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Riboflavin is an antioxidant: a review update

Nafisa Olfat[1], Marziyeh Ashoori[2] and Ahmad Saedisomeolia[3,4]*

[1]*Department of Nutrition, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Community Nutrition, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[3]*Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran*
[4]*School of Human Nutrition, McGill University, 21,111 Lakeshore, Ste. Anne de Bellevue, QC H9X 3V9, Canada*

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Abstract

Aerobic organisms need antioxidant defense systems to deal with free radicals which either are produced during aerobic respiration or may have an external origin. Oxidative stress, which is resulted from an imbalance between the production of free radicals and the ability of antioxidant defense mechanism to deactivate them, is involved in the development of many chronic diseases such as cancer, diabetes, CVD and some neurodegenerative diseases. Reinforcing the antioxidant potential of the body has been considered as a strategy that could prevent and manage such conditions. In the previous review article published by British Journal of Nutrition, in 2014, for the first time, we concluded that riboflavin could alleviate oxidative stress. Although riboflavin can serve as a prooxidant when exposed to ultraviolet irradiation, the literature is replete with studies that support its antioxidant properties. Furthermore, recent evidence suggests that riboflavin may have a therapeutic potential in many conditions in which oxidative stress is involved, although the therapeutic efficacy of riboflavin as an antioxidant requires further study under conditions of wellness and clinical disease.

Key words: **Riboflavin: Vitamin B_2: Oxidative stress: Antioxidant: Lipid peroxidation: Glutathione cycle**

Riboflavin, also known as vitamin B_2, is a water-soluble and heat-stable vitamin that plays a critical role in a wide range of biological pathways and processes, mainly serving as a coenzyme in the form of flavin mononucleotide and flavin adenine dinucleotide for a variety of flavoprotein enzymes[1]. Riboflavin is an essential nutrient for human health and needs to be included in the diet[2]. The rich sources of riboflavin are dairy product, meat, legumes, peas, liver, eggs and fortified grains and cereals[3–5]. Recommended daily dietary intake of riboflavin for women and men is 1·2 mg and 1·3 mg, respectively[6]. Riboflavin deficiency is prevalent all around the world, particularly in underdeveloped countries due to the low intake of dairy products and meats[7,8]. Relevant to clinical conditions, patients with certain types of cancers and heart disease, excessive alcohol intake, chronic diarrhoea, liver disorder and haemodialysis are at a greater risk of riboflavin deficiency[3,9]. Moreover, low levels of riboflavin can cause a variety of health problems including growth retardation, sore throat, cheilosis, anaemia, renal damage, neurodegeneration, gastrointestinal tract disorders, stomatitis of the mouth and tongue and skin disorders[10]. Riboflavin deficiency can also disrupt some biological processes such as Fe absorption, metabolism of tryptophan, mitochondrial function and metabolism of other nutrients[11].

Novel studies have addressed riboflavin-dependent cellular processes in various diseases[11]. One of the processes through that riboflavin could exert a substantial effect on human health is antioxidation[12]. Free radicals are produced by all aerobic cells playing the main role in ageing as well as in chronic diseases[13]. The toxicity of free radicals contributes to the damage of cellular macromolecules (such as DNA, proteins and lipids), inflammation, tissue damage and subsequent cellular apoptosis[14]. Therefore, as a result, oxidative stress is related to several chronic diseases such as cancer, diabetes, CVD[15–17] and some neurodegenerative disorders (such as Alzheimer's disease or Parkinson's disease)[18].The function of the antioxidant defense system of the body is to prevent any damage due to the action of free radicals[19].

In 2014, we reviewed the existing evidence regarding antioxidant properties of riboflavin for the first time, which was

Abbreviations: CAT, catalase; CK2, casein kinase 2; GPx, glutathione peroxidase; GR, glutathione reductase; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase.

* **Corresponding author:** Ahmad Saedisomeolia, email a_saedi@tums.ac.ir

published by British Journal of Nutrition[12]. Since that time, there have been several studies highlighting the antioxidant effects of riboflavin and revealing as well as novel aspects of its antioxidant function. In this review, we aim to update the current literature on riboflavin as an antioxidant nutrient. The reviewed studies are summarised in Table 1.

