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Riboflavin (vitamin B_2) and oxidative stress: a review

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Abstract

Oxidative stress is involved in the development of many chronic diseases. One of the main factors involved in oxidative stress reduction is increased antioxidant potential. Some nutrients such as vitamin C, vitamin E and carotenoids are known to act as antioxidants; however, riboflavin is one of the neglected antioxidant nutrients that may have an antioxidant action independently or as a component of the glutathione redox cycle. Herein, studies that have examined the antioxidant properties of riboflavin and its effect on oxidative stress reduction are reviewed. The results of the reviewed studies confirm the antioxidant nature of riboflavin and indicate that this vitamin can protect the body against oxidative stress, especially lipid peroxidation and reperfusion oxidative injury. The mechanisms by which riboflavin protects the body against oxidative stress may be attributed to the glutathione redox cycle and also to other possible mechanisms such as the conversion of reduced riboflavin to the oxidised form.

Key words: Riboflavin: Lipid peroxidation: Reperfusion oxidative injury: Glutathione peroxidase

Oxidative stress is defined as an increased generation of reactive oxygen species or a reduced ability to deactivate them. It is well known that oxidative stress is involved in the development of many chronic diseases such as cancer[1], CVD[2] and diabetes[3]. These diseases are considered as public health problems in both developed and developing countries. Many researches have focused on the relationship between oxidative stress and the above-mentioned diseases. One of the main factors involved in oxidative stress reduction is increased antioxidant potential. Numerous studies have investigated the antioxidant properties of some vitamins such as vitamin E[4], vitamin C[5] and carotenoids[6] and their effects on human health. One of the neglected antioxidant vitamins is riboflavin, which acts as a coenzyme for redox enzymes in FAD and FMN forms. Many studies have examined the effects of riboflavin on various diseases such as cataract[7], night blindness[8], some cancers (oesophageal, cervical and colorectal)[9–11], anaemia[12] and CVD[13]. One of the roles through which riboflavin can have a potential effect on human health is that as an antioxidant, which has not been investigated completely. Herein, studies that have examined the antioxidant properties of riboflavin and its effect on oxidative stress reduction are reviewed. PubMed and MEDLINE databases were searched for the published studies. The keywords used were riboflavin, oxidative stress, antioxidation, lipid peroxidation, antioxidant enzymes and glutathione peroxidase (GPx). The papers published before August 2012 are reviewed herein.

In this review, two aspects of the antioxidant properties of riboflavin are considered: (1) the role of riboflavin in the prevention of lipid peroxidation and (2) and the effect of riboflavin on the attenuation of reperfusion oxidative injury. Human and animal studies are summarised in Table 1.

Riboflavin at a glance

Riboflavin was discovered by Blyth in 1872 as a yellow fluorescent pigment in milk[14], but the vitamin property of this pigment was not established until the early 1930s[15]. Riboflavin is essential for nutrient metabolism and also for antioxidant protection[16]. Plants and some micro-organisms can synthesise riboflavin; however, it is an essential nutrient for human health and should be provided by the diet[15]. The Food and Nutrition Board (FNB) has recommended a daily intake of 0·3–0·4 mg riboflavin/d for infants, 0·5–0·9 mg riboflavin/d for children, 1·3 mg riboflavin/d for adolescents, and 1·4 mg riboflavin/d during pregnancy and 1·6 mg riboflavin/d during lactation for adults[16]. Riboflavin-rich foods are eggs, lean meat, milk and leafy vegetables[14,15].

Riboflavin deficiency is more prevalent in underdeveloped populations with a low intake of dairy products and meat; however, a higher prevalence of low serum riboflavin levels

Abbreviations: GPx, glutathione peroxidase; GR, glutathione reductase; MDA, malondialdehyde; SOD, superoxide dismutase.

