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Riboflavin Deficiency and Iron Absorption in Adult Gambian Men

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Key Words

Iron absorption
Riboflavin deficiency
Stable isotopes

Abstract

Iron absorption from 3.38 mg ⁵⁸Fe was measured in riboflavin-deficient Gambian men with haemoglobin (Hb) less than 11.5 g/dl before and after oral riboflavin therapy, and the results compared with a group not receiving riboflavin. Riboflavin status (as determined by erythrocyte glutathione reductase activation coefficient) and Hb increased in the riboflavin-supplemented but not placebo group. Plasma ferritin levels were low and did not change in either group. There was very wide variation in percentage iron absorption between individuals and also within single individuals on two separate occasions but no measurable change with riboflavin supplementation. The results of the study indicate that the efficiency of iron utilization is impaired in riboflavin deficiency, but that iron absorption is unaffected.

Introduction

Riboflavin deficiency interferes with iron utilisation. Studies in humans have demonstrated that correction of a riboflavin defi-

ciency enhances the haematological response to iron supplements in subjects with low haemoglobin values [1, 2]. The mechanism of action of the riboflavin supplement is not clear but there is evidence that the effect may

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be related to an improvement in iron absorption. Animal studies have shown that riboflavin deficiency reduces iron absorption, probably via an increased rate of turnover of the epithelial cells of the mucosa of the small intestine [3, 4]. Furthermore, the mobilisation of iron from ferritin has been shown to be dependent, at least in vitro, upon a flavoprotein, NADH-FMN oxidoreductase [5-8] which has implications for iron absorption and haem synthesis.

Previous studies in West Kiang, a subsistence farming region of The Gambia have shown riboflavin deficiency to be widespread all year round [9, 10]. Iron deficiency has been identified as a major cause of anaemia in this region [11].

The study described in this paper was conducted to test the hypothesis that riboflavin deficiency results in reduced iron absorption and that an improvement in riboflavin status will therefore increase iron absorption. Stable isotopes were employed rather than radioisotopes as in previous studies measuring iron absorption [12] because of ethical constraints relating to the possible hazards of ionising radiation.

Methods

The subjects participating in the study were adult males between 16 and 60 years from 3 villages in West Kiang, The Gambia. All men in the three villages were screened for low haemoglobin (Hb) levels and 37 men with Hb < 11.5 g/dl were recruited to the study. Subjects were allocated at random, matched for age and Hb to one of two groups. One group (treatment group) received 10 mg riboflavin daily for 6 days per week for 4 weeks the other group (control group) received no riboflavin. Tablets were administered each evening and the men were seen to swallow them.

All the subjects attended a clinic immediately prior to the initiation of supplementation (clinic 1), after 2 weeks of supplementation (clinic 2) and after a further 2 weeks of supplementation (clinic 3).

Clinic 1: a finger prick blood sample was collected into lithium heparin for the determination of plasma ferritin and erythrocyte glutathione reductase activation coefficient (EGRAC). An oral dose of ^{58}Fe was given to all subjects as part of the iron absorption test.

Clinic 2: 15 ml blood were collected into lithium heparin from the antecubital vein for the measurement of Hb, packed cell volume (PCV), ferritin, EGRAC, glucose-6-phosphate dehydrogenase activity (G6PD), and the ^{58}Fe enrichment of red blood cells. The second ^{58}Fe dose was given, as part of the post-supplement iron absorption test.

Clinic 3: A further 15-ml blood sample was collected for the measurement of Hb, PCV, ferritin, EGRAC, and ^{58}Fe enrichment of the red blood cells.

Biochemical and Haematological Measurements

The following measurements were made on fresh samples of whole blood: (a) Hb by the cyanmethaemoglobin method [13] (b) PCV using a microhaematocrit method (c) G6PD using a spectrophotometric method [14].

The remaining whole blood was centrifuged for separation into plasma and red blood cells. Aliquots were stored at -20°C for up to 3 months for the following assays: (a) EGRAC was measured on the Cobas Bio Autoanalyser using a method described previously [15]; (b) plasma ferritin was measured by radioimmunoassay using an Amersham kit (IM 1051).

