Riboflavin status, MTHFR genotype and blood pressure: current evidence and implications for personalised nutrition

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Clinical deficiency of the B-vitamin riboflavin (vitamin B2) is largely confined to developing countries; however accumulating evidence indicates that suboptimal riboflavin status is a widespread problem across the developed world. Few international data are available on riboflavin status as measured by the functional biomarker, erythrocyte glutathione reductase activation coefficient, considered to be the gold standard index. One important role of riboflavin in the form of flavin dinucleotide is as a co-factor for the folate-metabolising enzyme methylenetetrahydrofolate reductase (MTHFR). Homozygosity for the common C677T polymorphism in MTHFR, affecting over 10% of the UK and Irish populations and up to 32% of other populations worldwide, has been associated with an increased risk of CVD, and more recently with hypertension. This review will explore available studies reporting riboflavin status worldwide, the interaction of riboflavin with the MTHFR C677T polymorphism and the potential role of riboflavin in personalised nutrition. Evidence is accumulating for a novel role of riboflavin as an important modulator of blood pressure (BP) specifically in individuals with the MTHFR 677TT genotype, with results from a number of recent randomised controlled trials demonstrating that riboflavin supplementation can significantly reduce systolic BP by 5–13 mmHg in these genetically at risk adults. Studies are however required to investigate the BP-lowering effect of riboflavin in different populations and in response to doses higher than 1·6 mg/d. Furthermore, work focusing on the translation of this research to health professionals and patients is also required.

Riboflavin: Methylene:tetrahydrofolate reductase; MTHFR C677T; Blood pressure; Personalised nutrition

Riboflavin (vitamin B2) is a water-soluble B-vitamin defined chemically as 7,8-dimethyl-10-1′-D-ribityl isoalloxazine. It acts as a precursor for FMN and FAD1. Clinical riboflavin deficiency is not generally considered to be a problem in the developed world but in recent years evidence has shown that sub-optimal status may be more widespread than generally perceived based on studies reporting the functional biomarker, erythrocyte glutathione reductase activation coefficient (EGRac) generally considered as the gold standard index of status. Few international data are however available based on EGRac and reports on riboflavin status are more commonly based solely on dietary intake data. Although riboflavin is required for numerous metabolic reactions its role (in the form of FAD) as a cofactor for the folate-metabolising enzyme, methylenetetrahydrofolate reductase (MTHFR) has recently received particular attention. Homozygosity (MTHFR 677TT genotype) for a common polymorphism in MTHFR, affecting over 10% of the UK and Irish populations and up to 32% of other populations worldwide2, has been associated with an increased risk of CVD3 and more recently with hypertension4. Emerging evidence from intervention trials supports a novel role for riboflavin supplementation in protecting against hypertension specifically in individuals with the MTHFR 677TT genotype5–7. This genotype-specific effect of riboflavin potentially offers a personalised approach for the prevention and treatment of CVD.

Abbreviations: BP, blood pressure; EGRac, erythrocyte glutathione reductase activation coefficient; GP, general practitioners; MTHFR, methylenetetrahydrofolate reductase; NO, nitric oxide.

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hypertension. This review will explore available studies reporting riboflavin status in populations worldwide and consider its potential role in human health, with a particular emphasis on the interaction of riboflavin with the MTHFR C677T polymorphism. In this regard, the potential role of riboflavin in personalised nutrition and its translation to the management of patients with hypertension will also be discussed.

