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Małgorzata Szczuko*, Maciej Ziętek, Danuta Kulpa, Teresa Seidler

Riboflavin - properties, occurrence and its use in medicine

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Abstract: Riboflavin is built on an isoalloxazin ring, which contains three sixcarbon rings: benzoic, pyrazine and pyrimidine. Riboflavin is synthesized by some bacteria, but among humans and animals, the only source of flavin coenzymes (FAD, FMN) is exogenous riboflavin. Riboflavin transport in enterocytes takes place via three translocators encoded by the SLC52 gene. Deficiency of dietary riboflavin has wide ranging implications for the efficacy of other vitamins, the mechanism of cellular respiration, lactic acid metabolism, hemoglobin, nucleotides and amino acid synthesis. In studies it was found that, pharmacologic daily doses (100 mg) have the potential to react with light, which can have adverse cellular effects. Extreme caution should be exercised when using riboflavin as phototherapy in premature newborns. At the cellular level, riboflavin deficiency leads to increased oxidative stress and causes disorders in the glutathione recycling process. Risk factors for developing riboflavin deficiency include pregnancy, malnutrition (including anorexia and other eating disorders, vegetarianism, veganism and alcoholism). Furthermore, elderly people and athletes are also at risk of developing this deficiency. Widespread use of riboflavin in medicine, cancer therapy, treatment of neurodegenerative diseases, corneal ectasia and viral infections has resulted in the recent increased interest in this flavina.

Keywords: riboflavin; dietary supply; deficiency; application; properties.

***Corresponding author: Małgorzata Szczuko**, Department of Biochemistry and Human Nutrition, Pomeranian Medical University in Szczecin, Poland, E-mail: malgorzata.szczuko@pum.edu.pl

Maciej Ziętek: Clinic of Perinatology, Obstetrics and Gynecology Pomeranian Medical University in Szczecin, Poland

Danuta Kulpa: Department of Genetics, Plant Breeding and Biotechnology, West Pomeranian University of Technology in Szczecin, Poland

Teresa Seidler: Department of Human Nutrition, West Pomeranian University of Technology in Szczecin, Poland

Structure and properties of riboflavin

Riboflavin (7,8-Dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl] benzo[g]pteridine-2,4-dione) is built on an isoalloxazin ring, which contains three sixcarbon rings: benzoic, pyrazine and pyrimidine. The isoalloxazin ring is additionally connected with alcoholic sugar – ribitol. Riboflavin may create two co-enzymatic forms: as a flavin mononucleotide (FMN) and flavin dinucleotide (FAD) as well. The synthesis of FMN occurs by phosphorene connection and replacement in the hydroxy group site with catalytic enzyme activity – riboflavin kinase (RFK) [1]. FAD synthesis occurs as a result of further phosphorylation with the participation of ATP, which is presented on the figure 1. In its purest form, riboflavin creates orange-yellow crystals, without an acute melting point. At 240°C these crystals darken and melt in the temperature range of 274-282°C. Small yellow bitter-tasting riboflavin needles are sparingly soluble in water at room temperature, whereas at 100°C riboflavin solubility increases more than 18 times. Additionally, riboflavin dissolves well in a 10% urea solution, 30% p-aminobenzoic acid sodium salt aqueous solution and in nicotinamide aqueous solution. In fat and fatty solvents, it does not dissolve at all whereas in an acid environment it is fairly durable but in an alkaline environment, especially under the ultraviolet light influence, it easily decomposes [2, 3].

Absorption, transport and riboflavin excretion

Riboflavin is synthesized by bacteria but among humans and animals the only source of flavin coenzymes (FAD, FMN) is exogenous riboflavin [1]. Riboflavin is well absorbed in the stomach and small intestine where it is released from protein in food under the influence of hydrochloric acid. Absorption is largely carried out by diffusion; however, it has been reported that the absorption process of riboflavin, as well as its analogs

Figure 1: Metabolism of riboflavin [24]

(lumiflavin), can also take place in the colon [4]. In the intestinal epithelium, riboflavin undergoes ATP-dependent phosphorylation to generate the active form of FMN. Following another reaction of FMN with ATP (in which AMP is transferred to FMN) FAD is formed (Figure 1). Both FMN and FAD are prosthetic groups of oxidoreductive enzymes (i.e. flavoproteins). In the role of coenzymes, the isoalloxazine ring of flavoproteins undergoes a reversible reduction with subsequent creation of reduced form of FMNH₂ and FADH₂ [3]. Riboflavin transport in enterocytes takes place via three translocators: RFVT3 located on the apical membrane and RFVT1 and RFVT2 on the basolateral membrane [5]. RFVT1, however, is most strongly expressed in the placenta and RFVT2 is expressed in the brain, and all of them are encoded by the SLC52 gene [6].

Galactoflavin and chloriboflavin are riboflavin antagonists which enter the same binding site but do not display the same biological activity as riboflavin. Karande *et al.* have shown that *riboflavin* carrier protein (RCP) concentrations are dependent on estrogen activity [7]. This effect was discovered following observation of the fluctuating RCP blood concentration in healthy women before and after menopause as well as healthy women at different points of their menstrual cycles. This study found that the RCP concentration is regulated by estrogen activity due to physiological conditions and changes [7]. Therefore, it seems that taking oral contraceptives containing synthetic estradiol and progestogens may affect the body's supply of riboflavin. Excess riboflavin is mainly excreted in the urine, mostly in an unchanged form as free riboflavin. In some individuals with hypothyroidism

the disorder impedes the conversion of riboflavin into its coenzymatic form [8]. It has been proven that the thyroid hormone, thyroxine, regulates the enzymatic conversion of riboflavin to active forms of coenzyme in adult humans [9].