Effect of riboflavin on antioxidant enzymes activity and GSH

Aerobic organisms need antioxidant defense systems to deal with free radicals that either are produced during aerobic respiration or may have an external origin. Antioxidant enzymes, including catalase (CAT), superoxide dismutase, glutathione peroxidase (GPx) and glutathione reductase (GR), are the main parts of antioxidant defense system that deactivate reactive radicals mainly by reducing them to a more stable compound[20]. *Glutathione peroxidase* catalyses the reduction of hydrogen peroxide (H_2O_2)/lipid peroxide to H_2O/alcohol using reduced GSH as an electron donor[20] (Fig. 1). Through this reaction, GSH is converted to the oxidised form (GSSG). GR reduces GSSG and recovers its antioxidant properties in the reaction that requires riboflavin in the flavin adenine dinucleotide coenzyme form[4]. The detailed role of riboflavin in this reaction has been explained in our previous publication[12]. *Superoxide dismutase* (SOD) catalyses the dismutation of superoxide ion (O_2^-) to molecular oxygen (O_2) and H_2O_2 (a less reactive species)[20]. H_2O_2 could be converted to water by CAT or GPx[20] (Fig. 1). Considering the substantial role of antioxidant enzymes in maintaining redox homoeostasis of the cells, they are assessed as common biomarkers of the redox status of organisms[21]. As riboflavin has a key role in the glutathione redox cycle, it has been expected that riboflavin status could affect redox status, and consequently, glutathione content and activity of antioxidant enzymes. The effect of riboflavin status on antioxidant enzyme activity has been examined through several experimental studies using animal models of various diseases which have a connection with oxidative stress. Al-Harbi *et al* investigated the effect of riboflavin on lipopolysaccharides (LPS)-induced acute lung injury in rats[22]. In this study, riboflavin administration reversed the decline in GR and GPx activities and GSH content of lung induced by LPS. In another experimental study, riboflavin therapy significantly increased the activity of SOD, CAT, GR and GSH content of kidneys in renal toxicity rat model[23]. The improved antioxidant enzymes activity and GSH content of tissues have been also reported after riboflavin administration in rat models of migraine (GSH, microsomal GPx)[24], hepatotoxicity (CAT, SOD and GSH)[25,26], ulcerative colitis (SOD and GSH)[27], anticancer drug-induced toxicity (CAT, SOD and GSH)[28,29], spinal cord injury (SOD and GSH)[30], diabetes (GR, SOD, CAT and GSH)[31] and abdominal aortic aneurysm (SOD)[32].

Moreover, there are several animal studies addressing riboflavin and its antioxidant properties as a factor that may affect animal growth and quality and quantity of animal products. For example, Jiang *et al* examined the effect of a riboflavin-deficient diet on fish flesh quality indicating that riboflavin deficiency leads to a significant decrease in antioxidant enzymes activity (GPx, GR, SOD and CAT) and GSH content in the muscle, while riboflavin sufficient diet reverses all these negative effects[33]. Similarly, the reduced content of GSH and decreased activity of antioxidant enzymes in the intestine (decreased GR, GPx and SOD activity)[34] and gills (decreased GR, GPx, SOD and CAT activity)[35] of riboflavin-deficient fish have been reported. In another study on ducks, it has been shown that a riboflavin-deficient diet decreased the activity of SOD in plasma and liver compared with those ducks fed by a riboflavin-supplemented diet[36].

Although in most of the reviewed studies, riboflavin has shown a positive effect on antioxidant enzymes activity, there were also some limited exceptions. Nazıroğlu *et al* investigated the effect of Se and Se plus riboflavin on oxidative stress in rat headache model[37]. In this study, although GPx activity and GSH content in brain tissue showed a greater increase in Se + B_2 group than the Se group, this difference was not statistically significant[37]. Another study showed that after riboflavin administration in a rat model of headache, brain microsomal GPx activity was increased significantly; however, the increase in brain GPx was not statistically significant[24]. As the improvement in redox status of the brain has been observed in these studies, it seems that riboflavin exerts its antioxidant properties mainly through other antioxidant enzymes (which have not been assessed) or through mechanisms independent of the glutathione cycle or antioxidant enzymes, such as deactivation of reactive oxygen species (ROS) through the conversion of the reduced form of riboflavin to its oxidised form[12]. Another unexpected result was an increased activity of CAT in intestine of fish fed a riboflavin-deficient diet reported by Chen *et al*[34]. As intestine is an immune organ in fish[38], the increased activity of CAT may be a protective mechanism against the impaired intestinal immune function caused by increased levels of ROS which was concurrently observed in this study.

As mentioned previously, the role of riboflavin in the glutathione redox cycle could explain how riboflavin affects oxidative stress and antioxidant enzyme activities. However, the findings of the *in vitro* and experimental studies indicated that the mechanism by which riboflavin influences antioxidant enzymes is not limited to its role as the cofactor for GR. These investigators have revealed new pathways through which riboflavin status may have a regulatory effect on antioxidant enzymes gene expression. In an *in vitro* study on human keratoconic stromal cells (keratoconus is an eye disorder that oxidative stress may be implicated in its development), an increased gene expression of antioxidant enzymes (SOD, GPx and CAT) in riboflavin-treated cells (compared with untreated cells) was reported[39]. It has also been indicated that riboflavin deficiency decreases the mRNA level of antioxidant enzymes in muscle (GR, GPx, SOD and CAT)[33], intestine (GR, GPx and SOD)[34] and gills (GR, SOD)[35] of fish. The findings of these studies suggest that riboflavin may exert such regulatory effect through the Nuclear Factor E2-Related Factor 2 signalling pathway[33–35]. NRF2 is a transcriptional factor that activates the transcription of many antioxidant and detoxification enzymes and its abundance is tightly regulated at several points (i.e. transcriptional, post-transcriptional and post-translational)[40]. It has been shown that riboflavin deficiency decreases the NRF2 mRNA level in fish[33–35].

