ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2018 November 28; 24(44): 4959-5056

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 24 Number 44 November 28, 2018

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Wan-Long Chuang, MD, PhD, Doctor, Professor, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 642 experts in gastroenterology and hepatology from 59 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology (*WJG*) is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central and Directory of Open Access Journals. The 2018 edition of Journal Citation Reports® cites the 2017 impact factor for *WJG* as 3.300 (5-year impact factor: 3.387), ranking *WJG* as 35th among 80 journals in gastroenterology and hepatology (quartile in category Q2).

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORIAL OFFICE
Ze-Mao Gong, Director
World Journal of Gastroenterology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
November 28, 2018

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 November 28; 24(44): 4979-4988

DOI: 10.3748/wjg.v24.i44.4979

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Glutathione depleting drugs, antioxidants and intestinal calcium absorption

Luciana Moine, María Rivoira, Gabriela Díaz de Barboza, Adriana Pérez, Nori Tolosa de Talamoni

Luciana Moine, María Rivoira, Gabriela Díaz de Barboza, Adriana Pérez, Nori Tolosa de Talamoni, Laboratorio "Dr. Fernando Cañas", Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, INICSA (CONICET-Universidad Nacional de Córdoba), Córdoba 5000, Argentina

ORCID number: Luciana Moine (0000-0002-0350-8956); María Rivoira (0000-0002-7316-1564); Gabriela Díaz de Barboza (0000-0003-0617-8005); Adriana Pérez (0000-0002-5415-3139); Nori Tolosa de Talamoni (0000-0001-5570-2024).

Author contributions: Moine L, Rivoira M, Díaz de Barboza G, Pérez A and Tolosa de Talamoni NT participated in information collection, analysis, information organization, writing, figure design, and final editing.

Conflict-of-interest statement: No conflicts of interest, financial or otherwise, are declared by the authors.

Manuscript source: Invited manuscript

Corresponding author to: Nori Tolosa de Talamoni, PhD, Professor, Laboratorio "Dr. Fernando Cañas", Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, INICSA (CONICET-Universidad Nacional de Córdoba), Pabellón Argentina, 2do. Piso, Ciudad Universitaria, Córdoba 5000, Argentina. ntolosa@biomed.fcm.unc.edu.ar
Telephone: +54-351-4333024

Received: August 28, 2018
Peer-review started: August 28, 2018
First decision: October 9, 2018
Revised: October 24, 2018
Accepted: November 2, 2018

Article in press: November 2, 2018
Published online: November 28, 2018

Abstract

Glutathione (GSH) is a tripeptide that constitutes one of the main intracellular reducing compounds. The normal content of GSH in the intestine is essential to optimize the intestinal Ca^{2+} absorption. The use of GSH depleting drugs such as DL-buthionine-S,R-sulfoximine, menadione or vitamin K_3, sodium deoxycholate or diets enriched in fructose, which induce several features of the metabolic syndrome, produce inhibition of the intestinal Ca^{2+} absorption. The GSH depleting drugs switch the redox state towards an oxidant condition provoking oxidative/nitrosative stress and inflammation, which lead to apoptosis and/or autophagy of the enterocytes. Either the transcellular Ca^{2+} transport or the paracellular Ca^{2+} route are altered by GSH depleting drugs. The gene and/or protein expression of transporters involved in the transcellular Ca^{2+} pathway are decreased. The flavonoids quercetin and naringin highly abrogate the inhibition of intestinal Ca^{2+} absorption, not only by restoration of the GSH levels in the intestine but also by their anti-apoptotic properties. Ursodeoxycholic acid, melatonin and glutamine also block the inhibition of Ca^{2+} transport caused by GSH depleting drugs. The use of any of these antioxidants to ameliorate the intestinal Ca^{2+} absorption under oxidant conditions associated with different pathologies in humans requires more investigation with regards to the safety, pharmacokinetics and pharmacodynamics of them.

Key words: Glutathione; Transcellular and paracellular Ca^{2+}pathways; DL-buthionine-S,R-sulfoximine; Fructose rich diet; Menadione; Sodium deoxycholate; Glutamine; Ursodeoxycholic acid; Melatonin; Quercetin; Naringin

Core tip: The normal content of glutathione (GSH) in the intestine is essential to optimize the intestinal Ca^{2+} absorption. The use of GSH depleting drugs such as DL-buthionine-S,R-sulfoximine, menadione or vitamin K_3, sodium deoxycholate or diets enriched in fructose, which induce several features of the metabolic syndrome, produce inhibition of the intestinal Ca^{2+} absorption. The flavonoids quercetin and naringin highly abrogate the inhibition of intestinal Ca^{2+} absorption, not only by restoration of the GSH levels in the intestine but also by their anti-apoptotic properties. Ursodeoxycholic acid, melatonin and glutamine also block the inhibition of Ca^{2+} transport caused by GSH depleting drugs.

Moine L, Rivoira M, Díaz de Barboza G, Pérez A, Tolosa de Talamoni N. Glutathione depleting drugs, antioxidants and intestinal calcium absorption. *World J Gastroenterol* 2018; 24(44): 4979-4988 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i44/4979.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i44.4979

INTRODUCTION

Ca^{2+} absorption is one of the most important intestinal functions since the intestine is the only entrance gate of the cation into the body. This physiological process together with the renal Ca^{2+} reabsorption and the bone Ca^{2+} resorption maintain the Ca^{2+} homeostasis. An appropriate Ca^{2+} homeostasis preserves bone integrity, metabolic balance and avoids epithelial cancers such as breast, colon and prostate cancer[1-3]. A poor intestine absorption caused by infection, inflammation or a pathology in the intestine morphology may cause an adverse Ca^{2+} balance[4], which under chronic conditions leads to a deleterious bone mineralization. The intestinal Ca^{2+} absorption occurs along the entire intestine, but the small intestine is responsible for about 90% of overall Ca^{2+} absorption, and the order of Ca^{2+} absorption rate is duodenum > jejunum > ileum. The colon is only responsible for less than 10% of the total Ca^{2+} absorbed, but it appears to become important under pathological conditions[5].

Ca^{2+} is absorbed in the intestine by active and passive transport systems. The transcellular Ca^{2+} absorption is an active process and occurs *via* cation influx into the enterocyte, intracellular shuttling, and basolateral extrusion[6]. Ca^{2+} absorption can also occur *via* a passive, paracellular route, where the movement of the cation between epithelial cells is made through tight junction (TJ) proteins, which facilitate or block the Ca^{2+} movement[7]. The active transport of Ca^{2+} is mainly regulated by the biologically active form of vitamin D, $1,25(OH)_2D_3$ (calcitriol)[8], by previous activation of a vitamin D receptor (VDR)[9]. When VDR was deleted specifically in the intestine (VDR^{int-}) of mice, the intestinal Ca^{2+} absorption was decreased, the bone mineralization is inhibited and bone fractures were increased[10]. Thus, intestinal VDR is not only essential for intestinal Ca^{2+} absorption, but also for bone formation.

As previously reported, the transcellular Ca^{2+} movement involves the participation of transient receptor potential vanilloid type 6 (TRPV6) and transient receptor potential vanilloid type 5 in the step across the brush border membrane from enterocytes, calbindin D_{9k} (CB D_{9k}) as a ferry from one pole to the other pole of the cells and the plasma membrane Ca^{2+}-ATPase ($PMCA_{1b}$) and the Na^+/Ca^{2+} exchanger (NCX1) for cation extrusion[11]. The molecules involved in the paracellular Ca^{2+} movement are not completely known, but there is certain evidence that the proteins of the TJ such as claudin-2 and claudin-12 facilitate the Ca^{2+} transport[12,13]. In contrast, either gene or protein expression of cadherin-17 are decreased in mice´s and rat´s intestines during low Ca intake[14], as well as in Caco-2 cells after treatment with calcitriol[15].

When Ca^{2+} intake is low, the cation entry occurs through the transcellular pathway; whereas high luminal Ca^{2+} content (> 2-6 mmol/L) switches on the paracellular route due to a short sojourn time in the intestine and a down-regulation of molecules involved in the transcellular pathway[16,17]. The expression of paracellular TJ genes seems to be regulated by the calbindin protein, which suggests that the active and passive Ca^{2+} transport pathways may work cooperatively[18]. A reduction in more than 70% in the active intestinal Ca^{2+} absorption, 55% in CB D_{9k} expression and 90% in TRPV6 expression was observed in VDR null mice[19].

