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## Plasma riboflavin concentration as novel indicator for vitamin-B2 status assessment: suggested cutoffs and its association with vitamin-B6 status in women

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### Abstract

Riboflavin (vitamin B2), as the coenzymes flavin mononucleotide (FMN) and flavin dinucleotide (FAD), is essential for oxidation-reduction reactions and energy metabolism. Riboflavin also interacts with vitamin B12, B6 and folate in one-carbon metabolism, and is required for the conversion of dietary vitamin B6 forms to the coenzyme pyridoxal 5'-phosphate (PLP). Biochemical riboflavin status is rarely measured given the lack of convenient and accessible biomarkers. The current gold-standard marker is erythrocyte glutathione reductase activation coefficient (EGRac) that involves laborious sample processing. High prevalence of riboflavin deficiency (EGRac  $\geq$  1.4) and suboptimal status (EGRac of 1.3–1.39) have been reported in the UK and Ireland; yet the functional significance is unclear. Plasma riboflavin concentration may serve as an alternative indicator; its association with related metabolites has not yet been investigated. Secondary analysis was conducted to determine the change-point of plasma riboflavin with EGRac, to derive a reference interval for plasma riboflavin, and to determine the association of riboflavin status with plasma PLP, using data of 223 older adult women from a cross-sectional study. Fasting blood samples and sociodemographic, anthropometric and dietary data were available for a convenience sample of 223 older adult women. Plasma PLP and related metabolites were quantified using isotope-dilution liquid chromatography-tandem mass spectrometry. The change-point (95% CI) between EGRac and plasma riboflavin occurred at plasma riboflavin concentration of 26.5 (20.5; 32.5) nmol/L (with EGRac of 1.25). The median (IQR) plasma riboflavin concentration was 15.7 (11.2, 23.8); and the upper and lower limits (90%CI) of the central 95% reference interval were 6.70 (6.33, 7.79) and 64.2 (55.0, 74.6) nmol/L, respectively. Plasma PLP (geometric mean (95%CI)) was significantly lower in women with riboflavin deficiency, 54.0 (46.8, 62.2) nmol/L ( $n = 64$ ), and suboptimal riboflavin status, 56.1 (48.9, 64.3) nmol/L ( $n = 48$ ), compared to those with riboflavin adequacy, 135 (112, 161) nmol/L ( $n = 110$ ). Plasma PLP was positively associated with plasma riboflavin concentration after adjustment for total B6 intake, age, ethnicity, BMI, education, household income and C-reactive protein concentration [ $\beta$  (95% CI) = 1.92 (.670, 3.17) nmol/L;  $p = 0.003$ ]; a significant interaction between plasma riboflavin and total dietary B6 intake was observed ( $p = 0.024$ ). In conclusion, we are presenting for the first time a reference range for plasma riboflavin concentration and its change-point with EGRac in healthy women. Vitamin B6 status is strongly associated with riboflavin status; more research is needed to elucidate this relationship in a larger sample and ideally intervention study.

### Conflict of Interest

There is no conflict of interest