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Table 1. Summary of the human and animal studies reviewed

Investigators	Study type	Outcome measure	Main findings
Adelekan & Thurnham[38]	Experimental	Erythrocyte GPx and SOD activities were compared between four groups of rats (riboflavin-deficient and riboflavin-sufficient rats infected or not with *Plasmodium berghei* malaria)	Erythrocyte GPx activity was slightly, but not significantly lower in riboflavin-deficient rats. Erythrocyte SOD activity was not affected by riboflavin deficiency
Bates[27]	Experimental	Glutathione levels, GPx and SOD activities, and TBARS levels were assessed in riboflavin-deficient, riboflavin-repleted and control rats	TBARS levels were increased in riboflavin-deficient rats. Glutathione levels and GPx and SOD activities were unaffected
Brady[33]	Experimental	Liver, muscle and erythrocyte GPx activity and erythrocyte GSH content were assessed in riboflavin-deficient and riboflavin-sufficient pigs	Riboflavin deficiency decreased muscle and liver GPx activity, but did not affect erythrocyte GPx activity and GSH content in riboflavin-deficient pigs
Das *et al.*[43]	Human study	MDA levels in riboflavin-deficient and riboflavin-sufficient children suffering from *Plasmodium falciparum* malaria were compared	Increased plasma MDA levels were observed in malaria patients with riboflavin deficiency than in riboflavin-sufficient malaria-infected control children
Dutta *et al.*[24]	Experimental	Lens GSH levels were assessed in riboflavin-deficient and riboflavin-sufficient rats receiving adriamycin or saline	GSH levels in riboflavin-deficient rats receiving adriamycin or saline were decreased compared with those in the control rats
Dutta *et al.*[39]	Experimental	Liver GPx activity and GSH content were measured in riboflavin-deficient and riboflavin-sufficient rats with or without ethanol administration	Unchanged liver GPx activity and increased liver GSH content were observed in riboflavin-deficient rats. Ethanol decreased GPx activity and GSH levels in riboflavin-deficient rats
George & Ojegbemi[45]	Human study	Malaria patients were assigned to receive chloroquine (group A) or chloroquine + B_2 (group B), and healthy individuals receiving no drug served as controls. Serum LHP levels were measured	A reduction in serum LPH levels was observed in both A and B groups. The reduction was higher in the riboflavin-treated group
Hirano *et al.*[34]	Experimental	Serum and lens GPx, SOD and GST activities, and lipid peroxide levels were assessed in riboflavin-deficient rats and riboflavin-sufficient control rats	Serum and lens lipid peroxide levels were increased and lens GPx activity was decreased in riboflavin-deficient rats compared with those in the control rats. SOD and GST activities did not exhibit a significant change
Horiuchi *et al.*[25]	Experimental	Lens glutathione content was assessed in riboflavin-deficient rats and riboflavin-sufficient control rats	Lens glutathione content in riboflavin-deficient rats were decreased significantly compared with that in the control rats
Huang *et al.*[28]	Experimental	Juvenile groupers (a kind of fish) were fed graded levels of riboflavin. GSH levels, GST, GPx, SOD and catalase activities, and TBARS levels were assessed	Riboflavin status did not affect liver GSH levels and GPx and GST activities. SOD and catalase activities were decreased and TBARS levels were increased in fish fed low amounts of riboflavin compared with those in other groups
Kodentsova *et al.*[44]	Human study	Riboflavin intake and serum MDA levels were assessed in 4–15-year-old children	A significant negative linear correlation between serum MDA levels and riboflavin intake was observed
Lee *et al.*[37]	Experimental	Liver catalase activity was compared between riboflavin-deficient rats and riboflavin-sufficient control rats. This measurement was repeated after riboflavin therapy in riboflavin-deficient rats	Decreased liver catalase activity was observed in riboflavin-deficient rats than in the control rats. Catalase activity was increased after B_2 therapy
Levin *et al.*[40]	Experimental	Erythrocyte SOD, GPx and catalase activities and lipid peroxidation product levels were measured in riboflavin-deficient rats and riboflavin-sufficient control rats	Erythrocyte GPx activity and lipid peroxidation product levels were higher in riboflavin-deficient rats than in the control rats, while erythrocyte SOD and catalase activities were not affected
Liang *et al.*[23]	Experimental	Blood MDA and GSH levels and SOD activity were measured in riboflavin-deficient rats and riboflavin-sufficient control rats	Riboflavin-deficient rats had lower SOD activity and GSH levels and higher MDA levels than the control rats
Powers & Thurnham[26]	Human study	Erythrocyte glutathione levels were compared between riboflavin-deficient and normal subjects	No significant differences in erythrocyte glutathione levels were observed between riboflavin-deficient and normal subjects
Rao & Bhat[29]	Experimental	Lens MDA levels, ascorbate levels, GPx, catalase and SOD activities, and GSH levels were assessed in riboflavin-deficient rats and riboflavin-sufficient control rats	Ascorbate levels were lower, while GPx activity and MDA levels were higher in riboflavin-deficient rats than in the control rats. Lens SOD and catalase activities and glutathione levels remained unaltered
Taniguchi[41]	Experimental	Serum and liver MDA levels were assessed in riboflavin-deficient rats and riboflavin-sufficient control rats	Serum and liver MDA levels were significantly higher in riboflavin-deficient rats than in the control rats
Taniguchi & Hara[22]	Experimental	GSH and lipid peroxide levels and GPx activity were measured in riboflavin-deficient rats and riboflavin-sufficient control rats. These measurements were repeated after riboflavin injection	Liver GPx activity and lipid peroxide levels were higher, while GSH levels were lower in riboflavin-deficient rats than in the control rats. After riboflavin injection, GSH and lipid peroxide levels returned to the levels found in the control rats
Tumkiratiwong *et al.*[35]	Experimental	SOD, catalase and GPx activities were compared between four groups of rats: non-infected rats fed a control diet; non-infected rats fed a riboflavin-deficient diet; *Trichinella spiralis*-infected rats fed a control diet; *T. spiralis*-infected rats fed a riboflavin-deficient diet	Riboflavin deficiency caused a reduction in SOD, catalase and GPx activities compared with the control groups

