Blood Pressure in Treated Hypertensive Individuals With the *MTHFR* 677TT Genotype Is Responsive to Intervention With Riboflavin : Findings of a Targeted Randomized Trial

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Original Article

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Abstract—Intervention with riboflavin was recently shown to produce genotype-specific lowering of blood pressure (BP) in patients with premature cardiovascular disease homozygous for the 677C→T polymorphism (TT genotype) in the gene encoding the enzyme methylenetetrahydrofolate reductase (MTHFR). Whether this effect is confined to patients with high-risk cardiovascular disease is unknown. The aim of this randomized trial, therefore, was to investigate the responsiveness of BP to riboflavin supplementation in hypertensive individuals with the TT genotype but without overt cardiovascular disease. From an available sample of 1427 patients with hypertension, we identified 157 with the *MTHFR* 677TT genotype, 91 of whom agreed to participate in the trial. Participants were stratified by systolic BP and randomized to receive placebo or riboflavin (1.6 mg/d) for 16 weeks. At baseline, despite being prescribed multiple classes of antihypertensive drugs, >60% of participants with this genotype had failed to reach goal BP (≤140/90 mm Hg). A significant improvement in the biomarker status of riboflavin was observed in response to intervention ($P<0.001$). Correspondingly, an overall treatment effect of 5.6±2.6 mm Hg ($P=0.033$) in systolic BP was observed, with pre- and postintervention values of 141.8±2.9 and 137.1±3.0 mm Hg (treatment group) and 143.5±3.0 and 144.3±3.1 mm Hg (placebo group), whereas the treatment effect in diastolic BP was not significant ($P=0.291$). In conclusion, these results show that riboflavin supplementation targeted at hypertensive individuals with the *MTHFR* 677TT genotype can decrease BP more effectively than treatment with current antihypertensive drugs only and indicate the potential for a personalized approach to the management of hypertension in this genetically at-risk group.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: ISRCTN23620802. (***Hypertension*. 2013;61:00-00.**) • **Online Data Supplement**

Key Words: blood pressure ■ hypertension ■ MTHFR ■ personalized medicine ■ riboflavin

Hypertension affects more than 1 billion individuals globally[1] and is estimated to be responsible for 7.6 million premature deaths.[2] Drug[3–5] and lifestyle[6,7] interventions to lower blood pressure (BP), even by small amounts, have been proven to decrease cardiovascular disease (CVD) risk. Multiple lifestyle and genetic factors are thought to contribute to the development and progression of hypertension. A large number of genetic variants, each contributing modestly to BP variability, seem to be implicated.[8] Recent genome-wide association studies in >150 000 individuals have identified several genetic loci associated with BP variation.[9,10] One study identified an association between BP and common variants in 8 genetic loci, including one near the gene encoding the folate-metabolizing enzyme methylenetetrahydrofolate reductase (MTHFR).[10] In general agreement with this observation, evidence from a recent meta-analysis of observational studies shows a strong association of the *MTHFR* 677C→T polymorphism with hypertension.[11]

The frequency of the homozygous mutant *MTHFR* 677TT genotype is reported to be 10% worldwide, ranging from 4% to 18% in the United States, 20% in northern China to as high as 32% in Mexico.[12] The MTHFR enzyme catalyzes the conversion of 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate which, in turn, is required for the remethylation of homocysteine to methionine. The common 677C→T variant in *MTHFR* results in a thermolabile enzyme with decreased activity, typically leading to elevated plasma homocysteine in vivo.[13] Molecular studies demonstrate that the decreased activity of the variant enzyme is attributable to the loss of its riboflavin (ie, FAD; flavin adenine dinucleotide)