The physiological role of riboflavin

Human beings utilize riboflavin to metabolize fats, proteins and carbohydrates in order to generate energy but also as an antioxidant to maintain normal function of the immune system with the help of two co-enzymes. The flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are prosthetic groups of oxidoreductive enzymes which are widely distributed in the mammalian metabolic system. The isoalloxazine ring of flavoproteins is reversibly reduced to reduced forms of FMNH₂ and FADH₂, which may react with molecular oxygen and in contrast to nicotinamide coenzymes, may also be involved in reactions associated with the transfer of one (semichinon) or two electrons. Currently, over 100 flavin enzymes are known, some of which are listed below along with their functions:

- Glutathione reductase maintains glutathione in a reduced form on the pentose phosphate pathway in erythrocytes.
- α -amino acid oxidase is present in the liver and kidneys and participates in deamination of amino acids (release of ammonium with subsequent α -keto acid synthesis).

- D-amino acid oxidase degrades alien D-isomers of amino acids, formed as a result of biological activities of bacteria.
- Xanthine oxidase is involved in purine degradation (xanthine) to uric acid.
- Glucose oxidase catalyzes the oxidation of D-glucose to acid lactone D-gluconic.
- Pyridoxal phosphate oxidase is involved in the synthesis of coenzymatic form of vitamin B6.
- Dihydrofolate reductase is involved in the synthesis of coenzymatic forms of folic acid.
- Methemoglobin reductase maintains hemoglobin in a reduced form.
- Aldehyde dehydrogenase is involved in the degradation of highly toxic aldehydes.
- Glycerol-3-phosphate mitochondrial dehydrogenase transfers reducing equivalents from the cytoplasm to the mitochondria.
- Succinate dehydrogenase catalyzes the oxidation of succinic acid to fumarate in the Krebs Cycle.
- Succinyl dehydrogenase is involved in the citric acid cycle.
- Acyl-CoA dehydrogenase transfers electrons involved in the oxidation of fatty acids in their oxidation (β -oxidation).
- Dihydrolipoyl dehydrogenase participates in the oxidative decarboxylation (by dehydrogenation of the liponate) of pyruvate and α -ketoglutarate,
- NADH dehydrogenase, an important component of the respiratory chain in the Mitochondria, is involved in the transfer of electrons and protons from nicotinamide coenzymes to cytochrome C. NADH dehydrogenase therefore plays a key role in the processes of biological oxidation and energy production.

The activity of all these enzymes is impaired when riboflavin is deficient [2, 3]. The result of this deficiency is disturbances in cellular respiration, lactic acid metabolism, hemoglobin, nucleotides and amino acid (specifically glycine, valine and leucine) synthesis as well as conversion of tryptophan into niacin resulting in activation of pyridoxine [10]. Primary deficiency of dietary riboflavin has wide-reaching implications for the function of other vitamins because flavoenzymes are directly linked to the metabolism of both fat and water soluble vitamins, namely, vitamin B-12 (cobalamin), folic acid, niacin, pyridoxine, vitamin K and vitamin D [11]. Additional consequences of riboflavin deficiency include dysregulated transmission of hormonal and cellular information due to abnormal levels of steroid hormones.

Abnormal catabolism of adrenaline, noradrenaline, serotonin and detoxification of xenobiotics may also result from such a deficiency. Riboflavin is also implicated in biochemical changes in the retina which are essential for maintaining good eyesight. Furthermore, riboflavin aids the elimination of poisonous chemicals from the human body as a consequence of alcohol consumption and tobacco smoking. Elimination from such substances has been found to be important in the prevention of esophageal cancer. Riboflavin also functions in conjunction with vitamin A to regulate the activity of the mucous membranes of the gastrointestinal tract, respiratory tract, epithelium of blood vessels and skin. Levit *et al.* demonstrated the antiinflammatory effect of riboflavin following administration of *Lactobacillus plantarum* CRL2130, a strain that overexpresses riboflavin, in mice [12]. A previous study demonstrated a reduction in the neurotoxicity of chromium following the simultaneous administration of riboflavin (5 mg) in rats [13]. A well characterised functions of riboflavin is its role in the conversion of glutathione by NADH dependant (a reduced form of nicotinamide adenine dinucleotide phosphate) glutathione reductase, with riboflavin constituting its prosthetic group. In an attempt to defend against excessive accumulation of oxidized glutathione (GSSG), cells remove GSSG by active transport leading to a reduction of total intracellular glutathione. Glutathione is believed to be involved in the metabolism of leukotrienes which, as well as having antioxidative activity, are mediators of inflammatory processes [14]. Mulherin *et al.* demonstrated that in patients with rheumatoid arthritis with active synovitis, a stimulation of glutathione reductase (RG) activity occurs as well as riboflavin deficiency [15]. This riboflavin deficiency could be induced by both the increased demand placed on the body due to inflammation as well as insufficient intake. The aforementioned study also showed that riboflavin uptake influenced the persistence of inflammation [15]. However, results from Ullegaddi *et al.* indicate the benefits of increasing the volume of antioxidants in the form of supplements with vitamins C, E and B vitamins immediately after myocardial infarction, which reduces damage and has anti-inflammatory effects [16]. Free radicals, also known as reactive oxygen species (ROS), formed as a result of inflammation play an important role in negotiating cell apoptosis and necrosis, [17, 18].

