Influence of Nitrous Oxide Anesthesia, B-Vitamins, and *MTHFR* Gene Polymorphisms on Perioperative Cardiac Events

The Vitamins in Nitrous Oxide (VINO) Randomized Trial

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ABSTRACT

Background: Nitrous oxide causes an acute increase in plasma homocysteine that is more pronounced in patients with the methylenetetrahydrofolate reductase (*MTHFR*) C677T or A1298C gene variant. In this randomized controlled trial, the authors sought to determine whether patients carrying the *MTHFR* C677T or A1298C variant had a higher risk for perioperative cardiac events after nitrous oxide anesthesia and whether this risk could be mitigated by B-vitamins.

Methods: The authors randomized adult patients with cardiac risk factors undergoing noncardiac surgery, to receive nitrous oxide plus intravenous B-vitamins before and after surgery, or to nitrous oxide and placebo. Serial cardiac biomarkers and 12-lead electrocardiograms were obtained. The primary study endpoint was the incidence of myocardial injury, as defined by cardiac troponin I increase within the first 72 h after surgery.

Results: A total of 500 patients completed the trial. Patients who were homozygous for either *MTHFR* C677T, or A1298C gene variant (n = 98; 19.6%) had no increased rate of postoperative cardiac troponin I increase compared with wild-type and heterozygous patients (11.2 *vs.* 14.0%; relative risk 0.96; 95% CI, 0.85–1.07; $P = 0.48$). B-vitamins blunted the rise in homocysteine, but had no effect on cardiac troponin I increase compared with patients receiving placebo (13.2 *vs.* 13.6%; relative risk 1.02; 95% CI 0.78 to 1.32; $P = 0.91$).

Conclusions: Neither *MTHFR* C677T and A1298C gene variant, nor acute homocysteine increase are associated with perioperative cardiac troponin increase after nitrous oxide anesthesia. B-vitamins blunt nitrous oxide-induced homocysteine increase but have no effect on cardiac troponin I increase.

What We Already Know about This Topic

- Nitrous oxide increases circulating homocysteine concentration, and may do so more in patients with variants of the methylenetetrahydrofolate reductase gene
- Whether patients with these variants have increased cardiac risk from nitrous oxide exposure and whether this can be mitigated by vitamin B_{12} administration are not known

What This Article Tells Us That Is New

- In 500 patients with cardiac risk factors undergoing noncardiac surgery with nitrous oxide, methylenetetrahydrofolate reductase gene variant did not alter homocysteine concentration or the incidence of cardiac injury
- Vitamin B_{12}, although decreasing homocysteine concentration, did not alter the incidence of cardiac injury

IN use for more than 150 yr, nitrous oxide (laughing gas) is not only the oldest, but also one of the most widely used general anesthetics, worldwide. Because of its weak potency, nitrous oxide is typically used as an adjunct during general anesthesia, at a concentration of 50–70%. Its use during general anesthesia, particularly among patients with cardiac risk factors, has been associated with an increased risk for perioperative myocardial ischemia and infarction in some studies,[1–3] but not others.[4–8]

Nitrous oxide causes an acute increase in plasma homocysteine by irreversible inactivation of vitamin B_{12},[9–11] a

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side effect unrelated to its anesthetic action.[12,13] The acute increase in homocysteine has been proposed as the cause for the increased perioperative myocardial infarction risk.[14] Previously, we showed that patients homozygous for the C677T or A1298C variant in the methylenetetrahydrofolate reductase (*MTHFR*) gene, which is the most important genetic determinant of plasma homocysteine,[15–19] developed higher plasma homocysteine concentrations after nitrous oxide anesthesia.[20]

The purpose of the present investigation was to determine whether patients who were homozygous for the *MTHFR* C677T or A1298C variant had an increased risk for perioperative cardiac events after nitrous oxide anesthesia, and whether this risk could be mitigated by B-vitamins, which reliably lowers plasma homocysteine.[21–23] To answer this question, we conducted a double-blind, randomized placebo-controlled trial. In the trial, patients scheduled for nitrous oxide anesthesia were randomly allocated to receive B-vitamins or placebo.

Materials and Methods

Study Design and Oversight

The Vitamins in Nitrous Oxide trial was a single-center randomized, double-blind placebo-controlled trial of nitrous oxide and B-vitamins in patients with or at risk for coronary artery disease, undergoing noncardiac surgery at Barnes-Jewish-Hospital, St. Louis. The investigators were responsible for all aspects of the trial, including design, protocol, data collection, and analyses. The study was conducted in accordance to the protocol. A data and safety monitoring board monitored the trial. The study was approved by the Washington University institutional review board (St. Louis, Missouri), and all patients provided written, informed consent. The trial was registered at clinicaltrials.gov with the identifier NCT00655980.

