Lead Article

Cruciferous vegetables: rationale for exploring potential salutary effects of sulforaphane-rich foods in patients with chronic kidney disease

Ludmila F.M.F. Cardozo, Livia A. Alvarenga, Marcia Ribeiro, Lu Dai, Paul G. Shiels, Peter Stenvinkel, Bengt Lindholm, and Denise Mafra

Sulforaphane (SFN) is a sulfur-containing isothiocyanate found in cruciferous vegetables (Brassicaceae) and a well-known activator of nuclear factor-erythroid 2-related factor 2 (Nrf2), considered a master regulator of cellular antioxidant responses. Patients with chronic diseases, such as diabetes, cardiovascular disease, cancer, and chronic kidney disease (CKD) present with high levels of oxidative stress and a massive inflammatory burden associated with diminished Nrf2 and elevated nuclear transcription factor-κB-κB expression. Because it is a common constituent of dietary vegetables, the salutogenic properties of sulforaphane, especially it's antioxidative and anti-inflammatory properties, have been explored as a nutritional intervention in a range of diseases of ageing, though data on CKD remain scarce. In this brief review, the effects of SFN as a senotherapeutic agent are described and a rationale is provided for studies that aim to explore the potential benefits of SFN-rich foods in patients with CKD.

INTRODUCTION

Cruciferous vegetables, which belong to the Brassicaceae family, such as broccoli, broccoli sprouts, brussels sprouts, cabbage, cauliflower, kale, and green cabbage, are highly nutritious foodstuffs in the diet, as highlighted by numerous clinical[1–3] and epidemiological[4,5] studies. These vegetables are rich in vitamins, minerals, (poly)phenolics, and have sulfur-containing compounds, such as the isothiocyanate sulforaphane (SFN).[6]

Recent studies have demonstrated that SFN potentially has numerous essential roles as an antimicrobial, antioxidant, anti-inflammatory,[6–9] and anti-oncogenic[10] agent and as an epigenetic modulator.[11–13] Sulforaphane is a nuclear factor erythroid 2–related factor 2 (Nrf2) agonist and as such, it indirectly can influence the transcription of a battery of antioxidant enzymes.[14] Sulforaphane also exhibits cytoprotective properties, through increasing natural killer (NK) cell activity and *TP53* expression, suppression of nuclear transcription factor-κB (NF-κB), increase in inhibition

Affiliation: *L.F.M.F. Cardozo* and *D. Mafra* are with the Graduate Program in Cardiovascular Sciences, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil. *L.A. Alvarenga* and *D. Mafra* are with the Graduate Program in Medical Sciences, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil. *M. Ribeiro* and *D. Mafra* are with the Graduate Program in Nutrition Sciences, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil. *L. Dai, P. Stenvinkel* and *B. Lindholm* are with the Division of Renal Medicine and Baxter Novum, Department of Clinical Science, Technology and Intervention, Karolinska Institutet, Stockholm, Sweden. *P.G. Shiels* is with Wolfson Wohl Translational Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow, Scotland.

Correspondence: *D. Mafra*, Unidade de Pesquisa Clínica-UPC, Rua Marquês de Paraná, 303/4 andar, Federal Fluminense University, Niterói-Rio de Janeiro 24033-900, Brazil. E-mail: dmafra30@gmail.com.

Key words: chronic kidney disease, gut microbiota, inflammation, nrf2, senescence, sulforaphane.

doi: 10.1093/nutrit/nuaa129

of histone deacetylases, induction of apoptosis, as well as antimicrobial properties.[7] Sulforaphane has been reported to act as a bactericidal agent against *Helicobacter pylori* by inhibiting bacterial urease synthesis.[7] It has also been speculated that Nrf2 activators have a potential role in the treatment of coronavirus disease 2019 (COVID-19) viral pneumonia via benefiting the pulmonary antibacterial defense system.[15] Indeed, Müller et al[16]showed in a randomized controlled study that SFN-containing broccoli-sprout homogenates increased virus-induced, peripheral-blood, NK-cell granzyme B production in NK cells compared to alfalfa sprout homogenate; thus, the effects of broccoli sprouts on severity of COVID-19 infection need attention.

The beneficial properties of SFN have been extensively studied in the context of cancer.[17–20] In addition, SFN may contribute to prevention and mitigation of complications in several other diseases, such as diabetes, obesity, and cardiovascular and neurological diseases.[21–24] In this review, we summarize some of the potential beneficial effects of SFN in various diseases and then provide a rationale for studies exploring its potential also in chronic kidney disease (CKD).

CRUCIFEROUS VEGETABLES AS A SOURCE OF SULFORAPHANE

Sulforaphane (4-methyl-sulfinyl butyl isothiocyanate) is a naturally occurring, oily isothiocyanate found in cruciferous vegetables. Glucoraphanin (4-methyl-sulfinyl butyl) is the inactive and chemically stable biological precursor of SFN, which belongs to a group of phytochemicals termed glucosinolates (GLSs) that have a sugar component (ie, D-glucose or dextrose) built into their structure.[6,25]

When a cruciferous vegetable suffers tissue damage by a microbial attack, mechanical food processing, or chewing, the enzyme myrosinase, which usually is in the plant, is physically segregated from glucosinolates, released, and comes into contact with glucoraphanin. Myrosinase catalyzes the hydrolysis of glucoraphanin, releasing glucose and sulphate components, the latter forming stable intermediate products, of which the most reactive is SFN isothiocyanate (Figure 1).[26,27]

Broccoli, cabbage, cauliflower, and kale are vegetables rich in SFN. Among these vegetables, broccoli contains the highest concentration of SFN,[25] and broccoli sprouts contain even higher SFN levels (1153 mg/100 g dry weight) than mature broccoli (44–171 mg/100 g dry weight). Figure 2 shows the approximate glucosinolate concentration in 100 g of cruciferous fresh vegetables.[25,28,29] Sulforaphane is prevalent in fresh vegetable material but not in vegetable derivatives, such as powder and tablets, where it is not detectable.[25]

Total concentrations of GLS in cruciferous vegetables can be influenced by processing methods and heat treatment (>70°C), as in routine domestic-cooking procedures such as blanching, boiling, and freezing,[28,30] where the plant's myrosinase is inactivated, thus interrupting the formation of SFN.[30] Blanching reduces total GLS concentrations by 13.0% in white cauliflower and 30.0% in brussels sprouts and broccoli. In boiled vegetables, losses are more substantial, reaching 35.3% in white cauliflower and 72.4% in curly kale. For broccoli, the loss of glucoraphanin has been reported as 39.1% by blanching and 60.6% by boiling.[28] Apart from this, cold storage in a domestic refrigerator (4–8°C) for 7 days reduces the concentration of GLSs in broccoli (−27%), brussels sprouts (−20%), cauliflower (−11%), and green cabbage (−14%).[31] In contrast, microwaving and mild heating in the range of 40–60°C has been reported to increase the levels of glucoraphanin and SFN in broccoli compared to raw broccoli.[32] Although mammals do not possess myrosinases, the conversion of glucoraphanin to SFN still occurs and seems to be carried out by the intestinal microbiota; this is discussed later in this review.

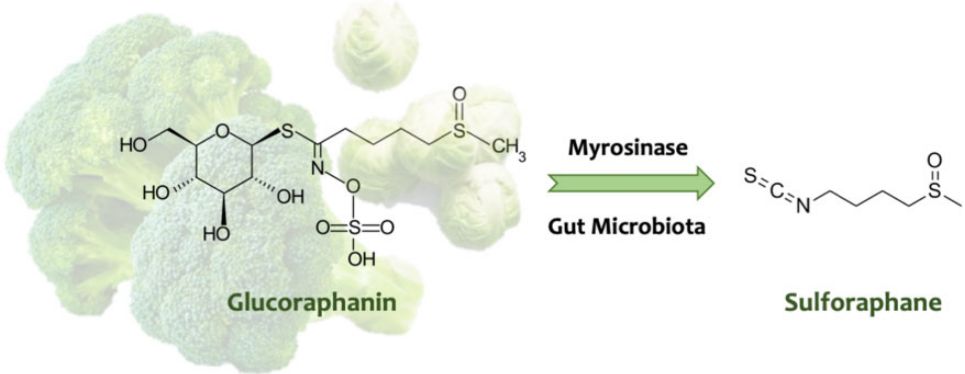

Figure 1 **Conversion of glucoraphanin to sulforaphane.**

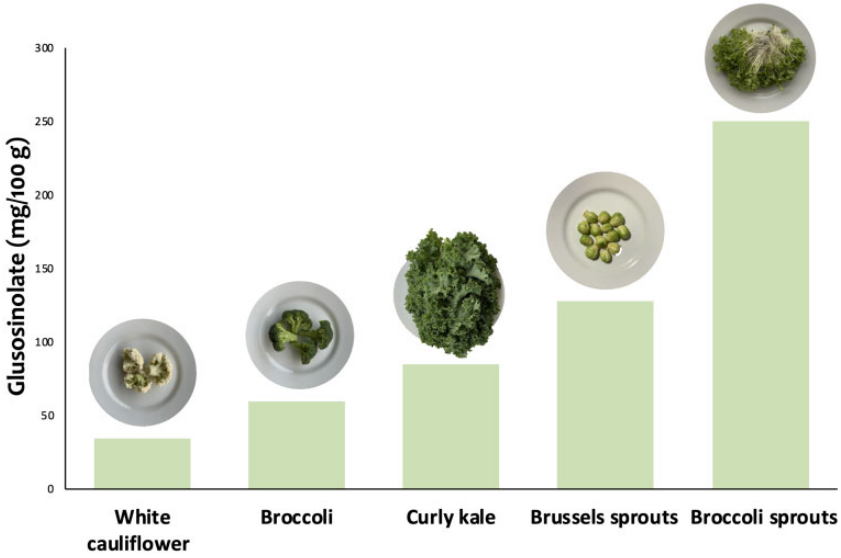

Figure 2 **Glucosinolate concentration in cruciferous fresh vegetables.**

The GLS fraction that is released from the plant matrix is bioaccessible as it is hydrolyzed by the myrosinase present in plants or by the myrosinase-like activity of the human gut microbiota. The underlying biochemistry of these processes is exemplified by the mercapturic acid pathway, where isothiocyanates are conjugated to glutathione (GSH) by a reaction catalyzed by glutathione transferase. Several cleavage reactions occur, giving rise to sulforaphane-*N*-acetylcysteine.[6,26] The isothiocyanate conjugates are then actively transported into the extracellular space by multidrug resistance–associated protein 1, 2, and P-glycoprotein.[33]

Isothiocyanate-glutathione conjugates dissociate in the blood, partly due to the low plasma GSH concentration and partly through further conjugation with serum albumin, which is a source of free thiol groups. Free isothiocyanate can be absorbed by peripheral organs, where it can accumulate in cells by reacting with thiol groups in GSH and other proteins, with both forms excreted mainly in the urine.[33,34]

Sulforaphane absorption and excretion in humans have been evaluated in several small clinical studies, and the evidence indicates absorption is affected by the way SFN is consumed.[35–38] SFN absorption was assessed in healthy individuals after a meal containing air-dried broccoli sprouts rich in myrosinase, broccoli powder lacking myrosinase, and a combination of both. The 24-hour urinary excretion of SFN was 74% for broccoli sprouts, 19% for broccoli powder, and 49% for the combination,[35] demonstrating that the presence of myrosinase appears to improve SFN absorption.[35,37] Higher levels of SFN were found in human blood and urine after raw broccoli was consumed (bioavailability of 37%), compared with cooked broccoli (bioavailability of 3.4%). The time to reach the peak of plasma SFN concentration was also shorter in raw broccoli (1.6 hours) compared with cooked broccoli (6 hours).[38]

In humans, a moderate dose of SFN appears safe; the metabolites are rapidly eliminated[39,40] and daily administration of broccoli-sprout extract for 3 months to patients with type 2 diabetes resulted in no severe adverse effects.[41] Notably, in vitro studies have reported that although low doses of SFN (0.25 μM) protected mesenchymal stem cells from cellular oxidative injuries and inhibited those cells from undergoing senescence (detected with β-galactosidase assay) and apoptosis, high doses of SFN (20 μM) exerted a cytotoxic effect by boosting DNA damage, resulting in cell-cycle arrest, senescence, and apoptosis.[42] This finding suggests a high dose of SNF potentially could be toxic and pro-oxidant by causing GSH depletion and superoxide production. Kubo et al[43] proposed that such toxic and pro-oxidant SFN activity is related to overactivation of NRF2-mediated Kruppel-like (Klf9) expression and downstream repression of peroxiredoxin 6 (Prdx6) (ie, Nrf2/Klf9/Prdx6 axis), inducing unfavorable oxidative stress and cell death.[43]

Sulforaphane has gained increased attention because of salutogenic effects mediated through the Nrf2 pathway,[14] especially in cancer, but also because of its potential preventive effects in diabetes and cardiovascular and neurological diseases.[21,23,24] Here, we discuss the potential effects of SFN as an anti-inflammatory agent, an antioxidant, an antioncogenic agent, and a senotherapeutic, all in the context of CKD.

SULFORAPHANE EFFECTS ON NRF2 AND INFLAMMATION

In a bibliometric review of the biological effects of SFN, activation of Nrf2 was the most cited pathway.[14] The Keap 1-Nrf2-ARE pathway is the primary regulator of cell cytoprotective responses to increased oxidative stress, through inducible expression of detoxification and antioxidant enzymes.[44] Nrf2 is a protein that contains 605 amino acids and is expressed in several tissues and cell types. It belongs to a subgroup of fundamental leucine zipper genes that share a conserved structural domain called Cap-N-Collar.[45,46] In the absence of oxidative stress, the cytosolic repressor protein Kelch-like ECH-associated protein 1 (Keap1), an adapter component of the E3 ubiquitin ligase complex based on Cullin 3 (Cul3), inhibits the Nrf2, which then undergoes ubiquitination and promotes Nrf2 proteasomal degradation.[46] Thus, Keap1 is a negative cysteine-rich Nrf2 regulator,[47] and these reactive cysteine residues act as sensors for oxidants and electrophiles.[48]

The main characteristic of SFN is its electrophilicity, which occurs because of the high chemical reactivity of the central carbon of the isothiocyanate group that reacts with nucleophiles containing a sulfur, nitrogen, or oxygen center.[48] Thus, the isothiocyanates promote modification of the thiol groups on Keap1, inducing the dissociation of the 2 proteins and a consequent increase in Nrf2 intracellular levels.[49] Nrf2 moves to the nucleus and interacts with small musculoaponeurotic fibrosarcoma proteins and coactivating proteins, activating antioxidant response elements in their promoter regions, activating the transcription of the target gene,[46,50] leading to the expression of more cytoprotective proteins with antioxidant and detoxifying functions, such as NAD(P)H quinone oxidoreductase 1 (NQO1) and heme oxygenase 1 (HO-1).[51,52] Nrf2 also binds to the regulatory regions of these inflammatory cytokine genes[53] and also can antagonize NF-κB, which coordinates the expression of inflammatory genes, by preventing the degradation of its cytosolic repressor (IκB).[54,55] In vitro studies have shown a positive regulation of phase II antioxidant enzymes, a downregulation of NF-κB, and a decrease of reactive oxygen species (ROS) production, and ICAM-1, VCAM-1, E-selectin, and monocyte adhesion to endothelial expression in cells treated with SFN.[56–60]

Sulforaphane also had anti-inflammatory activity in several contexts across a range of in vitro and in vivo studies[58,61–63] (Table 1).[8,9,41,58,59,61,62,64–108] Evidence indicates SFN not only activates Nrf2 but also targets other pathways associated with inflammation, including direct inhibitory activity on NF-κB and direct negative regulation of pro-inflammatory genes and inflammasomes.[109–113]

Sulforaphane modulates NF-κB activity by selective reduction of NF-κB target DNA binding with or without interfering with NF-κB nuclear translocation. Mechanistically, SFN interacts with thiol groups by the formation of dithiocarbamate, thus preventing reduction-oxidation–sensitive DNA binding and NF-κB transactivation. Sulforaphane appears to directly inactivate NF-κB subunits by binding to essential Cys residues or interacting with GSH or other reduction–oxidation regulators important to NF-κB function.[114] In an in vitro study, SFN induced phosphatidylinositol 3-kinase/protein kinase B (AKT) activity, with consequent phosphorylation of glycogen synthase kinase 3 β (GSK3β) leading to Nrf2 activation. Sulforaphane also prevented the interaction between NF-κB and its consensus sequence by modifying free thiols on NF-κB in lymphocytes.[115]

Wang et al[4] demonstrated that 0.5 mg/kg SFN administered subcutaneously for 5 days/week over 4 months in a mouse model of type 2 diabetes increased Nrf2 mRNA expression, with consequent increase in SOD-1 and HO-1 expression. Treated animals displayed reduced changes in wall thickness and structural derangement of the aorta. In addition, SFN decreased levels of some inflammatory markers, including TNF and VCAM-1. In keeping with these observations, Giacoppo et al[63] demonstrated that 10 mg/kg/day (R_s)-glucoraphanin bioactivated with myrosinase decreased NF-κB translocation to the nucleus, resulting in decreased expression of IL-1β, Bax, and caspase 3 in a murine model of multiple sclerosis, indicating the cytoprotective and antiapoptotic effect of such a treatment.

