RIBOFLAVIN STATUS IN LATE PREGNANCY, POSTPARTUM AND CORD BLOOD

Ning-Sing Shaw, PhD

Department of Agricultural Chemistry, National Taiwan University
Taipei, Taiwan

ABSTRACT

Riboflavin status of 61 pregnant Chinese women was investigated in a longitudinal study by functional assay of the coenzyme activation of erythrocyte glutathione reductase. Maternal nonfasting venous blood was collected at 26 and 36 gestational weeks and 6 weeks postpartum. At delivery, 27 pairs of maternal and cord blood samples were also collected. Dietary intake of riboflavin was estimated by 24-hour recall method at the 26th gestational week. Judged by an activation coefficient > 1.25 , the prevalence of riboflavin deficiency was 31% to 45% in late pregnancy and 34% postpartum. Cord blood exhibited better riboflavin status than the maternal blood, and there was significant correlation between the two. Of all the subjects, 47.5% had a daily riboflavin intake less than the recommended dietary allowances for pregnancy. Prevalence of deficiency was highest in subjects whose diet included neither milk nor prenatal vitamin supplement. Therefore, prenatal supplementation is recommended for pregnancy in order to guarantee an adequate supply of riboflavin.

Key words: Riboflavin status, Erythrocyte glutathione reductase, Pregnancy, Postpartum, Cord blood

INTRODUCTION

Riboflavin is an essential cofactor in cellular oxidation. It is necessary for fetal growth and development. Milk and dairy products are important riboflavin sources, but these foods are less frequently consumed as compared to Western countries by Chinese adults due to lactose intolerance. The riboflavin status of populations in Taiwan has been evaluated by dietary survey and the functional

assessment of the coenzyme activation of erythrocyte glutathione reductase (EGR, EC 1.6.4.2). A national dietary survey in Taiwan area conducted in 1980-81 (1) indicated that average daily riboflavin intake per person was 0.9 mg, reaching only 75% RDNA (Recommended Daily Nutrient Allowances, a Chinese version equivalent to RDA of U.S.A.)(2). At that same time, survey for pregnant women in rural and urban area resulted in a daily intake of 69% and 85% of RDNA respectively (3), and frequency of an activation coefficient (AC) of EGR > 1.2 was 58.5% and 45% respectively (3). In this past decade, Taiwan's economic level and living standard has risen significantly, and the general public has an increased knowledges in nutrition and health. A recent national dietary survey conducted in 1986-88 (4) indicated an increase of riboflavin intake to 1.03 mg/day, reaching 84% of the RDNA. People in general are aware of increased maternal nutrient demand during pregnancy. It was not known whether riboflavin status of pregnant women was improved parallelly. This study updated the understanding by investigating the riboflavin status in late pregnancy, postpartum and cord blood with the EGR assay.

MATERIALS AND METHODS

Subject

In August and September of 1990, 61 healthy pregnant women were selected randomly from the patients attending the Antenatal Clinic Of Veterans General Hospital, Taipei, to participate in this study. The study was reviewed and approved by the Committee on Clinical Research of the Veterans General Hospital-Taipei, and informed consent was obtained from each subject at the beginning of the study. The subjects were recruited at 26 ± 2 gestational weeks. Their average age was 29 ± 4 yrs, average pregestational weight was 50.5 ± 5.0 Kg, and average height was 158.7 ± 4.2 cm. Forty of them were primigravida, and all had uneventful full-term deliveries. None of the subjects was anemic in her first trimester, was not diagnosed with any clinical symptoms of deficiency and received no prescribed supplements. However, self-chosen prenatal supplements were not excluded, and 27 subjects took 40 mg iron supplement per day from 26th gestational week to term, and 21 subjects took prenatal vitamin and mineral supplement (New Prenatal) regularly, which contained 2 mg of riboflavin per tablet. Dietary data were collected by 24-hour dietary recall at 26 gestational weeks, and was computer analyzed using Chinese food composition tables (5, 6).

Blood collection and analysis

Nonfasting venous blood samples were drawn at 26 (26wk) and 36 (36wk) gestational weeks and 6 weeks postpartum (6PP), and 61, 43 and 41 samples were collected at each period respectively, among which 30 constituted true longitudinal samples. Twenty-seven pairs of maternal and cord

blood were drawn at delivery. Blood samples were collected in heparinized tubes and erythrocytes were washed thoroughly with saline after separation from plasma by centrifugation. The in vitro activation of EGR with FAD was measured by the procedures of Tollotson and Sauberlich (7). The change of absorbance at 340 nm was followed for 10 min in both the presence and absence of added FAD. The results were calculated and presented as AC, which is the ratio between absorbance change with FAD and that without FAD. A ratio > 1.25 is considered indicative of riboflavin deficiency, with values > 1.4 indicating severe deficiency and values between 1.25 and 1.4 indicating marginal deficiency (8, 9).