**Riboflavin and health**

*Roles of riboflavin in human health*

Riboflavin is a water-soluble B-vitamin, which acts as a precursor for the coenzymes FMN and FAD, the metabolically active vitamin forms(1). These coenzymes (FAD and FMN) participate in intermediary metabolism and catalyse numerous oxidation-reduction reactions, playing a fundamental role in the metabolism of energy, certain drugs and toxins and in antioxidant protection(2,3,4). Furthermore, riboflavin interacts with a number of other nutrients, including metabolically linked B-vitamins and iron. Early animal studies have linked riboflavin deficiency with impaired iron absorption, increased intestinal loss of iron, and/or impaired iron utilisation for the synthesis of Hb(5,6). Moreover, supplementation with riboflavin has been shown to enhance circulating Hb levels in human subjects and showed that riboflavin improved the response of iron deficiency anaemia to iron therapy(7). Irrefutable evidence has shown the metabolism of other B-vitamins is dependent on riboflavin coenzymes. Riboflavin is involved in vitamin B6 metabolism; the enzyme pyridoxine-phosphate oxidase requires FMN for the conversion of pyridoxine phosphate to its coenzyme form pyridoxal-5 phosphate(8). Historical evidence from animal studies reported that pyridoxine-phosphate oxidase activity is sensitive to changes in dietary riboflavin intake and thus riboflavin deficiency may alter pyridoxal 5′ phosphate activity(9). Research from our centre conducted a number of years ago confirmed the interrelationship between riboflavin and vitamin B6 in human subjects and showed that riboflavin supplementation of older adults not only improved biomarker status of riboflavin, but also enhanced blood pyridoxal-5 phosphate (vitamin B6) concentrations(10). Niacin synthesis is also reliant on the FAD-dependent enzyme kynurenine mono-oxygenase, which is required for the synthesis of the coenzymes NAD and nicotinamide adenine dinucleotide phosphate from tryptophan. Riboflavin deficiency decreases the conversion of tryptophan to NAD and nicotinamide adenine dinucleotide phosphate resulting in niacin deficiency(8). Work from our centre and others have demonstrated the key role riboflavin plays in C1 metabolism via its role as a co-factor for the MTHFR enzyme.

**Riboflavin absorption and transport**

Dietary riboflavin occurs mainly in the form of FAD and smaller amounts occur as FMN or as free riboflavin(11). Unlike free riboflavin, FAD and FMN must be hydrolysed in the intestinal lumen to yield free riboflavin prior to absorption. Animal studies have shown that the uptake of dietary riboflavin from the intestine is increased in riboflavin deficiency(12) and urinary excretion was found to increase linearly with increasing dietary intakes in individuals with optimal status(13). The transport of flavins in blood is by loose binding to albumin and tight binding to a number of immunoglobulins in serum particularly IgA, IgG and IgM(14). A number of physiological factors have been reported to influence the rate of intestinal absorption of riboflavin. Diets high in psyllium gum decrease the rate of intestinal absorption, whereas bile salts increase absorption(15). Alcohol is reported to interfere with the digestion of food flavins into riboflavin and the intestinal absorption of riboflavin(16). Notably, concentrations of riboflavin synthesised by bacterial metabolism in the human colon may be more than 6-fold higher than dietary intakes(17).

**Riboflavin requirements and sources**

Worldwide dietary recommendations for riboflavin range from 1-1 to 1-6 mg/d for adults, an increment of 0-3 mg/d is recommended during pregnancy to cover the increased tissue synthesis for fetal and maternal development and an additional 0-4–0-5 mg/d during lactation(18). Clinical signs of deficiency in human subjects appear at intakes <0-5–0-6 mg/d and urinary excretion of riboflavin is seen at intake levels of approximately 1 mg/d(19). Riboflavin is found in a wide variety of food but yeast extract and offal products especially those based on liver are the only rich sources, containing more than 2 mg/100 g(20). The latest National Diet and Nutrition Survey reported that milk and milk products, meat, and fortified breakfast cereal make the greatest contribution to riboflavin intake in a British diet(21) (Table 1). Unlike any European country, the USA has a mandatory riboflavin enrichment policy to replace the riboflavin lost from starch during milling (0-40 mg for wheat starch)(22). Similarly, a mandatory enrichment of starch with riboflavin (4 mg/kg for starch) is in place in Canada. No toxic effects have been reported in relation to intakes of riboflavin at doses higher than dietary recommendations(23,24), the absorption of riboflavin appears to be limited to approximately 30 mg at any one time(13) with apparently little or no absorption at higher doses(25).