Inflammasomes are multiprotein cytoplasmic complexes from the innate immune system; they are formed in response to stimuli of pathogen-associated and danger-associated molecular patterns, such as by infections, tissue damage, or cell stress. The inflammasomes promote the activation of caspase-1, which, through cleavage of IL-1β and IL-18, give rise to its mature forms, leading to local and systemic inflammatory reactions.[116,117] Surprisingly, inflammasome inhibition by SFN seems to be independent of the transcription factor Nrf2 and the antioxidant response-element pathway. Sulforaphane inhibits the autoproteolytic activation of caspase-1 and IL-1β maturation and reduces the activation of NLRP1 and NLRP3 inflammasome.[113] These findings contribute to a better understanding of the anti-inflammatory effects of SNF, because they indicate SFN can inhibit the inflammasomes and, consequently, the inflammatory process, by an alternative mechanism. Because SFN has been overlooked as a mediator of

Table 1 **Studies involving sulforaphane supplementation and its effects on nuclear factor erythroid 2–related factor 2 and inflammation pathways**

Reference	Sample/design	Intervention	Results
In vitro studies			
Townsend and Johnson (2016)[61]	Murine microglia cell line, BV2, neuroimmune system model	2.5 μM SFN	↑Nrf2 activity, ↑NQO1, HO-1, GCLM ↓ LPS-induced IL-1β, IL-6, iNOS
Matsui et al (2016)[64]	HUVECs	0.4 and 1.6 μM SFN	↓ MCP-1, ICAM-1, VCAM-1 gene expression
Lee et al (2016)[65]	BMDMs from C57BL/6 mice LPS induced	40 μM SFN	↓ activation of NLRP3, ↓ IL-1β, caspase-1 ↓ mitochondrial ROS
Qin et al (2016)[66]	PBMCs isolated from healthy volunteers treated with acrolein	Pretreatment with SFN at a concentration 5 μM for 24 h	↓ ROS, protein carbonyl, sulfhydryl content, lipid peroxide levels ↓ COX-2 levels ↑ Nrf-2, SOD, CAT, GST and GPX
Axelsson et al (2017)[41]	H4IIE cells, T2DM model	Pretreatment with SFN at 0.5 to 10 mM	↓ Glucose production ↑ NRF2 nuclear translocation
Carrasco-Pozo et al (2017)[67]	Min6 cells, high cholesterol–induced model	SFN at 10 μM	↓NF-κtranslocation to the nucleus; ↓IL-1β, TNFα, IFNγ ↑IL-4, IL-10, HO-1, SOD
de Oliveira et al (2018)[68]	Mitochondria obtained from human neuroblastoma SH- SY5Y cells exposed to H_2O_2	Pretreatment with SFN at 5 μM	↓ Lipid peroxidation, protein carbonylation, and protein nitration ↑ Cellular and mitochondrial GSH
de Oliveira et al (2018)[69]	Human neuroblastoma SH-SY5Y cells, pro-inflammatory state induced by H_2O_2	Pretreatment with SFN at 5 μM for 30 min before H_2O_2	↓ IL-1β, TNF-α; ↓ COX-2 ↓ NF-κB activity
Zhao et al (2018)[70]	N2a/APPswe cells (cellular model of Alzheimer's disease)	SFN 1.25 and 2.5 μM	↓ DNA methylation levels (DNMT1, DNMT3a , DNMT3b) ↑Nrf2, NQO1, HO-1 mRNA levels ↑ Nuclear translocation of Nrf2; ↑ SOD activity ↓ ROS, MDA levels, IL-1β, IL-6, NF-κB p65, COX-2, iNOS protein
Rakariyatham et al (2018)[58]	RAW 264.7 macrophages stimulated by LPS	Pretreatment with SFN at different doses	↑ Nrf2 (at 1 μM) and HO-1 (at 0.5 and 1 μM) ↓NF-κB p-p65 and p65 in the nucleus (at 0.5 μM) ↓ IL-1β (at 1 μM); ↓ ROS (at 0.25, 0.5, and 1 μM)
Qin et al (2018)[71]	Mouse microglial (BV-2) cell line, LPS induced	Pretreatment with SFN (5, 10, and 15 μM)	↓ TNF-α, IL-1β, IL-6, and iNOS ↓ MAPKs and p65 activation
Eren et al (2018)[72]	N9 microglial cells, LPS induced	Pretreatment with SFN 5 μM for 1 h	↑ Translocation of Nrf2 to the nucleus ↓TNF-α, IL-1β and IL-6; ↓NF-κB, AP-1
Liu et al (2019)[59]	Cultured human trabecular meshwork cells exposed to H_2O_2	Pretreatment with SFN at 20 μM	↓ Intracellular ROS ↑ Gene and protein expression of NQO-1, HO-1 enzymes, catalytic subunit of GCLC and GCLM modifying subunit by Nrf2 dependent pathway
Cox et al (2019)[73]	HUVECs, placental and endothelial model of oxidative stress	Treatment with SFN at different doses	↑ activation and nuclear translocation of NRF2 (20 μM) ↑ HO-1 (20 μM) ↓ VCAM1, ICAM1 and E-selectin (5, 10, and 20 μM)
Haodang et al (2019)[74]	Human monocytic THP-1cells stimulated with a mycoplasmal lipopeptide	SFN 0.5, 1.0, 5.0 μmol/L	↓TNF-α, IL-1β, and IL-8 ↓ IκB phosphorylation and degradation ↓ DNA-binding activity of NF-κB
Subedi et al (2019)[75]	LPS-activated BV2 microglia	Pretreatment or posttreatment with SFN at 5 or 10 μM	↓ MAPK phosphorylation levels ↓ NF-κB and AP-1; ↓ iNOS, COX-2, NO, PGE2 ↓ TNF-α, IL-6, and IL-1β ↑ Expression of Nrf2, HO-1, IL-10, IL-4

(continued)

Table 1 **Continued**

Reference	Sample/design	Intervention	Results
Liu et al (2020)[9]	PBMCs from healthy donors	SFN (2 μM and 5 μM) for 6 h SFN (5 μM) pretreatment + LPS stimulation	↑ NQO1, HO-1 after 6-h SF treatment ↓ COX-2, TNF-α, IL-, and IL-1β by up to 80% of the levels when compared with the controls
Chang et al (2020)[76]	Human ARPE-19 cell, oxidative stress-induced retinal injury	Pretreatment with SFN at different doses (0.5 μM, 1 μM, or 5 μM)	↓ ROS production (5 μM) In a dose-dependent manner: ↑ mRNA expression of NQO1, GR, and GPx1 ↑ protein expression of HO-1, NQO1, and GR ↓ ICAM-1 and MCP-1 ↑ DNA binding affinity of Nrf2 in the nucleus
Yang et al (2020)[77]	Human knee osteochondral composites, H_2O_2 induced	SFN at 7 μM	↓ iNOS; ↓ IL-6, TNF-α ↑ Nrf2, SOD1, CAT
Ali et al (2020)[78]	Human monocytic THP-1 cell line, macrophage infection model	SFN 10 μM	↑ Nrf2 nuclear translocation and activity ↓ ROS levels, IL-1β, IL-6, and TNF-α gene expressions
Deramaudt et al (2020)[79]	Human THP-1 monocytic cell line, *Staphylococcus aureus* infection model	SFN 10 μM	↓ IL-1β, IL-6, and TNF-α mRNA expression levels ↓ Activation of p38 and JNK ↑ Nrf2 protein levels
Nadeem et al (2020)[80]	PBMCs/monocytes isolated from blood of children with autism spectrum disorder, LPS induced	Incubated overnight with or without SFN 5 μM	↓ p-NF-κB, IL-6 expression, iNOS
Animal studies			
Dong et al (2016)[81]	BALB/c mice, acute pancreatitis model induced by cerulean hyperstimulation	5 mg/kg SFN for 3 consecutive days before AP	↑ Nrf2, HO-1;↑SOD, GPx ↓ NLRP3/NF-kB; ↓ MDA; ↓TNF-α, IL-1β, IL-6
Zhao et al (2016)[82]	Male Sprague-Dawley rats, subarachnoid hemorrhage model	SFN 50 mg/kg (intraperitoneal)	↑Nrf2, HO-1, NQO1 ↓IL-1β, IL-6, and TNF-α
Xu et al (2016)[83]	Male db/db mice, T2DM	BSE-low dose ($\sim$ 0.5 mg/kg SFN), BSE-high dose ($\sim$ 1.0 mg/kg SFN), SFN molecule (0.5 mg/kg) as a positive control for 3 mo	↑ Nrf2 ↑ NQO1, CAT, HO-1 ↓ TNF-α, VCAM-1, MDA ↓ 4-HNE, 3-nitrotyrosine (3-NT)
Holloway et al (2016)[84]	Male WT C57BL/6J mice, LPS induced	SFN administration (5 and 50 mg/kg i.p.)	↓ IFN-γ MCP-1, TNF-α serum levels ↑ IL-10 serum levels
Townsend and Johnson (2017)[85]	Adult Balb/c mice, LPS induced	SFN 50 mg/kg, i.p. for 3 d	↓ IL-1β, IL-6 mRNA ↑ NQO1, HO-1
Yu et al (2017)[86]	Adult male Sprague-Dawley rats, cerebral ischemic/reperfusion injury model	SFN 5 mg/kg (intraperitoneal)	↓ NLRP3 inflammasome expression ↓ IL-1β, IL-18 level
Yan et al (2017)[87]	C57/BL6 male mice, bleomycin-induced pulmonary fibrosis	Subcutaneous injection of SFN 5 mg/kg/d	↑ Nrf2 expression ↑ Protein levels and the mRNA levels of HO-1, NQO1, SOD1, and CAT
Wang et al (2017)[88]	C57BL/6J male mice, angiotensin II–induced testicular cell death	SFN 0.5 mg/kg 5 d/ wk for 3 mo	↓ Caspase-3 protein, ↓ IL-6, and VCAM-1 ↓ Nitrosative and oxidative damage (3-NT and 4-HNE) ↑ Nrf2, HO-1, NQO1
Bai et al (2017)[89]	Male Sprague-Dawley rats, doxorubicin-induced CHF	SFN subcutaneously administered at 0.5 mg/kg daily for 6 wk	↑ Nrf2, HO-1, NQO1 ↑ SOD1, SOD2, CAT, and GSH-Px activities ↓ MDA levels

(continued)

Table 1 **Continued**

Reference	Sample/design	Intervention	Results
Xin et al (2018)[90]	WT mice, angiotensin II–induced cardiomyopathy	SFN (0.5 mg/kg) 5 d/wk for 3 mo	↑ Nrf2 expression, CAT, NQO1, HO-1
Pu et al (2018)[91]	Male db/db mice	SFN (1 mg/kg) intraperitoneal for 28 d	In hippocampus: ↓ ROS/RNS levels ↑Nrf2, HO-1, NQO1
Moustafa et al (2018)[92]	Adult male Wistar rats, T2DM induced	SFN 1 mg/kg for 2 wk	↓ MDA, NO, IL-6, and MMP-2 and -MMP9 contents ↓ COX-2 and NF-κB p65 ↑ SOD and IL-10 contents
Ma et al (2018)[93]	New Zealand White rabbits, ascending aortic cerclage model of CHF	SFN 0.5 mg/kg for 5 d/wk for 12 wk	↓TNF-α, IL-6, MDA ↑SOD
Yang et al (2018)[94]	C57BL/6 mice, models of acute gout induced	SFN at different doses (1, 5, 10, 30 mg/ kg)	↓NLRP3 inflammasome activation ↓ degradation of pro-caspase-1 to caspase-1(p10) and of pro-IL-1b to IL- 1 b
Gong et al (2019)[95]	Female Sprague-Dawley rats, retinal ischemia/reperfusion injury model	SFN 10 and 20 mg/kg (gavage)	↓NLRP3, ASC, and caspase-1 ↓TNF-α and IL-1β
Saleh et al (2019)[96]	Adult male Wistar rats, intraperitoneally injected with D-galactosamine to induce liver aging	SFN at doses 0.5, 1 and 2 mg/kg/d, for 6 wk	↑ CAT, and GST ↓ MDA, NO, protein carbonyl, TNF-α, Keap-1 levels ↑ cytoplasmic and nuclear Nrf-2 levels ↑ HO-1
Nadeem et al (2019)[97]	BTBR mice, autism model	SFN 50 mg/kg, i.p. once daily for 7 d	↓ *p*-NF-κBp65, iNOS, and nitrotyrosine in neutrophils ↓ Lipid peroxides levels ↑ SOD1/GPx1 expression
Silva-Palacios et al (2019)[98]	Adult female Wistar rats, myocardial ischemia–reperfusion model	injection of SFN 500 μg/kg in the cavity of the left ventricle	↓ Carbonyl groups, MDA levels, and nitrotyrosine residues ↓ IL-1β, IL-6 ↑ AhR, HO-1, NQO1
Angulo et al (2019)[99]	Old male Sprague-Dawley rats	SFN, 10 μM	↑ Nrf2 and ↓ oxidative stress in arteries from aged rats
Wu et al (2019)[100]	BALB/c mice, a 2,4-dinitrochlorobenzene-induced atopic dermatitis mouse model	SFN at 2.5, 5, and 10 mg/kg	↓ IL-6, IL-1β, and TNF-α ↑ p-Nrf2, Nrf2, and HO-1
Wei et al (2020)[101]	Male Balb/c mice, 5-fluorouracil-induced intestinal injury model	SFN-Low group (SFN at 2 mg/kg/d) SFN-High group (SFN at 20 mg/kg BW/d)	SFN-High group: ↑ Nrf2, HO-1 in jejunum ↓ NF-κB in jejunum; ↓iNOS in colon
Liu et al (2020)[102]	Adult female Sprague–Dawley rats, sciatic nerve endometriosis model	SFN at 30 mg/kg/d (i.p.) for 28 d	↓ IL-6, IL-1β, and TNF-α ↓ COX2 and iNOS upregulation ↑ Keap1 and Nrf2
Wang et al (2020)[8]	Spontaneous T2DM db/db mice	0.5 mg/kg of SFN for 1 mo (i.p.)	↑ HO-1, CAT, and NQO1 ↑ Phosphorylation of Nrf2 ↓ Protein expression of NF-κB P65, TNF-α, PAI1, TGF-β1, Caspase-3, and caspase-1 ↑ Expression levels of Nrf2, CAT, HO-1, GPx, GST, and SOD ↓ TNF-α, TGF-β1