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cofactor,[14,15] whereas supplementation with riboflavin seems to restore MTHFR activity resulting in a significant homocysteine-lowering response in humans.[16] This effect is found only in individuals with the *MTHFR* 677TT genotype with no response evident in the homozygous wild-type CC or heterozygous CT genotype groups and, therefore, demonstrates a genotype-specific effect of riboflavin.[16] We first linked riboflavin status with BP in relation to this polymorphism in a cohort of premature patients with CVD.[17] Patients with the TT genotype had significantly higher BP compared with those without the polymorphism and, moreover, were highly responsive to riboflavin supplementation, an effect that was not found in patients with CC or CT genotypes.[17] When we followed these high-risk patients 4 years later, those with the TT genotype remained hypertensive, despite the occurrence (during the follow-up period) of marked changes in the number and type of drugs prescribed, and target BP levels were again achieved only in response to riboflavin.[18]

The aforementioned studies[17,18] focused on a highly selected cohort of patients with premature CVD who may not be representative of hypertensive individuals generally. It is not known whether riboflavin can produce genotype-specific lowering of BP in patients with hypertension without overt CVD. The aim of this study, therefore, was to investigate the potential of riboflavin as a targeted treatment for hypertension in individuals with the *MTHFR* 677TT genotype.

Methods

Participants

Individuals with hypertension prescreened for the *MTHFR* 677TT genotype were recruited from an ongoing observational study, namely the Trinity Ulster and Department of Agriculture (TUDA) aging cohort study (Figure 1). Potential participants were excluded if they had a history of gastrointestinal, hepatic, renal, or hematological disorders, or were taking B-vitamin supplements, anticonvulsant therapy, or any other drugs known to interfere with folate/B-vitamin metabolism. The minimum sample size to detect an effect of riboflavin on systolic BP (and accounting for a possible 10% dropout rate) was estimated to be 40 subjects per treatment group based on power calculations from a previous study.[18] The type I error rate was 0.05 with desired power of 0.80. Ethical approval was granted by the Office for Research Ethics Northern Ireland (ORECNI Ref: 09/NIR01/68), and all patients provided written informed consent.

Study Design

The study was conducted as a randomized controlled trial (Figure 1). Participants were stratified by systolic BP (low, ≤134; medium, ≥135–152; high, ≥153 mm Hg) and subsequently randomized within each stratum to receive either riboflavin (1.6 mg/d) or placebo for 16 weeks. To maximize compliance, patients were given supplements on an 8-weekly basis in 7-day pill boxes and asked to return these with any untaken pills remaining. Participants were invited to attend a total of 2 appointments, at the start and end of the trial. Relevant clinical and lifestyle information, including currently prescribed BP medication, were retrieved from records (collected as part of the TUDA study). At both sampling points, drug details and supplement usage were reconfirmed with participants.

Procedures

To minimize measurement variability, BP measurements were performed pre- and postintervention by the same researcher, at the same location for each participant and at the same time of day, approximately, in accordance with appropriate clinical guidelines,[19] using an A&D UA-787 digital BP monitor (Cardiac Services, Belfast, United Kingdom) and appropriate cuff. The participants, and the researcher conducting the BP measurements, were blind to treatment group allocation.

At each sampling time point, one 30-mL blood sample was collected. Sample preparation and fractionation were performed within 0.5 to 2.5 hours of the time of sampling, and fractions were stored at −80°C until analysis. Blood samples were analyzed by standard laboratory assays for plasma total homocysteine[20] and red cell folate.[21] Riboflavin status was determined using the erythrocyte glutathione reductase activation coefficient (EGRac), a functional assay which measures the activity of the enzyme glutathione reductase in washed red cells before and after in vitro reactivation with its prosthetic group FAD[22]; EGRac is calculated as a ratio of FAD-stimulated to unstimulated enzyme activity, with values ≥1.3 generally indicative of suboptimal riboflavin status. All samples were analyzed blind, in duplicate, within 2 years 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 using the SPSS statistical package for the social sciences (version 19.0, SPSS UK Ltd, Chersey, United Kingdom). Analyses were conducted with an intention to treat approach with the last observation carried forward method used to impute missing values for any dropouts (n=3). Outliers were determined via the box plot algorithm of SPSS (see the online-only Data Supplement) and were excluded from the subsequent parametric analysis to meet the assumptions of normal distribution (4 subjects for systolic BP and 4 for diastolic BP).

