Project Title:
Development of Riboflavin biomarkers to relate dietary sources with status, gene-nutrient Interactions and Validated health Effects in adult cohorts; DERIVE

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Level: PhD

Background to the project:
Hypertension, a major risk factor for heart disease and stroke, is the world’s leading cause of preventable, premature death. A common polymorphism (677C→T) in the gene encoding the folate metabolizing enzyme methylenetetrahydrofolate reductase (MTHFR) is associated with increased blood pressure, and there is accumulating evidence demonstrating that this phenotype can be modulated, specifically in individuals with the MTHFR 677TT genotype, by the B-vitamin riboflavin, an essential co-factor for MTHFR.

Although not generally considered to be an issue in the developed world, because of the reliance on nutrition surveys to report riboflavin status, accumulating evidence suggests that sub-optimal riboflavin status appears to be more widespread than is generally recognised. Biomarker status is rarely measured because the existing biomarker EGRac requires very specific pre-analysis processing, unfeasible in most settings. The DERIVE project which represents a collaboration between partners in Ireland and Canada will address this gap by developing accessible riboflavin biomarkers for use in population surveys globally (Canadian partners). At Ulster important functional, gene-nutrient and health effects of optimal riboflavin status in Canadian, Irish and UK cohorts will be demonstrated by investigating the role of riboflavin in modulating blood pressure via the novel gene-nutrient interactive effect mediated through MTHFR. This will build considerably on recent findings from RCTs conducted at this centre showing significant blood pressure-lowering effects of riboflavin in adults pre-screened to select those homozygous for the common C677T polymorphism in MTHFR.

Objectives of the research project:

- **OBJECTIVE 1:** To evaluate the health benefit of riboflavin (using direct and functional biomarkers) in modulating blood pressure via a novel gene-nutrient interactive effect

We anticipate that blood pressure will be significantly higher in adults with the MTHFR 677TT genotype than in those with the 677CC or 677CT genotypes within our population cohorts, and that higher biomarker riboflavin intake/status will neutralise the elevated blood pressure phenotype whilst low riboflavin will exacerbate it. Both functional (EGRac) and direct (plasma, FMN and FAD) riboflavin will be included in this analysis.
OBJECTIVE 2: To investigate the functional significance of riboflavin status by determining the biomarker response of a metabolically-dependent nutrient, vitamin B6, to intervention with riboflavin

We anticipate that an improved B6 status will be observed in response to intervention with riboflavin.

Methods to be used:

OBJECTIVE 1:

This analysis will be based on data from the two population cohorts; the National Adult Nutrition Survey (NANS) of Ireland and the Canadian BC Generations project and will include relevant health and lifestyle information which was obtained in face-to-face interviews conducted by trained researchers.

Blood samples collected from NANS and BC Generations Project participants were analyzed for routine laboratory markers in the participating local laboratories at the time of collection. Riboflavin status, as measured by EGRac, was previously analysed in the NANS cohort by Ulster and will be accessed. Samples (red blood cells) from the BC Generations Project cohort will be shipped to Ulster for EGRac analysis. Direct measures of riboflavin status analysed by the Canadian partners (plasma riboflavin, FMN and FAD) will be accessed for statistical analysis. Information on Blood pressure, which was measured in accordance with standard operating procedures and clinical guidelines in each population cohort will be accessed for analysis.

Statistical analysis

Statistical analysis will be performed using the Statistical Package for Social Sciences (SPSS, version 21, SPSS UK Ltd, Chertsey Road, Surrey, UK). Before statistical analysis, tests for normality will be performed and variables log-transformed as appropriate. Analysis will be limited to participants with available MTHFR genotype, valid blood pressure and riboflavin biomarker measurements. Participant characteristics by MTHFR genotype and differences between groups will be analyzed using one-way between-groups analysis of variance (ANOVA) for continuous variables and χ2 tests for categorical parameters. Logistic regression analysis will be performed to examine the association of MTHFR genotype and established risk factors with the risk of hypertension. Multinomial regression will be performed to assess the interactive effect of MTHFR genotype with riboflavin status (low versus normal) using both biomarkers on the risk of prehypertension and hypertension. Odds ratios will be calculated using MTHFR 677CC genotype combined with normal riboflavin status as the reference category.

Objective 2:

Samples will be accessed from randomised controlled intervention studies previously conducted at Ulster. In total we will have 537 pre- and post-intervention samples available from 4 previously conducted RCT’s. All studies conducted followed a similar protocol with riboflavin intervention at 1.6 or 10mg/d for a period of 14-16 weeks. Male and female participants, aged 18 years or above who were non-consumers of B-vitamin supplements were recruited from across Northern Ireland. Ethical approval for all studies was granted by either the Research Ethics committee at Ulster University or the Office for Research Ethics in NI. B6 analysis will be conducted to determine the biomarker response of a metabolically-dependent nutrient, vitamin B6, to intervention with riboflavin.

Skills required of applicant:

- The applicant should be highly motivated and willing to engage in teamwork and collaborative research.
- Excellent interpersonal skills
- Ability to work on own initiative
- Excellent written and oral communication skills
- Ability to complete a project within a specified time
- Willingness to learn new skills and techniques, including laboratory skills.
- Organisational skills and record keeping
References:


