Riboflavin requirements of Filipino children and non-pregnant, pregnant and lactating women: Studied by the erthrocyte glutathione reductase activation test

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Riboflavin requirements were studied in six non-pregnant, 12 pregnant, 11 lactating women, 20 children aged 4–6 years and 14 children aged 10–12 years. All subjects initially were riboflavin-deficient with erythrocyte glutathione reductase activation coefficient (EGR-AC) of ≥1.3, confined in a metabolic ward and repleted with increasing doses of riboflavin during four 10 or 8 day feeding periods. The repletion diet simulated the usual basal diet of the subjects but were modified to contain adequate energy and protein in the pregnant group. Minimum riboflavin requirements determined by regression analysis as intakes required to obtain an EGR-AC value of < 1.3 were: 0.72 ± 0.09 (non-pregnant); 1.36 ± 0.37 (pregnant); 1.31 ± 0.16 (lactating); 0.58 ± 0.10 (children 4–6 years), and 0.70 ± 0.18 mg/d (children 10–12 years). Riboflavin requirements in mg/d, mg/1000 kcal, mg/g protein or mg/kg metabolic body size for the groups varied. Because of the large variability, the use of a single value relating riboflavin requirement to either energy, protein requirements or metabolic body size to calculate requirements for different age groups or gender is deemed inappropriate. It is recommended that riboflavin requirements be expressed in mg/d as determined experimentally for different population groups. Estimated recommended dietary allowances for the groups studied, obtained by adding 30% to the mean minimum riboflavin requirement, are presented.

Key words: Filipino, riboflavin, Philippines, pregnant woman, lactating woman, child.

Introduction

Riboflavin deficiency is a common nutritional problem in pregnant women in the Philippines. An overall prevalence of 54.7% was reported by Natera *et al.* in 1984.[1] This high prevalence raises the need for a re-evaluation of the recommended dietary allowances (RDA) for riboflavin for this group which is a requisite to any analysis of possible etiologic factors for the deficiency.

During the past several years, there has been growing recognition of some serious deleterious effects of riboflavin deficiency in children, such as retarded intellectual development,[2] disturbances in psychomotor and physical performance,[3,4] poor and steadiness,[5] and possible growth retardation.[6] In view of these findings, there is need also for a direct and more precise determination of riboflavin RDA in children.

Riboflavin deficiency has been reported in neonates who undergo phototherapy for excessive jaundice, which suggests that infants are vulnerable to riboflavin deficiency and have no significant stores of the vitamin.[7] Because the only source of riboflavin in the newborn is milk, and need for a re-study of riboflavin requirement of lactating mothers has become apparent.

Previous RDA values for riboflavin in the Filipino population were published in 1970 and 1976 and were based on urinary excretion studies of the vitamin in normal adults.[8] No studies were performed on children, pregnant or lactating women. Values for these latter groups were obtained by extrapolation of adult requirements by relating them to the recommended energy intakes. The assumption was that riboflavin requirements are proportional to energy intakes for all groups.

In recent years, the determination of erythrocyte glutathione reductase activation coefficient (EGR-AC) has gained wide acceptance for the assessment of riboflavin nutriture. An increasing number of studies from different countries have used this biochemical parameter for establishing riboflavin requirments.[9–13] Erythrocyte glutathione reductase activation coefficient is considered more sensitive, specific and reproducible.

The aim of this study was to determine the minimum riboflavin requirements (MRR) of non-pregnant, pregnant and lactating women, and preschool and pre-adolescent children using the EGR-AC response of subjects deficient in riboflavin to varying dietary intakes of the vitamin under controlled metabolic ward conditions. A re-evaluation of riboflavin RDA is particularly timely because nutrition surveys in the Philippines have shown that of the nine essential nutrients studied, riboflavin intake was found to be the least adequate.[14,15] The data would be useful in updating the Philippine RDA for riboflavin, thus making it a reliable nutri-

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tion tool for use in the Philippine Food and Nutrition Program. In addition, the utility of expressing riboflavin requirement by relating it to energy and protein requirements as well as to metabolic body size (MBS) was critically examined in this study.