**Table 1. Food sources of riboflavin in the UK (NDNS)**(27)

<table>
<thead>
<tr>
<th>Sources</th>
<th>mg/average serving</th>
<th>mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>0-45</td>
<td>0-90</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>0-35</td>
<td>0-44</td>
</tr>
<tr>
<td>Eggs</td>
<td>0-26</td>
<td>0-52</td>
</tr>
<tr>
<td>Fortified breakfast cereal</td>
<td>0-22</td>
<td>0-74</td>
</tr>
<tr>
<td>Spinach</td>
<td>0-21</td>
<td>0-24</td>
</tr>
<tr>
<td>Chicken</td>
<td>0-15</td>
<td>0-15</td>
</tr>
<tr>
<td>Cheese</td>
<td>0-11</td>
<td>3-67</td>
</tr>
<tr>
<td>Bread (White)</td>
<td>0-09</td>
<td>0-36</td>
</tr>
</tbody>
</table>


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Furthermore, the Institute of Medicine in the USA did not establish a tolerable upper intake level for riboflavin when the RDA was revised in 1998\(^3\). A harmful side-effect of high-dose riboflavin intake is the increased intensity of urine colour to bright yellow.

**Riboflavin deficiency**

Isolated clinical riboflavin deficiency does not have unique or characteristic physical features. The classical symptoms, angular stomatitis, cheilosis and glossitis, are not specific to riboflavin deficiency and may be due to other vitamin deficiencies. Furthermore, after 3-8 months of inadequate riboflavin intake other symptoms are reported to appear including magenta tongue, seborrhoeic dermatitis, vascularisation of the cornea and nor- morchoic normocytic anaemia\(^1,8\). Other than dietary rheic dermatitis, vascularisation of the cornea and nor-

**Riboflavin status: the global picture**

In the developing world riboflavin deficiency is commonly acknowledged; less well recognised however is the evidence emerging to suggest that sub-optimal riboflavin status may also be more wide-spread in developed countries than previously considered. The majority of population-based studies report dietary intake data only for riboflavin and relatively few have included a biomarker of riboflavin status.

A number of European studies have identified low dietary intake of riboflavin\(^27,53,54\). Furthermore, Troesch et al.\(^55\), reported the percentage of men and women with dietary riboflavin intakes below the recommended nutrient intake using national dietary surveys; intakes were lowest in the Netherlands (25-50 % of men and >50-70 % of women) followed by the UK (5-25 % of men and >25-50 % of women), then Germany (5-25 % of men and women) and the USA (<5 % of men and 5-25 % of women). A systematic review including data from adults ≥65 years (n 28 000) in Europe, North America, Australia and New Zealand concluded that 41 % of males and 31 % of females had dietary intake values below the estimated average requirement, with riboflavin identified as one of six nutrients considered to be a possible public health concern\(^56\). Similarly in Asia, a number of large population-based studies and national dietary surveys have reported inadequate riboflavin intakes\(^57-59\). It has been suggested that the Chinese population tend to excrete very little riboflavin and thus their requirement may be lower than that of other populations\(^60\); however, this requires further investigation.

Far fewer studies have reported EGRac values and when they are reported they are typically limited to certain age and ethnic groups and lack standardised EGRac cut-off thresholds making comparisons between population groups difficult. Using a cut-off EGRac value of ≥1.40, one study of 311 children in Botswana reported riboflavin deficiency in up to 40 %\(^61\). In elderly free living adults in Guatemala (n 433) the prevalence of supplementation periods of at least 4 weeks with doses ranging from 1.0 to 5.0 mg. The EGRac assay reflects long-term riboflavin status; however a number of conditions are known to affect the performance of the assay, including deficiency of glucose-6-phosphate dehydrogenase, β-thalassemia, hypothyroidism and hyperthyroidism\(^67\).

