Is the Generation of Active Vitamin B6 Dependent upon Riboflavin Status? New Analysis of Data from RCTs of Riboflavin Supplementation †

Ryan Barlow 1,*, Helene McNulty 1, Catherine Hughes 1, Kristina Pentieva 1, Geraldine Horigan 1, Yvonne Lamers 2 and Mary Ward 1

1 Nutrition Innovation Centre for Food and Health (NICHE), Ulster University, Coleraine BT52 1SA, UK; h.mcnullty@ulster.ac.uk (H.M.); c.hughes@ulster.ac.uk (C.H.); k.pentieva@ulster.ac.uk (K.P.); gb.horigan@ulster.ac.uk (G.H.); mw.ward@ulster.ac.uk (M.W.)
2 Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC V6T 1Z4, Canada; yvonne.lamers@ubc.ca
* Correspondence: barlow-r@ulster.ac.uk

Abstract: Background and objectives: Riboflavin in the form flavin mononucleotide (FMN) acts as a cofactor for the pyridoxine phosphate oxidase required to generate pyridoxal 5′-phosphate (PLP), the active form of vitamin B6 in tissues. Few human studies have investigated this metabolic interaction between riboflavin and vitamin B6. The primary objective of this study was to examine the response of plasma PLP to riboflavin supplementation in individuals with the MTHFR 677TT genotype. A secondary objective was to consider whether the dose of riboflavin (1.6 mg/d vs. 10 mg/d) affects the PLP response. Methods: Data from four randomised controlled trials (RCTs) of riboflavin supplementation previously conducted at this centre were accessed to identify 209 participants of 19–60 years meeting the inclusion criteria (≤ 60 years, MTHFR 677TT genotype, not taking a vitamin B6 supplement). In the original RCTs, participants were randomly assigned to receive a placebo (n = 85) or 1.6 mg/d of riboflavin (n = 87) for 16 weeks. In one trial only, a higher riboflavin dose, 10 mg/d (n = 37), was administered. Plasma PLP was measured via reversed phase HPLC with fluorescence detection. Riboflavin status was assessed using the functional assay, erythrocyte glutathione reductase activation coefficient (EGRac). Results: riboflavin supplementation resulted in a decrease (p < 0.001) in the mean EGRac values, from 1.34 (1.32, 1.37) to 1.21 (1.19, 1.22). Correspondingly, PLP increased (p = 0.027), an effect driven by those with a sub-optimal riboflavin status at baseline (EGRac > 1.26), whereby PLP increased by 5.2 nmol/L, from 44.9 (40.3, 49.4) to 50.1 (44.6, 55.6) nmol/L (p = 0.042), while with the optimal baseline riboflavin (EGRac ≤ 1.26), there was no significant PLP response to the intervention. Although 10 mg/d vs. 1.6 mg/d of riboflavin resulted in a greater EGRac response (p = 0.012), there was no significant effect of riboflavin dose on the PLP response. Discussion: These results provide randomised trial evidence that optimising riboflavin status leads to an increase in plasma PLP, confirming the metabolic dependency of vitamin B6 on FMN. The findings indicate that riboflavin intake may need to be considered when setting dietary recommendations for vitamin B6 in adults. Further work is needed to explore the impact of the common MTHFR C677T polymorphism of the interrelationship of these B vitamins.

Keywords: riboflavin; vitamin B6; pyridoxal 5′-phosphate; one-carbon metabolism

Author Contributions: The authors’ contributions were as follows: H.M., M.W. and R.B. conceptualised and designed the study. All authors completed the acquisition, analysis and interpretation of the data. H.M. and M.W. obtained study funding. H.M., M.W., R.B. and K.P. were responsible for the methodology. H.M., M.W. and C.H. provided study supervision. R.B. drafted the original version of
the manuscript. All authors critically revised drafts of the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was completed as part of the DERiVE project, awarded under the JPI ERA-HDHL scheme for transnational research under the ‘Biomarkers for nutrition and health’ scheme: UK – Biotechnology and Biological Sciences Research Council (BBSRC, grant ref: BB/P028241/1).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.