Glutathione is found in many products but it can not be absorbed from all sources [19] and therefore must be produced by each cell separately. It seems, however, that consuming foods rich in glutathione increases its synthesis. This fact in combination with other research,

suggests that the glutathione concentration present in cells can be modeled according to its availability from food, where the richest products in GSH are vegetables, fruits and fresh meat [20]. Interestingly, manganese ions selectively inhibit RG activity and, in this way, may impair enzymatic defense pathways which are associated with oxidative stress [21]. However, in studies focused on the longevity of centenarians, it was found that the independent factor of long life incidence increases the antioxidant capacity of the blood, mainly due to the increased activity of glutathione reductase and catalase, which interact with tocopherols thereby delaying the aging process [22].

Toxicology

Riboflavin is non-toxic, however, in the presence of ultraviolet (UV) light or visible light it produces ROS, which causes a greater risk of tissue damage during phototherapy used, among others, during treatment of various tumors and cancers [23]. This effect may be additionally increased by the presence of Cu (II) in the Haber-Weiss reaction which necessitates the use of extreme caution when phototherapy is used to treat hyperbilirubinemia in premature newborn infants [24]. Pharmacological daily doses of riboflavin (100 mg) have the potential to react with light which can lead to adverse cellular effects. The photoreactive properties of the isoalloxazine ring causes free riboflavin to become a strong oxidizer by producing potentially toxic peroxides or other ROS and/or by forming an atypical tryptophan metabolite. The tryptophan-riboflavin adduct has been shown to exhibit hepato- and cytotoxic effects and to be particularly detrimental to the integrity of lens and retina proteins which are permanently exposed to light [11].

Occurrence in nature and food products

Riboflavin is synthesized *de novo* by many plants and microorganisms including *Schizophyllum commune* [25], yeast-like *Eremothecium ashbyii*, *Ashbya gossypii*, *Candida*, *Clostridium acetobutylicum*, *Micrococcus lactis*, *Brevibacterium ammoniagenes* and *Propionibacterium freudenreichii* [26]. Recent research indicates that spontaneously formed mutant *Propionibacterium freudenreichii* produces more riboflavin compared to wild-type. When rats were fed yogurt fermented

with this mutant *Propionibacterium freudenreichii*, arylboflavinose associated growth and developmental retardation, elevated glutathione reductase (EGRAC) activity and hepatomegaly was eliminated [27]. As a result, in an attempt to increase dietary riboflavin mutant *Propionibacterium freudenreichii* species are now widely used in the dietary industry [28] (e.g. riboflavin enriched breakfast cereals). The most important use of riboflavin in industrial applications in recent times has been its addition to food products to give the yellow-orange color (E101). Riboflavin has also been used in the cosmetics industry as a substance that accelerates the action of tyrosine in skin tanning products. Among the natural foods, the main sources of riboflavin are animal products (i.e. milk, eggs, fish and meat), mushrooms and some plants and their seeds (e.g. almonds, sesame seeds and pumpkin seeds) [28]. Interestingly, black sesame seeds which are widely used in traditional Chinese medicine have more health benefits than white sesame seeds due to differences in the biosynthesis of phenylpropanoids, tyrosine metabolism and riboflavin metabolism [29].

Riboflavin is a thermostable and due to its photolability, a decrease in riboflavin content can be detected in riboflavin containing products. It is possible to observe a noticeable decrease (up to 50%) in the riboflavin content of products which have been dried in sunlight. Significant losses of riboflavin may also occur during the grain milling process (rejection of bran), where up to 70% of vitamin B2 content is lost. Tryptophan is known to prevent riboflavin degradation due to exposure to sunlight. Wheat and corn have a low tryptophan content, therefore, other cereal species are actually better sources of vitamin B2. However, the best sources of tryptophan can be found in milk and milk products because they are widely consumed. Offal and red meat are also good sources of tryptophan. Unfortunately, milk is often stored in transparent bottles and exposed to sunlight leading to a loss of 26-64% of its riboflavin content within 5-45 minutes. This is also the case for milk which is stored in illuminated refrigerators [30]. Having analyzed 1218 menus (a nutritional interview covering 24 hours collected from volunteers), we found that from the 30 nutrients we analyzed using the “Diet 5d” -diet program recommended by the Polish National Institute of Food and Nutrition, the most significant correlations with riboflavin were as follows (data not published):

- in women’s diet: thiamine, phosphorus, protein, magnesium, potassium, fiber (Table 2).
- in men’s diet: protein, phosphorus, calcium, vitamin A, lactose, iron (Table 3).

[illegible]

Table 1: Correlation of the content of individual components in a 7-day diet of polish women (Spearman rank correlation coefficient; significance of correlation $r > 0.20$).