Although calcitriol is the main regulator of intestinal Ca^{2+} absorption, other hormones also contribute to altering this process as parathyroid hormone, glucocorticoids, estrogen, growth hormone, etc. In addition, many dietary and pharmacological compounds also modify the intestinal Ca^{2+} transport[20]. We have demonstrated that the normal content of the tripeptide glutathione (GSH) in enterocytes is essential for an optimal intestinal Ca^{2+} absorption, which was proved either in birds or in mammals[21,22]. GSH depletion produced by different ways generates a low GSH/glutathione disulfide (GSSG) ratio leading to oxidative stress and apoptosis of enterocytes by exacerbation of reactive oxygen species (ROS) production[23]. Clausen *et al*[24] have reported that GSH plays an important role in the opening of the TJ of intestinal epithelia enhancing the paracellular transport. In this review we will analyze the role of GSH in the intestine, the molecular mechanisms by which GSH depleting drugs inhibit the intestinal Ca^{2+} absorption and the prevention or restoration of these effects by drugs that act through normalization of intestinal GSH content.

GSH SYNTHESIS AND ITS PHYSIOLOGICAL ROLE IN THE INTESTINE

The intestinal mucosa comprises the surface monolayer

of self-renewing epithelial cells and the lamina propria with the vascular, immune and structural components[25]. In the small intestine there are invaginations called crypts of Lieberkuhn and prominences into the lumen called villi with differentiated cells. The crypts contain proliferative stem cells and Paneth cells responsible for the innate immunity and antibacterial defense and for providing essential signals to intestinal stem cells. The intestinal epithelium is a single heterogeneous layer of different cells: enterocytes (80% of total cells), enteroendocrine cells (1% of all epithelial cells), Goblet cells (4% in duodenum, secretory cells) and tuft cells (secretory cell type). They originate in the crypts, migrate toward the villi during differentiation and then suffer spontaneous apoptosis and shedding when reaching the villus tip after terminal differentiation[26]. Only enterocytes are involved in the Ca^{2+} transport from the intestinal lumen to the lamina propria. Although Ca^{2+} uptake occurs in all enterocytes, the mature cells from the tip and the middle part of the villi are mainly involved in the transcellular Ca^{2+} movement. Ca^{2+} uptake is stimulated by calcitriol or low Ca diets either in the mature enterocytes or in the undifferentiated cells from the crypt, but the most differentiated cells exhibit a higher response[27]. The GSH content in the intestine is in the millimolar range as occurs in other cells[28,29]; however, the tripeptide concentration varies according to the degree of maturation of cells. Surprisingly, mature enterocytes have lower GSH content than the immature cells[30]. GSH exists as the biologically active reduced-thiol form, and its oxidation to GSSG is associated with oxidative stress (OS). The GSH/GSSG ratio is around 100/1; when GSSG increases, this ratio decreases causing an oxidative shift in the cellular redox state[31]. Intestinal GSH redox homeostasis is maintained by *de novo* synthesis[32], regeneration from GSSG[33] and GSH uptake that derives from the dietary intake and mainly from the biliary output because the bile is enriched in GSH (1-2 mmol/L)[34,35]. The dietary GSH comes from fresh fruits, vegetables, and many types of meat, but the luminal GSH is lower (250 μmol/L in rats) than that from the intracellular compartment[36]. In the enterocytes and in the proximal tubular cells from kidney, the enzyme γ-glutamyl transpeptidase plays an important role in GSH homeostasis. It is located in the outer surface of plasma membranes of epithelial cells and cleaves the extracellular GSH to glutamate and cysteinyl-glycine, which is subsequently hydrolyzed by a dipeptidase to yield the constituent amino acids[37]. The biosynthesis of GSH occurs in the cytosolic compartment through two consecutive adenosine triphosphate (ATP)-dependent reactions. First, the glutamate cysteine ligase (GCL) catalyzes the formation of a dipeptide constituted by glutamate and cysteine, and then the GSH synthetase catalyzes the addition of glycine to form GSH[38], the former being the rate-limiting step. GCL has a catalytic subunit and a modulatory subunit. The control of GCL function is regulated at transcriptional levels of both subunits and through product feedback[39]. The γ-glutamyl cycle comprises the enzymatic reactions involved in the intracellular GSH synthesis and the extracellular GSH degradation, which could be considered as a mechanism to preserve cellular GSH homeostasis in transport epithelial cells. The reduction of GSSG by glutathione reductase (GR) to form GSH depends on the supply of the reductant nicotinamide adenine dinucleotide phosphate, which is provided by the pentose phosphate shunt[40]. The redox couple GSH/GSSG assures a redox environment that allows the maintenance of the gut microbiota, the adequate nutrient absorption, the reversal of oxidant-induced epithelium damage and the modulation of intestinal cell transformation and apoptosis[41]. The regulation of the GSH metabolism by the gut microbiota in mice has also been suggested[42], but it needs further investigation. GSH from the intestinal lumen plays different important roles such as reduction of dietary disulfides, detoxification of xenobiotics, metabolism of peroxidized lipids and maintenance of the mucus fluidity[29,34,41]. Tsunada *et al*[43] have shown that chronic administration of lipid peroxides interferes with the regulation of enterocyte death and proliferation *in vivo*; these disruptive effects were reversed by GSH supplementation after normalization of GSH/GSSG redox balance (Figure 1).

Intracellular GSH is distributed in different compartments. Cytosolic GSH is the source of GSH pool of the mitochondria, endoplasmic reticulum and nucleus. The GSH/GSSG ratio in the cytoplasm varies between 30/1 to 100/1, whereas in the endoplasmic reticulum (ER) is between 3/1 and 1/1, which indicates that in ER the system GSH/GSSH is more oxidized than that from the cytoplasm. The steady-state redox potential of the GSH/GSSG system is about -330 mmol/L to -300 mmol/L in the mitochondrial matrix and -260 mmol/L to -200 mmol/L in the cytoplasm. The endoplasmic reticular GSH/GSSG redox potential is about -150 mmol/L, which is in midway between cytoplasmic and plasma values[44]. The nuclear GSH/GSSG redox potential remains unknown but there is certain evidence that it can be somewhat more reducing than that from the cytoplasm. Proliferative cells have more negative steaty-state redox potential and differentiated cells have more positive cells and cells undergoing apoptosis and necrosis have a more oxidized steady-state potential (around -170 mmol/L to -150 mmol/L)[45]. In other words, the cellular compartments have different GSH/GSSG ratios, and the life cell cycle is also associated with different thiol redox potentials, which can alter the cellular functions. Since enterocytes show different degrees of differentiation and then suffer apoptosis and shedding in a range of 4-7 d, the redox potential of GSH/GSSG couple must change quite rapidly in order to facilitate the variety of functions of those cells during the lifespan.

GSH DEPLETION AND THE INTESTINAL CALCIUM ABSORPTION

Many years ago, Mårtensson *et al*[46] demonstrated that

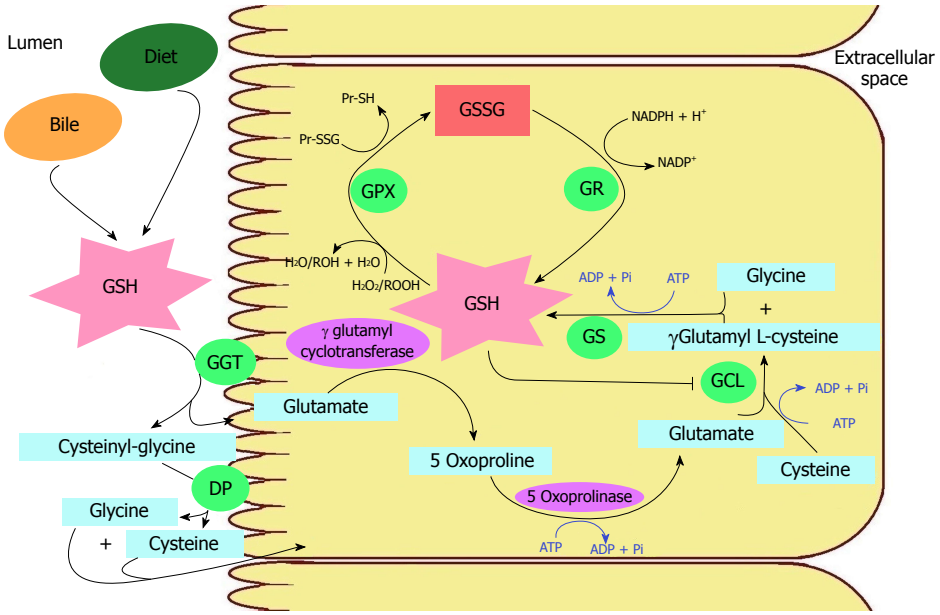

Figure 1 Intestinal glutathione metabolism. The γ-glutamyl cycle comprises the enzymatic reactions involved in the extracellular glutathione (GSH) degradation and the intracellular GSH synthesis: The enzymes γ-glutamyl transpeptidase and dipeptidase, localized in plasma membrane of enterocytes, cleave the extracellular GSH to their constituent amino acids. Within the enterocytes the GSH is synthesized *de novo* by two reactions that consume adenosine triphosphate that are catalyzed by glutamate cysteine ligase and glutathione synthetase, sequentially. In the redox couple that ensures the maintenance of the cellular redox state participates the enzymes glutathione peroxidase and glutathione reductase. GSH: Glutathione; GSSG: Glutathione disulfide; GGT: γ-glutamyl transpeptidase; GCL: Glutamate cysteine ligase; GS: Glutathione synthetase; GR: Glutathione reductase; GPX: Glutathione peroxidase; DP: Dipeptidase.

