids¹⁰. However, their anticarcinogenic and antioxidant potential has been attributed mainly to their high glucosinolate (GSL) content^{11,12}.

GSLs are thioglucosides that have one cyano group and one sulfate group, encompassing nearly 120 chemically stable compounds¹³. When the tissues of these plants are processed by cutting, cooking, freezing, or mastication, GSLs are exposed to an enzyme named myrosinase, present in the very vegetable, which hydrolyzes them to isothiocyanates, substances that are bioactive compounds¹⁴. Human intestinal microflora also has a myrosinase isoform¹⁵ and, although there are different types of GSLs and isothiocyanates in nature, glucoraphanin (GRA) and sulforaphane (SFN) are the most studied compound and the one that currently has the strongest evidence for beneficial effects, respectively¹⁶.

Broccoli, more especially its sprouts, is recognized as the best source of SFN, and GRA corresponds to 90% of the GSL content in some of its species¹⁷. SFN is considered a very promising compound because it was found to have properties that prevent, delay or reverse the development of preneoplastic lesions and to improve survival rates, acting on cancer cells as a therapeutic agent¹⁸⁻¹⁹.

SFN may interact with many molecular targets, but its well described mechanism of action is through nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Nrf2 is a transcription factor essential to the regulation of the cellular redox state that, in non-stimulated cells, remains bound to kelch-like ECH-associated protein 1 (Keap1), forming an inactive complex²⁰. When entering the cell, SFN may interact with Keap1 and disrupt the binding between Nrf2 and Keap1, which allows for Nrf2 activation and nuclear translocation²¹. In the nucleus, Nrf2 binds to the antioxidant response element (ARE), a DNA promoter region of genes co-difying antioxidant enzymes, such as NADPH quinone oxidoreductase (NQO1), heme-oxygenase-1 (HO-1), thioredoxin, and superoxide dismutase^{8,22}. Increased transcription of Nrf2 target genes leads to a strong cytoprotective response, which increases resistance to carcinogenesis and to other diseases whose pathophy-

biology involves oxidative stress^{6,20}. Additionally, with Nrf2 activation, SFN increases the activity of phase II enzymes involved in the elimination of xenobiotic compounds, such as glutathione S-transferase (GST) and quinone reductase²³.

Several studies have shown the protective effect of SFN against insults and diseases, most of the times using animal models and cell cultures. In view of the above, the present review aimed to investigate evidence already documented in human intervention studies with broccoli, GRA and SFN.

Methods

This systematic review was performed using a pre-determined protocol established according to the recommendations of the Cochrane Manual²⁴, and results were presented according to the criteria defined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement²⁵.

The review included clinical trials, controlled clinical trials, and randomized clinical trials (RCTs) on the possible effects of the consumption of broccoli, GRA or SFN on humans that were published in English after 2003. Research was conducted on the PubMed and Virtual Health Library (VHL) electronic databases from September to November 2013 and consisted of searching on article titles and abstracts for the following descriptors: “broccoli” or “glucoraphanin” or “sulforaphane”. In addition, one study was independently included due to its relevance. Outcomes of interest were clinical and molecular parameters of cell damage in study participants. The content, absorption, metabolism, and excretion of SFN metabolites were not considered outcomes of interest. Similarly, this review did not include studies conducted but not published, abstracts from scientific events (whether published or not), theses, and dissertations. It was established that the intervention should exclusively use broccoli, GRA or SFN in at least one group, in order to assess their isolated effects. The articles identified underwent a blinded independent evaluation by two authors of the present manuscript (AC and FF). Disagreements with regard to inclusion in the study were resolved by a third investigator (CGS).

Results

Seventeen articles were selected, based on the combination of the abovementioned descriptors and filters. The degree of agreement between reviewers was rated at $\kappa=1.0$. The flow chart of study selection is shown in figure 1. The impact factor of the selected journals ranged from 1.257 to 6.504, with most of them (65%) showing values above 3.0. The year of publication ranged from 2004 to 2013, with most articles being published in 2012.