Fe Absorption Test

In order to standardise the iron intake for 3 days prior to taking the test dose, all subjects attended a dining centre in the morning, at midday and in the evening to receive meals prepared from local foods. The meals were typical of meals eaten at this time of year and did not include any rich sources of iron. On the 4th day, after an overnight fast, each subject was given 100 ml cola drink containing 3.38 mg ^{58}Fe . The solution of isotope was prepared from a mixture of iron as supplied in several batches from AERE, Harwell, UK, of elemental iron (19.2 mg, 71.5 atom% ^{58}Fe , and 98.7 mg, 66.98 atom% ^{58}Fe , 74.4 mg, 61.08 atom% ^{58}Fe and 131.7 mg, 71.88 atom% ^{58}Fe) and Fe_2O_3 (24.9 mg iron 71.5 atom% ^{58}Fe) dissolved in aristar HCl and made up to an appropriate volume with distilled water. The ^{58}Fe concentration was determined by neutron activation analysis (NAA). Absorption of the ^{58}Fe was estimated from the ^{58}Fe enrichment of the red blood cells collected 14 days after ^{58}Fe dose [16].

Since the red cells have a relatively slow turnover, we made the assumption that the ^{58}Fe enrichment 14 days following the first test dose remained constant for

Table 1. Details of subjects at the beginning of the study: age (y), body weight (kg), Hb (g/l), PCV (%), plasma ferritin ($\mu\text{g/l}$) and EGRAC

	Group 1 (18)	Group 2 (19)
Age	44.7 \pm 10.7	39.9 \pm 14.8
Body mass index ¹	18.5 \pm 1.5	19.2 \pm 1.8
Body weight	54.1 \pm 5.9	54.4 \pm 6.1
PCV	38.6 \pm 3.7	39.1 \pm 3.7
Hb	107 \pm 14.0	106 \pm 14.0
Ferritin ²	14.4 \pm 13.2	21.1 \pm 20.0
EGRAC ³	2.174 \pm 0.388	2.075 \pm 0.362

Mean values \pm SD, number of observations are shown in parentheses.

¹ Weight/height².

² Results for 1 subject unacceptably high (possibly due to infection), excluded from statistical analysis.

³ Subjects with G6PD deficiency excluded from statistical analysis.

the duration of the study. This amount was deducted from the final enrichment figure 14 days after the second test to give the percent absorption from the second test dose. For the ⁵⁸Fe determination the red blood cells were thawed and weighed into large silica crucibles. They were dried slowly on a hot plate, care being taken not to let the viscous material foam over the top of the crucible. The dried material was covered and gradually heated to 480 °C in a muffle furnace, every precaution being taken to minimise the risk of further foaming. After 48 h, the crucibles were cooled and weighed, and the ash:red blood cell ratio calculated.

Portions of homogenised ash were analysed for iron by atomic absorption spectroscopy (AAS) and ⁵⁸Fe by as described previously [17]. Enrichment of the ashed red blood cells with ⁵⁸Fe was determined by deducting naturally occurring ⁵⁸Fe, 0.28 wt% of total iron (AAS), from total ⁵⁸Fe (NAA).

Calculation of Iron Absorption

The total volume of circulating blood was estimated from the subject's age and body weight by means of a nomogram. This was multiplied by the PCV (as a fraction) to obtain the volume of red blood cells and then multiplied by 1.0941 to obtain the total weight of red blood cells [18]. The ⁵⁸Fe enrichment of the ashed blood was multiplied by the ash:red blood cell ratio and total weight of red blood cells to obtain

the weight of ⁵⁸Fe absorbed from the test dose. This was then expressed as a fraction of the administered dose (3.38 mg).

Statistical Analysis

Differences between the riboflavin-supplemented and placebo groups for age, bodyweight, Hb, PCV, ferritin and EGRAC values were tested by means of Student's t tests. Subjects with demonstrable G6PD deficiency were excluded from any analyses involving EGRAC values [19]. The relationships between Hb and PCV, and Hb and ferritin were examined by regression analysis. Ferritin and EGRAC values in the two groups were subjected to two-way Anova with variables time and supplementation. Iron absorption from the first test dose differed between the two groups, therefore the effect of riboflavin supplementation was tested using paired t tests (dose 1 vs. dose 2) separately for each group. The correlations between iron absorption, EGRAC and ferritin at the time of the first test dose were tested by multiple regression analysis. The regression analyses and Anova were carried out using GENSTAT programs [20].

Ethical Considerations

The study was approved by the MRC Gambian Ethical Committee and the AFRC IFR Ethics Committee.