Methods

Subjects

The subjects consisted of 29 women (six non-pregnant, 12 pregnant and 11 lactating), 20 children aged 4–6 years, and 14 children aged 10–12 years. Subjects were selected so that their ages would fall within age range groupings given in the Philippine RDA.[16] Only subjects who were deemed riboflavin-deficient based on an initial blood EGR-AC ≥1.30 as determined by the method of Sauberlich *et al.*[17] were included. Each subject was weighed and the deviation from standard weight for height in adults and weight for age in children were calculated using local standards.[18,19] The subjects fell within ±15% of the standards. Informed consent was obtained from all subjects or their guardians.

For all subjects who qualified for participation, blood was extracted from the antecubital vein, which was analysed for haemoglobin,[20] the total serum protein and albumin.[21] Routine urinalysis, stool examination for parasites and blood counts were performed for each subject.

All subjects were resident of Metro Manila. The pregnant women were on their second or third trimester and had pregnancy weights ranging from 90 to 105% of the standard.[18] The lactating women were subjects of a recently completed study on protein requirements conducted in their community and who met the requirements for the riboflavin study.

Diet composition, analysis and preparation

Baseline information on the diets of the subjects were obtained by weighing food intakes for a 3 day period and the amount of nutrients calculated using the Philippine Food Compostion Table.[22] Based on the usual dietary pattern obtained, a basal diet (4 or 5 day menu cycle) was designed for each group. The basal diets in non-pregnant and four pregnant women were provided with protein and energy adequate to meet requirements recommended in the 1976 Philippine RDA.[23] Approximately 60% of the protein came from vegetable sources and 40% from animal sources. Food items such as fish, pork, beef, chicken and shrimps were preweighed in individual portions corresponding to the calculated amounts to satisfy RDA values and were kept frozen until required for meal preparation.

Actual chemical analysis of the basal diet was done from aliquots from each of the 4 or 5 one-day menus in the cycle. The energy content was determined by bomb calorimetry; protein, riboflavin and thiamin were analysed using Association of Official Analytical Chemists (AOAC) methods.[24]

Metabolic study protocol

The metabolic studies were all done in the Nutrition Evaluation Laboratory (NEL) of the Food and Nutrition Research Institute (FNRI), Manila, with the exception of four of the pregnant women. These four women were studied in a makeshift metabolic ward in an institution located at Marillac Hills, Alabang, Metro Manila, of the Department of Social Services and Development.

The study was divided into four feeding periods of equal numbers of days. Except in the child groups, the riboflavin content of the diet was designed to contain an amount of riboflavin equal to that found in their usual diet (period 1), 80% of the RDA (period 2), 100% of the RDA (period 3) and 120% of the RDA (period 4). The 1976 RDA values for each group were followed. In the child groups, the riboflavin contents of feeding periods 1 and 2 were those provided by the basal diet alone. In all groups, when the basal diet did not meet the desired amount of riboflavin intake, the subjects were given supplements of synthetic riboflavin (USP) in gelatin capsules to attain the necessary intake for the feeding period.

In the child groups, the lactating group and in some of the pregnant women, an 8 day feeding period, instead of 10 day as in the other groups, was deemed to be a sufficient length of time to effect a response in the EGR-AC levels based on the findings of other investigators.[17,25,26]

Water intake was controlled at 40 mL per kg body weight. The subjects were asked to consume all the food given at every meal. They were weighed daily in the morning after voiding the first urine and before taking their breakfast.

The effects of diets containing different riboflavin levels on EGR-AC was determined by examining the blood samples drawn in the morning before breakfast on the first day of the succeeding feeding period.

Estimation of minimum riboflavin requirements

The MRR was estimated for each subject by regression analysis of EGR-AC on riboflavin intake. The riboflavin intake needed to obtain an EGR-AC of < 1.30 was estimated from the regression equation derived. The riboflavin intakes were related to energy and to protein requirements as well as to MBS, except in the case of the pregnant women where MBS was not determined.

