



# Journal of Biological Sciences

ISSN 1727-3048

**science**  
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## Evaluation of Urinary Riboflavin Levels of Primary School Children in Rafsanjan, Iran

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**Abstract:** Riboflavin deficiency is one of the most common nutritional deficiencies, which has detrimental effects on physical growth of children. In the present study, the nutritional status of this vitamin in primary school children in Rafsanjan was investigated. In this cross-sectional study, the subjects were selected using multistage cluster random sampling and the general demographic data, including age and gender were collected by questionnaires. Urinary riboflavin level was measured as the indicator of riboflavin status. A moderate to severe riboflavin deficiency was found in half of the participants with a higher prevalence of severe deficiency in girls ( $p < 0.005$ ). Mean  $\pm$  SD of urinary riboflavin in male and female students were  $392 \pm 381$  and  $421 \pm 546 \mu\text{g g}^{-1}$  creatinine, respectively. Based on findings of this cross-sectional study, riboflavin deficiency is a serious nutritional problem in primary school children, especially among female students.

**Key words:** Urinary riboflavin, riboflavin deficiency, school-aged children

### INTRODUCTION

Riboflavin or vitamin B<sub>2</sub> (7,8-dimethyl-10-ribityl-isoalloxazine) was first synthesized in 1953. Riboflavin is a component of two important coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), both of which are essential for a number of oxidative enzyme systems involved in electron transport (Gibson, 2005).

Riboflavin deficiency has been implicated as a risk factor for some diseases and health alterations, including cancer (Siassi and Ghadirian, 2005; Powers, 2003), protein and DNA damage (Manthey *et al.*, 2006), ocular surface damage (Takami *et al.*, 2004) and preeclampsia (Wacker *et al.*, 2000). A detailed review of riboflavin and health was presented by Powers (2003).

Riboflavin deficiency presents with symptoms such as greasy dermatitis around the folds of the nose, nasolabial dyssebacia, cracking of lips at the center (cheilosis), glossitis, angular stomatitis, scrotal dermatitis and increased vascularization of the cornea (Srivastava, 2008). Riboflavin deficiency is particularly common in regions where intakes of dairy products and meat are low (Neumann *et al.*, 2003). Riboflavin deficiency has been described in several countries (El-Hazmi and Warsy, 1987;

Bates *et al.*, 1994; Blanck *et al.*, 2002; Gregory *et al.*, 2000; Rohner *et al.*, 2007). A national food consumption survey in Iran revealed that riboflavin intakes of 70% of households are lower than 80% of their requirements (Ghassemi *et al.*, 2002). Schoolchildren, in both developing and industrialized countries, are the age group at high risk for riboflavin deficiency (Powers, 2003).

Many of the earlier studies regarding riboflavin deficiency in Iran relied only on food consumption surveys, but this method assesses nutritional status in a subjective and indirect way. Biochemical methods have this potential to assess nutritional status in a direct and objective way. As there is limited data about riboflavin status in many parts of Iran, the aim of the present study was to determine urinary riboflavin levels in primary school aged children in Rafsanjan, located in the central region of Iran.

### MATERIALS AND METHODS

The study site was Rafsanjan, which is a district located in the central region of Iran. This cross-sectional study was carried out on 6-7 year old children in primary schools during 2004-2005. Complete information about the study was offered to the school directors. Informed

Table 1: Interpretive criteria for the urinary excretion of riboflavin ( $\mu\text{g g}^{-1}$  creatinine), from Gibson (2005)

Subjects	Less than acceptable (at risk)		
	Deficient (high risk)	Low (medium risk)	Acceptable (low risk)
Children			
1-3 year	<150	150-499	$\geq$ 500
4-6 year	<100	100-299	$\geq$ 300
7-9 year	<85	85-269	$\geq$ 270
10-15 year	<70	70-199	$\geq$ 200
Adults	<27	27-79	$\geq$ 80

written consents were obtained from the children's parents and the school directors. The study protocol was approved by the committee of ethics of Rafsanjan University of Medical Sciences.

District map at the main health center was used for clusters selection. A weighed random cluster sampling method was used. The district was divided into 5 parts and according to the population in each part, clusters were selected randomly. Because there was no previous data on approximate prevalence of riboflavin deficiency, it considered 50%. For  $\alpha = 0.05$ , at least 100 subjects were required to be examined. For more confidence, it was decided to recruit about 150 subjects. Totally, 147 subjects were recruited. A simple questionnaire was used for collecting general demographic data.