Table 1. Summary of the *in vitro*, animal and human studies included in the review

Investigators	Study type	Outcome measure	Main findings
Al-Harbi *et al.*[25]	Animal study	The effect of riboflavin therapy on liver function, inflammation and oxidative stress (MDA, GSH) biomarkers and liver histopathology in rat model of hepatotoxicity was evaluated.	A dose-dependent significant decrease in MDA level and increase in GSH content of liver were observed in riboflavin-administered groups compared with control group. Riboflavin also improved liver function and inflammation biomarkers and histopathological findings.
Al-Harbi *et al.*[22]	Animal study	The effect of riboflavin administration on GR, GSH, GPx and MDA was assessed in rat model of acute lung injury induced by lipopolysaccharides (LPS). The results were compared with dexamethasone administration.	Riboflavin significantly increased antioxidant enzymes activity (GR, GPx) and GSH content and decreased MDA in lung tissue of LPS-administered rats. The protective effects of riboflavin were similar to those of dexamethasone.
Alam *et al.*[31]	Animal study	The effect of riboflavin on oxidative stress (GSH, GR, SOD, CAT, MDA and PC), hyperglycaemia, DNA and tissue damage were investigated in diabetic rats.	Riboflavin administration significantly improved biomarkers of oxidative stress in a dose-dependent manner. FBS, tissue and DNA damage were also showed a marked decrease due to riboflavin administration.
Alhazza *et al.*[23]	Animal study	In renal toxicity rat model, markers of oxidative stress (MDA, PC, GSH, SOD, CAT and GR), renal function and tissue damage were compared between rats receiving riboflavin and control group.	Riboflavin significantly improved oxidative stress biomarkers, renal function and decreased tissue alteration induced by potassium bromate.
Bashandy *et al.*[26]	Animal study	The effect of riboflavin, nicotinamide and vitamin c (individually or in combination) on biomarkers of oxidative stress (GSH, SOD, CAT and MDA) and inflammation, liver function, lipid profile and tissue damage were evaluated in rat model of hepatotoxicity induced by thioacetamide.	Antioxidant enzymes activity and GSH level increased and MDA level decreased in liver of riboflavin-treated rats compared with control group. Riboflavin also improved biomarkers of liver function, inflammation, lipid profile and histopathological findings.
Butun *et al.*[24]	Animal study	In rat model of migraine, the effect of riboflavin administration on brain and brain microsomal GSH, GPx, MDA level and brain concentration of β-carotene, vitamin C, vitamin E and vitamin A was examined.	The brain and microsomal GSH and MDA were significantly improved in riboflavin-treated rats compared with the control group. Riboflavin increased microsomal GPx, while brain GPx did not show significant change. Brain concentrations of β-carotene, vitamin C, and vitamin E were also increased in rats treated by riboflavin.
Chen *et al.*[34]	Animal study	Intestinal immunity, tight junctions and antioxidant status (ROS, MDA, PC, GSH, GR, GPx, SOD and CAT) of fish fed with diets containing graded levels of riboflavin were assessed.	Riboflavin deficiency significantly increased intestinal ROS, MDA and PC and decreased GSH level and antioxidant enzymes activity (except for CAT). Riboflavin also led to a decrease in intestinal immunity and structural integrity of fish.
Chen *et al.*[35]	Animal study	The gill immunity, tight junction proteins, antioxidant status (ROS, MDA, PC, GSH, GR, GPx, SOD and CAT) of fish fed with graded levels of riboflavin were evaluated.	In fish fed a riboflavin-deficient ROS, MDA and PC was increased, while GSH level and antioxidant enzymes activity were decreased. Riboflavin deficiency also impaired gill immunity and changed tight junction proteins.
Cheung *et al.*[39]	*In vitro* study	The effect of riboflavin therapy on antioxidant enzymes gene expression in human keratoconic stromal cells was investigated.	An increased gene expression of SOD, GPx and CAT in riboflavin-treated cells (compared with untreated cells) was observed.
Yu *et al.*[4]	Animal study	Riboflavin photosensitisation treatment for inactivation of circulating HCT116 tumour cells was investigated.	Riboflavin photosensitisation treatment (RPT) could effectively decrease the proliferation of tumour cell in the peripheral blood
Makdoumi *et al.*[5]	*In vitro* study	Photodynamic UVA-riboflavin bacterial elimination in antibiotic-resistant bacteria was investigated.	UVA-riboflavin implicated in photoactivated chromophore for infectious keratitis (PACK)-collagen cross-linking (CXL) are effective in reducing both antibiotic-resistant and non-resistant bacteria.
Yoshimoto *et al.*[6]	*In vitro* study	Riboflavin plays a pivotal role in the UVA-induced cytotoxicity of fibroblasts as a key molecule in the production of H_2O_2 by UVA radiation was investigated.	They suggested that a riboflavin photosensitisation is elicited by various mechanisms depending on the vitamins and amino acids, cellular oxidative stress by producing H_2O_2 during UVA exposure.
Reynoso *et al.*[7]	*In vitro* study	Riboflavin-sensitised photooxidation of Ceftriaxone and Cefotaxime kinetic study and effect on *Staphylococcus aureus* was investigated.	Photoirradiation of aqueous solutions of Ceftriaxone (Cft) and Cefotaxime (Ctx) leads to degradation of both β-lactam antibiotics (Atbs) in the presence of riboflavin, which is a well-known pigment dissolved in natural aquatic systems.
Khan *et al.*[8]	*In vitro* study	Photocatalytic interaction of aminophylline–riboflavin leads to ROS-mediated DNA damage, and cell death was investigated.	Photocatalytic interaction of aminophylline with riboflavin renders aminophylline highly prooxidant caused a significant DNA damage resulting into apoptosis.
Khan *et al.*[9]	Animal study	Oxidative damage and cell death potential of photoilluminated aminophylline-riboflavin system in cancer lung cells of Swiss albino mice were examined.	They have demonstrated DNA damaging potential of this system in lung carcinoma cells which was associated with significant macromolecular damage and mitochondrial membrane disruption.