Results and discussion

Characteristics of the subjects

A summary of anthropometric characteristics, blood chemistry and EGR-AC values at entry of the different study groups is given in Table 1. Serum albumin levels were acceptable to low, except in some pregnant and lactating women and children who were rated as deficient.[27] No significant abnormal findings were obtained from routine urinalysis, stool examination for parasites and blood counts (data not presented).

Characteristics of the basal diet

This study was motivated in part by the result of surveys indicating that riboflavin deficiency is one of the common nutritional problems in the Philippines. In pregnant women, the incidence of deficiency ranges from 18.5% in Eastern Visayas to 49.2% in Western Visayas.[28] Riboflavin intake was found least adequate and met only 56.3 and 54.5% of the RDA levels in the 1982 and 1987 national nutrition surveys, respectively.[14,15]

In the present study, chemical analyses of the basal diets also showed inadequate riboflavin intakes that ranged from 32% (pregnant women, community) to 53.9% (children 4–6 years) of the 1976 RDA as shown in Table 2. Energy intakes met RDA requirements for all groups. In the child groups,

Table 1. Anthropometric characteristics, blood chemistry and EGR-AC values at entry of different study groups

	Women			Children	
Parameter	Non-pregnant	Pregnant	Lactating	4–6 years	10–12 years
No. subjects	6	12	11	20	14
Age (years)	24.2 ± 3.4[a]	26.9 ± 5.4	28.8 ± 5.0	5.1 ± 1.0	10.6 ± 0.9
	(20–29)[b]	(19–36)	(22–37)	(4–6.8)	
Body weight (kg)	46.0 ± 3.1	50.7 ± 4.7	45.7 ± 4.0	15.6 ± 1.5	29.8 ± 3.4
	(41–49.8)	(42.1–56.9)	(41.7–54.5)	(13.1–17.9)	(25.8–40.0)
Height (cm)	150.7 ± 3.1	150.4 ± 5.5	148.9 ± 5.1	104.4 ± 5.9	135.0 ± 7.3
	(148–155)	(140–158)	(139.5–157.0)	(91.4–115.4)	(127.0–150.0)
Weight/height (%)[c]	98.7 ± 5.2	96.8 ± 8.0	99.4 ± 9.4	93.8 ± 5.5	98.3 ± 5.9[d]
	(92.2–105.3)	(85–116)	(87.1–113.2)	(87–105)	(90–112)
Gestational age (week)		27.4 ± 3.4			
		(20–32)			
Lactational age (week)			7 ± 2		
			(4–10)		
Hemoglobin (g/dL)	13.7 ± 0.9	11.6 ± 0.8	13.1 ± 1.20	12.8 ± 1.0	14.1 ± 0.7
	(12.6–14.8)	(10.5–13.2)	(11.2–14.5)	(11.4–14.5)	(12.7–15.1)
Hematocrit (vol%)	40.0 ± 0.4	33.5 ± 2.8	37.2 ± 2.9	37.4 ± 2.0	40.7 ± 1.8
		(29.5–39.0)	33.8–41.2	(34–40.0)	(37.0–44.0)
Serum albumin (g/dL)	4.1 ± 0.4	3.1 ± 0.4	3.3 ± 0.8	3.0 ± 0.50	2.8 ± 0.5
	(3.4–4.7)	(2.3–3.7)	(2.0–4.0)	(2.1–3.9)	(1.8–3.6)
EGR-AC	1.43 ± 0.11	1.59 ± 0.20	1.53 ± 0.12	1.41 ± 0.07	1.42 ± 0.04
	(1.30–1.56)	(1.31–2.00)	(1.35–1.66)	1.33–1.60	(1.35–1.45)

[a]Mean ± SD; [b]actual range; [c]expressed as % standard weight for height; [d]expressed as % standard weight for age.

