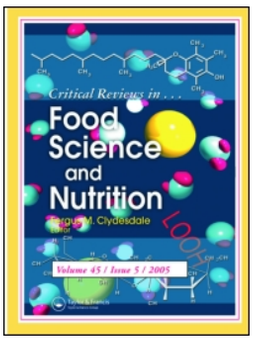

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Riboflavin and health: A review of recent human research

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ABSTRACT

There has lately been a renewed interest in Riboflavin owing to insight into its recognition as an essential component of cellular biochemistry. The knowledge of the mechanisms and regulation of intestinal absorption of riboflavin and its health implications has significantly been expanded in recent years. The purpose of this review is to provide an overview of the importance of riboflavin, its absorption and metabolism in health and diseased conditions, its deficiency and its association with various health diseases, and metabolic disorders. Efforts have been made to review the available information in literature on the relationship between riboflavin and various clinical abnormalities. The role of riboflavin has also been dealt in the prevention of a wide array of health diseases like migraine, anemia, cancer, hyperglycemia, hypertension, diabetes mellitus, and oxidative stress directly or indirectly. The riboflavin deficiency has profound effect on iron absorption, metabolism of tryptophan, mitochondrial dysfunction, gastrointestinal tract, brain dysfunction, and metabolism of other vitamins as well as is associated with skin disorders. Toxicological and photosensitizing properties of riboflavin make it suitable for biological use, such as virus inactivation, excellent photosensitizer, and promising adjuvant in chemo radiotherapy in cancer treatment. A number of recent studies have indicated and highlighted the cellular processes and biological effects associated with riboflavin supplementation in metabolic diseases. Overall, a deeper understanding of these emerging roles of riboflavin intake is essential to design better therapies for future.

KEYWORDS

Riboflavin; deficiency; risk; diseases; anemia; cataract; oxidative stress

Introduction

Each B-group vitamin acts in synergy to maintain the body's homeostasis by playing major roles in metabolic processes such as energy production and red blood cell formation. One of such essential vitamins, B_2 ("riboflavin") is an essential vitamin that generally acts as a cofactor; flavin adenine mononucleotide (FMN), and flavin adenine dinucleotide (FAD) in numerous enzymatic reactions in all forms of life and performs key metabolic functions by mediating the transfer of electrons in biological oxidation-reduction reaction (Fig. 1) (Alam et al., 2015). It is also involved in the metabolism of folate, vitamin B_{12}, vitamin B_6, and other vitamins, which explains why plasma riboflavin is a determinant of plasma homocysteine in certain genotypes associated with cardiovascular diseases, pregnancy complications, and cognitive impairment (Hustad et al., 2000; Hustad et al., 2002). It also helps to maintain the integrity of mucous membranes, skin, eyes, and the nervous system. During periods of dietary deprivation or physiological and pathological stress, humans are vulnerable to developing riboflavin deficiency (Alam et al., 2015). This may lead to a variety of clinical abnormalities, including growth retardation, anemia, skin lesions, renal damage, and degenerative changes in the nervous system which are discussed in later parts of review.

Riboflavin-deficient rat models have been used for many years to assess the biological effects of riboflavin. These models have indicated that riboflavin is important in the early postnatal development of the brain (Ogunleye et al., 1989), and gastrointestinal tract Williams et al., 1995, 1996; Yates et al., 2001, 2003). It is able to a modulate a host of metabolic activities, such as carcinogen induced DNA damage (Pangrekar et al., 1993; Webster et al., 1996), iron absorption and utilization (Powers et al., 1988, 1991; Butler et al., 1993; Powers et al., 1993, 1997), inflammatory (Lakshmi et al., 1991) and immune responses (Mazuret et al., 2013; Corbett et al., 2014; Schramm et al., 2014). These models also allow the extrapolation of data obtained in an animal model to human clinical data (Greene et al., 2014). Although requirement for riboflavin may be rare among bacteria, yet it is known to be an essential growth factor for many bacterial species (Koser, 1968). The biosynthetic deficiency correlates with the absence of riboflavin biosynthetic genes in the genomes of these organisms (Vitreschk et al., 2002). Ongoing research claims how gut microbiota of large intestine can be vitamin supplier to the body of host (Leblanc et al., 2012). In light of the recent research in the role of riboflavin and its health benefits and clinical uses, it is appropriate to arrange the information and reevaluate the important aspects of this particular vitamin and its public health significance.

Riboflavin absorption and metabolism

The knowledge of the mechanism and regulation of intestinal absorption of water-soluble vitamin is getting accumulated