(continued)

Table 1 **Continued**

Reference	Sample/design	Intervention	Results
Sun et al (2020)[103]	Male mice with global knockout of AMPKα2 gene (AMPKα2-KO), diabetes-induced cardiomyopathy model	SFN 0.5 mg/kg, 5 d/wk for 3 mo	↑ Nrf2 ↑ CAT, HO-1 ↑ PGC-1α, PPAR-α
Ruhee et al (2020)[104]	Male C57BL/6 mice, exhaustive exercise protocol model	SFN 50 mg/kg 2 h before the running test	↓ IL-1β, TNF-α ↑ mRNA expression of Nrf2, HO-1, SOD1, CAT, GPx
Lee et al (2020)[105]	Male C57BL/6 mice, LPS-induced lethal endotoxemia model	SFN 0.13, 0.26, or 0.39 mg/kg at 12 h after LPS injection	↓ TLR-4, ↓TNF-α, IL-6 ↓ Nuclear level of the NF-κB p65
Human studies			
Mirmiran et al (2012)[106]	Patients with T2DM	5 or 10 g/d broccoli sprouts powder for 4 wk	↓ hs-CRP concentration
Navarro et al (2014)[62]	Healthy, young individuals	Basal diet supplemented with 14 g/kg cruciferous vegetables (broccoli, cauliflower, kale, and radish sprouts) for 2 wk	↓ IL-6
Ushida et al (2015)[107]	Healthy individuals	Single administration of a broccoli supplement containing glucoraphanin (30 mg)	↑ GST and NQO1
López-Chillón et al (2019)[108]	Healthy, overweight individuals	Broccoli sprouts (30 g/d) for 10 wk	↓ IL-6 and CRP

Abbreviations: 4-HNE, 4-hydroxy-2-nonenal; AhR, aryl hydrocarbon receptor; BMDM, bone marrow–derived macrophage; BSE, broccoli sprout extract; BW, body weight; CAT, catalase; CHF, chronic heart failure; COX-2, cyclooxygenase-2; CRP, C-reactive protein; GCLC, glutamate-cysteine ligase; GCLM, glutamate-cysteine ligase; GPx, glutathione peroxidase; GSH, glutathione; GST, glutathione *S*-transferase; H_2O_2, hydrogen peroxide; HO-1, heme oxygenase 1; hs-CRP, high-sensitivity C-reactive protein; HUVEC, human umbilical vein endothelial cell; IL, interleukin; iNOS, inducible nitric oxide synthase; i.p., intraperitoneal; NF-κB, nuclear transcription factor-κB; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2–related factor 2; LPS, lipopolysaccharide; MDA, malondialdehyde; MMP, matrix metalloproteinase-2; NO, nitric oxide; PBMC, peripheral blood mononuclear cell; ROS, reactive oxygen species; SFN, sulforaphane; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor-α; WT, wild type.

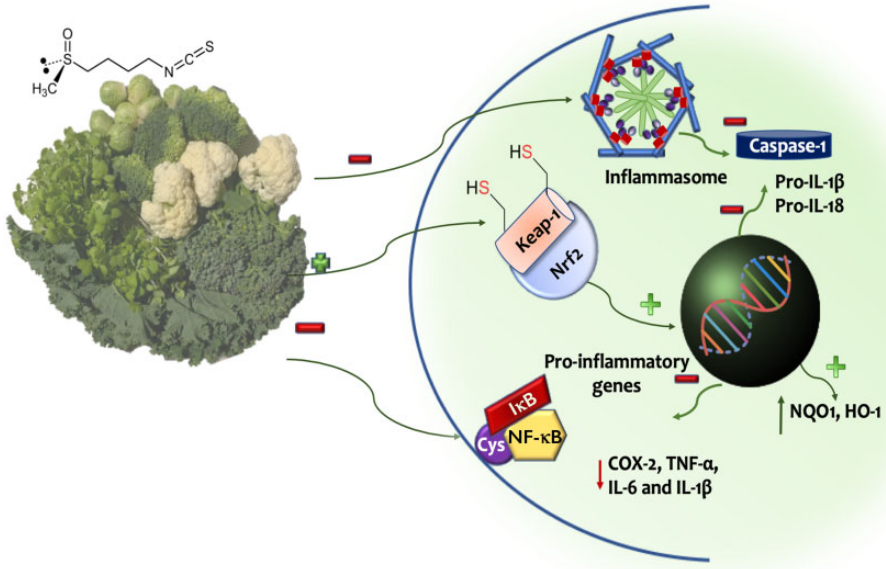

Figure 3 **Effects on Nrf2 and inflammatory factors in the cells.** Sulforaphane in cruciferous vegetables activates the nuclear factor erythroid 2–related factor 2 (Nrf2) and through this and other pathways, influences inflammatory factors in the cells. Sulforaphane modifies the thiol groups of Keap1, increases the availability of Nrf2 to the nucleus, binds to the essential Cys residues, inactivates NF-κB, and reduces the activation of NLRP3 inflammasome.

many inflammatory pathways, the Nrf2-independent anti-inflammatory effects of SFN deserve more study.[113]

Inflammation and oxidative stress are intrinsically involved in the pathogenesis of chronic noncommunicable diseases such as CVD, hypertension, obesity, CKD, diabetes, and cancer.[118] Accordingly, numerous nutritional strategies have been developed and applied in an attempt to decrease inflammation and oxidative stress, and to improve the quality of life of affected individuals.[118,119] According to studies summarized in this review, SFN treatment seems to be an efficient anti-inflammatory and antioxidant strategy, as shown the Figure 3. Clinical studies in humans should be encouraged because only a limited number of published reports dealing with this topic currently exist in the literature.

SULFORAPHANE AS A MODULATOR OF AGEING PROCESSES

Ageing is a process characterized by physiological decline and a diminished capacity to maintain physiological homeostasis due to "'the burden of wear and tear"'[121] resulting from allostatic (over)load over the life course.[120,121] As such, the human body becomes susceptible to exogenous or endogenous stress stimuli and cellular insults, predisposing individuals at high risk for development of chronic degenerative diseases (eg, CVD, CKD, diabetes, cancer, sarcopenia, neurodegenerative diseases).[122] One of the prominent features of premature ageing is the incremental increase in oxidative stress and the waning of the antioxidant defense system, which eventually lead to cumulative oxidative DNA damage and cellular senescence concomitant with chronic inflammation.[123] Accumulating evidence suggests that isothiocyanates, including SFN, can counteract aspects of the ageing process via a range of underlying mechanisms, from Nrf2-dependent or independent pathways to modification of the epigenetic landscape of ageing.[124]

Given the role of Nrf2 as a geroprotective agent and mediator of ageing processes,[125] it is tempting to speculate on the capacity of SFN to prevent or mitigate the progression of ageing-related diseases. Indeed, the Nrf2-dependent antiaging potential of SNF has been widely demonstrated for cardiovascular ageing in preclinical and clinical studies. Accordingly, SNF-mediated Nrf2 signaling has been demonstrated to hurdle endothelial cell activation in atherosclerotic plaque,[126] regulate vascular smooth-muscle proliferation,[127] and mitigate the inflammatory and thrombotic burden.[128] Such a protective and geroprotective role for SNF has also been described in various neuropathological diseases, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, and multiple sclerosis.[44,129–133] Recently, Saleh et al[96] showed that SFN could improve liver ageing and inhibit hepatic fibrosis via the Keap-1/Nrf2 pathway in D-galactose–induced liver ageing rats.

Apart from targeting Nrf2 signaling–mediated oxidative DNA damage and inflammation, low-dose treatment with SNF (1 μM, once per week) delayed the onset of cellular senescence by repressing cellular glucose uptake and downregulating glycolysis, thus exerting a caloric restriction mimetic-like response.[134] Such antisenescence activity has also been reported in mesenchymal stem cell culture under oxidative stress conditions (300 μM hydrogen peroxide [H_2O_2]), where a hormetic (ie, biphasic dose response) behavior of SNF was observed.[42]

SULFORAPHANE AND THE EPIGENOME

The epigenetic landscape comprises canonical features, such as DNA methylation and chromatin modification, as well as noncanonical features, such as reciprocal regulatory networks of noncoding RNAs.[135] This landscape enables rapid genomic responses to environmental changes without the requirement to fix these in the DNA sequence, which would otherwise take many generations.[136,137] Dysregulation of the epigenome can lead to multistage carcinogenesis, accelerated ageing, and the development of chronic diseases.[11,135,136,138]

Cancer cells also display several DNA alterations, such as site-specific DNA hypermethylation, altered cellular histone deacetylase (HDAC) activity, and altered microRNA (miRNA) expression. DNA methyltransferases (DNMTs), enzymes that methylate DNA cytosine residues, and HDACs function to enable stable gene repression.[139] In this context, SFN has been characterized as a modulator of epigenetic enzymes, via inhibition of HDAC expression, that may have a similar effect as trichostatin A, a pharmaceutical HDAC inhibitor.[139,140]

Abbaoui et al[13] showed in an in vitro study of bladder cancer that SFN inhibits HDAC, specifically HDACs 1, 2, 4, and 6, by decreased histone acetyltransferase activity. In addition, SFN decreases the phosphorylation status of histone H1 and increases phosphatase PP1β and PP2A activity. These data support the assertion that SFN modulates histone status through HDAC inhibition and increase of phosphatase activity.[13] Also, in a study in melanoma cell lines, SFN reduced cell viability and total histone deacetylase activity, and modulated the expression levels of histone deacetylases, and acetyl and methyltransferases. These results indicate SFN regulates the epigenetic response by modulation of acetylation and methylation in melanoma cells.[12]

In a study of colorectal cancer in rats, a single administration of 60 mg/kg SFN by gavage decreased HDAC3 expression and histone acetyltransferase activity, and increased γ-H2AX levels, a marker of DNA damage. These results have shown that SFN causes DNA damage in colon cancer cells and decreases their proliferation.[141] This hypothesis is an essential caveat for strategies using SFN to treat cancers, especially Nrf2-positive cancers, that dosing requirement and signs of any hormetic effects need more investigation, robust data, and data analysis.

In another in vitro study, SFN was described as affecting the hypomethylation of phosphatase and tensin homolog (PTEN) and retinoic acid receptor β 2 (RARβ2) promoters, which led to concomitant tumor suppressor gene upregulation. The PTEN and RARβ2 promoters are involved in the tumor suppressor genes that are silenced in breast cancer cells. It is thus essential to recognize that PTEN and RARβ2 promoters can decrease DNMT expression through negative regulation of the MAPKAP1 signaling pathway, an intracellular oncogenic pathway.[142] Another study in breast cancer cells showed that the anticancer effects of SFN were mediated by global DNA hypomethylation, decreased levels of DNMT1 and DNMT3B, and diminished N6-methyladenosine RNA methylation. In another study, SFN upregulated expression of 60 miRNAs and downregulated expression of 32 miRNAs.[143]

Another study using human hepatocellular carcinoma cells showed that SFN can downregulate DNA damage and modulate expression of histone deacetylases, leading to downregulated genes involved in inflammatory signaling (*HDAC5* and *HDAC11*) as well as upregulated and hypomethylated genes linked to the Nrf2 pathway, including *NQO1*, *HO-1*, glutamate-cysteine ligases, and thioredoxin reductase 1.[144] Epigenetic regulation of Nrf2 through SFN promotes the transcription of Nrf2 and its nuclear translocation and activation.[145] Also, in primary effusion lymphoma cells, SFN decreased cell viability and inhibited the phosphorylation of p38 mitogen-activated protein kinase and AKT, both of which are involved in the inflammatory response. Consequently, there was a reduction in cell growth and enhanced apoptosis.[146]

In a study in dendritic cells, which have a pivotal role in host immune responses, SFN inhibited the lipopolysaccharide-induced *HDAC6*, *HDAC10*, and *DNMT3a* gene expression. Moreover, SFN upregulated the expression of the *DNMT1* gene and inhibited global HDAC activity. Sulforaphane altered the induction of toll-like 4 receptor gene expression, consequently regulating the toll-like 4 receptor–induced activity of transcription factor NF-κB and leading to decreased proinflammatory cytokine secretion.[147]

Another epigenetic action of SFN is by inhibition of telomerase reverse transcriptase (hTERT) expression and activity. Levels of hTERT, a catalytic subunit of telomerase responsible for changes in chromatin structure and composition,[148] are elevated in 90% of cancers and

essential for their proliferation.[149] Sulforaphane mediates changes in histone post-translational modifications levels,[148] which is pertinent because HDAC1 regulates hTERT mRNA levels and expression.[149] Moreover, SFN downregulates telomerase protein expression levels and enzymatic activity. These effects lead to inhibition of cell viability and induce apoptosis of the colorectal cancer cells. In in vitro studies, Chen et al[150] observed antitumor activities of SFN in nasopharyngeal carcinoma and concluded that SFN could be useful in suppressing the development of neural progenitor cells through the DNMT1/WIF1 axis pathways.

Nutraceutical combinations have been evaluated as a potent treatment for colon cancer, and among these substances, SFN has been evaluated for its antioncogenic capabilities in HT-29 and Caco-2 colon cancer cells, wherein the combination with dihydrocaffeic acid was more effective. Subsequently, this combinatorial therapy has been proposed as a basis for the creation of effective food products in the prevention and even cotreatment of colon cancer.[151] Corroborating this, Lan et al[152] evaluated the apoptotic potential of SFN in colon cancer cells. SW480 cells with *P53* deficiency were treated with varying concentrations of SFN (5, 10, 15, and 20 μM). All studied concentrations of SFN were able to cause apoptosis, and the authors concluded that SFN might be a therapeutic strategy in the cotreatment of patients with p53-deficient colon cancer.

Lewinska et al[143] have observed the effects of SFN (concentrations of 5, 10, and 20 μM) on breast cancer cells. Sulforaphane at 5 and 10 μM was effective in stopping the cell cycle, increasing the levels of p21 and p27. and inducing cellular senescence, whereas at 20 μM, SFN induced apoptosis. They also observed nitro-oxidative stress, genotoxicity, reduced AKT signaling, negative regulation of miRNAs, and a significant reduction in the levels of miR-23b, miR-92b, miR-381, and miR-382 in 3 types of cancer cells, showing that SFN can exert its effect via the epigenetic landscape.