Nutrients	Energy [kcal]	Protein [g]	Fat [g]	NKT [g]	JNKT[g]	WNKT [g]	Cholesterol [mg]	Carbohydrates [g]	Sacharose [g]	Lactose [g]	Dietary fiber[g]	Na [mg]	K [mg]	Ca [mg]	P [mg]	Mg [mg]	Fe [mg]	Zn [mg]	Cu [mg]	Mn [mg]	Vit. A [µg]	Vit. D [µg]	Vit. E [mg]	Vit. B1 [mg]	Vit. B2 [mg]	Niacin [mg]	Vit. B6 [mg]	Folate [µg]	Vit. B12 [µg]	Vit. C [mg]
Cu	0,55	0,51	0,42	0,38	0,36	0,41	0,31	0,53	0,34	0,28	0,60	0,35	0,63	0,35	0,63	0,72	0,82	0,86	1,00											
Mn	0,37	0,32	0,24	0,19	0,21	0,22	0,19	0,37	0,22	0,13	0,51	0,26	0,46	0,19	0,43	0,51	0,65	0,70	0,68	1,00										
Vit. A	0,12	0,12	0,09	0,03	0,09	0,18	0,41	0,13	0,28	-0,05	0,36	0,05	0,16	0,03	0,22	0,21	0,50	0,28	0,30	0,19	1,00									
Vit. D	0,14	0,22	0,23	0,14	0,22	0,28	0,34	0,02	0,09	0,10	-0,03	0,22	0,04	0,01	0,15	0,09	0,05	0,06	0,08	0,08	0,06	1,00								
Vit. E	0,55	0,44	0,66	0,35	0,68	0,89	0,27	0,41	0,29	0,10	0,51	0,32	0,46	0,27	0,49	0,55	0,31	0,34	0,48	0,22	0,19	0,18	1,00							
Vit B1	0,53	0,63	0,46	0,39	0,44	0,39	0,41	0,48	0,32	0,20	0,57	0,50	0,57	0,35	0,60	0,52	0,50	0,54	0,43	0,35	0,19	0,07	0,36	1,00						
Vit. B2	0,53	0,65	0,39	0,35	0,33	0,35	0,49	0,53	0,38	0,43	0,60	0,39	0,61	0,48	0,71	0,64	0,57	0,54	0,54	0,40	0,39	0,16	0,37	0,74	1,00					
Niacin	0,31	0,40	0,23	0,16	0,21	0,23	0,18	0,19	0,03	0,01	0,28	0,32	0,42	0,16	0,41	0,42	0,68	0,72	0,69	0,55	0,16	0,04	0,19	0,46	0,42	1,00				
Vit. B6	0,47	0,52	0,37	0,24	0,38	0,40	0,24	0,46	0,35	0,14	0,60	0,35	0,72	0,20	0,51	0,62	0,37	0,35	0,44	0,37	0,12	0,07	0,38	0,67	0,67	0,49	1,00			
Folate	0,41	0,40	0,27	0,21	0,25	0,25	0,25	0,37	0,15	0,14	0,46	0,25	0,48	0,31	0,48	0,51	0,82	0,79	0,77	0,65	0,32	0,07	0,26	0,37	0,47	0,74	0,32	1,00		
Vit B12	0,02	0,13	0,03	-0,05	0,03	0,14	0,40	-0,01	0,09	0,06	0,13	0,10	0,04	0,00	0,20	0,15	0,51	0,36	0,33	0,25	0,79	0,39	0,13	0,18	0,46	0,26	0,07	0,45	1,00	
Vit C	0,16	0,18	-0,03	-0,01	-0,05	-0,01	-0,12	0,19	0,07	0,08	0,26	0,12	0,30	0,23	0,22	0,30	0,20	0,09	0,15	0,14	0,02	-0,13	0,04	0,16	0,12	0,19	0,18	0,28	-0,09	1,00

the most important correlations
less significant correlations
own research - data not published

Nutrients	Energy [kcal]	Protein [g]	Fat [g]	NKT [g]	JNKT[g]	WNKT [g]	Cholesterol [mg]	Carbohydrates [g]	Saccharose [g]	Lactose [g]	Dietary fiber[g]	Na [mg]	K [mg]	Ca [mg]	P [mg]	Mg [mg]	Fe [mg]	Zn [mg]	Cu [mg]	Mn [mg]	Vit. A [µg]	Vit. D [µg]	Vit. E [mg]	Vit. B1 [mg]	Vit. B2 [mg]	Niacin [mg]	Vit. B6 [mg]	Folate [µg]	Vit.B12 [µg]	Vit. C [mg]				
Energy	1,00																																	
Protein		0,80	1,00																															
Fat			0,87	0,57	1,00																													
SFA				0,69	0,44	0,92	1,00																											
MUFA				0,84	0,51	0,97	0,85	1,00																										
PUFA				0,75	0,61	0,60	0,30	0,56	1,00																									
Choles-terol				0,52	0,43	0,68	0,73	0,66	0,13	1,00																								
Carbohyd-rates								0,89	0,79	0,56	0,32	0,54	0,76	0,18	1,00																			
Saccha-rose									0,67	0,47	0,61	0,46	0,59	0,65	0,13	0,63	1,00																	
Lactose										0,04	0,47	-0,23	-0,21	-0,31	0,03	-0,16	0,24	-0,02	1,00															
Dietary fiber									0,56	0,68	0,28	0,17	0,18	0,57	-0,03	0,73	0,38	0,45	1,00															
Na											0,73	0,63	0,68	0,53	0,69	0,50	0,44	0,60	0,30	-0,08	0,37	1,00												
K												0,63	0,75	0,40	0,31	0,27	0,58	0,22	0,70	0,45	0,46	0,87	0,36	1,00										
Ca													0,59	0,79	0,43	0,40	0,34	0,39	0,39	0,53	0,43	0,55	0,41	0,33	0,56	1,00								
P														0,69	0,92	0,43	0,36	0,32	0,55	0,30	0,74	0,42	0,66	0,78	0,44	0,82	0,83	1,00						
Mg															0,60	0,70	0,33	0,28	0,18	0,55	0,05	0,72	0,37	0,54	0,92	0,34	0,92	0,55	0,86	1,00				
Fe																0,72	0,74	0,53	0,40	0,46	0,63	0,25	0,74	0,47	0,31	0,75	0,59	0,76	0,63	0,76	0,75	1,00		
Zn																	0,75	0,78	0,54	0,46	0,45	0,52	0,31	0,79	0,50	0,40	0,74	0,61	0,74	0,63	0,85	0,80	0,79	1,00