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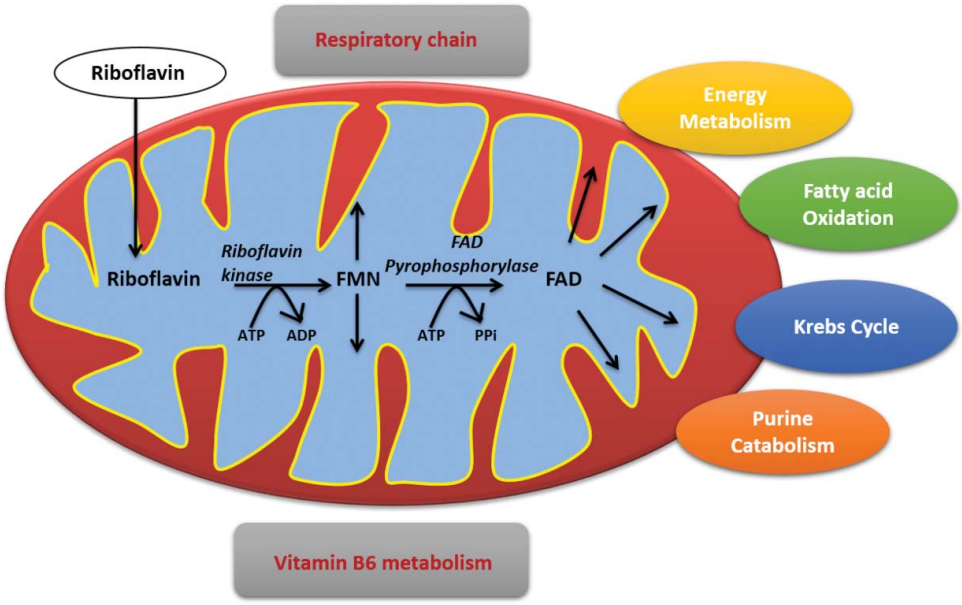

Figure 1. Conversion of riboflavin into active forms (FAD) and (FMN) and involvement in Metabolic pathways.

significantly with time. The intestinal absorption of riboflavin via specific carrier-mediated processes is well established (Fig. 2). These processes are regulated by a variety of factors and conditions, and the regulation involves transcriptional and/or post-transcriptional mechanisms (Glatzle et al., 1970). Studies have identified the role of riboflavin in the maintenance of normal intestinal homeostasis (Nakano et al., 2011). Thus, disturbance in normal riboflavin homeostasis is expected to lead to negative health consequences (Fig. 3) (Rivilin, 2010; Said and Ross, 2011). Previous studies have shown that the small intestinal and colonic riboflavin uptake process is specific and carrier mediated, and that all the three recently cloned riboflavin transporters (RFVT-1, 2, and 3; products of the SLC52A1, SLC52A2, and SLC52A3 genes, respectively) are expressed in the human gut, with expression of RFVT-3 being the predominant (Yao et al., 2010; Said, 2011; Said and Ross, 2011; Subramanian et al., 2011; Yonezawa and Inui, 2013; Said and Trebble, 2015). Utilizing rats fed on riboflavin deficient and oversupplemented (OS) diets as well as human intestinal epithelial (Caco-2 and NCM460) cells maintained under riboflavin deficient and OS conditions, a significant induction in intestinal riboflavin uptake was observed in deficiency while suppression was observed upon OS (Said and Mohammadkhani, 1993; Said and Ma, 1994; Said et al., 2000). An efficient and specific Na+-independent carrier-mediated mechanism located at the apical membrane domain is involved for riboflavin uptake in the small and large intestines. Many mutations in hRFT-2 have been identified in patients with Brown–Vialetto–Van Laere syndrome, a rare neurological disorder caused by mutations in this transporter, and are associated with riboflavin deficiency and suboptimal levels (Green et al., 2010; Bosch et al. 2011). In another study, thiol-group-specific reagents were also used to indicate the possible involvement of such groups in the function of its intestinal uptake process (Bates, 1993). Various

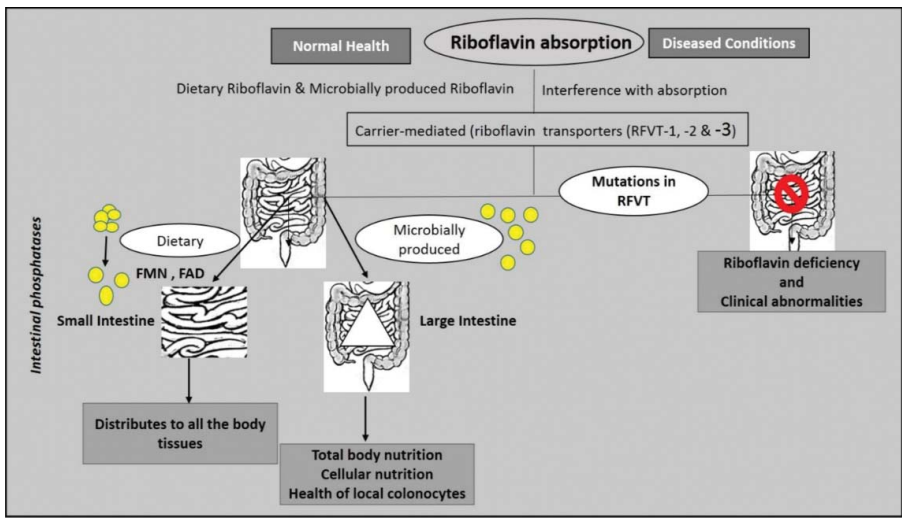

Figure 2. Intestinal absorption of dietary and microbially produced riboflavin.