Patients

Adult patients diagnosed with or at risk for coronary artery disease (combination of at least two risk factors, such as smoking history, hypertension, hypercholesterolemia, peripheral vascular disease, diabetes, stroke/transient ischemic attack), who were scheduled for elective noncardiac surgery under general anesthesia lasting more than 2 h, were assessed for eligibility. Patients were ineligible: if they had a contraindication to the use of nitrous oxide (*e.g.,* pneumothorax, bowel obstruction, laparoscopic surgery, increased intracranial pressure, middle-ear occlusion); clinically significant pulmonary disease requiring supplemental oxygen; patients not expected to survive 24 h; patients taking supplemental vitamin B_{12} or folic acid; allergy or hypersensitivity to cobalamins; Leber disease or a seizure disorder.

Randomization and Intervention

The Vitamins in Nitrous Oxide trial consisted of two randomized arms, with a total sample size of 500 patients, all of whom received nitrous oxide throughout surgery at a concentration of 60%. Patients were randomized to receive either 1 mg vitamin B_{12} and 5 mg folic acid (in 100 ml of normal saline) before and after surgery (nitrous oxide/B-vitamin group; n = 250), or a placebo infusion (100 ml normal saline; nitrous oxide/placebo group; n = 250). After initiation of the trial, the addition of a nitrous oxide-free nonrandomized reference group (n = 125) was recommended during scientific review at the National Institutes of Health, to determine if nitrous oxide had an effect on perioperative cardiac events independent of homocysteine increase. Patients in this reference cohort were drawn from the same population, had identical inclusion and exclusion criteria, but received nitrous oxide-free anesthesia and no B-vitamins. The protocol was amended, and the first patient in the reference group was enrolled in May 2010 (around study midpoint). No changes to the primary and secondary study outcomes were made. Patients in the reference group received an identical intraoperative oxygen concentration (40% oxygen; 60% nitrogen). All other aspects of intraoperative management were at the discretion of the anesthesia team.

The B-vitamin/placebo-infusion was covered with aluminum foil by an independent clinical pharmacist to prevent unblinding. Randomization was performed by our clinical pharmacy service as well and was in blocks of random sizes, using the Moses–Oakford algorithm[24]; no member of the study team had access to the randomization sequence until study enrolment finished. Patients, all members of the clinical care team (anesthesia team, surgeons, nurses),

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all members of the study team, and outcome assessors were blinded to the group assignment and genotype, which was determined after the patient had completed the study. The administration of nitrous oxide was open label due to practical reasons.

Outcomes

For outcome assessments, patients had five serial blood collections and electrocardiograms at the following time points: preoperative (baseline), end of surgery, and postoperative days 1, 2, and 3. Plasma total homocysteine (tHcy), serum troponin I (TnI; Siemens Dimension RxL; Siemens, Tarrytown, NY), and high-sensitivity troponin T (hs-cTnT; Roche Elycsys 2010; Roche Diagnostics, Indianapolis, IN) were measured at all time points; serum folate and vitamin B_{12} were measured at baseline and on postoperative day 1. *MTHFR* genotypes (rs1801131, rs1801133) were determined by the Sequenom MassARRAY (Sequenom, San Diego, CA) system.

The primary study endpoint was the incidence of myocardial injury, as defined by cardiac troponin I (cTnI) increase within the first 72 h after surgery. Secondary endpoints were the incidence of myocardial infarction within the first 72 h after surgery, and a composite of 30-day mortality and nonfatal myocardial infarction, which were assessed by a 30-day follow-up interview and medical records review. For the assessment of the primary study outcome, a Food and Drug Administration-cleared troponin I assay was used (Siemens Dimension RxL; limit of detection: 0.04 μg/l; 99th percentile: 0.07 μg/l; 10% coefficient of variation: 0.14 μg/l).[25] During the course of the trial, a novel high-sensitivity troponin T assay became available (Roche Elecsys; limit of detection: 5.0 ng/l; 99th percentile: 14 ng/l; 10% coefficient of variation: 13 ng/l),[25] which we additionally used due to its markedly increased sensitivity in the detection of circulating troponins (currently not cleared by the U.S. Food and Drug Administration). Cardiac troponin increase was defined as a peak postoperative cardiac troponin I concentration more than 99th percentile (>0.07 μg/l). Myocardial infarction was defined according to the universal definition (troponin I increase >99th percentile plus electrocardiogram changes indicative of myocardial ischemia and/or clinical symptoms).[26] Electrocardiograms were read and analyzed by an expert assessor blinded to the group assignment. Adverse events were assessed by the study team from patient medical records.

