

## Red cell glutathione reductase saturation obtained with oral riboflavin supplementation

### Saturação da glutathione redutase eritrocitária obtida pela suplementação oral com riboflavina

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**ABSTRACT.** The erythrocyte glutathione reductase (E-GR) saturation with its coenzyme flavin adenine dinucleotide (FAD) affords the nutritional status regarding the vitamin B2, as FAD proceeds from riboflavin intake. E-GR normally exhibits physiological partial saturation with its coenzyme. **OBJECTIVE:** To study the maximum *in vivo* saturation which could be reached by increasingly oral vitamin supplementation. **METHODS:** Three groups of normal volunteers were given 3 mg ( group A ), 20 mg ( group B ) and 50 mg ( group C ) riboflavin daily during twenty days. The enzyme activities were assayed before and after supplementation, with and without coenzyme addition to the reagent system. **RESULTS:** 77%, 83% and 87% saturation were observed in the groups A, B and C respectively. **CONCLUSION:** Increasing *in vivo* saturation was observed with increasing oral doses of riboflavin supplementation, but total saturation was not observed even with the highest oral 50 mg riboflavin supplementaion.

**KEY WORDS.** red cell glutathione reductase, riboflavin, vitamin B2.

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## INTRODUCTION

There are red cell enzymes which are vitamin dependent, and so their activities are correlated with the specific vitamin nutritional condition<sup>2</sup>. As an example, glutathione reductase needs the coenzyme flavin adenine dinucleotide for accomplish with its catalytic function, which in turn requires riboflavin (vitamin B2) for its biosynthesis<sup>7</sup>. The red cell glutathione activity assay with or without FAD in the reagent system may be useful in affording the riboflavin physiological saturation. When adding FAD to the reagent system it saturates completely the enzyme loci for FAD, reaching maximum activity. Thus, a high increase of GR basal activity obtained when FAD is added to the reagent system indicates that the GR is deprived of sufficient vitamin B2 intake. Conversely, a slight activity increase obtained with FAD means a fairly good vitamin B2 nutritional status. This correlation is linear and may be used to evaluate the vitamin intake<sup>10</sup>.

An index has been proposed for measuring the nutritional vitamin status for all vitamin dependent enzymes, the activity coefficient (AC), which is the ratio of the activity after coenzyme addition/activity before coenzyme addition. Several studies have reported minimum AC normal threshold values for glutathione reductase of 1.2,<sup>5,13</sup> 1.3,<sup>9,12</sup> 1.4,<sup>11</sup> 1.5,<sup>1</sup> and values above these figures indicate vitamin deficiency.

As the enzyme coenzyme saturation is always partial in individuals with current normal diet<sup>3</sup>, the daily vitamin supplementation for a long period could lead to total coenzyme saturation.

In order to investigate which would be the vitamin B2 daily dosis for reaching an hypothetical total saturation, increasing oral supplementation riboflavin doses were given to normal volunteers.

## METHODS

Normal volunteers, from 24 to 54 years old, of both sexes, non smokers, not taking any medicine or vitamin supplementation were separated in the following groups:

### Group A:

15 individuals were given 3 mg riboflavin daily / 20 days

### Group B:

15 individuals were given 20 mg riboflavin daily / 20 days

### Group C:

18 individuals were given 50 mg riboflavin daily / 20 days

10 ml of blood were drawn and collected in EDTA, kept at 4° C for up to 2 days. The samples were washed in saline at 4° C for 4 times, and lysed in deionized water and freeze-and-thawing in acetone/dry ice bath. The suspension was centrifuged at 10,000 g and the supernatant was used for enzymatic assays.

The red cell glutathione reductase activity assays, with and without FAD, were done according to Beutler<sup>4</sup>, and were performed just before and just after treatment. The activity

coefficient (AC) is calculated as the ratio of the enzyme activity after coenzyme addition / activity before coenzyme addition.

Glutathione reductase saturation with its coenzyme was ascertained by the ratio enzyme activity before in vitro coenzyme addition / enzyme activity after in vitro coenzyme addition in the reagent system x 100.

Mann-Whitney statistical method for non-parametric data was employed with 5% of significance.

## RESULTS

Tables 1, 2 and 3 depict mean glutathione reductase activity values before and after treatment. It can be observed that there is a significant GR activity increase without FAD in the reagent systems, after 20 days treatment in all groups .

On the other hand, when FAD was added to the reagent systems in samples from riboflavin supplemented individuals, no significant activity increase (  $p > 0.05$  ) was observed.

In all groups a significant saturation increase after treatment (  $p < 0.05$  ) was observed. The highest observed saturation was 87% (AC: 1.15), obtained with 50mg riboflavin intake (Table 3).

## DISCUSSION

The GR activity increase without addition FAD in the reagent systems after treatment with riboflavin in all groups of this work has been also reported by other authors<sup>2, 3, 6, 8</sup>.

However, when FAD was added in the reagent system from riboflavin supplemented individuals samples, it was not observed significant GR activity increase , what suggests the physiological saturation was enhanced by the vitamin intake.

Increasing *in vivo* saturation was observed with increasing oral doses of riboflavin supplementation (Figure 1), but total saturation was not observed even with the highest oral 50 mg riboflavin supplementation. What would limit this saturation might be the slow riboflavin input into the red cells, or the existence of an absorption and or a metabolism regulatory mechanism.