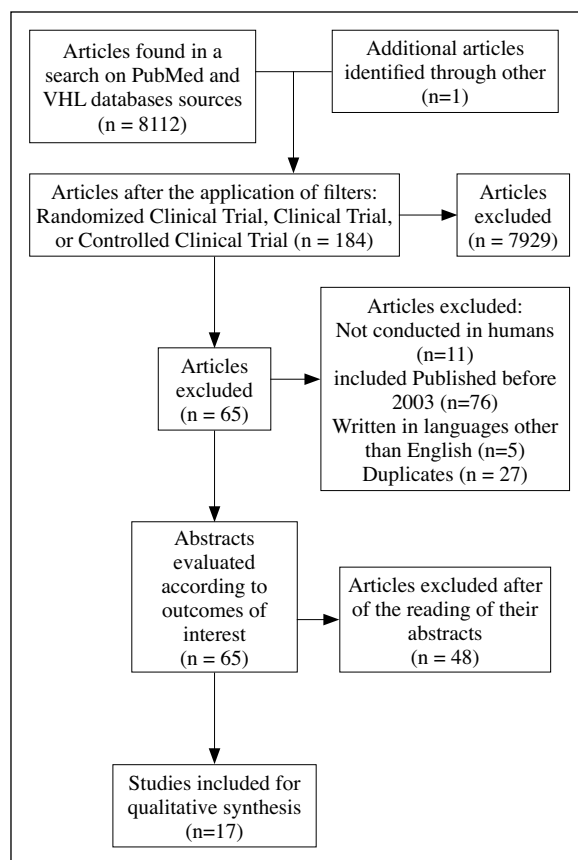


Fig. 1.—Flow chart of study selection.

Mean sample size was 48 ± 44 individuals, and mean intervention time was 41 ± 86 days. The predominant intervention included broccoli sprouts, used in their powdered (29% of the studies), fresh (18%), and homogenized (0.6%) forms or in the form of infusions or beverage (12%), totaling 11 studies. The other six studies used broccoli commercially cultivated or technologically developed to have high levels of GSL when cooked, raw, or prepared in soups. Study samples comprised healthy individuals, smokers or nonsmokers, individuals at risk for the development of cardiovascular diseases or cancer, and patients under oncologic follow-up, with type 2 diabetes, or infected with *Helicobacter pylori* (*H. Pylori*).

The results of the search are shown in table I. For a better understanding of the results, they were grouped into the three categories below, according to the main outcomes of the studies.

Type 2 diabetes and cardiovascular diseases - clinical parameters

Four studies analyzed the effects of broccoli intake on diabetes parameters in patients with type 2 diabetes mellitus (DM2)²⁶⁻²⁹. All studies performed the same intervention: consumption of 5 or 10 g of broccoli sprout

Table I
Studies that assessed the effect of the intake of broccoli, GRA and SFN on clinical and molecular parameters in humans

<i>Authors and year</i>	<i>Type of study and sample</i>	<i>Intervention</i>	<i>Parameters evaluated</i>	<i>Results</i>
Bahadoran et al. (2012) ²⁶	Double-blind RCT with type 2 diabetes patients (n=63)	5 or 10 g/day broccoli sprout powder containing nearly 22.5 μ mol/g SFN vs. placebo (corn starch stained with chlorophyll) for 4 weeks	Fasting blood glucose, plasma insulin concentration, insulin sensitivity (fasting blood glucose/insulin), insulin resistance (HOMA-IR index)	Decreased fasting blood glucose in both intervention groups compared with baseline values. Decreased insulin concentration and HOMA-IR index only in the group that received 10 g in comparison with the placebo group. There was no difference in the index of insulin sensitivity in both intervention groups.
Mirmiran et al. (2012) ²⁷	Double-blind RCT with type 2 diabetes patients (n=63)	5 or 10 g/day broccoli sprout powder containing nearly 22.5 μ mol/g SFN vs. placebo (corn starch stained with chlorophyll) for 4 weeks	Fasting blood glucose, serum C-reactive protein concentration, IL-6, TNF- α	Decreased fasting blood glucose and C-reactive protein in both intervention groups compared with baseline values. Non-significant decrease in IL-6 and TNF- α levels in both intervention groups compared with baseline. Decreased C-reactive protein and IL-6 levels in the group that received 10 g/day in comparison with the placebo group.
Bahadoran et al. (2012) ²⁸	Double-blind RCT with type 2 diabetes patients (n=72)	5 or 10 g/day broccoli sprout powder containing nearly 22.5 μ mol/g SFN vs. placebo (corn starch stained with chlorophyll) for 4 weeks	Fasting blood glucose, TC, TG, LDL, HDL, and oxidized LDL, oxidized LDL/LDL ratio, atherogenic index of plasma (log TG/HDL), TC/HDL ratio, and LDL/HDL ratio	Decreased fasting blood glucose, TC, and LDL levels in both intervention groups compared with baseline values. Decreased TG levels, oxidized LDL/LDL ratio and atherogenic index of plasma with the consumption of 10 g of broccoli sprouts compared with baseline values. Decreased HDL levels in comparison with baseline only in placebo group and in the intervention group that received 5 g of broccoli sprouts; in the group that consumed 10 g of sprouts, the HDL concentration remained the same; no changes in the remaining parameters.
Bahadoran et al. (2011) ²⁹	Double-blind RCT with type 2 diabetes patients (n=63)	5 or 10 g/day broccoli sprout powder containing nearly 22.5 μ mol/g SFN vs. placebo (corn starch stained with chlorophyll) for 4 weeks	Oxidative stress parameters (serum total antioxidant capacity, total oxidant status, oxidative stress index, serum malondialdehyde concentration, and oxidized LDL)	Decreased malondialdehyde concentration and increased total antioxidant capacity with both interventions compared with placebo. Decreased oxidized LDL and oxidative stress index with both interventions compared with baseline values. No effect was found on total oxidant status.
Armah et al. (2013) ³⁰	RCT with individuals with moderate risk for the development of cardiovascular diseases (n=48)	400 g high-GSL broccoli (21.6 μ mol/g GRA) vs. 400 g standard broccoli (6.9 μ mol/g GRA) vs. 400 g steamed peas/week for 12 weeks	Systolic and diastolic blood pressure, TC, HDL, LDL, oxidized LDL, TG, C-reactive protein, pulse wave velocity, and arterial pulse stiffness assessed by the augmentation index	No changes in any of the parameters assessed.
Christiansen et al. (2010) ³¹	RCT with hypertensive patients (n=40)	10 g/day broccoli sprout powder containing nearly 30.3 μ mol/g GRA vs. usual diet for 4 weeks	Blood pressure, endothelial function (flow mediated dilation), TC, HDL, LDL.	No significant changes were observed in the parameters assessed.