GSH was required for intestinal function. They observed that chronic depletion of mucosal GSH by buthionine sulfoximine (BSO), a specific inhibitor of GCL[47], caused severe degeneration of epithelial cells from jejunum and colon, which was prevented by oral GSH or GSH monoester. We have shown that BSO alters the Ca^{2+} transfer from intestinal lumen-to-blood in vitamin D supplemented chicks but does not affect that of vitamin D-deficient chicks, which indicate that the effects of BSO on intestinal Ca^{2+} absorption were dependent on the vitamin D status of the animal. The reversibility of this inhibition was proved by adding GSH monoester, an indication that intestinal GSH is essential to have an optimal intestinal Ca^{2+} absorption[21]. At that time, the molecular mechanisms involved in the effect of BSO on the intestinal Ca^{2+} absorption remained unknown. Later on, we have demonstrated that GSH depleting drugs inhibit the intestinal Ca^{2+} transport not only in birds but also in mammals[48]. The tripeptide depletion might increase the oxidation of sulfhydryl groups that are important to maintain the functionality of proteins involved in the Ca^{2+} transport, such as occurs with the $PMCA_{1b}$[21]. In addition, other GSH depleting drug such as vitamin K_3 or menadione (MEN) causes inhibition of intestinal Ca^{2+} absorption, which is related to OS, as judged by a decrease in GSH content and an increment in the total carbonyl group content. This inhibitory effect of MEN on intestinal Ca^{2+} absorption begins in half an hour, lasts for several hours and finishes after 10 h of treatment[49]. The quinone inhibits two enzymes presumably involved in Ca^{2+} transcellular pathway such as the intestinal alkaline phosphatase (IAP), located in the brush border membrane, and $PMCA_{1b}$, protein that carries Ca^{2+} outside the cell, as mentioned above. The enzyme inhibition might be due to alterations produced by ROS, which are triggered by GSH depletion caused by its consumption in the redox cycle of the quinone[50]. When MEN is metabolized, it may undergo one or two-electron reduction. If it suffers one-electron reduction, there is formation of a very unstable semiquinone radical, which reacts rapidly with molecular oxygen resulting in regeneration of the parent compound and production of a superoxide anion that yields H_2O_2 *via* enzymatic or spontaneous dismutation. Two-electron reduction of MEN produces a hydroquinone, a pathway that constitutes a detoxification mechanism[51]. In both cases, GSH is the electron donor, which explains the tripeptide depletion after MEN treatment. Since the intestinal Ca^{2+} absorption is an active process, which requires ATP that is mainly provided by the mitochondria, we have analyzed the functionality of these organelles in the intestinal mucosa when animals were treated with MEN. In fact, we have detected that MEN produces mitochondrial dysfunction caused by GSH depletion, which alters the mitochondrial permeability resulting in the release of cytochrome c and DNA fragmentation, biomarkers of apoptosis through the intrinsic pathway. In other words, mitochondrial dysfunction is also involved in the mechanisms by which MEN inhibits transiently the intestinal Ca^{2+} absorption[49]. Later on, the system FASL/FAS/caspase-3, indicators of apoptosis *via* the extrinsic pathway, was also demonstrated to be activated by MEN[52].

Sodium deoxycholate (NaDOC) is another GSH depleting drug that produces OS, as indicated by ROS generation and mitochondrial swelling leading to inhibition of intestinal Ca^{2+} absorption[53]. The effect of

Table 1 Glutathione depletion and the intestinal calcium absorption

	Oxidative stress markers	Effect on intestinal Ca^{2+} absorption
BSO	↓GSH	↓Ca^{2+} transfer from lumen-to-blood
MEN	↓GSH ↑ROS and protein carbonyl ↑Mn^{2+}-SOD and GPX Mitochondrial dysfunction	↓Ca^{2+} transfer from lumen-to-blood ↓IAP and PMCA1b activities
NaDOC	↓GSH ↑ROS Mitochondrial swelling. ↑SOD, CAT and GPX ↑NO• ↑iNOS protein	↓Intestinal Ca^{2+}absorption ↓mRNA PMCA1b ↓PMCA1b, CB_{D28k} and NCX1 protein expression
FRD	↓GSH ↑O_2^- ↑protein carbonyl and nitrotyrosine content ↓SOD and CAT ↑NO•	↓Intestinal Ca^{2+} absorption ↓IAP activity ↓TRPV6, PMCA1b, CBD9k, CLDN 2, CLDN12 and VDR protein expression

BSO: DL-buthionine-*S*,*R*-sulfoximine; CAT: Catalase; CB_{D28k}: Calbindin D28k; CB_{D9k}: Calbindin D9k; CLDN 2: Claudin 2; CLDN12: Claudin 12; FRD: Fructose rich diet; GPX: Glutathione peroxidase. GSH: Glutathione; IAP: Intestinal alkaline phosphatase. MEN: menadione. Mn^{2+}-SOD: Mn^{2+}-superoxide dismutase. NaDOC: sodium deoxycholate.NCX1: Na^+/Ca^{2+} exchanger; NO•: Nitric oxide; PMCA1b: Plasma membrane Ca^{2+}ATPase; SOD: Superoxide dismutase; TRPV6: Transient receptor potential vanilloid type 6; VDR: Vitamin D receptor.

NaDOC is time and dose-dependent and is higher in mature enterocytes[53,22]. NaDOC exerts its effect altering the transcellular Ca^{2+} pathway; the gene and protein expression of $PMCA_{1b}$ and the protein expressions of calbindin D_{28k} (CB D_{28k}) and NCX1 are decreased by this hydrophobic bile salt. NaDOC is a major component of the bile; in high concentrations it provokes liver damage during cholestasis and promotes colon carcinogenesis in experimental animals[54,55]. The molecular mechanisms by which NaDOC alters the protein expression of molecules involved in the intestinal Ca^{2+} absorption seem to be also related to nitrosative stress, as indicated by increment in NO• content and in induced nitric oxide synthase (iNOS) protein expression, and apoptosis, as shown by enhancement of the system FASL/FAS/caspase-8/caspase-3. In addition, an increase in acidic vesicular organelles (AVOs) and in LC3 Ⅱ protein expression produced by NaDOC means that autophagy might be another mechanism triggered by this bile salt associated with the inhibition of the intestinal Ca^{2+} absorption[22] (Table 1).

The administration of fructose rich diets (FRD) to normal rats, which induce several features of the metabolic syndrome, inhibit the intestinal Ca^{2+} absorption and induce vitamin D insufficiency[56,57]. In our laboratory, we have found that the VDR protein expression is also diminished by the FRD[58]. Since $1,25(OH)_2D_3$ and its receptor are depleted, the inhibition in the cation transport could be explained due to $1,25(OH)_2D_3$ is the main stimulator of the intestinal Ca^{2+} absorption. FRD alter both the transcellular and the paracellular pathways. The protein expression of TRPV6, CB D_{9k} and $PMCA_{1b}$ as well as the enzyme activity of IAP are lower in animals fed FRD than in rats fed a normal diet. Similarly, the protein expressions of Claudin-2 and Claudin-12, molecules located in intestinal TJ, are also decreased in rats fed FRD[58]. The intestinal GSH levels are decreased by the FRD, which would explain the increment in the superoxide anion and in the protein carbonyl content. This scenario is worsened by a decrease in the activities of superoxide dismutase and catalase, enzymes of the antioxidant defense, which result in impairment of the redox equilibrium contributing to altering the intestinal Ca^{2+} absorption. Other authors have also demonstrated that FRD decrease the GSH content and the antioxidant enzyme activities as well as vitamin C and vitamin E levels in rat liver and skeletal muscle[59]. In addition, we have demonstrated that FRD increase the NO• content and the nitrosylation of proteins of 22 and 38 kDa from rat intestine. He *et al*[60] have demonstrated that FRD increase the expression of inducible NO• synthase in the liver. Kannappan *et al*[59] have shown that FRD augment nitrosothiols in the plasma, liver and skeletal muscle. All these findings indicate that an intake rich in fructose triggers nitrosative stress in a variety of tissues.