Results

The mean age, body mass index (BMI), Hb, PCV, ferritin and EGRAC values of the two groups of subjects at the start of the study are given in table 1. There were no differences in the means and SDs of the two groups. When data from all subjects were examined together, there were significant correlations between Hb and PCV ($R = 0.40$, $p < 0.05$), and Hb and ferritin ($r = 0.36$, $p < 0.05$). The mean BMI was just below the 'ideal' range of 20–25 as classified by the Health and Welfare Canada [21]. The Hb levels were indicative of iron deficiency anaemia since the lower limit of normality is taken to be 130 g/l, but the ferritin levels, although low, were not in the deficiency range of $< 12 \mu\text{g/l}$.

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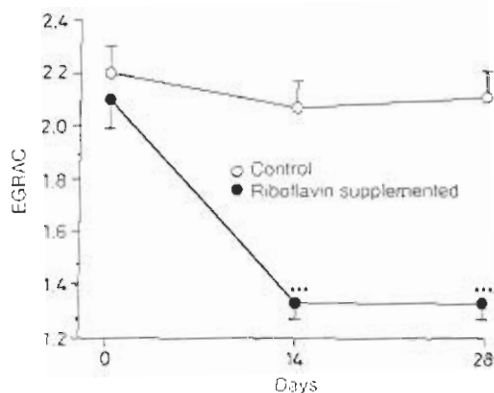


Fig. 1. EGRAC values in riboflavin-supplemented (●; n = 17) and control (○; n = 18) groups of subjects after 0, 14 and 28 days. Values are means with SE bars. Riboflavin-supplemented group significantly different from placebo; *** $p < 0.001$.

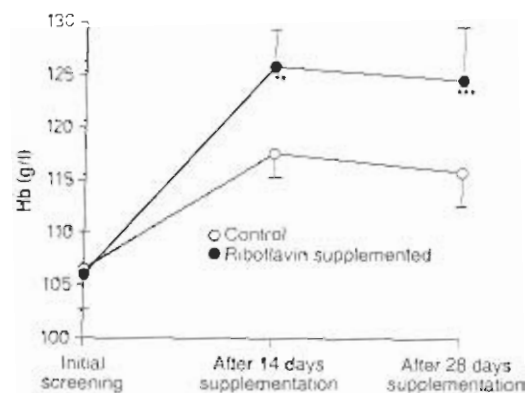


Fig. 2. Hb concentration (g/l) in riboflavin supplemented (●; n = 19) and control (○; n = 18) groups of subjects at initial screening and 14 and 28 days. Values are means with SE bars. Riboflavin-supplemented group significantly different from placebo: ** $p < 0.01$, *** $p < 0.001$.

Table 2. Blood PCV (%) and plasma ferritin (µg/l) in control subjects given riboflavin supplements for 28 days

	Placebo			Riboflavin-supplemented			Residual mean square ¹		
	initial	14 days	28 days	initial	14 days	28 days	time	supp	time·supp
PCV	38.6 (18)	40.0 (18)	38.8 (18)	39.1 (19)	40.3 (19)	40.4 (19)	15.7	17.8	4.4
Ferritin	14.4 (17) ²	21.7 (18)	12.7 (18)	21.1 (19)	21.0 (19)	22.5 (18) ²	157.4	746.8	265.5

Values are means, number of observations in parentheses.

¹ $SED = \sqrt{RMS ((1/n_1) + (1/n_2))}$, $t = (mean_1 - mean_2)/SED$.

² Results for 1 subject unacceptably high (infection?) so excluded from statistical analysis.

G6PD deficiency interferes with the EGRAC test for riboflavin status [19]. Two were identified as deficient in G6PD and were excluded from analyses involving EGRAC.

Riboflavin supplementation caused a decrease in EGRAC values ($p < 0.001$) and an

increase in Hb concentrations ($p < 0.001$), as illustrated in figures 1 and 2. In both cases, the effect was seen 14 days after starting the supplementation and no further change was observed over the next 14 days. There were no changes in mean values for PCV or ferritin, as shown in table 2.

