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The effects of vitamin supplementation and MTHFR (C677T) genotype on homocysteine-lowering and migraine disability

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Abstract

Background: Migraine is a prevalent and debilitating disease that may, in part, arise due to disruption in neurovascular endothelia caused by elevated homocysteine. This study examined the homocysteine-lowering effects of vitamin supplementation on migraine disability, frequency and severity and whether MTHFR_{C677T} genotype influenced treatment response.

Methods: This was a randomized, double-blind placebo, controlled trial of 6 months of daily vitamin supplementation (ie. 2mg of folic acid, 25mg vitamin B₆, and 400µg of Vitamin B₁₂) in 52 patients diagnosed with migraine with aura.

Findings: Vitamin supplementation reduced homocysteine by 39% (~4 µmol/L) compared to baseline, a reduction that was greater than placebo (P = 0.001). Vitamin supplementation also reduced the prevalence of migraine disability from 60% at baseline to 30% after 6 months (P=0.01), whereas no reduction was observed for the placebo group (P>0.1). Headache frequency and pain severity were also reduced (P<0.05), whereas there was no reduction in the placebo group (P>0.1). In this patient group the treatment effect on both homocysteine levels and migraine disability was associated with MTHFR_{C677T} genotype whereby carriers of the C allele experienced a greater response compared to TT genotypes (P<0.05).

Interpretation: This study provides some early evidence that lowering homocysteine via vitamin supplementation reduces migraine disability in a subgroup of patients. Larger trials are now warranted to establish whether vitamin therapy is a safe, inexpensive and effective prophylactic option for treatment of migraine and whether efficacy is dependent on MTHFR_{C677T} genotype.

Introduction

Migraine is a debilitating neurovascular disease that affects approximately 12% of the Caucasian population. It is characterised by nausea and vomiting, photophobia and phonophobia, and severe recurrent headache. Migraine is currently diagnosed based on criteria specified by the International Headache Society (IHS) [1]. The IHS has defined two major classes of the disease - migraine without aura (MO) and migraine with aura (MA). The two subtypes have substantial symptomatic overlap, but approximately 1 in 4 sufferers experience distinguishing neurological disturbances (the aura) that usually precedes the headache phase of an attack [1]. Susceptibility to migraine is multifactorial with variation in the clinical endpoints influenced by both genetic factors and environmental triggers.

The pathophysiology of migraine is not completely understood. For MA, a dramatic reduction in cerebral blood flow is associated with a depolarisation wave that propagates across the brain cortex (cortical spreading depression; CSD) [2]. The characteristic head pain that is common to both MA and MO may arise due to dilation of cerebral blood vessels following activation of the trigeminovascular system (TVS). The CSD can activate the TVS, providing a possible link between migraine aura and headache [3]. Therefore, biochemical factors that have the potential to disrupt vascular endothelial function and cerebral blood flow, leading to CSD and/or affecting the TVS, are candidates for involvement in migraine pathology [4].

Homocysteine is a highly reactive amino acid and has been shown to produce endothelial cell injury in both experimental animal and cell culture studies [5,6]. The pathophysiological consequences of homocysteine-related endothelial injury may include impaired release of nitric oxide (NO) [7]. In turn, reduced bioavailability of NO may cause abnormal reactions between the vessel wall, platelets, and macrophages [8] leading to significant alterations in neurovascular function and the coagulant properties of the blood [9,10]. Thus, it is plausible that homocysteine-

related endothelial dysfunction may be involved in the initiation and maintenance of a migraine episode. Moreover, studies have demonstrated that the firing rate of trigeminal neurons responding to pain increases with the application of D,L-homocysteic acid, a substance that mimics the effect of homocysteine, [11,12].

There is a large body of evidence linking mild hyperhomocysteinaemia to increase risk of atherosclerotic vascular disease [13]. Migraine sufferers, particularly those with aura, have an increased risk for vascular brain lesions and ischaemic stroke [14-16]. Whether homocysteine levels are raised in migraineurs is uncertain [17] but the CSD, which is characteristic of MA, as well as changes in cerebral blood flow and headache, can also occur during a stroke episode [18]. Based on the comorbidity of migraine and stroke and the putative role homocysteine plays in disturbing in the cerebrovascular system, it is plausible that elevated homocysteine may be a partial determinant for the neuro and/or vascular pathophysiologies underlying both MA and stroke.

Plasma homocysteine levels can be lowered with a simple, nontoxic and inexpensive therapeutic intervention in the form of vitamin supplementation with folic acid (vitamin B₉), vitamin B₁₂ and B₆. A meta-analysis of clinical trials has shown that folic acid supplementation can effectively reduce risk of stroke [19]. Folic acid and vitamins B₁₂ and B₆ act to reduce homocysteine via methylenetetrahydrofolate reductase (MTHFR).