The inflammation is another mechanism triggered by FRD in rat intestine, as suggested by an increment in the intestinal protein expression of nuclear factor (NF)-κB and interleukin (IL)-6. NF-κB is a transcription factor that controls over 100 genes activated direct or indirectly by inflammation[61]; IL-6 is a cytokine, whose gene has a promotor region with a site of binding for NF-κB. In addition, IL-6 has been suggested to be associated with metabolic syndrome and each of its components and it could be added as a biomarker of progression of that condition[62]. Therefore, the inhibition of the intestinal Ca^{2+} absorption by FRD is also mediated through the enhancement of inflammatory molecules[58].

In conclusion, any drug or disease associated with intestinal GSH depletion causes inhibition of intestinal Ca^{2+} absorption. This response is mediated by OS/nitrosative stress and inflammation, which could lead to cell death of enterocytes with capability to transport Ca^{2+} across the cells and in the paracellular route.

REVERSION/PREVENTION OF THE INHIBITION OF INTESTINAL CALCIUM ABSORPTION CAUSED BY GSH DEPLETION

The first approaches to revert or prevent the inhibition

Table 2 Antioxidants that preserve/ restore the inhibition of intestinal Ca^{2+} absorption caused by glutathione depletion

Drugs	Normalized OS markers	Effect on inhibition of ICaA	Normalized component of ICaA	Apoptosis markers	References
GSH monoester	GSH total	Restoration			Tolosa de Talamoni *et al*[21]
MEL	GSH, $^{\cdot}O^{2-}$ Protein carbonyl SOD, CAT and GPX activities iNOS gene and protein expression	Prevention restoration	PMCA1b, CB_{D9k}, NCX1, CLDN 2 and CLD 12 protein expression	↓TUNEL index ↓Caspase 3 activity/ protein expression	Carpentieri *et al*[80] Areco *et al*[81]
QT	GSH total. GPX activity	Prevention		↓Caspase 3 activity ↓ FAS, ↓FASL. Blocks mit swelling	Marchionatti *et al*[52]
GLN	GSH, $^{\cdot}O_2^{-}$. Protein carbonyl SOD and CAT activity	Prevention restoration	CB_{D28k} and PMCA1b protein expression.	↓TUNEL index. ↓FAS, ↓FASL ↓Caspase-3 activity	Moine *et al*[88]
NAR	GSH, $^{\cdot}O^{2-}$ NO• Protein carbonyl and nitrotyrosine content. SOD and CAT activity	Prevention	IAP activity (partially) PMCA1b, CB_{D9k}, NCX1, VDR, CLDN2 and CLD12 protein expression		Rodríguez *et al*[58]
UDCA	GSH, NO• protein carbonyl SOD activity iNOS protein expression	Increase restoration	IAP activity ↑PMCA1b, CB_{D28k}, NCX1, VDR gene and protein expression	↓Mit swelling ↓FAS, ↓FASL gene/ protein content Caspase 8 protein content Caspase 3 activity	Rodríguez *et al*[22,48]

CAT: Catalase; CB_{D28k}: Calbindin D28k; CB_{D9k}: Calbindin D9k; CLDN 2: Claudin 2; CLDN12: Claudin 12; GLN: Glutamine; GPX: Glutathione peroxidase; GSH: Glutathione; IAP: Intestinal alkaline phosphatase; ICaA: Intestinal Ca^{2+} absorption; MEL: Melatonin; MEN: Menadione; Mit: Mitochondrial; NAR: Naringin; NCX1: Na^{+}/Ca^{2+}exchanger; NO: Nitric oxide; OS: Oxidative stress; PMCA1b: Plasma membrane Ca^{2+}-ATPase; QT: Quercetin; SOD: Superoxide dismutase; TRPV6: Transient receptor potential vanilloid type 6; UDCA: Ursodeoxycholic acid; VDR: Vitamin D receptor.

of intestinal Ca^{2+} absorption caused by GSH depletion consisted in the use of GSH monoester in order to replenish the intestine with the tripeptide[21]. In fact, this treatment leads to the normalization of the intestinal Ca^{2+} absorption. In addition, other strategies were also assayed because the intestinal GSH depletion could be generated not only by drugs but also by pathological conditions such as cholestasis and metabolic syndrome[58,63].

Since GSH depletion causes exacerbation of ROS production, inflammation, apoptosis and autophagy, the reversion or prevention was thought to be blocked by flavonoids, molecules derived from natural sources with antioxidant, anti-inflammatory and antiapoptotic properties. Flavonoids are a class of phenolic metabolites produced by plants and fungi[64]. Among them, quercetin (QT) is largely present in fruit, red wine, tea, vegetables and aromatic plants[65], and exhibits all the biological properties mentioned above. Hence, QT is considered as a potential therapeutic agent for different diseases such as cancer, hypertension, inflammation, diabetes, thrombosis[66-68]. Inoue *et al*[69] have suggested that QT might improve the intestinal Ca^{2+} absorption because they have demonstrated in Caco-2 cells that QT increases the gene expression of TRPV6, which is a VDR target gene. We did not find that QT alone ameliorates the Ca^{2+} transport in the intestine, but we demonstrated that QT blocks the inhibition of the intestinal Ca^{2+} absorption caused by MEN *via* GSH depletion. Similarly, QT by itself does not change the intestinal GSH content, but it prevents the GSH depletion produced by the quinone[52]. Boots *et al*[70] have also observed that the effects of QT supplementation in patients with sarcoidosis appear to be more pronounced when the baseline levels of oxidative (malondialdehyde) and inflammatory (tumor necrosis factor α, IL-8, IL-10) markers are increased. So, it appears that the extent of the QT effects depends on the baseline of OS and inflammation. The protective mechanisms of QT on the intestinal Ca^{2+} absorption under oxidant conditions could be summarized in: (1) Normalization of intestinal redox state, (2) blockage of alterations in the mitochondrial membrane permeability (swelling), and (3) interference with the FASL/FAS/caspase-3 cascade activation. Taken together, it could be concluded that QT might be useful to prevent the inhibition of intestinal Ca^{2+} absorption caused by pro-oxidants or conditions that deplete GSH leading to OS and apoptosis[52].

Naringin (NAR) is another flavonoid that abrogates the inhibition of intestinal Ca^{2+} absorption caused by oxidant conditions such as an experimental metabolic syndrome produced in rats by FRD[58]. NAR is chemically known as naringenin 7-O-neohesperidoside and is present in different citrus being responsible for the bitterness in grapefruit, which is one of the richest sources of this flavonoid[71]. NAR has been demonstrated to increase the GSH content either in the liver or in the intestine from mice exposed to whole-body irradiation[72]. A meta-analysis also showed that NAR restores the GSH content in different parts of brain in various neurological ailments in rodents[73]. We have demonstrated that NAR (40 mg/kg bw) can protect the intestinal Ca^{2+} absorption by blocking all the alterations in the redox state of the intestinal mucosa caused under oxidant conditions such as the intake of FRD by rats[58]. With regard to GSH, NAR

not only blocked its depletion produced by FRD but also increased almost twice the normal intestinal GSH content by a mechanism that needs to be clarified. NAR was able to abrogate all the described alterations provoked by FRD in rats through its anti-oxidant, anti-nitrergic and anti-inflammatory properties. The use of NAR to ameliorate the intestinal Ca^{2+} transport under oxidant conditions associated with different pathologies holds a remarkable potential, but there are some obstacles in NAR clinical translation related to the extensive *in vivo* metabolism, low bioavailability and irregular absorption[74]. On the other hand, it has been demonstrated that various phenolic antioxidants exhibit pro-oxidant properties at high doses[75]. In fact, both flavonoids, quercetin and naringin, have antioxidant and pro-oxidant effects[76].