Table 1. *Continued*

Investigators	Study type	Outcome measure	Main findings
Wang *et al.*[32]	Experimental	SOD activity and MDA levels were compared in three groups: control rats fed a control diet; diabetic rats fed a control diet; diabetic rats fed a riboflavin-supplemented diet	Diabetic rats fed the riboflavin-supplemented diet had higher SOD activity and lower MDA levels than diabetic rats fed the control diet
Yagi *et al.*[42]	Experimental	MDA levels were assessed in three groups fed riboflavin-sufficient diet + linoleic acid, riboflavin-deficient diet + linoleic acid, and riboflavin-deficient diet + linoleic acid hydroperoxide	MDA levels were higher in riboflavin-deficient rats than in the riboflavin-sufficient rats. Linoleic acid hydroperoxide administration increased lipid peroxidation in riboflavin-deficient rats
Betz *et al.*[53]	Experimental	Brain oedema was evaluated after ischaemia in rats with and without riboflavin pre-treatment	Brain oedema was lower in riboflavin-treated rats than in the control rats
Iwanaga *et al.*[54]	Experimental	MDA, MPO and TNF-α (as markers of reperfusion injury) levels were assessed after heterotopic cardiac transplantation in riboflavin-treated rats and saline-treated control rats	Riboflavin reduced MDA, MPO and TNF-α levels
Mack *et al.*[55]	Experimental	Effect of riboflavin on reoxygenation-induced lactate dehydrogenase was assessed in isolated rabbit hearts	Riboflavin decreased reoxygenation-induced lactate dehydrogenase levels
Seekamp *et al.*[56]	Experimental	Indicators of lung injury (increases in vascular permeability, alveolar haemorrhage and neutrophil accumulation) and MDA levels were evaluated in three lung injury models with and without riboflavin therapy	Riboflavin administration decreased the indicators of lung injury in all the three lung injury models and decreased MDA levels in one of the models (induced by cobra venom factor)