SULFORAPHANE AND MITOCHONDRIA

Mitochondria are the most important providers of energy to the cell through cellular adenosine triphosphate (ATP) and metabolic intermediaries and participates in several signaling processes leading to ROS production. As an adaptation against stress, mitochondria are dynamic, and they can build extensive interorganelle networks among themselves and between isolated organelle fragments.[153] Maintenance of mitochondrial mass is an essential homeostatic function within the cell to optimize cellular metabolic capacity.[154] Mitochondrial biogenesis is responsible for the increase in mitochondrial mass, which is mediated by the nuclear respiration factors (including Nrf1), not to be confused with Nrf2. Nuclear respiration factors are activated by the peroxisome proliferator–activated receptor-γ coactivator 1-α (PGC-1α).[155] The reorganization of the mitochondrial dynamics (ie, fusion and fission) is carried out by mitofusins 1 and 2 and the optic atrophy protein-1. Any associated mitochondrial spoilage is attended to via mitophagy, mediated by the protein p62, and followed by recruitment to the autophagosome by light-chain protein 3. In the autophagosome, mitochondria are hydrolyzed and their components are recycled.[154]

In this context, some studies have reported that SFN acts as a protector for mitochondrial function and proteins and enzymes involved in mitochondrial biogenesis. This is supported by data from in vitro studies in which SFN induced mitochondrial biogenesis and stabilized Nrf2.[68,156,157] Mitochondrial biogenesis has also been reported to play an essential role in cancer cell death.[156] Furthermore, the induced knockdown of NRF1 has been reported to attenuate SFN activity in cancer cells.[156] These data indicate SFN can induce Nrf2 directly or through a transient increase in ROS production. This is in keeping with a role for Nrf2 as an inducer of Nrf1 expression, because Nrf1 activation leads to the production of proteins involved in mitochondrial biogenesis, such as mitochondrial transcription factor-A (TFAM).[156]

A range of studies have indicated SFN treatment affects mitochondrial biology with consequential effects on cell metabolism and viability. de Oliveira et al[68] showed that the pretreatment of mitochondria from human neuroblastoma cells exposed to H_2O_2, with SFN, prevented the loss of viability in these cells and decreased lipid peroxidation, protein carbonylation, and protein nitration in mitochondrial membranes. Furthermore, SFN increased the levels of cellular and mitochondrial GSH, maintained the mitochondrial bioenergetics state, and increased the expression of Nrf2. The authors concluded that SFN abrogated mitochondrial impairment in a Nrf2-dependent manner. Sulforaphane has also been reported to decrease the oxidative stress and mitochondrial apoptosis induced by angiotensin II in vitro, through ROS scavenger, induction of Nrf2 activation, and expression.[157]

Sulforaphane induces the nuclear expression of Nrf2 and activates HO-1 enzyme. The increase of antioxidant response by Nrf2/HO-1 leads to preservation of mitochondrial function, with a consequent decrease of ROS production from damaged mitochondria.[158,159] In addition, SFN induces the expression of PGC-1α, NRF1, and TFAM, which leads to the improvement of mitochondrial biogenesis and keeps the mitochondrial membrane potential of ATP.[160] Also, the antioxidant

and anti-inflammatory effects of SFN improve mitochondrial bioenergetic function. Consequently, there is a prevention of cholesterol alterations by the improvement of mitochondrial respiration, ATP turnover, and avoiding the impairment of the electron flow at complexes I, II, and IV.[67]

SULFORAPHANE AND MICROBIOTA

Among the food components known as essential modulators of the gut microbiota, dietary fibers and phytonutrients stand out.[161,162] Consequently, the consumption of cruciferous vegetables can alter the composition of the gut microbiota and lead to the growth of specific bacteria that increase the production of SFN,[163,164] because gut microbiota metabolize GLS to SFN.[162,165] The evidence for this mechanism is supported by data indicating the suppression of intestinal microbiota with antibiotics and mechanical cleaning of the intestine made the conversion of GLS into SFN insignificant.[26] In a pioneering study on the effect of brussels sprouts, inulin, and fermented milk on the fecal microbiota diversity of human microbiota–associated rats, Humblot et al[166] observed that brussels sprouts consumption led to an increase in the abundance of salutogenic bacteria, including *Lactobacillus* levels, associated with increase in levels of butyrate and acetate.

Liu et al[164] observed that the consumption of broccoli altered the composition of the caecal microbiota in rats, especially the genera of the phylum Clostridiales (eg, *Blautia, Clostridium, Dorea,* Ruminococcaceae*, Oscillospira*), and led to an increase in the hydrolysis of glucoraphanin (the main glycosinolate of broccoli) to bioactive isothiocyanate. Following these observations, Wu et al[167] evaluated the effects of broccoli ingestion on the hydrolytic action of myrosinase, the NQO1 enzyme, and on the diversity and composition of the intestinal microbiota of rats fed diets containing cooked and hydrolyzed broccoli. At the end of the study, they observed that the ingestion of broccoli for 6 weeks increased the activity of the hydrolytic action of myrosinase present in the colon and cecum and intensified the NQO1 activity of the colon mucosa. Broccoli ingestion also strongly interfered in the bacterial composition of the rat microbiota; both cooked broccoli and hydrolyzed broccoli led to a significant increase in the populations from the Bacteroidetes and Firmicutes and significantly decreased the Proteobacteria population.[167] Members of the Bacteroidetes and Firmicutes can hydrolyze glucosinolates to isothiocyanate,[168] and members of Proteobacteria are associated with diseases sustained by inflammation.[169]

Butyric and isobutyric acids are essential short-chain fatty acids that act as a source of energy used by intestinal cells and help in the formation of the intestinal barrier and in the development of the intestinal epithelium.[170,171] Sulforaphane treatment can have a beneficial effect on these aspects of intestinal health. Rats treated with SFN had increased levels of butyric acid and isobutyric acid in their colon and positive regulation of the expression of junction proteins and GLP2 after lesion of the mucosal epithelium of the colon and cecum.[172] The rats also had reduced levels of IL-6 and secretory immunoglobulin A, and a near-normative intestinal microbiota. The study authors also observed a significant increase in *Bacteroides fragilis*, one of the most abundant species in the mucosa that helps maintain a normative immune system, and an increase in *Clostridium* cluster I, a group of bacteria that aid in the degradation of carbohydrates and production of butyric acid.

Corroborating these findings, Xu et al[173] evaluated the impact of glucoraphanin from broccoli seeds on the intestinal microbiota and lipid parameters of mice fed a high-fat diet, noting that glucoraphanin present in the broccoli reduced the Firmicutes and Bacteroidetes fraction in the microbiota and reduced levels of total cholesterol, triglycerides, and LDL-cholesterol. They also observed a reduction in the weight of the liver and visceral fat, a reduction in the concentrations of inflammatory markers and in the actions of the *FAS* gene, in addition to a significant increase in the hepatic expression of the *PPARα*, *CPT1*, and *ACOX* genes. Finally, Xu et al[173] emphasized that glucoraphanin can be a potent adjuvant in preventing obesity and can be used as a functional food in the form of flour made from the seeds of cruciferous vegetables.

In human studies, Li et al[163] have conducted a randomized, crossover, controlled study with 17 participants who received for 14 days a regular diet with a low content of phytochemicals and fiber (ie, they ate refined grains without fruits or vegetables) and the researchers compared findings with that of a diet rich in cruciferous vegetables (14 g/kg of weight). At the end of the study, the ingestion of cruciferous vegetables had modified the bacterial composition of the intestinal microbiota, especially *Eubacterium hallii*, *Phascolarctobacterium faecium*, *Alistipes putredinis*, and *Eggerthella* spp. These bacteria use the glycosinolate present in cruciferous vegetables as a metabolic substrate.[163,174]

In a randomized crossover study of the relationship between consumption of a diet rich in brassicaceous vegetables and a decrease in sulfate-reducing bacteria, Kellingray et al[175] showed that consumption of a diet rich in brassicaceous vegetables (consisting of 6 portions of 84 g of broccoli, 6 84-g portions of cauliflower, and 6 300-g portions of a broccoli and sweet potato soup) for 2 weeks interfered with the bacterial

composition of the microbiota of 10 healthy adults. Specifically, in the brassicaceous vegetable–rich diet group, there was a significant reduction in the proportions of 5 bacterial taxa (4 members of the Clostridiales and 1 member of the Bacteroidales).

In a controlled and randomized study, Kaczmarek et al[162] demonstrated that broccoli can be a crucial piece in the modulation of the intestinal microbiota and, consequently, health promotion. They analyzed the effects of the intervention of 200 g of cooked broccoli and 20 g of raw radish per day on the intestinal microbiota of 18 healthy individuals in a study consisting of 2 18-day sessions, interspersed with a 24-day washout period. At the end of the analyses, they found that the intervention with broccoli modulated the intestinal microbiota of these individuals, leading to a significant reduction in members of the Firmicutes and a significant increase in the Bacteroidetes members.[162]

SULFORAPHANE IN CHRONIC KIDNEY DISEASE

Chronic inflammation and oxidative stress are common findings and are associated with the uremic phenotype in patients with CKD.[176] These patients have decreased Nrf2 expression and translocation to the nucleus, with a consequent decrease in the production of antioxidant enzymes and greater ROS production.[177] The increased ROS concentration is 1 of the triggers for the overexpression of NF-κB, which generates greater production of pro-inflammatory cytokines.[176] This process generates a vicious cycle of oxidative stress and inflammation in CKD.[46,176,178] The presence of inflammation and oxidative stress worsens the underlying, unregulated ageing process, mitochondrial dysfunction, and gut dysbiosis in CKD and increases the risk of premature cardiovascular events and death.[125,138,179,180]

Patients with CKD have a gut microbiota imbalance (uremic dysbiosis) that leads to overproduction of bacteria species responsible for the production of uremic toxins, such as indoxyl sulfate, *p*-cresyl sulfate, and indole-3 acetic acid.[181] The accumulation of these uremic toxins is linked to changes in the gut barrier, contributing to an increase of lipopolysaccharide production and of local and systemic inflammation and oxidative stress.[182] Mitochondrial dysfunction is linked to CKD pathogenesis.[183–185] Therefore, in CKD, there is an overproduction of ROS, a decrease in ATP generation, loss of inner mitochondrial membrane potential and cytochrome C and PGC-1α release.[186] Together, these factors lead to cell apoptosis or cell injury and DNA mitochondrial damage, which stimulates the activation of toll-like receptor and inflammation in CKD.[187,188] Both acute kidney injury (AKI) and CKD are associated with defects in mitochondrial biogenesis, demonstrated by low levels of PGC-1α, TFAM, and mitofusin 2.[184,189] Mitochondrial dysfunction is linked with CKD progression, muscle dysfunction, and sarcopenia in CKD.[185,186,190,191]

Several epigenetic alterations are linked with the uremic state, including hypermethylation of the RAS protein activator like 1 gene induced by the overproduction and retention of uremic toxins.[192] This leads to kidney fibrosis and suppression of Klotho activity, which is a significant regulator of anti-ageing defenses.[192,193] Another factor in the epigenetic changes in CKD is the *MTHFR* gene, which leads to methyl radical synthesis and provides methyl groups for global genomic methylation. This factor is associated with an increased CVD risk and an increase in biological age.[192]

Studies using dietary components and their potent bioactive compounds to improve the alterations found in patients with CKD have been conducted. Several studies have shown that bioactive compounds such as curcumin, prebiotics, and Brazil nuts, among others, can be an adjuvant therapy for patients with CKD[117,192,194–196] and the findings indicate food as medicine would be an attractive novel treatment option for this patient group.[197]

Although SFN is a promising nutritional therapy in several diseases, no studies have investigated the effects of SFN in CKD, to our knowledge. However, some in vivo and in vitro studies have investigated the effects of SNF in the kidney and renal cells as a renoprotective agent in CKD and AKI (Table 2).[154,198–221]

Sulforaphane prevented cell death and renal and mitochondrial damage induced by cisplatin treatment.[198,222] Moreover, SFN improved the nuclear translocation of Nrf2 in the cells, attenuating processes leading to renal dysfunction, structural damage, oxidative/nitrosative stress, and GSH depletion; and decreased the activity of catalase, GSH peroxidase, and glutathione *S*-transferase.[198,223] Pretreatment with SFN can prevent renal injury and attenuate activation of inflammation pathway signaling in cisplatin-induced nephropathy.[202] Another study demonstrated, in vitro and in vivo, that Nrf2 plays a protective role against intravascular hemolysis-mediated AKI caused by hemoglobin/heme-induced renal damage. Sulforaphane activated Nrf2 expression, which conferred protection against hemoglobin toxicity in mice and cultured tubular epithelial cells, leading to amelioration of kidney injury, cell stress and death, and improvement in renal function.[218] Some animal studies and in vitro studies in diabetic nephropathy showed that SFN decreases the production of ROS and inflammation (IL-6 and caspase 3) in kidney tissues by activation of Nrf2-HO-1/NQO-1 and reduction in the activity of the GSK3β signaling pathway, with the improvement of renal function and

Table 2 **Studies involving sulforaphane supplementation and its effects on models of renal injury and disease**

References	Sample/design	Intervention	Results
Guerrero-Beltrán et al (2010)[198]	In vivo: Wistar rats, cisplatin-induced nephrotoxicity In vitro: Porcine renal epithelial cells (LLC-PK1 cells), CDDP-induced cell death	In vivo: SFN (500 μg/kg i.v.) twice (before and after CDDP-injection) In vitro: Preincubation of LLC-PK1 cells with 0.5–5 μM SFN by 24 h	In vivo: ↓renal dysfunction, ↓structural damage, oxidative/nitrosative stress, GSH depletion, urinary H_2O_2 excretion ↑CAT, GPX, GST In vitro: ↓CDDP-induced cell death ↑Nuclear Nrf2 translocation
Zheng et al (2011)[199]	Mice with streptozotocin-induced diabetic nephropathy	12.5 mg/kg SFN 3×/wk for 16 wk	Restored normal morphology of glomeruli ↓Albuminuria, 8-oxo-dG ↑Nrf2, NQO1, γ-GCS
Senanayake et al (2012)[200]	Spontaneously hypertensive stroke-prone rats	10 μmol/kg BW of SFN by gavage for 4 mo	↓Blood pressure; ↓protein nitration in kidney ↑γ-glutamyl-cysteine ligase catalytic subunit protein ↑Methylated deoxycytosine levels
Cui et al (2012)[201]	Type 1 diabetic mouse model	0.5 mg/kg SFN daily for 3 mo	Prevented diabetes-induced renal fibrosis, inflammation (PAI-1, TNF- α), and oxidative stress (3-nitrotyrosine, 4-hydroxy-2-nonenal)
Guerrero-Beltrán et al (2012)[202]	Male Wistar rat, cisplatin-induced nephropathy	SFN 500 μg/kg via jugular vein two times (24 h before and 24 h after 7.5 mg/kg cisplatin injection)	↓NF-κB p53 ↓TNF-α levels, NF-κB activation in kidney ↓ICAM-1, VCAM-1
González-Guerrero et al (2013)[203]	MCT proximal tubular epithelial cells, calcineurin inhibitors induce inflammatory responses	SFN 20 μM	↑Nrf2 and HO-1 levels ↓IκBα phosphorylation/activation ↓MCP1, Rantes, and IL-6 mRNA synthesis
Cekauskas et al (2013)[204]	Male Brown Norway rats, kidney injury after transplantation model	D, L-sulforaphane 4.4 mg/kg BW i.v. to the recipients 24 and 1 h before, and 6 h after transplantation	↓Cr and urea ↓Tubular damage Preserved mitochondrial microstructure ↑SOD 2 expression
Ryoo et al (2014)[205]	Human kidney tubular epithelial HK2 cell line	SFN 5 μM	↑GSH reductase, GSH ↓ROS; ↓fibronectin-1, and collagen 1A1
Saito et al (2014)[206]	Male Sprague Dawley (SD) rats, ischemia/reperfusion-induced AKI	SFN (5 mg/kg) orally administered to rats 24 and 1 h before and 24 h after renal I/R treatment	↓Cr and urea ↓IS levels ↑Nrf2 expression in kidney
Shang et al (2015)[207]	Male Sprague–Dawley rats, diabetic nephropathy streptozotocin induced	SFN at 5 mg/kg BW daily for 12 wk, i.p. injection	↓Renal injury (Cr level, ratio of urine albumin/urine creatinine) ↓8-oxo-dG; ↓TGF-β1, collagen IV and fibronectin mRNA ↑NQO1, HO-1 mRNA levels
Shokeir et al (2015)[208]	Sprague–Dawley rats, renal ischemia model	500 μg/BW kg i.v. SFN was administered to the rats 1 h before clamping the renal pedicle	↓Cr and urea ↑Nrf2, HO-1, NQO-1 expression ↓TNF-α, IL-1, ICAM-1, caspase–3, MDA ↑Levels of GSH, SOD activities
Wu et al (2015)[209]	Type 2 diabetes-induced diabetic nephropathy rats	0.5 mg/kg SFN 5 d/wk for 4 mo	↓Urinary albumin-to-creatinine ratio ↓Renal hypertrophy ↑Nrf2 protein and NQO-1 expressions
Ebihara et al (2016)[210]	Human renal mesangial cells, TNF-α-stimulated	10 μM SFN	↑HO-1, NQO1; ↑Nrf2 activation ↓MCP-1 and IL-6; ↓NF-κB
Zhao et al (2016)[211]	Adult Sprague–Dawley rats, contrast-induced nephropathy	5 mg/kg SFN for consecutive 5 d	↓Renal histological damage; ↓Cr and urea; ↓MDA levels in renal tissues ↑SOD levels in renal tissues ↑Nrf2, NQO-1, and HO-1 mRNA expressions
Zhang et al (2016)[212]	Rat vascular smooth-muscle cell, vascular calcification in end-stage renal disease model	5 μM SFN pretreatment	↑Nrf-2 expression in nuclear ↓ROS production
Atilano-Roque et al (2016)[213]	Human proximal tubule cells and human embryonic kidney 293 cells, cisplatin-induced	SFN 5 μM, pretreatment	↑GCLC, NQO1 mRNA expressions

