Riboflavin offers a targeted strategy for managing hypertension in patients with the *MTHFR* 677TT genotype: a 4-y follow-up[1–3]

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ABSTRACT

Background: We recently reported that the elevated blood pressure (BP) observed in patients with cardiovascular disease who are homozygous for the 677C→T polymorphism (*TT* genotype) in the gene encoding methylenetetrahydrofolate reductase (MTHFR) was responsive to supplementation with riboflavin—the cofactor for MTHFR.

Objective: The objective was to investigate the effect of riboflavin on BP targeted at patients with the *TT* genotype 4 y after initial investigation, during which time major changes in the clinical guidelines for antihypertensive therapy were introduced.

Design: A total of 83 patients (representing all 3 genotypes) who participated in a placebo-controlled riboflavin intervention for 16 wk in 2004 agreed to take part. Nested within this follow-up, those with the *TT* genotype (n = 31) proceeded to intervention with riboflavin (1.6 mg/d for 16 wk) or placebo, conducted in a crossover style whereby the 2004 treatment groups were reversed.

Results: At follow-up in 2008, as in 2004, patients with the *TT* genotype had higher systolic BP ($P < 0.01$), with a nonsignificant trend noted for higher diastolic BP ($P = 0.051$). Despite the marked changes in antihypertensive therapy that had occurred, BP remained unchanged in patients with the *TT* genotype at the time of follow-up. Riboflavin supplementation (administered in 2004 and 2008) produced an overall decrease in systolic (-9.2 ± 12.8 mm Hg; $P = 0.001$) and diastolic (-6.0 ± 9.9 mm Hg; $P = 0.003$) BP.

Conclusions: Optimizing riboflavin status offers a low-cost targeted strategy for managing elevated BP in this genetically at-risk group. These findings, if confirmed in the general population, could have important implications for the prevention of hypertension. *Am J Clin Nutr* 2012;95:766–72.

INTRODUCTION

Elevated BP[4] is the leading risk factor for death worldwide (1) and is the most important predictor of vascular mortality (2). The exact pathophysiology of hypertension, however, remains unclear and in most cases no single cause is identifiable. Research into the genetic basis of hypertension has enabled a more detailed understanding of the biological processes underlying BP control (3). One genetic variant under recent investigation is the 677C→T polymorphism in the gene encoding the folate-metabolizing enzyme MTHFR. This polymorphism previously received much attention as the main genetic determinant of homocysteine concentrations (4, 5), but it has also been associated with elevated BP (6, 7) and an increased risk of stroke (8, 9). The homozygous mutant (*TT*) genotype produces an MTHFR enzyme with decreased activity (4) and has a reported frequency of 10% worldwide, ranging from 4% to 18% in the United States, 20% in Northern China, and to as high as 32% in Mexico (10). The B-vitamin riboflavin, in the coenzymatic form FAD, is required as a cofactor for MTHFR and the decreased enzyme activity evident in individuals with the *TT* genotype results from the loss of its riboflavin cofactor (11). However, supplementation with riboflavin appears to stabilize the variant enzyme in vivo (12). In an investigation of 181 CVD patients, we recently reported that this gene-nutrient interaction played a novel role in BP (13). At baseline, a graded relation between the *T* allele and BP was noted; furthermore, intervention with riboflavin (1.6 mg/d for 16 wk) produced a significant lowering of BP only in those patients with the *TT* genotype. This intervention was conducted in 2004—a time during which β-blockers were used as a first choice for antihypertensive therapy (14). In 2006, however, a major review of the National Institute for Health and Clinical Excellence guidelines in the United Kingdom occurred, which omitted β-blockers as a first-line treatment of hypertension and advocated a switch from monotherapy to polytherapy (15). Although our previous intervention showed a modulating effect of riboflavin on BP in those with the *TT* genotype (13), it was not clear whether this effect was independent of antihypertensive therapy concurrently administered and whether it would still be evident 4 y later, considering any changes in the drug management of hypertension over this period.

