Evidence of a Role for One-Carbon Metabolism in Blood Pressure: Can B Vitamin Intervention Address the Genetic Risk of Hypertension Owing to a Common Folate Polymorphism?

Helene McNulty, JJ Strain, Catherine F Hughes, Kristina Pentieva, and Mary Ward
Nutrition Innovation Centre for Food and Health, School of Biomedical Sciences, Ulster University, Coleraine, Northern Ireland, United Kingdom

ABSTRACT
Hypertension in adulthood is recognized as the leading risk factor contributing to mortality worldwide, primarily from cardiovascular disease, whereas hypertension in pregnancy leads to serious adverse fetal and maternal outcomes. This article explores the under-recognized role of one-carbon metabolism in blood pressure (BP) and the potential for folate-related B vitamins to protect against hypertension. Genome-wide association studies and clinical studies provide evidence linking the 677C → T polymorphism in the gene encoding methylenetetrahydrofolate reductase (MTHFR) with BP and increased risk of hypertension and hypertension in pregnancy. A novel role for riboflavin (the MTHFR cofactor) has recently emerged, however, with evidence from randomized trials that supplemental riboflavin can lower BP specifically in adults with the variant MTHFR 677TT genotype. Further studies are required to elucidate the biological mechanisms linking one-carbon metabolism with BP and explore the effect of riboflavin in modulating the genetic risk of hypertension in early and later life. 

Introduction
Hypertension is typically defined as a systolic/diastolic blood pressure (BP) of ≥ 140/90 mm Hg. It is the leading risk factor contributing to mortality worldwide, primarily from cardiovascular disease (CVD). Hypertension in pregnancy is of concern because it can lead to serious hypertensive disorders with major adverse consequences for fetal and maternal health. The development of hypertension through the lifecycle is linked with a number of well-recognized nutrition and lifestyle factors. There is now considerable evidence from genetic and clinical studies pointing to the role of one-carbon metabolism in BP, albeit this is largely overlooked in treatment of, or prevention strategies for, hypertension. This article reviews the under-recognized role of one-carbon metabolism and the potential for related B vitamins to exert a beneficial effect in maintaining healthier BP, both in patients and in subpopulations genetically at risk of developing hypertension owing to a common folate polymorphism leading to impaired one-carbon metabolism. The global health impacts of a novel gene–nutrient interaction in preventing and treating hypertension and hypertension in pregnancy will be considered, along with identification of future research priorities in this area.

Global Burden of Hypertension and Hypertension in Pregnancy
Hypertension in adulthood is a global public health issue, estimated to affect > 1 billion people (1), and is the leading risk factor contributing to mortality worldwide, primarily from CVD, particularly stroke (1–3). Each 20-mm Hg rise in systolic BP (or 10-mm Hg rise in diastolic BP) is associated with a doubling in the risk of CVD (4). The economic impacts for countries worldwide are

Keywords: blood pressure, hypertension, hypertension in pregnancy, one-carbon metabolism, MTHFR, folate, riboflavin, single nucleotide polymorphism, gene–nutrient interaction, personalized nutrition

Copyright © American Society for Nutrition 2019. All rights reserved. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Manuscript received May 7, 2019. Initial review completed July 23, 2019. Revision accepted August 30, 2019. Published online September 16, 2019.

The research described in this review was supported in part by governmental funding from the Irish Department of Agriculture, Food and the Marine and the Health Research Board (under the Food Institutional Research Measure initiative) and from the Northern Ireland Department for Employment and Learning (under its Strengthening the All-Island Research Base initiative); and by DSM Nutritional Products.

Author disclosures: CFH and KP, no conflicts of interest. HM, JJS, and MW hold an international patent on the use of riboflavin in the treatment of blood pressure. None of the entities providing support were involved in the writing of this article.

Address correspondence to HM (e-mail: h.mcnulty@ulster.ac.uk).

Abbreviations used: 5-methylTHF, 5-methyltetrahydrofolate; BP, blood pressure; CVD, cardiovascular disease; EGRac, erythrocyte glutathione reductase activation coefficient; eNOS, endothelial nitric oxide synthase; GWAS, genome-wide association study; MTHFR, methylenetetrahydrofolate reductase; NO, nitric oxide; PLP, pyridoxal 5’phosphate; PPO, pyridoxine-phosphate oxidase; RBC, red blood cell; THF, tetrahydrofolate.
substantial. In the United States alone, the direct and indirect health care costs associated with hypertension are estimated at $48.6 billion and predicted to increase to $274 billion by 2030 (5).

Hypertension affects 10–15% of pregnancies and can lead to serious hypertensive disorders of pregnancy, which are recognized as the major causes of fetal and maternal morbidity and mortality worldwide (6, 7). In a notable systematic review with meta-analysis using data from 55 studies in 25 different countries, chronic hypertension (i.e., pregestational hypertension of any cause) was associated with significantly higher risks of pre-eclampsia and all other pregnancy complications, cesarean delivery, and perinatal death (8). Women with chronic hypertension in US studies were estimated to have an ~3-fold increased risk of preterm delivery (i.e., before 37 weeks of gestation), birth weight <2500 g, and neonatal intensive care admission, and a 4-fold increased risk of perinatal death compared with the US general pregnancy population (8). Pre-eclampsia (hypertension combined with proteinuria developing in later pregnancy) is one of the most severe hypertensive disorders of pregnancy, posing a high risk of life-threatening outcomes for both mother and child (9).