Continued **Table 2:** Correlation of the content of individual components in a 7-day diet polish men (Spearman rank correlation coefficient; correlation significance $r > 0.38$).

Nutrients	Energy [kcal]	Protein [g]	Fat [g]	NKT [g]	JNKT[g]	WNKT [g]	Cholesterol [mg]	Carbohydrates [g]	Sacharose [g]	Lactose [g]	Dietary fiber[g]	Na [mg]	K [mg]	Ca [mg]	P [mg]	Mg [mg]	Fe [mg]	Zn [mg]	Cu [mg]	Mn [mg]	Vit. A [µg]	Vit. D [µg]	Vit. E [mg]	Vit. B1 [mg]	Vit. B2 [mg]	Niacin [mg]	Vit. B6 [mg]	Folate [µg]	Vit. B12 [µg]	Vit. C [mg]	
Cu	0,52	0,69	0,32	0,27	0,16	0,59	0,07	0,60	0,40	0,51	0,90	0,31	0,93	0,56	0,83	0,94	0,75	0,71	1,00												
Mn	0,42	0,45	0,10	0,01	0,01	0,46	-0,32	0,67	0,32	0,36	0,86	0,27	0,63	0,29	0,62	0,82	0,63	0,71	0,71	1,00											
Vit. A	0,35	0,53	0,38	0,39	0,25	0,34	0,37	0,26	0,26	0,43	0,54	0,30	0,71	0,43	0,58	0,58	0,51	0,50	0,66	0,24	1,00										
Vit. D	0,24	0,14	0,34	0,31	0,36	0,21	0,13	0,11	0,16	-0,08	-0,04	0,26	-0,18	0,22	0,15	-0,04	0,09	0,26	-0,10	0,09	-0,06	1,00									
Vit. E	0,69	0,63	0,60	0,39	0,53	0,88	0,13	0,66	0,69	0,10	0,63	0,37	0,60	0,48	0,59	0,57	0,55	0,46	0,64	0,47	0,44	0,20	1,00								
Vit B1	0,71	0,65	0,64	0,51	0,61	0,57	0,25	0,64	0,48	0,10	0,66	0,64	0,58	0,41	0,55	0,55	0,74	0,59	0,60	0,47	0,41	-0,10	0,59	1,00							
Vit. B2	0,48	0,78	0,33	0,26	0,27	0,31	0,40	0,46	0,13	0,68	0,54	0,43	0,62	0,72	0,78	0,54	0,65	0,58	0,58	0,24	0,71	0,01	0,36	0,54	1,00						
Niacin	0,37	0,63	0,10	-0,05	0,13	0,30	0,06	0,49	0,04	0,36	0,53	0,27	0,48	0,32	0,51	0,38	0,42	0,24	0,40	0,27	0,16	-0,10	0,36	0,38	0,54	1,00					
Vit. B6	0,41	0,68	0,09	-0,10	0,07	0,48	-0,06	0,61	0,13	0,49	0,77	0,30	0,74	0,39	0,64	0,66	0,63	0,42	0,66	0,54	0,45	-0,18	0,47	0,48	0,64	0,84	1,00				
Folate	0,72	0,79	0,47	0,30	0,44	0,57	0,33	0,74	0,28	0,37	0,71	0,59	0,74	0,56	0,74	0,67	0,79	0,66	0,63	0,51	0,58	-0,02	0,56	0,66	0,80	0,62	0,77	1,00			
Vit B12	0,15	0,29	0,23	0,23	0,22	0,04	0,39	0,02	-0,14	0,37	0,04	0,24	0,12	0,26	0,29	0,08	0,14	0,26	0,04	-0,11	0,55	0,42	-0,02	0,00	0,58	0,00	0,13	0,31	1,00		
Vit C	0,54	0,43	0,46	0,35	0,45	0,40	0,43	0,42	0,31	-0,03	0,31	0,43	0,36	0,44	0,42	0,32	0,46	0,43	0,31	0,22	0,26	0,25	0,46	0,30	0,36	0,18	0,21	0,62	0,07	1,00

the most important correlations
less significant correlations
own research - data not published

Table 3: The daily consumption reference values for riboflavin in various regions of the world.