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[4] Abbreviations used: BP, blood pressure; CVD, cardiovascular disease; EGRac, erythrocyte glutathione reductase activation coefficient; FAD, flavin adenine dinucleotide; MTHFR, methylenetetrahydrofolate reductase; NO, nitric oxide.

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The aim of this study was to investigate the effect of the *MTHFR* 677C→T polymorphism on hypertension over a 4-y period, during which antihypertensive drug treatment was likely to have changed considerably, and to confirm the response of BP to riboflavin supplementation targeted at patients with the *TT* genotype.

SUBJECTS AND METHODS

Participants

We followed-up a cohort of premature patients with CVD (n = 181) previously recruited from the Cardiology Unit at Altnagelvin Area Hospital (Western Health and Social Care Trust, Northern Ireland), who had completed a 16-wk, randomly assigned, double-blind, placebo-controlled intervention trial with riboflavin (1.6 mg/d) in 2004 (13). As part of the original study, patients were prescreened to identify those with the *MTHFR* 677TT genotype and were then appropriately matched to patients with the *CC* and *CT* genotypes (**Figure 1**).

At the time of follow-up in 2008, the original exclusion criteria (13) were reconfirmed. Ethical approval was granted by the Office for Research Ethics Northern Ireland (ORECNI ref no. 08/NIR03/40), and all patients provided written informed consent before participation.

Study design

This study comprised an observational 4-y follow-up investigation of BP across the 3 *MTHFR* 677C→T genotype groups and a nested crossover intervention trial in those patients with the *TT* genotype (Figure 1). All patients who completed the original (2004) intervention and had indicated a willingness to be recontacted regarding future studies were approached. Patients with the *TT* genotype, in addition, were invited to participate in a 16-wk placebo-controlled trial, conducted in a crossover design style so that those who had received riboflavin (1.6 mg/d) in the 2004 investigation were given placebo in 2008 and vice versa.

The sample size for the intervention was estimated by using data from our original study investigating the BP-lowering effect of riboflavin in those patients with the *MTHFR* 677TT genotype (13). The sample size was calculated on the basis of the mean (±SD) systolic BP response to riboflavin treatment (1.6 mg/d for 16 wk) compared with placebo, and it was estimated that a total of 31 participants were required to take part in the current follow-up study. Participants were given supplements on a monthly basis in 7-d pill boxes and asked to return these with any untaken pills remaining to monitor compliance. BP, blood samples, anthropometric measurements, medication history, and general health and lifestyle information were collected at the start and end of the intervention.

Procedures

BP measurements for both the observational and intervention components were performed by the same researcher, and BP was measured according to the appropriate clinical guidelines (15) by using an A&D UA-787 digital BP monitor (Cardiac Services) and appropriate cuff. Both the participants and the researchers conducting the BP measurements were blind to treatment group allocation. The reference arm was identified (the arm with the