Apart from the immediate adverse consequences of hypertension during pregnancy, also of concern are the long-term impacts on the mother’s health. One report estimated a 65% increased risk of early adult all-cause and cause-specific mortality in women with a history of hypertension in pregnancy compared with women normotensive during pregnancy (10). There may also be implications of hypertension in pregnancy in relation to the longer-term health of the offspring. The ALSPAC (Avon Longitudinal Study of Parents and Children) reported that systolic and diastolic BP were higher in adolescent offspring (n = 4438) of mothers with gestational hypertension compared with mothers without hypertensive disorders of pregnancy, after adjustment for potential confounders (11).

Preventing and Treating Hypertension

Given the significant consequences of untreated hypertension on health throughout life, effective management of hypertension should be a public health priority (12). A number of potentially modifiable factors are recognized to contribute to the development and progression of hypertension, including poor diet, obesity, smoking, alcohol, and physical inactivity (13). Thus, obvious targets for public health strategies to prevent hypertension involve interventions aimed at promoting weight reduction, increased physical activity, decreased alcohol consumption, dietary sodium restriction, or whole dietary approaches such as the Dietary Approaches to Stop Hypertension (DASH) diet (14, 15). In hypertensive patients, BP is much more effectively lowered through pregnancy with important impacts for maternal and child (9).

Evidence Linking One-Carbon Metabolism with Hypertension-Related Health Outcomes

Functional roles of B vitamins within one-carbon metabolism

Folate is required for one-carbon metabolism, a network of pathways involved in the transfer and utilization of one-carbon units required for DNA and RNA biosynthesis, amino acid metabolism, and methylation processes. Reduced folates in various cofactor forms interact closely with vitamin B-12, vitamin B-6, and riboflavin within this network (19). Upon entering the one-carbon cycle, tetrahydrofolate (THF) acquires a carbon unit from serine in a vitamin B-6–dependent reaction to form 5,10 methylenetetrahydrofolate, a folate cofactor that once generated has various fates. It is either converted to 5-methyltetrahydrofolate (5-methylTHF), or serves as the one-carbon donor in the synthesis of nucleic acids, where it is required by thymidylate synthetase in the conversion of deoxyuridine to deoxythymidine for pyrimidine biosynthesis or is converted to other folate cofactor forms required for purine biosynthesis.

Methylenetetrahydrofolate reductase (MTHFR) is the riboflavin-dependent enzyme that catalyzes the reduction of 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate. The latter folate form is required by methionine synthase for the vitamin B-12–dependent conversion of homocysteine to methionine with the generation of unsubstituted THF. Once formed, methionine is activated by ATP to generate S-adenosylmethionine, typically referred to as “the universal methyl donor,” because it is required for numerous methylation reactions by donating its methyl group to >100 methyltransferases involved in the regulation of essential biological processes, including the methylation of DNA, proteins, neurotransmitters, and membrane phospholipids (19). To summarize, effective folate functioning within the one-carbon cycle requires essential interaction of folate with vitamin B-12, vitamin B-6, and riboflavin. Suboptimal status of any of these B vitamins, or polymorphisms in related genes, can lead to impaired one-carbon metabolism, even if dietary folate intakes are adequate.
offspring health. Historical reports from the early 1930s documenting the discovery of human folate deficiency first highlighted the importance of folate at this stage of the lifecycle and described a fatal anemia in pregnant women in India; this was subsequently proven to be responsive to treatment with food sources of the vitamin (20). It is now known that clinical deficiency of folate causes megaloblastic anemia (characterized by immature, enlarged blood cells reflecting impaired DNA synthesis), a condition that can be reversed with folic acid treatment (21). It is estimated that up to 24% of pregnancies in Asia, Africa, and South America are affected by folate-related anemia in the absence of maternal supplementation with folic acid (22), whereas supplementation prevents the decline in maternal folate concentrations that typically occurs throughout pregnancy (23, 24) and thus prevents the occurrence of megaloblastic anemia of pregnancy (25, 26). Because folate is required for the remethylation of homocysteine to methionine, plasma homocysteine concentrations are invariably found to be elevated with deficient or low folate status and are effectively lowered in response to intervention with folic acid alone or in combination with related B vitamins (27).

There is considerable evidence to link low folate status (and/or elevated homocysteine concentrations) with an increased risk of adverse pregnancy outcomes including gestational hypertension (28), pre-eclampsia (29–31), placental abruption (32), pregnancy loss (33, 34), low birth weight, and intrauterine growth restriction (35). Some (28, 36, 37), but not all (38), studies suggest benefits of folic acid supplementation in preventing gestational hypertension and pre-eclampsia. Of note, a recent report provided evidence from a randomized multicenter trial that supplementation with high-dose folic acid beyond the first trimester has no beneficial effect on pre-eclampsia in women at high risk of this condition (39). Inconsistencies in the evidence may however be explained, at least to some extent, by genetic differences between populations. As discussed below, there is emerging evidence from this center and elsewhere showing that the common C677T polymorphism in the gene encoding the folate-metabolizing enzyme MTHFR is implicated in the development of hypertension and hypertension in pregnancy (40, 41).

**One-carbon metabolism and CVDs**

Considerable evidence, accumulated over many years, links one-carbon metabolism with CVD, especially stroke (42). Most relevant studies in this area have focused on plasma homocysteine as the relevant risk factor leading to CVD, but it is likely that folate and metabolically related B vitamins have protective roles in CVD that are independent of their homocysteine-lowering effects. Population data (43) and randomized trials (44) provide strong evidence that intervention with folic acid can significantly reduce stroke risk, particularly in people with no previous history of stroke (45).