Riboflavin status can also be assessed by urinary excretion although this is influenced by age, physical activity, body temperature, treatment with certain drugs and negative nitrogen balance\(^48\). Riboflavin excretion is reduced to 40 µg/24 h during deficiency compared with 120 µg/24 h when optimal status is achieved\(^49\). A number of studies have used biological samples particularly plasma and erythrocytes to measure riboflavin status directly but the results are inconsistent\(^50,51\). FMN is generally regarded as a more useful marker of status than FAD, which appears to be relatively unresponsive to riboflavin intakes\(^52\).

**Assessment of biomarker status of riboflavin**

Riboflavin status can be measured in a range of biological samples, including urine, plasma and erythrocytes. The method for the estimation of riboflavin status, which is regarded as the gold standard is EGRac, a functional assay that measures the activity of glutathione reductase before and after in vitro reactivation with its prosthetic group FAD\(^44\). EGRac is calculated as a ratio of FAD stimulated to unstimulated enzyme activity, with higher values reflective of lower riboflavin status. However currently there is no consensus as to the appropriate EGRac cut-off values to indicate low/high status, with studies reporting deficiency ranges from >1.2, >1.3 or >1.4\(^11,45,56\). Recent changes made to the assay methodology resulted in the acceptance of a cut-off ≥1.3 although others have suggested that this cut-off value should be further increased\(^68\). A systematic review by Hoey et al.\(^44\) identified EGRac to be an effective biomarker of riboflavin status at a population level with severe deficient-to-normal riboflavin status. This conclusion was drawn from randomised controlled trials and found EGRac to be sensitive to changes in
Riboflavin deficiency was reported to range from 50 to 75% using a cut-off EGRac value of >1.3, and status was found to be strongly correlated with milk consumption\(^{(62)}\). Consistent with this evidence, a larger study conducted in an elderly Taiwanese population (\(n=2379\)) reported that one in four had marginal riboflavin deficiency based on EGRac >1.2\(^{(63)}\). More recently, biomarker status of riboflavin was investigated in women of child bearing age in Cambodia; 89–92% of women were reported to be deficient or have suboptimal status based on an EGRac value \(\geq 1.3\). For comparison purposes in the latter study, a small convenient sample of women in urban Vancouver Canada (\(n=49\); two-thirds European descent and one-third Chinese), were investigated and somewhat surprisingly 70% were reported to have suboptimal or deficient riboflavin status\(^{(64)}\).

The UK is one of the very few countries worldwide to report biomarker data for riboflavin in a population-based survey. The most recent National Diet and Nutrition Survey indicates that all age/sex groups had a mean EGRac >1.30, with the poorest status reported in 11–18 year olds (EGRac values of 1.47 reported for boys and 1.53 for girls). However, 21% of girls reported dietary riboflavin intakes below the lower reference nutrient intake\(^{(65)}\). The high prevalence of biochemical riboflavin deficiency in the UK population is not fully understood as dietary intakes for riboflavin are sufficient, with the exception of 11–18 year olds with mean intakes of 1.97 mg/d for men and 1.50 mg/d for women reported.

Based on the available evidence sub-optimal riboflavin status appears to be common in many populations. The significance of these findings is not clear; however, it is possible that marginal riboflavin status in the absence of clinical deficiency may have adverse functional effects and long-term consequences for health. A standardised method of EGRac assessment is required and consideration of the current threshold (EGRac \(\geq 1.3\)) needs to be reinvestigated to better reflect functional impairment. The intake and requirements of populations requires further investigation based on robust biomarker data.

### Riboflavin and C\(_1\) metabolism

The B-vitamins folate, vitamins B\(_{12}\) and B\(_{6}\) and riboflavin are fundamental for C\(_1\) metabolism, the metabolic process involving the transfer and utilisation of C\(_1\) units in a network of biochemical pathways required for DNA and RNA biosynthesis, amino acid metabolism and methylation reactions. Riboflavin in its co-factor form FAD is critical for the folate metabolising enzyme MTHFR, required for the irreversible conversion of 5, 10-methylenetetrahydrofolate to the predominant circulatory and cellular form of folate, 5-methyltetrahydrofolate, which then serves as a methyl donor for the remethylation of homocysteine to methionine. A common variant in the MTHFR gene is the 677C \(\rightarrow\) T polymorphism, which involves a point mutation, in which cytosine (C), localised at nucleotide 677 of the gene, is replaced by thymidine (T), in turn producing an alanine to valine substitution in the enzyme. This results in a thermolabile form of MTHFR with approximately 30% decreased enzyme activity and elevated homocysteine concentration \(\text{in vivo}\)\(^{(65)}\). The prevalence of the MTHFR 677TT genotype is reported to be 10% worldwide, but this varies in different geographical regions and ethnic groups; ranging from 4 to 26% in Europe, 4 to 18% in USA, 20% in Northern China to as high as 36% in Mexico\(^{(2)}\).

