Institution: Ulster University

Unit of Assessment: Allied Health Professions, Dentistry, Nursing and Pharmacy (3)

Title of case study: ICS-6 Vitamin B2 (riboflavin) as a novel, non-drug, intervention for prevention and treatment of high blood pressure in genetically at-risk adults

Period when the underpinning research was undertaken: 2002 - 2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Professor Mary Ward	Professor of Nutrition and Dietetics	1998-present
Professor Helene McNulty	Professor of Nutritional Sciences	1992-present
Professor JJ (Sean) Strain	Professor of Human Nutrition	1981-present
Professor Kristina Pentieva	Professor in Human Nutrition	1998-present
Dr Catherine Hughes	Lecturer in Public Health Nutrition	2010-present
Dr Liadhan McAnena	Scientific Officer	2001-present

Period when the claimed impact occurred: 2014 - 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

Underpinning research at Ulster identified a novel role for the B-vitamin, riboflavin, as a non-drug solution for high blood pressure (hypertension) in genetically at-risk adults. Since 2014, a patent filing, protected in 12 countries worldwide, resulted in significant licensing agreements with:
Impact 1: Aprofol, a Swiss life science start-up, to develop a new product for eye health.
Impact 2: DSM, the world's leading producer of vitamins, to develop a new product - a drug-nutrient combination - to treat hypertension.

The research was also used by the European Food Safety Authority (EFSA) to:
Impact 3: establish EU dietary recommendations for riboflavin to deliver health benefits for populations.
Impact 4: substantiate health claims used by food manufacturers.

2. Underpinning research

Early underpinning research conducted at Ulster (2002-10) discovered that intervention with riboflavin resulted in: (i) improved status of the vitamin as indicated by a response in the gold standard biomarker (erythrocyte glutathione reductase activation coefficient; EGRac) **(R1),** and more importantly, (ii) restoring the activity of the enzyme methylene tetrahydrofolate reductase (MTHFR) in individuals with impaired folate metabolism owing to a common genetic variant (i.e. *MTHFR* 677TT; aka 'TT genotype' in the *MTHFR* gene) (**R2**).

Our landmark research **(R3)** was a randomised controlled trial which showed, for the first time, that riboflavin intervention lowered blood pressure (BP) in patients with this specific genetic variant (TT genotype) that had previously been associated with a higher risk of hypertension. We investigated over 400 premature cardiovascular disease patients, pre-screened for *MTHFR* genotype, to select those with the genotype of interest (TT *n*=54) and compared them to those without this genetic variant. At baseline, we observed that target BP (< 140/90 mmHg) was achieved in only 37% of patients with the TT genotype compared with 62% in those with the non-TT genotypes. Some 179 patients completed the intervention, receiving either 1.6 mg per

day riboflavin or placebo for 16 weeks. Riboflavin intervention reduced mean BP in those with the TT genotype from 144/87 to 131/80 mmHg, with no response observed in those without the genetic variant. Notably, this 13mmHg decrease in systolic BP occurred even though over 80% of the patients were taking one or more antihypertensive drugs at recruitment. The magnitude of BP response achieved compares very favourably with typical decreases from other interventions such as dietary salt reduction of 3g/d (3.6/1.9 mmHg) and 6g/d (7.1/3.9 mmHg).

In a separate study in hypertensive patients without overt cardiovascular disease (CVD), those with the TT genotype were found to have higher BP and poorer BP control on antihypertensive therapy **(R4)**. Once again, BP responded significantly to 1.6mg/d of riboflavin for 16 weeks in the TT genotype group. This trial confirmed that the novel BP findings were not confined to high-risk cardiovascular disease patients but also applied to hypertensive patients generally and that the effect was independent of the antihypertensive drugs co-administered (**R4**). Furthermore, for patients with the TT genotype, the addition of supplemental riboflavin was shown to greatly enhance the achievement of goal BP with routine antihypertensive drugs.

The biological mechanism linking this gene-nutrient interaction and BP had not previously been considered; however we recently reported that it is mediated via the potent vasodilator nitric oxide as outlined in **R5,** greatly strengthening the underpinning research. Notably, we have recently shown in a large cross-sectional cohort of approximately 6,000 Irish adults that the variant TT genotype is associated with higher BP from 18 years and predisposes to an increased risk of hypertension and poorer BP control in response to antihypertensive treatment, throughout adulthood, whilst better riboflavin status is associated with a reduced genetic risk **(R6).** We have also confirmed BP differences by genotype in adulthood for the first time using the recognised NICE (National Institute for Health and Care Excellence) method for diagnosing hypertension and we are currently investigating the role of this novel gene-nutrient interaction as a determinant of BP in pregnancy. This new research, funded by the Public Health Agency in Northern Ireland, is important given the high rates of maternal and infant morbidity and mortality worldwide associated with hypertensive disorders of pregnancy (e.g. pre-eclampsia). Moreover, we are exploring further interactions between riboflavin and the metabolically linked B-vitamins in research funded by the Biotechnology and Biological Sciences Research Council (BBSRC) under the European Joint Partners Initiative (resultant papers not cited here).

