# Riboflavin deficiency in pregnant women in a public institution of São Paulo city, SP, Brazil

# Avaliação da deficiência de riboflavina em gestantes de uma instituição pública da cidade de São Paulo, SP, Brasil

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**ABSTRACT**
In a prospective study, the riboflavin nutritional status was evaluated in pregnant women to whom varied vitamin doses were given and in women who did not receive any vitamin supplementation during gestation**.** Riboflavin nutritional determination was assessed by means of glutathione reductase activation coefficient (GR-AC)**,** and the value higher than 1.5 was considered as indicative of riboflavin deficiency. The pregnant women were divided into four groups: 123 pregnant women to whom any vitamin supplementation were not given, 25 who daily received 2.5 to 3.5mg of riboflavin, 53 who were daily supplemented with 1.0 to 1.7mg of riboflavin, and 22 to whom were daily given <0.85mg of riboflavin**.** The women group daily supplemented with 2.5 to 3.5mg riboflavin showed the lowest riboflavin deficiency, and the other groups (64.5%) presented vitamin deficiency with GR-AC higher than 1.5. These data suggest that the vitamin concentrations found in the commercially available vitamin supplemented – preparations**,** as well as those found in ingested food were insufficient to provide a good nutritional status for pregnant women. These findings strongly suggest that riboflavin deficiency has in fact been a relevant public health problem in São Paulo city**.**
**Key word**s. Riboflavin malnutrition, riboflavin deficiency, pregnant women, glutathione reductase

**RESUMO**
Neste estudo prospectivo foi avaliado o estado nutricional em riboflavina nas parturientes que faziam uso da suplementação vitamínica de variadas concentrações de riboflavina, bem como naquelas que não fizeram uso de qualquer suplementação durante o período gestacional. O recurso utilizado para a avaliação nutricional foi a determinação do coeficiente de ativação da glutationa redutase (CA-GRE). Os valores de CA-GRE acima de 1,5 foram considerados como indicativos de deficiência de riboflavina. Foram analisados quatro grupos de gestantes: 123 parturientes sem suplementação vitamínica; 25 que utilizaram formulação contendo de 2,5 a 3,5mg de riboflavina; 63 parturientes que utilizaram fármacos contendo 1,0 a 1,7mg de riboflavina e o quarto grupo constituído de 22 mulheres que receberam formulação contendo < 0,85mg de riboflavina. O grupo de parturientes que apresentou menor índice de deficiência de riboflavina foi aquele que fez o uso da suplementação vitamínica com as taxas entre 2,5 a 3,5mg de vitamina $B_2$. Os demais grupos, com a inclusão daquele que não complementou a dieta com suplementação vitamínica, apresentaram índices de deficiências bem maiores**.** Esses achados indicam que as quantidades de riboflavina apresentadas nesses fármacos, bem como na dieta alimentar, foram insuficientes para atingir níveis bioquímicos semelhantes ao do grupo controle. No geral, foi encontrada uma incidência de deficiência de riboflavina de 64,4%, o que permite sugerir que a arriboflavinose é um importante problema de Saúde Pública na cidade de São Paulo.
**Palavras-chave**. desnutrição, Riboflavina, gestantes, deficiência, glutationa redutase.

## INTRODUTION

Riboflavin or vitamin $B_2$ deficiency during gestational period has been reported to induce some disturbances as anemia and pre-eclampsia, and is also related to the newborn birthweight[1,2,3].

It is very important in several metabolic pathways and its daily need during gestation increases about 27 % when compared with non pregnant women[4], due to increased maternal and foetal tissue synthesis and increase in energy requirement[5].

Riboflavin deficiency during gestation has been reported in several countries[1,2,6,7,8] and a plenty of causes may trigger it, as mal nutrition, mal absorption, hemodilution and increasing foetal needs along the pregnancy[2]. However, the most important factor responsible for the maternal deficiency is a poor diet in nutrients, which has been treated by vitamin supplementation, and better intake of food rich in ribolflavin as milk, cheese, meat, liver, cereals, eggs and fresh vegetables[8].

In order to assess the nutritional status in riboflavin, the degree of activation of erythrocyte glutathione reductase (E-GR) by its coenzyme flavin adenine dinucleotide (FAD) has been employed. Riboflavin is a FAD precursor, and FAD concentration depends directly of riboflavin intake[9]. That being so, initially E-GR is assayed without any FAD addition, what gives the basal activity; when FAD is added to the reagent system, the more activation is obtained the more it is deficient in riboflavin. Aiming to assess the riboflavin deficiency, an activation coefficient (AC) has been designed (AC), calculated by the reason E-GR activity with FAD and E-GR without FAD [10]. The greater the figures obtained, the greater is the deficiency.