Since it was first described in 1995 (46), the common C677T polymorphism in *MTHFR* has been linked with several adverse health outcomes, including an increased risk of CVD by up to 40% (40). There is, however, a large geographical variation in the extent of excess CVD risk associated with this folate polymorphism (47–49), pointing to the involvement of a gene–environment interaction, and the evidence generally shows stronger associations for studies investigating the risk of stroke than for those on heart disease (40, 50, 51).

**One-carbon metabolism, MTHFR, and BP**

Folate and related B-vitamins, through functioning as one-carbon donors, may be protective against CVD through mechanisms that are not necessarily related to homocysteine. In particular, as discussed below, emerging evidence suggests that riboflavin, the MTHFR cofactor, plays an important role in modulating BP.

A link between the *MTHFR* C677T polymorphism and BP was first reported >20 y ago in a small study which found higher mean systolic/diastolic BP in middle-aged Japanese men with the variant TT genotype (147/91 mm Hg) than in those with the CT (134/81 mm Hg) or CC (133/79 mm Hg) genotypes (52). This evidence was largely overlooked for many years, perhaps because homocysteine was the phenotype of interest. More recently, GWASs and clinical studies have provided separate lines of evidence to link one-carbon metabolism—and specifically MTHFR—with BP. In 1 GWAS, 2.5 million single nucleotide polymorphisms were tested and 8 genetic loci identified as being associated with BP, including a region near the *MTHFR* gene (16), a finding confirmed in subsequent GWASs (53, 54). As reviewed elsewhere (40), the available evidence from meta-analyses of clinical studies indicates that the *MTHFR* 677C→T polymorphism is associated with an increased risk of hypertension and hypertension in pregnancy by up to 87% (41, 55, 56), with reported ORs from meta-analyses ranging from 1.36 (95% CI: 1.20, 1.53) to 1.87 (95% CI: 1.31, 2.68), for worldwide and Chinese populations, respectively (41, 57, 58).

The variant *MTHFR* 677TT genotype has a reported frequency of 10% worldwide, but this shows marked geographical variation, ranging from 4–18% in the United States, 4–26% in European populations (increasing north to south), and 20% in northern China to as high as 32% in Mexico, whereas the lowest TT genotype frequencies are found in populations of African ancestry (59). There is also marked ethnic variation within countries; thus, in certain ethnic groups within China and Mexico, even higher TT genotype frequencies are reported (60, 61), which are, in turn, associated with higher rates of hypertension (62).

### Relevant Nutrient–Nutrient and Gene–Nutrient Interactions within One-Carbon Metabolism

**Riboflavin**

Riboflavin has 2 cofactor forms, FMN and FAD, which are essential for numerous oxidation-reduction reactions and thus required in the metabolism of energy, certain drugs, and toxins and in supporting cellular antioxidant potential (63). Of note, riboflavin-dependent metabolism involves interaction with a number of other nutrients including iron (64). Of particular relevance to one-carbon metabolism is the close metabolic interaction of riboflavin with vitamin B-6 (65), where riboflavin is required (as FMN) for the generation in tissues of the active vitamin B-6 coenzyme form pyridoxal 5’ phosphate (PLP) from pyridoxine phosphate by pyridoxine-phosphate oxidase (PPO). In animals, PPO activity was shown to be responsive to changes in riboflavin intake and lower PLP concentrations were found with riboflavin deficiency (65). In humans, the metabolic dependency of vitamin B-6 on riboflavin status was demonstrated in a study from our center showing that riboflavin supplementation of older adults not only improved the biomarker status of riboflavin, but also led to increased PLP concentrations (66).
Also within one-carbon metabolism, riboflavin plays an important role in folate recycling where it acts in the form of FAD as a cofactor for MTHFR in the conversion of 5,10-methyleneTHF to 5-methylTHF. The importance of riboflavin within the one-carbon metabolic network is perhaps most evident in individuals with the variant 677TT genotype in MTHFR, resulting in a thermolabile enzyme with reduced activity (46). Molecular studies demonstrated that the loss of MTHFR activity that occurs with the TT genotype is the result of an increased propensity for the variant enzyme to dissociate from its FAD cofactor (67, 68). In humans, the typical phenotype in adults with the variant TT genotype is one of elevated plasma homocysteine (46), along with low folate concentrations (69), and studies show that the homocysteine phenotype is most pronounced when the TT genotype occurs in combination with low folate (70) or riboflavin (71, 72) status. Marked lowering of plasma homocysteine however occurs in response to riboflavin supplementation specifically in adults with the TT genotype, an effect not found in those with the CC or CT genotypes (73). The genotype-specific responsiveness of homocysteine to riboflavin intervention may indicate that optimizing riboflavin status can stabilize the variant enzyme and thus restore MTHFR activity in vivo. Of greater relevance to public health, however, is emerging evidence that riboflavin interacts with MTHFR to influence BP and hypertension risk.

**Novel genotype-specific role of riboflavin in BP**

An entirely novel role of riboflavin as an important modulator of BP has emerged in recent years, specifically in genetically at-risk individuals owing to the C677T polymorphism in MTHFR (40). Studies from our center show significantly higher systolic and diastolic BP in adults homozygous for this polymorphism but this phenotype appears to be highly responsive to riboflavin intervention (74–76).