Table 1. (*Continued*)

Investigators	Study type	Outcome measure	Main findings
Yeo *et al.*[10]	*In vitro* study	Riboflavin-sensitised photooxidation of low-density lipoprotein (LDL) cholesterol was investigated.	Riboflavin caused the riboflavin-sensitised photooxidation of human LDL particles thereby increase in the conjugated dienes, indicating that this could serve as a major culprit in the development of CVD.
Howe *et al.*[52]	Human study (cross-sectional)	The association between B vitamin intake and urinary monomethyl arsenic and 15-F2t-isoprostane among US adults was investigated.	Dietary riboflavin intake was inversely associated with urinary 15-F2t-Isoprostane; however, this association did not remain significant after adjusting for confounders.
Jiang *et al.*[33]	Animal study	The impact of dietary riboflavin on fish flesh quality, muscle antioxidant status and antioxidant gene expression was examined.	Riboflavin deficiency reduced the flesh quality and impaired antioxidant defense of fish muscles (increased ROS, MDA and PC and decreased SOD, CAT, GR, GPx and GSH). The flesh quality and mRNA level of antioxidant enzymes were also decreased in riboflavin-deficient fish.
Karakoyun *et al.*[27]	Animal study	In rat model of ulcerative colitis, the effect of riboflavin therapy (immediately after the colitis induction (AA+ RF group) or 1 week before the colitis induction (RF+ AA+RF group)) on oxidative stress (8-OHdG, GSH and SOD) and severity of tissue damage was assessed.	In both riboflavin-treated rat groups, 8-OHdG was reduced (compared with control group). GSH content of colon was significantly increased in RF+AA+RF group than control group. Riboflavin therapy also decreased the severity of colonic injury.
Lee *et al.*[46]	*In vitro* study	The effect of riboflavin depletion on intestinal cell function was investigated.	Riboflavin depletion in intestinal cells led to overproduction of ROS in these cells. It also significantly decreased intracellular ATP concentration and made an irreversible loss of cell proliferative potential.
Naseem *et al.*[28]	Animal study	The effect of riboflavin administration on oxidative stress (SOD, CAT, GSH, MDA and PC) and liver and kidney function were assessed by gender in cisplatin toxicity mice model.	Riboflavin improved biomarkers of oxidative stress especially among female mice (decreased MDA and PC; increased SOD, CAT, GSH). It also exerted more ameliorative effects in liver and kidney function biomarker in female mice.
Nazıroğlu *et al.*[37]	Animal study	The impact of Se or with/without riboflavin on brain and brain microsomal lipid peroxidation (MDA) and antioxidant status (GSH, GPx, vitamin A, β-carotene, vitamin C and vitamin E) was evaluated in migraine rat model.	Compared with Se alone, riboflavin + Se did not cause further improvement in MDA level or antioxidant status except for vitamin E.
Pan *et al.*[47]	Animal study	The effects of N-nitrosomethylbenzylamine and riboflavin deficiency (individually or in combination) on DNA damage (8-OHdG, γH2AX) and oesophageal tumourigenesis were examined in rats.	Riboflavin deficiency significantly increased DNA damage and oesophageal tumourigenesis.
Peraza *et al.*[45]	Animal study	The effect of riboflavin and pyridoxine on brain oxidative stress (GSH, TBARS and H_2O_2) induced by 3-nitropropionic acid (3-NPA) in rats.	In rats receiving riboflavin alone (without 3-NPA), H_2O_2 significantly decreased in one region of the brain (cerebellum/medulla oblongata) compared with B2+ 3-NPA. However, riboflavin did not improve other markers of oxidative stress in none of brain regions.
Sakarcan *et al.*[30]	Animal study	In rat model of spinal cord injury, markers oxidative stress (MDA, SOD and GSH), DNA damage (8-OHdG) and tissue damage were compared between rats receiving riboflavin and control group.	Riboflavin significantly improved MDA, SOD, GSH, 8-OHdG in both spinal cord and kidney tissues. Riboflavin therapy also led to an improvement in spinal cord tissue damage and motor function of rats.