\(\text{In vitro}\) evidence suggests that the reduced activity of the variant enzyme is the result of an increased propensity to dissociate from its FAD cofactor\(^{(66,67)}\). Early animal studies showed that MTHFR enzyme activity is lower in the livers of riboflavin deficient rats than in controls\(^{(68)}\). These findings were confirmed by Bates and Fuller\(^{(69)}\) who reported a dose-dependent relationship between riboflavin status and MTHFR activity. More recently in human studies, riboflavin supplementation was shown to lower plasma homocysteine specifically in individuals with the MTHFR 677TT genotype\(^{(70)}\), suggesting that the variant enzyme can be stabilised by optimising riboflavin status. A number of studies have identified riboflavin as an important determinant of homocysteine among individuals with the TT genotype, which is independent of folate status\(^{(70,71,72)}\). This evidence confirms the modulating role of riboflavin in determining homocysteine concentration in individuals with the TT genotype.

### Riboflavin, C\(_1\) metabolism and CVD risk

In addition to its role as the main genetic determinant of plasma homocysteine concentration, the C677T polymorphism in MTHFR has been independently associated with a higher risk of CVD, certain cancers, neural tube defects and most recently with hypertension. Of particular interest, extensive evidence has led to a number of meta-analyses reporting a strong association between this polymorphism and CVD, particularly stroke\(^{(73-78)}\).

It has been estimated that individuals with the MTHFR 677TT polymorphism have a 14–21% increased risk of CVD\(^{(75,77,78)}\). Of note, these meta-analyses have identified important geographical influences on the extent of excess CVD risk due to this polymorphism, strongly suggesting that environmental factors may have a modulating effect on the phenotype and thus CVD risk.