Table 2. Energy and nutrient composition and adequacy of dietary intake during metabolic period for each population group

	Energy			Protein			Thiamin			Riboflavin		
Population group	RDA	Intake kcal/d	Intake %RDA	RDA	Intake g/d	Intake %RDA	RDA	Intake mg/d	Intake %RDA	RDA	Intake mg/d	Intake %RDA
Women												
Non-pregnant	1920	1902 ± 140	102.0 ± 2.0	54	53.2 ± 3.4	102.0 ± 2.0	0.5 mg/ 1000 kcal	1.0 ± 0.09	104.0 ± 8.3	0.5 mg/ 1000 kcal	0.46 ± 0.05	47.0 ± 5.0
Pregnant												
Marillac Hills	+430	2387 ± 240	102.6 ± 4.7	+14	66.3 ± 5.0	99.0 ± 3.5	+0.4	0.62 ± 0.99	46.7 ± 5.5	+0.4	0.54 ± 0.12	39.0 ± 9.3
Community	+430	2261 ± 143	92.5 ± 6.2	+14	71.8 ± 15.8	101.7 ± 22.5	+0.4	0.78 ± 0.09	58.9 ± 9.3	+0.4	0.42 ± 0.06	32.0 ± 5.4
Lactating												
1st 6 months	+550	2328 ± 199	101.6 ± 4.5	+28	73.2 ± 16.5	105.7 ± 23.6	+0.3	0.82 ± 0.12	67.8 ± 8.7	+0.6	0.45 ± 0.11	33.7 ± 9.6
2nd 6 months	+440	2328 ± 199	101.6 ± 4.5	+16	73.2 ± 16.5	105.7 ± 23.6	+0.3	0.82 ± 0.12	67.8 ± 8.7	+0.4	0.45 ± 0.11	33.7 ± 9.6
Children												
4–6 years	1600	1352 ± 153	97.7 ± 6.0	32	22.9 ± 6.0	82.4 ± 19.6	0.5 mg/ 1000 kcal	0.51 ± 0.11	101.9 ± 21.3	0.5 mg/ 1000 kcal	0.27 ± 0.07	53.9 ± 14.6
10–12 years	M2090 F19010	1816 ± 193	100.8 ± 4.7	45 45	36.9 ± 7.4	88.5 ± 16.0	0.5 mg/ 1000 kcal	0.36 ± 0.09	72.4 ± 18.7	0.5 mg/l 1000 kcal	0.36 ± 0.09	52.3 ± 11.8

protein intakes were between 80 and 90% of the RDA. However, regression analysis using the Statistical Package for the Social Sciences (SPSS)[29] showed that protein intake at the levels used in this study did not affect EGR-AC values ($P > 0.05$).

Except in the non-pregnant women and children 4–6 years, it is notable that thiamin intakes fell short of RDA values in four of the study groups, although to a lesser degree compared with riboflavin intakes (Table 2). This raises the question of whether thiamin intakes that fell short of RDA values would have adversely affected EGR-AC values. To help answer this question, a simple regression of thiamin intakes on EGR-AC values was done in children aged 10–12 years. The result showed no significant correlation ($r = –0.216$, $P > 0.05$) suggesting no effect of thiamin intake on EGR-AC. Multiple regression also showed that the thiamin intake had no significant effect. Furthermore, Horwitt concluded that because of the relatively large safety factor incorporated in current RDAs, there has been no report of clinical deficiency of water-soluable vitamins including that for thiamin in healthy individuals consuming at least 75% of the RDA.[30] Nonetheless, the current data suggest there should be increased awareness of the possibility that a deficiency of thiamin might be commonly associated with riboflavin deficiency. Regression analysis of riboflavin/1000 kcal on thiamin/1000 kcal intakes in the basal diets of children 10–12 years revealed a significant positive correlation ($r = 0.35$, $P < 0.01$). It is possible that this association is due to a similar distribution of these two vitamins in foods in the basal diets used in this particular study.