(continued)

Table 2 **Continued**

References	Sample/design	Intervention	Results
Lv et al (2018)[214]	F344 rat kidneys were orthotopically transplanted into Lewis rat, chronic renal allograft dysfunction models	1.5 mg/kg SFN i.p. once daily for 24 wk	↓MDA, 8-isoprostane, ox-LDL and 8-OHdG ↑SOD, CAT, GPx, GR, and γ-GCS ↑Levels of Nrf2, HO-1, and NQO-1
Briones-Herrera et al (2018)[215]	AKI rat model	1 mg/kg of SFN each day for 4 ds before AKI induction	↓Proteinuria; ↓MDA and 4-hydroxy-nonenal ↓Mitochondrial H_2O_2 generation Prevented alterations in mitochondrial bioenergetics
Shin et al (2019)[216]	Human proximal renal tubule cell, HG-induced EMT model	SFN 0.63 μm for 1 h prior to D-glucose stimulation	↑Nrf2-HO-1 protein levels ↓ROS, HG-induced attenuation in EMT markers
Kim et al (2019)[217]	Human renal tubule cells HK-2, HG model on autophagy	1.25 μm SFN 1 h before D-glucose stimulation	Modulates autophagy and ↓apoptosis; ↓ROS levels ↑HO-1 ↓Protein 1 LC3, and beclin-1
Rubio-Navarro et al (2019)[218]	In vivo: WT C57BL/6 mice, intravascular hemolysis–mediated AKI model In vitro: Proximal MCT cells, Hb/heme stimulated	In vivo: SFN (12.5 mg/ kg BW) i.p. 48, 24, and 2 h before phenylhydrazine injection In vitro: SFN, 2 μM pretreatment	In vivo: ↑Nrf2 phosphorylation and HO-1 in kidney Improved renal function ↓Tubular injury markers; ↓oxidative stress In vitro: ↓ROS production, H_2O_2, mitochondrial superoxide, total superoxide ↑mRNA and protein HO-1 expression, GSH
Khaleel et al (2019)[219]	Adult male Wistar rats, streptozotocin-induced diabetes and CIN	SFN 3 mg/kg, i.p., pretreatment	In CIN rat: ↓Cr and urea restored histopathological features ↓Renal MDA and 8-OHdG levels ↑Nrf2 protein levels; ↑HO-1 mRNA ↓IL-6, caspase 3 expression level
Thangapandiyan et al (2019)[220]	Male albino Wistar rats, arsenic-induced renal damage	SFN (80 mg/kg BW) daily for 28 d	↓ROS, OHdG, lipid peroxidation, DNA damage ↓Urea, uric acid, Cr, and bilirubin; ↓ICAM ↑ SOD, CAT, GPx, GST, and GR
Briones-Herrera et al (2020)[154]	AKI rat model	1 mg/kg of SFN each day for 4 d before AKI induction	↓Proteinuria; ↓4-hydroxy-nonenal ↑Nrf1, Nrf2, PGC1α, and TFAM
Gigliotti et al (2020)221	Gstm1 knockout mouse line, subtotal nephrectomy–induced CKD model	SFN-rich broccoli powder mixed with powdered chow at a 1:1 ratio	In vivo (animal) ↓Renal levels of superoxide, albumin-to-Cr ratio, kidney histopathology scores

Abbreviations: 8-oxo-dG, 8-oxo-deoxyguanosine; AKI, acute kidney injury; BW, body weight; CAT, catalase; CKD, chronic kidney disease; Cr, creatinine; CIN, contrast-induced nephropathy; EMT, epithelial-to-mesenchymal transition; GCLC, GCLC, glutamate-cysteine ligase; GSH, glutathione; GPX, glutathione peroxidase; GST, glutathione-*S*-transferase; H_2O_2, hydrogen peroxide; Hb, hemoglobin; HG, high glucose; HO-1, heme oxygenase 1; IL, interleukin; IS, indoxyl sulfate; i.p., intraperitoneally; i.v., intravenous; LC3, light-chain 3; MCT, murine cortical tubular renal epithelial cell; MDA, malondialdehyde; mRNA, messenger RNA; NF-κB, nuclear transcription factor-κB; Nrf2, nuclear factor erythroid 2–related factor 2; NQO1, NAD(P)H quinone oxidoreductase 1; OHdG, hydroxydeoxyguanosine; ROS, reactive oxygen species; SFN, sulforaphane; SOD, superoxide dismutase; TFAM, mitochondrial transcription factor-A; TNF-α, tumor necrosis factor-α.

prevention of fibrosis and tubular atrophy.[207,214,216,217,219]

Current evidence suggests an essential role Nrf2 renal expression in SFN action to prevent renal damage and that the most critical effect of SFN on chemical or ischemia-induced renal damage is exerted by the induction of Nrf2.[224–226] Sulforaphane also reduces ROS production and increases cytoprotective enzymes, quinone oxidoreductase 1 (NQO1), and γ-glutamyl cysteine ligase. Consistent with its role as a cytoprotectant, SFN improved renal function, increased Nrf2 expression, and reduced inflammation and the expression of apoptotic markers in an animal model of renal injury. Moreover, animals receiving SFN had a significant increase in glutathione and SOD activities, with a decrease in malondialdehyde levels in renal tissues.[70,208] SFN-mediated renoprotection was abolished in diabetic Nrf2-null mice, confirming a central role for Nrf2 in

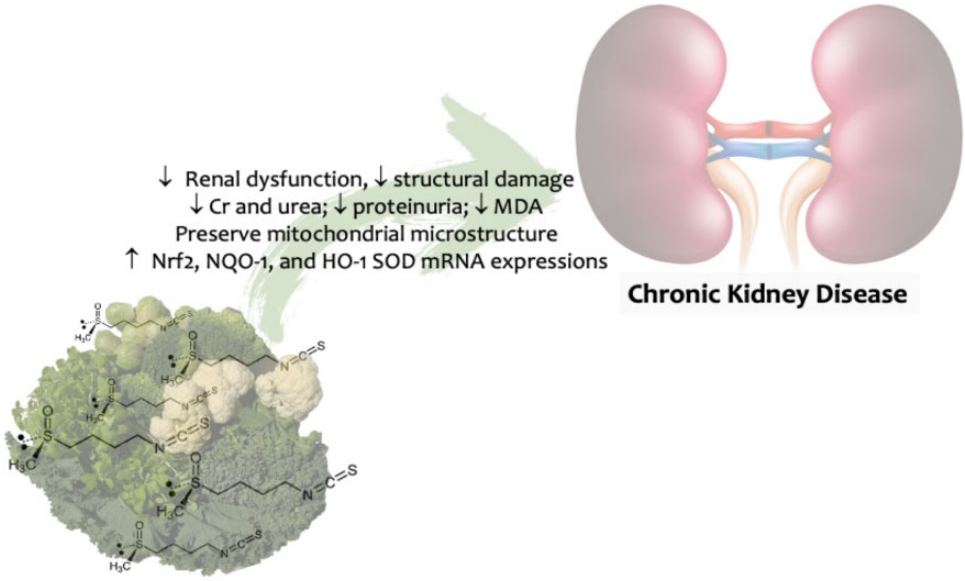

Figure 4 **Possible effects of sulforaphane in chronic kidney disease**. Sulforaphane seems to prevent structural damage in the kidney and reduce renal dysfunction, including proteinuria by mitigating inflammation; increasing nuclear factor erythroid 2–related factor 2 (Nrf2), NAD(P)H quinone oxidoreductase 1 (NQO-1), heme oxygenase 1 (HO-1) and superoxide dismutase (SOD) messenger RNA expression; and reducing oxidative stress. *Abbreviations:* Cr, creatinine; MDA, malondialdehyde.

SFN-mediated benefits.[199,209] In maleic acid–induced nephropathy, SFN has been reported to induce avoidance of the decrease in fatty acid–related oxygen consumption rate, oxidative phosphorylation on proximal kidney tubule, and mitochondrial membrane potential, with consequent better control of the respiratory index and decreased mitochondrial production of H_2O_2.[215]

Cekauskas et al[204] have shown that SFN can decrease kidney injury in transplanted rats. Sulforaphane decreased reperfusion damage in the kidney and decreased serum urea nitrogen and creatinine serum levels. Moreover, mitochondrial microstructure was preserved and there was an increase in *SOD2* gene expression.

To our knowledge, no clinical studies have explored the effects of SFN supplementation in CKD. However, Gigliotti et al[221] showed that supplementation with broccoli powder improved kidney injury in a glutathione *S*-transferase m-1 (GSTM1) knockout-mice CKD model. Findings from the African American Study of Kidney Disease and Hypertension Trial and the Atherosclerosis Risk in Communities study suggest that deletion of GSTM1 (part of the superfamily of phase 2 antioxidant enzymes) is linked to CKD progression.[221] This could provide a mechanism whereby high vs low consumption of cruciferous vegetables is associated with fewer kidney failure events and suggests an effect of protective metabolites from dietary intake when there is GSTM1 deficiency.[227] It seems conceivable that SFN can activate the Nrf2 signaling pathway directly and induce phase-2 detoxification enzymes indirectly.[221]

Taken together, study findings indicate SFN could act in several pathways in renal injuries, especially in ameliorating inflammation and oxidative stress (Figure 4). Sulforaphane may represent an alternative strategy for improving the prognosis of patients with CKD by preventing progression of CKD and targeting common complications such as CVD in this patient population. More studies on the effects of SFN in CKD are warranted.

CONCLUSION

Sulforaphane is an important bioactive compound present in cruciferous vegetables. Throughout scientific studies in different diseases, several beneficial functions of the SFN have been observed in chronic noncommunicable diseases. An extensive literature has shown that the main route of action of SFN is by its antioxidant potential and activation of the transcription factor Nrf2, which has a key role in the antioxidant response. In addition, SFN acts as a geroprotectant, modulates the epigenetic landscape, protects against mitochondrial damage, and helps maintain a normative gut microbiota, thus suggesting a promising role for SFN in the control of several diseases.

In this context, patients with CKD, a disease characterized by inflammation, oxidative stress, gut dysbiosis, mitochondrial dysfunction, and an altered epigenetic machinery, may be an ideal patient group for using food as medicine as a novel treatment strategy. Although there are no clinical studies demonstrating an

effect of SFN in CKD, findings of studies in other patient groups suggest SFN could be a promising adjunctive therapy also in CKD. Notably, SFN therapy has already been shown to improve renal function in a range of preclinical models of renal damage. Clinical studies with patients with CKD using SFN should thus be encouraged to promote improvement in patients' quality of life.

Acknowledgments

We thank Karla T.R. Teixeira for her excellent graphic work and pictures in Figure 2.

Funding. D.M. is supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro. P.S. is supported by the Heart and Lung Foundation, Karolinska Institutet Diabetic Theme Centre, INTRICARE, CaReSyAn, and Njurfonden. Baxter Novum is the result of a grant from Baxter Healthcare to Karolinska Institutet.

Author contributions. L.F.M.F., P.G.S., B.K., P.S., and D.M. coordinated all the steps of review and contributed to the data interpretation and restructuring and revision of the manuscript. L.A.A., M.R., and L.D. contributed to writing and revision of the manuscript.

Declaration of interests. P.G.S. is funded by Industrial PhD Partnership awards, 4D Phrma (United Kingdom), and Constant Pharma (United States). The other authors have no conflicts of interest to declare.

Abbreviations

ATP	adenosine triphosphate;
CKD	chronic kidney disease;
DNMT	DNA methyltransferase;
GLS	glucosinolate;
GSTM1	glutathione S-transferase m-1;
HO-1	heme oxygenase 1;
H2O2	hydrogen peroxide;
HDAC	histone deacetylase;
HTERT	telomerase reverse transcriptase;
Keap1	Kelch-like ECH-associated protein 1;
miRNA	mitochondrial RNA;
NQO1	NAD(P)H quinone oxidoreductase 1;
Nrf2	nuclear factor-erythroid 2-related factor 2;
NF-κB	nuclear factor-κB;
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator 1-α;
PTEN	phosphatase and tensin homolog;
RARβ2	retinoic acid receptor β 2;
ROS	reactive oxygen species;
SFN	sulforaphane;
TFAM	mitochondrial transcription factor-A

REFERENCES

1. Chang YW, Jang JY, Kim YH, et al. The effects of broccoli sprout extract containing sulforaphane on lipid peroxidation and *Helicobacter pylori* infection in the gastric mucosa. *Gut Liver*. 2015;9:486–493.
2. Charron CS, Vinyard BT, Ross SA, et al. Absorption and metabolism of isothiocyanates formed from broccoli glucosinolates: effects of BMI and daily consumption in a randomised clinical trial. *Br J Nutr*. 2018;120:1370–1379.
3. Chartoumpekis DV, Ziros PG, Chen JG, et al. Broccoli sprout beverage is safe for thyroid hormonal and autoimmune status: results of a 12-week randomized trial. *Food Chem Toxicol*. 2019;126:1–6.
4. Wang X, Ouyang Y, Liu J, et al. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ*. 2014;349:g4490.
5. Riboli E, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr*. 2003;78:559–569.
6. Vanduchova A, Anzenbacher P, Anzenbacherova E. Isothiocyanate from broccoli, sulforaphane, and its properties. *J Med Food*. 2019;22:121–126.
7. Houghton CA. Sulforaphane: its "coming of age" as a clinically relevant nutraceutical in the prevention and treatment of chronic disease. *Oxid Med Cell Longev*. 2019;2019:1–27.
8. Wang M, Pu D, Zhao Y, et al. Sulforaphane protects against skeletal muscle dysfunction in spontaneous type 2 diabetic db/db mice. *Life Sci*. 2020;255:117823.
9. Liu H, Zimmerman AW, Singh K, et al. Biomarker exploration in human peripheral blood mononuclear cells for monitoring sulforaphane treatment responses in autism spectrum disorder. *Sci Rep*. 2020;10:5822.
10. Calcabrini C, Maffei F, Turrini E, et al. Sulforaphane potentiates anticancer effects of doxorubicin and cisplatin and mitigates their toxic effects. *Front Pharmacol*. 2020;11:doi: 10.3389/fphar.2020.00567.
11. Hyun TK. A recent overview on sulforaphane as a dietary epigenetic modulator. *Excli J*. 2020;19:131–134.
12. Mitsiogianni M, Trafalis DT, Franco R, et al. Sulforaphane and iberin are potent epigenetic modulators of histone acetylation and methylation in malignant melanoma. *Eur J Nutr*. 2020; online doi: 10.1007/s00394-020-02227-y.
13. Abbaoui B, Telu KH, Lucas CR, et al. The impact of cruciferous vegetable isothiocyanates on histone acetylation and histone phosphorylation in bladder cancer. *J Proteomics*. 2017;156:94–103.
14. Paunkov A, Chartoumpekis DV, Ziros PG, et al. A bibliometric review of the Keap1/Nrf2 pathway and its related antioxidant compounds. *Antioxidants*. 2019;8:353.
15. Lin CY, Yao CA. Potential role of NRF2 activators with dual antiviral and anti-inflammatory properties in the management of viral pneumonia. *Infect Drug Resist*. 2020;3:1735–1741.
16. Müller L, Meyer M, Bauer RN, et al. Effect of broccoli sprouts and live attenuated influenza virus on peripheral blood natural killer cells: a randomized, double-blind study. *PLoS One*. 2016;11:E0147742.
17. Myzak MC, Karplus PA, Chung F-L, et al. A novel mechanism of chemoprotection by sulforaphane. *Cancer Res*. 2004;64:5767–5774.
18. Kensler TW, Chen JG, Egner PA, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo Township, Qidong, People's Republic of China. *Cancer Epidemiol Biomarkers Prev*. 2005;14:2605–2613.
19. Fimognari C, Hrelia P. Sulforaphane as a promising molecule for fighting cancer. *Mutat Res Rev Mutat Res*. 2007;635:90–104.
20. Lenzi M, Fimognari C, Hrelia P. Sulforaphane as a promising molecule for fighting cancer. *Cancer Treat Res*. 2014;159:207–223.
21. Yagishita Y, Fahey JW, Dinkova-Kostova AT, et al. Broccoli or sulforaphane: is it the source or dose that matters? *Molecules*. 2019;24:3593.
22. Martins T, Colaço B, Venâncio C, et al. Potential effects of sulforaphane to fight obesity. *J Sci Food Agric*. 2018;98:2837–2844.
23. Bai Y, Wang X, Zhao S, et al. Sulforaphane protects against cardiovascular disease via Nrf2 activation. *Oxid Med Cell Longev*. 2015;2015:1–13.
24. Klomparens E, Ding Y. The neuroprotective mechanisms and effects of sulforaphane. *Brain Circ*. 2019;5:74–83.
25. Nakagawa K, Umeda T, Higuchi O, et al. Evaporative light-scattering analysis of sulforaphane in broccoli samples: quality of broccoli products regarding sulforaphane contents. *J Agric Food Chem*. 2006;54:2479–2483.
26. Shapiro TA, Fahey JW, Wade KL, et al. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. *Cancer Epidemiol Biomarkers Prev*. 1998;7:1091–1100.
27. Gu Z-X, Guo G-H, Gu Y-J. Factors influencing glucoraphanin and sulforaphane formation in *Brassica* plants: a review. *J Integr Agric*. 2012;11:1804–1816.

