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The MTHFR C677T polymorphism, B-vitamins and blood pressure

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High blood pressure (BP) and elevated homocysteine are reported as independent risk factors for CVD and stroke in particular. The main genetic determinant of homocysteine concentrations is homozygosity (TT genotype) for the C677T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene, typically found in approximately 10% of Western populations. The B-vitamins folate, vitamin B_{12} and vitamin B_6 are the main nutritional determinants of homocysteine, with riboflavin more recently identified as a potent modulator specifically in individuals with the TT genotype. Although observational studies have reported associations between homocysteine and BP, B-vitamin intervention studies have shown little or no BP response despite decreases in homocysteine. Such studies, however, have not considered the MTHFR C677T polymorphism, which has been shown to be associated with BP. It has been shown for the first time that riboflavin is an important determinant of BP specifically in individuals with the TT genotype. Research generally suggests that 24 h ambulatory BP monitoring provides a more accurate measure of BP than casual measurements and its use in future studies may also provide important insights into the relationship between the MTHFR polymorphism and BP. Further research is also required to investigate the association between specific B-vitamins and BP in individuals with different MTHFR genotypes in order to confirm whether any genetic predisposition to hypertension is correctable by B-vitamin intervention. The present review will investigate the evidence linking the MTHFR C677T polymorphism to BP and the potential modulating role of B-vitamins.

MTHFR C677T polymorphism: B-vitamins: Homocysteine: Blood pressure

CVD is one of the leading causes of death worldwide. High blood pressure or hypertension, defined as a blood pressure (BP) of >140/90 mmHg[1], is a major risk factor for CVD, with subjects with uncontrolled hypertension being at approximately three times greater risk of developing CVD compared with subjects who are normotensive[2]. Furthermore, hypertension is seen as the strongest predictor of stroke risk[3]. The relationship between BP and CVD risk is continuous across a wide range of values, with evidence indicating that the association with CVD mortality is apparent down to BP levels of 115/75 mmHg[4]. BP reduction, through the use of antihypertensive therapy, has been shown to reduce cardiovascular events, in particular stroke[5], and it can effectively reduce the recurrence of stroke even in individuals with normal BP values[6]. The prevalence of hypertension is estimated to be as high as 40% in the UK population but its detection and management are often suboptimal[7]. The exact pathophysiology of hypertension is unclear but in the majority of instances no single cause is identifiable, with various factors such as age, obesity, diet, physical activity and genetic factors being thought to play a role.

Evidence has accumulated in recent years to suggest that elevated homocysteine, itself considered to be an independent risk factor for CVD[8], may also be associated with hypertension. Homocysteine concentrations are determined by both nutritional and genetic factors. The main nutritional determinants are the B-vitamins, in particular

Abbreviations: BP, blood pressure; MTHFR, methylenetetrahydrofolate reductase.

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folate[9] and to a lesser extent vitamin B_{12}[10] and vitamin B_6[11], and the most common genetic determinant is homozygosity for the C677T polymorphism in the gene that encodes the methylenetetrahydrofolate reductase (MTHFR) enzyme[12]. Meta-analyses have concluded that individuals with the C677T polymorphism have a higher risk of heart disease compared with those without the polymorphism[13–15]. Some evidence also suggests that this polymorphism may be linked to BP[16,17].

The aim of the present review is to investigate emerging evidence that links the MTHFR C677T polymorphism to BP and the potential modulating role of B-vitamins.

Homocysteine as a risk factor for CVD

Homocysteine is an amino acid that is produced from the demethylation of dietary methionine. The MTHFR enzyme is indirectly involved in homocysteine metabolism as it is required for the formation of 5-methyltetrahydrofolate (the metabolically-active form of folate), which acts as a methyl donor in the conversion of homocysteine to methionine. Homozygosity for the C677T polymorphism, termed the TT genotype, is present in approximately 10% of the population[18] and results in reduced activity of the MTHFR enzyme and correspondingly higher homocysteine concentrations[12]. Apart from this genetic factor, the B-vitamins folate, vitamin B_{12} and vitamin B_6 are the main nutritional determinants of homocysteine concentrations. Of these B-vitamins, folate appears to be the most important determinant[19] and supplementation with folic acid, the synthetic form of the vitamin, is the most effective treatment to lower elevated homocysteine concentrations in the population generally[10,20]. However, the B-vitamin riboflavin, which is required as a cofactor for the MTHFR enzyme, has recently been shown to be an important modulator of homocysteine concentrations specifically in individuals with the C677T polymorphism[21,22].