Discriminator*	reference values for women	reference values for men
France	1.5	1.6
Spain	1.4	1.8
Portugal	1.4	1.6
Sweden, Norway, Finland	1.3	1.8
Italy, Greece, Ireland, Belgium	1.3	1.6
Austria, Switzerland, Germany	1.2	1.4
Netherlands	1.1	1.5
UK, USA	1.1	1.3
WHO	1.1	1.3
Poland since November 2008	1.1	1.3
Poland before November 2008	1.6	2.4
Directive dated the 28th of October 2008/100/WE	1.4	

* daily reference values in several countries, Scientific Committee on Food, the 5th of March 2003.

These data indicate that riboflavin sources in women's diets differs from men where meat and dairy products dominated. Regarding riboflavin content in plants, its presence is marked in *Erythroxylon coca*, which appears to be one of the most famous medicinal plants of South America. Its young leaves contain up to 1.22% of alkaloids and a lot of vitamins, especially thiamine, riboflavin and vitamin C. Chewing whole or powdered leaves is an age-old custom practiced by Indians in the Andes and the western Amazon Basin. The leaves of *Erythroxylon coca* are often eaten with bread and used to prepare sweet pastries.

Riboflavin deficiency

At the molecular level, the deficiency of riboflavin causes damaged to DNA, due to protein and DNA-strand oxidation, which may lead to the arrest of the G1 phase of the cell cycle [31]. According to Werner's *et al.* (2005) tissue loses its ability to restore itself when arrest of the G0/G1 stage occurs as a result of riboflavin deficiency [32]. At the cellular level, the increased activity of ROS in riboflavin deficiency conditions results in an accumulation of proteins with the incorrect conformation in the cell's endoplasmic reticulum. This leads to an activation of ubiquitin and non-lysosomal proteolysis, the so-called misfolded proteins, that contribute to the pathogenesis of Alzheimer's disease (protein A), Parkinson's disease (synuclein) and Huntington's disease (huntingtin). This

is also the case for spongiform encephalopathies (prP protein).

At the cellular level, riboflavin deficiency leads to increased oxidative stress and causes disorders in the glutathione recycling process. Camporeale *et al.* (2003) have proven that some tissues are more sensitive to ariboflavinosis than others and the most resistant are lymphoid cells [33]. In comparison the most sensitive cells for riboflavin deficiency are HepG2 liver cancer cells, where riboflavin deficiency signs are observed within 4 days of cell culture *in vitro* [32]. Monocytes and macrophages that lose their ability to phagocytose pathogens are also sensitive to riboflavin deficiency [34]. Furthermore, effector functions such as signaling events that facilitate myelin destruction as a consequence of the synthesis of proinflammatory cytokines and chemokines are impaired [35].

Riboflavin is found in high concentrations in the retina of the eye of mammals, urine, semen, liver and kidneys, therefore, dysfunctions of these organs as a result of its deficit are expected. Recent research has also shown that that riboflavin deficit leads to liver hypertrophy with lipid accumulation and increased saturated fatty acid (SFA) concentrations leading to proinflammatory responses [36]. The mechanism of this reaction is related to the involvement of proteins in the biosynthesis of triglycerides and cholesterol with a simultaneous decrease of proteins concentrations, involved in β -oxidation of fatty acids and the mitochondrial electron transport chain. Chronic

riboflavin deficiency may predispose individuals to oesophageal cancer development due to the interactions with various carcinogens [37]. It still remains unclear at what stage oncogenesis occurs (i.e. initiation, promotion or tumor development) but the earliest detected neoplastic changes are associated with esophageal and stomach epithelial atrophic changes, followed by the occurrence of hyperplasia and hyperkeratosis (hypertrophy and keratosis). Additionally, riboflavin deficiency often correlates with body mass index (BMI) [38]. Patients with clinical manifestation of riboflavin deficiency have also been found to have a lower body weight compared to people with a normal vitamin supply, which may be associated with malnutrition [39]. Riboflavin deficiency is a frequent cause of visual impairment, light hypersensitivity as well as migraine headaches. Riboflavin and co-enzymes Q10 are involved in the maintenance of the normal function of the mitochondrial respiratory chain, thus improving brain energy metabolism and are effective in the prophylaxis of migraines [40]. Deficiencies also contribute to anemia, caused by the shortened lifespan of erythrocytes and reduced reticulocyte count, neuropathy, hyperactivity, immunodeficiency and depression. Multiple sclerosis (MS) is an inflammatory demyelinating central nervous system disease in which a deficiency of riboflavin as a xanthine oxidase co-factor, increases uric acid deficit, leading to myelin degeneration [41]. Other studies have found that the levels of riboflavin in pregnant women influences the birth weight of newborn babies. The hemoglobin, hematocrit, and glutathione concentrations in mothers correlate with their concentrations in newborns, wherein the level of absolute values in children is always higher [42, 43]. In addition, Sanchez *et al.* (1999) have proven that both maternal and infants' status of riboflavin correlates with embryonic development of the child (i.e. birth weight, body length and labor duration) [43]. During pregnancy there may be deficiency of thiamine, riboflavin and pyridoxine [43, 44]. The deficiency of these vitamins during such an important period of development may adversely affect the health of both pregnant women and her fetus. Hypovitaminosis is now somewhat recognised as a physiological condition which should be monitored during pregnancy [45], but in the context of potential health effects, this does not seem to be the right approach. Considering the above, due to an increased process of erythropoiesis, the riboflavin supplementation during pregnancy and lactation seems to be indispensable [46 - 48].