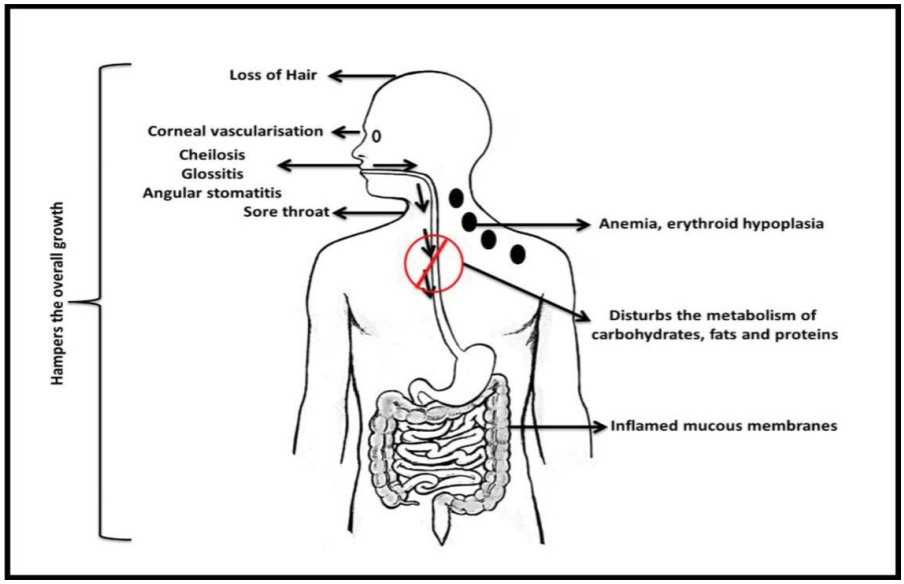

Figure 3. Implications of riboflavin deficiency on health.

studies have been conducted using cultured intestinal epithelial cells and rats where riboflavin deficiency leads to a significant upregulation in riboflavin uptake, whereas over supplementation with riboflavin causes a significant downregulation (Bates, 1993; Said and Khani, 1993; Said 2011).

The human intestine is exposed to two sources of riboflavin, one being dietary which is absorbed in the small intestine and the other bacterial which is supplied by the normal microflora of the large intestine and is absorbed in that region of the intestinal tract (Fig. 2) (Wrong et al., 1981). The bacterially produced riboflavin presents a considerable amount of free (absorbable) form (Iinuma, 1995). This source may contribute to total body vitamin nutrition, and especially toward the cellular nutrition and health of the local colonocytes. In the diet, a very small amount of riboflavin exists in the free form as isoalloxazine ring bound to a ribitol side chain, while major forms are FMN and FAD (Powers, 2003). The latter two forms are hydrolyzed to free riboflavin prior to absorption by intestinal phosphatases (Daniel et al., 1983). Being as a water-soluble vitamin, it does not stay in body for longer periods and hence it necessitates daily intake of vitamin. It is present in red blood cells, and seems to bind to a subfraction of immunoglobulins in plasma. When riboflavin intake meets the minimal daily requirement, it is only about 10-20% of total intake, which appears in the urine (Roughead et al., 1991). McCormick has described riboflavin metabolism in microbes and animals, and the biosynthesis and turnover of flavocoenzymes in pro- and eukaryotic organisms (McCormic, 2003).

Riboflavin deficiency

Generally, a balanced diet meets the riboflavin Recommended Daily Allowance corresponding to 1.4 mg/day for an adult man (Food and Nutrition Board, 1998) as well as human gut microbiota act as riboflavin supplier through *in situ* production (O'Brien et al., 2001). Although present in a wide variety of foods, such as dairy products, meat, eggs, and certain green vegetables, riboflavin deficiency (ariboflavinosis) still occurs in both developing and industrialized countries (Bamji, 1983; Blank et al., 2002). Riboflavin deficiency is one of the most common vitamin deficiencies in developing countries especially those with rice as the staple food coupled with insufficient milk and meat intake (Leblanc et al., 2011). It is usually due to dietary inadequacy but also occurs most frequently in people with long-standing infections, liver disease, and alcoholism. Consequences of ariboflavinosis in humans include sore throat, hyperemia, edema of oral and mucous membranes, cheilosis, and glossitis, which further leads to loss of hair, inflammation of the skin, cataract development, migrane prophylaxix, and decrease in hemoglobin (hb) status (Fig. 3). Worsening symptoms include a swollen tongue, seborrheic dermatitis, anemia, and impaired nerve function. Clinical symptoms of riboflavin deficiency are rarely apparent in developed countries but the subclinical stage of deficiency, characterized by a change in biochemical indices, is seen in a significant portion of the population (O'Brien et al., 2001). Though severe cases of ariboflavinosis are uncommon in most societies, yet subclinical manifestations are frequent amongst all subpopulation groups. Subclinical riboflavin deficiencies are only detectable by measuring the vitamin concentration in body fluids such as blood plasma and serum (Mensink et al., 2013). A study suggests that the current intakes of vitamins from foods lead to a relatively low risk of low intakes in all age and sex groups across European countries (Fabian et al., 2008). However, the same authors found that riboflavin intakes were more than 5% below the Lower Reference Nutrient Intake, young women and seniors being the most affected (Fabian et al., 2008). Flyn et al., (2003) reported that a considerable percentage (60%) of elderly people was at risk for riboflavin deficiencies. In western countries, poor riboflavin status seems to be of the most concern for elderly, alcoholic, unhealthy people specially suffering from kidney problem after dialysis and people having absorption problem, malnourish kids, strict vegetarians, athletes, pregnant women, lactating women, infants, and adolescents. Ariboflavinosis may not only occur due to dietary inadequacy, but also by the concurrent effects of some drugs, alcohol consumption, or

an increased requirement due to specific physiological conditions, such as pregnancy or breastfeeding, childhood, and elderly (Boisvert et al., 1993; Powers, 2003).