A polymorphism in the Methylenetetrahydrofolate reductase gene (MTHFR_{C677T}) is associated with enzyme function whereby carriers of the TT genotype exhibit ~50% reduction in enzyme activity [20]. The MTHFR_{C677T} variant has been linked to MA in multiple independent studies and a meta-analysis of 2961 migraine patients has provided convincing evidence that the TT genotype, specifically, increases disease risk [21].

Here we report the results of a clinical trial, which was designed to test the hypothesis that supplementation with folic acid, B₆ and B₁₂, reduces plasma homocysteine in migraine patients, which in turn serves to reduce migraine disability. Furthermore, we test whether the homocysteine-lowering effect is dependent on MTHFR_{C677T} genotype.

Methods

Study Design: This was a randomised, double blind, placebo controlled clinical trial of daily vitamin supplementation on migraine disability conducted over a 6-month period. The trial was designed using the guidelines for controlled trials of drugs in migraine [22].

Patient Groups: The study involved Caucasian adult subjects who had been previously enrolled in migraine research at the Genomics Research Centre (GRC) at Griffith University, Australia. Patients were included if they; were long time migraine sufferers (>20 years), had a current diagnosis of migraine with aura (>90% of their migraine attacks were associated with aura), and a 1-year history of severe, long lasting attacks (at least 4 attacks lasting more than 48 hrs), had a family history of migraine. Confirmation of migraine diagnosis was carried out by a qualified clinical neurologist (John Macmillan) using the International Headache Society (IHS) criteria [1]. Patients were excluded if they were currently taking vitamin supplementation, were pregnant, or had been diagnosed with a clinically recognized cardiovascular or neuropsychiatric condition.

Randomization and Blinding: Sixty patients meeting the inclusion criteria were randomized assigned into two treatment groups - placebo and vitamin. A blocked random allocation sequence was generated using Microsoft Excel. Group sizes were split 1:2 respectively, with the vitamin group (n=40) being deliberately larger than the placebo (n=20) to allow post treatment analysis stratified by C677T genotype subgroup. Specialised staff who were not involved in the study

allocated and labeled treatment containers with the participants unique sequence number. Patients and primary investigators were blinded to the randomization and group allocation.

Treatment: Patients received either VITAPOPs tablets (containing 2mg of folic acid, 25mg vitamin B₆, and 400µg of Vitamin B₁₂), or the placebo tablet. The vitamin and placebo tablets, produced by Blackmores®, were indistinguishable. Patients were instructed to take one tablet daily for 6 months.

Baseline Assessment: Prior to treatment all patients were assessed for migraine disability using the Migraine Disability Assessment Score (MIDAS) instrument, which provides a measure of productive days lost to migraine headache in the previous 3 months (ie. migraine disability), as well as headache frequency and pain severity [23,24]. Patients were asked to complete a daily diary during the treatment period to record details of migraine symptoms eg. duration, frequency and severity, as well as treatment compliance. Patients were instructed to take their usual migraine treatment for acute attacks. A blood sample was collected for baseline measurement of plasma homocysteine (µmol/L), folate (nmol/L), vitamins B₁₂ and B₆ (pmol/L) concentration. A small sample of blood was taken for genomic DNA extraction from white cells.

Follow-up Assessment: Patients were contacted after 3 months for headache diary and compliance checking. After the 6 month treatment period participants were re-assessed at the GRC clinic which included formal questioning regarding 6-month migraine history and re-collection of blood samples for measurement of homocysteine, folate, B₁₂ and B₆ concentration.

Clinical Outcome Measures: The primary clinical outcome was migraine disability which was assessed by the MIDAS instrument. Studies have shown that this is a valid and clinically useful instrument for assessing health related quality of life in migraineurs [23,24]. Based on the 5-question MIDAS rating patients were arbitrarily categorised into "high" and "low" disability groups

by the following criteria; Low = MIDAS rating of 0-10 and High = MIDAS rating >11, whereby patients falling into the "high" category were considered to suffer *severe* migraine disability. Secondary outcome variables, which are partly captured within the primary outcome, were migraine frequency and head pain severity. These were measured as number of days with headache (over a 3 month period) and a pain score (based on a scale of 1-10), respectively [23,24].