Melatonin (MEL) is one of the natural human antioxidant that has gained increasing attention. MEL is a hormone secreted by the pineal gland and other tissues such as bone marrow, skin and gastrointestinal tract. MEL is a lipophilic antioxidant of broad spectrum that has a high membrane permeability[77]. The molecular mechanisms triggered by MEL seem to be different from those of the classical antioxidants such as vitamin C, vitamin E and GSH; however, MEL synergizes with them in the scavenging of free radicals. The classical antioxidants undergo redox cycling so they have the potential to promote oxidation or prevent it. In contrast, MEL does not display redox cycling, thus, it does not stimulate oxidation; therefore it could be considered as a suicidal or terminal antioxidant. MEL may interact with free radicals forming several stable end products, which are excreted in the urine[78]. MEL content is 400 times larger in the intestine than in the pineal gland[79], but the physiological significance of this is not very clear. Similarly to QT, MEL alone does not affect the intestinal Ca^{2+} absorption, but it avoids or reverses the inhibitory effect of MEN or BSO[80,81]. The GSH depletion caused by MEN was also prevented by MEL, counteracting the oxidative stress and apoptosis provoked by the quinone. MEL protects either the intestinal transcellular Ca^{2+} pathway or the paracellular Ca^{2+} route, but only under oxidant conditions. The modulation of transporters of Ca^{2+} by MEL has also been reported in pancreatic acinar cells[82] and in rat pituitary GH3 cells[83]. In conclusion, MEL could be a drug for reversal of impaired intestinal Ca^{2+} absorption produced by OS and apoptosis that occurs under pathophysiological conditions such as aging, celiac disease, intestinal bowel disease, cancer or other gut disorders, or by GSH depleting drugs (Table 2).

Since the amino acid glutamine (GLN) in the intestine is a fuel and a source of glutamate, substrate for GSH synthesis[84,85], and it has antioxidant and antiapoptotic properties[86], and has the advantage of being an oral nutritional supplement[87], we have thought that it could be used to prevent or reverse the intestinal Ca^{2+} absorption inhibited by GSH depleting drugs. Similarly to other antioxidants, GLN alone does not modify the intestinal Ca^{2+} absorption but it reverses the inhibition of the intestinal cation transport caused by MEN. The GLN protective action is dose and time dependent and also occurs when it is administered previous to MEN treatment. The normalization of the protein expression of CB D_{28k} and $PMCA_{1b}$ by GLN indicates that this amino acid protects the transcellular Ca^{2+} pathway. The protection may be achieved because GLN restores the intestinal GSH content, normalizes the enzymatic activities of the antioxidant defense system and decreases the activation of the apoptotic pathway FASL/FAS/Caspase-3[88]. In other words, the antioxidant and antiapoptotic properties of GLN facilitate the normalization of the intestinal Ca^{2+} absorption under oxidant conditions. Whether GLN alters or not the intestinal Ca^{2+} paracelluar route and/or other mechanisms are involved in the protection of the intestinal Ca^{2+} absorption is under investigation.

Ursodeoxycholic acid (UDCA) is a minor component of the bile and has hydrophilic properties[48]. It is known that UDCA blocks the reactive oxygen species formation, the mitochondrial dysfunction and the death receptor induced apoptosis[89]. It has been widely used for treatment of cholestatic liver diseases, mainly primary biliary cirrhosis (PBC)[90]. In our laboratory, we have explored the possibility that UDCA could prevent the inhibition of intestinal Ca^{2+} absorption caused by NaDOC, a hydrophobic bile acid that causes GSH depletion in the duodenum, as mentioned in the previous section. Verma *et al*[91] have demonstrated that UDCA therapy enhances fractional Ca^{2+} absorption in PBC. In agreement with these data, we have observed that UDCA alone improves the intestinal Ca^{2+} absorption by increasing the amount of Ca^{2+} transporters involved in the transcellular Ca^{2+} pathway *via* activation of the VDR gene and protein expression. The effect of UDCA on Ca^{2+} uptake by enterocytes has been shown to depend on the degree of differentiation of these cells, being higher in mature enterocytes. When UDCA is given simultaneously with NaDOC, the intestinal Ca^{2+} absorption is similar to that from the control animals, which means that UDCA prevents the inhibition in the Ca^{2+} transport caused by NaDOC. Although UDCA alone decreases FASL and FAS protein expression without changing the Caspase-8 protein expression and caspase-3 activity, it avoids the apoptotic effects of NaDOC through normalization of the protein expression of FASL, FAS, Caspase-8 and the enzyme activity of Caspase-3. Similarly, UDCA *per se* does not alter the intestinal NO• content, but it abrogates the increase in NO• and in iNOS protein expression provoked by NaDOC. In addition, UDCA avoids efficiently the enhancement in LC3Ⅱ protein expression and in the number of AVOs in enterocytes caused by NaDOC, which means that UDCA attenuates the biomarkers of autophagy[22]. The physiological significance of this response is not quite clear and needs to be clarified.

CONCLUSION

The Ca^{2+} entrance to the organism is very important to maintain the Ca^{2+}-dependent functions and the correct mineralization of the skeleton. An optimal intestinal

Ca^{2+} absorption is reached when the GSH content in the intestine is in the normal range. Conditions associated with intestinal GSH depletion arising from administration of certain drugs or different diseases may inhibit the intestinal Ca^{2+} transport. This response could be prevented or restored by using flavonoids (QT, NAR), MEL, UDCA or GLN, which block the effects of the GSH depletion mainly through their antioxidant, antiapoptotic and anti-inflammatory properties. However, the use of these drugs to improve the intestinal Ca^{2+} absorption under oxidant conditions associated with different pathologies in humans requires more investigation with regards to the safety, pharmacokinetics and the pharmacodynamics of them.

REFERENCES

1 **Cianferotti L**, Gomes AR, Fabbri S, Tanini A, Brandi ML. The calcium-sensing receptor in bone metabolism: from bench to bedside and back. *Osteoporos Int* 2015; **26**: 2055-2071 [PMID: 26100412 DOI: 10.1007/s00198-015-3203-1]

2 **Bartlett PJ**, Gaspers LD, Pierobon N, Thomas AP. Calcium-dependent regulation of glucose homeostasis in the liver. *Cell Calcium* 2014; **55**: 306-316 [PMID: 24630174 DOI: 10.1016/j.ceca.2014.02.007]

3 **Farfariello V**, Iamshanova O, Germain E, Fliniaux I, Prevarskaya N. Calcium homeostasis in cancer: A focus on senescence. *Biochim Biophys Acta* 2015; **1853**: 1974-1979 [PMID: 25764980 DOI: 10.1016/j.bbamcr.2015.03.005]

4 **Krupa-Kozak U**, Markiewicz LH, Lamparski G, Juśkiewicz J. Administration of Inulin-Supplemented Gluten-Free Diet Modified Calcium Absorption and Caecal Microbiota in Rats in a Calcium-Dependent Manner. *Nutrients* 2017; **9** [PMID: 28684691 DOI: 10.3390/nu9070702]

5 **Jongwattanapisan P**, Suntornsaratoon P, Wongdee K, Dorkkam N, Krishnamra N, Charoenphandhu N. Impaired body calcium metabolism with low bone density and compensatory colonic calcium absorption in cecectomized rats. *Am J Physiol Endocrinol Metab* 2012; **302**: E852-E863 [PMID: 22275757 DOI: 10.1152/ajpendo.00503.2011]

6 **Beggs MR**, Alexander RT. Intestinal absorption and renal reabsorption of calcium throughout postnatal development. *Exp Biol Med* (Maywood) 2017; **242**: 840-849 [PMID: 28346014 DOI: 10.1177/1535370217699536]

7 **Alexander RT**, Rievaj J, Dimke H. Paracellular calcium transport across renal and intestinal epithelia. *Biochem Cell Biol* 2014; **92**: 467-480 [PMID: 25386841 DOI: 10.1139/bcb-2014-0061]

8 **Norman AW**. Intestinal calcium absorption: a vitamin D-hormone-mediated adaptive response. *Am J Clin Nutr* 1990; **51**: 290-300 [PMID: 2407100 DOI: 10.1093/ajcn/51.2.290]

9 **Uenishi K**, Tokiwa M, Kato S, Shiraki M. Stimulation of intestinal calcium absorption by orally administrated vitamin D3 compounds: a prospective open-label randomized trial in osteoporosis. *Osteoporos Int* 2018; **29**: 723-732 [PMID: 29273827 DOI: 10.1007/s00198-017-4351-2]

10 **Lieben L**, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Baatsen P, Lafage-Proust MH, Dresselaers T, Feng JQ, Bonewald LF, Meyer MB, Pike JW, Bouillon R, Carmeliet G. Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization. *J Clin Invest* 2012; **122**: 1803-1815 [PMID: 22523068 DOI: 10.1172/JCI45890]

11 **Diaz de Barboza G**, Guizzardi S, Tolosa de Talamoni N. Molecular aspects of intestinal calcium absorption. *World J Gastroenterol* 2015; **21**: 7142-7154 [PMID: 26109800 DOI: 10.3748/wjg.v21.i23.7142]