GPx, glutathione peroxidase; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; GSH, reduced glutathione; MDA, malondialdehyde; LHP, lipid hydroperoxide; GST, glutathione *S*-transferase; MPO, myeloperoxidase.

has also been reported in some developed countries such as the USA and the UK[12]. People with some cancers, congenital heart disease and excessive alcohol intake are at a greater risk of riboflavin deficiency[14]. As riboflavin is destroyed by UV light exposure, UV therapy in infants with hyperbilirubinaemia could cause riboflavin deficiency[16]. When riboflavin supplementation is needed, an amount that is five to ten times the daily recommended amount is appropriate[14]. No toxic or adverse effects of intake of high riboflavin doses by humans have been reported so far[14,16]. However, it can be suggested that a high dose of riboflavin could cause an imbalance in the antioxidant state of human body. However, there is no strong evidence in this area, recommending further investigations to clarify the possible adverse effects of riboflavin intake in high amounts.

Role of riboflavin in the prevention of lipid peroxidation

Riboflavin as the glutathione reductase coenzyme

Glutathione reductase (GR) requires riboflavin in the FAD coenzyme form for its activity[16]. GR converts oxidised glutathione to the reduced form[17] (Fig. 1). FAD transports hydrogen from NADPH to oxidised glutathione to convert it into the reduced form[18] (Fig. 2). Reduced glutathione acts as an endogenous antioxidant in different cell types[19] and deactivates reactive oxygen species. Through its action, this peptide is deactivated as it is converted to the oxidised form[17]. Therefore, oxidised glutathione should be reduced by GR again to recover its antioxidant properties, the process in which riboflavin has a key role. Consequently, there is a possibility that riboflavin deficiency could affect the antioxidant properties of glutathione and lead to an impaired antioxidant potential of cells. One of the most important antioxidant activities of glutathione is the deactivation of peroxides such as hydroperoxide. This activity of glutathione is mediated by the action of GPx[20]. GPx transfers a hydrogen ion from reduced glutathione to lipid peroxide and produces oxidised glutathione and alcohol[21]. According to the mentioned mechanisms, it is expected that riboflavin deficiency could increase lipid peroxidation.

Effect of riboflavin status on glutathione content in tissues

The effect of riboflavin status on reduced glutathione content in tissues has been investigated in a limited number of studies. Taniguchi & Hara[22] reported that liver reduced glutathione

Fig. 1. Conversion of oxidised glutathione (GSSG) to the reduced form (GSH) by glutathione reductase. Glutathione reductase requires riboflavin in the FAD coenzyme form for its activity. G-6P-D, glucose-6-phosphate dehydrogenase.

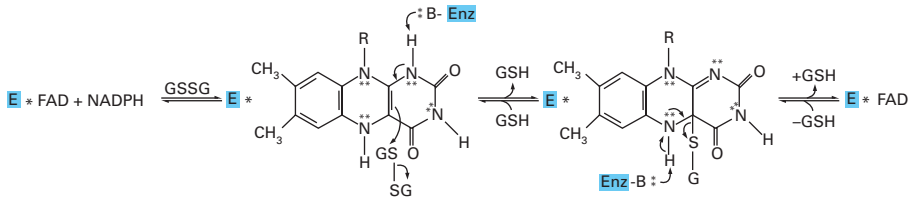

Fig. 2. Transportation of hydrogen from reduced NADPH to oxidised glutathione (GS–SG; GSSG) by FAD for conversion into the reduced form (GSH). Enz, enzyme. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