Implications of riboflavin deficiency on health

Migraine

With limited effectiveness of current preventive therapies, the use of complementary and alternative medicine has been increasing in headache management (Bajwa and Sabahat, 2008). It is not clear how riboflavin might prevent migraine headaches because no single theory or hypothesis can explain all the phenomena that occur with migraine (Maizels et al., 2004). One of the most frequently referenced mechanisms of action suggests that a mitochondrial dysfunction resulting in an impaired oxygen metabolism may play a role in migraine pathogenesis, and riboflavin can reverse this and therefore may be effective in the prevention of migraine (Schoenen et al., 1998; Sandor et al., 2000; Sandor et al., 2005). Sandor et al., (2000) suggested that a mitochondrial deficit in energy metabolism could play a role in the pathophysiology of migraine headaches. Riboflavin acts as a precursor in the mitochondrial electron transport chain and also serves as a cofactor in the Krebs cycle which supplies energy (Depeint et al., 2006) (Fig. 1). Hence, the hypothesis is that an increase in riboflavin availability might improve brain mitochondrial functions and help in migraine prevention (Sparaco et al., 2006). Studies indicate a potential role for riboflavin in migraine prophylaxis. Despite being safe, well tolerated, and inexpensive (Sherwood et al., 2014), there seems to be gender and age differences in response to riboflavin (Condo et al., 2009), possibly owing to differences in pharmacokinetics and riboflavin serum levels. Pharmacogenetic studies are going on, and there might be certain haplotypes of mitochondria that respond to riboflavin more readily (Foley et al., 2014). In previous reports, riboflavin reduced the frequency of migraine attacks and the number of headache days (Sandor et al., 2000). When it was given with magnesium and there was no difference between the experimental group and the placebo group in reduction of migraines or reduction of migraine days (Sandor et al., 2005). Evidences for the use of riboflavin in children are scanty. One retrospective study reported decreased migraine frequency in younger patients and decreased intensity in male patients (Condo et al., 2009).

Childhood neuropathy

In a study, a new gene responsible for Brown-Vialetto-Van Laere syndrome mSLC52A2 encoding riboflavin transporter RFVT2 was found (Fairweather-Tair et al., 1992). Authors demonstrated the reduction in riboflavin uptake and decline in riboflavin transporter protein expression because of the SLC52A2 mutations and the response to high-dose oral riboflavin therapy in patients with this mutations led to significant and sustained clinical and biochemical improvements. Hence, this study favored riboflavin supplementation to combat with the progression of this neurodegenerative condition.

Anemia

Riboflavin plays a role in erythropoiesis, improves iron absorption, and aids in the mobilization of ferritin from tissues (Boisvert et al., 1993). In previous studies, it was reported that riboflavin deficiency interferes with iron utilization, but not absorption and observed anemia was not due to a lack of iron but to impairment of hemoglobin synthesis (Fishman et al., 2003). The study revealed that, as hemoglobin concentration increased with riboflavin supplementation, there was no subsequent change in iron absorption. In animal studies, riboflavin has been shown to enhance iron absorption (Powers et al., 1993), and riboflavin deficiency can significantly increase the rate of gastrointestinal iron loss as well as decrease the mobilization of iron from stores (Powers et al., 1983). In humans, riboflavin deficiency has been shown to negatively affect iron utilization (Shi et al., 2014). The recent data from the Jiangsu Nutrition Study from China shows that inadequate riboflavin intake was associated with an increased risk of persistent anemia. There was a positive association between riboflavin intake and anemia among women, particularly in those below 50 years. Significant interaction between riboflavin intake and iron intake in relation to anemia risk was observed. Cross-sectional population studies show a relationship between riboflavin intake and anemia but prospective population studies are limited (Powers et al., 2011). Animal studies correlate riboflavin deficiency with decreased iron absorption and increased iron loss, and conflict with current human studies. More human studies are needed to ascertain the role of riboflavin in iron absorption and iron loss associated with riboflavin deficiency. Iron supplementation may not be the best way to prevent anemia in the population when riboflavin intake is inadequate. Riboflavin alone without additional iron supplement has been shown to improve hematologic status in young women in the United Kingdom (Srivastava et al., 1973). Therefore, correcting riboflavin deficiency can be one of the components in the prevention of anemia, and population-based measurement and intervention trials are required for this purpose (Powers et al., 2011).