Table I (cont.)
Studies that assessed the effect of the intake of broccoli, GRA and SFN on clinical and molecular parameters in humans

<i>Authors and year</i>	<i>Type of study and sample</i>	<i>Intervention</i>	<i>Parameters evaluated</i>	<i>Results</i>
Murashima et al. (2004) ³²	Pilot study with healthy individuals (n=12)	100 g/day fresh broccoli sprouts for 7 days	TC, HDL, LDL, TG, uric acid, urea, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, natural killer cell activity, plasma amino acid concentration (total, essential, branched-chain, glycine, cystine, and glutamate) and oxidative stress markers (plasma PCOOH concentration, CoQ10H2/CoQ10 ratio, urinary 8-isoprostane, urinary 8-OHdG)	Decreased TC and LDL levels in men and increased HDL levels in women compared with baseline levels. Increased plasma concentration of the amino acid cystine and no changes in the remaining amino acids. Decreased plasma PCOOH concentration, increased CoQ10H2/CoQ10 ratio, decreased urinary 8-isoprostane and urinary 8-OHdG compared with baseline. No changes in the remaining parameters assessed.
Kensler et al. (2012) ³³	Crossover RCT with individuals from a community with a high incidence of hepatocellular carcinoma (n=50)	Beverages made with lyophilized broccoli sprouts diluted with mango juice and containing 800 μ mol GRA or 150 μ mol SFN/day vs. mango juice for 7 days	Urinary excretion of metabolites from airborne pollutants (mercapturic acids of acrolein, crotonaldehyde, ethylene oxide and benzene)	Increased levels of excretion of glutathione-derived conjugates of acrolein, crotonaldehyde and benzene compared with pre-treatment values in individuals who received beverages containing SFN or GRA. No differences were observed between the effects of SFR and GRA.
Riso et al. (2010) ³⁴	Crossover RCT with adult smokers (n=27)	250 g/day steamed broccoli vs. usual diet without cruciferous vegetables for 10 days	DNA damage and repair biomarkers	Decreased DNA oxidation in blood mononuclear cells compared with pre-treatment values. Increased resistance to H(2)O(2)-induced DNA strand breaks compared with pre-treatment. No changes either in the activity of the repair enzyme OGG1 or in the expression levels of the enzymes OGG1, nucleoside diphosphate linked moiety X-type motif 1, and heme-oxygenase 1 (HO-1).
Riso et al. (2009) ³⁵	Crossover RCT of adult smokers and nonsmokers (n=20)	200 g/day steamed broccoli vs. usual diet without cruciferous vegetables for 10 days	Biomarkers of endogenous oxidative DNA damage, biomarkers of cancer risk (HDAC activity) in lymphocytes, serum IGF-1 levels.	Decreased oxidized DNA bases in smokers compared with baseline values. Increased resistance to H(2)O(2)-induced DNA strand breaks compared with pre-treatment in smokers and nonsmokers. No changes in HDAC activity or in serum IGF-1 levels.
Riedl et al. (2009) ³⁶	Dose escalation clinical trial of healthy individuals (n=57)	25, 100, 125, 150, 175 or 200 g of homogenized broccoli sprouts containing nearly 0.283 μ mol/ml SFN vs. 200 g alfalfa sprouts for 3 days	Expression of phase II antioxidant enzymes (glutathione-S-transferase M1, glutathione-S-transferase P1, NADPH quinone oxidoreductase, and HO-1) in nasal lavage cells	Dose-dependent increase in enzyme expression, with maximal enzyme induction observed with the intake of 200 g of broccoli sprouts compared with baseline. Increased enzyme expression with the intake of 200 g of broccoli sprouts compared with control.