Predictor variables: Treatment (vitamin supplementation vs placebo) was the primary predictor variable for this study. Secondary predictor variables included plasma homocysteine level and the C677T polymorphism of the MTHFR gene, grouped by TT and CT/CC genotypes. Plasma homocysteine was measured in an Australian accredited pathology laboratory and genotyping of C677T was undertaken in GRC laboratories using previously published methods [25].

Statistical Analysis: At baseline, group means were compared using unpaired samples T tests, medians were compared using Mann-whitney U tests and proportions compared using the chi-squared test of independence. To test our primary hypothesis that vitamin supplementation reduces migraine disability we compared proportions of "high" disability migraineurs before and after 6 months in both the vitamin-treated and placebo groups. Proportion changes were compared using the chi-squared test of independence. Mean changes were compared (ie. before and after treatment) using paired samples T-tests and median changes compared using non-parametric Wilcoxin sign ranked tests for related samples. Pearson's correlation tests were used to assess relationships among biochemical variables at baseline. Where means for treatment and placebo groups were compared at 6 months post treatment unpaired samples T testing was conducted. An α level of 0.05 was set as the significance threshold and unadjusted P-values are reported. Power estimates indicated suggested that n=20 patients in each treatment group (n=40 in total) was required to detect the primary outcome variable as statistically significant at $\alpha = 0.05$.