Cataract

Riboflavin does appear to play an essential role in prevention of cataract formation. Riboflavin acts as a cofactor for glutathione reductase (GR), which is linked to cataract formation by decreased glutathione levels in the lens, and cataract formation increases in the elderly with riboflavin deficiencies. Riboflavin deficiency is implicated in the formation of cataracts due to the concentration of reduced glutathione in the lens and its ability to protect the tissue from oxidative damage (Srivastava et al., 1973). Decreased enzymatic activity of GR, the enzyme responsible for the production of glutathione is associated with riboflavin deficiency (Beutler, 1969). Glutathione exists in high concentrations in the lens in the reduced forms (GSH) and lenticular GSH is diminished in all the forms of human cataract (Skalka and Prchal, 1981). GSH protects the lens proteins against oxidative damage and hence again visual opacifaction of the lens. The enzyme (GR) reduces oxidized glutathione (GSSG) back to GSH. The levels of GR are also decreased in

cataractous lenses (Skalka and Prchal, 1981). Being a precursor of FAD, which is the coenzyme of GR, riboflavin is associated with decreased activity of GR (Beutler, 1969). In a study examining the B-vitamin status of cataract patients, it was revealed that 80% of the cataract patients had a riboflavin deficiency (Bhat, 1987). Another study supports a link between low levels of riboflavin and cataracts, as determined by EGRAC (Leske et al., 1995). A large, randomized, double-blind, controlled trial in China studied nutrient effects on cataract formation given a multivitamin/mineral or placebo for 5–6 years. The most protective effect was noted in the 65- to 74-year age group taking riboflavin (3 mg)/niacin (940 mg) daily (Sperduto et al., 1993). The connection between riboflavin and cataracts was supported from University of Georgia where a series of case reports revealed improvement in lens opacity with 15 mg riboflavin daily (Werbach and Moss, 1999). According to this study, the relationship between riboflavin deficiency and late-stage cataract formation was established ((Werbach and Moss, 1999). The authors concluded that riboflavin deficiency in the general public does not appear to be cataractogenic, although they did find an association between riboflavin deficiency and cataract formation in the elderly (Skalka and Prchal, 1981). Some studies have shown that riboflavin deficiency is linked with cataract formation (Ono et al., 1976), while other studies have opposed it (Srivastava and Beutler, 1972). Further studies are warranted to determine the exact relationship between riboflavin deficiency and risk for cataract formation. Recent study has shown that riboflavin can effectively penetrate into the corneal stroma through the endothelium after intracameral injection; it can even achieve the enrichment effect similar to that after the instillation on a deepithelialized corneal surface (Li et al., 2015).

Oxidative stress

Riboflavin is one of the neglected antioxidant that may have antioxidant role independently by conversion of reduced riboflavin to oxidized form or as a component of glutathione redox cycle (Ashoori and Saedisomeolia, 2014). There are studies, which confirm the two aspects of antioxidant nature of riboflavin through which this vitamin can protect the body against oxidative stress, especially by lipid peroxidation and repurfusion oxidative injury. The main antioxidant activity of riboflavin arises from its involvement in the conversion of glutathione. Glutathione is a very important antioxidant in the body that it is only effective when in the reduced form. However, GR enzyme requires riboflavin in the FAD coenzyme form for the conversion of oxidized glutathione to reduce form shown in Fig. 4 (Dringen et al., 2000; Gallagher, 2012). Reduced glutathione serves as an endogenous antioxidant in different cell types (Pompella et al., 1982). It also deactivates reactive oxygen species and peroxides such as hydroperoxides. Its activity is mediated by GPx (Hayes and McLellan, 1999). In some studies, it is reported that riboflavin deficiency led to reduction in liver glutathione levels in rats (Taniguchi and Hara, 1983). However, there are very limited human studies, which show the effect of riboflavin on reduced glutathione (Ashoori and Saedisomeolia, 2014). According to precious review reports, it is indicated the riboflavin also affects the levels of other antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase. Some studies have found that either riboflavin deficiency, or supplementation with riboflavin, affect levels of these antioxidant enzymes. According to previous studies it is confirmed that riboflavin can increase SOD activity in heart tissue (Wang et al., 2014), decrease in liver and muscle GPx activity in riboflavin deficient pigs (Brady et al., 1979), significant reduction in SOD and catalase activities of riboflavin deficient fish (Huang et al., 2010). There are studies, which indicate that riboflavin could affect the activity of antioxidant enzymes but some studies do not agree with this statement (Ashoori and Saedisomeolia, 2014). Various animal studies show the negative effect of riboflavin deficiency on lipid peroxidation (Taniguchi and Hara, 1983; Horiuchi et al., 1984; Levin et al., 1990; Huang et al., 2010) as well as desirable effects of its supplementation on it. A very few human studies have been reported for the similar effect. Repurfusion injury is the tissue damage due to ischemia (Carden and Granger, 2000). Free radicals (Ambrosio et al., 1991; Bolli et al., 1995) and inflammatory cytokines (Kukielka, 1995) (94) play important role in this process. The reports suggest that riboflavin can alleviate oxidative injuries by scavenging the free radicals (Iwanaga et al., 2007). Riboflavin is important for the antioxidant status within cell systems, both by itself and as part of the GR and xanthine oxidase system. However, most of the investigations in this area are limited to experimental studies and therefore, interventional studies in human population are required.