Minimum riboflavin requirements

Riboflavin requirements have been measured previously by relating dietary intakes to riboflavin concentration in red cells,[31] urinary riboflavin excretion[32] and more recently to the EGR-AC values.[9] In this study, the EGR-AC method was followed[17] but instead of the suggested cut-off point of 1.2,[15,26] a value of 31.30 was adopted as indicative of riboflavin deficiency based on the findings of a previous study in pregnant Filipinos by Natera *et al.*[1]

Table 3 summarizes the mean riboflavin intakes during the four feeding periods in the different population groups studied. It can be grossly discerned from the corresponding mean EGR-AC values obtained at the end of feeding period 3, except in the case of pregnant women from Marillac Hills,

Table 3. Riboflavin intake and EGR-AC value on each level of intake of different population groups

		Population group			Riboflavin intake	
	mg/d	mg/1000 kcal	EGR-AC	mg/d	mg/1000kcal	EGR-AC
Women		Period 1			Period 2	
Non-pregnant	0.45 ± 0.05	0.24 ± 0.02	1.21 ± 0.14	0.73 ± 0.05	0.39 ± 0.01	1.30 ± 0.04
Pregnant M	0.53 ± 0.12	0.23 ± 0.06	1.8 ± 0.07	1.04 ± 0.09	0.44 ± 0.01	1.58 ± 0.06
Pregnant C	0.31 ± 0.04	0.14 ± 0.02	1.60 ± 0.20	1.05 ± 0.18	0.46 ± 0.08	1.44 ± 0.14
Lactating	0.30 ± 0.05	0.13 ± 0.02	1.52 ± 0.14	1.10 ± 0.10	0.48 ± 0.05	1.40 ± 0.09
Children						
4–6 years	0.25 ± 0.03	0.19 ± 0.02	1.40 ± 0.06	0.32 ± 0.04	0.24 ± 0.03	1.36 ± 0.03
10–12 years	0.34 ± 0.05	0.20 ± 0.02	1.39 ± 0.05	0.42 ± 0.06	0.23 ± 0.02	1.35 ± 0.06
Women		Period 3			Period 4	
Non-pregnant	0.91 ± 0.06	0.48 ± 0.02	1.16 ± 0.04	1.09 ± 0.07	0.58 ± 0.01	1.04 ± 0.02
Pregnant M	1.29 ± 0.12	0.56 ± 0.01	1.33 ± 0.15	1.56 ± 0.14	0.67 ± 0.01	1.22 ± 0.16
Pregnant C	1.32 ± 0.12	0.58 ± 0.04	1.28 ± 0.10	1.55 ± 0.14	0.67 ± 0.04	1.23 ± 0.09
Lactating	1.36 ± 0.08	0.59 ± 0.04	1.30 ± 0.02	1.60 ± 0.10	0.68 ± 0.02	1.23 ± 0.04
Children						
4–6 years	0.73 ± 0.09	0.52 ± 0.04	1.25 ± 0.03	0.95 ± 0.09	0.69 ± 0.05	1.18 ± 0.05
10–12 years	0.93 ± 0.10	0.51 ± 0.04	1.23 ± 0.05	1.21 ± 0.12	0.65 ± 0.03	1.15 ± 0.07

Table 4. MRR and estimate of RDA for different population groups

Population			MRR		Estimated RDA		Philippine (1989)	RDA[a]
group	No.	X	SD	CV (%)	MRR + 30%	mg/d	mg/1000 kcal	mg/d
Women								
Non-pregnant	6							
mg/d		0.72	0.09	12.5	0.94	0.94		
mg/1000 kcal		0.38	0.04	10.5	0.49	0.94	0.5	1.0
mg/g protein		0.014	0.001	7.6	0.018	0.87		
mg/kg MBS		0.041	0.004	8.9	0.053	0.98		
Pregnant	12							
mg/d		1.36	0.37	24.1	1.77	1.77		
mg/1000 kcal		0.58	0.18	31.0	0.75	1.70		+0.6
mg/g protein		0.019	0.004	23.4	0.025	1.53		
Lactating	10							
mg/d		1.31	0.16	12.1	1.70	1.70		
mg/1000 kcal		0.60	0.007	11.2	0.78	1.86		+0.4
mg/g protein		0.017	0.002	9.2	0.022	1.47		
mg/kg MBS		0.076	0.007	10.1	0.099	1.82		
Children 4–6 years	20							
mg/d		0.58	0.10	17.2	0.75	0.76		
mg/1000 kcal		0.43	0.07	15.6	0.56	0.91	0.5	0.8
mg/g protein		0.024	0.003	11.5	0.03	0.98		
mg/kg MBS		0.074	0.011	15.2	0.096	0.80		
Children 10–12 years	14							
mg/d		0.70	0.18	25.7	0.91	0.91		
mg/1000 kcal		0.38	0.09	23.7	0.49	1.04	0.5	1.0
mg/g protein		0.018	0.003	16.7	0.023	1.05		

[a]Phil RDA (1989)	Body weight (kg)	Energy (kcal)	Protein (g)	US RDA (1989)	FAO/WHO 1967
Children 4–6 years	18	1600	32	0.6 mg/1000 kcal	0.55 mg/1000 kcal
10–12 years	32	2090	45		
Females 20–39 years	49	1900	52	0.6 mg/1000 kcal	0.55 mg/1000 kcal
Pregnancy (2nd & 3rd)		+300	+9	+0.3 mg/d	
Lactation 1st 6 months		+500	+16	+0.5 mg/d	
2nd 6 months			+12	+0.4 mg/d	

that the mean activation coefficients have 'nomalized' by attaining a value ≤ 1.30. The riboflavin intakes during period 3, as indicated earlier, were calculated to meet 100% of the 1976 riboflavin RDA.

A more precise determination of the MRR was obtained by regression analysis of each of the subject's riboflavin intake that would attain an EGR-AC of < 1.30 by extrapolation using the linear regression equation derived. While currently many countries and FAO/WHO[33] express riboflavin requirements in relation to energy intake, the validity of such practice has been questioned. Horwitt has strongly argued that riboflavin requirement be related to that of protein, rather than to energy requirements.[30] On the other hand, at one time the US Food and Nutrition Board in 1968 related the requirements to MBS.[34] The question is still considered unresolved.[13] Because the riboflavin, energy and protein content have been assayed to determine the actual intakes of the subjects in this study, the MRR (intakes giving < 1.3 EGR-AC values) were expressed as riboflavin in mg/d, mg/1000 kcal, and mg/g protein. Since the data on the weights of the subjects were also available, the riboflavin requirements in mg/kg MBS were calculated. The data obtained are compared in Table 4.

The MRR in mg/d (Table 4), as expected, are higher in pregnant and lactating mothers and the values for the two groups are similar.

When MRR were compared, the values varied from 0.38 (non-pregnant) to 0.60 (lactating mothers) per 1000 kcal or a difference of 36.7%. Considering only the values for non-pregnant women and the child groups, the agreement improved greatly with only a 13% difference between 0.38 (non-pregnant women) and 0.43 (children 4–6 years) per 1000 kcal. It should be noted that the figures for pregnant and lactating women, using EGR-AC as a parameter, already took into account the cost of pregnancy as well as that of lactation, respectively.

When the MRR were expressed in mg/g protein intake, the values showed greater variation with the lowest value of 0.014 mg/g protein (non-pregnant women), varying by 41.7% from the highest value of 0.024 (children 4–6 years).

When expressed in relation to MBS, the MRR varied from 0.041 (non-pregnant women) to 0.076 mg/kg (lactating women), or a difference of 46.1% between the two values. No calculation was made on the MBS of pregnant women.

Thus, it can be seen from the different modes of relating MRR per day in this particular study that the least variability between groups was obtained when requirements were expressed per 1000 kcal. This variability was further decreased when pregnant and lactating mothers were excluded.