### Novel role of MTHFR genotype and blood pressure

Globally, hypertension accounts for 16.5% of deaths each year (9.4 million); an estimated 45% of deaths due to heart disease and 51% of deaths due to stroke are a result of hypertension\(^{(79)}\). High blood pressure (BP), even within the normal range substantially increases the risk of CVD and death, while a lowering of systolic BP by as little as 2 mmHg can decrease CVD risk by as much as 10%\(^{(80)}\). Hypertension is a polygenic disease that occurs as a result of a complex interaction of diverse environmental conditions and genetic factors. Risk factors include high dietary sodium intake,
National studies have reported an association between the 677C → T polymorphism and hypertension, a number of observational studies have identified several genetic loci associated with BP variation in European populations, including one near the gene encoding the folate metabolising enzyme MTHFR (methylenetetrahydrofolate). This finding was also replicated in non-European cohorts including Chinese, Japanese, Indian and US populations. Generally consistent with these findings from genome-wide association studies, there is a growing body of evidence from observational studies to support a specific association between the 677C → T polymorphism in the MTHFR gene and BP variation. As the evidence has accumulated numerous meta-analyses were conducted, all reporting significant associations of the MTHFR 677C → T polymorphism with hypertension (Table 2). Although there is strong evidence linking this polymorphism with hypertension, a number of observational studies have reported conflicting or inconclusive results. Many of the studies that failed to detect significant associations used small sample sizes or suffered possible selection bias. Some studies have reported a gender specific association; one large population study in >3000 Japanese individuals reported that the MTHFR 677C → T polymorphism was associated with a 42% increased risk of hypertension in women but not in men. In contrast, in a Spanish cohort, the TT genotype was found to be a significant predictor of hypertension in men but not in women, however, only 26% of the sample were females. Recent work conducted at our centre has considered the BP-lowering effect of riboflavin supplementation in individuals with the MTHFR 677TT genotype. The first of these trials was conducted in premature CVD patients (mean age 54 years) and demonstrated that riboflavin (1.6 mg/d for 16 weeks) decreased systolic BP (−13.2 (sd 15.0) mmHg; P = 0.02) and diastolic BP (−7.5 (sd 12.0) mmHg; P = 0.02) specifically in individuals with the TT genotype, while no BP response was observed in those with CC or CT genotype. These findings were later confirmed in a 4-year follow-up cross-over design study, which demonstrated in the same cohort (mean age 59 years) that riboflavin (at the same dose and duration of intervention) significantly lowered systolic BP (−9.2 (sd 12.8) mmHg; P = 0.001) and diastolic (−6.0 (sd 9.9) mmHg; P = 0.003) BP specifically in the TT genotype group. These findings were subsequently confirmed in hypertensive patients without overt CVD aged 70 years (5.6 (sd 2.6) mm Hg lowering in systolic BP; P = 0.033). The extent of response to riboflavin supplementation observed in these trials appears to lessen with increasing age. Based on the available evidence and in agreement with preliminary findings from a large population-based study, it appears that age is a significant factor in relation to the BP phenotype and its responsiveness to riboflavin and should be considered in future studies. To date, a low-dose supplementation level (1.6 mg/d) of riboflavin has been used and the effect of higher doses is not known. Thus, it remains possible that greater BP-lowering could be achieved with a larger dose of riboflavin. Of note, in all of the aforementioned trials the BP-lowering effect of riboflavin was shown to be independent of the number and type of antihypertensive drugs being currently administered.

**Mechanism of MTHFR C677T polymorphism, riboflavin and blood pressure**

The exact mechanism by which the MTHFR C677T polymorphism affects BP (and riboflavin modulates the relationship) has not been clearly identified; however, there are a number of plausible explanations which could explain these effects. In two separate studies involving patients undergoing coronary artery bypass graft surgery, it was identified that those with the MTHFR 677TT genotype had reduced vascular concentrations of 5-methyltetrahydrofolate which in turn were associated with deregulation of nitric oxide (NO); a potent vasodilator known to play a key role in BP. The riboflavin supplementation observed in these trials appears to lessen with increasing age. Based on the available evidence and in agreement with preliminary findings from a large population-based study, it appears that age is a significant factor in relation to the BP phenotype and its responsiveness to riboflavin and should be considered in future studies. To date, a low-dose supplementation level (1.6 mg/d) of riboflavin has been used and the effect of higher doses is not known. Thus, it remains possible that greater BP-lowering could be achieved with a larger dose of riboflavin. Of note, in all of the aforementioned trials the BP-lowering effect of riboflavin was shown to be independent of the number and type of antihypertensive drugs being currently administered.

<table>
<thead>
<tr>
<th>MTHFR, methylenetetrahydrofolate.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A number of these meta-analyses have considered hypertension in pregnancy, only results for hypertension have been considered in this review.</em></td>
</tr>
<tr>
<td>Odds ratio refers to MTHFR CC v. TT genotypes.</td>
</tr>
</tbody>
</table>

### Table 2. Meta-analyses of association of C677T polymorphism in MTHFR with hypertension

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample size (n)</th>
<th>Populations</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qian et al.</td>
<td>2814 cases</td>
<td>Caucasian</td>
<td>1.24 (1.02, 1.50)</td>
</tr>
<tr>
<td>Niu et al.</td>
<td>1520 cases</td>
<td>Chinese</td>
<td>1.87 (1.31, 2.68)</td>
</tr>
<tr>
<td>Yang et al.</td>
<td>6584 cases</td>
<td>Chinese</td>
<td>1.36 (1.20, 1.53)</td>
</tr>
<tr>
<td>Wu et al.</td>
<td>5207 cases</td>
<td>Worldwide</td>
<td>1.62 (1.32, 1.99)</td>
</tr>
<tr>
<td>Yang et al.</td>
<td>5418 cases</td>
<td>Worldwide</td>
<td>1.59 (1.32, 1.92)</td>
</tr>
</tbody>
</table>