Statistical Analysis

Patients were grouped according to their *MTHFR* genotype in homozygotes (*MTHFR* 677TT and 1298CC) and wild-type or heterozygotes (*MTHFR* 677CC and CT, and 1298AA and AC) on the basis of our previous observation, suggesting a significantly higher homocysteine increase after nitrous oxide anesthesia among *MTHFR* homozygous patients.[20] The sample size determination was planned for the two randomized trial arms and did not include the reference group. Extrapolated from incidences observed in patients undergoing vascular surgery and our previous study,[20] we expected an incidence rate of cardiac troponin increase among *MTHFR* homozygous patients (expected proportion 20% of the study population) of 24% (with B-vitamins) and 47% (placebo), and at 20% for nonhomozygous patients (B-vitamins) and 32% (placebo).

On the basis of these assumptions, a sample size of n equals to 500 was sufficient to detect a statistically significant difference between *MTHFR* homozygotes with an α = 0.05 and β = 0.30, and between *MTHFR* nonhomozygotes with an α = 0.05 and β = 0.22. Using continuous data, we could detect at a power of 0.7, a difference of 0.5 of a SD between *MTHFR* homozygotes, and a difference of 0.28 SDs between nonhomozygotes. All power calculations are based on two-tail tests, with a 0.05 significance level, using the program STPLAN (M.D. Anderson Cancer Center, University of Texas, Houston, TX).

Data were entered into a Research Electronic Data Capture (REDCap) database[27] and exported into SAS (Cary, NC). Descriptive statistics for each group were computed, including the calculation of the median and 25th and 75th percentiles because many of the distributions were skewed or means ± SD. When analyzing dichotomous outcomes, chi-square tests and logistic regression were used. For continuous values, which were obtained at multiple time points, a repeated measures ANOVA was computed, and appropriate contrasts from the time*group effect constructed to compare changes from baseline to peak values between groups. Analyses were also repeated using stratification by *MTHFR* genotype. Where regression diagnostics raised concerns, results were confirmed using transformations (*e.g.,* logarithmic) or rank order transformations. Significance level was set at 0.05, two-tailed.

Results

Patients

From March 2008 to December 2011, we enrolled 687 patients into the study; sixty-two patients were withdrawn after randomization but before surgery, resulting in a final intention-to-treat sample of 625 patients. The most common causes for withdrawal were cancelled surgeries or refusal by the anesthesia team (fig. 1). Of the final study sample, 250 patients were randomly assigned to the nitrous oxide/B-vitamin group (5 patients did not receive nitrous oxide), and 250 to the nitrous oxide group/placebo (3 patients did not receive nitrous oxide). One hundred twenty-five patients were assigned to the nonrandomized reference group. Baseline characteristics between the two randomized study arms were well balanced (table 1). The *MTHFR* A1298C allele (minor allele frequency 0.29) was in Hardy–Weinberg equilibrium, but the C677T allele (minor allele frequency 0.28) was not. The TT variant had an observed prevalence of: 10.6% (expected: 7.8%; $P < 0.001$).