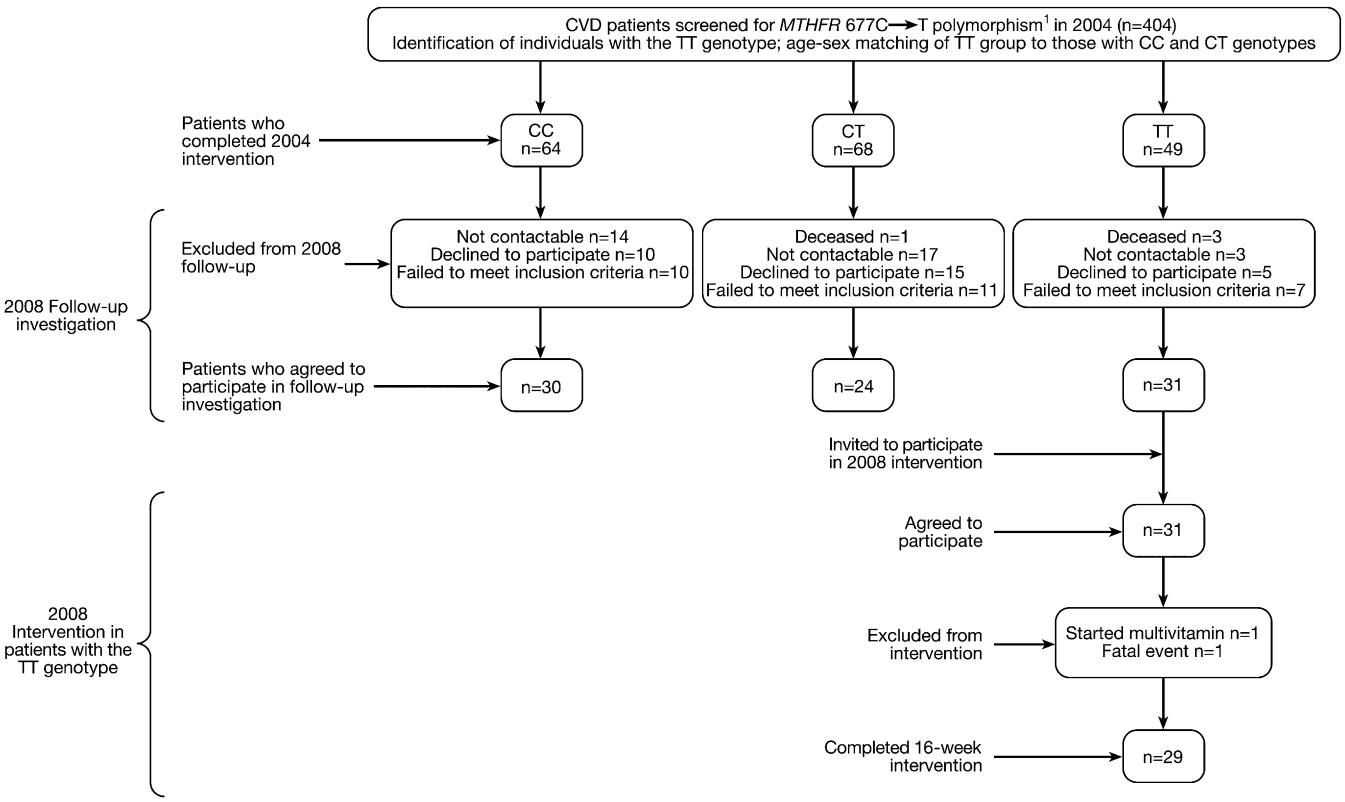

FIGURE 1. Flow diagram of study design and completion rates. [1]*CC* (wild-type), *CT* (heterozygous), and *TT* (homozygous) genotypes for the *MTHFR* 677 C→T polymorphism. CVD, cardiovascular disease; MTHFR, methylenetetrahydrofolate reductase.

highest BP), and 2 measurements from this arm were taken 10 min apart and the mean calculated. However, if a difference of >5 mm Hg in diastolic BP or >10 mm Hg in systolic BP was observed, then a third measurement was taken and the 2 measurements in closest agreement were used to calculate the mean.

Blood samples were collected for the purposes of measuring riboflavin status. Riboflavin status was determined by using EGRac—a functional assay that measures the activity of the enzyme glutathione reductase before and after in vitro reactivation with its prosthetic group FAD (16); EGRac is calculated as the ratio of FAD-stimulated to unstimulated enzyme activity, with values ≥1.3 generally indicative of suboptimal riboflavin status. This assay required washed red blood cells, which were prepared from a fresh EDTA sample that had been centrifuged at 719 × *g* (2000 rpm) for 15 min at 4°C within 0.5–2.5 h after the initial blood sample was taken. Once the plasma and buffy layers were removed, the remaining red blood cells were washed 3 times with phosphate-buffered saline. The saline and buffy layers were removed after each centrifugation, and the resultant washed red blood cells were stored at −70°C for batch analysis of EGRac determination at the end of the study. Samples were analyzed blind, in duplicate, within 6 mo of collection, and quality controls were provided by repeated analysis of pooled samples covering a wide range of values.