A disposable cup was given to each child for taking a random urine sample. After obtaining the sample, 10 mL was transferred into an oxalic acid treated test tube, which was covered in aluminum foil in order to be protected from light. Samples were maintained frozen ( $-70^{\circ}\text{C}$ ) until the time of laboratory analysis.

Urinary riboflavin was measured by fluorometric method (Ferrebee, 1940) using Jenway fluorometer (Model 6280, United Kingdom) and expressed as  $\mu\text{g g}^{-1}$  creatinine. Urinary creatinine was assayed by the Jaffé method (Cook, 1975). Interpretive criteria for the urinary excretion of riboflavin are presented in Table 1 (Gibson, 2005).

Statistical analysis were done by SPSS (Version 10). Continuous quantitative variables were tested by Student t-test. Chi-square test was used for other variables. A  $p < 0.05$  were considered as statistically significant level.

## RESULTS AND DISCUSSION

From 147 samples, 4 samples were excluded because they were outliers and no detectable amounts of riboflavin were found in them. It can probably result from exposure to light through transportation. In the study 143 subjects who involved, 72 male and 71 females. Sixty seven (46.85%) and 76 (53.15%) of subjects were attended grades 1 or 2, respectively. According to

Table 2: Prevalence of riboflavin deficiency in male and female students in Rafsanjan, Iran

Status	Male		Female		Total	
	No.	%	No.	%	No.	%
Severe deficiency*	9	12.5	20	28.2	29	20.3
Moderate or mild deficiency*	29	40.3	13	18.3	42	29.4
Normal	34	47.2	38	53.5	72	50.3
Total	72	100.0	71	100.0	143	100.0

\* $p < 0.005$  (between male and female)

current national regulations, 6 year old children start primary school as grade 1.

Frequency distribution of riboflavin deficiency is shown in Table 2. Table 2 shows that half of the participants had riboflavin deficiency.

Mean $\pm$ SD of urinary riboflavin in male and female students were  $392 \pm 381$  and  $421 \pm 546 \mu\text{g g}^{-1}$  creatinine, respectively. The difference between sexes was not statistically significant. In Table 2, prevalence of riboflavin deficiency in males and females is presented, separately. Prevalence of severe riboflavin deficiency was higher among female students and this difference was statistically significant ( $p < 0.005$ ).

This study showed high prevalence of riboflavin deficiency in primary school children in Rafsanjan, Iran; with higher prevalence of severe deficiency among females. Although, the mean of urinary riboflavin was different between male and female students, but this difference was not statistically significant. In addition, no significant association was observed between riboflavin status and grades. Overall, only half of the participants had urinary riboflavin levels in the normal range. It seems that riboflavin deficiency is not limited to some parts of Iran, because few earlier studies in different parts of the country had showed similar results (Esmailzadeh *et al.*, 2008; Ghassemi *et al.*, 2002). Many of the previous studies had assessed the riboflavin status by dietary intakes methods (Ghassemi *et al.*, 2002), which have a few sources of errors. Biochemical indicators are potentially more reliable and more objective in nutritional assessment (Lee and Nieman, 2003).

Because there is an established relationship between urinary riboflavin and riboflavin intakes, findings of this study reveal that at least half of the participants did not have adequate dietary riboflavin intakes. Prevalence of severe riboflavin deficiency in female students was more than twice than male students. Because little riboflavin is stored as such, the urinary excretion reflects dietary intake and catabolic and photodegradative events (McCormick, 2006). As there are no reasons for any differences between male and female participants with regard to catabolic and photodegradative events, such difference can be contributed to dietary intakes.

Riboflavin deficiency is an important source of concern in primary school children. A recent study has shown increase of learning ability of such students following treatment by oral riboflavin intakes (Jiang, 2006). Riboflavin is involved in glutaryl CoA dehydrogenase activity, which in turn has an important role in energy metabolism (Chalmers *et al.*, 2006). Therefore, riboflavin has role in growth, serum protein levels and learning ability.

Findings of this study showed high prevalence of riboflavin deficiency in primary school children in Rafsanjan, Iran. Severe riboflavin deficiency was more prevalent in female students. Attention of health authorities to this nutritional problem is strongly recommended.

#### ACKNOWLEDGMENT

We thank for financial support of Rafsanjan University of Medical Sciences.