Odds ratio refers to MTHFR CC v. TT genotypes.

excess weight, excessive alcohol consumption and lack of physical activity. Notably, twin studies have reported the heritability of BP variation to be as much as 50%. In the past decade, genome-wide association studies have identified several genetic loci associated with BP variation in European populations, including one near the gene encoding the folate metabolising enzyme MTHFR. This finding was also replicated in non-European cohorts including Chinese, Japanese, Indian and US populations. Generally consistent with these findings from genome-wide association studies, there is a growing body of evidence from observational studies to support a specific association between the 677C → T polymorphism in the MTHFR gene and BP variation. As the evidence has accumulated numerous meta-analyses were conducted, all reporting significant associations of the MTHFR 677C → T polymorphism with hypertension (Table 2). Although there is strong evidence linking this polymorphism with hypertension, a number of observational studies have reported conflicting or inconclusive results. Many of the studies that failed to detect significant associations used small sample sizes or suffered possible selection bias. Some studies have reported a gender specific association; one large population study in >3000 Japanese individuals reported that the MTHFR 677C → T polymorphism was associated with a 42% increased risk of hypertension in women but not in men. In contrast, in a Spanish cohort, the TT genotype was found to be a significant predictor of hypertension in men but not in women, however, only 26% of the sample were females. Recent work conducted at our centre has considered the BP-lowering effect of riboflavin supplementation in individuals with the MTHFR 677TT genotype. The first of these trials was conducted in premature CVD patients (mean age 54 years) and demonstrated that riboflavin (1.6 mg/d for 16 weeks) decreased systolic BP (−13.2 (sd 15.0) mmHg; P = 0.02) and diastolic BP (−7.5 (sd 12.0) mmHg; P = 0.02) specifically in individuals with the TT genotype, while no BP response was observed in those with CC or CT genotype. These findings were later confirmed in a 4-year follow-up cross-over design study, which demonstrated in the same cohort (mean age 59 years) that riboflavin (at the same dose and duration of intervention) significantly lowered systolic BP (−9.2 (sd 12.8) mmHg; P = 0.001) and diastolic (−6.0 (sd 9.9) mmHg; P = 0.003) BP specifically in the TT genotype group. These findings were subsequently confirmed in hypertensive patients without overt CVD aged 70 years (5.6 (sd 2.6) mm Hg lowering in systolic BP; P = 0.033). The extent of response to riboflavin supplementation observed in these trials appears to lessen with increasing age. Based on the available evidence and in agreement with preliminary findings from a large population-based study, it appears that age is a significant factor in relation to the BP phenotype and its responsiveness to riboflavin and should be considered in future studies. To date, a low-dose supplementation level (1.6 mg/d) of riboflavin has been used and the effect of higher doses is not known. Thus, it remains possible that greater BP-lowering could be achieved with a larger dose of riboflavin. Of note, in all of the aforementioned trials the BP-lowering effect of riboflavin was shown to be independent of the number and type of antihypertensive drugs being currently administered.

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those with the TT genotype or by increasing 5-methyltetrahydrofolate and thus decreasing BP irrespective of BP-lowering drugs\(^{105}\). Further work is required to investigate mechanisms linking this polymorphism with BP and the potential for riboflavin to provide a targeted option to treat elevated BP in this genotype group.

**Implications for the use of riboflavin as a personalised blood pressure management option**

The concept that nutrient recommendations require differentiation for specific subgroups of the population is not a new concept and was described as far back as the 1970s\(^{108}\). Many definitions for personalised nutrition exist; however recently, Ronteltap et al.\(^{105}\) defined personalised nutrition at three levels, where one level builds on the foundations of another and level 3 is regarded as the ultimate personalisation, with advice based on the individual’s diet, phenotypic parameters and genetic profile. Given that evidence is accumulating to support the role of riboflavin in modulating BP in individuals with the MTHFR 677TT genotype, translating this research, both to health professionals responsible for the management of BP and those genetically at risk, should be considered. However, limited evidence is available regarding the attitude of health professionals towards riboflavin as a targeted treatment option for BP management.