Estimation of RDAs for the different study groups

The importance of the data obtained in this study is that they may serve as the basis for possible estimations of RDA for riboflavin for the Filipino population. The RDAs for the different study groups were calculated by adding to the experimentally determined MRR a safety factor equal to 30% to cover individual variations (Table 4). This is in consonance with current practice, wherein according to Beaton,[35] the rule of thumb evolved on empirical evidence is that for many biological systems variability among individuals has a coefficient of variation (CV) of about 15%. Twice the CV represents two standard deviations. This safety factor is generally accepted since in many nutrient requirement studies, the number of subjects is usuallly considered too few to adequately define the population standard deviation. In the present study, the CV for the groups mean MRR values in mg/d varied from a low value of 12.1% (lactating women) to a high value of 25.7% (children 10–12 years). Between groups the CV were particularly large in the pregnant women and children 10–12 years. Because of the limited number of subjects and the wide range of standard deviations observed, in this study a CV of 15% for all groups was adopted. The RDA values thus estimated for the different groups, expressed in four ways, are shown in Table 4. In order to estimate the daily intake of riboflavin (in mg) by extrapolation, the determined MRR values (Table 4, expressed per 1000 kcal), were multiplied by the corresponding RDA for energy for each group as shown in Table 4 and the calculated values are shown. Similar estimates were done using MRR expressed in mg/g protein using the RDA for protein in grams given in Table 4. The estimates using MBS were similarly obtained from the reference body weights for each population group.

Comparison of the estimated RDA in relation to energy, protein or MBS

Statistical comparison (using Duncan's Multiple Range test) of the amounts of riboflavin (mg) required per day in non-pregnant women calculated from the riboflavin requirements expressed per 1000 kcal, per g protein, and per kg MBS were not significantly different from the value of 0.94 mg/d arrived at from the riboflavin contents of the diet (Table 4). The statistical comparisons are shown in Table 5. Similarly, the RDA estimates for pregnant women were comparable, as well as those for children 10–12 years. The RDA estimate of 1.47 mg riboflavin/d (obtained on the basis of protein intake) for lactating women was significantly lower than the other values derived for this group. However, values obtained on the basis of per 1000 kcal (0.91) or per g protein (0.98) on children 4–6 years were significantly higher than the other values obtained in mg/d or in mg/kg MBS. Overall, the RDA values estimated using MBS were most closely comparable to corresponding experimentally determined riboflavin requirement in mg/d. In the four groups where MBS was determined, the RDA estimates were all statistically comparable with the corresponding RDAs determined as riboflavin in mg/d.

Table 5. Comparison of estimate of RDA[a] for riboflavin expressed as mg/d, mg/1000 kcal, mg/g protein and mg/kg MBS by Duncan's multiple range test

Women				
Non-pregnant	0.873[d]	0.936[b]	0.939[c]	0.983[e]
Pregnant	1.53[d]	1.70[c]	1.77[b]	
Lactating	1.47[d]	1.70[b]	1.820[e]	1.862[c]
Children 4–6 years	0.760[b]	0.80[e]	0.91[c]	0.98[d]
Children 10–12 years	0.911[b]	0.962[e]	1.055[d]	1.04[c]

Means underscored by the same line are not significantly different at P = 0.05.

[a] Basis; [b]regression of EGR-AC on riboflavin intake (mg/d); [c]regression of EGR-AC on riboflavin intake (mg/1000 kcal); [d]regression of EGR-AC on riboflavin intake (mg/g protein); [e]regression of EGR-AC on riboflavin intake (mg/kg MBS).