Statistical analysis

All statistical analyses were performed by using the SPSS statistical package for the Social Sciences (version 15.0; SPSS UK Ltd). Tests for skewness showed a departure from normality for systolic BP. Data for this variable were therefore transformed by using logarithmic transformations. Subsequent analyses for systolic BP using transformed and untransformed data produced comparable results; therefore, untransformed scores were used in all analyses.

One-factor ANOVA with Tukey's post hoc test was used to compare preintervention characteristics among the genotype groups, and chi-square tests were used to compare categorical data. Comparisons of the number and type of antihypertensive drugs used at both time points, in the cohort as a whole and among the genotype groups, were performed by using chi-square tests. ANOVA was used to investigate differences in the achievement of goal BP (<140/90 mm Hg) (17) among the genotype groups in those patients prescribed antihypertensive therapy. Systolic and diastolic BP responses to the crossover riboflavin intervention (2004 and 2008 combined) were examined by using a within-between, repeated-measures ANOVA with Bonferroni adjustment. The between-patient factor was intervention group (placebo compared with riboflavin) and the within-patient factor was time (before compared with after). Post hoc independent *t* tests were carried out to determine significant differences between groups for interaction effects. In all analyses, *P* values <0.05 were considered significant.

RESULTS

Follow-up observational investigation

A total of 83 patients, all of whom were white, agreed to participate in this follow-up study. Relevant preintervention characteristics of this cohort in 2004 compared with 2008 are shown in **Table 1**. No significant differences were found among the genotype groups in any relevant variable at either time point, apart from age in 2008 (when participants with the *CC* genotype were found to be older than those with the *CT* genotype).

Preintervention systolic and diastolic BP in 2004 and 2008 are shown in **Figure 2**. Patients with the *TT* genotype had significantly higher systolic BP than did those without the polymorphism at both time points ($P = 0.004$ in 2004 and $P = 0.006$ in 2008) and a trend toward higher diastolic BP in 2008, which approached statistical significance ($P = 0.057$).

An increase in the number of antihypertensive medications prescribed between 2004 and 2008 was evident (**Table 2**). In 2004, most of the patients (45%) were taking only one medication, typically a β-blocker, and just 5% of patients were taking

TABLE 1
Preintervention characteristics in 2004 and 2008 of follow-up patients with cardiovascular disease by *MTHFR* 677C→T genotype[1]

	MTHFR genotype: 2004				*MTHFR* genotype: 2008			
	All[2]	*CC*	*CT*	*TT*	All	*CC*	*CT*	*TT*
n	83	30	24	29	83	30	24	29
Age (y)	53.9 ± 5.6[3]	55.3 ± 5.8	52.3 ± 4.2	53.7 ± 6.2	58.2 ± 5.6	60.1 ± 5.5[a]	56.3 ± 4.3[b]	57.6 ± 6.1[a,b]
Male (%)	84	83	88	82	84	83	88	82
BMI (kg/m^2)	29.2 ± 4.9	30.1 ± 4.1	29.3 ± 3.8	28.8 ± 4.7	29.8 ± 5.2	29.6 ± 5.9	30.1 ± 4.1	29.7 ± 5.4
Waist (cm)	96.4 ± 13.0	96.8 ± 13.0	97.8 ± 10.5	94.6 ± 15.1	104.8 ± 13.6	104.8 ± 13.6	105.5 ± 12.8	104.2 ± 14.6
Patients receiving antihypertensive therapy (%)	88	87	96	83	95	93	96	97
Treated patients achieving goal BP (%)[4]	51	58	61	32	65	71	70	54

[1] Significance for comparison between genotype groups (at either time point) was determined by 1-factor ANOVA or chi-square test as appropriate. Values within a row with different superscript letters are significantly different ($P < 0.05$) between genotype groups by Tukey's post hoc test. BP, blood pressure; MTHFR, methylenetetrahydrofolate reductase [*CC* (wild type), *CT* (heterozygous), and *TT* (homozygous mutant) genotypes for the *MTHFR* 677C→T polymorphism].