In summary, **R3** was the critical research output for the filing of the patent and studies **R4, R5** and **R6** provided additional scientific evidence to attract industry partners and formed the basis of our licensing agreements and the substantiation of health claims. In addition, this evidence base was utilized by the European Food Safety Authority (EFSA) in revising the dietary riboflavin recommendations for health. Our novel research also contributes to the new paradigm of using a nutrient in the treatment (as opposed to prevention) of a disease.

3. References to the research

R1 Hoey L, McNulty H and Strain JJ. Studies of biomarker responses to intervention with riboflavin: a systematic review. The American Journal of Clinical Nutrition. 2009, 89 (6): 1960S-1980S. DOI: https://doi.org/10.3945/ajcn.2009.27230B

R2 McNulty H, McKinley MC, Wilson B, McPartlin J, Strain JJ, Weir DG and Scott JM. Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. The American Journal of Clinical Nutrition. 2002, 76 (2) 436-441. DOI: https://doi.org/10.1093/ajcn/76.2.436

R3 Horigan G, McNulty H, Ward M, Strain JJ, Purvis J and Scott JM. Riboflavin lowers blood pressure in cardiovascular disease patients homozygous for the 677C→ T polymorphism in MTHFR. Journal of Hypertension. 2010, 28 (3) 478-486. DOI: 10.1097/HJH.0b013e328334c126

R4 Wilson CP, McNulty H, Ward M, Strain JJ, Trouton, TG. Hoeft BA, Weber P, Roos FF, Horigan G, McAnena L and Scott JM. 2013. Blood pressure in treated hypertensive individuals

with the MTHFR 677TT genotype is responsive to intervention with riboflavin: findings of a targeted randomized trial. Hypertension. 2013, 61 (6) 1302-1308
DOI: https://doi.org/10.1161/HYPERTENSIONAHA.111.01047

R5 McNulty H, Strain JJ, Hughes CF and Ward M. Riboflavin, MTHFR genotype and blood pressure: a personalized approach to prevention and treatment of hypertension. Molecular Aspects of Medicine. 2017, 53 2-9 (2017) DOI: https://doi.org/10.1016/j.mam.2016.10.002

R6 Ward M, Hughes C, Strain S, Reilly R, Cunningham C, Molloy A, Horigan G, Casey M, McCarroll K, O'Kane M, Gibney M, Flynn A, Walton J, McNulty B, McCann A, Kirwan L, Scott J & McNulty H. Impact of the common MTHFR 677C→T polymorphism on blood pressure in adulthood and role of riboflavin in modifying the genetic risk of hypertension: evidence from the JINGO project. BMC Med 18, 318 (2020). DOI: https://doi.org/10.1186/s12916-020-01780-x

Key research grants:

- M Ward, H McNulty, C Hughes, JJ Strain. *Blood pressure lowering with riboflavin and related B-vitamins to promote health through the lifecycle; OptiPREG study*. Funder: DSM Nutritional Products Ltd**: GBP944,733** (2011-2021; 8 consecutive awards).
- M Ward, H McNulty, K Pentieva, L McAnena, JJ Strain. *Biomarkers for Nutrition and Health 2* – the DERIVE project. Funder: BBSRC (JPI-ERA-HDHL): **GBP207,000** (2017-2020).
- H McNulty**,** M Ward, K Pentieva**.** *Optimal nutrition for prevention of hypertension in pregnancy using a personalised approach (OptiPREG).* Funders: HSC R&D Public Health Agency Enabling Award**: GBP40,000** (2017-2018)**.**
- D Lees-Murdock, M Ward. EpiRiboSH: *Epigenetic Effects of Riboflavin Supplementation in a Randomised Control Trial of Hypertensive patients stratified by MTHFR genotype.* Funder: Northern Ireland Chest Heart and Stroke Association: **GBP60,595** (2016-20).
- JJ Strain, H McNulty, M Ward. Joint Irish Nutrigenomics Organisation 'JINGO' project. Funder: Irish Department of Agriculture, Food & the Marine and Health Research Board; *FIRM* initiative: **GBP578,758** (2008-16).
- M Ward, H McNulty, J Purvis, JJ Strain. *The homocysteine-lowering effect of riboflavin in CVD patients with different MTHFR C677T genotypes.* Funder: Northern Ireland Chest Heart and Stroke Association: **GBP36,490** (2004-06).