Table I (cont.)
Studies that assessed the effect of the intake of broccoli, GRA and SFN on clinical and molecular parameters in humans

<i>Authors and year</i>	<i>Type of study and sample</i>	<i>Intervention</i>	<i>Parameters evaluated</i>	<i>Results</i>
Traka et al. (2008) ³⁷	RCT with adult and elderly men under urologic follow-up (n=22)	400 g of cooked broccoli vs. 400 g of cooked peas/ week following usual diet for 12 months	Markers of prostate growth (PSA) and signaling pathways of cell proliferation for prostate carcinoma	No difference in PSA levels between the groups before and after treatment. Modulation of cell proliferation pathways reduced the chance of tumorigenesis
Gasper et al. (2007) ³⁸	Crossover RCT with healthy individuals (n=16)	A single 150-ml dose of soup made with standard broccoli or with high-GSL broccoli containing retrospectively 682.6 μ mol/L and 2295.9 μ mol/L of SFN, vs. water	Expression of genes related to xenobiotic metabolism and cell cycle control in gastric mucosa	Up-regulation of xenobiotic metabolizing genes, including thioredoxin reductase, aldoketoreductases, and glutamate cysteine ligase modifier subunit in the group that consumed high-GSL broccoli compared with baseline. Suppressed expression in four genes (nuclear receptor subfamily 1 group D member 2, MAX 1 dimerization protein, thyrotroph embryonic factor, and basic helix-loop-helix domain class B2) in both groups who consumed broccoli soup.
Hakooz and Hamdan (2007) ³⁹	Pilot study with healthy individuals (n=10)	500 g of raw broccoli vs. 500 g of cooked broccoli for 6 days	Activity of cytochrome P450 enzymes (CYP2A6 and CYP1A2) related to xenobiotic metabolism	Increased CYP2A6 and CYP1A2 activity compared with baseline.
Kensler et al. (2005) ⁴⁰	RCT with individuals from a community with a high incidence of hepatocellular carcinoma (n=200)	125 ml of an infusion of broccoli sprouts containing 400 μ mol of GRA vs. placebo for 2 weeks	Excretion of carcinogen biomarkers (urinary aflatoxin and metabolites of trans, anti-phenanthrene tetraol polycyclic aromatic hydrocarbons [trans, anti-PheT])	Although urinary levels did not differ between the groups at the end of the treatment, there was an inverse correlation between dithiocarbamate concentrations (marker of SFN excretion) and aflatoxin-N7-guanine and trans, anti-PheT in the intervention group.
Galan et al. (2004) ⁴¹	Pilot study with adults infected with <i>H. pylori</i> (n=9)	Broccoli sprouts (14, 28 or 56 g) twice daily for 7 days	Biomarkers of <i>H. pylori</i> colonization (HpSA and UBT)	Seven of the nine patients were negative for HpSA after 7 days (the three patients from the 14 g group, two from the 28 g group, and two 56 g group) and six remained negative after 35 days (the three patients from the 14 g group, one from the 28 g group, and two from the 56 g group). UBT was performed in six patients, with negative results in two of them. <i>H. pylori</i> eradication was observed in one patient from each of the three groups (14, 28 and 56 g broccoli sprouts/day).
Yanaka et al. (2009) ⁴²	Pilot study with adults infected with <i>H. pylori</i> (n=48)	70 g/day of broccoli sprouts containing nearly 6 μ mol/g of GRA (420 μ mol GRA/portion) vs. alfalfa sprouts (control) for 8 weeks	Biomarkers of <i>H. pylori</i> colonization (HpSA, UBT) and biomarkers of gastric inflammation (serum PGI e PGII concentrations, PGI/PGII ratio)	Reduced serum PGI and PGII concentrations, increased PGI/PGII ratio, decreased HpSA and UBT values in comparison with baseline values. No differences compared with the control group. The levels returned to baseline values after 2 months.