Riboflavin deficiency in Brazil has been scarcely ascertained, and in seventies Wilson et al.[11] reported clinical signs of deficiency in 31% of a population, and Barretto et al.[12] reported that 81% of pregnant women were found to be deficient through AC determination.

Riboflavin deficiency has also been related to cancer[14,15,16], homocisteinemia[13,17], postpartum depression[18], and anaemia[19,20] . This present prospective survey has been designed to investigate the nutritional status in pregnant women who were being supplemented with several concentrations of riboflavin and in another group who was not receiving any vitamin supplementation.

## CASUISTICS AND METHODS

A total of 233 pregnant women who looked for a public hospital at labour was investigated. They were 27 ± 6 years old and the gestation was between 35 and 41 weeks.

The women were grouped in the following categories:

- Group A – 25 pregnant women who were taking 2.5 to 3.5 mg riboflavin daily
- Group B- 63 pregnant women who were taking 1.0 to 1.7 mg riboflavin daily
- Group C- 22 pregnant women who were taking < 0.85 mg riboflavin daily
- Group D – 123 pregnant women who were not taking vitamins at all
- A group of 34 non pregnant women ranging from 23 to 48 years old who were not supplemented with vitamins but were fed regularly with meat, milk, cereals and fresh vegetables was taken a control.

The pregnant women were attended at Hospital e Maternidade Leonor Mendes de Barros, a public hospital set at East side of São Paulo city, state of São Paulo, Brazil. Blood was drawn by venous puncture and collected 3:1 in ACD (citric acid, citrate and dextrose) before delivery and kept at 4º C for enzyme assays up to 4 days after drawing.

Blood was centrifuged a 1000 g at 4º C for 10 minutes, the plasma was discarded, and the red cells were washed with saline three times at 1000 g at 4º C. The buffy coat was discarded in order to avoid leucocytes contamination. The packed red cells were lysed 1:20 with hemolysing solution {0.7µM 2-mercaptoethanol, 2µM nicotinamide adenine dinucleotide phosphate (NADP), 2.7µM ethylene diamine tetraacetic acid (EDTA) pH 7.0}, submitted to freeze and thawing 3 times and centrifuged at 10000g at 4ºC for 20 minutes, and the supernatant was employed for enzyme assay .

Glutathione reductase activity at 38ºC per minute with and without FAD was assayed according to Beutler[21], by the glutathione (GSH) oxidation to oxidized glutathione (GSSG), observed by nicotinamide adenine dinucleotide phosphate (NADP) reduction to NADPH at 340 nm. The activity coefficient (AC) is obtained the following formula:

$$AC = \frac{\text{E-GR activity with FAD}}{\text{E-GR activity without FAD}}$$

The non-parametric Mann-Whitney statistical method was utilized, with a significance level of 5%.

## RESULTS

Table 1 depicts the AC means obtained in the studied groups compared to the control group. This figure was obtained with a control group of non pregnant civil servants of the laboratory who were not taking any vitamin supplementation but were fed with milk, meat, cereals and fresh vegetables. This procedure was necessary as there is a fair diversity of figures in the literature, with thresholds which varies from figures indicating deficiency higher than 1.7[22], 1.5[23], 1.4[6], 1.3[2,8,12] to 1.2[24]. According to our findings, which disclosed a mean value of AC as 1.5 in the control group, we considered deficients the individuals who presented figures higher than AC 1.5 for analyzing the data obtained in studying the pregnant women.

Table 2 shows the frequency of riboflavin deficiency taking AC>1.5 as indicative of deficiency.

**Table 1**. Glutathione reductase activation coefficient (GR-AC) means in the studied groups and comparison with the control group.

| | | Pregnant women who were given riboflavin supplementation | | | |
|---|---|---|---|---|---|
| | Control group | Group A 2.5 - 3.5 mg | Group B 1.7 - 1.0 mg | Group C < 0.85mg | Group with no supplementation |
| n | 34 | 25 | 63 | 22 | 123 |
| AC | 1.5 ± 0.14 | 1.49 ± 0.32 | 1.71 ± 0.49 | 1.79 ± 0.55 | 1.76 ± 0.46 |
| p-value | | 0.841 | 0.018* | 0.004* | 0.001* |

*significant p<0,05.