12 **Fujita H**, Sugimoto K, Inatomi S, Maeda T, Osanai M, Uchiyama Y, Yamamoto Y, Wada T, Kojima T, Yokozaki H, Yamashita T, Kato S, Sawada N, Chiba H. Tight junction proteins claudin-2 and -12 are critical for vitamin D-dependent Ca2+ absorption between enterocytes. *Mol Biol Cell* 2008; **19**: 1912-1921 [PMID: 18287530 DOI: 10.1091/mbc.E07-09-0973]

13 **Chiba H**, Osanai M, Murata M, Kojima T, Sawada N. Transmembrane proteins of tight junctions. *Biochim Biophys Acta* 2008; **1778**: 588-600 [PMID: 17916321 DOI: 10.1016/j.bbamem.2007.08.017]

14 **Benn BS**, Ajibade D, Porta A, Dhawan P, Hediger M, Peng JB, Jiang Y, Oh GT, Jeung EB, Lieben L, Bouillon R, Carmeliet G, Christakos S. Active intestinal calcium transport in the absence of transient receptor potential vanilloid type 6 and calbindin-D9k. *Endocrinology* 2008; **149**: 3196-3205 [PMID: 18325990 DOI: 10.1210/en.2007-1655]

15 **Christakos S**, Dhawan P, Ajibade D, Benn BS, Feng J, Joshi SS. Mechanisms involved in vitamin D mediated intestinal calcium absorption and in non-classical actions of vitamin D. *J Steroid Biochem Mol Biol* 2010; **121**: 183-187 [PMID: 20214989 DOI: 10.1016/j.jsbmb.2010.03.005]

16 **Bronner F**. Mechanisms of intestinal calcium absorption. *J Cell Biochem* 2003; **88**: 387-393 [PMID: 12520541 DOI: 10.1002/jcb.10330]

17 **Wasserman RH**. Vitamin D and the dual processes of intestinal calcium absorption. *J Nutr* 2004; **134**: 3137-3139 [PMID: 15514288 DOI: 10.1093/jn/134.11.3137]

18 **Hwang I**, Yang H, Kang HS, Ahn C, Hong EJ, An BS, Jeung EB. Alteration of tight junction gene expression by calcium- and vitamin D-deficient diet in the duodenum of calbindin-null mice. *Int J Mol Sci* 2013; **14**: 22997-23010 [PMID: 24264043 DOI: 10.3390/ijms141122997]

19 **Xue Y**, Fleet JC. Intestinal vitamin D receptor is required for normal calcium and bone metabolism in mice. *Gastroenterology* 2009; **136**: 1317-1327, e1-e2 [PMID: 19254681 DOI: 10.1053/j.gastro.2008.12.051]

20 **Areco V**, Rivoira MA, Rodriguez V, Marchionatti AM, Carpentieri A, Tolosa de Talamoni N. Dietary and pharmacological compounds altering intestinal calcium absorption in humans and animals. *Nutr Res Rev* 2015; **28**: 83-99 [PMID: 26466525 DOI: 10.1017/S0954422415000050]

21 **Tolosa de Talamoni N**, Marchionatti A, Baudino V, Alisio A. Glutathione plays a role in the chick intestinal calcium absorption. *Comp Biochem Physiol A Physiol* 1996; **115**: 127-132 [PMID: 8916550]

22 **Rodríguez VA**, Rivoira MA, Pérez Adel V, Marchionatti AM, Tolosa de Talamoni NG. Ursodeoxycholic and deoxycholic acids: Differential effects on intestinal Ca(2+) uptake, apoptosis and autophagy of rat intestine. *Arch Biochem Biophys* 2016; **591**: 28-34 [PMID: 26707246 DOI: 10.1016/j.abb.2015.12.006]

23 **Xiao Y**, Cui J, Shi YH, Sun J, Wang ZP, Le GW. Effects of duodenal redox status on calcium absorption and related genes expression in high-fat diet-fed mice. *Nutrition* 2010; **26**: 1188-1194 [PMID: 20444574 DOI: 10.1016/j.nut.2009.11.021]

24 **Clausen AE**, Kast CE, Bernkop-Schnürch A. The role of glutathione in the permeation enhancing effect of thiolated polymers. *Pharm Res* 2002; **19**: 602-608 [PMID: 12069161 DOI: 10.1023/A:1015345827091]

25 **Neish AS**. Redox signaling mediated by the gut microbiota. *Free Radic Res* 2013; **47**: 950-957 [PMID: 23937589 DOI: 10.3109/10715762.2013.833331]

26 **van der Flier LG**, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol* 2009; **71**: 241-260 [PMID: 18808327 DOI: 10.1146/annurev.physiol.010908.163145]

27 **Centeno VA**, Díaz de Barboza GE, Marchionatti AM, Alisio AE, Dallorso ME, Nasif R, Tolosa de Talamoni NG. Dietary calcium deficiency increases Ca2+ uptake and Ca2+ extrusion mechanisms in chick enterocytes. *Comp Biochem Physiol A Mol Integr Physiol* 2004; **139**: 133-141 [PMID: 15528161 DOI: 10.1016/j.cbpb.2004.08.002]

28 **Meister A**, Anderson ME. Glutathione. *Annu Rev Biochem* 1983; **52**: 711-760 [PMID: 6137189 DOI: 10.1146/annurev.bi.52.070183.003431]

29 **Circu ML**, Aw TY. Intestinal redox biology and oxidative stress. *Semin Cell Dev Biol* 2012; **23**: 729-737 [PMID: 22484611 DOI: 10.1016/j.semcdb.2012.03.014]

30 **Venoji R**, Amirtharaj GJ, Kini A, Vanaparthi S, Venkatraman A, Ramachandran A. Enteral glutamine differentially regulates Nrf 2 along the villus-crypt axis of the intestine to enhance glutathione levels. *J Gastroenterol Hepatol* 2015; **30**: 1740-1747 [PMID: 26095579 DOI: 10.1111/jgh.13019]

31 **Smith IC**, Vigna C, Levy AS, Denniss SG, Rush JW, Tupling AR. The effects of buthionine sulfoximine treatment on diaphragm contractility and SERCA pump function in adult and middle aged rats. *Physiol Rep* 2015; **3** [PMID: 26371231 DOI: 10.14814/phy2.12547]

32 **Aw TY**, Wierzbicka G, Jones DP. Oral glutathione increases tissue glutathione in vivo. *Chem Biol Interact* 1991; **80**: 89-97 [PMID: 1913980 DOI: 10.1016/0009-2797(91)90033-4]

33 **Shan XQ**, Aw TY, Jones DP. Glutathione-dependent protection against oxidative injury. *Pharmacol Ther* 1990; **47**: 61-71 [PMID: 2195557 DOI: 10.1016/0163-7258(90)90045-4]

34 **Aw TY**. Biliary glutathione promotes the mucosal metabolism of luminal peroxidized lipids by rat small intestine in vivo. *J Clin Invest* 1994; **94**: 1218-1225 [PMID: 8083363 DOI: 10.1172/JCI117439]

35 **Samiec PS**, Dahm LJ, Jones DP. Glutathione S-transferase in mucus of rat small intestine. *Toxicol Sci* 2000; **54**: 52-59 [PMID: 10746931 DOI: 10.1093/toxsci/54.1.52]

36 **Jones DP**, Coates RJ, Flagg EW, Eley JW, Block G, Greenberg RS, Gunter EW, Jackson B. Glutathione in foods listed in the National Cancer Institute's Health Habits and History Food Frequency Questionnaire. *Nutr Cancer* 1992; **17**: 57-75 [PMID: 1574445 DOI: 10.1080/01635589209514173]

37 **Circu ML**, Aw TY. Glutathione and modulation of cell apoptosis. *Biochim Biophys Acta* 2012; **1823**: 1767-1777 [PMID: 22732297 DOI: 10.1016/j.bbamcr.2012.06.019]

38 **Wu G**, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr* 2004; **134**: 489-492 [PMID: 14988435 DOI: 10.1093/jn/134.3.489]

39 **Soltaninassab SR**, Sekhar KR, Meredith MJ, Freeman ML. Multi-faceted regulation of gamma-glutamylcysteine synthetase. *J Cell Physiol* 2000; **182**: 163-170 [PMID: 10623879 DOI: 10.1002/(SICI)1097-4652(200002)182:2<163::AID-JCP4>3.0.CO;2-1]

40 **Meister A**, Tate SS. Glutathione and related gamma-glutamyl compounds: biosynthesis and utilization. *Annu Rev Biochem* 1976; **45**: 559-604 [PMID: 9027 DOI: 10.1146/annurev.bi.45.070176.003015]