In recent years, there is increasing public interest in genetic testing in the health field, in a study investigating attitudes to genetic testing among 2000 individuals, 81 % of respondents believed that knowing their genetic risk could lead to better control of their lives\(^{108}\). A number of studies have reported that individuals identified as having a higher disease risk through genetic testing may be more motivated to change dietary habits\(^{109-111}\). Nevertheless a number of concerns towards genetic testing have been reported including cost, privacy, misuse of genetic information and fear that results could influence insurance companies and job opportunities\(^{107,108,110,112}\). Despite these criticisms many believe that genetic testing has the potential to motivate consumers to adopt changes that aim to prevent the onset and development of diseases\(^{113,114}\). Currently however, personal genetic testing is only easily obtained by Direct-to-Consumer genetic testing kits without interpretation by a healthcare professional\(^{115}\). Furthermore, very few diet–gene–health relationships have been tested for causality in human intervention studies\(^{109}\). This has resulted in concerns regarding the analytical validity and clinical utility of the genetic testing for general consumer purposes.

**Potential role of the health professional in personalised medicine**

Given the important role of general practitioners (GP) in BP management their attitude towards riboflavin as a treatment option for hypertension in individuals with the MTHFR 677TT genotype is important for the translation of this novel role for riboflavin. A number of studies have highlighted the role of GP in genetic testing of their patients\(^{120,121}\); however, few have considered the attitude of GP towards targeted treatment options for diseases and the potential role of GP in the delivery of personalised medicine. One study of Canadian oncologists, cardiologists and family physicians (n 363) reported that although the majority of respondents agreed that personalised medicine could influence treatment plans and improve outcomes, a number of barriers were perceived, including lack of clinical guidelines, limited provider knowledge and the lack of evidence-based clinical information. These Canadian physicians recognised that they lacked the education, information and support they needed to practice personalised medicine effectively and that they required national strategies, resources and training\(^{121}\). Thus, although many health care professionals recognise the potential of nutrigenomics in the prevention and treatment of diseases, many feel pessimistic about incorporating this new concept into their practice as they do not believe it provides sufficient information to adequately advise patients\(^{122,123}\). A number of challenges have been identified in the delivery of this information to the patient and it is clear that increasing genomics education in the training of health care professionals is required. Such intervention was previously found to improve both self-reported and assessed genomics knowledge among medical students\(^{124}\). Further work is clearly needed to investigate attitudes, particularly of GP, towards riboflavin as a treatment option for hypertension in patients identified with the MTHFR 677TT genotype.

**Conclusion and future work**

There is emerging evidence that sub-optimal riboflavin status is a problem not confined to developing countries, but also evident in the developed world. Apart from the widely recognised roles of riboflavin in human health, a novel and important role of riboflavin in modulating BP specifically in individuals with the MTHFR 677TT genotype is emerging. Optimal riboflavin status may therefore be particularly important in maintaining health for the 10 % of individuals worldwide (and up to 30 % in some populations) who share this genetic characteristic and are thus at increased risk of developing hypertension. Riboflavin potentially offers a personalised approach to the prevention and treatment of hypertension in these genetically at risk individuals. Further studies are however required to further investigate the BP-lowering effect of riboflavin in different populations and in response to doses higher than 1-6 mg/d. Furthermore, work focusing on the translation of this research to health professionals and patients is also required.

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Conflicts of Interest
There is a patent granted in Europe and pending elsewhere by M. W., H. McN. and J. J. S. and on the use of riboflavin in the treatment of hypertension.

Authorship
E. McA. drafted the manuscript. M. W., H. McN., C.H. and J. J. S. critically revised the manuscript for important intellectual content. All the authors have read and approved the final manuscript.

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