For >40 years homocysteine has been linked with vascular disease, yet, there is still substantial controversy in terms of whether the observed relationship is causal. While meta-analyses have identified homocysteine as an independent risk factor for CVD[8,13], there appears to be an even stronger relationship between homocysteine and stroke in particular[23–25]. Randomised controlled trials investigating the impact of homocysteine lowering on the secondary prevention of CVD have failed to demonstrate a corresponding decrease in CVD events generally[26–28]. However, the most promising evidence of a beneficial effect of B-vitamin intervention and homocysteine lowering appears to be in terms of stroke[28]. A recent meta-analysis has concluded that lowering homocysteine (via folic acid supplementation) reduces the risk of stroke by 18% overall and by 25% in those trials involving individuals with no previous history of stroke[29]. This finding is further supported by a population-based study that has observed an accelerated decline in stroke mortality in the USA and Canada after the full implementation of folic acid fortification, but not in England where population-based fortification had not been introduced[30].

Homocysteine and blood pressure

Evidence has accumulated to suggest that elevated homocysteine may also play a role in the development of hypertension, and as such may provide a potential mechanism linking elevated homocysteine with vascular disease. Experimental evidence has shown that elevated homocysteine has negative effects on the potent vasodilator NO[31], vascular smooth muscle cell proliferation[32], impaired endothelial function[33], altered elasticity of the vascular wall[34] and renal function[35]. Given that physiological factors such as peripheral resistance, arterial stiffness and kidney function are also strong determinants of BP, it would therefore seem reasonable to predict an association between homocysteine and BP.

Many studies have investigated the relationship between homocysteine and BP but have produced conflicting findings[36–39]. The Hordaland Homocysteine Study, a large cross-sectional study in >16 000 individuals without CVD, has observed a positive linear association between homocysteine and BP. However, the strength of this relationship appears small given the <1 μmol/l difference in homocysteine concentrations between individuals with a diastolic BP ≥100 mmHg compared with those with a diastolic BP <70 mmHg[40]. The Systolic Hypertension in the Elderly Program has observed higher homocysteine concentrations in older individuals with isolated systolic hypertension compared with controls, and the association between homocysteine and systolic BP remains after adjustment for confounders[41]. Associations between homocysteine and systolic BP have also been noted at the other end of the age spectrum. A weak positive association between homocysteine and systolic BP has been observed in 3524 American schoolchildren, which remains after adjustment for vitamin supplement use[42]. Cross-sectional data from the Third National Health and Nutrition Examination Survey show a positive relationship between homocysteine and BP, and it was noted that men and women in the highest quintile of homocysteine had a 2-fold and 3-fold increase in risk of hypertension respectively compared with those in the lowest quintile, but this trend is only significant in women[43]. However, analysis of more-recent National Health and Nutrition Examination Survey data from the post-folic-acid-fortification period has detected an association between homocysteine and systolic BP only, showing that individuals with systolic BP ≥129 mmHg have homocysteine levels that are 0·57 μmol/l higher than those with systolic BP <108 mmHg[44]. In contrast, preliminary analysis of the Framingham Offspring cohort, which included 1960 individuals, has found no relationship between homocysteine and BP but does note that homocysteine concentrations are higher in individuals using antihypertensive medication[45]. Furthermore, a follow-up study of 2104 participants from the original Framingham Heart Study cohort has observed no significant relationship between homocysteine and either the incidence of hypertension or the progression towards hypertension after adjustment for important covariates[38]. Although a correlation between homocysteine and both systolic BP and diastolic BP has been observed in African-American women, this relationship does not remain after adjustment

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for age and BMI[46]. Similarly, several case–control studies have failed to observe a relationship between homocysteine and BP in patients who have had a stroke[47], individuals with coronary artery disease[39] or patients who have had a cerebral infarct[48].

Methionine-loading studies, which induce hyperhomocysteinaemia, have also generated conflicting results in terms of an observed effect on BP. Although one study has observed that hyperhomocysteinaemia induced in this way results in a marked increase in pulse pressure in fourteen healthy men[49], other studies have observed no significant effect on either systolic BP or diastolic BP[50] or arterial stiffness[51] despite marked increases in homocysteine (of >12 μmol/l). The acute haemodynamic effects of hyperhomocysteinaemia on BP therefore require further investigation.