Salman1 *et al.*[29]	Animal study	The effect of riboflavin (under photo illumination or not) as an adjuvant for cisplatin on cisplatin-induced toxicities and its anti-cancer activity was investigated in mice model of skin cancer.	In in liver, kidney and skin of mice treated with riboflavin (with/without photo illumination), the MDA level was significantly lower and GSH content and GST activity were significantly higher than the control group.
Tang *et al.*[36]	Animal study	The impacts of the graded level (from 0 to 7 mg/kg) dietary riboflavin on growth performance and antioxidant status (SOD, MDA) of Pekin ducks were examined.	Adding riboflavin to the ducks' diet significantly increased SOD activity and reduced MDA level in serum and liver compared with riboflavin-deficient diet.
von Martels *et al.*[53]	Human study (quasi-experimental)	In patients with Crohn's disease, serum biomarkers of inflammation and redox status (plasma-free thiols) and clinical disease activity were assessed before and after riboflavin supplementation (100 mg/d for 3 weeks).	Plasma-free thiols significantly increased after riboflavin supplementation. Some biomarkers of inflammation (IL2, CRP and TNF-α) and clinical disease activity were also improved after intervention.
Yu *et al.*[32]	Animal study	The formation of abdominal aortic aneurysm (AAA) and markers of oxidative stress (ROS, 8-OHdG and SOD) were compared between riboflavin-treated rat and control group.	Riboflavin significantly improved aortic wall oxidative stress (ROS, 8-OHdG and SOD) and prevents AAA formation in rats.
Adakul *et al.*[54]	Animal study	The potential protective effect of pretreatment with riboflavin against renal ischaemia/reperfusion injury was examined. MDA, GSH and 8-OHdG were compared in riboflavin-treated rats and control group	Pretreatment with riboflavin significantly decreased renal MDA and 8-OHdG level and increased GSH content of kidney. It also reduced ischaemia/reperfusion-induced renal injury.

Table 1. (*Continued*)

Investigators	Study type	Outcome measure	Main findings
Ertas, *et al.*[55]	Animal study	The potential protective effects of riboflavin in ischaemia reperfusion injury in the rat bladder were investigated. MDA and GSH were assessed in riboflavin-treated rats and control group.	Ischaemia reperfusion led to an increase in MDA and a decrease in GSH level. Riboflavin significantly improved both GSH and MDA level in the bladder.
Li *et al.*[57]	Animal study	The effect of pretreatment with riboflavin on marker of oxidative stress (MDA and SOD activity in serum and liver) and liver function (AST and ALT activity in serum) were assessed in rat model of hepatic ischaemia/reperfusion injury.	Ischaemia/reperfusion injury significantly increased LAT and AST activity in serum decreased the SOD activity and elevated MDA level in liver. Riboflavin treatment improved all these biomarkers and alleviated the alteration of liver structure
Sanches *et al.*[56]	Animal study	In rat model of liver ischaemia/reperfusion injury, marker of oxidative stress (GSH, SOD) and liver function and the histological damage score were compared between riboflavin-treated rat and control rats.	Riboflavin treatment significantly reduced liver tissue damage and alleviated biomarkers of oxidative stress (GSH, SOD) and liver function (AST, ALT).

GPx, glutathione peroxidase; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; GSH, reduced glutathione; MDA, malondialdehyde; GST, glutathione S-transferase; TNF-α, tumour necrosis factor alpha; 8-OHdG, 8-hydroxydeoxyguanosine; ROS, reactive oxygen species; PC, protein carbonyl; FBS, fasting blood sugar; IL2, interleukin-2; CRP, C-reactive protein; ALT, alanine transaminase; AST, aspartate transaminase.