Diabetes mellitus

Increasing evidences (Alam et al., 2015) in both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of type-2 diabetes mellitus. The study has suggested that supplementation with dietary riboflavin might help in the reduction of diabetic complications. The decrease in the renal function test and liver function test markers in the diabetic mice upon riboflavin treatment suggested that the riboflavin treatment reduces the risk of diabetic complications by reducing inflammation caused by oxidative stress. In this study, the liver and kidney from untreated diabetic mice showed extensive damage in the hepatic microstructure including altered contour of hepatocytes, sparse presence of cell organelles and poorly maintained hepatic cords, the renal tubule and the glomeruli were also affected severely. These changes were greatly reduced by administration of riboflavin to the animals. Moreover, authors concluded that the altered lipid peroxidation, protein carbonylation, antioxidant levels, and tissue damage in the diabetic condition were ameliorated by riboflavin treatment through reduction in reactive oxygen species induced inflammation. In another study recent study, it has been reported that by altering gut microbiota artificial sweetener induces glucose intolerance (Suez et al., 2014). The reduction in hyperglycemia points toward the role of riboflavin in the absorption of sugar from intestine and thus indicting the role of microflora in supplementing vitamins. This is the first ever study to associate riboflavin with hyperglycemia. However, much has to be deciphered in this direction to support this observation.

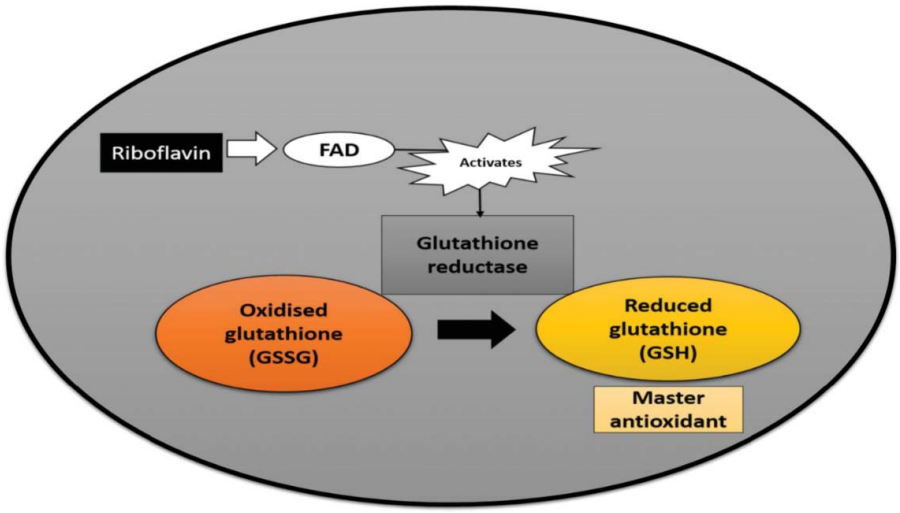

Figure 4. FAD activates the GR for conversion of GSSG to GSH.

Hypertension

Hypertension is the leading risk factor for mortality worldwide (Lopez et al., 2006), and is responsible for an estimated 8 million premature deaths per year (Lawes et al., 2008). The exact pathophysiology of hypertension remains unclear, and multiple lifestyle and genetic risk factors have been identified. The genome-wide association studies have identified several genetic loci associated with BP variation (Levy et al., 2009; Newton-Cheh et al., 2009). One such locus is near the gene encoding methylenetetrahydrofolate (MTHFR), the folate metabolizing enzyme required for the formation of 5-MTHFR.

Riboflavin acts as a cofactor (FAD) for MTHFR, and the decreased enzyme activity in individuals with the TT genotype results from a greater propensity for loss of FAD (Guenther et al., 1999; Yamada et al., 2001). Supplementation with riboflavin lowers homocysteine concentrations, specifically in individuals with the TT genotype (McNulty et al., 2006), and therefore, appears to overcome the increased tendency for loss of FAD from the active site. This study has suggested the role for riboflavin in modulating blood pressure, specifically in individuals with the MTHFR 677TT genotype. Supplementation with riboflavin can lower blood pressure in hypertensive individuals with the TT genotype but not in those with the CC or CT genotype (Horigan et al., 2010). By stabilizing the variant MTHFR enzyme, it is possible that riboflavin supplementation could restore 5-MTHFR concentrations in vascular cells, and improve nitric oxide availability, which, in turn, would improve endothelial function, and lower blood pressure.

Cancer

There have been several studies, which relate the riboflavin deficiency with the inhibition in tumor growth in experimental animals and possibly in man, but the precise mechanisms involved have not been revealed. Some studies indicate that riboflavin deficiency increases the risk of cancer at certain sites, while others suggest a possible attenuating effect of riboflavin in the presence of some carcinogens (Rivlin, 1973). Riboflavin has gained much less attention, but there is increasing interest since the role of flavins in folate metabolism and possible synergy of a protective effect between these two vitamins is well known (Powers, 2005). Folate has a key role to play in the mechanisms like DNA synthesis, repair, methylation, which are the basic of mechanistic explanation for a putative role of folate in cancer prevention. The activity of folate is suggested to be modulated by genotype for the common C677T thermolabile variant of methylene tetrahydrofolate reductase (*MTHFR*), homozygosity for which leads to lower enzyme activity, lower plasma and red blood cell folate, and elevated plasma homocysteine. Riboflavin, as FAD, is a cofactor for MTHFR provides the evidence for some interactions among riboflavin status, folate status, and genotype in determining plasma homocysteine, a functional marker of folate status. The *MTHFR* C677T polymorphism appears to interact with folate and riboflavin in modulating cancer risk and this interaction varies according to the cancer site. Most evidences points to a protective effect of this polymorphism for risk of colorectal cancer, but the effect on cervical cancer risk is not clear (Powers, 2005).