It is interesting that glucose-6-phosphate dehydrogenase deficient, it does not matter their nutrition condition, present 95%<sup>6,14</sup> saturation. In these individuals there is high red cell FAD concentration but low serum level, and this deficiency could increase the red cell FAD concentration and facilitate the FAD linkage with glutathione reductase<sup>6,11,14</sup>. Cyrrhosis and severe uremia patients present 90%<sup>14</sup> saturation, and although presenting higher red cell FAD concentration, exhibit high serum FAD level<sup>14</sup>.

The data herein presented and together with some reported findings suggest there are factors which avoid that all apoenzymes linking sites may be saturated with FAD.

It is known that red cells remain in circulation for 120 days, and after many metabolic and membrane changes, mostly by oxidative damage, they are deeply affected and are trapped by spleen. It is possible that even red cell ageing may affect the receptors for riboflavin or FAD

As the individuals in this study were given vitamin supplementation for 20 days with high vitamin doses, it is feasible to suggest that riboflavin was preferably used by young and mature red cells, which present functioning metabolic pathways. On the other hand, the senescent erythrocytes which exhibit the referred changes, hardly accomplish with the metabolic needs and would jeopardize the riboflavin utilization. The lack of maximum saturation, of about 13%, could be ascribed

to the percentage of old cells, which comprise those cells older than 105 days, which are not capable to absorb riboflavin and or to convert riboflavin to FAD.

Another approach would to supplement volunteers for 120 days, what would afford constant riboflavin availability for all red cells produced during this period, so that all young cells could be saturated by riboflavin and keep its coenzyme throughout their life span, reaching FAD total saturation in all cells.

**Table 1.** Group A. Mean glutathione reductase activity (IU.g Hb-1. min<sup>-1</sup> a 37°C), without and with FAD addition to the reagent system, and Activity Coefficients (AC). 15 individuals with 3 mg riboflavin daily supplementation for 20 days

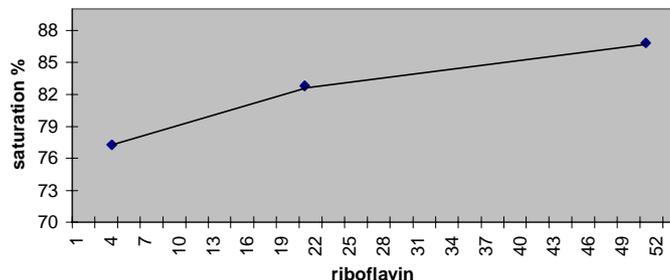
	Without FAD	With FAD	AC	% saturation
Before treatment	7.36 ± 0.6	11.0 ± 0.9	1.5 ± 0.1	66.4 ± 6.2
After treatment	8.5 ± 0.6	11.1 ± 0.9	1.3 ± 0.1	77.3 ± 5.7
<i>P</i>	<0.0001	0.1909	<0.0001	<0.0001

**Table 2.** Group B. Mean glutathione reductase activity (IU.g Hb-1. min<sup>-1</sup> at 37°C), without and with FAD addition to the reagent system, and Activity Coefficients (AC). 15 individuals with 20 mg riboflavin daily supplementation for 20 days

	Without FAD	With FAD	AC	% saturation
Before treatment	7.2 ± 0.7	10.7 ± 0.9	1.5 ± 0.1	68.1 ± 6.2
After treatment	8.8 ± 0.5	10.7 ± 0.9	1.2 ± 0.1	82.8 ± 6.4
<i>P</i>	0.0001	1.0000	0.0001	0.0001

**Table 3.** Group C. Mean glutathione reductase activity (IU.g Hb-1. min<sup>-1</sup> at 37°C), without and with FAD addition to the reagent system, and Activity Coefficients (AC). 18 individuals with 50 mg riboflavin daily supplementation for 20 days

	Without FAD	With FAD	AC	% saturation
Before treatment	7.6 ± 0.8	10.2 ± 1.0	1.4 ± 0.1	74.4 ± 6.8
After treatment	9.0 ± 1.1	10.3 ± 1.1	1.1 ± 0.1	86.8 ± 3.4
<i>P</i>	<0.0001	0.3529	<0.0001	<0.0001



**Figure 1** - Percentage of glutathione reductase saturation achieved by increasing riboflavin supplementation

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**RESUMO.** A atividade da glutatona redutase eritrocitária (GR-E) é dependente da coenzima flavina adenina dinucleotídeo (FAD), derivada da vitamina B2. Em condições nutricionais normais a saturação desta enzima por esta coenzima é parcial. **OBJETIVO:** Estudar se *in vivo* a máxima saturação da GR-E pode ser observada através da administração oral crescente de riboflavina. **MATERIAL E MÉTODOS:** Voluntários normais com idade entre 24 e 54 anos ingeriram por 20 dias consecutivos 3 mg (grupo A), 20 mg (grupo B) e 50 mg (grupo C) de riboflavina. A determinação da atividade enzimática foi realizada antes e após a ingestão da riboflavina, com e sem adição da coenzima no sistema reagente. **RESULTADOS:** Foram observadas saturações de 77%, 83% e de 87% nos grupos A, B e C respectivamente. **CONCLUSÃO:** O aumento crescente da saturação *in vivo* foi observado à medida que a dose de suplementação foi sendo aumentada. A saturação total “*in vivo*” não foi observada mesmo com a maior dose de 50 mg de riboflavina.

**PALAVRAS-CHAVE.** glutatona redutase eritrocitária, riboflavina, vitamina B2, glóbulo vermelho.

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