In the first of 3 randomized trials, premature CVD patients (mean age of 53 y) with the variant MTHFR 677TT genotype were found before intervention to have significantly higher systolic/diastolic BP (143/86 mm Hg) than age- and sex-matched patients with the CC (131/80 mm Hg) or CT (133/83 mm Hg) genotypes (74). In response to supplementation with riboflavin (1.6 mg/d for 16 wk), however, BP decreased by a mean of 14 mm Hg (systolic BP) specifically in patients with the TT genotype, with no BP response in the CC or CT genotype groups (74). When this cohort of premature CVD patients was followed up 4 y later, those with the TT genotype were hypertensive at baseline, despite marked changes in the number and type of antihypertensive drugs being prescribed since the initial investigation (following changes in clinical guidelines for hypertension), and goal BP (i.e., systolic/diastolic BP ≤140/90 mm Hg) was achieved only in response to riboflavin intervention (75). In a third trial, hypertensive adults without overt CVD were investigated (76). Despite being prescribed multiple classes of antihypertensive drugs, >60% of participants with the variant TT genotype in MTHFR were found to be hypertensive at baseline, but after riboflavin intervention for 16 wk (during which time antihypertensive drug treatment remained unchanged), BP significantly decreased and there was a marked improvement in BP control (76). Together these trials show that targeted riboflavin supplementation of genetically at-risk adults can effectively lower BP and improve BP control in hypertensive patients, with or without overt CVD, independently of concurrent antihypertensive drug use.

**Do other relevant B vitamins have a role in BP?**

Red blood cell folate (RBC) concentrations are found to be significantly lower in people with the MTHFR 677TT genotype, perhaps indicating a higher requirement for folate in order to normalize folate metabolism in these individuals (69). It is possible that in people with the TT genotype, supplementation with the folate derivative 5-methylTHF may achieve a better folate biomarker response than that obtained with an equivalent dose of folic acid (the synthetic vitamin form), because 5-methylTHF would bypass the relevant MTHFR-dependent step in folate metabolism, but there is no direct evidence for such an effect in human studies. Moreover, given the dependency of MTHFR on not only folate, but also riboflavin, the combined effect of these vitamins may have a greater effect on the biomarker response than intervention with folate alone. It is also possible that any corresponding effect on the BP phenotype arising from correcting MTHFR activity in TT individuals (among whom normal folate recycling is impaired) that occurs with riboflavin only could be further enhanced by combining riboflavin with 5-methylTHF. This remains entirely speculative, however, because no human study to date has investigated the genotype-specific effect on BP of riboflavin and 5-methylTHF in combination.

Apart from any genotype-specific BP-lowering effects, it is unlikely that supplementation with folate-related B vitamins in adults generally is beneficial in maintaining healthy BP. Numerous randomized trials involving intervention with folic acid (typically in combination with vitamin B-12 and vitamin B-6) aimed at lowering homocysteine concentrations and CVD showed little or no corresponding BP response (77, 78).

**Mechanisms explaining the role of one-carbon metabolism in BP**

The biological mechanisms linking one-carbon metabolism with BP and explaining the modulating role of riboflavin are unclear. In individuals with the MTHFR 677TT genotype, MTHFR enzyme activity appears to be particularly sensitive to changes in riboflavin status (71–73). It could be speculated that in TT-genotype individuals there is a higher capacity to replace inactivated enzyme with optimal riboflavin than with low riboflavin status, or that higher riboflavin status prevents the FAD cofactor from leaving the active site or allows its quick replacement, thus stabilizing the variant form of the enzyme. Riboflavin requirements may therefore be higher in individuals with the TT genotype in order to sustain normal MTHFR activity (71, 73), although this remains to be specifically demonstrated.

As reviewed elsewhere (40), the MTHFR–BP relation is likely to involve the potent vasodilator nitric oxide (NO) via an effect on endothelial function. Vascular tissue concentrations of 5-methylTHF (the product of the MTHFR reaction) were associated with NO bioavailability and improved endothelial function in patients undergoing coronary artery bypass graft surgery, and were found to be lower in those patients with the MTHFR 677TT genotype (79, 80). Stabilizing the variant MTHFR enzyme in vivo with supplemental riboflavin (73) could at least partially restore 5-methylTHF concentrations in vascular cells in the TT genotype. This in turn would improve NO availability and endothelial function, and thus lower BP specifically in individuals with the TT genotype. Restoration of cellular concentrations of 5-methylTHF with riboflavin supplementation may also correct...
any imbalance of THF derivatives owing to this polymorphism, and specifically the accumulation of formylTHF relative to 5-methylTHF that is reported in RBCs in individuals with the TT genotype (81). The impaired folate metabolism that occurs in this polymorphism may also affect the activity of dihydrofolate reductase which, along with dihydrobiotin reductase, contributes to the generation of tetrahydrobiotin from dihydrobiotin (82). Tetrahydrobiotin is an essential cofactor in the coupling of reduction and oxidation reactions catalyzed by nitric oxide synthase (eNOS). When coupled with tetrahydrobiotin, eNOS produces NO from arginine and NAD(P)H, inducing vasodilation, but with accumulation of dihydrobiotin, eNOS activity leads to the generation of superoxide (83, 84). In summary, by correcting MTHFR activity with riboflavin supplementation in the TT genotype, endothelial function could be improved through increased bioavailability of NO, via increased vascular concentrations of 5-methylTHF and/or tetrahydrobiotin.

Whatever the mechanism explaining the role of one-carbon metabolism in BP, it is unlikely to involve homocysteine. Although significant associations of plasma homocysteine with BP have been reported in several observational studies, no BP response was found in trials designed to lower homocysteine as a means to reduce CVD (77, 78). Mechanistic studies are required to elucidate the biological perturbation that leads to higher BP with the common MTHFR C677T polymorphism and to understand how riboflavin can rescue this phenotype and thus potentially protect against the development of hypertension.