Rao et al. (1999) conducted a study that evaluated plasma levels of riboflavin carrier protein (RCP), an estrogen-inducible protein, in patients suffering from breast adenocarcinoma (Rao et al., 1999). According to this study, the serum RCP level of >1.0 ng/ml was highly predictive of the presence of breast cancer, proclaiming that the levels of RCP may be useful as new markers for breast cancer, even in early stages. In another study, animal studies have shown that riboflavin deficiency can lead to disruption of the integrity of the epithelium of the esophagus (Foy et al., 1972) and some epidemiologic studies have recognized a relation between esophageal cancer and diets low in riboflavin Foy et al., 1972). Nevertheless, not all studies support this relation (Siassi et al., 2000). Riboflavin deficiency in rats exposed to hepatocarcinogens leads to increased DNA strand breakage as well as carcinogen binding to DNA is increased in riboflavin-deficient rats (Pangrekar et al., 1993). Poor riboflavin status has also been implicated as a risk factor for cervical dysplasia, a precursor condition for invasive cervical cancer (Liu et al., 1993). Tsao et al. (2007) carried out a study to examine the oxidative stress and B vitamins status in non–small-cell lung cancer patients at different stages. The

reduced levels of vitamin B_2 and B_6 in red cells, inversely correlated with plasma ghrelin, underline the importance of these vitamins in patients with lung cancer (Tsao et al., 2007). Riboflavin deficiency has been related to increased susceptibility to cancer (Bareford et al., 2005). On the other hand, high doses of riboflavin supplementation were able to reverse the effects of hepato-carcinogens in rodents (Webster et al., 1996). The results obtained so far are not convincing but are optimistic. To understand the role of vitamins and other micronutrients in the prevention and treatment of cancer, more studies are needed to clarify the mechanism of action (Mamede et al., 2011).

Riboflavin: A promising adjuvant in cancer treatment

Various contemporary investigations strongly suggest that this vitamin has tremendous potential to be used in improving the chemotherapeutic potential of major anticancer drugs (Naseem et al., 2015). Cisplatin (CP), though one of the most valued anticancer drugs against various forms of cancer has limitation because of many side effects (Hassan et al., 2013). One study suggests that a moderate amount of riboflavin can persuade the extrinsic pathway of apoptosis, while higher amounts can activate additional cell death mechanisms, such as the intrinsic pathway of apoptosis by downregulating many antiapoptotic factors as well as upregulating many other apoptosis inducing factors as shown in Fig. 5 (Hassan et al., 2013). Interestingly, its anti-inflammatory and antipain properties have also been documented and these are highly desired requisites for better cancer treatment (Bertollo et al., 2006). CP exerts most of its toxic effects by oxidative and nitrosative stress so, clubbing ribophototherapy with chemotherapy involving CP can shift the redox status favoring better cancer treatment. Riboflavin is either directly involved in decreasing both types of the stress by replenishment of cellular reductants and antioxidant enzymes or, riboflavin does not allow CP to cause any organ injury by halting the derogatory inflammatory response (Hassan et al., 2012). Thus, riboflavin can induce apoptosis as well as autophagy and can be useful for management of cancer treatment under chemoradiotherapy. According to Hassan et al. (2013) this strategy can be used to address both aspects of the disease by facilitating CP to bind DNA in the cancerous cells on one hand, while decreasing the cellular stress level favoring the apoptosis induction on the other hand (Hassan et al., 2013). The recent study has investigated the ameliorative potential of riboflavin on cisplatin-induced toxicity in mice (Naseem et al., 2015).

Controlled inactivation of recombinant viruses with riboflavin

Inactivated viruses are important tools for vaccine development and gene transfer. 8-Methoxypsoralen and long-wavelength ultraviolet irradiation (LWUVI) inactivates many viruses. Toxicity limits its use in animals and humans. Toxicological and photosensitizing properties of riboflavin make it suitable for virus inactivation in preparations for biological use.

DNA and RNA viruses can be inactivated by riboflavin and LWUVI and used in physiological systems sensitive to other photochemicals (Sakurai et al., 2007). Riboflavin is a molecule capable of intercalation and oxidation of DNA and RNA in the presence of UV light (Kumar et al., 2004). Unlike psoralen, commonly used for inactivation of adenovirus, it significantly altered the activity of hepatic CYP3A2, an enzyme that plays a key role in drug metabolism. Riboflavin, a reagent with similar properties but which does not alter CYP3A2 was found to successfully inactivate a variety of recombinant viruses with minimal changes in capsid structure and an elemental dietary component with a low toxicity profile (Corbin, 2002). Riboflavin in combination with UV light has been found to inactivate a large number of pathogens such as extra- and intracellular human immunodeficiency virus (HIV), pseudorabies virus, West Nile virus, parvovirus, Gram positive bacteria (*Straphylococcus epidermidis*), Gram negative bacteria (*Escherichia coli*), and *Leishmania* protozoa without inducing adverse side effects (Corbin, 2002; Ruane et al., 2004; Cardo et al., 2006; Pelletier et al., 2006). This property of riboflavin can serve as an important tool in preclinical pharmacological and toxicological assessment for many vaccination and gene therapy protocols and is inexpensive, nontoxic and poses no risk to biological systems, medical personnel, and the environment.