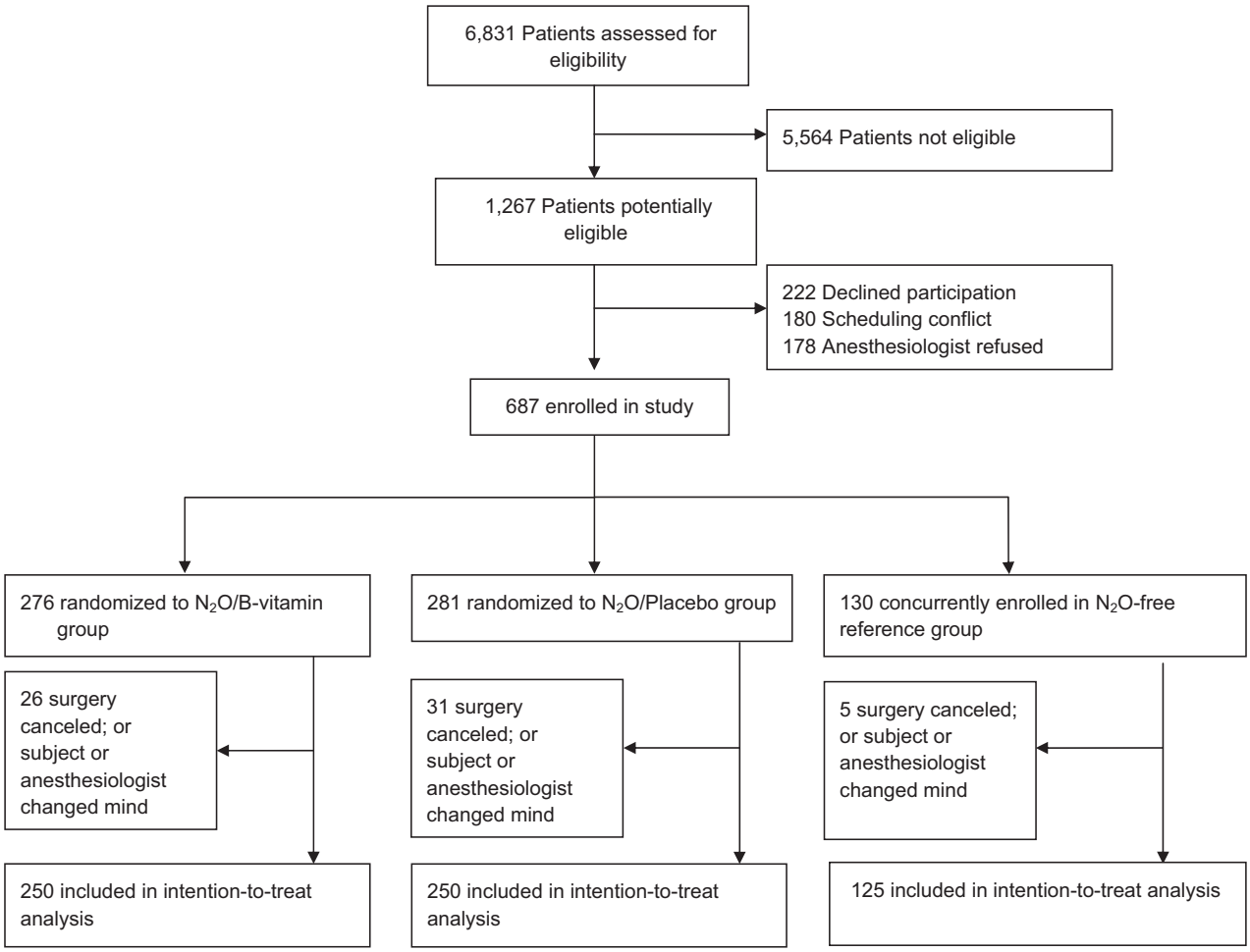

Fig. 1. Enrollment and randomization. N_2O = nitrous oxide.

Study Outcomes—Homocysteine

Patients in the nitrous oxide/B-vitamin group and nitrous oxide/placebo group had similar median exposures to nitrous oxide (measured as the product of duration of nitrous oxide anesthesia in hours and fraction of inspiratory nitrous oxide concentration: 1.7 ± 1.0 percent*hours *vs.* 1.7 ± 1.0 percent*hours (P = 0.62). Patients in the nitrous oxide/B-vitamin group had significantly higher postoperative vitamin B_{12} and folate concentrations compared with baseline and to both the other groups (see table, Supplemental Digital Content 1, http://links.lww.com/ALN/A937). Plasma total homocysteine concentrations rose in all patients who received nitrous oxide, regardless of MTHFR genotype. The increase was significantly blunted in patients receiving B-vitamins (fig. 2 and table 2). The *MTHFR* C677T and A1298C allele status did not influence the extent of plasma total homocysteine increase. In the B-vitamin group, homozygous patients had a median increase of 2.1 μM [interquartile range, 0–5.3] and wild-type/heterozygous patients had 2.1 μM [−0.2 to 5.5]; in the placebo group, homozygous patients had a 3.1-μM increase [interquartile range, 1.4–7.3] compared with 3.8 μM [interquartile range, 0.6–6.8] for wild-type/heterozygous patients.