Differences in baseline characteristics between the treatment groups were analyzed using independent *t* tests for linear variables and χ^2 tests for categorical parameters. Responses to intervention were examined using repeated measures ANOVA. The time × treatment interaction was used to assess the effect of treatment versus placebo over time, the main outcome of the trial. The between-patient factor was the intervention group (placebo versus riboflavin), and the within-patient factor was time (pre and post). χ^2 Tests were used to compare the proportion of patients achieving goal BP (≤140/90 mm Hg) between the treatment groups. Descriptive statistics are expressed as mean±SEM or SD throughout the article. In all analyses, *P* values <0.05 were considered significant.

Results

A total of 157 individuals were identified with the *MTHFR* 677TT genotype from an available sample of 1427 participants with hypertension enrolled in the TUDA study (11% frequency of the TT genotype). Some 91 of the 157 hypertensive individuals with the TT genotype agreed to participate in this trial (Figure 1). Examination of baseline characteristics (Table 1) showed that participants were predominantly male with a mean age of 69 years and had a mean body mass index in the obese category. Despite almost all participants (>90%) being prescribed antihypertensive therapy at the time of sampling, only one third of the participants had achieved goal BP (≤140/90 mm Hg) at baseline.

A detailed breakdown of antihypertensive drug use and drug combinations by treatment group is shown (Table 2), with 35% of patients identified as taking ≥3 medications. Achievement of goal BP was low even in those individuals taking multiple medications. Of those participants taking ≥3 antihypertensive medications, 44% achieved goal BP.

The biomarker and BP responses to riboflavin intervention are shown in Table 3. A significant time × treatment interaction was observed for the biomarker EGRac (repeated measures ANOVA; $P<0.001$), indicating a significant improvement in riboflavin status (ie, a decrease in EGRac) in response to