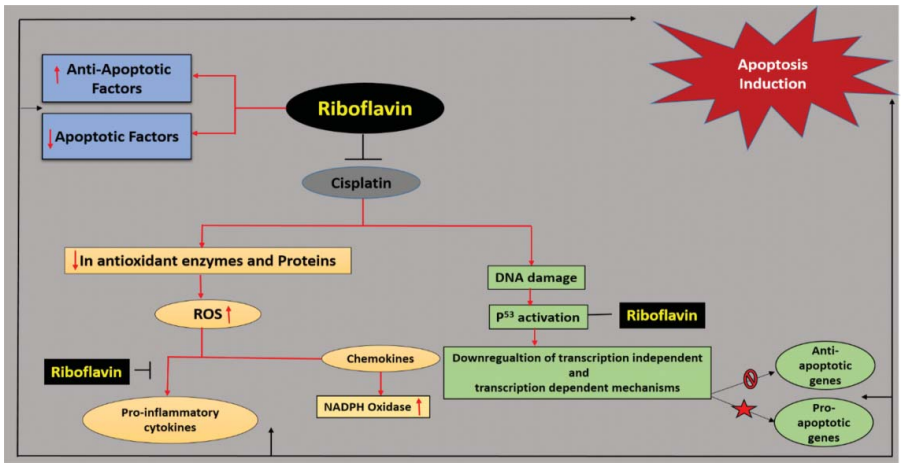

Figure 5. Role of riboflavin as an adjuvant in cisplatin based chemo radiotherapy (modified and adapted from Hassan et al., 2013).

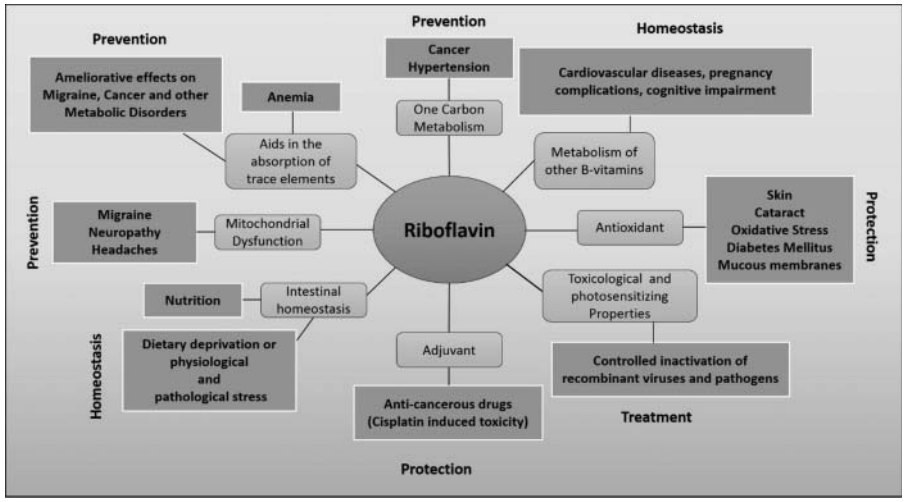

Figure 6. Physiological functionality of Riboflavin.

Riboflavin produced by food-grade microorganisms

In the last years, significant advances have been achieved in the field of *in situ* bacterial overproduction of the B group vitamins, including riboflavin. Now, driven by economic and environmental considerations, the fermentation-based methods are taking place of chemical synthesis of riboflavin (Stahmann et al., 2000). There are numbers of reports which showed the riboflavin production by various lactic acid bacteria (LAB) (Leblanc et al., 2005, 2006; Jayashree et al., 2010; Russo et al., 2014; Valle et al., 2014; Thakur and Tomar, 2015a; Thakur et al., 2015; Thakur and Tomar, 2015b). Further research, exploration, and development of riboflavin producing LAB for food applications should be encouraged. More knowledge is required about riboflavin forms produced and their intestinal absorption and riboflavin-producing microorganisms. By using proper selection strategies, the riboflavin production ability of microbial strains used in the production of fermented milk starters, formulations may be optimized for natural enhancement of the riboflavin content in food products. Such products would provide economic benefits to food manufacturers since increased "natural" riboflavin concentrations would be an important value-added effect without increasing production costs. At the same time, consumers would also be benefited from such products since they can boost their riboflavin intakes by consuming such products that form part of their normal lifestyle.

Conclusions

There has been an increase in evidences in both experimental and clinical studies that suggest that riboflavin plays a major role in the prevention and treatment of many diseased conditions (Fig. 6). However, systemically designed more human trials are required to deeply decipher the mechanism of action of riboflavin with respect to various health disorders as well as to further evaluate the effects of riboflavin on overall human health. Riboflavin deficiency is endemic in population with certain age group, gender, diseased conditions, and certain food habits. Thus, it may be advisable to supplement their daily intake of riboflavin. Development of novel fortified and functional riboflavin enriched foods for consumers appears to be healthy proposition. Furthermore, an increasing awareness of the consumers prompts the food makers to implement alternative environment-friendly solutions in the production processes.

Conflicts of interest

There is none to declare.

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