Results

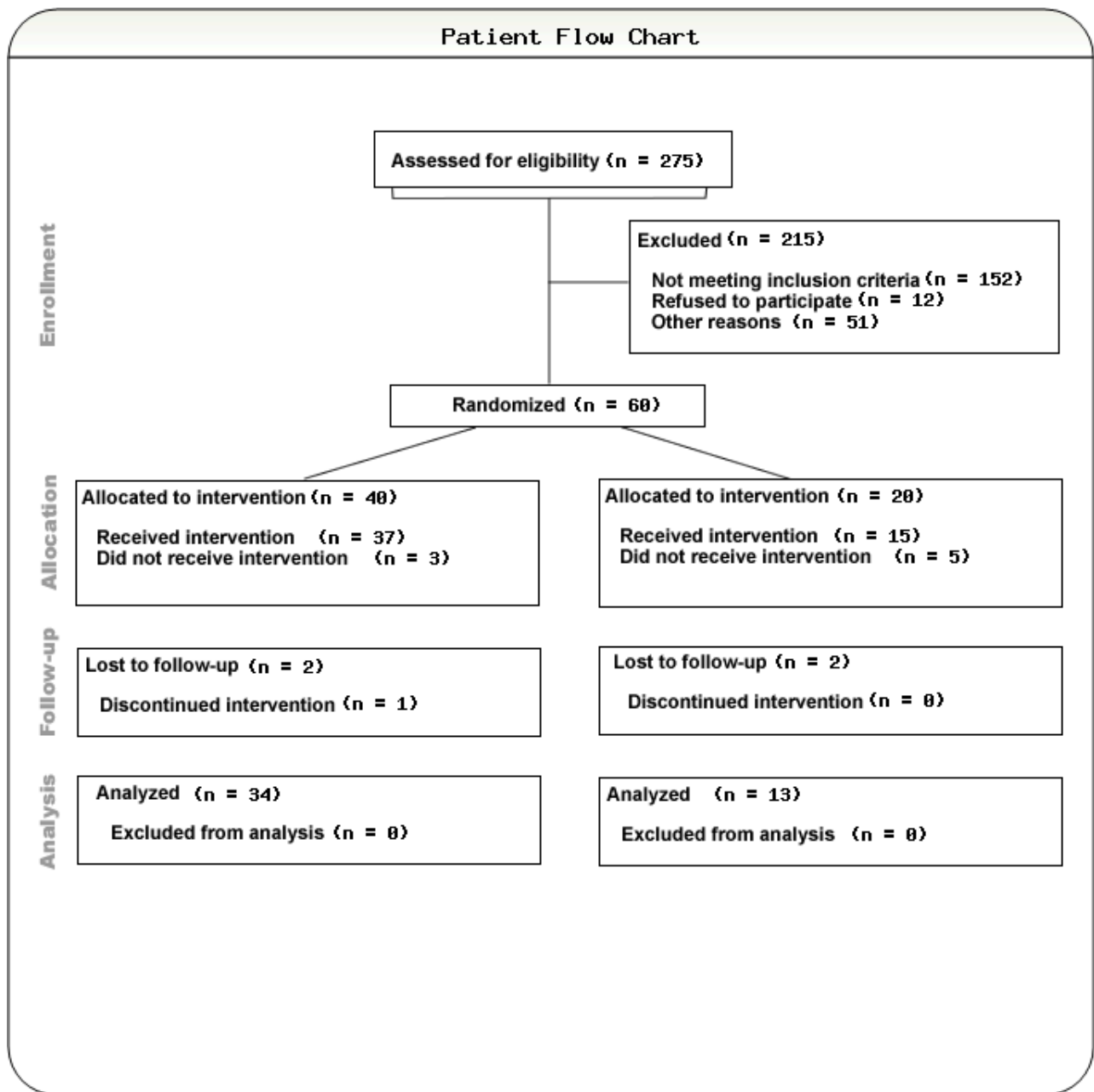


Figure 1. Patient flow chart for the trial

Figure 1 illustrates the patient flow through the trial from Jan 2006 to Jan 2007. Sixty migraine patients were initially enrolled in the trial but eight patients dropped out prior to commencement because they moved interstate or otherwise could not commit to the trial (Figure 1). The remaining 52 patients (39 females and 13 males) received full baseline assessment and commenced treatment.

Thirty seven of the patients received vitamin supplementation, whilst the remainder (n=15) received the placebo. Five patients were lost to follow-up due to lack of compliance. Forty seven patients completed the trial (13 placebo:34 treatment).

1. Baseline analysis

Table 1 shows the baseline clinical characteristics of migraine patient groups. For the total migraine group (n=52), mean folate concentration was 11.8 $\mu\text{mol/L}$, which is below the average for a general Caucasian population replete for folate (13.7 $\mu\text{mol/L}$) [26]. The mean plasma homocysteine concentration for the migraine group was 10.8 $\mu\text{mol/L}$ which is above average for a general Caucasian population (8.9 $\mu\text{mol/L}$) [26]. Five participants (2 males and 3 females) had hyperhomocysteinemia at baseline (ie homocysteine concentration > 15 $\mu\text{mol/L}$). For the total group, plasma homocysteine concentration was negatively correlated with plasma folate (Pearson's $r = -0.43$, $P = 0.002$), vitamin B₁₂ (Pearson's $r = -0.37$, $P = 0.008$) and vitamin B₆ (Pearson's $r = -0.28$, $P = 0.044$). There were no statistically significant differences between the vitamin and placebo groups for the test variables at baseline.

Baseline concentration of B₆ and B₁₂ vitamins between C677T genotype groups (ie. TT vs CT/CC) were not different ($P > 0.05$). The mean homocysteine concentration was marginally higher in the TT genotype group (11.7 $\mu\text{mol/L}$) compared to CT/CC (10.4 $\mu\text{mol/L}$), although this difference was not statistically significant ($P = 0.36$).

Table 1. Clinical characteristics of patient groups at baseline

Variable	Total	Vitamin	Placebo	P-value*
N	52	37	15	
Age in years - mean (SD)	52 (13)	53 (13)	48 (13)	0.20
Female (%)	75			
Folate - mean (SD)	11.8 (5.9)	11.2 (5.2)	13.2 (7.5)	0.29
B12 - mean (SD)	472.4 (142)	479.2 (138)	456.2 (154)	0.60
B6 - mean (SD)	42.3 (51)	41.2 (52)	44.9 (48)	0.81
Homocysteine - mean (SD)	10.8 (4.1)	10.7 (4.2)	10.9 (3.9)	0.84
Age of Disease Onset - median (range)	15 (3-51)	15.5 (3-51)	15 (5-44)	0.83
High Migraine Disability (%) [#]	59	61	53	0.61
Attack Frequency - median (range) [#]	4.5 (1-90)	6 (1-45)	4 (1-90)	0.20
Head pain score - median (range) [#]	6 (1-10)	6 (1-10)	7 (3-10)	0.47
MTHFR _{C677T} (TT) genotype (%)	40	38	46	0.62

*All P-values are two-tailed, [#] based on 3-month history

2. Six-month follow-up analysis

Forty seven patients completed the trial and were re-assessed after 6 months. Treatment was well tolerated with no reports of adverse events. Treatment compliance was high with patients reporting an average of only 3 days of treatment missed over the 6 month period (range 0-12 days). Figure 2 shows the post-treatment change in plasma folate, B₁₂, B₆ and homocysteine concentration in vitamin and placebo groups. At follow-up the vitamin treated group (n=34) had marked increases in folate, B₁₂ and B₆ concentration compared to baseline and the placebo group (P < 0.001). In the vitamin-treated group mean homocysteine reduced by 39% after 6 months (10.7 μ mol/L to 6.5 μ mol/L, P=0.001) compared to a 20% reduction observed for the placebo group (P >0.05).