#### REFERENCES

- Bates, C.J., P.H. Evans, G. Allison, B.J. Sonko, S. Hoare, S. Goodrich and T. Aspray, 1994. Biochemical indices of neuromuscular function tests in rural Gambian children given a riboflavin or multivitamin plus iron, supplement. *Br. J. Nutr.*, 72: 601-610.
- Blanck, H.M., B.A. Bowman, M.K. Serdula, L.K. Khan, W. Kohn and B.A. Woodruff, 2002. Angular stomatitis and riboflavin status among adolescent Bhutanese refugees living in southeastern Nepal. *Am. J. Clin. Nutr.*, 76: 430-435.
- Chalmers, R.A., M.D. Bain and J. Zschocke, 2006. Riboflavin-responsive glutaryl CoA dehydrogenase deficiency. *Mol. Genet. Metab.*, 88: 29-37.
- Cook, J.G.H., 1975. Factors influencing the assay of creatinine. *Ann. Clin. Biochem.*, 12: 219-232.
- El-Hazmi, M.A. and A.S. Warsy, 1987. Riboflavin status in a Saudi population: A study in Riyadh. *Ann. Nutr. Metab.*, 31: 253-258.
- Esmailzadeh, A., S. Samareh and L. Azadbakht, 2008. Dietary patterns among pregnant women in the West-North of Iran. *Pak. J. Biol. Sci.*, 11: 793-796.
- Ferrebee, J.W., 1940. The urinary excretion of riboflavin fluometric methods for its estimation. *J. Clin. Invest.*, 19: 251-256.
- Ghassemi, H., G. Harrison and K. Mohammad, 2002. An accelerated nutrition transition in Iran. *Public Health Nutr.*, 5: 149-155.
- Gibson, R.S., 2005. Principles of Nutritional Assessment. 2nd Edn., Oxford University Press, Oxford, New York, ISBN-10: 0195171691, pp: 554-562.
- Gregory, J., S. Lowe, C.J. Bates, A. Prentice, L.V. Jackson, G. Smithers, R. Wenlock and M. Farron, 2000. National Diet and Nutrition Survey: Young People Aged 4 to 18 Years. Vol. 1, The Stationary Office, London, ISBN: 9780116212658.
- Jiang, Y.Y., 2006. Effect of B vitamins-fortified foods on primary school children in Beijing. *Asia Pac. J. Public Health*, 18: 21-28.
- Lee, R.D. and D.C. Nieman, 2003. Nutritional Assessment. 3rd Edn., McGraw-Hill, Boston, ISBN: 9780072927313, pp: 303-337.
- Manthey, K.C., R. Rodriguez-Melendez, J.T. Hoi and J. Zempleni, 2006. Riboflavin deficiency causes protein and DNA damage in HepG2 cells, triggering arrest in G1 phase of the cell cycle. *J. Nutr. Biochem.*, 17: 250-256.
- McCormick, D.B., 2006. Riboflavin, In: Modern Nutrition in Health and Disease, Shils, M.E., M. Shike, A.C. Ross, B. Caballero and R.J. Cousins (Eds.). Lippincott Williams and Wilkins, Baltimore, ISBN: 0-7817-4133-5, pp: 434-441.
- Neumann, C.G., N.O. Bwibo, S.P. Murphy, M. Sigman, S. Whaley, L.H. Allen, D. Guthrie, R.E. Weiss and N.W. Demment, 2003. Animal source foods improve dietary quality, micronutrient status, growth and cognitive function in Kenyan school children: Background, study design and baseline findings. *J. Nutr.*, 133: 3941S-3949S.
- Powers, H.J., 2003. Riboflavin (vitamin B-2) and health. *Am. J. Clin. Nutr.*, 77: 1352-1360.
- Rohner, F., M.B. Zimmermann, R. Wegmueller, A.B. Tschannen and R.F. Hurrell, 2007. Mild riboflavin deficiency is highly prevalent in school-age children but does not increase risk for anaemia in Côte d'Ivoire. *Br. J. Nutr.*, 97: 970-976.
- Siassi, F. and P. Ghadirian, 2005. Riboflavin deficiency and esophageal cancer: A case control-household study in the Caspian Littoral of Iran. *Cancer Detect. Prev.*, 29: 464-469.
- Srivastava, N., 2008. Vitamins. In: Basics of Clinical Nutrition, Joshi, Y.K. (Ed.). Jaypee Brothers Medical Publishers, New Delhi, ISBN: 978-81-8448-213-3.
- Takami, Y., H. Gong and T. Amemiya, 2004. Riboflavin deficiency induces ocular surface damage. *Ophthalmic Res.*, 36: 156-165.
- Wacker, J., J. Frühauf, M. Schulz, F.M. Chiwora, J. Volz and K. Becker, 2000. Riboflavin deficiency and preeclampsia. *Obstet. Gynecol.*, 96: 38-44.