Health Impacts, Challenges, and Research Priorities

Health impacts of a novel gene–nutrient interaction in BP
Hypertension in adulthood continues to be the major risk factor for cardiovascular death in every region of the world (85). Effective lowering of BP is, however, proven to be highly beneficial in reducing cardiovascular mortality (5, 86, 87). In 2017, the American Heart Association published new guidelines for the prevention, detection, and management of elevated BP in US adults and lowered the threshold defining hypertension, from the existing BP level (systolic/diastolic) of >140/90 mm Hg to >130/80 mm Hg. These changes were introduced primarily as a result of compelling findings from the SPRINT (Systolic Blood Pressure Intervention Trial) study, a large multicenter study that reported lower rates of fatal and nonfatal major cardiovascular events and death from any cause, in response to intensive compared with standard BP-lowering treatment (88). This evidence has led to calls for newer approaches, including novel combination therapies and nonpharmacological solutions (89).

Hypertensive disorders of pregnancy are internationally categorized into 4 clinical categories: gestational hypertension (pregnancy-induced hypertension after 20 weeks of gestation); chronic hypertension (pregestational hypertension of any cause); pre-eclampsia/eclampsia; and chronic hypertension with superimposed pre-eclampsia. The combined impact of these disorders is estimated to account for 14% of maternal deaths worldwide (9). Pre-eclampsia in particular remains a leading cause of maternal and perinatal mortality and morbidity globally, particularly in low- and middle-income countries (7). In combination with a rising maternal age in many countries, hypertension in pregnancy puts the lives of women and their infants at risk and imposes an increasing health care burden on society. There have been recent calls therefore for effective, safe, and affordable treatments and prevention strategies (90).

Given the considerable burdens of hypertension and hypertonstension in pregnancy and the proven benefits of effective treatment, important health impacts throughout the lifecycle for relevant subpopulations globally could arise from implementing interventions based on the evidence reviewed here, showing a novel and genotype-specific role for riboflavin in lowering BP. The magnitude of BP lowering demonstrated in the variant MTHFR genotype in response to riboflavin (by a mean decrease of 6–14 mm Hg in systolic BP across 3 separate trials) (74–76) is clinically significant, considering that each 2-mm Hg lowering of systolic BP is estimated to decrease CVD risk by 10% (4). Furthermore, the genotype-specific BP response to riboflavin occurs independently of antihypertensive drugs (74–76), and, as currently prescribed, these appear to be associated with poorer BP control in patients with the MTHFR 677TT genotype, whereas achievement of target BP can be greatly enhanced in these at-risk patients with supplemental riboflavin (76). Other genetic factors are implicated in the development of hypertension (16, 53, 54), but the common MTHFR C677T polymorphism is the only genetic factor linked with hypertension that offers a personalized management option, via optimizing riboflavin, the MTHFR cofactor.

Optimizing riboflavin status—a global concern
Riboflavin deficiency is a significant problem in developing countries (91). Suboptimal riboflavin status on a more widespread basis may also exist across the developed world, but this is generally unrecognized because riboflavin biomarkers are rarely measured in human studies (92). The United Kingdom and Ireland are among the very few countries worldwide to have included a riboflavin biomarker as part of their population-based nutrition surveys (93, 94). In both national nutrition surveys, the biomarker status of riboflavin was measured using the erythrocyte glutathione reductase activation coefficient (EGRac) assay, widely considered to be the gold-standard measure of status (95). This coefficient is expressed as the ratio of glutathione reductase activity in lysed red cells with, to without, the in vitro addition of FAD and provides a measure of enzyme saturation with its riboflavin-derived cofactor. A low EGRac value is generally considered to be normal, whereas higher values indicate suboptimal status, but there is no universal agreement as to the precise EGRac cutoffs to categorize deficient and low riboflavin status.

Some concern has however been expressed regarding the high proportion of healthy adults with EGRac values indicative of suboptimal biomarker status of riboflavin, as assessed in both the British and Irish population-based surveys. On the limited available evidence, therefore, suboptimal riboflavin status may be more widespread than is generally recognized in populations globally because of the current reliance on dietary data only in nutrition surveys in most countries (including in the United States and Canada), without corresponding riboflavin biomarkers. There is a need to measure the biomarker status of riboflavin in population surveys, and to demonstrate the functional and health effects of riboflavin, covering the range from deficient to suboptimal to optimal status.
Conclusions

The evidence was reviewed linking one-carbon metabolism and related B vitamins with BP, risk of hypertension, and hypertension in pregnancy. These are significant public health concerns for populations worldwide. Homozygosity for the C677T polymorphism in MTHFR, affecting 1 in 10 adults globally, is associated with higher BP, but emerging evidence shows that riboflavin (the MTHFR cofactor) exerts an important modulating effect on the BP phenotype, with riboflavin supplementation proven to lower BP in adults with the variant TT genotype. The finding that the BP phenotype associated with this common folate polymorphism is modifiable by riboflavin may have important clinical and public health impacts. For hypertensive patients or subpopulations worldwide with the variant MTHFR 677TT genotype, enhancing riboflavin status could offer a personalized option to treat, delay, or prevent the development of high BP. The health impacts of intervention with riboflavin can be anticipated to be greatest in those countries worldwide, including Mexico and northern China, with the highest reported frequencies of the variant genotype. Other relevant genes for BP have been identified in GWAS, but this folate polymorphism is the only genetic factor linked with hypertension that offers a personalized management option, via optimizing riboflavin. Further investigations are required to explore the underpinning biological mechanisms linking one-carbon metabolism with BP and to more fully investigate the effect of riboflavin and other relevant B vitamins in preventing hypertension and hypertension in pregnancy in adults genetically at risk owing to this polymorphism. Large-scale studies are also required to investigate the long-term health outcomes, particularly in relation to CVD and hypertensive disorders of pregnancy, of targeted B vitamin intervention at various stages of the lifecycle.