The differences among periods and among food groups were evaluated with Duncan's multiple range test. Correlation was evaluated by Pearson's correlation test.

RESULT

The distribution of AC EGR at each period is presented in figure 1. The average AC at 26wk and 36wk and 6PP was 1.21 ± 0.18 , 1.21 ± 0.15 and 1.22 ± 0.13 respectively, among which there is no significant difference ($p > 0.05$). The mean AC for the 30 subjects followed at each of the three periods showed similar results. Judged by AC, the frequency of marginal deficiency was 16% at 26wk, 33% at 36wk and 22% at 6PP; the frequency of severe deficiency was 15% at 26wk, 12% at 36wk and 12% at 6PP; and total frequency of deficiency was 31% at 26wk, 45% at 36wk and 34% at 6PP. Among these three periods the highest incidence of deficiency occurred in 36wk.

At delivery, AC was 1.27 ± 0.19 for maternal blood and 1.07 ± 0.10 for cord blood. About 59% of maternal AC was > 1.25 (26% > 1.4 and 33% between 1.25 and 1.4). The AC of the cord blood was significantly lower than that of maternal blood from both in late pregnancy and at delivery ($p < 0.01$). Out of 27 cord blood samples, none exhibited severe deficiency, and 4 were very close to marginal deficiency, of which all can be traced to mothers with severe deficiency. Correlation coefficients are listed in table 1. Significant correlation existed between all periods of sampling ($p < 0.001 - 0.05$) except between cord blood and 6PP.

The daily riboflavin intake and prevalence of deficiency are listed in table 2. In order to further investigate the effect of riboflavin sources on the status, the subjects were divided into four dietary groups according to whether milk or prenatal vitamin supplement was consumed or not: group A did not consume either milk or supplement; group B consumed milk; group C did not consume milk but took supplement; group D consumed both milk and supplement. Results for these four groups are listed in table 2.

Fig. 1
Distribution And Mean of Activation Coefficient of Erythrocyte Glutathione Reductase (EGR) of 26 (I) and 36 (II) Gestational Weeks, 6 Weeks Postpartum (IV), and Maternal (III) and Cord Blood (V) at Delivery.

TABLE 1

Correlation Analysis of Erythrocyte Glutathione Reductase Activation Coefficient in Late Pregnancy, Postpartum and Cord Blood

	Gestational weeks		At delivery	
	26 wk	36 wk	maternal	cord
Gestation 36 wk	0.3788 ^c (43)			
At delivery maternal	0.6535 ^a (27)	0.6556 ^a (22)		
cord	0.3942 ^c (27)	0.6370 ^b (22)	0.6826 ^a (27)	
Postpartum 6 PP	0.5002 ^b (41)	0.6458 ^a (32)	0.6352 ^b (17)	0.3355 (17)

(n) indicates number of subjects; a: $p < 0.001$, b: $p < 0.01$, c: $p < 0.05$

TABLE 2

Daily Riboflavin Intake and Prevalence of Deficiency at 26th Gestational Week

	Total	Dietary groups			
		A diet	B + milk	C + supplement	D + milk + supplement
Subject numbers	61	8	23	12	18
Daily riboflavin intake (mg)					
mean $\pm$ s.d.	2.24 $\pm$ 1.37	0.99 $\pm$ 0.32 ^b	1.14 $\pm$ 0.49 ^b	3.41 $\pm$ 1.35 ^a	3.24 $\pm$ 0.76 ^a
range	0.24-6.03	0.39-1.41	0.24-2.09	1.69-6.03	2.09-5.05
< 1.4 mg*	47.5%	75%	56.6%	0%	0%
< 0.7 mg [#]	8.2%	12.5%	17.4%	0%	0%
Erythrocyte glutathione reductase activation coefficient					
mean $\pm$ s.d.	1.22 $\pm$ 0.18	1.38 $\pm$ 0.16 ^a	1.25 $\pm$ 0.17 ^b	1.21 $\pm$ 0.19 ^b	1.13 $\pm$ 0.11 ^c
> 1.4	15%	25%	22%	17%	0%
1.25-1.4	16%	75%	17%	0%	11%
< 1.25	69%	0%	61%	83%	89%

* RDNA, # 50% RDNA

a, b, c: different letters in the same row indicate significant difference at $p < 0.05$

Although daily intake averaged 2.24 mg, large individual variation (ranging from 0.24 to 6.03 mg) existed due to difference in dietary selection and utilization of supplements. Nearly half (47.5%) of the subjects studied had an intake lower than the RDNA; among these, 5 were lower than 50% of the RDNA.