The diets of pregnant women living in Nepal, in which a chronic deficiency of this vitamin occurs, is supplemented with riboflavin [47]. Another risk group with a deficient supply of riboflavin or its increased

demand are malnourished people, especially those with anorexia or other eating disorders [49], vegetarians [50], vegans, the elderly [51], athletes [52, 53] and alcoholics [54]. In the last three groups, the increased oxidative stress leads to increased demand for this vitamin. When considering the problem of riboflavin deficiency, it should be noted that riboflavin deficiency is more likely to affect population groups rather than individuals. In African and Asian countries, riboflavin deficiency affects 20-80% of the population but this deficiency is not always related to its low content in food.

Interestingly malaria, which is endemic in parts of Africa and Asia, the deficiency of riboflavin actually inhibits the proliferation of the single-celled parasitic protozoa, *Plasmodium spp.*, the causative agent of different forms of malaria. In this case, riboflavin deficiency should be considered as an evolutionary adaptation and not necessarily a problem. Moreover, riboflavin deficiency is rarely associated with clinical symptoms of arboflavinosis in individuals living in Africa and Asia [55]. The topic of riboflavin deficiency in malaria patients infection is complex because on the one hand, restricting the access of riboflavin to *Plasmodium spp.* prevents its multiplication, but the use of antimalarial drugs like quinine also affects the depletion of riboflavin stores in the body [56]. Therefore, it seems that the deficiency of riboflavin in malaria endemic areas may play a role as a favorable adaptation of the organism to frequently occurring infections. Notably, drugs that use the same transport pathways (e.g. penicillin), as well as riboflavin, do not always cause a decrease the levels of vitamin's in the body [57]. The increase in RG activity and thus the increased requirement by the body for riboflavin was found during studies on trichinellosis infection [58], as well as respiratory infections caused by *Klebsiella pneumoniae* [59]. As noted by Brijlal *et al.*, infection with this gram-negative bacteria, mobilizes riboflavin surge from the liver and kidneys into the blood and consequently increases its excretion in the urine [59]. In addition, such infections are more intense in the presence of a riboflavin deficit due to its low supply. Therefore, it is believed that both low intake of this vitamin and the accompanying frequent respiratory infections may play an important role in the etiology of riboflavin deficiency. Frequent respiratory infections are a non-diet factor contributing to subclinical riboflavin deficiency [60]. It seems that this condition, as some other above mentioned conditions, is associated with more general vitamin deficiency and not specific to riboflavin. Chronic immune activation and ROS production may lead to a decline of the oxidation labile vitamins [61, 62].

The riboflavin transporters deficiency

In addition, riboflavin deficiency may result from abnormalities, associated with its absorption and transport. It was demonstrated that mutations in the riboflavin transporter genes SLC52A2 (coding for RFVT2) and SLC52A3 (coding for RFVT3) may cause a neurodegenerative disorder formerly known as Brown-Vialetto-Van Laere (BVVL) syndrome [63, 64]. These riboflavin transporter deficiencies present in the clinic as weakness, cranial nerve deficits including hearing loss, sensory symptoms including sensory ataxia, feeding difficulties and respiratory difficulties which are caused by a sensory motor axon neuropathy and cranial neuropathy [65]. The deficiency of type 2 transporter is also found in patients with Charcot-Marie-Tooth disease, where it manifests by function, strength and sensation impairment of upper limbs [66]. In both cases, supplementation with riboflavin in doses of 10-100 mg/kg/day improves the patient's condition [64].

The state of organism supply with riboflavin

The results of epidemiological studies, including those made in Guatemala, showed that the dietary requirement for riboflavin in people aged over 60 years old were not significantly different when compared to younger adults. In contrast, the Western diet model with high fat and low complex carbohydrates content in comparison with typical Guatemalan diet inversely characterized by low fat and high complex carbohydrates content, affects the state of supply of the body for this component [67]. However, some scientists are of the opinion that there is an age-dependent increased requirement for riboflavin in humans, as a result of decreased efficiency of its absorption by enterocytes and an increased demand in the course of many diseases [68].

RG activity has been used in the diagnosis of liver diseases, especially malignant neoplastic lesions. And also in the assessment of supply along with diet and in studies of genetically conditioned enzyme deficiency states. The activity factor of glutathione reductase (EGRAC) cannot be used to assess levels of riboflavin in the body for people who are deficient in enzymes of the pentose phosphate pathway, such as pyruvate kinase and glucose-6-phosphate dehydrogenase (G6PD) is the case

in hemolytic anemia [69]. Red blood cells of people with G6PD activity deficiency, do not produce an adequate amount of NADPH, which is essential for the regeneration of GSH from GSSG. This metabolic block impairs the blood cells ability to defend against H_2O_2 and oxygen radicals, which results from cell lysis [3]. To assess the supply of the organism for riboflavin in these individuals, an appropriate method is to utilize the fluorescent properties of galactoflavin using the HPLC methods [49]. However, the best method to assess riboflavin deficiencies in potentially healthy humans is measurement of glutathione reductase activity [30, 67]. It is assumed that the EGRAC index value above 1.2 is a clinical proof of riboflavin deficiency, and values ranging between 1.0 and 1.2, suggest possible riboflavin deficiency. The riboflavin saturation degree in an organism may be influenced by many factors, not related to dietary supply. We found that the dietary riboflavin intake in women is 1.43mg and 1.8mg in men, led to EGRAC index decrease below 1.0 [70]. We have therefore established that the daily intake of riboflavin in both women and men groups in many countries seems to be too low (Table 3).