Figure 3 shows that for the vitamin-treated group the frequency of severe migraine disability decreased from 61% to 30% following 6 months of supplementation ($P=0.01$). The reduction in the placebo group was not statistically significant ($P=0.3$). Figure 4 shows the vitamin-treated group also reported a decrease in headache frequency, from a median of 4 to 1 ($P=0.04$), and a decrease in pain severity, from a median score 6 to 4.5 ($P=0.002$), whereas the placebo group reported no change ($P>0.1$).

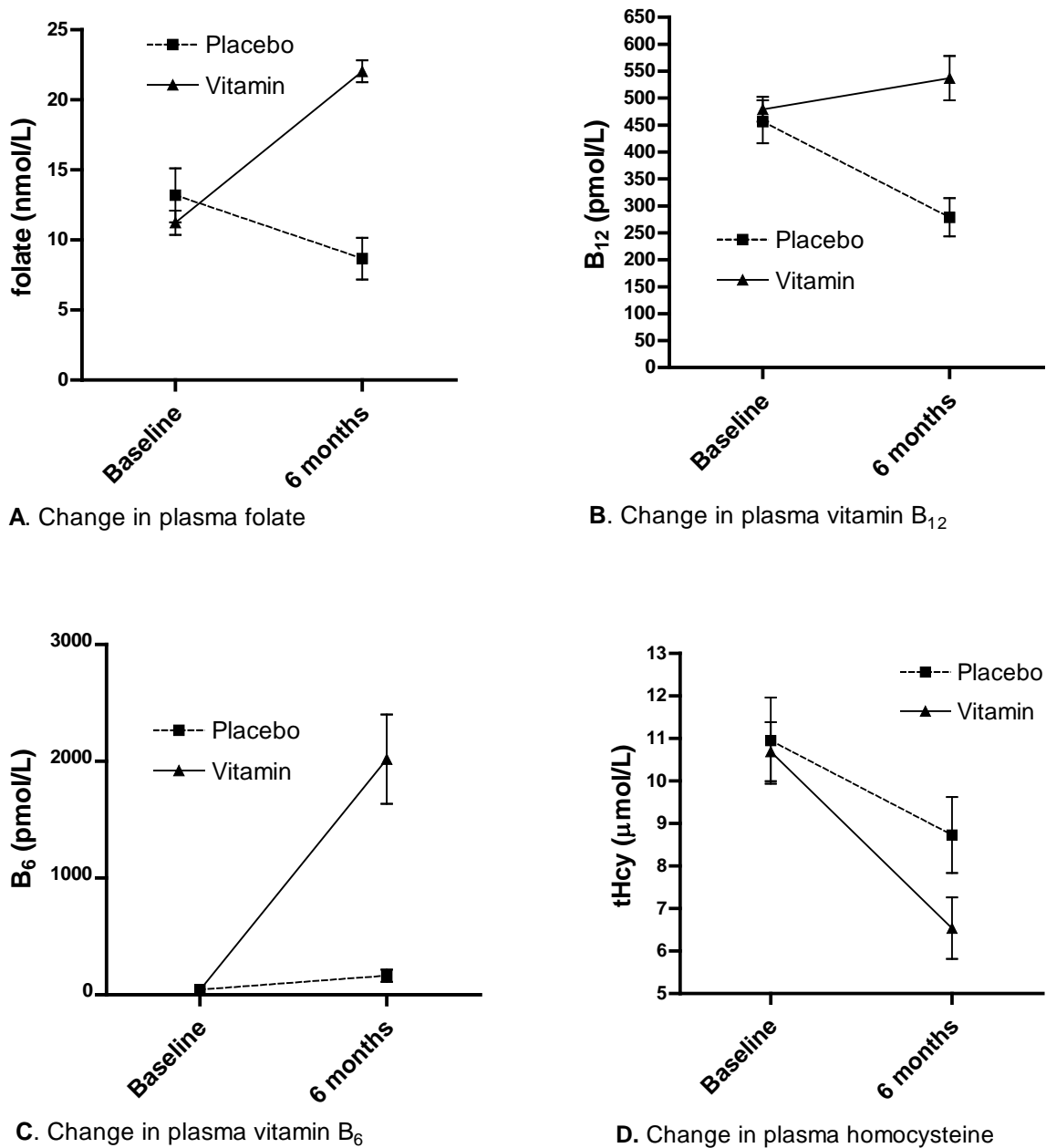


Figure 2. Change in plasma folate (A) vitamin B₁₂ (B), vitamin B₆ (C) and homocysteine (D) concentration over the treatment period in the vitamin and the placebo groups. Values are mean \pm SEM.