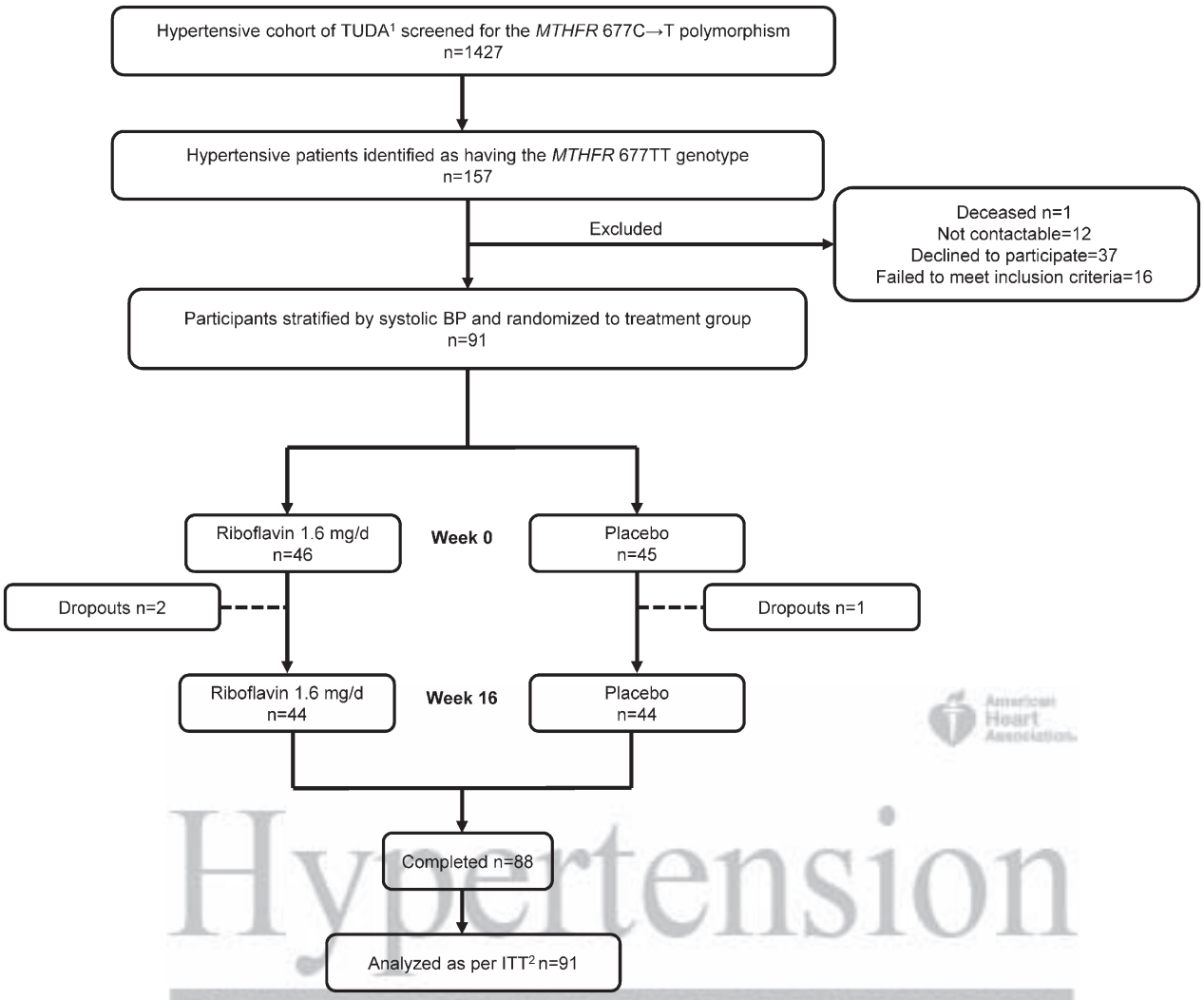

Figure 1. Flow diagram of study design and completion rates. Trinity Ulster Department of Agriculture (TUDA) study[1]. Analyzed as per intention to treat (ITT)[2]. BP indicates blood pressure.

treatment and providing confirmation of the generally excellent compliance of participants with the intervention protocol (estimated by pill-counting to be 99%). Correspondingly, a significant time × treatment interaction was observed for systolic BP (P=0.033), with pre- and postintervention values of 141.8±2.9 and 137.1±3.0 mm Hg (treatment group) and 143.5±3.0 and 144.3±3.1 mm Hg (placebo group). The decrease in diastolic BP was not significant (P=0.291). After intervention, a greater proportion (P=0.052) of participants in the treatment group (57%) compared with the placebo group (30%) had achieved goal BP. Responses to intervention were compared between the treatment and placebo groups and overall treatment effects of −0.131±0.029 (P<0.001) for EGRac, and correspondingly of 5.6±2.6 mm Hg (P=0.033) for systolic BP, were observed (Figure 2).

Discussion

This study demonstrates for the first time that BP in treated individuals with hypertension with the *MTHFR* 677TT genotype, but without overt CVD, is responsive to riboflavin. In these patients taking routine antihypertensive drugs, hypertension control rates increased (from 32% preintervention to almost 60% postintervention) in response to riboflavin intervention. The findings, therefore, demonstrate that the achievement of goal BP (≤140/90 mm Hg) in this genetically at-risk group may be greatly enhanced by the coadministration of riboflavin with routine antihypertensive drug therapy.

The *MTHFR* 677C→T polymorphism has previously been investigated in relation to BP. Although some observational studies have failed to show any relationship, the totality of evidence seems to suggest that there is a significant association between this polymorphism and BP,[11,23,24] with more recent evidence from intervention trials indicating that this association is riboflavin-dependent.[17,18] The findings reported here contribute to the emerging evidence by showing that the modulating effect of riboflavin on BP in those with *MTHFR* 677TT genotype is not confined to high-risk patients with CVD but may apply to individuals with hypertension generally with this genetic variant. Despite the fact that 92% of participants were taking antihypertensive therapy, with the majority being prescribed ≥2 medications, mean systolic BP at baseline was found to be 143 mm Hg, with only 32% of participants achieving the target of ≤140 mm Hg. This observation compares with hypertension control rates of 50% among