Thus, there is contradictory evidence in relation to the association between homocysteine and BP in the general population. Nevertheless, if a direct relationship exists, then it would be expected that patients with hypertension would have higher homocysteine concentrations than individuals who are normotensive. Again, there is inconsistency in the literature, as several studies have observed higher concentrations of homocysteine in individuals with hypertension compared with controls[52–55], whereas others have failed to observe any significant difference[56,57] and one study has even observed markedly lower concentrations of homocysteine in patients with hypertension compared with controls[58]. It must be recognised, however, that many case–control studies of patients with hypertension have involved recruitment from specialised hypertension clinics and, by definition, such patients are a highly-selected group that may not be representative of the relationship between homocysteine and uncomplicated hypertension in the general population.

Evidence also suggests that elevated homocysteine may exert an additive effect on the occurrence of atherosclerosis and cardiovascular events when hypertension is present, but this effect has not been found in all studies[41,59]. In a follow-up to the Caerphilly Study in >2000 men an interaction was noted between homocysteine and diastolic BP, with elevated homocysteine having a much stronger impact on risk of ischaemic stroke in individuals with hypertension compared with individuals who are normotensive[60]. Similarly, the European Concerted Action Project, which included 750 patients with atherosclerotic vascular disease and 800 controls, has observed that elevated homocysteine has a multiplicative and interactive effect[61]. In contrast, a 10-year follow-up study in 878 elderly men has found that high homocysteine concentrations at baseline are associated with increased prevalence of stroke and increased risk of death from cerebrovascular disease only in subjects who are normotensive[62]. However, the latter study was conducted in elderly individuals, which may limit its interpretation as both homocysteine and BP change with increasing age. Similar findings observed in subgroup analysis of a nested case–control within the Physicians' Health Study indicate that elevated homocysteine is predictive of ischaemic stroke only in individuals who are normotensive[63]. However, in this particular analysis the sample size may be a limitation as there were only 133 individuals with hypertension compared with 403 individuals who were normotensive.

Despite the fact that there are plausible biological mechanisms to explain how homocysteine could increase BP, it is unclear whether homocysteine is truly associated with BP, as many studies have failed to accurately and consistently identify or adjust for confounders. Reverse causality, the possibility that elevated BP itself can elevate homocysteine concentrations, must be considered as a potential explanation because high BP is associated with increased renal dysfunction, which can in turn increase homocysteine concentrations. The majority of studies have not taken renal function into account and those that have considered it have used serum creatinine levels, which are a poor indicator of mild renal impairments[64]. Accurate adjustments should therefore be made for renal function in order to establish whether homocysteine is an independent risk factor in the development of hypertension. Of note, the use of antihypertensive medications has been shown to affect homocysteine concentrations[65,66] and remains unaccounted for or even unconsidered in many studies. Furthermore, in some studies hypertension is defined not only in terms of a BP cut-off but can also include those taking antihypertensive medications irrespective of current BP, which may have been normalised in response to this treatment. This factor, in turn, can complicate the interpretation of these results by leading to an underestimation of the relationship between homocysteine and BP. Smoking has also been reported to have a strong additive effect on the relationship between homocysteine and BP[67], yet many studies have failed to account or adjust for this effect. Interpretation of case–control studies can also be problematic given that individuals with hypertension are more likely to have had a previous vascular event, which in itself is likely to have elevated homocysteine concentrations. Methodological differences such as the BP cut-off value used to define hypertension, the measurement method of BP, use of fasting or non-fasting blood samples and the use of data-derived cut-offs, alongside unaccounted genetic and nutritional factors, can further hinder the interpretation of many studies.

Homocysteine lowering (via B-vitamin supplementation) and blood pressure

Although a positive association has been reported between homocysteine and BP[46,68,69], studies examining the effect of homocysteine lowering on BP have yielded generally disappointing and inconsistent results. If homocysteine is causally linked to BP then it would be expected that homocysteine lowering (via B-vitamin supplementation) would result in a decrease in BP. Several large-scale randomised controlled trials in high-risk population groups have used various combinations and doses of B-vitamins that have produced decreases in homocysteine but have failed to consider or report a BP response[27,28]. Those trials that have considered BP have produced conflicting findings (Table 1). The Vitamin Intervention for Stroke Prevention trial, which compared a high-dose v. a low-dose

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Table 1. Studies investigating the effect of homocysteine (Hcy) lowering (via B-vitamin supplementation) and the reported blood pressure (BP) response