28. Cieslik E, Leszczynska T, Filipiakflorkiewicz A, et al. Effects of some technological processes on glucosinolate contents in cruciferous vegetables. *Food Chem*. 2007;105:976–981.
29. Maldini M, Baima S, Morelli G, et al. A liquid chromatography-mass spectrometry approach to study "glucosinoloma" in broccoli sprouts. *J Mass Spectrom*. 2012;47:1198–1206.
30. Van Eylen D, Oey I, Hendrickx M, et al. Kinetics of the stability of broccoli (*Brassica oleracea* cv. Italica) myrosinase and isothiocyanates in broccoli juice during pressure/temperature treatments. *J Agric Food Chem*. 2007;55:2163–2170.
31. Song L, Thornalley PJ. Effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables. *Food Chem Toxicol*. 2007;45:216–224.
32. Lu Y, Pang X, Yang T. Microwave cooking increases sulforaphane level in broccoli. *Food Sci Nutr*. 2020;8:2052–2058.
33. Kim YJ, Lee DH, Ahn J, et al. Pharmacokinetics, tissue distribution, and antilipogenic/adipogenic effects of allyl-isothiocyanate metabolites. *PLoS One*. 2015;10: E 0132151.
34. Oliviero T, Verkerk R, Dekker M. Isothiocyanates from *Brassica* vegetables—effects of processing, cooking, mastication, and digestion. *Mol Nutr Food Res*. 2018;62:E1701069.
35. Cramer JM, Jeffery EH. Sulforaphane absorption and excretion following ingestion of a semi-purified broccoli powder rich in glucoraphanin and broccoli sprouts in healthy men. *Nutr Cancer*. 2011;63:196–201.
36. Cramer JM, Teran-Garcia M, Jeffery EH. Enhancing sulforaphane absorption and excretion in healthy men through the combined consumption of fresh broccoli sprouts and a glucoraphanin-rich powder. *Br J Nutr*. 2012;107:1333–1338.
37. Atwell LL, Hsu A, Wong CP, et al. Absorption and chemopreventive targets of sulforaphane in humans following consumption of broccoli sprouts or a myrosinase-treated broccoli sprout extract. *Mol Nutr Food Res*. 2015;59:424–433.
38. Vermeulen M, Klöpping-Ketelaars IWAA, van den Berg R, et al. Bioavailability and kinetics of sulforaphane in humans after consumption of cooked versus raw broccoli. *J Agric Food Chem*. 2008;56:10505–10509.
39. Hanlon N, Coldham N, Gielbert A, et al. Repeated intake of broccoli does not lead to higher plasma levels of sulforaphane in human volunteers. *Cancer Lett*. 2009;284:15–20.
40. Shapiro TA, Fahey JW, Dinkova-Kostova AT, et al. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study. *Nutr Cancer*. 2006;55:53–62.
41. Axelsson AS, Tubbs E, Mecham B, et al. Sulforaphane reduces hepatic glucose production and improves glucose control in patients with type 2 diabetes. *Sci Transl Med*. 2017;9:Eaah4477.
42. Zanichelli F, Capasso S, Di Bernardo G, et al. Low concentrations of isothiocyanates protect mesenchymal stem cells from oxidative injuries, while high concentrations exacerbate DNA damage. *Apoptosis*. 2012;17:964–974.
43. Kubo E, Chhunchha B, Singh P, et al. Sulforaphane reactivates cellular antioxidant defense by inducing Nrf2/ARE/Prdx6 activity during aging and oxidative stress. *Sci Rep*. 2017;7:1–17.
44. Suzuki T, Motohashi H, Yamamoto M. Toward clinical application of the Keap1-Nrf2 pathway. *Trends Pharmacol Sci*. 2013;34:340–346.
45. Kaspar JW, Niture SK, Jaiswal AK. Nrf2: INrf2 (Keap1) signaling in oxidative stress. *Free Radic Biol Med*. 2009;47:1304–1309.
46. Pedruzzi LM, Stockler-Pinto MB, Leite M, et al. Nrf2-keap1 system versus NF-κB: the good and the evil in chronic kidney disease? *Biochimie*. 2012;94:2461–2466.
47. McMahon M, Itoh K, Yamamoto M, et al. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J Biol Chem*. 2003;278:21592–21600.
48. Dinkova-Kostova AT, Fahey JW, Kostov RV, et al. KEAP1 and done? Targeting the NRF2 pathway with sulforaphane. *Trends Food Sci Technol*. 2017;69:257–269.
49. Kensler TW, Egner PA, Agyeman AS, et al. Keap1-Nrf2 signaling: a target for cancer prevention by sulforaphane. *Top Curr Chem*. 2013;329:163–177.
50. Takaya K, Suzuki T, Motohashi H, et al. Validation of the multiple sensor mechanism of the Keap1-Nrf2 system. *Free Radic Biol Med*. 2012;53:817–827.
51. Lee S, Hu L. Nrf2 activation through the inhibition of Keap1–Nrf2 protein–protein interaction. *Med Chem Res*. 2020;29:846–867.
52. Ma Q. Role of Nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol*. 2013;53:401–426.
53. Kobayashi EH, Suzuki T, Funayama R, et al. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. *Nat Commun*. 2016;7:11624.
54. Antunes F, Han D. Redox regulation of NF-κB: from basic to clinical research. *Antioxidants Redox Signal*. 2009;11:2055–2056.
55. Kim J, Cha YN, Surh YJ. A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders. *Mutat Res Fundam Mol Mech Mutagen*. 2010;690:12–23.
56. Angeloni C, Leoncini E, Malaguti M, et al. Modulation of phase II enzymes by sulforaphane: implications for its cardioprotective potential. *J Agric Food Chem*. 2009;57:5615–5622.
57. Leoncini E, Malaguti M, Angeloni C, et al. Cruciferous vegetable phytochemical sulforaphane affects phase II enzyme expression and activity in rat cardiomyocytes through modulation of Akt signaling pathway. *J Food Sci*. 2011;76:H175–81.
58. Rakariyatham K, Wu X, Tang Z, et al. Synergism between luteolin and sulforaphane in anti-inflammation. *Food Funct*. 2018;9:5115–5123.
59. Liu Y, Liu P, Wang Q, et al. Sulforaphane attenuates H 2 O 2 -induced oxidant stress in human trabecular meshwork cells (HTMCs) via the phosphatidylinositol 3-kinase (PI3K)/serine/threonine kinase (Akt)-mediated factor-e2-related factor 2 (Nrf2) signaling activation. *Med Sci Monit*. 2019;25:811–818.
60. Huang CS, Lin AH, Liu CT, et al. Isothiocyanates protect against oxidized LDL-induced endothelial dysfunction by upregulating Nrf2-dependent antioxidation and suppressing NFκB activation. *Mol Nutr Food Res*. 2013;57:1918–1930.
61. Townsend BE, Johnson RW. Sulforaphane induces Nrf2 target genes and attenuates inflammatory gene expression in microglia from brain of young adult and aged mice. *Exp Gerontol*. 2016;73:42–48.
62. Navarro SL, Schwarz Y, Song X, et al. Cruciferous vegetables have variable effects on biomarkers of systemic inflammation in a randomized controlled trial in healthy young adults. *J Nutr*. 2014;144:1850–1857.
63. Giacoppo S, Galuppo M, Iori R, et al. Protective role of (RS)-glucoraphanin bioactivated with myrosinase in an experimental model of multiple sclerosis. *CNS Neurosci Ther*. 2013;19:577–584.
64. Matsui T, Nakamura N, Ojima A, et al. Sulforaphane reduces advanced glycation end products (AGEs)-induced inflammation in endothelial cells and rat aorta. *Nutr Metab Cardiovasc Dis*. 2016;26:797–807.
65. Lee J, Ahn H, Hong EJ, et al. Sulforaphane attenuates activation of NLRP3 and NLRC4 inflammasomes but not AIM2 inflammasome. *Cell Immunol*. 2016;306–307:53–60.
66. Qin W, Sen Deng YH, Cui FC. Sulforaphane protects against acrolein-induced oxidative stress and inflammatory responses: modulation of Nrf-2 and COX-2 expression. *Arch Med Sci*. 2016;4:871–880.
67. Carrasco-Pozo C, Tan KN, Gotteland M, et al. Sulforaphane protects against high cholesterol-induced mitochondrial bioenergetics impairments, inflammation, and oxidative stress and preserves pancreatic β-cells function. *Oxid Med Cell Longev*. 2017;2017:1–14.
68. de Oliveira MR, de Bittencourt Brasil F, Fürstenau CR. Sulforaphane promotes mitochondrial protection in SH-SY5Y cells exposed to hydrogen peroxide by an Nrf2-dependent mechanism. *Mol Neurobiol*. 2018;55:4777–4787.
69. de Oliveira MR, Brasil FB, Fürstenau CR. Sulforaphane attenuated the pro-inflammatory state induced by hydrogen peroxide in SH-SY5Y cells through the Nrf2/HO-1 signaling pathway. *Neurotox Res*. 2018;34:241–249.
70. Zhao F, Zhang J, Chang N. Epigenetic modification of Nrf2 by sulforaphane increases the antioxidative and anti-inflammatory capacity in a cellular model of Alzheimer's disease. *Eur J Pharmacol*. 2018;824:1–10.
71. Qin S, Yang C, Huang W, et al. Sulforaphane attenuates microglia-mediated neuronal necroptosis through down-regulation of MAPK/NF-κB signaling pathways in LPS-activated BV-2 microglia. *Pharmacol Res*. 2018;133:218–235.
72. Eren E, Tufekci KU, Isci KB, et al. Sulforaphane inhibits lipopolysaccharide-induced inflammation, cytotoxicity, oxidative stress, and miR-155 expression and switches to Mox phenotype through activating extracellular signal-regulated kinase 1/2-nuclear factor erythroid 2-related factor 2/an. *Front Immunol*. 2018;9: doi: 10.3389/fimmu.2018.00036
73. Cox AG, Gurusinghe S, Abd Rahman R, et al. Sulforaphane improves endothelial function and reduces placental oxidative stress in vitro. *Pregnancy Hypertens*. 2019;16:1–10.
74. Haodang L, Lianmei Q, Ranhui L, et al. HO-1 mediates the anti-inflammatory actions of sulforaphane in monocytes stimulated with a mycoplasmal lipopeptide. *Chem Biol Interact*. 2019;306:10–18.
75. Subedi L, Lee J, Yumnam S, et al. Anti-inflammatory effect of sulforaphane on LPS-activated microglia potentially through JNK/AP-1/NF-κB inhibition and Nrf2/HO-1 activation. *Cells*. 2019;8:194.
76. Chang HY, Lin CW, Yang CM, et al. Nrf-2 activator sulforaphane protects retinal cells from oxidative stress-induced retinal injury. *J Funct Foods*. 2020;71:104023.
77. Yang J, Song X, Feng Y, et al. Natural ingredients-derived antioxidants attenuate H2O2-induced oxidative stress and have chondroprotective effects on human osteoarthritic chondrocytes via Keap1/Nrf2 pathway. *Free Radic Biol Med*. 2020;152:854–864.
78. Ali M, Bonay M, Vanhee V, et al. Comparative effectiveness of 4 natural and chemical activators of Nrf2 on inflammation, oxidative stress, macrophage polarization, and bactericidal activity in an in vitro macrophage infection model. *PLoS One*. 2020;15:E0234484.
79. Deramaudt TB, Ali M, Vinit S, et al. Sulforaphane reduces intracellular survival of Staphylococcus aureus in macrophages through inhibition of JNK and p38 MAPK-induced inflammation. *Int J Mol Med*. 2020;45:1927–1941.
80. Nadeem A, Ahmad SF, AL-Ayadhi LY, et al. Differential regulation of Nrf2 is linked to elevated inflammation and nitrative stress in monocytes of children with autism. *Psychoneuroendocrinology*. 2020;113:104554.
81. Dong Z, Shang H, Chen YQ, et al. Sulforaphane protects pancreatic acinar cell injury by modulating Nrf2-mediated oxidative stress and NLRP3 inflammatory pathway. *Oxid Med Cell Longev*. 2016;2016:7864150.