Table 1. Baseline Characteristics of Participants

Variable	All (n=91)	Placebo (n=45)	Riboflavin (n=46)	*P* Value
Age, y	69.1 (6.6)	68.5 (6.3)	69.8 (7.0)	0.34
Men, %	67	69	65	0.88
BMI, kg/m^2	30.0 (4.7)	30.1 (4.2)	29.9 (5.2)	0.81
Smoker, %	6	4	7	1.00
Alcohol consumer, %	64	67	61	0.72
Diabetes mellitus, %	15	13	17	0.81
Blood pressure				
Systolic BP, mm Hg	142.6 (19.5)	143.5 (19.7)	141.8 (19.4)	0.69
Diastolic BP, mm Hg	83.9 (10.9)	86.1 (10.7)	81.9 (10.8)	0.07
Antihypertensive medication use, %	92	98	87	0.12
Participants achieving goal BP, %*	32	23	40	0.16
B-vitamin status				
EGRac	1.34 (0.20)	1.31 (0.11)	1.37 (0.26)	0.15
Plasma homocysteine, μmol/L	17.1 (6.3)	17.1 (6.5)	17.0 (6.2)	0.95
Red cell folate, nmol/L	726 (347)	714 (336)	737 (361)	0.75

Data expressed as mean (±SD) unless otherwise indicated. Statistical significance for comparison between treatment groups by independent *t* tests or χ^2 as appropriate. BMI indicates body mass index; BP, blood pressure; and EGRac, erythrocyte glutathione reductase activation coefficient (biomarker of riboflavin status; a higher value indicates lower status).

*The treatment of hypertension is aimed at achieving the goal BP of ≤140/90 mm Hg.[19]

treated adults in general in the United States.[25] Riboflavin supplementation lowered mean systolic BP by >5 mm Hg in these hypertensive individuals and changed the mean BP from being within the hypertensive category at baseline to achievement of goal BP postintervention. This suggests that the excess risk of hypertension linked with this genetic factor can be overcome by riboflavin, either by improving the clinical responsiveness to routine antihypertensive therapy or by an unrelated mechanism.

There is a continuous relationship between BP and CVD mortality, with 1 meta-analysis of 61 prospective studies (including 1 million adults), estimating that a 2-mm Hg decrease in systolic BP is associated with a 10%-reduction in stroke risk.[26] Thus, the 5-mm Hg decrease in systolic BP that we observed in response to riboflavin is clinically relevant and could translate into a reduction in stroke risk by >20%, specifically in individuals with the *MTHFR* 677TT genotype. The extent of response to riboflavin observed here is less marked than the 9.2-mm Hg decrease in systolic BP that we previously reported in patients with premature CVD with this genotype.[18] This difference in response is not unexpected, given that we previously investigated highly selected patients with CVD (identified by a previous myocardial infarction or angina), who might not have been representative of patients with hypertension generally and were on average 15 years younger than the current participants. With increasing age, the genetic contribution to hypertension (and its responsiveness to targeted treatment) may be attenuated to some extent by other age-related factors.

The annual cost of antihypertensive medication represents a large and increasing proportion of healthcare expenditure

Table 2. Antihypertensive Medication Use Among Participants

Drug Treatment*	All Participants, n (%)	% Achieving Goal BP	Treatment, n (%)	Placebo, n (%)
Drug class				
Diuretic	43 (51)		20 (49)	23 (52)
CCB	40 (47)		24 (59)	16 (39)
ACE inhibitors	35 (41)		15 (37)	20 (45)
β-Blockers	32 (38)		16 (39)	16 (36)
ARB	26 (31)		13 (32)	13 (30)
α-Blocker	11 (13)		2 (5)	9 (20)
Drug combination				
No medication	6 (7)	4	5 (11)	1 (2)
1 medication	29 (32)	26	12 (26)	17 (38)
2 medications	24 (26)	26	14 (30)	10 (22)
≥3 medications	32 (35)	44	15 (33)	17 (38)

ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blockers; BP, blood pressure; and CCB, calcium-channel blockers.