Study			Subjects			Intervention		Mean response	
Reference	Country	Participants	Gender	*n*	Age (years)	B-vitamin combination	Duration	Hcy (μmol/l)	BP (mmHg)
Toole *et al.*[26]	USA, Canada, Scotland	Cerebral infarct	M+F	3649	≥35	High dose *v.* low dose: folic acid 2·5 mg, 20 μg; vitamin B_6 25 mg, 200 μg; vitamin B_{12} 0·4 mg, 6 μg respectively	2 years	↓2·3‡	No effect
van Dijk *et al.*[70]†	The Netherlands	Siblings of patients with premature atherothrombosis	M+F	130	18–65	Folic acid 5 mg, vitamin B_6 250 mg	2 years	↓7·8*	↓3·7 SPB* ↓1·9 DPB*
McMahon *et al.*[72]†	New Zealand	Individuals with Hcy ≥13 μmol/l	M+F	249	≥65	Folate 1 mg, vitamin B_6 10 mg, vitamin B_{12} 500 μg	2 years	↓4·4*	No effect
Mangoni *et al.*[71]†	UK	Smokers	M+F	24	21–58	Folic acid 5 mg	4 weeks	↓2·6*	↓8 SPB* ↓4 DPB*

M, male; F, female; ↓, decrease; SBP, systolic BP; DBP, diastolic BP.
Mean response was significant: *$P<0·05$.
†Randomised double-blind placebo-controlled trial.
‡Difference in mean response between high-dose *v.* low-dose groups.

B-vitamin combination over a 2-year period in >3000 patients who had had a cerebral infarct, has observed a greater reduction in homocysteine (by 2·3 μmol/l) with the high-dose combination compared with the low-dose treatment[26]. No significant BP response was observed in either treatment group, with mean BP differences of ≤1 mmHg in both systolic BP and diastolic BP post intervention in the high-dose treatment group[26]. In contrast, a few studies have reported a significant effect of B-vitamin intervention on BP. In a placebo-controlled intervention in 130 individuals supplementation with folic acid and vitamin B_6 was found to result in a significant homocysteine lowering of 7·8 μmol/l alongside a modest (but significant) decrease in systolic BP and diastolic BP of 3·7 mmHg ($P=0·02$) and 1·9 mmHg ($P=0·04$) respectively[70]. Similarly, a significant decrease in systolic BP and diastolic BP, by 8 mmHg and 4 mmHg respectively ($P<0·01$ in both cases), has also been observed in response to folic acid supplementation but the homocysteine lowering reported in this study is modest in comparison (2·6 μmol/l)[71]. Further multiple regression analysis indicates that the folic acid independently predicts this BP response and not the change in homocysteine. In contrast, a study conducted in 249 individuals with elevated homocysteine concentrations at baseline (≥13 μmol/l) has shown a significant decrease of 4·4 μmol/l in homocysteine, from 16·8 μmol/l to 12·4 μmol/l in response to B-vitamin supplementation, but no effect was observed on either systolic BP or diastolic BP[72]. It is interesting that of these four studies the two that have observed significant BP responses both used the highest dose of folic acid (5 mg/d). Furthermore, it is also noteworthy that the study reporting the greatest decrease in homocysteine[70] does not report the greatest decrease in BP, as would be expected if homocysteine is causatively related to the BP response. Interestingly, the study that reports the most significant decrease in BP[71] was performed in a small number of smokers, and given that smoking has been shown to elevate both homocysteine and BP[67], care should be taken when interpreting this finding.

The mandatory fortification of grain foods with folic acid in the USA and Canada may explain, to some extent, the heterogeneity in study results as folate status and consequently homocysteine concentrations would already be improved at a population-wide level, thus diminishing any homocysteine-lowering response to B-vitamin supplementation. Forming a consensus of opinion on the effect of homocysteine lowering on BP is problematic as the studies have been conducted in different population groups and have used different doses, combinations and durations of B-vitamin supplementation.

It is important to note that folic acid, independently of its homocysteine-lowering effect, may itself influence BP, with several studies observing positive effects of folic acid on endothelial function[73–75], antioxidant potential[76] and generation of the vasodilator NO[77]. A recent meta-analysis examining the effect of high-dose folic acid supplementation (5 mg/d) on BP and endothelial function has concluded that folic acid has a modest yet significant effect on systolic BP (mean decrease of 2·03 mmHg; $P=0·04$) but the authors conclude that the most-clinically-relevant finding is its ability to improve endothelial function[78].