Acknowledgments

The authors’ responsibilities were as follows—HM: drafted the initial manuscript; MW, JJS, CFH, and KP: critically revised the manuscript for important intellectual content; and all authors: read and approved the final manuscript.

References

1. World Health Organization. A global brief on hypertension. WHO/DCO/WHD/2013.2. Geneva: WHO; 2013.
2. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2224–60 [Erratum in: Lancet 2013;381:628, 1276].
3. NCD Risk Factor Collaboration. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19–1 million participants. Lancet 2017;389:37–55.
4. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002;360:1903–13.
5. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després J-P, Fullerton HJ, et al. Heart Disease and Stroke Statistics—2016 update: a report from the American Heart Association. Circulation 2015;133:e38–48.
6. American College of Obstetricians and Gynecologists. Hypertension in pregnancy: executive summary. Report of the American College of Obstetricians and Gynecologists’ Task Force on Hypertension in Pregnancy. Obst Gynecol 2013;122:1122–31.
7. Payne RA, Hutcheon JA, Ansermino JM, Hall DR, Bhutta ZA, Bhutta ZS, Biryabarema C, Grobman WA, Groen H, Haniff F, et al. A risk prediction model for the assessment and triage of women with hypertensive disorders of pregnancy in low-resourced settings: the miniPIERS (Pre-eclampsia Integrated Estimate of Risk) multi-country prospective cohort study. PLoS Med 2014;11:e1001589.
8. Bramham K, Parnell B, Nelson-Piercy C, Seed PT, Poston L, Chappell LC. Chronic hypertension and pregnancy outcomes: systematic review and meta-analysis. BMJ 2014;348:g2301.
9. Say L, Chou D, Gemmill A, Tuncalp O, Moller A-B, Daniels J, Gülmezoglu AM, Temmerman M, Alkema L. Global causes of maternal death: a WHO systematic analysis. Lancet Glob Health 2014;2:e323–33.
10. Theilen LH, Fraser A, Hollingshaus MS, Sliepke KC, Varner MW, Smith KR, Esplin MS. All-cause and cause-specific mortality after hypertensive disease of pregnancy. Obstet Gynecol 2016;128:238–44.
11. Fraser A, Nelson S, Macdonald-Wallis C, Satter N, Lowal D. Hypertensive disorders of pregnancy and cardiometabolic health in adolescent offspring. Hypertension 2013;62:614–20.
12. Williams B, Mancia G, Spiwier W, Rosei EA, Azizi M, Burnier M, Clement DL, Coca A, De Simone G, Dominiczak A, et al. 2018 ESC/ESH guidelines for the management of arterial hypertension. Eur Heart J 2018;39:3021–10.
13. Yusuf PS, Hawken S, Oumpu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study); case-control study. Lancet 2004;364:937–52.
14. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, et al. A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med 1997;336:1117–24.
15. Appel LJ, Champagne CM, Harsha DW, Cooper LS, Obarzanek E, Elmer PJ, Stevens VJ, Vollmer WM, Lin P-H, Svetkey LP, et al. Effects of comprehensive lifestyle modification on blood pressure control. JAMA 2003;289:2083–93.
16. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bohuch M, Coin L, Najjar SS, Zhao JH, Heath SC, Eyrehamd S, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet 2009;41:666–76.
17. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet 2009;41:677–87.
18. Kattah AG, Garovic VD. The management of hypertension in pregnancy. Adv Chronic Kidney Dis 2013;20:229–39.
19. Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory JF, Mills JL, Pfeiffer CM, Faczli Z, Zhang M, Ueland PM, et al. Biomarkers of nutrition for development—folate review. J Nutr 2015;145:1636S–805.
20. Wills L. Treatment of “pernicious anaemia of pregnancy” and “tropical anaemia” with special reference to yeast extract as a curative agent. Br Med J 1931;1:1059–64.
21. Chanarin I. Folate and cobalamin. Clin Haematol 1985;14:629–41.
22. Chanarin I. Folate and cobalamin. Clin Haematol 1985;14:629–41.
23. Hall MH, Pirani BBK, Campbell D. The cause of the fall in serum folate in normal pregnancy. Br J Obstet Gynaecol 1976;83:132–6.
24. McNulty B, McNulty H, Marshall B, Ward M, Molloy AM, Scott JM, Dornan J. Pentieva K. Impact of continuing folic acid after the first trimester of pregnancy: findings of a randomized trial of folic acid supplementation in the second and third trimesters. Am J Clin Nutr 2013;98:92–1117.
25. Foger T, Aellig F, Ellumpeen A, Prankerd T, Brandt H, Menzies D. The value of folic acid supplements in pregnancy. J Obstet Gynaecol Br Commonwealth 1971;78:781–5.
26. Blot I, Papiernik E, Kaltwasser JP, Werner E, Tchernia G. Influence of routine administration of folic acid and iron during pregnancy. Gynecol Obstet Invest 1981;12:294–304.
27. Homocysteine Lowering ‘Trialists’ Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr 2005;82:806–12.

28. De Ocampo MPG, Araneta MRG, Macara CA, Alcaraz JE, Moore TR, Chambers CD. Folic acid supplement use and the risk of gestational hypertension and preeclampsia. Women Birth 2018;31(2):e77–83.