*The treatment of hypertension is aimed at achieving the goal blood pressure of ≤140/90 mm Hg.[19]

Table 3. Response to Riboflavin Intervention in Patients With the *MTHFR* 677TT Genotype

Response Indicator	Placebo (n=45)	Riboflavin (n=46)	*P* Value*
EGRac†			
Before	1.31 (0.03)	1.37 (0.03)	
After	1.32 (0.02)	1.25 (0.02)	<0.001
Systolic BP, mm Hg‡			
Before	143.5 (3.0)	141.8 (2.9)	
After	144.3 (3.1)	137.1 (3.0)	0.033
Diastolic BP, mm Hg‡			
Before	86.1 (1.7)	81.9 (1.6)	
After	86.7 (1.7)	80.9 (1.6)	0.291
Attainment of goal BP, %			
Before	23	41	0.131§
After	30	57	0.052§

Data expressed as mean (±SEM). BP indicates blood pressure; and EGRac, erythrocyte glutathione reductase activation coefficient.

*Time × treatment interaction (repeated measures ANOVA, comparing the effect of treatment vs placebo over time).

†EGRac, biomarker of riboflavin status; a higher value indicates lower status.

‡Individual BP values are the average of 2 readings from the reference arm measured according to clinical guidelines[19]; if a difference of >5 mm Hg in diastolic BP or >10 mm Hg in systolic BP was observed, a third measurement was taken and the 2 measurements in closest agreement were used.

§χ^2 test was used to compare differences in the achievement of goal BP (≤140/90 mm Hg) between the treatment groups.

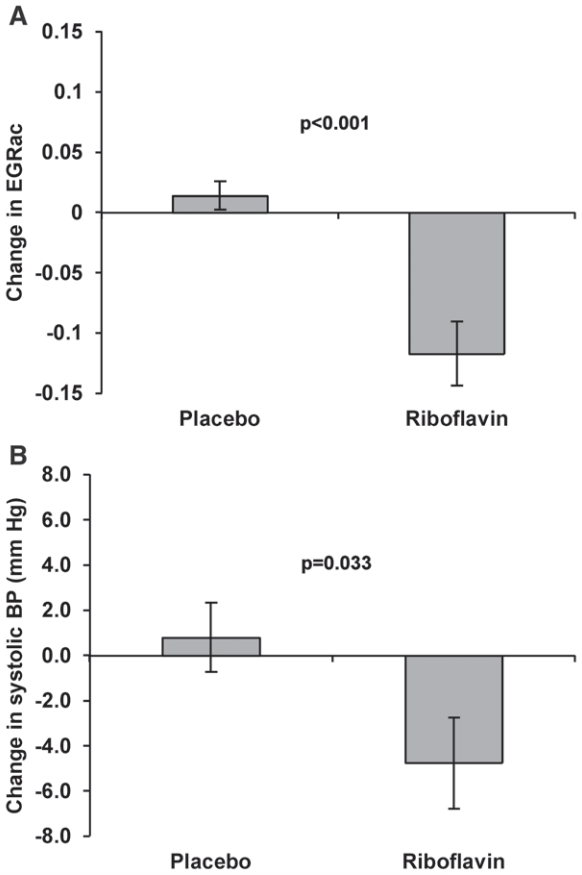

Figure 2. Comparison of responses of erythrocyte glutathione reductase activation coefficient (EGRac; **A**) and systolic blood pressure (**B**) to intervention with riboflavin (1.6 mg/d) or placebo in patients with the *MTHFR* 677TT genotype over 16 weeks. Riboflavin status was measured as EGRac, a functional biomarker assay for riboflavin, with higher values indicating lower status. Values are mean changes (±SEM) between preintervention and postintervention. The *P* values refer to the treatment × time interaction of the repeated measures ANOVA, that is, the efficacy of the treatment vs placebo over time. BP indicates blood pressure.