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Thus, at this time, the evidence supporting a potentially causal role between elevated homocysteine and BP appears to be weak. Although the observed lowering of homocysteine in response to folic acid fortification in the USA and Canada has been associated with a decrease in stroke mortality, sensitivity analysis of other stroke risk factors such as hypertension remains relatively unchanged[30]. This outcome would not be the case if a causal relationship between homocysteine and BP exists. Nonetheless, further studies are required to investigate more fully the relationship between BP and homocysteine, but evaluation of the evidence to date suggests that it is unlikely that homocysteine lowering *per se* (independently of a B-vitamin effect) can lower BP.

The interaction between the MTHFR polymorphism, homocysteine and blood pressure

Given that the main genetic determinant of homocysteine is the MTHFR polymorphism several observational and case–control studies have considered both these factors in order to investigate their relationship with BP. In a hypertension case–control study in Asian-Indians plasma homocysteine was observed to be higher and the frequency of the T allele was found to be increased in patients with hypertension compared with controls[79]. It was concluded that the MTHFR polymorphism is significantly associated with hypertension (OR 3·91 (95% CI 2·60, 6·05)). In contrast, a hypertension case–control study in adolescents and adults has failed to observe a significant difference in MTHFR genotype frequency between individuals with hypertension and controls[80]. Furthermore, while a recent study has reported higher mean homocysteine concentrations in individuals with hypertension compared with controls, no significant difference in genotype frequency between the groups was evident[81]. However, in a subgroup analysis of individuals with a BMI $<30\,kg/m^2$ individuals with the T allele were found to have both higher homocysteine and increased risk of hypertension compared with those with the CC genotype. There are several limitations in this latter study that must be considered, one of which is that hypertension is only defined on the use of antihypertensive therapy and second the authors do not statistically control for BMI (but rather split by BMI), which may confound the interpretation of these findings. While patients with the TT genotype who are hypertensive have been observed to have higher homocysteine concentrations compared with patients without the polymorphism who are hypertensive, no significant association between either homocysteine or the MTHFR polymorphism and BP was found[82]. Several other studies have failed to observe any significant relationship between the MTHFR polymorphism or homocysteine and BP[83,84].

Evidence of an independent relationship between the MTHFR polymorphism and BP has been noted[85]. While mean homocysteine concentrations were found to be significantly higher in men with hypertension, with homocysteine concentrations $\geq 15\,\mu mol/l$ being associated with increased risk of hypertension, interestingly this outcome was not observed in men without the TT genotype but with similar homocysteine concentrations. The MTHFR genotype was only found to account for 2% of the variance in homocysteine levels and it was concluded that homocysteine concentrations alone do not account fully for the increased risk associated with the TT genotype.

While the evidence to date is somewhat inconsistent, it does suggest that individuals with both the TT genotype and elevated homocysteine may be at increased risk of developing hypertension. Furthermore, methodological and population differences make it difficult to ascertain to what extent the strength of the relationship is a result of homocysteine or the MTHFR polymorphism or their interaction. However, because significant decreases in homocysteine have not been mirrored by a consistent BP response, and given that there is an association between the TT genotype and BP in individuals with normal homocysteine concentrations, the possibility of an independent association between the MTHFR polymorphism and BP rather than a homocysteine-driven BP effect is strengthened.

Evidence of an independent effect of the MTHFR polymorphism on blood pressure

A number of recent studies have focused on investigating the effect of the MTHFR polymorphism on BP in different populations. A graded relationship between the number of T alleles and BP has been observed in Japanese men[86]. Mean systolic and diastolic BP (mmHg) of 133/79, 134/81 and 147/91 for the CC, CT and TT genotypes respectively were observed but only the diastolic BP was significantly higher in individuals with the TT genotype ($P<0·05$) but no significant difference in the prevalence of hypertension was observed between the genotype groups. A study investigating BP response to angiotensin-converting enzyme inhibitors in patients with hypertension with different MTHFR genotypes has also observed that individuals with the TT genotype have significantly higher diastolic BP at baseline compared with those without the genotype ($P<0·05$)[87]. In contrast, an observational study of 716 Spanish men and women has failed to observe this significant relationship between diastolic BP and MTHFR genotype, but has reported a graded association between the number of T alleles and systolic BP in men, with a mean systolic BP (mmHg) of 122, 125 and 128 for CC, CT and TT genotypes respectively[88]. A study performed in >3000 Japanese individuals has failed to observe an association between the TT genotype and hypertension in men but has reported that women with the TT genotype have a significantly higher diastolic BP, with an all-adjusted OR for hypertension in women with the TT genotype of 1·42 (95% CI 1·01, 1·99)[89]. It was also noted that individuals with the TT genotype have higher homocysteine concentrations. A case–control study in individuals with hypertension has observed an over-representation of the T allele in patients with hypertension that approaches significance ($P = 0·07$), with the T allele conferring a significant increase in the risk of hypertension (OR 1·57 (95% CI 1·04, 2·37)) after adjusting for BMI[16]. Similarly, a case–control study of 221 patients with chronic renal failure has observed an increase in the