levels are decreased in riboflavin deficiency in rats. In an experimental study, a reduction in reduced glutathione levels was observed in rats fed a riboflavin-deficient diet for over 6 weeks[23]. Similar results have been reported by some other studies[24,25]. However, to our knowledge, there are a limited number of human studies that have examined the effect of riboflavin on reduced glutathione status. The only human study published has reported no significant difference in erythrocyte glutathione concentrations between normal and riboflavin-deficient subjects[26]. Furthermore, some animal studies also did not report any change in glutathione content in different tissues (such as the liver, lens and erythrocytes) in riboflavin-deficient animals[27–29]. A mechanism for the unchanged tissue glutathione content in riboflavin deficiency could be the increased biosynthesis of glutathione from its precursor amino acids as a compensatory action[30]. Another possible mechanism could be the ability of GR to maintain reduced glutathione concentrations in tissues even at low activity levels[31].

Effect of riboflavin status on the activity of antioxidant enzymes

As expected, some reports have indicated that riboflavin status can affect the activity of antioxidant enzymes including GPx, superoxide dismutase (SOD) and catalase. The results of a study that has investigated the effect of riboflavin therapy on diabetic cardiomyopathy indicated that riboflavin can increase SOD activity in the heart tissue[32]. A reduction in liver and muscle GPx activity in riboflavin-deficient pigs was reported by Brady *et al.*[33]. However, erythrocyte GPx activity was not found to be affected by riboflavin deficiency in this study. Another study that has investigated the effect of dietary riboflavin on the antioxidant defence mechanism of fish has reported a significant reduction in the SOD and catalase activities of riboflavin-deficient (12 weeks) fish compared with those of the control fish[28]. Moreover, some other animal studies have reported a similar effect of riboflavin on SOD[23,34,35], GPx[35,36] and catalase[35,37] activities. However, some animal studies did not observe any association between riboflavin status and antioxidant enzyme activity[38,39]. The results of the study carried out by Adelekan & Thurnham[38] indicated that the mean erythrocyte GPx activity in riboflavin-deficient rats was slightly, but not significantly lower than that in the control rats. In a study carried out by Dutta *et al.*[39], although riboflavin deficiency alone did not affect the activities of GPx and GR and increased the content of glutathione in the liver, ethanol administration (which can exacerbate oxidative stress) significantly decreased the activities of these enzymes and the content of glutathione in the liver of riboflavin-deficient rats compared with those in the liver of the riboflavin-sufficient control rats. However, there is a controversy regarding the results of different studies. In these studies, an increased GPx activity in the liver[22], lens[29] and blood cells[40] due to riboflavin deficiency has been reported. Increased lipid peroxidation has also been observed concurrent with an increased GPx activity. Therefore, it has been suggested that increased GPx activity could be a response to increased lipid peroxidation, resulting from riboflavin deficiency[29]. The possible allosteric effect of reactive oxygen species on GPx enzyme can lead to an increased activity through increased lipid peroxidation[40].

Finally, results indicate that riboflavin status could affect the activity of antioxidant enzymes, but some studies do not agree with this. Furthermore, all studies in this area have been limited to animals, recommending further investigations in human populations.

Effect of riboflavin status on lipid peroxidation

Animal studies

The results of several animal studies indicate not only the adverse effects of riboflavin deficiency on lipid peroxidation but also the desirable effects of riboflavin administration on it[22,23,27–29,32,34,40–42]. In studies in which riboflavin deficiency was induced in animals using a riboflavin-deficient diet, lipid peroxidation in different tissues was found to be significantly increased compared with that in the control groups[22,23,27–29,34,40–42]. In another study, it was shown that riboflavin administration could reduce the production of lipid peroxides (such as malondialdehyde (MDA)) or/and protein carbonyls in diabetic rats[32]. However, only one study[27] has reported that riboflavin deficiency increased MDA levels in the lens but did not affect MDA content in the liver in rats.