**Table 2.** Glutathione reductase activation coefficient (GR-AC) frequence among the studied groups.

| Pregnant women | n | GR-AC < 1,5 | GR-AC ≥ 1,5 |
|---|---|---|---|
| Group A (2.5 – 3.5mg) | 25 | 16 (64%) | 9 (36%) |
| Group B (1.7 – 1.0 mg) | 63 | 21 (33.3%) | 42 (66.7%) |
| Group C (< 0.85 mg) | 22 | 6 (27.3%) | 16 (72.7%) |
| Total (with supplementation) | 110 | 43 (39.1%) | 67 (60.9%) |
| Group D (no supplementation) | 123 | 40 (32.5%) | 83 (67.5%) |

## DISCUSSION AND CONCLUSION

In general terms, the lesser is the vitamin concentration in the vitamins supplemention, the greater are the ACs, showing that the degree of GR activation depends on the riboflavin doses. In a matter of fact, the riboflavin supplementation given to groups B and C was insufficient to show the same AC of the control group.

On the other hand, the Table 2 shows that group A who was given 2.5 to 3.5mg exhibited AC similar to the control group, what suggests that this daily supplementation was enough to afford good nutritional status in terms of vitamin $B_2$ for 64% of this group, but was not sufficient to provide it for 36% . This lack of good response to these doses may be accounted for biological variation among the individuals, to low adherence to the treatment.

However it was occasionally found AC <1.5 in members of groups B, C and D, possibly due to a good intake of food rich in vegetables, milk not reported in the query, due to misunderstanding of low educational level of some women.

The Brazilian Agência Nacional de Vigilância Sanitária (ANVISA) adopted the Food and Nutrition Board of the National Research Council from United States of 1.6 mg recommendation for daily riboflavin need, but recently the ANVISA published the RDC no. 269/2005[25] in which there is a recommendation of 1.4mg riboflavin daily (Ingestão Mínima Recomendada–IDR) for pregmant women in Brazil.

As the higher deficiency rates were found in the women who were taking less than 1.7 mg (groups C and D), it is reasonable to suggest that the IDR proposed by ANVISA is not enough to protect the Brazilian pregnant women from vitamin deficiency , as many authors have found 1.6mg daily insufficient[7] . In our country Barretto et al.[12] have already reported that even when were given 3 mg daily for pregnant women along the gestational period an expressive percentage of pregnant women still presented ACs smaller than 1.3, the used lower threshold. It is possible that these figures could be different whether these authors would have used AC of 1.5 instead of 1.3, but the data herein presented agree with their findings, as the only group which presented better figures was that who were given 2.5 to 3.5mg daily. In this report 64.4 %

out the 233 investigated pregnant women presented AC> 1.5, what indicates a high riboflavin deficiency rate, a similar figure found in Mexico[8] but higher than in Phillipines[7] and Thailand[26], Italy[27], Spain[2], Nepal[6, 28] and Zimbabwe[1].

The riboflavin deficiency may be due to poor intake of food rich in this vitamin. Velásquez-Meléndez et al.[29] studied the intake of some micronutrients, among them the riboflavin in a community of the metropolitan area of São Paulo city, and among other deficiencies the riboflavin was found to be 1.1mg daily among non pregnant women ranging from 20 to 39 years old, approximatedly 21% less than the 1.4mg recommended by ANVISA[25].

In this study the 43 % of pregnant women were not participating of any prenatal assistencial program, a very high rate which did not agree with Ministerio da Saude data reports, between 1995 and 2002 the rate of women who did not receive prenatal medical assistence decreased from 10,73% to 3,77%[30].

It is well known that maternal and foetal deaths are related to gestation period and labour complications which may be avoided by a careful and efficient pre-natal program[31]. In Rio Grande do Sul state 42% of deaths by complications during the gestation or by the delivery were observed in women who did not have prenatal medical care[32].

Some surveys have found correlation between poverty and lack of prenatal care, although other factors as maternal school level may also influence the enrolment of pregnant women to prenatal medical care[31,33].

These factors, poverty and school level, might account for the high rates of prenatal care lack in this study, as most of the population herein presented were poor and even street (dwellers) lodgers.

Although the Programa de Humanização do Pré-Natal e Nascimento do Ministério da Saúde in Brasil has been settled since 2000, low governamental attention on prenatal care, delivery, puerperium as well as to newborn may account for the high rates of neonatal and maternal mortality[30].

Taking the data obtained in this survey, it is possible to suggest that most of the pregnant women presented riboflavin deficiency, based on the minimum AC of 1.5 to define this deficiency. A minimum of 2.5 to 3.5mg daily riboflavin may be acceptable to give enough protection in terms of riboflavin nutrition, although higher dosages may be also indicated. It is very sad however that a very high rate of women did not receive prenatal medial care.

In conclusion, it must be pointed out that the maternal nutritional status is fundamental for herself as well as for the foetus, and that the prenatal medical care since the conception is essential to provide them a good health.

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