[2] At the time of the original investigation, individuals with the *TT* genotype were age- and sex-matched to those with the *CC* or *CT* genotype.

[3] Mean ± SD (all such values).

[4] The aim of hypertension treatment was achievement of the goal BP of <140/90 mm Hg (17).

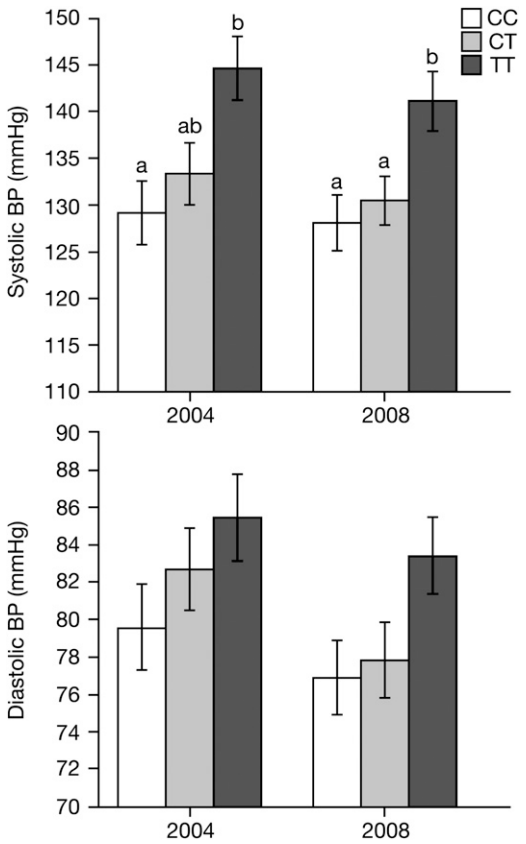

FIGURE 2. Mean (±SEM) systolic and diastolic BP in 2004 and 2008 by *MTHFR* 677C→T genotype. Differences between genotype groups at each time point were determined by 1-factor ANOVA; the different lowercase letters indicate significant differences ($P < 0.05$) between any 2 values by Tukey's post hoc test. *CC* (wild-type), *CT* (heterozygous), and *TT* (homozygous) genotypes for the *MTHFR* 677C→T polymorphism. The aim of hypertension treatment was achievement of the goal BP of <140/90 mm Hg (17). BP, blood pressure; MTHFR, methylenetetrahydrofolate reductase.

≥3 medications. By 2008, however, the latter figure had increased to 28%. Although a higher percentage of patients with the *TT* genotype were taking ≥3 medications (data not shown), only 32% had achieved goal BP in 2004 and 54% in 2008, compared with ~60% (in 2004) and 70% (in 2008) for patients not homozygous for the *T* allele (*CC* and *CT* genotypes combined) (Table 1).

Follow-up intervention trial with riboflavin

Mean compliance was estimated (by pill counting) to be ≥98%. The BP and riboflavin biomarker (EGRac) responses to the crossover intervention combining the 2004 and 2008 riboflavin trials are shown in **Table 3**. A significant time × treatment interaction for both systolic BP ($P = 0.028$) and diastolic BP ($P = 0.018$) was evident. Post hoc analysis showed that riboflavin supplementation resulted in a significant decrease in both systolic ($P = 0.001$) and diastolic ($P = 0.003$) BP. Riboflavin supplementation was associated with a significant increase in riboflavin status, as reflected by a marked decrease in EGRac ($P = 0.006$) in the riboflavin treatment group; however, the change in riboflavin status (before compared with after) was not found to be significant between the 2 groups. No significant response in these variables was noted in the placebo group.