4. Details of the impact

Impacts 1 and 2: Our research has direct application in the prevention and treatment of high blood pressure (hypertension), the number one cause of death globally, primarily from heart disease and stroke. Globally an estimated 26% of the world's population (972 million people) have hypertension and this is expected to increase to 29% by 2025. Our research has resulted in the generation of a University Patent Portfolio entitled "Use of riboflavin in the treatment of hypertension" **(C1 EP2139488A1)** in collaboration with Western Health and Social Care Trust and Trinity College Dublin and in turn resulted in two Intellectual Property License Agreements (IPLAs) **(C2, C3)** during the current impact period. The technology is currently protected under patents in China, Mexico, Japan, Canada, Ireland, UK, Italy, Spain, France, Netherlands, Germany and Switzerland.

The health implications of these findings in preventing hypertension are considerable for the 1 in 10 adults worldwide (and much higher in some populations e.g. 32% in Mexico) who carry this genetic risk factor. In particular, patients with existing hypertension will benefit, as documented in a video investigating the patient experience showing that riboflavin intervention was not only highly effective at lowering BP, but was also very well received by patients **(C4)**. Riboflavin supplementation thus offers a novel, non-drug treatment (and/or the potential to be combined with an antihypertensive drug) to effectively lower BP, most likely via an effect on nitric oxide (**R5)** and improve BP control in genetically at-risk adults.
Two IP licensing agreements were signed during the current impact period **(C2, C3).**

- The first is a significant agreement for the development of a new product for eye health, Ocufolin, designed by US ophthalmologists to reduce the risk of age-related macular degeneration and diabetic retinopathy by decreasing homocysteine and consequently

reducing microcirculation in eye diseases. The license with Aprofol AG, Switzerland (effective date 28-06-2016; date of variation 26-01-2018) provides exclusive use of 'personalised nutrition' claims related to degenerative eye disease in the European patent with the option to negotiate a patent license in China. Aprofol's investment in Ocufolin to date is estimated at > EUR3M. Aprofol is using the license for sales of a 'medical food' (as defined by the US FDA) and Ocufolin products (for eye health) **(C2)**.

The second license **(C3)** is with DSM Nutritional Products AG, Switzerland (effective date 06-02-2018). The license provides exclusive use of pharmaceutical claims under patent rights in China, Mexico, Japan, Canada and Europe and has realised an upfront payment to the University of EUR100,000 (02-2018) and significant company investment (additional to DSM's research investment in grant income to the University of almost GBP1M, as detailed above). DSM has negotiated an agreement (confidential at this stage) with a global pharma company regarding the development of an antihypertensive-riboflavin combination for the prophylaxis and treatment of elevated BP, building upon the existing patent. In parallel, DSM has produced outputs focusing on hypertension treatment, including a white paper which presents an in-depth look at the science behind riboflavin as a treatment for hypertension and the latest research developments from Ulster **(C5).** The company has also run several promotions using both social graphics and a blog, and promoted a hypertension monograph to a global pharma audience **(C6)**. DSM launched a campaign to promote the concept to a targeted list of pharma companies globally and has used this information successfully to attract the current pharma partner. DSM also engaged a human data science company (IQVIA) to conduct a global survey of doctors (n=736 from US, Germany, France, China) in order to understand the market potential of the concept of a riboflavin-antihypertensive combination (see Figure 1 below). As a result of our licensing agreement (**C3)** with DSM, the company has filed a new international patent **PCT/EP2019/077270** entitled "Medicaments containing riboflavin exhibiting improved flowability" WIPO Patent Application WO/2020/094319 **(C7).**