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frequency of the TT genotype in the subgroup with hypertension, the prevalence of the TT genotype being 46·7 % compared with 17·6 % in controls[17]. In contrast, a case–control study in a Czech population (*n* 1199) has observed an association between MTHFR polymorphism (T allele) and coronary artery disease in combination with essential hypertension but no association in those patients with hypertension only[90]. However, a recent meta-analysis that has investigated the association between the MTHFR polymorphism and hypertension in twenty-five studies of Asians and Caucasians has observed a significant pooled OR for the T allele and hypertension ($P = 0{\cdot}042$)[91]. Caution should be used when interpreting this finding as significant heterogeneity was noted in certain aspects of their analysis.

Overall, there does appear to be an association between the MTHFR polymorphism and BP. The generally-found graded association between the number of T alleles and BP, alongside the over-representation of the T allele in individuals with hypertension, would suggest an effect of this polymorphism on BP. A genetic link with hypertension is not surprising given that approximately 50 % of the variability in BP is attributable to heritability[92]. Furthermore, a genome-wide association study in 34 433 individuals from the Global BPgen Consortium that tested $>2{\cdot}5 \times 10^6$ single nucleotide polymorphisms for an association with BP has identified the MTHFR loci as one of the eight loci that are associated with BP[93]. It is recognised, of course, that there may be other unknown or unconsidered genetic variants linked to BP that may also be located in this region.

As both the frequency of the MTHFR polymorphism and the prevalence of hypertension varies in different population groups, alongside the lack of genotype-driven recruitment (reflected by the small number of individuals with the TT genotype), it is not surprising that an association between the MTHFR polymorphism and BP has not been consistently observed. It is also possible that the effect of the polymorphism is masked by the presence of other risk factors that also affect BP. For example, BMI is known to affect BP, and several studies have observed an association between the polymorphism and BP only after adjustment for the effect of BMI[16,81]. This finding highlights the importance of accurately identifying and appropriately adjusting for relevant confounders that may have been previously unconsidered.

The MTHFR polymorphism and riboflavin: a gene–nutrient interaction with a potential role in blood pressure

While meta-analyses have confirmed that supplementation with folic acid or a combination of B-vitamins results in a significant decrease in homocysteine[10,20], a specific genotype–nutrient interaction has become apparent, with individuals that have the TT genotype being particularly sensitive to B-vitamin status[94]. It has been observed that individuals with the TT genotype have increased folate requirements reflected in the fact that they have lower erythrocyte folate concentrations compared with those without the polymorphism[95]. Evidence also suggests that riboflavin status is particularly important in individuals with the TT genotype. *In vitro* evidence has shown that reduced activity of the MTHFR enzyme evident in individuals with the TT genotype can be explained by the inappropriate loss of the riboflavin cofactor from the enzyme[96,97]. Higher concentrations of homocysteine have been observed in individuals with the TT genotype, particularly in those with poor riboflavin status[21,22]. Furthermore, it has been shown that riboflavin supplementation can lower homocysteine concentrations specifically in individuals with the TT genotype, and that the homocysteine-lowering effect of riboflavin is particularly pronounced in those individuals with the lowest riboflavin status at baseline[98].

There is a paucity of evidence investigating the effect of B-vitamin supplementation on BP in individuals with different MTHFR genotypes, and those that have been conducted are opportunistic in nature, given the lack of genotype-driven recruitment. Folic acid supplementation (5 mg/d for 3 weeks) has been observed to decrease pulse pressure and arterial stiffness and it was concluded that the effect is independent of MTHFR genotype or homocysteine concentrations; however, only five individuals had the TT genotype[99]. Another folic acid intervention study (5 mg/d for 4 weeks) in individuals with hypertension has observed that while the T allele is associated with increased risk of hypertension, decreases in homocysteine are not accompanied by any BP response[100]. Once again, interpretation is limited by the inclusion of only eight individuals who were homozygous for the MTHFR polymorphism.