The BP pattern over the 4-y period, including both of the 16-wk riboflavin intervention trial periods, is shown in **Figure 3**. Those patients randomly assigned to placebo in 2004 showed no change in systolic BP over the 16-wk intervention period, and their systolic BP was found to be relatively unchanged 4 y later, with preintervention values of 142.4 ± 16.0 mm Hg and 140.4 ± 16.3 mm Hg in 2004 and 2008, respectively. Only when riboflavin treatment was introduced in 2008 was there a decrease of 6.0 ± 9.4 mm Hg in systolic BP and of 3.9 ± 7.59 mm Hg in diastolic BP. Those individuals randomly assigned to riboflavin treatment in 2004 showed a decrease of 13.2 ± 15.0 mm Hg in systolic BP and of 8.5 ± 12.0 mm Hg in diastolic BP; however, at follow-up, the systolic BP of this 2004 riboflavin treatment group had increased back to preintervention values. At neither time point was there a significant difference in antihypertensive medication use between the riboflavin and placebo groups (data not shown).

DISCUSSION

Over a 4-y follow-up period, CVD patients with the *MTHFR* 677TT genotype remained hypertensive despite marked changes in the number and type of antihypertensive treatments prescribed. In this genetically at-risk group, significant lowering of BP was achieved only with riboflavin treatment. The current results not only confirm the BP-lowering effect of riboflavin previously reported by us in patients with the *MTHFR* 677TT genotype (13) but show that the modulating effect of riboflavin in this genotype group is independent of the drug or drugs concurrently prescribed.

In the current trial, conducted in a crossover manner (albeit with an extended 4-y washout period), significant decreases of 9.2 mm Hg in systolic BP and of 6.0 mm Hg in diastolic BP (combined analysis for the 2 interventions) in response to riboflavin were observed. Despite being prescribed multiple classes of antihypertensive medication by 2008, nearly 50% of patients with the *TT* genotype had failed to achieve goal BP and, as such, remained at an increased and sustained vascular risk. Although we did not conduct repeated monitoring of BP throughout the 4-y period, our results suggest that the only time at which BP reached target levels in this genotype group was when riboflavin was introduced. To put these results into context, it would take ~10 kg of weight loss or an exercise regimen that burned 4200 kcal/wk to achieve comparable decreases in BP (18). Furthermore, dietary interventions to lower BP require considerable effort by both patients and health care staff as well as sustained follow-up, and long-term compliance is often poor, even with such support. In contrast, optimization of riboflavin status could be achieved in individuals through the use of low-dose supplements or the consumption of foods naturally rich in (or fortified with) riboflavin or in populations via food fortification. Such approaches could lower BP in those genetically at risk (~10% of populations globally; 10) without causing harm to those who are not. The concept of vitamin therapy to correct decreased enzyme activity in people with genetic variation is not novel (19). Our finding of beneficial effects from intervention at recommended dietary intakes, however, is more relevant to emerging nutrition policy. Any beneficial effects of enhancing riboflavin status might be greatest in countries with a high frequency of the *MTHFR* 677TT genotype and low riboflavin

TABLE 2
Antihypertensive medication use in the follow-up cohort (all participants)[1]

	Patients	
Drug treatment	2004 (n = 83)	2008 (n = 83)
	%	
No medication	12	5
One medication		
β-Blockers	21	8
ACE inhibitors/ARBs	19	8
Other	5	4
Two medications		
ACE inhibitors/ARBs and β-blocker	24	34
ACE inhibitors/ARBs and diuretic/CCBs	6	12
Other combination	8	1
Three medications		
ACE inhibitors/ARBs and β-blocker and diuretic/CCBs	4	14
Other combination	1	6
Four medications		
ACE inhibitors and ARBs and β-blocker and diuretic/CCBs	0	4
Other combination	0	4

[1] ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; CCBs, calcium channel blockers.

intakes. Although food fortification with riboflavin has been mandatory for many years in the United States, this does not necessarily mean that riboflavin status is optimized, particularly in individuals with the *TT* genotype, who may have increased riboflavin requirements to stabilize the variant enzyme (20, 21).