82. Zhao X, Wen L, Dong M, et al. Sulforaphane activates the cerebral vascular Nrf2–ARE pathway and suppresses inflammation to attenuate cerebral vasospasm in rat with subarachnoid hemorrhage. *Brain Res*. 2016;1653:1–7.
83. Xu Z, Wang S, Ji H, et al. Broccoli sprout extract prevents diabetic cardiomyopathy via Nrf2 activation in db/db T2DM mice. *Sci Rep*. 2016;6:30252.
84. Holloway PM, Gillespie S, Becker F, et al. Sulforaphane induces neurovascular protection against a systemic inflammatory challenge via both Nrf2-dependent and independent pathways. *Vascul Pharmacol*. 2016;85:29–38.
85. Townsend BE, Johnson RW. Sulforaphane reduces lipopolysaccharide-induced proinflammatory markers in hippocampus and liver but does not improve sickness behavior. *Nutr Neurosci*. 2017;20:195–202.
86. Yu C, He Q, Zheng J, et al. Sulforaphane improves outcomes and slows cerebral ischemic/reperfusion injury via inhibition of NLRP3 inflammasome activation in rats. *Int Immunopharmacol*. 2017;45:74–78.
87. Yan B, Ma Z, Shi S, et al. Sulforaphane prevents bleomycin-induced pulmonary fibrosis in mice by inhibiting oxidative stress via nuclear factor erythroid 2-related factor-2 activation. *Mol Med Rep*. 2017;15:4005–4014.
88. Wang Y, Wu H, Xin Y, et al. Sulforaphane prevents angiotensin II-induced testicular cell death via activation of NRF2. *Oxid Med Cell Longev*. 2017;2017:1–12.
89. Bai Y, Chen Q, Sun YP, et al. Sulforaphane protection against the development of doxorubicin-induced chronic heart failure is associated with Nrf2 Upregulation. *Cardiovasc Ther*. 2017;35:E12277.
90. Xin Y, Bai Y, Jiang X, et al. Sulforaphane prevents angiotensin II-induced cardiomyopathy by activation of Nrf2 via stimulating the Akt/GSK-3ß/Fyn pathway. *Redox Biol*. 2018;15:405–417.
91. Pu D, Zhao Y, Chen J, et al. Protective effects of sulforaphane on cognitive impairments and AD-like lesions in diabetic mice are associated with the upregulation of Nrf2 transcription activity. *Neuroscience*. 2018;381:35–45.
92. Moustafa PE, Abdelkader NF, El Awdan SA, et al. Extracellular matrix remodeling and modulation of inflammation and oxidative stress by sulforaphane in experimental diabetic peripheral neuropathy. *Inflammation*. 2018;41:1460–1476.
93. Ma T, Zhu D, Chen D, et al. Sulforaphane, a natural isothiocyanate compound, improves cardiac function and remodeling by inhibiting oxidative stress and inflammation in a rabbit model of chronic heart failure. *Med Sci Monit*. 2018;24:1473–1483.
94. Yang G, Yeon SH, Lee HE, et al. Suppression of NLRP3 inflammasome by oral treatment with sulforaphane alleviates acute gouty inflammation. *Rheumatology*. 2018;57:727–736.
95. Gong Y, Cao X, Gong L, et al. Sulforaphane alleviates retinal ganglion cell death and inflammation by suppressing nlrp3 inflammasome activation in a rat model of retinal ischemia/reperfusion injury. *Int J Immunopathol Pharmacol*. 2019;33:205873841986177.
96. Saleh DO, Mansour DF, Hashad IM, et al. Effects of sulforaphane on D-galactose-induced liver aging in rats: role of keap-1/nrf-2 pathway. *Eur J Pharmacol*. 2019;855:40–49
97. Nadeem A, Ahmad S F, Al-Harbi N O, et al.. Nrf2 activator, sulforaphane ameliorates autism-like symptoms through suppression of Th17 related signaling and rectification of oxidant-antioxidant imbalance in periphery and brain of BTBR T+tf/J mice. *Behavioural Brain Research*. 2019;364:213–224. 10.1016/j.bbr.2019.02.031
98. Silva-Palacios A, Ostolga-Chavarría M, Sánchez-Garibay C, et al. Sulforaphane protects from myocardial ischemia-reperfusion damage through the balanced activation of Nrf2/AhR. *Free Radic Biol Med*. 2019;143:331–340.
99. Angulo J, El Assar M, Sevilleja-Ortiz A, et al. Short-term pharmacological activation of Nrf2 ameliorates vascular dysfunction in aged rats and in pathological human vasculature. A potential target for therapeutic intervention. *Redox Biol*. 2019;26:101271.
100. Wu W, Peng G, Yang F, et al. Sulforaphane has a therapeutic effect in an atopic dermatitis murine model and activates the Nrf2/HO-1 axis. *Mol Med Rep*. 2019;20:1761–1771.
101. Wei L, Wang J, Yan L, et al. Sulforaphane attenuates 5-fluorouracil induced intestinal injury in mice. *J Funct Foods*. 2020;69:103965.
102. Liu Y, Zhang Z, Lu X, et al. Anti-nociceptive and anti-inflammatory effects of sulforaphane on sciatic endometriosis in a rat model. *Neurosci Lett*. 2020;723:134858.
103. Sun Y, Zhou S, Guo H, et al. Protective effects of sulforaphane on type 2 diabetes-induced cardiomyopathy via AMPK-mediated activation of lipid metabolic pathways and NRF2 function. *Metabolism*. 2020;102:154002.
104. Ruhee RT, Ma S, Suzuki K. Protective effects of sulforaphane on exercise-induced organ damage via inducing antioxidant defense responses. *Antioxidants*. 2020;9:136.
105. Lee C, Yang S, Lee BS, et al. Hepatic protective effects of sulforaphane through the modulation of inflammatory pathways. *J Asian Nat Prod Res*. 2020;22:386–396.
106. Mirmiran P, Bahadoran Z, Hosseinpanah F, et al. Effects of broccoli sprout with high sulforaphane concentration on inflammatory markers in type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *J Funct Foods*. 2012;4:837–841.
107. Ushida Y, Suganuma H, Yanaka A. Low-dose of the sulforaphane precursor glucoraphanin as a dietary supplement induces chemoprotective enzymes in humans. *Food Nutr Sci*. 2015;06: 1603–1612.
108. López-Chillón MT, Carazo-Díaz C, Prieto-Merino D, et al. Effects of long-term consumption of broccoli sprouts on inflammatory markers in overweight subjects. *Clin Nutr*. 2019;38:745–752.
109. Hennig P, Fenini G, Di Filippo M, et al. Electrophiles against (skin) diseases: more than Nrf2. *Biomolecules*. 2020;10:271.
110. Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. *Cancer Lett*. 2008;269:291–304.
111. Cheung KL, Kong AN. Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. *Aaps J*. 2010;12:87–97.
112. Liu H, Dinkova-Kostova AT, Talalay P. Coordinate regulation of enzyme markers for inflammation and for protection against oxidants and electrophiles. *Proc Natl Acad Sci USA*. 2008;105:15926–15931.
113. Greaney AJ, Maier NK, Leppla SH, et al. Sulforaphane inhibits multiple inflammasomes through an Nrf2-independent mechanism. *J Leukoc Biol*. 2016;99:189–199.
114. Heiss E, Herhaus C, Klimo K, et al. Nuclear factor κB is a molecular target for Sulforaphane-mediated anti-inflammatory mechanisms. *J Biol Chem. Published Online* 2001;276:32008–32015.
115. Checker R, Gambhir L, Thoh M, et al. Sulforaphane, a naturally occurring isothiocyanate, exhibits anti-inflammatory effects by targeting GSK3β/Nrf-2 and NF-κB pathways in T cells. *J Funct Foods*. 2015;19:426–438.
116. Kelley N, Jeltema D, Duan Y, et al. The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *Int J Mol Sci*. 2019;20:3328.
117. Alvarenga L, Cardozo LFMF, Borges NA, et al. Can nutritional interventions modulate the activation of the NLRP3 inflammasome in chronic kidney disease? *Food Res Int*. 2020;136:109306.
118. Mazarakis N, Snibson K, Licciardi PV, et al. The potential use of L-sulforaphane for the treatment of chronic inflammatory diseases: a review of the clinical evidence. *Clin Nutr*. 2020;39:664–675.
119. Tan SM, de Haan JB. Combating oxidative stress in diabetic complications with Nrf2 activators: how much is too much? *Redox Rep*. 2014;19:107–117.
120. Stenvinkel P, Shiels PG. Long-lived animals with negligible senescence: clues for ageing research. *Biochem Soc Trans*. 2019;47:1157–1164.
121. Shiels PG, Buchanan S, Selman C, et al. Allostatic load and ageing: linking the microbiome and nutrition with age-related health. *Biochem Soc Trans*. 2019;47:1165–1172.
122. López-Otín C, Blasco MA, Partridge L, et al. The hallmarks of aging. *Cell*. 2013;153:1194–1217.
123. Franceschi C, Garagnani P, Parini P, et al. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol*. 2018;14:576–590.
124. Santín-Márquez R, Alarcón-Aguilar A, López-Diazguerrero NE, et al. Sulforaphane - role in aging and neurodegeneration. *GeroScience*. 2019;41:655–670.
125. Dai L, Schurgers LJ, Shiels PG, et al. Early vascular ageing in chronic kidney disease: impact of inflammation, vitamin K, senescence and genomic damage. *Nephrol Dial Transplant*. 2020;35:li31–ii37.
126. Zakkar M, Van der Heiden K, Luong LA, et al. Activation of Nrf2 in endothelial cells protects arteries from exhibiting a proinflammatory state. *Arterioscler Thromb Vasc Biol*. 2009;29:1851–1857.
127. Kwon J-S, Joung H, Kim YS, et al. Sulforaphane inhibits restenosis by suppressing inflammation and the proliferation of vascular smooth muscle cells. *Atherosclerosis*. 2012;225:41–49.
128. Jayakumar T, Chen W-F, Lu W-J, et al. A novel antithrombotic effect of sulforaphane via activation of platelet adenylate cyclase: ex vivo and in vivo studies. *J Nutr Biochem*. 2013;24:1086–1095.
129. Liddell J. Are astrocytes the predominant cell type for activation of Nrf2 in aging and neurodegeneration? *Antioxidants*. 2017;6:65.
130. Morroni F, Tarozzi A, Sita G, et al. Neuroprotective effect of sulforaphane in 6-hydroxydopamine-lesioned mouse model of Parkinson's disease. *Neurotoxicology*. 2013;36:63–71.
131. Jang M, Cho I-H. Sulforaphane ameliorates 3-nitropropionic acid-induced striatal toxicity by activating the Keap1-Nrf2-ARE pathway and inhibiting the MAPKs and NF-κB pathways. *Mol Neurobiol*. 2016;53:2619–2635.
132. Li B, Cui W, Liu J, et al. Sulforaphane ameliorates the development of experimental autoimmune encephalomyelitis by antagonizing oxidative stress and Th17-related inflammation in mice. *Exp Neurol*. 2013;250:239–249.
133. Zhang J, Zhang R, Zhan Z, et al. Beneficial effects of sulforaphane treatment in Alzheimer's disease may be mediated through reduced HDAC1/3 and increased P75NTR expression. *Front Aging Neurosci*. 2017;9.
134. Hariton F, Xue M, Rabbani N, et al. Sulforaphane delays fibroblast senescence by curbing cellular glucose uptake, increased glycolysis, and oxidative damage. *Oxid Med Cell Longev*. 2018;2018:1–16.
135. Shiels PG, McGuinness D, Eriksson M, et al. The role of epigenetics in renal ageing. *Nat Rev Nephrol*. 2017;13:471–482.
136. Tortorella SM, Royce SG, Licciardi PV, et al. Dietary sulforaphane in cancer chemoprevention: the role of epigenetic regulation and HDAC inhibition. *Antioxidants Redox Signal*. 2015;22:1382–1424.