RCT: randomized clinical trial; SFN: sulforaphane; GRA: glucoraphanin; GLS: glucosinolate; TC: total cholesterol; TG: triglycerides; LDL: low-density lipoprotein; HDL: high-density lipoprotein; IL-6: interleukin-6; TNF- α : tumor necrosis factor- α ; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; OGG1: HO-1:heme-oxygenase 1; HpSA: *H. pylori* stool antigen; UBT: urea breath test; PGI: pepsinogen I; PGII: pepsinogen II; HDAC: histone deacetylase; IGF-1: insulin-like growth factor-1; PSA: prostate-specific antigen; PCOOH: phosphatidylcholine hydroperoxide; CoQ10H2: reduced form of coenzyme Q10; CoQ10: coenzyme Q10; 8-OHdG: 8-hydroxydeoxyguanosine.

powder vs. corn starch stained with chlorophyll (placebo) for 4 weeks. The number of participants ranged from 63 to 72 (mean age 51.8 ± 6.5 years).

In three of the studies that measured fasting blood glucose, there was a significant mean decrease of 30.34 ± 11.94 mg/dl in this parameter after interventions as compared with baseline values²⁶⁻²⁸, and one of the studies²⁹ found a significant decrease of 62 mg/dl only in the group that consumed 10 g of broccoli sprout powder per day. Only one study measured insulin concentration and the Homeostatic Model Assessment for Insulin Resistance (HOMA IR) index, which decreased respectively from 5.2 ± 4.11 to 4.35 ± 3.11 mU/l and from 2.21 ± 2.04 to 1.55 ± 1.32 in comparison with baseline values ($p < 0.05$) with the intake of 10 g of broccoli sprouts²⁶.

One study evaluated patients' complete lipid profile (total cholesterol [TC], low-density lipoprotein [LDL], high-density lipoprotein [HDL], and triglycerides [TG]) and found a decrease in TC and LDL levels after both interventions as compared with baseline. However, there was an increase in HDL and a decrease in TG only in the group that consumed 10 g of broccoli sprouts²⁸.

Only one of the studies evaluated the inflammation parameters interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor α (TNF- α). Although there was a decrease in these parameters in both intervention groups compared with baseline (2.81 vs. 2.74 pg/ml; 3.33 vs. 2.38 ng/ml; 13.53 vs. 11.8 pg/ml in the group that received 10 g of broccoli sprouts, and 3.99 vs. 3.59 pg/ml; 4.37 vs. 3.24 ng/ml; 13.08 vs. 10.38 pg/ml in the group that received 5 g of broccoli sprouts, respectively), only CRP reached statistical significance ($p < 0.05$), which occurred also when the group that received 10 g was compared with the placebo group²⁷.

Similarly, only one study evaluated oxidative stress parameters, finding a decrease of 18% in lipid peroxidation and an increase of 14% in total antioxidant capacity in both intervention groups as compared with the placebo group ($p < 0.01$). There was also a decrease of 14% in the oxidative stress index with the consumption of 10 g of broccoli sprouts and of 8% with the consumption of 5 g in comparison with baseline values ($p < 0.01$)²⁹.

Five studies analyzed cardiovascular disease (CVD) parameters²⁸⁻³². Two of them were conducted with diabetic patients^{28,29}, one with individuals with moderate risk for CVD³⁰, one with hypertensive individuals³¹, and one with healthy individuals³². The interventions used were 400g of high-GSL broccoli vs. 400g of standard broccoli vs. 400g of peas (control)³⁰, 5 or 10 g broccoli sprout powder vs. corn starch stained with chlorophyll (placebo)^{28,29}, 10 g of broccoli sprout powder + usual diet vs. usual diet³¹, or 100g of fresh broccoli sprouts/day³². The follow-up time ranged from 7 days to 12 weeks, and the number of participants ranged from 12 to 72.

As for the studies with diabetic patients, one of them evaluated patients' complete lipid profile, as previously described, in addition to oxidized LDL, oxidized

LDL/LDL ratio, atherogenic index of plasma, TC/HDL ratio, and LDL/HDL ratio²⁸, while the other one evaluated only oxidized LDL²⁹, which decreased only in the group that received 10 g of broccoli sprouts as compared with baseline (5%) ($p < 0.05$). Similarly, only this intervention was able to decrease oxidized LDL/LDL ratio (from 3.16 to 2.63) and the atherogenic index of plasma (0.049 to 0.041) in comparison with initial values ($p < 0.05$)²⁸.

The studies with individuals with moderate risk for CVD and with hypertensive individuals^{30,31} measured blood pressure and lipid profile and did not find any change in these parameters after interventions. One of these studies also measured oxidized LDL, CRP, pulse wave velocity, and arterial pulse stiffness assessed by the augmentation index³⁰, while the other also measured flow mediated dilation³¹, and no changes were observed in these variables as well.

The study with healthy individuals evaluated their complete lipid profile and found a decrease in TC and LDL levels (from 178 ± 25 to 160 ± 28 mg/dl and from 94 ± 22 to 75 ± 40 mg/dl respectively) in males and an increase in HDL levels (7%) in females as compared with baseline values ($p < 0.05$)³².