Despite the genotype-specific response in homocysteine lowering with riboflavin supplementation, there are currently no published studies that have investigated the effect of riboflavin supplementation on BP in individuals with the TT genotype. A recent randomised placebo-controlled intervention study involving 181 patients with premature CVD, pre-screened for MTHFR genotype, has found that individuals with the TT genotype have higher systolic and diastolic BP at baseline compared with those without the polymorphism and this effect is driven by those individuals with the TT genotype who have the lowest riboflavin status[101]. Furthermore, riboflavin intervention (1·6 mg/d for 16 weeks) was shown to result in a decrease in BP specifically in those individuals with the TT genotype (*n* 49), with systolic BP decreasing by 13 mmHg and diastolic BP by approximately 8 mmHg, yielding post-intervention BP values similar to those of individuals with the CC genotype. The homocysteine response was found to be modest yet significant, decreasing from 11·3 μmol/l to 10·0 μmol/l ($P = 0{\cdot}014$). The effect of riboflavin supplementation on BP, specifically in those individuals with the TT genotype, which is likely to be mediated through the stabilisation of the variant MTHFR enzyme, represents a novel gene–nutrient interaction that affects BP in an already-at-risk group. It is also noteworthy that the dosage of the B-vitamin used is at a physiological level, as opposed to the previously-mentioned B-vitamin intervention studies that often use therapeutic levels of B-vitamins in order to observe any clinically-significant findings.

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However, further intervention trials, with genotype-driven recruitment, are required to confirm this finding and also to investigate whether the gene–nutrient interaction affects BP in individuals with hypertension generally.

Considerations in the measurement of blood pressure

The accurate measurement of BP is essential given that it is the basis for the diagnosis, treatment and management of hypertension. Casual BP measurements, taken in a clinical setting, have been the traditional method used to measure BP. However, this conventional method is subject to several sources of potential error[102–105] and can be affected by factors such as the selection of the arm from which BP is measured, external temperature, time of day and whether the patient is at rest both before and during the measurement. Many external factors can therefore influence the BP reading obtained, and if they are not considered it can lead to substantial measurement errors. Recent evidence suggests that ambulatory BP monitoring, which measures BP over a 24 h period, can overcome many of these issues and thus provide a more accurate measurement of BP. This greater accuracy is reflected in the fact that ambulatory BP monitoring is a much stronger predictor of CVD morbidity and mortality than casual BP measurements[106]. It has been observed that BP follows a normal circadian pattern, being highest during the day and gradually decreasing at night. However, it has been noted that there is often a circadian variation from this normal pattern, especially in individuals with hypertension[3,102]. Abnormal variations in the circadian pattern of BP result in increased risk of cardiac mortality[106,107] and these differences in BP profile can only be determined using ambulatory BP monitoring.

Studies to date that have investigated the relationship between the MTHFR polymorphism and BP have not considered the use of ambulatory BP monitoring, which would enable a more accurate determination of the relationship between this polymorphism and BP and would allow accurate investigation of the hypothesis that this polymorphism is a predisposing factor in the development of hypertension. Furthermore, because ambulatory BP monitoring provides a wealth of data, including the circadian profile of BP as well as mean night-time and daytime BP, it may help to elucidate potential mechanisms to explain the increased vascular risk observed in individuals with the TT genotype.

Conclusion

Overall, the evidence linking homocysteine to BP appears to be weak and there is mounting evidence to suggest that the MTHFR C677T polymorphism, previously only considered in its role as a determinant of homocysteine concentrations, may itself be independently linked to BP. Given the high prevalence of the TT genotype, further studies are required to confirm the role of this polymorphism in the development of hypertension, and also to confirm the responsiveness of BP to interventions with B-vitamins specifically in those individuals with the TT genotype. Such findings would have important implications for the management, treatment and long-term prognosis in this genetically-predisposed group because BP reduction does translate into a decrease in vascular end points[4]. Furthermore, recent evidence suggests that the reduction in cardiovascular events in response to BP lowering occurs irrespective of either baseline BP or the existence of previous vascular disease[108], which highlights the relevance and population-wide benefits of decreasing BP.

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