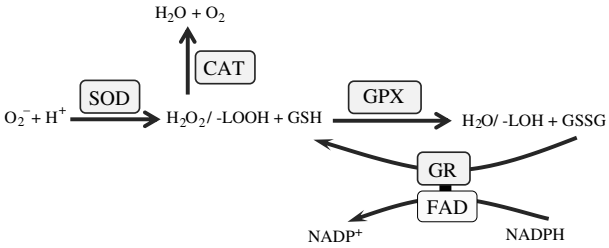

Fig. 1. The reactions catalysed by antioxidant enzymes. CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidised glutathione; FAD, flavin adenine dinucleotide.

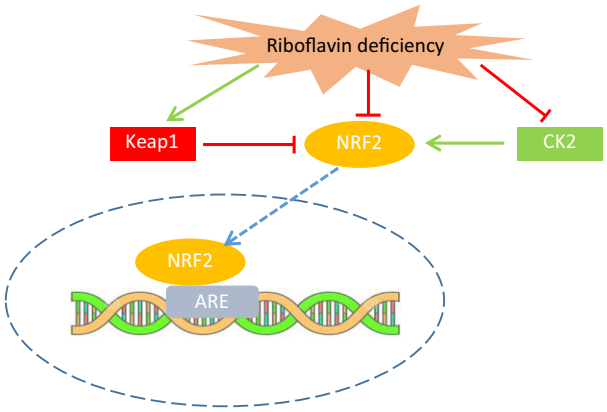

Fig. 2. Riboflavin deficiency may decrease the antioxidant enzymes activity through up-regulation of Keap1 and down-regulation of CK2, resulting in prevention of NRF2 nuclear translocation and activation. Riboflavin may also down-regulate NRF2 gene expression. CK2, casein kinase 2; NRF2, nuclear factor E2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; ARE, antioxidant response element.

Moreover, the NRF2 mRNA level showed a positive correlation with the mRNA level of antioxidant enzymes in these studies[33–35]. Riboflavin deficiency also led to a significant increase in Kelch-like ECH-associated protein 1(Keap1)[33–35] and decreased casein kinase 2 (CK2) mRNA level[33]. Keap1, as a suppressor of NRF2, binds to the NRF2 and prevents its translocation to the nucleus[41]. On the other hand, phosphorylation of NRF2 by CK2 leads to its nuclear translocation and activation of NRF2 transcriptional function[42]. Therefore, the results of the studies on fish suggest that riboflavin deficiency decreases the antioxidant enzymes mRNA level through, at least in part, the down-regulation of NRF2 gene expression, up-regulation of Keap1 and down-regulation of CK2, resulting in the prevention of NRF2 nuclear translocation and activation (Fig. 2). Nevertheless, further research is needed to clarify the exact mechanisms through which riboflavin regulates these signalling molecules.

Finally, reviewing studies published in recent years (2014–2021) indicated that riboflavin supplementation in conditions that are accompanied by increased oxidative stress could improve the antioxidant enzymes activity; and its deficiency has shown an adverse effect on the activity of these enzymes in animals. However, to the best of our knowledge, no human research has addressed the effect of riboflavin on antioxidant enzymes activity, warranting further human studies.

Effect of riboflavin on reactive oxygen species and oxidative damage of the lipids, DNA and proteins

Reactive oxygen species (ROS) is a collective term referring to the several oxygen metabolites, which could have both exogenous and endogenous origin and include superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$) radicals and hydrogen peroxide (H_2O_2)[43]. Most of these oxygen metabolites are free radicals (H_2O_2 is a non-radical ROS, but can easily be converted to free radicals), which have one or more unpaired electrons making them highly reactive[44]. Accumulation of these compounds causes tissue damage through oxidative damage of cellular structures, that is, proteins, membrane lipids and nucleic acids[44]. Some specific products resulted from the oxidation of protein, lipids and DNA are assessed as the biomarkers of oxidative stress[21].

In some experimental studies, the protective effects of riboflavin on ROS have also been reported[32,33,35,45]. In these studies, the beneficial effect of riboflavin supplementation[32,33,45] and

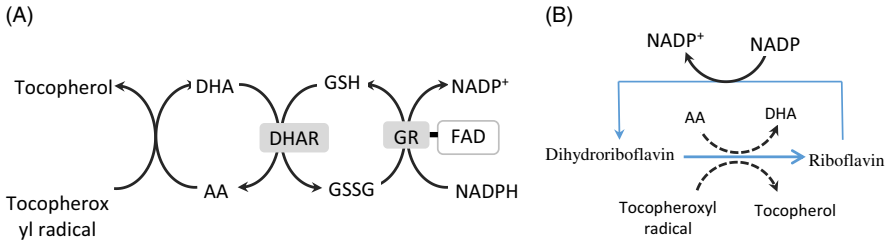

Fig. 3. The suggested mechanisms through which riboflavin reinforces the antioxidant effect of vitamin C and vitamin E. Riboflavin may recover the antioxidant activity of these vitamins through its role in glutathione redox cycle (A) or directly through its conversion from reduced to oxidized form (B). AA, ascorbate; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GSH, reduced glutathione; GSSG, oxidised glutathione; GR, glutathione reductase.

the deleterious effect of its deficiency[33,35] on ROS levels in different tissues have been indicated. An *in vitro* study also has shown that riboflavin depletion in intestinal cells could lead to overproduction of ROS in these cells[46].