A strength of the current study was its follow-up design. Patients with the *MTHFR* 677TT genotype were found to have significantly higher systolic BP, both at initial sampling in 2004 and 4 y later. We found a graded relation between the *T* allele and BP at both time points, with a significantly higher systolic BP (by 16 mm Hg) in patients with the *TT* genotype than in those with the *CC* genotype in 2004—a difference that remained significantly higher (by 13 mm Hg) in 2008. Similar, although nonsignificant, trends were noted for diastolic BP at both time points. Although previous studies investigated associations between the *MTHFR* 677C→T polymorphism and BP with inconsistent findings (22, 23), none has done so over a follow-up period. Studies reporting a significant relation with BP have predominantly been conducted in Asian countries (24, 25), where the prevalence of both the polymorphism (10) and hypertension (26) is high. The greater differences in BP by *MTHFR* genotype found in the current study than in previous studies may be explained by our study design, which involved initial prescreening of several hundred patients to identify those with the *TT* genotype who were then matched with those with the *CC* or *CT* genotype, which ensured comparable patient numbers across the 3 genotypes. Previous studies did not use such an approach and may have underestimated the size of the genotype effect as a result of too few participants with the *TT* genotype.

In 2004, β-blockers were recommended as first line therapy for managing hypertension (14), but changes in clinical guidelines in the United Kingdom in 2006 introduced a preference for other classes of antihypertensive medications often used in combination (15). Consequently, changes in both the type and number of antihypertensive medications over the 4-y period were observed, which provided a unique opportunity to investigate the effect of this polymorphism on BP in the context of these changes. Despite the use of polytherapy, individuals with the *TT* genotype remained hypertensive and had significantly higher BP at both time points compared with those without this

TABLE 3
Blood pressure response to riboflavin intervention in patients with the *MTHFR* 677TT genotype with cardiovascular disease[1]

	Combined 2004 and 2008 interventions[2]	
	Placebo (n = 29)	Riboflavin (n = 29)
Systolic blood pressure (mm Hg)		
Before	142.3 ± 17.2	143.5 ± 18.9
After	142.0 ± 19.6	134.3 ± 17.2[3]
Change[4]	0.25 ± 16.9	−9.2 ± 12.8[5–7]
Diastolic blood pressure (mm Hg)		
Before	83.3 ± 11.7	85.5 ± 12.0
After	84.4 ± 12.9	79.5 ± 9.6[3]
Change[4]	1.1 ± 12.1	−6.0 ± 9.9[6,7]
EGRac		
Before	1.39 ± 0.17	1.38 ± 0.18
After	1.35 ± 0.19	1.29 ± 0.09[3]
Change	−0.04 ± 0.15	−0.09 ± 0.17

[1] All values are means ± SDs. EGRac, erythrocyte glutathione reductase activation coefficient (biomarker of riboflavin status; a higher EGRac value indicates lower riboflavin status); MTHFR, methylenetetrahydrofolate reductase.

[2] Those assigned to riboflavin in 2004 were given placebo in 2008 and vice versa.

[3] $P < 0.05$ (post hoc paired t test).

[4] Probability values refer to interactions obtained from a 2-factor repeated-measures ANOVA with Bonferroni adjustments for multiple comparisons.

[5] Significant time effect.

[6] Significant time × treatment interaction.

[7] $P < 0.05$ (independent t tests were used to compare responses to treatment).

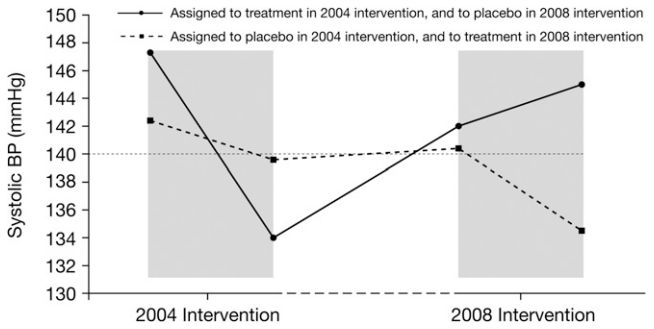

FIGURE 3. Systolic BP response to intervention (crossover) with riboflavin in patients with the *MTHFR* 677TT genotype. The shaded area denotes riboflavin intervention periods (1.6 mg/d for 16 wk); concurrent antihypertensive drugs are listed in Table 2. For full statistical analysis of the overall BP response to treatment, *see* Table 3. In 2004, $n = 13$ in the riboflavin treatment group and $n = 16$ in the placebo group; in 2008, $n = 16$ in the riboflavin treatment group and $n = 13$ in the placebo group. The dotted line indicates the suggested clinical goal for systolic BP of <140 mm Hg (17). BP, blood pressure; MTHFR, methylenetetrahydrofolate reductase.