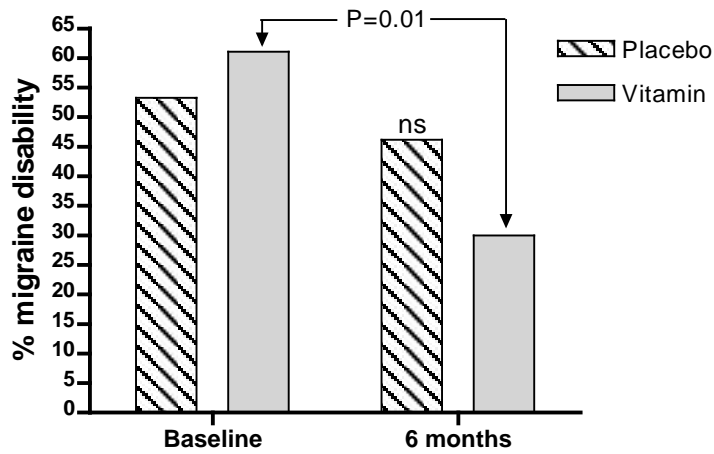
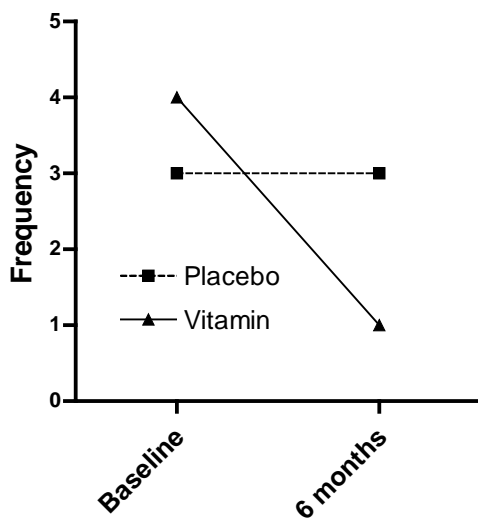
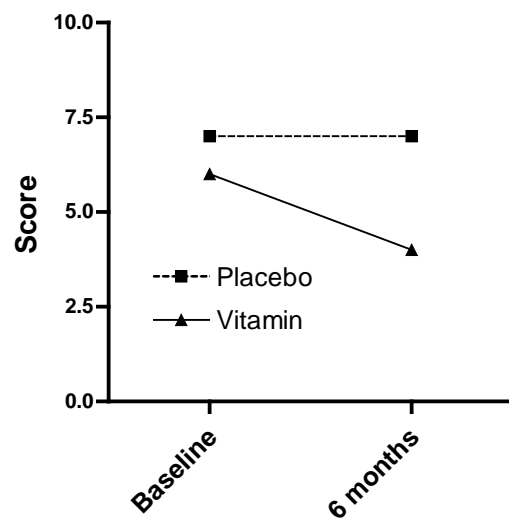


Figure 3. Change in primary clinical outcomes ie. frequency of high level migraine disability (MIDAS >11) over the treatment period in vitamin and placebo groups.



A. Change in headache frequency



B. Change in head pain severity score

Figure 4. Change in secondary clinical outcomes ie. migraine frequency (**A**) and average pain score (**B**) over the treatment period for vitamin and placebo groups. Values are medians. Quartiles not shown but reductions are statistically significant ($P < 0.05$).

3. Treatment response by MTHFR C677T genotype

When the vitamin-treated group was stratified by MTHFR_{C677T} genotype the mean homocysteine reduction was 31.4% for TT carriers compared to 47.7% for CT/CC carriers (P =0.047). For the vitamin-treated group there was a reduction in migraine disability for the CC/CT genotypes with the % of highly disabled migraineurs decreasing from 76% to 28% (P =0.002). There was no apparent reduction in migraine disability for the TT genotype (P >0.1).

Discussion

Improved health-related quality of life is a major goal of migraine therapy and has the potential to reduce the global burden of disease on individuals and society. Several mainstream pharmacotherapies exist which can successfully treat migraine symptoms (eg. selective serotonin agonists) or reduce attacks (eg. beta blockers, NSAIDs). However, current treatments are not always efficacious, are often expensive or are associated with adverse effects. Thus, safe, effective and inexpensive alternatives are desirable to combine with, or replace, current treatments. Elevated homocysteine seems to represent a modifiable risk factor for certain neurovascular-related disorders. This study examined whether folic acid and vitamin supplementation could effectively reduce plasma homocysteine and migraine disability, as well as the modifying effect of MTHFR_{C677T} genotype on the treatment response.