41 **Circu ML**, Aw TY. Redox biology of the intestine. *Free Radic Res* 2011; **45**: 1245-1266 [PMID: 21831010 DOI: 10.3109/10715762.2011.611509]

42 **Mardinoglu A**, Shoaie S, Bergentall M, Ghaffari P, Zhang C, Larsson E, Bäckhed F, Nielsen J. The gut microbiota modulates host amino acid and glutathione metabolism in mice. *Mol Syst Biol* 2015; **11**: 834 [PMID: 26475342 DOI: 10.15252/msb.20156487]

43 **Tsunada S**, Iwakiri R, Fujimoto K, Aw TY. Chronic lipid hydroperoxide stress suppresses mucosal proliferation in rat intestine: potentiation of ornithine decarboxylase activity by epidermal growth factor. *Dig Dis Sci* 2003; **48**: 2333-2341 [PMID: 14714622 DOI: 10.1023/B:DDAS.0000007872.66693.6c]

44 **Jones DP**, Go YM. Redox compartmentalization and cellular stress. *Diabetes Obes Metab* 2010; **12** Suppl 2: 116-125 [PMID: 21029308 DOI: 10.1111/j.1463-1326.2010.01266.x]

45 **Go YM**, Ziegler TR, Johnson JM, Gu L, Hansen JM, Jones DP. Selective protection of nuclear thioredoxin-1 and glutathione redox systems against oxidation during glucose and glutamine deficiency in human colonic epithelial cells. *Free Radic Biol Med* 2007; **42**: 363-370 [PMID: 17210449 DOI: 10.1016/j.freeradbiomed.2006.11.005]

46 **Mårtensson J**, Jain A, Meister A. Glutathione is required for intestinal function. *Proc Natl Acad Sci USA* 1990; **87**: 1715-1719 [PMID: 2308931 DOI: 10.1073/pnas.87.5.1715]

47 **Nishizawa S**, Araki H, Ishikawa Y, Kitazawa S, Hata A, Soga T, Hara T. Low tumor glutathione level as a sensitivity marker for glutamate-cysteine ligase inhibitors. *Oncol Lett* 2018; **15**: 8735-8743 [PMID: 29928324 DOI: 10.3892/ol.2018.8447]

48 **Rodríguez V**, Rivoira M, Marchionatti A, Pérez A, Tolosa de Talamoni N. Ursodeoxycholic and deoxycholic acids: A good and a bad bile acid for intestinal calcium absorption. *Arch Biochem Biophys* 2013; **540**: 19-25 [PMID: 24096173 DOI: 10.1016/j.abb.2013.09.018]

49 **Marchionatti AM**, Perez AV, Diaz de Barboza GE, Pereira BM, Tolosa de Talamoni NG. Mitochondrial dysfunction is responsible for the intestinal calcium absorption inhibition induced by menadione. *Biochim Biophys Acta* 2008; **1780**: 101-107 [PMID: 18053815 DOI: 10.1016/j.bbagen.2007.10.020]

50 **Marchionatti AM**, Díaz de Barboza GE, Centeno VA, Alisio AE, Tolosa de Talamoni NG. Effects of a single dose of menadione on the intestinal calcium absorption and associated variables. *J Nutr Biochem* 2003; **14**: 466-472 [PMID: 12948877 DOI: 10.1016/S0955-2863(03)00078-0]

51 **Chiou TJ**, Tzeng WF. The roles of glutathione and antioxidant enzymes in menadione-induced oxidative stress. *Toxicology* 2000; **154**: 75-84 [PMID: 11118672 DOI: 10.1016/S0300-483X(00)00321-8]

52 **Marchionatti AM**, Pacciaroni A, Tolosa de Talamoni NG. Effects of quercetin and menadione on intestinal calcium absorption and the underlying mechanisms. *Comp Biochem Physiol A Mol Integr Physiol* 2013; **164**: 215-220 [PMID: 23000882 DOI: 10.1016/j.cbpa.2012.09.007]

53 **Rivoira MA**, Marchionatti AM, Centeno VA, Díaz de Barboza GE, Peralta López ME, Tolosa de Talamoni NG. Sodium deoxycholate inhibits chick duodenal calcium absorption through oxidative stress and apoptosis. *Comp Biochem Physiol A Mol Integr Physiol* 2012; **162**: 397-405 [PMID: 22561666 DOI: 10.1016/j.cbpa.2012.04.016]

54 **Lamireau T**, Zoltowska M, Levy E, Yousef I, Rosenbaum J, Tuchweber B, Desmoulière A. Effects of bile acids on biliary epithelial cells: proliferation, cytotoxicity, and cytokine secretion. *Life Sci* 2003; **72**: 1401-1411 [PMID: 12527037 DOI: 10.1016/S0024-3205(02)02408-6]

55 **Farhana L**, Nangia-Makker P, Arbit E, Shango K, Sarkar S, Mahmud H, Hadden T, Yu Y, Majumdar AP. Bile acid: a potential inducer of colon cancer stem cells. *Stem Cell Res Ther* 2016; **7**: 181 [PMID: 27908290 DOI: 10.1186/s13287-016-0439-4]

56 **Douard V**, Asgerally A, Sabbagh Y, Sugiura S, Shapses SA, Casirola D, Ferraris RP. Dietary fructose inhibits intestinal calcium absorption and induces vitamin D insufficiency in CKD. *J Am Soc Nephrol* 2010; **21**: 261-271 [PMID: 19959720 DOI: 10.1681/ASN.2009080795]

57 **Douard V**, Patel C, Lee J, Tharabenjasin P, Williams E, Fritton JC, Sabbagh Y, Ferraris RP. Chronic high fructose intake reduces serum 1,25 (OH)2D3 levels in calcium-sufficient rodents. *PLoS One* 2014; **9**: e93611 [PMID: 24718641 DOI: 10.1371/journal.pone.0093611]

58 **Rodríguez V**, Rivoira M, Guizzardi S, Tolosa de Talamoni N. Naringin prevents the inhibition of intestinal Ca^{2+} absorption induced by a fructose rich diet. *Arch Biochem Biophys* 2017; **636**: 1-10 [PMID: 29122589 DOI: 10.1016/j.abb.2017.11.002]

59 **Kannappan S**, Palanisamy N, Anuradha CV. Suppression of hepatic oxidative events and regulation of eNOS expression in the liver by naringenin in fructose-administered rats. *Eur J Pharmacol* 2010; **645**: 177-184 [PMID: 20655900 DOI: 10.1016/j.ejphar.2010.07.015]

60 **He S**, Rehman H, Wright GL, Zhong Z. Inhibition of inducible nitric oxide synthase prevents mitochondrial damage and improves survival of steatotic partial liver grafts. *Transplantation* 2010; **89**: 291-298 [PMID: 20145519 DOI: 10.1097/TP.0b013e3181c99185]

61 **Boone DL**, Dassopoulos T, Lodolce JP, Chai S, Chien M, Ma A. Interleukin-2-deficient mice develop colitis in the absence of CD28 costimulation. *Inflamm Bowel Dis* 2002; **8**: 35-42 [PMID: 11837936 DOI: 10.1097/00054725-200201000-00005]

62 **Srikanthan K**, Feyh A, Visweshwar H, Shapiro JI, Sodhi K. Systematic Review of Metabolic Syndrome Biomarkers: A Panel for Early Detection, Management, and Risk Stratification in the West Virginian Population. *Int J Med Sci* 2016; **13**: 25-38 [PMID: 26816492 DOI: 10.7150/ijms.13800]

63 **Wang P**, Gong G, Wei Z, Li Y. Ethyl pyruvate prevents intestinal inflammatory response and oxidative stress in a rat model of extrahepatic cholestasis. *J Surg Res* 2010; **160**: 228-235 [PMID: 19628226 DOI: 10.1016/j.jss.2009.03.027]

64 **Xiao J**. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? *Crit Rev Food Sci Nutr* 2017; **57**: 1874-1905 [PMID: 26176651 DOI: 10.1080/10408398.2015.1032400]

65 **Patel RV**, Mistry BM, Shinde SK, Syed R, Singh V, Shin HS.