Human studies

A limited number of human studies have investigated the effect of riboflavin status on lipid peroxidation and confirmed the mentioned effect of riboflavin on lipid peroxidation. In a case–control study[43] on Indian children, it was found that plasma MDA levels of malaria patients with riboflavin deficiency were significantly higher than those of malaria-infected children with normal riboflavin status. However, in healthy children without malaria infection, no difference in

MDA levels was observed between riboflavin-deficient and riboflavin-sufficient children. Furthermore, in this study, riboflavin status was found to be inversely correlated with plasma MDA levels in malaria patients but not with those in healthy subjects. A possible mechanism explained by the researchers is the increased oxidative stress in malaria-infected patients, which causes a reduction in glutathione levels[43]. In a cross-sectional study carried out in Moscow, it was shown that there is a significant negative linear correlation between serum MDA levels and riboflavin intake[44]. The results of the study carried out by George & Ojegbemi[45] confirm these findings.

According to the findings of animal and human studies, riboflavin status appears to have an effect on the oxidative state of the body, in particular, on lipid peroxidation. However, it seems that the mechanism through which riboflavin can exert its antioxidant effect cannot be limited to the glutathione redox cycle and its relevant antioxidant enzymes. Although in most of the reviewed studies riboflavin status was found to be inversely related to lipid peroxidation, only in some of these studies, this relationship could be attributed to the role of riboflavin in the activities of glutathione reductase and related antioxidant enzymes. In these cases, a change in lipid peroxidation was found to occur simultaneously with an inverse change in the activity of antioxidant enzymes[23,32,34]. In spite of increased lipid peroxidation due to riboflavin deficiency, any change in the activity of antioxidant enzymes was not observed in the study carried out by Huang *et al.*[28]. In this study, increased liver MDA levels were observed in riboflavin-deficient fish without any change in liver GR and GPx activities and glutathione content. However, in this study, riboflavin deficiency was found to lead to reduced SOD and catalase activities. As H_2O_2 and superoxide (O_2^-) ions have been reported to have an inhibitory effect on SOD and catalase activities, respectively, the authors have suggested that riboflavin deficiency may lead to an overproduction of reactive oxygen species (obviously through a mechanism independent of the glutathione redox cycle) beyond the scavenging ability of SOD and catalase. Therefore, SOD and catalase could be inhibited by these free radicals. Bate *et al.*[27] also reported elevated lipid peroxidation in riboflavin deficiency with no change in glutathione levels and GPx activity. Moreover, in some studies, an increased GPx activity was observed simultaneously with increased lipid peroxidation in riboflavin deficiency[22,29,40]. As has been mentioned previously, this increased GPx activity, most probably, is a compensatory response to increased lipid peroxidation occurring in riboflavin deficiency.

It has been suggested that riboflavin may have antioxidant properties independent of its action in the glutathione redox cycle. Durusoy *et al.*[46] reported that the antimutagenic effect of riboflavin is independent of antioxidant enzyme activity. They suggested that the antimutagenic effect of riboflavin can, at least in part, result from its direct scavenging activity on free radicals produced by mutagens. *In vitro* studies have also indicated that riboflavin itself has an antioxidant nature, independent of its action as the GR coenzyme[47]. The suggested mechanism could be the deactivation of hydroperoxide through the reversion of riboflavin from the reduced form (dihydroriboflavin) to the oxidised form (Fig. 3) [48]. It is also possible that riboflavin exerts its antioxidant effect by reinforcing the effect of other antioxidants such as vitamin C[29]. In this study carried out by Rao & Bhat[29], a reduced concentration of vitamin C in the lens of riboflavin-deficient rats was reported, which is concurrent with increased lipid peroxidation. However, there is no strong evidence about the effect of riboflavin on other dietary antioxidants, suggesting further investigations.