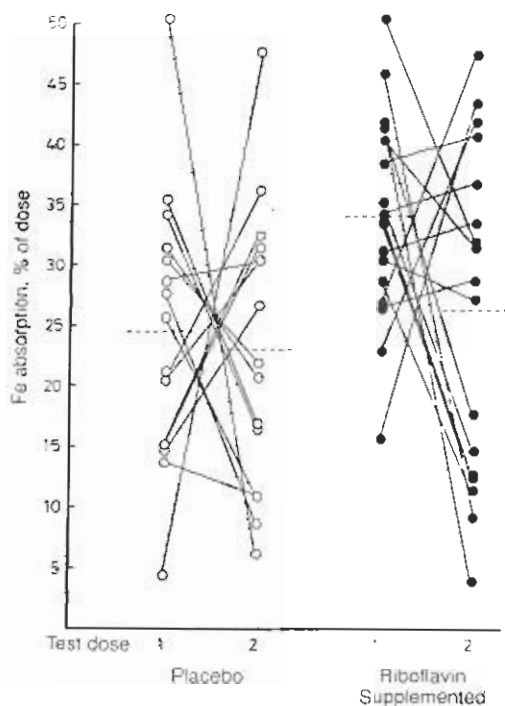


Fig. 3. Fe absorption (% of administered dose) in riboflavin supplemented (●; n = 17) and control (○; n = 15) groups of subjects initially and after 14 days supplementation. Values are individual results with group means (---).

Individual results for iron absorption are shown in figure 3. Two subjects were excluded from the analysis because they did not appear to absorb any of the test dose on one occasion, and a further 3 were rejected as outliers because Anova showed large residuals [23]. There was a very wide inter-individual variation in % absorption from the test dose. Ferritin and EGRAC values were not correlated with iron absorption from the first test dose when tested by multiple regression analysis. There was no effect of riboflavin supplementation on percentage iron absorption from the test dose.

Discussion

Previous studies in rats have shown that riboflavin deficiency is associated with a reduction in iron absorption and an increase in rate of loss of iron after absorption [3]. The origin of the iron loss is under investigation, but recent evidence suggests an increased rate of turnover of epithelial cells of the mucosa of the small intestine [4]. Correction of riboflavin deficiency should concomitantly result in an increase in iron absorption and a reduction in the rate of loss of Fe, thereby leading to an improvement in iron status.

The increase in blood Hb concentrations seen in the present study after only 14 days riboflavin supplementation agrees with results from previous studies [1, 2], and reflects a greater supply of iron to the erythroid marrow. The iron may come directly from the diet or from mobilisation of iron stores, mainly deposited in the liver. We were, however, unable to demonstrate a difference in iron absorption between the riboflavin-supplemented and placebo groups at the end of the study, or that an improvement in riboflavin status was associated with altered iron absorption. Nor was there any correlation between percentage iron absorption from the first test dose and plasma ferritin or blood Hb levels.

In the previous animal work, the riboflavin-deficient rats absorbed approximately 15% less iron than the controls. The variances in the present human study were much greater than in the rat studies, and may have confounded our attempt to detect an effect of riboflavin status on iron absorption. A major methodological problem in iron absorption studies is the enormous variability in the percentage absorption. This variation is comprised of day-to-day differences within the same subject and of biological differences between subjects [24]. The size of the variation

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depends to some extent upon dose, the smaller the test dose the greater the variability [25]. Experimental data are not available to indicate the variation in studies using stable isotopes, but it has been shown with radioisotopes that the variation in absorption on different days within a single individual given 5 mg doses is $\pm 35\%$ (coefficient of variation) [25]. In order to minimise the problem of variation of absorption, test doses should be administered on several occasions and the mean figure for percentage iron absorption taken as representative of the individual's capacity to absorb iron under the given experimental conditions. However, it was not possible to follow this type of protocol in the present study.

The haematological profile of the subjects was interesting. The initial mean Hb concentration of 106 g/l indicated iron deficiency anaemia, yet the ferritin values were not low enough to support this conclusion. It would appear that the riboflavin-deficient state had altered the efficiency of iron utilization and that the anaemia was not primarily due to iron lack but to an impairment in Hb synthesis. Once the riboflavin deficiency had been corrected, Hb levels returned to normal. Riboflavin has been implicated in the mechanism for iron utilisation by the erythroid mar-

row for haem synthesis [26, 27], but it has yet to be proven that riboflavin plays a role in haem synthesis, via an FMN oxidoreductase system. However, a mechanism of this nature would mean that correcting a riboflavin deficiency would increase the efficiency of iron utilisation.

The present study confirms that the correction of a subclinical riboflavin deficiency can increase Hb concentration but with no detectable change in efficiency of iron absorption. The failure to demonstrate an association between riboflavin status and iron absorption may be due to the very large variability in iron absorption between the different subjects. An improved methodology for determining iron absorption would enable us to make a more reliable assessment as to whether or not small changes in iron absorption (as seen in rats) play any role in the observed increase in Hb concentration following riboflavin supplementation in man.

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