137. Gianfredi V, Vannini S, Moretti M, et al. Sulforaphane and epigallocatechin gallate restore estrogen receptor expression by modulating epigenetic events in the breast cancer cell line MDA-MB-231: a systematic review and meta-analysis. *J Nutrigenet Nutrigenomics*. 2017;10:126–135.
138. Kooman JP, Kotanko P, Schols AMWJ, et al. Chronic kidney disease and premature ageing. *Nat Rev Nephrol*. 2014;10:732–742.
139. Watson G, Beaver L, Williams D, et al. Phytochemicals from cruciferous vegetables, epigenetics, and prostate cancer prevention. *Aaps J*. 2013;15:951—961.
140. Biersack B. Non-coding RNA/microRNA-modulatory dietary factors and natural products for improved cancer therapy and prevention: alkaloids, organosulfur compounds, aliphatic carboxylic acids and water-soluble vitamins. *Non-Coding RNA Res*. 2016;1:51–63.
141. Okonkwo A, Mitra J, Johnson GS, et al. Heterocyclic analogs of sulforaphane trigger DNA damage and impede DNA repair in colon cancer cells: interplay of HATs and HDACs. *Mol Nutr Food Res*. 2018;62:E1800228.
142. Lubecka-Pietruszewska K, Kaufman-Szymczyk A, Stefanska B, et al. Sulforaphane alone and in combination with clofarabine epigenetically regulates the expression of DNA methylation-silenced tumour suppressor genes in human breast cancer cells. *J Nutrigenet Nutrigenomics*. 2015;8:91—101.
143. Lewinska A, Adamczyk-Grochala J, Deregowska A, et al. Sulforaphane-induced cell cycle arrest and senescence are accompanied by DNA hypomethylation and changes in microRNA profile in breast cancer cells. *Theranostics*. 2017;7:3461–3477.
144. dos Santos PW, da S, Machado ART, et al. Transcriptome and DNA methylation changes modulated by sulforaphane induce cell cycle arrest, apoptosis, DNA damage, and suppression of proliferation in human liver cancer cells. *Food Chem Toxicol*. 2020;136:111047.
145. Su X, Jiang X, Meng L, et al. Anticancer activity of sulforaphane: the epigenetic mechanisms and the Nrf2 signaling pathway. *Oxid Med Cell Longev*. 2018;2018:5438179.
146. Ishiura Y, Ishimaru H, Watanabe T, et al. Sulforaphane exhibits cytotoxic effects against primary effusion lymphoma cells by suppressing p38MAPK and AkT phosphorylation. *BiolPharmaceut Bull*. 2019;42:2109–2112.
147. Qu X, Pröll M, Neuhoff C, et al. Sulforaphane epigenetically regulates innate immune responses of porcine monocyte-derived dendritic cells induced with lipopolysaccharide. *PLoS One*. 2015;10:E0121574.
148. Abbas A, Hall JA, Patterson WL, et al. Sulforaphane modulates telomerase activity via epigenetic regulation in prostate cancer cell lines1. *Biochem Cell Biol*. 2016;94:71–81.
149. Martin SL, Kala R, Tollefsbol TO. Mechanisms for the inhibition of colon cancer cells by sulforaphane through epigenetic modulation of microRNA-21 and human telomerase reverse transcriptase (hTERT) down-regulation. *Curr Cancer Drug Targets*. 2018;18:97–106.
150. Chen L, Chan LS, Lung HL, et al. Crucifera sulforaphane (SFN) inhibits the growth of nasopharyngeal carcinoma through DNA methyltransferase 1 (DNMT1)/Wnt inhibitory factor 1 (WIF1) axis. *Phytomedicine*. 2019;63:153058.
151. Santana-Gálvez J, Villela-Castrejón J, Serna-Saldívar SO, et al. Synergistic combinations of curcumin, sulforaphane, and dihydrocaffeic acid against human colon cancer cells. *Int J Mol Sci*. 2020;21:3108.
152. Lan H, Yuan H, Lin C. Sulforaphane induces p53-deficient SW480 cell apoptosis via the ROS-MAPK signaling pathway. *Mol Med Rep*. 2017;16: 7796–7804.
153. Bhargava P, Schnellmann RG. Mitochondrial energetics in the kidney. *Nat Rev Nephrol*. 2017;13:629–646.
154. Briones-Herrera A, Ramírez-Camacho I, Zazueta C, et al. Altered proximal tubule fatty acid utilization, mitophagy, fission and supercomplexes arrangement in experimental Fanconi syndrome are ameliorated by sulforaphane-induced mitochondrial biogenesis. *Free Radic Biol Med*. 2020;153:54–70.
155. Palikaras K, Tavernarakis N. Mitochondrial homeostasis: the interplay between mitophagy and mitochondrial biogenesis. *Exp Gerontol*. 2014;56:182–188.
156. Negrette-Guzmán M, Huerta-Yepez S, Vega MI, et al. Sulforaphane induces differential modulation of mitochondrial biogenesis and dynamics in normal cells and tumor cells. *Food Chem Toxicol*. 2017;100:90–102.
157. Zhang M, Xu Y, Jiang L. Sulforaphane attenuates angiotensin II-induced human umbilical vein endothelial cell injury by modulating ROS-mediated mitochondrial signaling. *Hum Exp Toxicol*. 2020;39:734–747.
158. Lin CF, Chueh TH, Chung CH, et al. Sulforaphane improves voiding function via the preserving mitochondrial function in diabetic rats. *J Formos Med Assoc* 2019;119:1422–1430.
159. Chung SD, Lai TY, Chien CT, et al. Activating Nrf-2 signaling depresses unilateral ureteral obstruction-evoked mitochondrial stress-related autophagy, apoptosis and pyroptosis in kidney. *PLoS One*. 2012;7:E47299.
160. Lei P, Tian S, Teng C, et al. Sulforaphane improves lipid metabolism by enhancing mitochondrial function and biogenesis in vivo and in vitro. *Mol Nutr Food Res*. 2019;63:E1800795.
161. Holscher HD, Guetterman HM, Swanson KS, et al. Walnut consumption alters the gastrointestinal microbiota, microbially derived secondary bile acids, and health markers in healthy adults: a randomized controlled trial. *J Nutr*. 2018;148:861–867.
162. Kaczmarek JL, Liu X, Charron CS, et al. Broccoli consumption affects the human gastrointestinal microbiota. *J Nutr Biochem*. 2019;63:27–34.
163. Li F, Hullar MAJ, Schwarz Y, et al. Human gut bacterial communities are altered by addition of cruciferous vegetables to a controlled fruit- and vegetable-free diet. *J Nutr*. 2009;139:1685–1691.
164. Liu X, Wang Y, Hoeflinger JL, et al. Dietary broccoli alters rat cecal microbiota to improve glucoraphanin hydrolysis to bioactive isothiocyanates. *Nutrients*. 2017;9:262.
165. Angelino D, Dosz EB, Sun J, et al. Myrosinase-dependent and –independent formation and control of isothiocyanate products of glucosinolate hydrolysis. *Front Plant Sci*. 2015;6: doi: 10.3389/fpls.2015.00831
166. Humblot C, Bruneau A, Sutren M, et al. Brussels sprouts, inulin and fermented milk alter the faecal microbiota of human microbiota-associated rats as shown by PCR-temporal temperature gradient gel electrophoresis using universal, *Lactobacillus* and *Bifidobacterium* 16S rRNA gene primers. *Br J Nutr*. 2005;93:677–684.
167. Wu Y, Shen Y, Zhu Y, et al. Broccoli ingestion increases the glucosinolate hydrolysis activity of microbiota in the mouse gut. *Int J Food Sci Nutr*. 2019;70:585–594.
168. Brabban AD, Edwards C. Isolation of glucosinolate degrading microorganisms and their potential for reducing the glucosinolate content of rapemeal. *FEMS Microbiol Lett*. 1994;119:83–88.
169. Rizzatti G, Lopetuso LR, Gibiino G, et al. Proteobacteria: a common factor in human diseases. *Biomed Res Int*. 2017;2017:1–7.
170. Earel JK, VanOosten RL, Griffith TS. Histone deacetylase inhibitors modulate the sensitivity of tumor necrosis factor-related apoptosis-inducing ligand-resistant bladder tumor cells. *Cancer Res*. 2006;66:499–507.
171. Wang D, Wang Z, Tian B, et al. Two hour exposure to sodium butyrate sensitizes bladder cancer to anticancer drugs. *Int J Urol*. 2008;15:435–441.
172. He C, Huang L, Lei P, et al. Sulforaphane normalizes intestinal flora and enhances gut barrier in mice with BBN-induced bladder cancer. *Mol Nutr Food Res*. 2018;62:E1800427.
173. Xu X, Dai M, Lao F, et al. Effect of glucoraphanin from broccoli seeds on lipid levels and gut microbiota in high-fat diet-fed mice. *J Funct Foods*. 2020;68:103858.
174. Clavel T, Henderson G, Alpert CA, et al. Intestinal bacterial communities that produce active estrogen-like compounds enterodiol and enterolactone in humans. *Appl Environ Microbiol*. 2005;71:6077–6085.
175. Kellingray L, Tapp HS, Saha S, et al. Consumption of a diet rich in *Brassica* vegetables is associated with a reduced abundance of sulphate-reducing bacteria: a randomised crossover study. *Mol Nutr Food Res*. 2017;61:1600992.
176. Himmelfarb J, Stenvinkel P, Ikizler TA, et al. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia tients [3, 4] more important than these traditional risk. *Kidney Int*. 2002;62:1524–1538.
177. Stenvinkel P, Meyer CJ, Block GA, et al. Understanding the role of the cytoprotective transcription factor nuclear factor erythroid 2–related factor 2—lessons from evolution, the animal kingdom and rare progeroid syndromes. *Nephrol Dial Transplant*. 2019;gfz120. doi: 10.1093/ndt/gfz120.
178. Pedruzzi LM, Cardozo LFMF, Daleprane JB, et al. Systemic inflammation and oxidative stress in hemodialysis patients are associated with down-regulation of Nrf2. *J Nephrol*. 2015;28:495–501.
179. Mafra D, Borges NA, Lindholm B, et al. Mitochondrial dysfunction and gut microbiota imbalance: an intriguing relationship in chronic kidney disease. *Mitochondrion*. 2019;47:206–209.
180. Glorieux G, Gryp T, Perna A. Gut-derived metabolites and their role in immune dysfunction in chronic kidney disease. *Toxins (Basel)*. 2020;12:245.
181. Cigarran Guldris S, González Parra E, Cases Amenós A. Gut microbiota in chronic kidney disease | Microbiota intestinal en la enfermedad renal crónica. *Nefrologia*. 2017;37:9–19.
182. Mafra D, Fouque D. Gut microbiota and inflammation in chronic kidney disease patients. *Clin Kidney J*. 2015;8:332–334.
183. Mafra D, Gidlund EK, Borges NA, et al. Bioactive food and exercise in chronic kidney disease: targeting the mitochondria. *Eur J Clin Invest*. 2018;48:E13020.
184. Fontecha-Barriuso M, Martin-Sanchez D, Martinez-Moreno JM, et al. The role of PGC-1α and mitochondrial biogenesis in kidney diseases. *Biomolecules*. 2020;10:347.
185. Bai M, Chen H, Ding D, et al. MicroRNA-214 promotes chronic kidney disease by disrupting mitochondrial oxidative phosphorylation. *Kidney Int*. 2019;95:1389—1404.
186. Enoki Y, Watanabe H, Arake R, et al. Potential therapeutic interventions for chronic kidney disease-associated sarcopenia via indoxyl sulfate-induced mitochondrial dysfunction. *J Cachexia Sarcopenia Muscle*. 2017;8:735—747.
187. Galvan DL, Green NH, Danesh FR. The hallmarks of mitochondrial dysfunction in chronic kidney disease. *Kidney Int*. 2017;92:1051—1057.
188. Duann P, Lin PH. Mitochondria damage and kidney disease. *Adv Exp Med Biol*. 2017;982:529–551.
189. Su Z, Klein JD, Du J, et al. Chronic kidney disease induces autophagy leading to dysfunction of mitochondria in skeletal muscle. *Am J Physiol Ren Physiol*. 2017;312:F1128–F1140.
190. Gamboa JL, Billings FT, Bojanowski MT, et al. Mitochondrial dysfunction and oxidative stress in patients with chronic kidney disease. *Physiol Rep*. 2016;4:E12780.

191. Liu C, Gidlund EK, Witasp A, et al. Reduced skeletal muscle expression of mitochondrial-derived peptides humanin and MOTS-C and Nrf2 in chronic kidney disease. *Am J Physiol Ren Physiol*. 2019;317:F1122–F1131.
192. Mafra D, Esgalhado M, Borges NA, et al. Methyl donor nutrients in chronic kidney disease: impact on the epigenetic landscape. *J Nutr*. 2019;149:372–380.
193. Kato M, Natarajan R. Epigenetics and epigenomics in diabetic kidney disease and metabolic memory. *Nat Rev Nephrol*. 2019;15:327—345.
194. Cardozo LFMF, Stockler-Pinto MB, Mafra D. Brazil nut consumption modulates Nrf2 expression in hemodialysis patients: a pilot study. *Mol Nutr Food Res*. 2016;60: doi: 10.1002/mnfr.201500658
195. Esgalhado M, Kemp JA, Azevedo R, et al. Could resistant starch supplementation improve inflammatory and oxidative stress biomarkers and uremic toxins levels in hemodialysis patients? A pilot randomized controlled trial. *Food Funct*. 2018;9:6508–6516.
196. Alvarenga L, Salarolli R, Cardozo LFMF, et al. Impact of curcumin supplementation on expression of inflammatory transcription factors in hemodialysis patients: a pilot randomized, double-blind, controlled study. *Clin Nutr*. 2020;30110–30112.
197. Mafra D, Borges NA, Linholm B, et al. Food as medicine in chronic kidney disease. *Nat Rev Nephrol*. 2020.doi.org/10.1038/s41581-020-00345-8
198. Guerrero-Beltrán CE, Calderón-Oliver M, Tapia E, et al. Sulforaphane protects against cisplatin-induced nephrotoxicity. *Toxicol Lett*. 2010;192:278–285.
199. Zheng H, Whitman SA, Wu W, et al. Therapeutic potential of Nrf2 activators in streptozotocin-induced diabetic nephropathy. *Diabetes*. 2011;60:3055–3066.
200. Senanayake GVK, Banigesh A, Wu L, et al. The dietary phase 2 protein inducer sulforaphane can normalize the kidney epigenome and improve blood pressure in hypertensive rats. *Am J Hypertens*. 2012;25:229–235.
201. Cui W, Bai Y, Miao X, et al. Prevention of diabetic nephropathy by sulforaphane: possible role of Nrf2 upregulation and activation. *Oxid Med Cell Longev*. 2012;2012:1–12.
202. Guerrero-Beltrán CE, Mukhopadhyay P, Horváth B, et al. Sulforaphane, a natural constituent of broccoli, prevents cell death and inflammation in nephropathy. *J Nutr Biochem*. 2012;23:494–500.
203. González-Guerrero C, Ocaña-Salceda C, Berzal S, et al. Calcineurin inhibitors recruit protein kinases JAK2 and JNK, TLR signaling and the UPR to activate NF-κB-mediated inflammatory responses in kidney tubular cells. *Toxicol Appl Pharmacol*. 2013;272:825–841.
204. Cekauskas A, Bruns H, Manikas M, et al. Sulforaphane decreases kidney injury after transplantation in rats: role of mitochondrial damage. *Ann Transplant*. 2013;18:488–496.
205. Ryoo IG, Ha H, Kwak MK. Inhibitory role of the KEAP1-NRF2 pathway in TGFbβ1-stimulated renal epithelial transition to fibroblastic cells: a modulatory effect on SMAD signaling. *PLoS One*. 2014;9:E93265.
206. Saito H, Yoshimura M, Saigo C, et al. Hepatic sulfotransferase as a nephropreventing target by suppression of the uremic toxin indoxyl sulfate accumulation in ischemic acute kidney injury. *Toxicol Sci*. 2014;141:206–217.
207. Shang G, Tang X, Gao P, et al. Sulforaphane attenuation of experimental diabetic nephropathy involves GSK-3 beta/Fyn/Nrf2 signaling pathway. *J Nutr Biochem*. 2015;26:596–606.
208. Shokeir AA, Barakat N, Hussein AM, et al. Activation of Nrf2 by ischemic preconditioning and sulforaphane in renal ischemia/reperfusion injury: a comparative experimental study. *Physiol Res*. 2015;64:313–323.
209. Wu H, Kong L, Cheng Y, et al. Metallothionein plays a prominent role in the prevention of diabetic nephropathy by sulforaphane via up-regulation of Nrf2. *Free Radic Biol Med*. 2015;89:431–442.
210. Ebihara S, Tajima H, Ono M. Nuclear factor erythroid 2-related factor 2 is a critical target for the treatment of glucocorticoid-resistant lupus nephritis. *Arthritis Res Ther*. 2016;18:139.
211. Zhao Z, Liao G, Zhou Q, et al. Sulforaphane attenuates contrast-induced nephropathy in rats via Nrf2/HO-1 pathway. *Oxid Med Cell Longev*. 2016;2016:1–12.
212. Zhang W, Li Y, Ding H, et al. Hydrogen peroxide prevents vascular calcification induced ROS production by regulating Nrf-2 pathway. *Ren Fail*. 2016;38:1099–1106.
213. Atilano-Roque A, Wen X, Aleksunes LM, et al. Nrf2 activators as potential modulators of injury in human kidney cells. *Toxicol Reports*. 2016;3:153–159.
214. Lv D, Zhou Q, Xia Y, et al. The association between oxidative stress alleviation via Sulforaphane-induced Nrf2-HO-1/NQO-1 signaling pathway activation and chronic renal allograft dysfunction improvement. *Kidney Blood Press Res*. 2018;43:191–205.
215. Briones-Herrera A, Avila-Rojas SH, Aparicio-Trejo OE, et al. Sulforaphane prevents maleic acid-induced nephropathy by modulating renal hemodynamics, mitochondrial bioenergetics and oxidative stress. *Food Chem Toxicol*. 2018;115:185—197.
216. Shin JH, Kim KM, Jeong JU, et al. Nrf2-heme oxygenase-1 attenuates high-glucose-induced epithelial-to-mesenchymal transition of renal tubule cells by inhibiting ROS-mediated PI3K/Akt/GSK-3β signaling. *J Diabetes Res*. 2019;2019:1–8.
217. Kim JH, Kim KM, Jeong JU, et al. Nrf2-heme oxygenase-1 modulates autophagy and inhibits apoptosis triggered by elevated glucose levels in renal tubule cells. *Kidney Res Clin Pract*. 2019;38:318–325.
218. Rubio-Navarro A, Vázquez-Carballo C, Guerrero-Hue M, et al. Nrf2 plays a protective role against intravascular hemolysis-mediated acute kidney injury. *Front Pharmacol*. 2019;10:doi 10.3389/fphar.2019.00740
219. Khaleel SA, Raslan NA, Alzokaky AA, et al. Contrast media (meglumine diatrizoate) aggravates renal inflammation, oxidative DNA damage and apoptosis in diabetic rats which is restored by sulforaphane through Nrf2/HO-1 reactivation. *Chem Biol Interact*. 2019;309:108689.
220. Thangapandiyan S, Ramesh M, Miltonprabu S, et al. Sulforaphane potentially attenuates arsenic-induced nephrotoxicity via the PI3K/Akt/Nrf2 pathway in albino Wistar rats. *Environ Sci Pollut Res*. 2019;26:12247–12263.
221. Gigliotti JC, Tin A, Pourafshar S, et al. GSTM1 deletion exaggerates kidney injury in experimental mouse models and confers the protective effect of cruciferous vegetables in mice and humans. *J Am Soc Nepherol*. 2020;31:102–116.
222. Guerrero-Beltrán CE, Calderón-Oliver M, Martínez-Abundis E, et al. Protective effect of sulforaphane against cisplatin-induced mitochondrial alterations and impairment in the activity of NAD(P)H: quinone oxidoreductase 1 and γ glutamyl cysteine ligase: studies in mitochondria isolated from rat kidney and in LLC-PK1 cells. *Toxicol Lett*. 2010;199:80—92.
223. Kim T, Kim YJ, Han IH, et al. The synthesis of sulforaphane analogues and their protection effect against cisplatin induced cytotoxicity in kidney cells. *Bioorganic Med Chem Lett*. 2015;25:62–66.
224. Dadras F, Khoshjou F. NF-E2-related factor 2 and its role in diabetic nephropathy. *Iran J Kidney Dis*. 2013;7:346–351.
225. Choi BH, Kang KS, Kwak MK. Effect of redox modulating NRF2 activators on chronic kidney disease. *Molecules*. 2014;19:12727–12759.
226. Cui W, Min X, Xu X, et al. Role of nuclear factor erythroid 2-related factor 2 in diabetic nephropathy. *J Diabetes Res*. 2017;2017:1–14.
227. Tin A, Scharpf R, Estrella MM, et al. The loss of GSTM1 associates with kidney failure and heart failure. *J Am Soc Nepherol*. 2017;28:3345–3352.