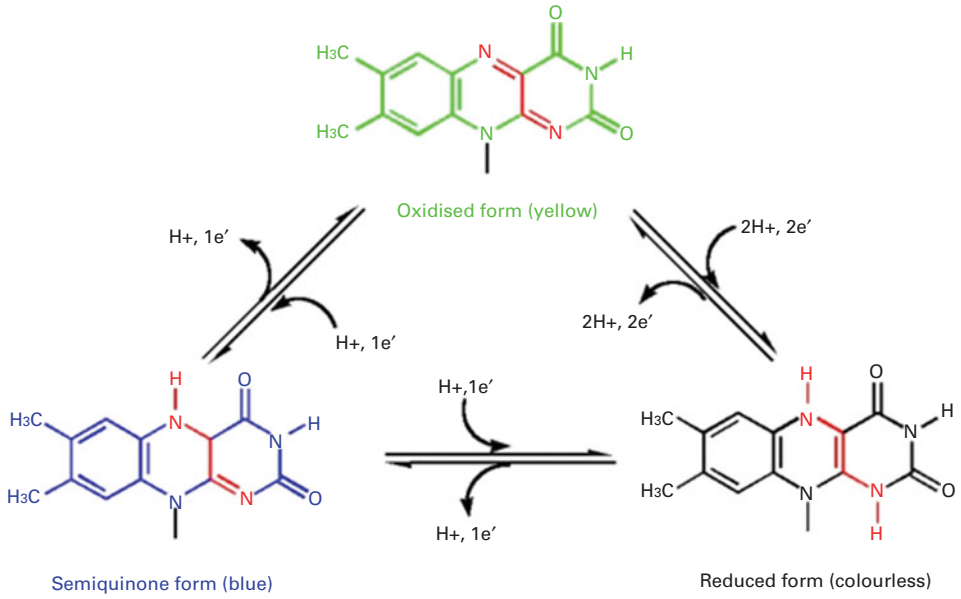

Fig. 3. Conversion of reduced riboflavin to the oxidised form – a possible mechanism for its antioxidant nature. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

Effect of riboflavin on the attenuation of reperfusion oxidative injury

Reperfusion injury is the tissue damage that occurs when blood flows into the tissue after a period of ischaemia[49]. It has been shown that free radicals[50,51] and inflammatory cytokines[52] have a key role in reperfusion injury process. Some reports[53–56] have indicated that riboflavin can alleviate oxidative injuries in these situations, probably through its ability to scavenge free radicals. Mack *et al.*[55] reported that riboflavin decreases reoxygenation injury in the heart of rabbits. It has also been shown that riboflavin could decrease oedema and brain injury after cerebral ischaemia[53]. The protective effect of riboflavin against reperfusion oxidative injury in other organs such as the lung and after cardiac allotransplantation in animal models has also been reported[54,56].

This protective effect of riboflavin could be attributed to dihydroriboflavin, produced by the flavin reductase activity of NADPH-dependent methaemoglobin reductase[57]. Dihydroriboflavin has been indicated to have the ability to reduce oxidised Fe in haemoproteins[58,59], which have been implicated in the oxidative damage of cells including reperfusion oxidative injury[57]. Although studies that have investigated the effect of riboflavin on reperfusion injury have obtained consistent results from experimental models, there are no firm data about its action in human body. Therefore, this needs to be clarified. Aside from this possible therapeutic effect of riboflavin, these findings could also introduce the riboflavin-dependent mechanism by which cells could protect themselves from oxidative damage in normal conditions[60] and provide further support for the antioxidant nature of riboflavin.

It is concluded that riboflavin could act as an antioxidant against oxidative stress, especially lipid peroxidation and reperfusion oxidative injury. The mechanisms by which riboflavin protects the body against oxidative stress may be attributed to the glutathione redox cycle and also to other possible mechanisms such as conversion of reduced riboflavin to the oxidised form. However, most of the investigations in this area are limited to experimental studies and, therefore, further investigations should examine this effect of riboflavin through observational and interventional studies in human populations.

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All authors contributed to the work and have approved the content of the submitted manuscript.

None of the authors has any conflicts of interest to declare.

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