When ranked in a high to low order, the daily riboflavin intake was $C = D > B = A$, which paralleled the reduction of AC; the frequency of an intake less than RDNA was $A > B > C = D$, and the prevalence of deficiency was $A > B > C = D$. Significant inverse correlation existed between the AC and riboflavin intake from either the supplement ($r = -0.4289$, $p < 0.001$) or the total intake ($r = -0.4678$, $p < 0.001$), indicating reduced risk of deficiency with increasing intake, and that prenatal supplement is more effective than milk in supporting the riboflavin nutriture of pregnant women.

DISCUSSION

In developed western societies, riboflavin deficiency is not a common nutritional problem for the general population due to comparatively higher consumption of milk and dairy products and fortification of cereal products. However, by measurement of AC EGR, reports from western societies have indicated the possibility of a high occurrence of riboflavin deficiency in expectant mothers as compared to nonpregnant women (10, 11), and an increasing need for riboflavin with the progression of pregnancy (12). Although national data indicated an improvement in riboflavin status in general population in Taiwan (1, 4), the prevalence of deficiency in late pregnancy evaluated with AC EGR was still more than 30% in this study. This was slightly better than the previous report of 45% in urban area (3), but is still high. This biochemical result was consistent with a recent dietary survey for pregnant women, which indicated an daily B_2 intake of only 64% RDNA during gestation (13). The prevalence of deficiency remained as high even in postpartum, this probably reflected the average prepregnant level of riboflavin nutriture. The riboflavin status for pregnant women has not been effectively improved, regardless of improved living standard. It is very unlikely that the reduced enzyme activity resulted from general or protein malnutrition because the dietary records of the subjects studied indicated an energy intake of 1815 ± 407 kcal/day and a protein intake of 78 ± 26 g/day. Analysis of the riboflavin sources for the pregnant women in this study have provided some clues of the problem.

For subjects consuming no milk and no supplement, the diet provided an daily average of 0.99 mg riboflavin. The majority of the subjects in this group had an intake less than the RDNA, and all of them were either marginally or severely deficient by the criterion of AC. If milk was consumed, daily riboflavin intake increased slightly to 1.14 mg, and AC decreased significantly with a prominent reduction in deficiency prevalence. However, the range of riboflavin intake (0.24 to 2.09 mg/day) in this group is wide, reflecting the wide variation in individual tolerance of milk, and deficiency in this group (39%) is not negligible. When supplement was taken, the riboflavin intake was about 3 times that from the diet, and both AC and deficiency prevalence were effectively reduced. It is not clear why $AC > 1.25$ still existed in some subjects of adequate riboflavin intake. To provide adequate riboflavin for pregnancy, it is apparent that Chinese

diet alone is not sufficient, and milk has a limited effect, while supplement is most effective. Since there is no national practice of riboflavin fortification, and subjects might not be aware of moderate deficiency due to lack of clinical symptoms, nutritional education and dietary guidance need to be emphasized.

In Chinese custom, Postpartum diets consist of large amounts of muscle and organ meat, and riboflavin intake in one study was estimated to reach 193% of the RDNA (13). The dietary pattern for the subjects in this study is similar to the one mentioned above, yet the riboflavin status showed no significant improvement in postpartum compared to that in late pregnancy. Since meat is usually well cooked in soups, it is speculated that loss of riboflavin or change of bioavailability from these food may contribute to such results. Although riboflavin is heat stable, it is liable to oxidation during food preparation and storage. Riboflavin availability from Chinese cuisine has not been studied.

It is noted that in this study the highest AC average (1.27) and highest frequency of AC > 1.25 (59%) both occurred at delivery. Although it was possible that riboflavin status further deteriorated toward term, other mechanisms are worthy of consideration since Vir et al. (11) also reported a higher incidence of AC > 1.20 in early (3 days) postpartum compared to 2nd and 3rd trimester, which cannot be accounted for by progressive deterioration of the riboflavin nutriture with the advancement of pregnancy. It is speculated that erythrocyte riboflavin may be depleted by high oxygen consumption due to strenuous labor, and its repletion is not accomplished immediately after delivery. Investigation of this possible mechanism is warranted.

Many previous reports (10, 14-19) have indicated that riboflavin status in newborns, as measured by riboflavin concentration, EGR activity, or AC EGR, is usually better than maternal status. The same result was observed in this study that AC of cord blood was significantly lower than that of mother's blood. One mechanism to protect the fetus from mild maternal deficiency is placenta conversion of FAD from maternal blood to free form for transplacenta passage and further metabolic trapping by conversion to FMN in fetus (20). Two other mechanisms: a better apoenzyme-coenzyme binding (17) or an active mechanism of placenta to favor fetal uptake (21) might also contribute to this protection. However, this protection is not without the danger that severe deficiency may result in fetal deficiency, as reported in underdeveloped countries (22).

It is concluded that riboflavin status of Chinese pregnant women but not newborns was impaired by inadequate dietary intake, and that prenatal vitamin supplement was effective in alleviating the deficiency and should be recommended to expectant mothers.

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