Human requirements for riboflavin

Dear Sir:

While greatly respecting Dr Horwitt's contributions to the subject of human requirements for vitamins, I would like to record an alternative viewpoint from his (1, 2) about the usefulness of the glutathione reductase test for riboflavin status.

In my experience, the activation coefficient of erythrocyte glutathione reductase (EGRAC) is well correlated with riboflavin intakes and with several other indices of riboflavin status in experimental animals and man. We showed in rats (3) an almost linear relationship between dietary riboflavin (0–30 μg/g diet) and EGRAC (3.7–1.3) which remained constant within diet groups for long periods and correlated precisely with tissue flavin levels and with other riboflavin-requiring enzyme activities.

Dr Horwitt (1) has queried the interpretation of EGRAC values < 1.5 in human studies and has requested data on the relationship between EGRAC and riboflavin concentration in the erythrocytes. Figure 1 shows the relationship between EGRAC and red cell riboflavin (μg/g hemoglobin) pooled from individual measurements on ~1300 blood samples from Gambian and UK adults, whose intakes ranged from < 0.4 to ~4.0 mg/d. Clearly the range of EGRAC values over which red cell riboflavin changes most rapidly is 1.0–1.5, whereas for EGRAC > 1.8 there is very little relation between the two indices (and significantly less than that predicted from a simple mathematical relationship). This might be due to individual variations in EGRAC unconnected with tissue status, or it might indicate a region of EGRAC sensitivity that is not shared by riboflavin levels. It may, however, help explain why some previous studies have failed to detect a significant relationship between the two, and it also helps to validate changes in EGRAC over the range 1.0–1.5 in terms of another status index.

Changes in EGRAC during controlled supplementation have, in my experience, always been accompanied by corresponding changes in red cell riboflavin. In a study of 30 lactating Gambian women (4) whose home food provided ~0.5 mg riboflavin/d, a supplement of 2.0 mg/d reduced mean EGRAC from 1.6 to 1.2 and increased red cell riboflavin from 0.38 to 0.58 μg/g hemoglobin. (Incidentally, in this study the EGR activity with saturating FAD increased from 5.56 to 6.95 IU/g hemoglobin, an increase of 25%, suggesting increase in apoenzyme level as well as increased saturation of existing apoenzyme. However in reply to recent comments [5, 6], we have always observed a close correspondence between EGRAC and unstimulated EGR levels, except where hypocromic anemia may perhaps confuse the picture. EGRAC, is of course, less sensitive to such confounding factors.)

I tend to prefer EGRAC over urinary excretion for the following reasons:

1) EGRAC is very sensitive, as indicated by percent change per unit increment in dietary intake, over the most critical range between severe and moderate undersaturation. This was well illustrated by our Gambian study (7, 8), in which an increase in riboflavin intake from ~0.5 mg to 1.3–1.5 mg/d in pregnant or lactating women reduced EGRAC from ~1.8 to ~1.4 and virtually abolished clinical signs of deficiency (8). Although urine levels weren't measured because even the supplemented women generally weren't saturated, it is likely that their fasting urinary riboflavin excretions would have been low. Thus while agreeing with Dr Horwitt that a substantial urinary excretion is likely to be proof of sufficiency, I suspect that a very low level can be compatible with either severe, or with mild-to-moderate deficiency, and it is important to be able to distinguish these.

2) EGRAC, by its nature, tends to integrate and hence dampen short-term status fluctuations and this must help reduce measurement noise.

3) With modern equipment we find the EGRAC test to be extremely robust, sensitive, simple, and reproducible to perform. It is within the sensitivity of a finger-prick blood sample, is stable for many months at -25°C, and in our hands given an intra-assay CV of 1.5% on a Cobas Bio centrifugal analyzer (9).

The concept of minimum requirements for nutrients is in some ways as difficult to define as that of recommended dietary allowances, because different indices inevitably give different cutoff points. Thus the cutoff for clinical deficiency correction is very low, that for change in slope of urinary excretion is higher, and that for saturation of erythrocyte enzymes like EGR is higher still. There are insufficient data available to allow for a pref-
ference between the biochemical indices on purely functional grounds.

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References


Reply to letter by Bates

Dear Sir:

There is no doubt that under controlled conditions of dietary intake in experiments conducted for sufficient time periods, one can obtain good correlation between riboflavin intake and the activation coefficient of erythrocyte glutathione reductase (EGRAC). The statements made in our papers (1, 2) were intended to highlight the fact that there have been several reports of studies that claimed deficiencies of riboflavin in human subjects based upon the EGRAC test despite the fact that the amounts of riboflavin in the urine of these subjects were quite large. It should be axiomatic that one cannot have a deficiency of riboflavin when a healthy kidney excretes substantial amounts of riboflavin. Continue to use the EGRAC test, if you will, but in survey studies when deficiency is suspected as a result of this test, it should be confirmed with a rather simple analysis of a urine sample for its riboflavin content. Recent increased consumption of as much as 0.5 mg of riboflavin causes minimal increases in urinary riboflavin in true riboflavin deficiency.

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References