Most results in this category point out to an improvement in the parameters assessed in intervention groups from baseline to the end of intervention, but few differences were found between these groups and control or placebo groups.

Toxicity, oxidant/antioxidant response, and cancer

This review found nine studies analyzing broccoli intake with regard to parameters related to toxicity, oxidant/antioxidant response, and cancer development³²⁻⁴⁰. Participants selected for these studies included communities with a high incidence of hepatocellular carcinoma^{33,40}, smokers^{34,35}, nonsmokers³⁵, patients under urologic follow-up³⁷, and healthy individuals^{36,38,39}. The interventions used were the following: beverage containing lyophilized broccoli sprouts + mango juice vs. mango juice (placebo)³³, steamed broccoli (250 or 200g) vs. usual diet without cruciferous vegetables^{34,35}, homogenized broccoli sprouts vs. alfalfa sprouts (control)³⁶, cooked broccoli (400g) vs. cooked peas (control)³⁷, standard broccoli soup vs. high-GSL broccoli soup vs. water³⁸, raw broccoli vs. steamed broccoli³⁹, infusion containing broccoli sprouts vs. placebo infusion⁴⁰. The follow-up time ranged from 6 hours to 12 months, sample size ranged from 10 to 200 individuals, participants' age ranged from 18 to 70 years, and the majority of subjects were female (55%).

One of the studies measured urinary excretion of metabolites of airborne pollutants derived from acrolein, crotonaldehyde and benzene, and observed an increase of respectively 36%, 17% and 62% in urinary excretion of these metabolites compared with their

pre-treatment values in groups receiving GRA or SFN, with no differences in the potential for increased excretion between GRA and SFN³³.

Two studies evaluated biomarkers of cancer risk and of DNA damage and repair; both of them found a decrease in DNA oxidation (mean of 42%) and an increase in the resistance to DNA breaks (22%) compared with baseline, although no changes were observed in the activity of damage or repair enzymes^{34,35}.

Two studies investigated cellular redox state through the expression or activity of antioxidant enzymes³⁶ and through oxidative stress markers³². The first study found an increase in antioxidant defense due to a dose-dependent increase in the expression of the enzymes glutathione S-transferase M1 (GSTM1), glutathione S-transferase P1 (GSTP1), NQO1, and HO-1, with maximal enzyme induction observed at the dose of 200g of broccoli sprouts (increase of 119%, 101%, 199% and 121% compared with baseline values respectively). An increase was also observed in comparison with the control group ($p < 0.05$)³⁶. The other study evaluated the oxidative stress markers phosphatidylcholine hydroperoxide, 8-isoprostane, and 8-hydroxydeoxyguanosine, and found a decrease of 17%, 39% 25% respectively in their levels and an increase of 50% in reduced/oxidized coenzyme Q ratio compared with pre-intervention values ($p < 0.05$). This study also evaluated the toxicity of bioactive compounds from broccoli by assessing liver function tests (transaminases), uric acid levels, urea levels, and natural killer cell activity, and did not find any difference in their values after treatment³².

One of the studies evaluated gene expression in the gastric mucosa 6 hours after the intake of broccoli soup and found an increase in the expression of genes involved in the metabolism of xenobiotics and in antioxidant activity, such as thioredoxin reductase³⁸. Another study found an increase in the activity of phase II enzymes (cytochrome P450) after the consumption of 500g of broccoli, with CYP2A6 enzyme activity increasing 48% in women and 216% in men, and CYP1A2 enzyme activity increasing 32% in women and 148% in men compared with baseline values ($p < 0.05$)³⁹.

Two other studies measured variables of cell growth (prostate specific antigen [PSA] and insulin-like growth factor-I [IGF-1]) and the pathways of cell proliferation. These studies did not find any change in PSA and IGF-1 levels but observed a modulation in proliferation pathways that reduced the chance of tumorigenesis^{35,37}.

Finally, one study evaluated urinary excretion of carcinogen biomarkers present in foods and in the air. Although these biomarkers did not differ between intervention and placebo groups at the end of the treatment, there was an inverse correlation between the marker of SFN excretion and the carcinogen biomarkers aflatoxin-N(7)-guanine ($R = 0.31$ and $P = 0.002$) and trans, anti-phenanthrene tetraol (PheT) ($R = 0.39$ and $P = 0.001$) in the intervention group⁴⁰.

In this category, only one study found differences between the results from intervention and control groups in only one of the parameters evaluated³⁶. The other studies observed an improvement in the results from intervention groups only in comparison with their own pre-treatment values.