The current practice of relating riboflavin requirement to energy intake makes use of a single factor that is applied to all age groups and gender, with additional 'corrections' for pregnancy and lactation. This practice has been adopted mainly because most of the previous studies of riboflavin requirements were performed in normal adults and in the absence of data for other population groups, some basis for extrapolating the riboflavin requirements of the latter had to be used. Thus, calculations of riboflavin requirements in the 1976 Philippine RDA[23] were based on the requirement of 0.5 mg/1000 kcal for all groups plus an additional 0.4 mg for pregnancy (2nd and 3rd trimesters) and 0.6 mg for the first 6 months and 0.4 mg for the next 6 months of lactation. The US RDA uses a value of 0.6 mg/1000 kcal applied to all age groups and gender with an additional 0.3 mg/d for pregnant and 0.5 mg/d for lactating women (1st 6 months).[36] In Australia, an additional 0.8 mg/d is recommended during lactation.[37] The values used differ in the case of FAO/WHO.[33] The factor of 0.55 mg/1000 kcal was used for all without additional increase in riboflavin over that supplied by the increase in energy intake for pregnancy and lactation. The differing factors used reflect the uncertainty of the relationship between riboflavin and energy requirements. Also, the difference in increments for pregnancy and lactation recommended by the various institutions arise from the difficulty of estimating additional riboflavin needs to support milk production and the cost of pregnancy. The determination of additional riboflavin requirements for these two groups to cover the cost of pregnancy or lactation is obviated by the use of the EGR-AC technique.

While on a practical basis the use of a single factor related to energy requirements conveniently facilitates the calculation of riboflavin allowances, the present study shows that to approximate the riboflavin requirement directly determined in mg/d, one has to use a *different factor* for the different population groups. Similarly, relating riboflavin requirements to protein requirement or MBS also necessitates the use of *different factors* for the different groups. When such *different factors* are used, this study also shows that indeed one can arrive at statistically comparable estimates of RDA in the different groups that are comparable to the experimentally determined requirement of riboflavin in mg/d. In this context, the present data is in agreement with the concept that allowances estimated by relating riboflavin requirements to energy, protein or MBS may not be significantly different.[38] It is to be emphasized, however, that our data shows that the use of a single factor relating riboflavin requirement to energy (or to protein or MBS) applied to all the different population groups would obviously give different RDA estimates. In order to calculate the RDA needs of different population groups, using a single factor is rather simplistic and not justified from the results of the present study. In view of our findings, we recommend that riboflavin requirements be expressed in terms of mg/d experimentally determined for the different population groups. To our knowledge, our studies are the first to present riboflavin requirement data for child groups of ages 4–6 and 10–12 years using the EGR-AC method. It is likely that data would be forthcoming for the population groups considering the availability and ease of use of the EGR-AC method in determining riboflavin requirements. When such data become complete for the different groups, there will be no need for 'extrapolation' procedures currently used to determine riboflavin RDAs.

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Riboflavin requirements of Filipino children and non-pregnant, pregnant and lactating women: Studied by the erthrocyte glutathione reductase activation test

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用紅細胞谷胱甘肽還原酶激活法研究了未懷孕、懷孕和授乳的菲律賓婦女和 4 - 6 歲及 10 — 12 歲兒童的核黃素需要量

摘　要

該文研究了六位未懷孕、十二位已懷孕、十一位授乳的婦女和二十位 4 至 6 歲、十四位 10 — 12 歲兒童的核黃素需要量。所有研究對象均有初期的核黃素缺乏，其紅細胞谷胱甘肽還原酶激活系數（ EGR — AC ）≥1.3 ，他們入住代謝病房並接受核黃素四個 10 天或八天的喂養治療。補充核黃素的膳食與研究對象的基本膳食類似，但在孕婦組增加足夠的能量和蛋白質，當維持病人的 EGR — AC 值<1.3 時，用回歸分析得出最低每日核黃素需要量（ MRR ）爲： 0.72±0.09 毫克 / 日(未懷孕組)， 1.36±0.37 毫克 / 日(懷孕組)， 1.31±0.16 毫克 / 日(授乳組)， 0.58±0.10 毫克 / 日(四—六歲兒童組)，和 0.72±0.09 毫克 / 日(10 —12 歲兒童組)，以毫克 / 日、毫克 / 1000 仟卡，毫克 / 每克蛋白質或毫克 / 公斤體重計算核黃素的需要量各組差異很大，由於有巨大的差異，因此用單一能量，蛋白質需要或體重對不用組別或性別計算核黃素的需要量相信是不合適的。作者認爲核黃素需要量（毫克 / 日）應根據不同人群的實驗決定。估計每日核黃素的需要量（ RDA ）爲各組最低需要量（ MRR ）加上 30%。

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