Influence of MTHFR *Genotype on Perioperative Cardiac Events*

Among the 500 patients within the randomized trial, neither the *MTHFR* genotype, nor the B-vitamin treatment had an effect on cardiac study outcomes. Patients who were homozygous for the *MTHFR* C677T or A1298C gene variant (n = 98; 19.6%) had an 11.2% incidence rate of postoperative cTnI increase compared with 14.0% for wild-type and heterozygous patients (relative risk, 0.96; 95% CI, 0.85–1.07; P = 0.48; table 3). B-vitamins had no effect on cTnI increase compared with patients receiving placebo (13.2 *vs.* 13.6%; relative risk, 1.02; 95% CI, 0.78–1.32; P = 0.91), regardless of *MTHFR* genotype.

Patients who were homozygous for the *MTHFR* C677T or A1298C gene variant had a 3.1% incidence rate of perioperative myocardial infarction (within 3 days after surgery) compared with 4.7% for wild-type and heterozygous patients (relative risk, 0.93; 95% CI, 0.78–1.10; P = 0.48). The incidence of postoperative myocardial infarction was 6.0% in the nitrous oxide/placebo group and 2.8% in the nitrous oxide/B-vitamin group (relative risk, 1.60; 95% CI, 0.87–2.98; P = 0.09).

The extent of myocardial injury or necrosis, as indicated by the rise in high-sensitivity troponin T (Δhs-cTnT), was

Table 1. Baseline Characteristics

	Nitrous Oxide/B-Vitamins (n = 250)	Nitrous Oxide /Placebo (n = 250)
Mean age, yr	64.7 ± 10.6	65.2 ± 10.7
Mean weight, kg	90.3 ± 22.7	88.9 ± 20.8
Female sex, no. (%)	92 (36.8)	97 (39.0)
Race, no. (%)		
White	197 (79.1)	200 (80.0)
Black	51 (20.5)	49 (19.6)
Other	1 (0.4)	1 (0.4)
Smoking history, no. (%)	195 (79.0)	175 (70.0)
Diabetes, no. (%)	96 (38.6)	88 (35.5)
Hypertension, no. (%)	200 (80.0)	204 (81.6)
Chronic renal failure, no. (%)	16 (6.5)	35 (14.1)
On hemodialysis, no. (%)	1 (0.4)	3 (1.2)
Coronary artery disease, no. (%)	140 (56.0)	148 (59.2)
Congestive heart failure, no. (%)	27 (10.8)	31 (12.4)
Peripheral vascular disease, no. (%)	81 (32.5)	85 (34.4)
ASA status, no. (%)		
I	1 (0.4)	0
II	45 (18.0)	42 (16.9)
III	199 (79.6)	196 (79.0)
IV	5 (2.0)	10 (4.0)
Lee revised cardiac risk index, mean	1.9 ± 0.8	2.0 ± 0.9
Median serum vitamin B_{12}, pg/ml (IQR)	415 (317–581)	405 (300–564)
Median serum folate, ng/ml (IQR)	16.1 (12.1–23.7)	16.5 (13.2–25.4)
Mean plasma total homocysteine, μM	13.5 ± 5.7	13.4 ± 5.8
Median serum high-sensitivity troponin T, ng/l (IQR)	11.9 (7.8–18.2)	11.5 (8.4–19.6)
MTHFR C677T (rs1801133)		
677CC	137 (56.6%)	130 (53.1%)
677CT	76 (31.4%)	89 (36.3%)
677TT	29 (12.0%)	26 (10.6%)
MTHFR A1298C (rs1801131)		
1298AA	133 (54.7%)	120 (49.2%)
1298AC	86 (35.4%)	105 (43.0%)
1298CC	24 (10.0%)	19 (7.8%)
Medications		
Aspirin	126 (50.8%)	137 (54.8%)
Clopidogrel	42 (16.8%)	45 (18.1%)
Warfarin	28 (11.2%)	21 (8.4%)
β-blocker	119 (47.6%)	141 (56.6%)
ACE-inhibitor	94 (37.6%)	88 (35.2%)
Statin	130 (52.0%)	146 (58.4%)
Diuretic	79 (31.6%)	95 (38.0%)
Surgical procedure		
Vascular	76 (30.4%)	87 (34.8%)
Orthopedic	88 (35.2%)	81 (32.4%)
ENT	22 (8.8%)	20 (8.0%)
Gynecology	26 (10.4%)	21 (8.4%)
Urology	22 (8.8%)	22 (8.8%)
Neurosurgery	16 (6.4%)	19 (7.6%)

ACE = angiotensin converting enzyme; ASA = American Society of Anesthesiologists; ENT = ear-nose-throat; IQR = interquartile range; *MTHFR* = methylenetetrahydrofolate reductase gene.