In our previous review, we cited studies that concluded riboflavin can alleviate lipid peroxidation[12]. Recent studies have corroborated these results[22–26,28,29,31,33–36]. In a recently published animal study, malondialdehyde (MDA, a marker of lipid peroxidation) is decreased after riboflavin administration in rat model of renal toxicity[23]. Similarly, Butun *et al* indicated that riboflavin significantly reduces the MDA levels in the brain of migraine rat model[24]. A significantly decreased MDA level following riboflavin therapy has also been reported in experimental models of several other diseases such as acute lung injury[22], hepatotoxicity[25,26], diabetes[31] and cisplatin- (a chemotherapy medication) induced toxicities[28,29]. Similarly, in other animal investigations, increased MDA has been reported in riboflavin-deficient animals which decreased following riboflavin administration[33–36].

In addition to lipid peroxidation, riboflavin has been shown to reduce the oxidative damage of DNA[27,32,47] and proteins[23,28,31]. Yu *et al* investigated the effect of riboflavin on abdominal aortic aneurysm formation in rats[32]. This study indicated that riboflavin could significantly decrease 8-hydroxydeoxyguanosine (a biomarker for oxidative DNA damage) in aneurysm walls[32]. In another experimental study reporting the protective effect of riboflavin in ulcerative colitis, the colonic 8-hydroxydeoxyguanosine level has been reported to be declined in riboflavin-treated rats compared with the control group[27]. Moreover, N-nitroso-N-methylbenzylamine (an environmental carcinogen) has caused a greater increase in oxidative DNA damage (8-hydroxydeoxyguanosine) and oesophageal tumourigenesis in riboflavin-deficient rats compared with the sufficient ones[47]. Protein carbonyl, a product of protein oxidative damage, has also been shown to be decreased by riboflavin administration in the rat models of diabetes[31], renal toxicity[23] and anticancer drug-induced toxicities[28], while its elevated level has been reported in riboflavin-deficient fishes[33,34].

Overall, results of reviewed experimental studies suggest that riboflavin could alleviate oxidative stress in the terms of lipid peroxidation and oxidative damage of DNA and proteins. However, there are a few exceptions in the findings of these studies. For example, Nazıroğlu *et al*. showed that supplementation with riboflavin and Se compared with Se alone caused a greater decrease in lipid peroxidation in the brain of migraine rat model, but this decrease was not statistically significant[37]. In another study, riboflavin led to an increased lipid peroxides in different regions of rats' brain[45]. Although the exact reason for such conflicting results is not clear, specific features of brain energy metabolism[48], concurrent supplementation with other antioxidants or probable technical issues may be involved in producing these results.

The observed protective effect of riboflavin against oxidative damage of lipids, proteins and nucleic acids could be mediated by the enhanced activity of antioxidant enzymes which have been reported to occur concurrent with the reduced levels of MDA[22–24,26,28,29,33,36], 8-hydroxydeoxyguanosine[27,30] and protein carbonyl[23,28,33]. However, the mechanism through which riboflavin acts as an antioxidant may not be limited to its effects on antioxidant enzymes. As discussed in our previous review[12], in some cases, riboflavin has led to a decrease in oxidative stress, while the activity of antioxidant enzymes has not increased concurrently, a finding that has been reported in recent studies as well[24]. It has been suggested that riboflavin has a direct free radical scavenging activity through conversion from reduced (dihydroriboflavin) to oxidised form[49]. NADPH-dependent methemoglobin reductase has been suggested to catalyse the reduction of oxidised riboflavin and recover its antioxidant activity[50]. Moreover, in the reviewed studies, riboflavin has been reported to increase vitamin E[24,37] and vitamin C[24] levels in the brain of rats, which highlights another path through which riboflavin may exert its antioxidant properties. The connection between GSH, vitamin C and vitamin E is illustrated in Fig. 3. GSH reduces oxidised vitamin C (dehydroascorbate) and recovers its antioxidant capability. Vitamin C, which is a powerful free radical scavenger, could regenerate active vitamin E from its radical form (tocopheroxyl radical) as well[51] (Fig. 3). In addition to its role in the glutathione redox cycle, riboflavin may directly act as an electron donor and reduce dehydroascorbate and tocopheroxyl radical to their active forms, the possible role which needs to be confirmed by future studies.