29. Sanin JE, Miranda J, Monterrosa Á, Dudbridge F, Diaz LA, Saldarriaga W, Quintero-Lesmes DC, Casas JP, Becerra-Bayona S, Mesa CM, et al. Association of pre-eclampsia risk with maternal levels of folate, homocysteine and vitamin B12 in Colombia: a case-control study. PLoS One 2018;13:e0208137.

30. Cotter AM, Molloy AM, Scott JM, Daly SF. Elevated plasma homocysteine in early pregnancy: a risk factor for the development of severe preeclampsia. Am J Obstet Gynecol 2001;185:781–5.

31. Cotter AM, Molloy AM, Scott JM, Daly SF. Elevated plasma homocysteine in early pregnancy: a risk factor for the development of nonsevere preeclampsia. Am J Obstet Gynecol 2003;189:391–4.

32. Goddijn-Wessel TA, Wouters MG, vandeMolen EF, Spuijbroek MD, Cotter AM, Molloy AM, Scott JM, Daly SF. Elevated plasma homocysteine and blood pressure: a personalized approach to prevention and treatment of hypertension. Proc Nutr Soc 2012;71:213–21.

33. Nelen WLDM, Blom HJ, Steegers EAP, DenHeijer M, Eskes TKAB. Hyperhomocysteinemia and risk of pre-eclampsia: a meta-analysis. Fertil Steril 2000;74:1196–9.

34. Dodds L, Fell DB, Dooley KC, Armson BA, Allen AC, Nassar BA, Perkins S, Joseph KS. Effect of homocysteine concentration in early pregnancy on gestational hypertensive disorders and other pregnancy outcomes. Clin Chem 2008;54:326–34.

35. Vollset SE, Refsum H, Ingens LM, Emblem BM, Tverdal A, Gjessing HK, Lise A, Mensen B, Ueland PM. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study. Am J Clin Nutr 2000;71:962–8.

36. Hernández-Díaz S, Weiler MM, Louik C, Mitchell AA. Risk of gestational hypertension in relation to folic acid supplementation during pregnancy. Am J Epidemiol 2002;156:806–12.

37. Bulloch RE, Lovell AL, Jordan VMB, McCowan LME, Thompson JMD, Wall CR. Maternal folic acid supplementation for the prevention of pre-eclampsia: a systematic review and meta-analysis. Paediatr Perinat Epidemiol 2012;26:259–67.

38. Sanin JE, Miranda J, Monterrosa Á, Dudbridge F, Diaz LA, Saldarriaga W, Quintero-Lesmes DC, Casas JP, Becerra-Bayona S, Mesa CM, et al. Elevated plasma homocysteine and blood pressure: a personalized approach to prevention and treatment of hypertension. Proc Nutr Soc 2012;71:213–21.

39. Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H, McNulty H, Strain JJ, Pentieva K, Ward M. Riboflavin, one-carbon metabolism and blood pressure. Eur J Hum Genet 2007;15:1239–45.

40. McNulty H, Strain JJ, Hughes CF, Ward M. One-carbon metabolism and blood pressure. Lancet 2012;380:300–6.

41. Liu W, Fatta-Sinha D, Pericak-Vance MA, Foroud T, Nilsson ME, Sedgwick JD, et al. Identifying multiple causative variants in MTHFR (rs1801133) affecting homocysteine and risk of cardiovascular disease. PLoS Genet 2013;9:e1003183.

42. Powers HJ, Hill MH, Mushtaq S, Dainty JR, Majsak-Newman G, Williams EA. Correcting a marginal riboflavin deficiency improves hematology.
status in young women in the United Kingdom (RIBOFEM). Am J Clin Nutr 2011;93:1274–84.

65. Rasmussen KM, Barsa PM, McCormick DB. Pyridoxamine (pyridoxine) S-phosphate oxidase activity in rat tissues during development of riboflavin or pyridoxine deficiency. Proc Soc Exp Biol Med 1979;161:527–30.

66. Madigan SM, Tracey F, McNulty H, Eaton-Evans J, Coulter J, McCartney H, Strain JJ. Riboflavin and vitamin B-6 intakes and status and biochemical response to riboflavin supplementation in free-living elderly people. Am J Clin Nutr 1998;68:389–95.

67. Yamada K, Chen Z, Rozen R, Matthews RG. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proc Natl Acad Sci U S A 2001;98:14853–8.

68. Pejchal R, Campbell E, Guenther BD, Lennon BW, Matthews RG, Ludwig ML. Structural perturbations in the Δα→Val polymorphism of methylenetetrahydrofolate reductase: how binding of folates may protect against inactivation. Biochemistry 2006;45:4808–18.

69. Molloy AM, Daly S, Mills JL, Kirke PN, Whitehead AS, Ramsbottom D, Conley MR, Weir DG, Scott JM. Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: implications for folate intake recommendations. Lancet 1997;349:1591–3.

70. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg JH, Selhub J, Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996;93:7–9.

71. McNulty H, McKinley MC, Wilson B, McPartlin J, Strain JJ, Weir DG, Scott JM. Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. Am J Clin Nutr 2002;76:636–41.

72. Hospa S, Middotou O, Schneebe J, Vollset SE, Grotmol T, Ueland PM. The methylenetetrahydrofolate reductase 677C→T polymorphism as a modulator of a B-vitamin network with major effects on homocysteine metabolism. Am J Hum Genet 2007;80:846–55.

73. McNulty H, Dowey LRC, Strain JJ, Dunne A, Ward M, Molloy AM, McAnena LB, Hughes JP, Hanlon-Fletcher M, Scott JM. Riboflavin lowers homocysteine in individuals homozygous for the MTHFR 677C→T polymorphism. Circulation 2006;113:74–80.