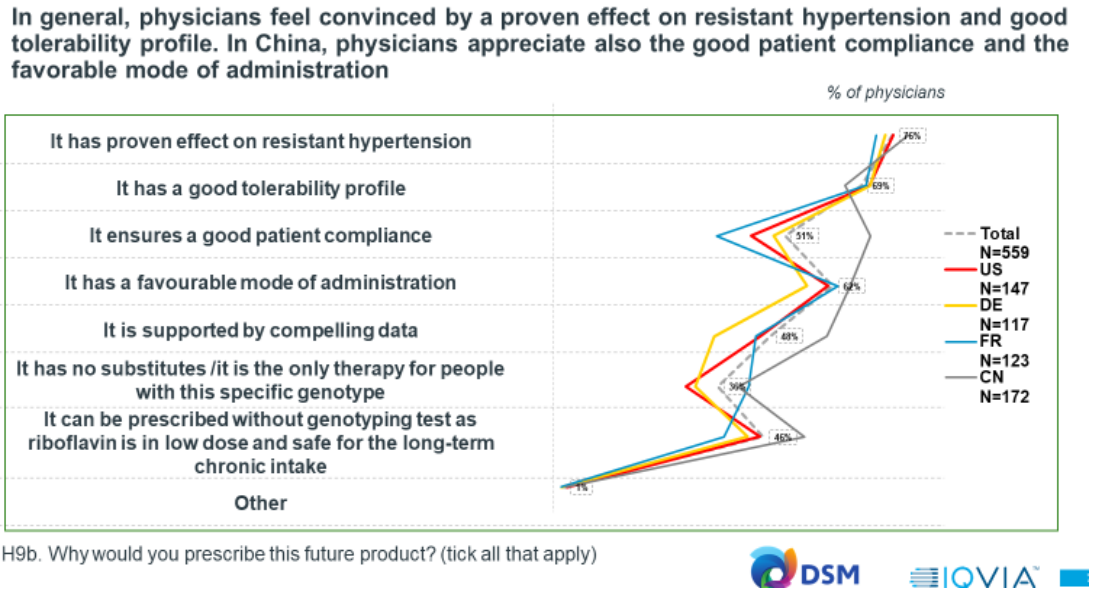

Figure 1: Global survey of physicians demonstrating their appreciation and knowledge of the proven effect of a riboflavin anti-hypertensive combination on resistant hypertension.

Impacts 3 and 4: In addition to the commercial impact, the underpinning research has impacted nutrition and health policy. Of note, the level of riboflavin (1.6 mg/day) found in our research trials to have beneficial effects on BP is within the range of what is achievable through normal dietary intakes, which is important given that our most recent research demonstrated that sub-optimal riboflavin status is associated with a three-fold increase in hypertension across adulthood **(R6)**. Furthermore, riboflavin is entirely safe with no evidence of adverse effects, even at doses 100-fold higher than the 1.6 mg/day proven to be effective. In 2017, the European Food Safety Authority (EFSA) published its scientific opinion on "Dietary Reference Values for Riboflavin". EFSA acknowledged for the first time among policymakers that riboflavin requirements were influenced by the *MTHFR* TT genotype

based solely on the research from Ulster and **R1-R4** were cited in the EFSA opinion **(C8).** Health professionals and food manufacturers in Europe use these dietary reference values daily in their work **(C8).** The underlying research **(R1)** was also cited in a health claim which was substantiated by EFSA and is authorised for use by the food / pharma industry on dietary supplements and any food product containing 15% or more of the reference dietary value for riboflavin, as well as being used by industry to promote their products.

In summary, the impacts are both commercial and health related. The patent portfolio has resulted in close engagement with global industry partners which has led to the development of a novel antihypertensive-riboflavin combination with a leading pharma company **(C1-C7).** The public awareness of the health benefits of riboflavin and their promotion by health professionals and industry are now on a global scale **(C8-C9)** through EFSA Dietary Reference Values for Riboflavin and the on-going promotion of products citing the EU generic health claim.

5. Sources to corroborate the impact

C1: U104 Patent Portfolio entitled "Use of Riboflavin in the Treatment of Hypertension". Collaboration with Ulster University, Western Health and Social Care Trust (WHSCT) and Trinity College Dublin (TCD). Inventors: Mary Ward (Ulster), Helene McNulty (Ulster), Geraldine Horigan (Ulster), Sean (JJ) Strain (Ulster), John Purvis (WHSCT) and John Scott (Deceased, TCD). EP2139488A1.

C2: License agreement between Innovation Ulster Limited and Aprofol AG, Switzerland (effective date 28-06-2016; date of variation 26-01-2018). Email from Founder and CEO of Aprofol. Ocufolin webpage: 'Introducing Ocufolin'.

C3: License Agreement between Innovation Ulster Limited and DSM Nutritional Products AG (effective date 06-02-2018). License provides exclusive use of pharmaceutical claims of the Patent Rights, in particular for the prophylaxis and treatment of elevated blood; Patent Rights i.e. patents in China, Mexico, Japan, Canada and Europe (validated in Ireland, UK, Italy, Spain, France, Netherlands, Germany and Switzerland). Option: use of the Patent Rights in the field of medical foods applications subject to Ulster being contractually free to do so. DSM webpage: 'DSM Nutritional Products'.

C4: DSM patient video for marketing purposes, showing riboflavin was not only highly effective at lowering BP, but was also well received by participants.

C5: DSM, "DSM Pharmaceutical Solutions Monograph, Dec 2019: The role of riboflavin in hypertension"?

C6: DSM LinkedIn page and blog.

C7: International patent application number: WIPO Patent Application WO/2020/094319; ("Medicaments containing riboflavin exhibiting improved flowability"). International Filing Date: 09-10-2019.

C8: Dietary Reference Values for Riboflavin (European Food Safety Authority), 2017.

C9: Hypertension *In* Manual of Dietetic Practice, 6th Edition, Wiley-Blackwell on behalf of the BDA, 2019. (Key textbook for Dietitians in UK and across Europe).