in many countries and was estimated several years ago at $15 billion in the United States alone.[27] The majority of individuals with hypertension often require ≥2 antihypertensive agents from different drug classes,[28–30] but BP control remains low among treated patients globally.[31] The health consequences of undertreating hypertension are considerable and estimated to account for 34% of strokes in older adults.[32] The present results support the case for personalized medicine in hypertension, first proposed many years ago,[33] but now possible only with the availability of molecular tools to identify small population subgroups with similar genetic characteristics who are more likely to respond favorably to targeted therapeutic interventions.[34] Individuals homozygous for this polymorphism (up to 30% in populations worldwide) are likely to respond suboptimally to present antihypertensive drugs but may benefit from targeted treatment with riboflavin.

Elucidation of a mechanism to explain the role of this gene-nutrient interaction in BP is not yet clear but must in some way involve MTHFR through loss of function in the variant form and the ability of supplemental riboflavin to restore normal enzyme activity in vivo,[16] consistent with the known molecular characteristics from in vitro studies.[14,15] A plausible mechanism linking MTHFR with BP, in turn, might involve the potent vasodilator nitric oxide (NO). Concentrations of 5-methyltetrahydrofolate (the product of the MTHFR reaction) in vascular tissue seem to be associated with NO regulation and endothelial function and are lower in patients with the *MTHFR* 677TT genotype.[35,36] By stabilizing the variant MTHFR enzyme, it is possible that riboflavin supplementation could restore 5-methyltetrahydrofolate concentrations in vascular cells, improve NO bioavailability, and in turn lower BP, specifically in patients with the TT genotype. Further investigation of mechanisms is clearly required, but at this time, we think it unlikely that the elevated homocysteine phenotype typically associated with this polymorphism (and shown previously to be responsive to riboflavin)[16,17] is implicated. Despite the significant associations between plasma homocysteine and BP reported in several observational studies, intervention studies to lower homocysteine have shown little or no corresponding BP response[37] and suggest that there is no causative link between homocysteine concentrations per se and hypertension.

An unanswered question is the extent to which early optimization of riboflavin status (ie, before commencing routine antihypertensive treatment) can prevent the progression toward hypertension in this genetically at-risk group, and whether individuals with the TT genotype who have optimal riboflavin status are less likely to develop hypertension compared with individuals with the same genotype who have poor riboflavin status. Furthermore, a riboflavin dose of 1.6 mg/d for 16 weeks was used in this and in our previous trials, therefore further work is required to determine whether a greater BP-lowering response can be achieved with a higher dose or

longer treatment duration, and whether riboflavin in combination with folic acid or 5-methyltetrahydrofolate could be more effective than riboflavin alone.

In conclusion, despite being prescribed multiple classes of antihypertensive medication, the majority of individuals with hypertension with the *MTHFR* 677TT genotype failed to have well-controlled BP and, as such, remained at an increased and sustained cardiovascular risk. Supplementation with riboflavin in this genotype group was effective in lowering in BP and consequently improved BP control rates. Riboflavin may be a safe and effective means of obtaining goal BP in patients with hypertension with the *MTHFR* 677TT genotype, with potentially important implications for the primary prevention of stroke; however, large clinical trials are required to investigate the translation of these novel findings to disease end points.