74. Horigan G, McNulty H, Ward M, Strain J, Purvis J, Scott JM. Riboflavin lowers blood pressure in cardiovascular disease patients homozygous for the 677C→T polymorphism in MTHFR. J Hypertens 2010;28:478–86.

75. Wilson CP, Ward M, McNulty H, Strain JJ, Trouton TG, Horigan G, Purvis J, Scott JM. Riboflavin offers a targeted strategy for managing hypertension in patients with the MTHFR 677TT genotype: a 4-y follow-up. Am J Clin Nutr 2012;95:766–72.

76. Wilson CP, McNulty H, Ward M, Strain JJ, Trouton TG, Hoefi BA, Weber P, Roos FF, Horigan G, McAnena L, et al. 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;61:1302–8.

77. Toole JF, Malinow MR, Chambliss LE, Spence JD, Pettigrew LC, Howard VI, Sides EG, Wang CH, Stamper M. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. JAMA 2004;291:565–75.

78. Wilson CP, McNulty H, Scott JM, Strain JJ, Ward M. The MTHFR C677T polymorphism, B-vitamins and blood pressure. Proc Nutr Soc 2010;69:156–65.

79. Antoniades C, Shiroudaria C, Warrick N, Cai S, De Boni J, Lee J, Leeson P, Neubauer S, Ratnatunga C, Pillai R, et al. 5-Methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and eNOS coupling. Circulation 2006;114:1193–201.

80. Antoniades C, Shiroudaria C, Leeson P, Baarlo OA, Van-Asseche T, Cunnington C, Pillai R, Ratnatunga C, Tousoulis D, Stefanadis C, et al. MTHFR 677 C→T polymorphism reveals functional importance for 5-methyltetrahydrofolate, not homocysteine, in regulation of vascular redox state and endothelial function in human atherosclerosis. Circulation 2009;119:2507–15.

81. Bagley PJ, Selhub J. A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci U S A 1998;95:13217–20.

82. Schmidt K, Kolesnik B, Gorren ACF, Werner ER, Mayer B. Cell type-specific recycling of tetrahydrobiopterin by dihydrofolate reductase explains differential effects of 7,8-dihydrobiopterin on endothelial nitric oxide synthase uncoupling. Biochem Pharmacol 2014;90:246–53.

83. Crabtree MJ, Tatham AL, Al-Waked Y, Warrick N, Hale AB, Cai S, Channon KM, Alp NJ. Quantitative regulation of intracellular endothelial nitric-oxide synthase (eNOS) coupling by both tetrahydrobiopterin-eNOS stoichiometry and biopterin redox status: insights from cells with TET-regulated GTP cyclohydrolase I expression. J Biol Chem 2009;284:1136–44.

84. Crabtree MJ, Channon KM. Synthesis and recycling of tetrahydrobiopterin in endothelial function and vascular disease. Nitric Oxide 2011;25:81–8.

85. Fisher ND, Curnan G. Hypertension—a public health challenge of global proportions. JAMA 2018;320:1757–9.

86. Gaziano TA, Bitton A, Anand S, Weinstein MC. The global cost of nonoptimal blood pressure. J Hypertens 2009;27:1472–7.

87. Falaschetti E, Mindell J, Knott C, Poulter N. Hypertension management in England: a serial cross-sectional study from 1994 to 2011. Lancet 2014;383:1912–19.

88. Wright JT, Williamson JD, Whelton PK, Snyder JK, Sink KM, Rocco MV, Reboussin DM, Rahman M, Oparil S, Lewis CE, et al. A randomized trial of intensive versus standard blood-pressure control. N Engl J Med 2015;373:2103–16.

89. Xie X, Atkins E, Lv J, Bennett A, Neal B, Ninomiya T, Woodward M, MacMahon S, Turnbull F, Hillas GS, et al. Effects of intensive blood pressure lowering on cardiovascular and renal outcomes: updated systematic review and meta-analysis. Lancet 2016;387:435–43.

90. Myers JE, Green M, Chappell LC. Why is the search for pre-eclampsia prevention so elusive? BMJ 2018;362:k3536.

91. Whitfield KC, Karakochuk CD, Liu Y, McCann A, Talukder A, Kroeun H, Ward M, McNulty H, Lynd LD, Kitts DD, et al. Poor thiamin and riboflavin status is common among women of childbearing age in rural and urban Cambodia. J Nutr 2015;145:628–33.

92. McAuley E, McNulty H, Hughes C, Strain JJ, Woodward M, MacMahon S, Turnbull F, Hillas GS, et al. Effect of riboflavin on cardiovascular and renal outcomes: updated systematic review and meta-analysis. Lancet 2016;387:435–43.

93. Public Health England. National Diet and Nutrition Survey Rolling Programme (NDNS) Supplementary Report: blood folate results for the UK as a whole, Scotland, Northern Ireland (years 1 to 4 combined) and Wales (years 2 to 5 combined). Revised 2017. London: Public Health England; 2017.

94. Keohel L, Walton J, Hopkins SM, McNulty BA, Nugent AP, McNulty H, Ward M, Flynn A. Intake, status and dietary sources of riboflavin in a representative sample of Irish adults aged 18–90 years. Proc Nutr Soc 2018;77:E66.

95. Hoey L, McNulty H, Strain JJ. Studies of biomarker responses to intervention with riboflavin: a systematic review. Am J Clin Nutr 2009;89:1960S–80S.