genetic variant. Thus, although the overall percentage of patients who achieved goal BP increased over the 4 y, only half of the patients with the *TT* genotype had reached goal BP by 2008, despite the fact that a greater percentage of these patients was taking $\geq$3 medications by this time.

The mechanism through which the *MTHFR* 677C→T polymorphism affects BP is not clear. Vascular concentrations of 5-methyltetrahydrofolate (the product of the MTHFR-catalyzed reaction and the metabolically active folate form) were recently reported to be decreased in those with the *TT* genotype and were shown to be a key regulator of the potent vasodilator NO (27). If riboflavin supplementation can correct the decreased MTHFR activity evident in those with the *TT* genotype (12), it may restore 5-methyltetrahydrofolate concentrations. In addition, restoring 5-methyltetrahydrofolate concentrations may protect against the oxidative breakdown of tetrahydrobiopterin (28, 29), an essential NO cofactor that, in turn, would increase NO bioavailability and thereby lower BP. Alternatively, carotid intima media thickness, itself a modest predictor of stroke (30), has been observed to be increased in individuals with the *TT* genotype in some (31), but not all (32), studies and could be responsive to riboflavin. The decreased methylation status reported in those with the *TT* genotype (33) may also respond to riboflavin and could also play a role in BP control. The direct involvement of homocysteine may offer another mechanism to explain these effects and cannot be ruled out, but we think that this is unlikely. Although elevated homocysteine is highly responsive to riboflavin specifically in those with the *TT* genotype (12), the finding by large-scale trials (typically involving folic acid intervention) of marked lowering of homocysteine but no BP response (34) does not support a homocysteine-driven effect as the explanation.

Despite the potential importance of the findings, this study had some limitations—the primary one being the small sample size. The available sample size was finite by the very nature of its follow-up design, and, although the study as designed was adequately powered, the number of participants who completed the study was marginally below the target. The use of a crossover approach for the intervention, however, maximized the statistical power of the limited sample size. In addition, the study involved a cohort of high-risk CVD patients and, as such, future work would be required to evaluate whether riboflavin can lower BP in individuals with the *MTHFR* 677TT genotype. Furthermore, the period of intervention examined was just 16 wk, and it remains to be seen how the duration of intervention (and subsequent withdrawal of riboflavin) affects BP. How riboflavin intervention translates to vascular endpoints can only be investigated in large clinical trials.

In conclusion, we showed that the *MTHFR* 677TT genotype remained a significant determinant of BP over a 4-y follow-up period. Specifically in this genotype group, we confirmed that the elevated BP observed was responsive to riboflavin and, relative to riboflavin intervention, conventional antihypertensive therapy appeared largely ineffective over the 4-y period. Thus, riboflavin could offer a low-cost strategy for managing elevated BP in this genetically predisposed group.

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The authors' responsibilities were as follows—MW, HM, JJS, and JMS: planned and designed the study; CPW: collected the study data; CPW, MW, HM, JJS, TGT, and JMS: analyzed and interpreted the data; CPW, MW, and HM: drafted the manuscript; CPW, MW, HM, JJS, TGT, JP, GH, and JMS: critically revised the manuscript for important intellectual content; and MW and HM: supervised the study. All authors read and approved the final manuscript. There is a patent pending by all authors (except for CPW and TGT) on the use of riboflavin for the treatment of hypertension. There were no other conflicts of interest. Neither of the funding entities was involved in the design, implementation, analysis, or interpretation of the data.

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