Vitamin supplementation at the recommended daily dosages was well tolerated with no reports of adverse events. At baseline the migraine patients in the study had mildly elevated homocysteine on average when compared to a general Caucasian population [26], an observation which is consistent with some, but not all, other studies of homocysteine in migraine [17,28]. Results of our trial showed a significant treatment effect with plasma homocysteine levels dropping by an average of 4 $\mu\text{mol/L}$, an effect size which is consistent with the expectation for these dosages [27].

The major finding of this study was that the migraine group receiving daily vitamin supplementation showed a marked reduction (2-fold) in migraine disability according to the MIDAS instrument scores. This decrease was greater overall compared to the placebo effect which was not statistically significant (Figure 2). Vitamin-treated migraineurs also reported substantial decreases in headache frequency and pain severity. These results provided compelling evidence that lowering plasma homocysteine levels via folic acid coupled with B₆ and B₁₂ vitamin supplementation improved health-related productivity and therefore quality of life for these patients.

Our findings are supported by a recent open labelled study by Rosa et al (2007) who conducted a 6-month trial of daily folic acid (5mg) in a small group of children with migraine. These researchers reported a complete resolution of migraine attacks in 60% of patients and a reduction of migraine headaches in the remaining patients after treatment [28]. Almost 40 years ago Kopjas published a small clinical study examining the use of folic acid for treatment of acute vascular migraine [29]. Kopja's study involved injecting 31 patients who were experiencing a migraine attack with 15mg of folic acid. Sixty % of these patients "responded satisfactorily to the first injection" with headache disappearing after 1 hour. The remainder of the patients experienced marked reduction in migraine headache intensity. Kopjas concludes his paper by stating that "folic acid therapy may not cure migraine, but certainly deserves a high place among the therapeutic agents for treatment, because of its safe, rapid and lasting pain relieving properties" [29].

The TT genotype of the MTHFR_{C677T} variant has been associated with increased risk of migraine with aura [21]. A secondary objective of this study was to assess whether the MTHFR_{C677T} variant may represent a genetic modifier of the treatment effect. We found that C allele carriers responded better to treatment compared to TT genotypes in terms of homocysteine and migraine reduction. This finding may be explained by the idea that TT genotypes are genetically slower homocysteine

metabolisers. That is, if all patients received the same vitamin dosage for the same period of time it would be expected that those with TT genotypes, having a reduced enzymatic rate, would metabolise less homocysteine over the treatment period compared to C allele carriers, thus resulting in a smaller reduction in homocysteine and consequent migraine symptoms. Indeed, it may be that TT genotypes although having a higher risk of disease actually require a larger dosage of vitamins to exhibit the same effect as C alleles. Further clinical trials of much larger patient cohorts are required to test this hypothesis.

There is convincing epidemiological evidence indicating an association between migraine with aura and stroke [14-16]. It is possible that higher than normal levels of homocysteine, or lower tolerance at normal levels, may lead to temporary cerebral thrombosis and/or altered blood flow allowing less oxygen into the brain and manifesting the symptoms common to MA and ischaemic stroke. Whilst our study was not designed to investigate the comorbidity of migraine and stroke, our findings lead us to postulate that elevated homocysteine may represent a non-coincidental causal risk factor for both diseases and that homocysteine-lowering in early onset migraine may also influence stroke risk in later life.

Conclusions

We have provided initial evidence that homocysteine-lowering via vitamin supplementation may reduce migraine disability in a subgroup of patients. Our data suggest a larger clinical trial is warranted to establish whether vitamin supplementation including folic acid is a safe, inexpensive and effective preventative treatment for increasing the quality of life of migraineurs more generally and also whether such treatment should be based on MTHFR genotype.

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References

1. Headache Classification Committee of The International Headache Society. The International Classification of Headache Disorders (second edition). *Cephalalgia* 2004;24(Suppl 1):1-160.
2. Goadsby PJ. Current concepts of the pathophysiology of migraine. *Neurol Clin* 1997;15(1):27-42.
3. Ferrari M. Migraine. *The Lancet* 1998;351:1043-1051.
4. Tzourio C, El Amrani M, Poirier O, Nicaud V, Bousser MG, Alperovitch A. Association between migraine and endothelin type A receptor (ETA -231 A/G) gene polymorphism. *Neurology* 2001;22;56(10):1273-7.
5. Harker LA, Ross R, Slichter SJ, Scott CR. Homocysteine-induced arteriosclerosis: the role of endothelial cell injury and platelet response in its genesis. *J Clin Invest.* 1976;58:731-741.
6. Wall RT, Harlan JM, Harker LA, Striker GE. Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. *Thromb Res.* 1980;18:113-121.

7. Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, Loscalzo J. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest.* 1993;91:308-318.
8. Cooke JP, Tsao PS. Is NO an endogenous antiatherogenic molecule? *Arterioscler Thromb.* 1994;14:653-655.
9. Lentz SR, Sobey CG, Piegors DJ, Bhopatkar MY, Faraci FM, Malinow MR, et al. Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. *J Clin Invest.* 1996;98:24-29.
10. Hering-Hanit R, Gadoth N, Yavetz A, Gavendo S, Sela B. Is blood homocysteine elevated in migraine. *Headache* 2001;41:779-781.
11. D. Bouassira, D. L. Bars and L. Villanueva. Heterotopic activation of A delta and C fibres triggers inhibition of trigeminal and spinal convergent neurones in the rat. *J. Physiol* 1987. 389 pp. 301-317.
12. Storer RJ and Goadsby PJ. Microiontophoretic application of serotonin (5HT)1B/1D agonists inhibits trigeminal cell firing in the cat. *Brain* 1997;120:2171-2177.
13. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ* 2002;325(7374):1202.
14. Tzourio C, Iglesias S, Hubert JB, Visy JM, Alperovitch A, Tehindrazanarivelo A, Biouesse V, et al. Migraine and risk of ischaemic stroke: a case-control study. *BMJ* 1993;31;307(6899):289-92.

15. Merikangas KR, Fenton BT, Cheng SH, Stolar MJ, Risch N. Association between migraine and stroke in a large-scale epidemiological study of the United States. *Arch Neurol* 1997;54(4):362-8.
16. Kurth T. Migraine and ischaemic vascular events. *Cephalalgia* 2007;27:965-75.
17. Scher AI, Terwindt GM, Verschuren WM, Kruit MC, Blom HJ, Kowa H, Frants RR, van den Maagdenberg AM, van Buchem M, Ferrari MD, Launer LJ. Migraine and MTHFR C677T genotype in a population-based sample. *Ann Neurol*. 2006 Feb;59(2):372-5.
18. Silberstein SD. Shared mechanisms and comorbidities in neurologic and psychiatric disorders. *Headache* 2001;41 Suppl 1:S11-7.
19. Wang X, Qin X, Demirtas H, Li J, Mao G, Huo Y, Sun N, Liu L, Xu X. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet*. 2007 Jun 2;369(9576):1876-82. Review.
20. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10:111-113.
21. Rubino E, Ferrero M, Rainero I, Binello E, Vaula G, Pinessi L. Association of the C677T polymorphism in the MTHFR gene with migraine: a meta-analysis. *Cephalalgia*. 2007 Aug 21; [Epub ahead of print]
22. Tfelt-Hansen P, Block G, Dahlof C, Diener H-C, Ferrari MD, Goadsby PJ, et al. Guidelines for controlled trials of drugs in migraine: second edition. *Cephalalgia* 2000;20:765-786.

23. Stewart WF Lipton RB et al. An international study to assess reliability of the Migraine Disability Assessment (MIDAS) score. *Neurology*. 1999; 53: 988-994.
24. Stewart WF Lipton RB et al. Reliability of the migraine disability assessment score in a population-based sample of headache sufferers. *Cephalalgia*. 1999; 19: 107-114.
25. Lea RA, Ovcacic M, Sundholm J, MacMillan J, Griffiths LR. The methylenetetrahydrofolate reductase gene variant C677T influences susceptibility to migraine with aura. *BMC Med*. 2004 Feb 12;2:3.
26. Selhub J, Jacques PF, Rosenberg IH, Rogers G, Bowman BA, Gunter EW, Wright JD, Johnson CL. Serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey (1991-1994): population reference ranges and contribution of vitamin status to high serum concentrations. *Ann Intern Med*. 1999 Sep 7;131(5):331-9.
27. Ho GY, Eikelboom JW, Hankey GJ, Wong CR, Tan SL, Chan JB, Chen CP. Methylenetetrahydrofolate reductase polymorphisms and homocysteine-lowering effect of vitamin therapy in Singaporean stroke patients. *Stroke*. 2006 Feb;37(2):456-60.
28. Di Rosa G, Attinà S, Spanò M, Ingegneri G, Sgrò DL, Pustorino G, Bonsignore M, Trapani-Lombardo V, Tortorella G. Efficacy of folic acid in children with migraine, hyperhomocysteinemia and MTHFR polymorphisms. *Headache*. 2007 Oct;47(9):1342-4.
29. Kopjas TL. The use of folic acid in vascular headache of the migraine type. *Headache*. 1969 Jan;8(4):167-70.