Perspectives

Riboflavin offers a novel, targeted approach to lower BP in patients with hypertension homozygous for a common genetic variant in folate metabolism, namely the 677C→T polymorphism in *MTHFR*. Combination antihypertensive therapy as currently prescribed seems to be associated with poor control rates in patients with this genotype, whereas the addition of supplemental riboflavin can greatly enhance the achievement of goal BP. The link between *MTHFR* genotype and hypertension may help to explain how this common genetic trait results in an excess risk of CVD, especially stroke, whereas the genotype-specific responsiveness of BP to riboflavin offers an explanation for the known variability in the excess CVD risk according to geographical location, consistent with differences in riboflavin status between populations. Dietary intakes (and biomarker status) of riboflavin vary greatly from countries, such as the United States, with generally higher intakes attributable to mandatory food fortification with riboflavin to Asian countries where intakes are generally low. The precise mechanism linking this polymorphism to hypertension remains to be established. The present results, however, suggest that the biological perturbation that leads to higher BP is modifiable by correcting the variant MTHFR enzyme through enhancing riboflavin status. Neither the genetic predisposition to hypertension nor its responsiveness to riboflavin are well-recognized in relation to the *MTHFR* 677C→T polymorphism but could be important in an era of personalized medicine whereby treatment can be tailored to patient subgroups based on genetic characteristics.

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Disclosures

There is a patent granted in Europe and pending elsewhere by all authors (except for Drs Wilson, Trouton, McAnena, Hoeft, Weber, and Roos) on the use of riboflavin in the treatment of hypertension. The other authors have no conflicts to report.

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Novelty and Significance

What Is New?

- Blood pressure in treated hypertensive individuals with the *MTHFR* 677TT genotype responds significantly to riboflavin.
- The results offer a personalized nondrug approach to blood pressure treatment targeted at a patient subgroup sharing a common genetic characteristic.

What Is Relevant?

- Failure to optimize riboflavin, specifically in this genotype group, is likely to limit the achievement of goal blood pressure with antihypertensive treatment, placing these patients at increased cardiovascular risk.

Summary

Supplementation with riboflavin lowers blood pressure in treated hypertensive individuals with the *MTHFR* 677TT genotype.

ONLINE SUPPLEMENT

BLOOD PRESSURE IN TREATED HYPERTENSIVE INDIVIDUALS WITH THE *MTHFR* 677TT GENOTYPE IS RESPONSIVE TO INTERVENTION WITH RIBOFLAVIN: FINDINGS OF A TARGETED RANDOMIZED TRIAL

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Boxplots, hinges, and quartiles

Technote (troubleshooting)

Problem(Abstract)

I am using SPSS and my boxplot appears incorrect; either the edges of the box do not coincide with the 25th and 75th percentiles, or the whiskers are not drawn to the limits I would expect based on the interquartile range. What is happening?

Resolving the problem

The boxplot was developed by John Tukey and presented in his book Exploratory Data Analysis. SPSS follows his definition of the plot, where the upper and lower limits of the box are the Tukey hinges H1 and H2. These values approximate, but in general do not match, the 25th and 75th percentiles reported by SPSS. The fences F1 and F2 are defined as:

F1 = H1 - 1.5*(H2 - H1) and F2 = H2 + 1.5*(H2 - H1).

The two fences provide upper limits for the whisker length; each whisker is drawn to that data value which is furthest from the median but still within the corresponding fence's distance from it.

Tukey's book emphasized techniques that can be done by hand with a minimum of calculation.. A simple example shows how the hinges are calculated. If the sorted variable X has 26 cases:

14 17 20 21 22
24 25 26 26 26
27 30 31 31 32
35 42 43 46 48
52 54 54 63 67
83

the median is the average of the 13th and 14th cases, or 31. Tukey would say that the median is at depth 13.5. He defines the hinges as those values that lie at

a depth midway between the median and the two extremes, or at depth (TRUNC(13.5) + 1)/2 = 7 counting in from each extreme, that is, at depths 7 and 20. Notice the use of the truncate function to avoid having to deal with fractions other than .5. For these data, the two hinges are 25 and 48, and the fences are at 25 - 1.5*(48 - 25) = -9.5 and 48 + 1.5*(48 - 25) = 82.5. The boxplot as a result identifies the value 83 as an outlier and draws the upper whisker to the next highest value, 67. The EXAMINE command with the /PERCENTILES HAVERAGE option reports the 25th and 75th percentiles as 24.75 and 49.

As an aside, Tukey appears to have invented the stem-and-leaf plot as a simple method of enumerating in a sorted fashion a batch of numbers in order to facilitate finding which data value is at a given depth.

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