Helicobacter pylori

The present review identified two studies that used broccoli sprouts in the treatment of adults infected with *H. pylori*, according to the following intervention plans: 14, 28 or 56g twice daily⁴¹ or 70g/day vs. alfalfa sprouts (control)⁴² for 7 days or 8 weeks, including 9 and 48 participants, respectively. Both studies evaluated the following markers of *H. pylori* colonization: *H. pylori* stool antigen (HpSA) and urea breath test (UBT).

One of the studies⁴² found a decrease in these parameters from baseline to 8 weeks after intervention ($p < 0.05$), but no difference was observed with regard to the control group. This study also evaluated the pepsinogen I (PGI)/pepsinogen II (PGII) ratio, which increased during intervention, indicating reduced inflammation. None of the subjects showed negative UBT results in any of the study periods; however, 32% of intervention group subjects had HpSA levels below the cutoff point at the end of the 8-week treatment. Nonetheless, 8 weeks after stopping the consumption of broccoli sprouts, these values increased again in 75% of the subjects.

Conversely, the other study⁴¹ found negative HpSA results in seven of the nine patients (78%) after the seven days of intervention and six remained negative at day 35. Of these six patients, two showed negative UBT results at day 35. One of the patients with an indeterminate UBT underwent gastric biopsy, which showed negative results for *H. pylori*. The consumption of broccoli sprouts was associated with the eradication of *H. pylori* in 33% of the patients, i.e., one patient for each dosage group.

Discussion

Data obtained in this review allowed to analyze the consumption of broccoli in different forms and amounts, indicating that the most consistent results in humans are those related to the clinical parameters blood glucose and lipid profile^{26-28,29,32} and to molecular parameters of oxidative stress^{29,32,34-36,38}, either by increasing antioxidant defenses or by decreasing insult markers. The findings from the present study also indicated that there was a decrease in low-grade chronic inflammation²⁷ and in *H. pylori* colonization^{41,42}, as well as a higher protection against cancer due to the inhibition of tumorigenesis pathways³⁷ or to the excretion of potentially carcinogen metabolites^{33,40}. Additionally, two studies found that their treatment protocols

did not have any effect on the parameters assessed^{30,31}, and the only study that evaluated toxicity parameters after the consumption of broccoli did not observe any change in this regard³².

The decrease in fasting blood glucose among DM2 patients, which was found in four of the studies analyzed here²⁶⁻²⁹, is one of the most relevant results of this investigation. The only study to measure insulin concentration and HOMA-IR index observed a decrease in these parameters²⁶. DM2 is a progressive disease that requires a growing number of oral hypoglycemic agents, which leads 50% of diabetic individuals to need exogenous insulin within a 10-year period after disease onset⁴³. The investigation of the compounds that may help in the control of diabetes is relevant, and the data obtained here suggest that SFN should be considered a possible complementary treatment to control diabetes and prevent its complications in the long term and reinforce the recommendation to consume cruciferous vegetables in our regular diet. These results corroborate findings from animal models, which observed higher glucose tolerance and lower insulin resistance after the treatment with SFN⁴⁴.

With regard to lipid profile, the studies analyzed in this investigation found somewhat controversial results. Three studies showed a decrease in TC, LDL, oxidized LDL, and TG levels and in the atherogenic index of plasma and/or an increase in HDL levels^{28,29,32}, while two other studies did not observe any change in these parameters after intervention^{30,31}. However, due to the methodological differences between the studies and to the fact that the number of positive results was greater than that of negative results, the findings on lipid profile may be considered relevant. Recent data from our research group also showed a significant improvement in lipid profile among diabetic rats treated with SFN for 21 days (Souza et al., data not published). It is known that changes in plasmatic lipids through SFN result from its capacity to induce genes through Nrf2 and that this induction vary from individual to individual and according to the time when it was measured after SFN intake⁴⁵. Nevertheless, the effects observed in the present study are of potential interest and require further investigation, since changes in lipid metabolism are common in the general population, especially in diabetic patients, contributing to increased risk of CVDs, which account for 60% of deaths in these patients and are the main cause of mortality worldwide⁴⁶.

Although clinical parameters are more palpable health outcomes in terms of improvement in individuals' prognosis, molecular parameters are also very important, since they work by modulating aspects that will present as clinical in the long term. In this sense, nine of the studies found in this review focused on molecular parameters³²⁻⁴⁰, both on increasing antioxidant response^{29,32,36} and minimizing oxidative damage^{29,34,35}, although oxidative damage as measured in these studies had included different parameters (lipid peroxidation, DNA damage, and quantification of reactive

species and urine metabolites of reactive species). It is important to highlight that the improvement in antioxidant response was limited to the increase in phase II enzymes^{36,38} with no effect on DNA repair enzymes^{34,35}. The antioxidant action of SFN is very relevant in humans, since nearly 200 diseases have been associated with oxidative stress, especially chronic non-communicable diseases⁴⁷, which evidences the undeniable role of reactive species in the pathophysiology of these diseases. An association between molecular and clinical parameters obtained in this study pointed out that individuals with hyperglycemia showed increased production of reactive species, which favors atherogenesis by stimulating LDL oxidation, a response similar to that of euglycemic individuals exposed to oxidative stress^{48,49}. Therefore, it is possible to believe that individuals who had their clinical parameters measured could show an improvement in molecular parameters as well, and vice versa.