Prooxidative effect of riboflavin

Although riboflavin has been widely examined as an antioxidant in experimental studies, it can also have a prooxidant effect when exposed to ultraviolet irradiation. Riboflavin itself is non-toxic in the darkness, although upon absorption of UVA and blue light, it can act as a photosensitiser[2]. The photoreactivity of riboflavin is

due to the isoalloxazine moiety[2]. Through photosensitisation, the isoalloxazine ring of riboflavin absorbs energy from light and induces chemical reactions in nearby molecule through type I and type II pathways[52]. In the type I pathway, riboflavin (in the excited triplet state) can abstract electrons or hydrogen ion from the substrates to produce radicals. However, this photosensitiser, in the type II pathway, can transfer its high energy to triplet oxygen and form singlet oxygen[52] and convert it to an ROS. Therefore, in the light-exposed tissues, that is, eyes and skin, riboflavin may induce oxidative damage to the macromolecules. Other dietary antioxidants such as polyphenols, vitamin E and carotenoids have been indicated to protect these tissues against riboflavin photosensitising effect by different mechanisms[53,54]. Polyphenols and vitamin E can quench the triplet state of riboflavin through direct electronic energy transfer[53], while carotenoids scavenge-free radicals and ROS resulting from type I and type II photosensitisation[54]. On the other hand, photosensitising characteristics of riboflavin make it a potential target for antimicrobial/anticancer photodynamic therapy[55,56], a multi-stage treatment that involves light and photosensitiser to destroy abnormal cells.

Human studies

Although animal studies have focused on the riboflavin antioxidant properties and its application in the management and/or prevention of various diseases so far, human research in this area is very scarce. In a cross-sectional study examining the association between dietary B vitamins intake and urinary monomethylarsenic and oxidative stress marker 15-F_{2t}-Isoprostane[57], dietary riboflavin intake was inversely associated with urinary 15-F_{2t}-Isoprostane in US adults; however, this association did not remain significant after adjusting for confounders. In the only available interventional research in this area, the effect of riboflavin supplementation in patients with Crohn's disease was investigated[58]. The results of this study indicated that redox status (plasma free thiols) was improved after three weeks of riboflavin supplementation in the patient with severe disease. Riboflavin also led to a significant decrease in inflammatory biomarkers and clinical symptoms of the disease[58]. Although this research was a quasi-experimental study (without a control group) in which the results are just before–after comparisons, its findings highlight the possible beneficial effects of this neglected antioxidant in various conditions which have a connection with oxidative stress, the area which remains to be elucidated through further randomised controlled trials.

Clinical outcomes

The beneficial effects of riboflavin on biochemical[22,23,26,31] and histopathological findings[22,23,25–27,30–32] and clinical symptoms[30,32], reported concurrent with alleviated oxidative stress in animals, suggest that riboflavin antioxidant activity may lead to promising clinical outcomes in these conditions. For example, improved histopathological findings in diabetes (liver and kidney)[31], hepatotoxicity[25,26] acute lung injury[22], renal toxicity[23], ulcerative colitis[27], spinal cord injury[30] and abdominal aortic aneurysm[32] have been indicated following riboflavin administration. Moreover, beneficial effects of riboflavin on renal function biomarkers in renal toxicity[23], biomarkers of liver function, inflammation and lipid profile in hepatotoxicity[26], biomarkers of renal and liver function and fasting blood glucose in diabetes[31] and markers of pulmonary permeability in lung injury[22] have been reported. Furthermore, as discussed in our previous review[12], riboflavin has also shown to have a protective effect against reperfusion oxidative injury (the tissue damage occurs after a period of ischaemia as a result of blood reperfusion) of different organs in experimental models, a finding which has been supported by the recent findings[59–62]. Therefore, the antioxidant effects of riboflavin could make it a potential therapeutic agent in mentioned conditions and other diseases in which oxidative stress is involved. However, a limited number of human studies have examined the effect of riboflavin as a therapeutic approach, warranting further human research in this regard[58].

Finally, the findings of reviewed studies provide further support for the antioxidative effects of riboflavin, although human studies are still very scarce. These effects of riboflavin could be mediated by antioxidant enzymes (through its role as an enzyme cofactor and as a gene expression regulator as well) and may be exerted through its direct free radical scavenging capacity. Riboflavin could also influence the concentration of other antioxidants such as vitamin C and vitamin E. It may also show peroxidative effect under photo illumination. Given the serious shortcoming in available human evidence, further research with stronger designs (i.e. randomised controlled trials or longitudinal studies) regarding probable beneficial effects of riboflavin in the prevention and management of diseases associated with oxidative stress are highly recommended.

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