Application in medicine

The deficiency of riboflavin may be manifested by anemia, cataracts, and thyroid gland dysfunction [10] or *vice versa*, riboflavin deficiency may develop as a consequence of the underlying pathomechanism of these symptoms. Recent reports suggest its association with occurrence of neurological disorders such as migraine, Parkinson's disease and multiple sclerosis [71]. Interestingly, elevated homocysteine concentrations are an independent risk factor for Alzheimer's disease and cardiovascular diseases. However, in studies analyzing plasma riboflavin and homocysteine correlations, despite their metabolic relationship, no correlation has been found [72]. It is believed that increased concentrations of homocysteine may constitute a marker of deficiency of three other B vitamins: pyridoxine, cobalamin and folic acid [73]. Riboflavin plays an important role in pathogenesis of neurodegenerative diseases and is related to impairment of antioxidant processes, in particular lipid peroxidation and oxidative damage after reperfusion, formation of myelin, mitochondrial function and iron metabolism [74]. Riboflavin promotes the gene and protein levels of brain-derived neurotrophic factor (BDNF) in the CNS of the SM animal model, suggesting that BDNF mediates the beneficial effects of riboflavin on neurological

motor disability [41]. Riboflavin acts neuroprotectively against cytokines and overwhelming production of ROS and nitrogen oxide (NO) responsible for myelin sheath and neurons injuries by enhancement of IL-6 and BDNF genes expression [74]. Riboflavin deficiency may increase the risk of some cancers. It was found that in colon cancer (CRC) the expression level of the riboflavin gene and translocators changes, the content of RFVT1 decreases both at the level of protein and mRNA, while the expression level of RFVT2 and RFVT3 gene increased with a simultaneous reduction the amount of riboflavin [5]. RFVT2 and RFVT3 riboflavin transporters are highly expressed in brain and intestinal tissues and RFVT1 expressed in placenta may play an important role in acyl-CoA dehydrogenase deficiencies (MADD) and may contribute to the MADD occurrence, although its primary cause is associated with genes mutations of two enzymes: dehydrogenase (ETFDH) and phosphoprotein (ETF) [75]. Mutations in the SLC52A2 and SLC52A3 genes encoding riboflavin transporters are often associated with chronic, inflammatory demyelination and sensory-motor polyneuropathy [76]. Nevertheless, riboflavin therapy terminates the disease progression in majority of patients with riboflavin transporter disorders [77]. It also affects the clinical and biochemical improvement in patients diagnosed with MADD, especially in patients with late disease onset, qualified as type III [78]. Type III is milder, more variable and characterized by recurrent episodes of hypoglycaemia, metabolic acidosis, vomiting and muscular weakness during catabolic stress [79].

In cancer therapy, therapeutic agents (DPI Diphenyleneiodonium chloride) are also used to block mitochondrial respiration by inhibiting flavin-containing enzymes (FMN and FAD-dependent). This method allows to functionally induce ATP-depletion without cell apoptosis stimulation in order to avoid toxic side effects [80].

Another medical use is utilisation of thiolated riboflavin gold nanoassembly for targeted induction of apoptosis in cancer cells by photophysically *in vitro* DNA intercalation [81]. It has been noted that impoverishment of riboflavin contributes to development of cancer of HEK293T and NIH3T3 cells and may be a risk factor for cancer development. The riboflavin deficiency induced the expression of genes related to the cell cycle, accelerating it from 1.5 to 2 times [82].

Riboflavin properties are also used in the treatment process by inactivation of viruses in the plasma by photochemical method. The effect of nucleic acid damage in blood-borne viruses has been demonstrated, which may be useful in transfusion medicine [83]. Antiretroviral

induced lactic acidosis and hepatic steatosis are rare syndromes caused by the Nucleoside Reverse Transcriptase Inhibitor (NRTI) class of antiretrovirals. Posteraro *et al.* (2001) described a case report of NRTI lactic acidosis, reversed by administration of riboflavin in high doses [84]. Interestingly, the strains of *Lactobacillus plantarum* CRL2130 showed better protective effect than the riboflavin supplementation for inflammatory changes in intestinal mucosa, while the bacterium had no influence on effectiveness of chemotherapy against Caco-2 intestinal cancer cell cultures [12]. Mastropasqua *et al.* (2015) have found another use of the riboflavin solution by using it for the treatment of corneal ectasia which is gradual corneal narrowing caused by collagen matrix in the stroma [85]. The disease is treated by cross-linking corneal fibers, after removal of the surface epithelium, 0.1% riboflavin is used for 30 minutes, and the cornea is treated with UVA radiation for the next 30 minutes, which allows its reconstruction [85]. In conclusion, the growing use of this vitamin in medicine and industry shows its huge health potential.

Conflict of interest: Authors state no conflict of interest

Informed consent: Not applicable

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