It is also important to comment on the following findings: the anti-inflammatory effect of SFN resulting from the decrease in IL-6, PCR e TNF- α levels, which were evaluated in only one study²⁷; the modulation of the pathways of cell proliferation, which leads to a lower stimulation of tumorigenesis and was evaluated by one study³⁷; the increased excretion of airborne pollutants and toxic metabolites coming from the diet^{33,40}; and the decrease in *H. pylori* colonization^{41,42}. With regard to anti-inflammatory mechanisms and decreased tumorigenesis, SFN is believed to inhibit cell signaling pathways, e.g., nuclear factor kappa-B (NF- κ B) inflammatory pathway, through Nrf2 or through the increase in phase II enzymes, which have been classically described as participating in the stimulation of tumorigenesis. However, although Nrf2 is the best described mechanism of action of SFN, its effects cannot be attributed only to this pathway. Agyeman et al.⁵⁰ compared gene expression modulated by SFN in Keap1 knockdown cells and found that only 14% of the genes modulated by SFN were similarly modulated by Keap1 knockdown, which indicates that most SFN-regulated genes appear not to be regulated through the Keap1/Nrf2 pathway. Conversely, the increased excretion of carcinogenic metabolites in urine is possibly related to the high detoxification capacity of SFN, due to the higher elimination of mercapturic acids through urine, a pathway through which SFN is also eliminated⁵¹.

Although consistent epidemiological evidence associates the consumption of cruciferous vegetables with a lower risk of cancer development, there were few intervention studies on this topic in humans, with the most convincing evidence about intervention coming from two studies that evaluated *H. pylori* infection^{41,42}. *H. pylori* infections are common and may cause gastroduodenal inflammation and peptic ulcer, in addition to increasing the risk of gastric neoplasm. Conventional treatment consists of triple therapy with two antibiotics and proton-pump inhibitor. However, eradication rates are far from ideal, possibly due to the

increase in the number of antibiotic-resistant *H. pylori* strains and to the several treatment side effects, such as nausea, diarrhea, dyspepsia, headache, and changes in gastrointestinal microflora. These problems point out to the need of new strategies for *H. pylori* prevention and eradication⁵², and the two studies found in this review reinforce the bactericidal potential of SFN, suggesting that it can be used in a possible diet therapy⁵³.

The mechanism of SFN antibacterial activity is related to urease inactivation. The ability of *H. pylori* to thrive in the unfavorable acid environment of the stomach depends on the generation of great amounts of the enzyme urease. Through the production of ammonia from urea provided by the host, urease neutralizes gastric acidity, promotes inflammation, and favors *H. pylori* proliferation. Due to its ability to inactivate urease, SFN could reduce gastric colonization and associated inflammation⁵⁴. However, studies with longer intervention times, a control group, and a larger sample size may obtain better results and solidify them, since one of the studies evaluated in this review showed a great limitation in this regard⁴¹.

An important limitation for achieving result consistency is the scarcity of studies on the same topics or evaluating similar outcomes, in addition to the variability in the parameters measured in each study and to the heterogeneity in interventions, follow-up times, and sample sizes. Not all studies determined the amount of GRA or SFN present in the intervention performed, which makes it difficult to draw a conclusion about which dosage or concentration should be recommended. Furthermore, the non-quantification of cruciferous vegetable intake in the usual diet of study subjects is also a limitation, since the frequent intake of these vegetables may be an influencing factor in the response to intervention, especially for those who were already adapted to a greater intake of these foods.

Conclusion

There are potential benefits from broccoli consumption with regard to clinical and molecular parameters in humans, especially fasting blood glucose, lipid profile, and oxidative stress, permeated by the intake of GRA and SFN. However, evidence for these benefits is still limited.

It is necessary to conduct more interventions studies that are well controlled, less heterogeneous, and that analyze the dosage of these compounds of interest, in order to evaluate outcomes more consistently and reach better grounded conclusions.

Acknowledgements

The authors would like to thank Fundo de Incentivo a Pesquisa e Eventos (FIPE) of Hospital de Clínicas de Porto Alegre (HCPA) for its financial support.

Conflict of interest statement

The authors report no conflict of interest to disclosure.

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