Therapeutic potential of quercetin as a cardiovascular agent. *Eur J Med Chem* 2018; **155**: 889-904 [PMID: 29966915 DOI: 10.1016/j.ejmech.2018.06.053]

66 **Kasmi S**, Bkhairia I, Harrabi B, Mnif H, Marrakchi R, Ghozzi H, Kallel C, Nasri M, Zeghal K, Jamoussi K, Hakim A. Modulatory effects of quercetin on liver histopathological, biochemical, hematological, oxidative stress and DNA alterations in rats exposed to graded doses of score 250. *Toxicol Mech Methods* 2018; **28**: 12-22 [PMID: 28679351 DOI: 10.1080/15376516.2017.1351507]

67 **Zhou J**, Fang L, Liao J, Li L, Yao W, Xiong Z, Zhou X. Investigation of the anti-cancer effect of quercetin on HepG2 cells in vivo. *PLoS One* 2017; **12**: e0172838 [PMID: 28264020 DOI: 10.1371/journal.pone.0172838]

68 **Kleemann R**, Verschuren L, Morrison M, Zadelaar S, van Erk MJ, Wielinga PY, Kooistra T. Anti-inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in human in vitro and in vivo models. *Atherosclerosis* 2011; **218**: 44-52 [PMID: 21601209 DOI: 10.1016/j.atherosclerosis.2011.04.023]

69 **Inoue J**, Choi JM, Yoshidomi T, Yashiro T, Sato R. Quercetin enhances VDR activity, leading to stimulation of its target gene expression in Caco-2 cells. *J Nutr Sci Vitaminol* (Tokyo) 2010; **56**: 326-330 [PMID: 21228504 DOI: 10.3177/jnsv.56.326]

70 **Boots AW**, Drent M, de Boer VC, Bast A, Haenen GR. Quercetin reduces markers of oxidative stress and inflammation in sarcoidosis. *Clin Nutr* 2011; **30**: 506-512 [PMID: 21324570 DOI: 10.1016/j.clnu.2011.01.010]

71 **Cavia-Saiz M**, Busto MD, Pilar-Izquierdo MC, Ortega N, Perez-Mateos M, Muñiz P. Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin: a comparative study. *J Sci Food Agric* 2010; **90**: 1238-1244 [PMID: 20394007 DOI: 10.1002/jsfa.3959]

72 **Jagetia GC**, Reddy TK. Modulation of radiation-induced alteration in the antioxidant status of mice by naringin. *Life Sci* 2005; **77**: 780-794 [PMID: 15936352 DOI: 10.1016/j.lfs.2005.01.015]

73 **Viswanatha GL**, Shylaja H, Moolemath Y. The beneficial role of Naringin- a citrus bioflavonoid, against oxidative stress-induced neurobehavioral disorders and cognitive dysfunction in rodents: A systematic review and meta-analysis. *Biomed Pharmacother* 2017; **94**: 909-929 [PMID: 28810519 DOI: 10.1016/j.biopha.2017.07.072]

74 **Lavrador P**, Gaspar VM, Mano JF. Bioinspired bone therapies using naringin: applications and advances. *Drug Discov Today* 2018; **23**: 1293-1304 [PMID: 29747006 DOI: 10.1016/j.drudis.2018.05.012]

75 **Wong WS**, McLean AE. Effects of phenolic antioxidants and flavonoids on DNA synthesis in rat liver, spleen, and testis in vitro. *Toxicology* 1999; **139**: 243-253 [PMID: 10647924 DOI: 10.1016/S0300-483X(99)00136-5]

76 **Vargas AJ**, Burd R. Hormesis and synergy: pathways and mechanisms of quercetin in cancer prevention and management. *Nutr Rev* 2010; **68**: 418-428 [PMID: 20591109 DOI: 10.1111/j.1753-4887.2010.00301.x]

77 **Yu H**, Dickson EJ, Jung SR, Koh DS, Hille B. High membrane permeability for melatonin. *J Gen Physiol* 2016; **147**: 63-76 [PMID: 26712850 DOI: 10.1085/jgp.201511526]

78 **Tan DX**, Manchester LC, Reiter RJ, Qi WB, Karbownik M, Calvo JR. Significance of melatonin in antioxidative defense system: reactions and products. *Biol Signals Recept* 2000; **9**: 137-159 [PMID: 10899700 DOI: 10.1159/000014635]

79 **Bubenik GA**. Gastrointestinal melatonin: localization, function, and clinical relevance. *Dig Dis Sci* 2002; **47**: 2336-2348 [PMID: 12395907 DOI: 10.1023/A:1020107915919]

80 **Carpentieri A**, Marchionatti A, Areco V, Perez A, Centeno V, Tolosa de Talamoni N. Antioxidant and antiapoptotic properties of melatonin restore intestinal calcium absorption altered by menadione. *Mol Cell Biochem* 2014; **387**: 197-205 [PMID: 24234419 DOI: 10.1007/s11010-013-1885-2]

81 **Areco V**, Rodriguez V, Marchionatti A, Carpentieri A, Tolosa de Talamoni N. Melatonin not only restores but also prevents the inhibition of the intestinal Ca(2+) absorption caused by glutathione depleting drugs. *Comp Biochem Physiol A Mol Integr Physiol* 2016; **197**: 16-22 [PMID: 26970583 DOI: 10.1016/j.cbpa.2016.03.005]

82 **Huai J**, Shao Y, Sun X, Jin Y, Wu J, Huang Z. Melatonin ameliorates acute necrotizing pancreatitis by the regulation of cytosolic Ca2+ homeostasis. *Pancreatology* 2012; **12**: 257-263 [PMID: 22687382 DOI: 10.1016/j.pan.2012.02.004]

83 **Yoo YM**, Jeung EB. Melatonin-induced calbindin-D9k expression reduces hydrogen peroxide-mediated cell death in rat pituitary GH3 cells. *J Pineal Res* 2010; **48**: 83-93 [PMID: 20041988 DOI: 10.1111/j.1600-079X.2009.00730.x]

84 **Wang AL**, Niu Q, Shi N, Wang J, Jia XF, Lian HF, Liu Z, Liu CX. Glutamine ameliorates intestinal ischemia-reperfusion Injury in rats by activating the Nrf2/Are signaling pathway. *Int J Clin Exp Pathol* 2015; **8**: 7896-7904 [PMID: 26339354]

85 **Newsholme P**, Lima MM, Procopio J, Pithon-Curi TC, Doi SQ, Bazotte RB, Curi R. Glutamine and glutamate as vital metabolites. *Braz J Med Biol Res* 2003; **36**: 153-163 [PMID: 12563517 DOI: 10.1590/S0100-879X2003000200002]

86 **Jiang Q**, Chen J, Liu S, Liu G, Yao K, Yin Y. l-Glutamine Attenuates Apoptosis Induced by Endoplasmic Reticulum Stress by Activating the IRE1α-XBP1 Axis in IPEC-J2: A Novel Mechanism of l-Glutamine in Promoting Intestinal Health. *Int J Mol Sci* 2017; **18** [PMID: 29206200 DOI: 10.3390/ijms18122617]

87 **Chaudhry KK**, Shukla PK, Mir H, Manda B, Gangwar R, Yadav N, McMullen M, Nagy LE, Rao R. Glutamine supplementation attenuates ethanol-induced disruption of apical junctional complexes in colonic epithelium and ameliorates gut barrier dysfunction and fatty liver in mice. *J Nutr Biochem* 2016; **27**: 16-26 [PMID: 26365579 DOI: 10.1016/j.jnutbio.2015.08.012]

88 **Moine L**, Díaz de Barboza G, Pérez A, Benedetto M, Tolosa de Talamoni N. Glutamine protects intestinal calcium absorption against oxidative stress and apoptosis. *Comp Biochem Physiol A Mol Integr Physiol* 2017; **212**: 64-71 [PMID: 28732794 DOI: 10.1016/j.cbpa.2017.07.006]

89 **Perez MJ**, Briz O. Bile-acid-induced cell injury and protection. *World J Gastroenterol* 2009; **15**: 1677-1689 [PMID: 19360911 DOI: 10.3748/wjg.15.1677]

90 **Úriz M**, Sáez E, Prieto J, Medina JF, Banales JM. Ursodeoxycholic acid is conjugated with taurine to promote secretin-stimulated biliary hydrocholeresis in the normal rat. *PLoS One* 2011; **6**: e28717 [PMID: 22194894 DOI: 10.1371/journal.pone.0028717]

91 **Verma A**, Maxwell JD, Ang L, Davis T, Hodges S, Northfield TC, Zaidi M, Pazianas M. Ursodeoxycholic acid enhances fractional calcium absorption in primary biliary cirrhosis. *Osteoporos Int* 2002; **13**: 677-682 [PMID: 12181628 DOI: 10.1007/s001980200092]

P- Reviewer: Kan L, Marcos M, Polonikov AV, Yu XJ
S- Editor: Wang XJ **L- Editor**: A **E- Editor**: Yin SY

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

ISSN 1007-9327