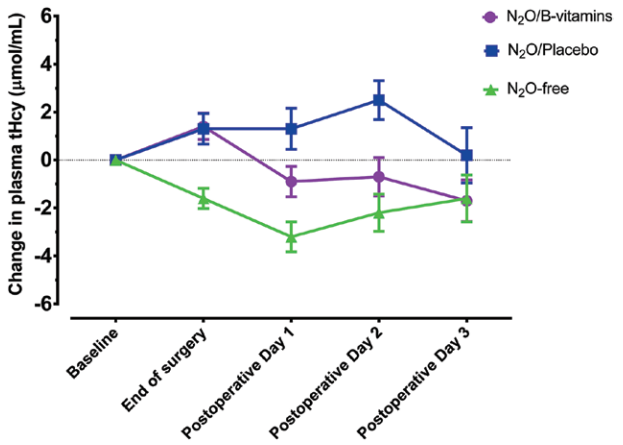

Fig. 2. Relative change in plasma homocysteine per study group over time. *Error bars* indicate 95% CI. tHcy = plasma total homocysteine.

not different among *MTHFR* homozygous and wild-type/heterozygous patients and patients who received B-vitamins or placebo (table 2 and fig. 3).

Acute homocysteine increase was not correlated with cardiac troponin release, as indicated by the rise in hs-cTnT (Δhs-cTnT; Spearman correlation coefficient = 0.003; P = 0.94). At 30 days, three patients (1.2%) had died in the nitrous oxide group and none in the B-vitamin group.

Nonrandomized Nitrous Oxide-free Reference Group

In addition to the randomized trial cohort, an additional, nonrandomized reference group (n = 125) of patients, receiving nitrous oxide-free anesthesia, were enrolled. Nine patients inadvertently received nitrous oxide during emergence from anesthesia and four patients for the duration of the case. The baseline characteristics of the nitrous oxide-free reference group were very similar to the randomized cohort (see table, Supplemental Digital Content 2, http://links.lww.com/ALN/A938). Patients in the nitrous oxide-free reference group had similar incidence rates of cardiac troponin I increase (13.6 *vs.* 13.4%) and a slightly increased rate of myocardial infarction (6.4 *vs.* 4.4%). The median Δhs-cTnT was similar to the randomized cohort, indicative of a similar magnitude of perioperative myocardial injury between patients receiving nitrous oxide and nitrous oxide-free anesthesia (see tables, Supplemental Digital Content 3, http://links.lww.com/ALN/A939, and Supplemental Digital Content 4, http://links.lww.com/ALN/A940).

Safety

No adverse events were related to the administration of intravenous B-vitamins (table 4). Within the first 3 postoperative days, 16 patients (6.4%) in the nitrous oxide/B-vitamin group had a cardiovascular event compared with 25 (10.0%) in the nitrous oxide/placebo group (table 4; see table, Supplemental Digital Content 5, http://links.lww.com/ALN/A941).

Discussion

This trial resulted in several findings: First, the prophylactic use of vitamin B_{12} and folic acid successfully blunted the nitrous oxide-induced increase in plasma homocysteine but had no effect on perioperative cardiac outcomes. Second, patients who are homozygous for the *MTHFR* C677T and A1298C gene variants had no increased risk for perioperative cardiac events after nitrous oxide anesthesia. Third, the acute increase in plasma homocysteine caused by nitrous oxide was not associated with perioperative cardiac troponin increases.

Several points deserve comment. Consistent with earlier evidence,[9] our study showed that nitrous oxide causes an acute increase in plasma homocysteine, which lasts several days. Contrary to previous suggestions, however, the observed plasma homocysteine increase was not associated with troponin increases or myocardial infarction. The administration of B-vitamins before and after nitrous oxide anesthesia clearly lowered plasma homocysteine levels, but did not decrease the rate and magnitude of postoperative cardiac troponin increase, or myocardial infarction. We

Table 2. Peak and Change in Plasma Total Homocysteine and High-sensitivity Troponin T According to *MTHFR* Genotype and Intervention Group

	MTHFR Genotype	Nitrous Oxide/ B-Vitamins (n = 250)	Nitrous Oxide/Placebo (n = 250)
Peak tHcy, µM	Homozygous	16.5 [13.3–21.2]	17.4 [12.8–21.1]
	Wild-type/heterozygous	14.8 [11.8–19.4]	16.2 [12.8–20.7]
Change tHcy, µM	Homozygous	2.1 [0–5.3]	3.1 [1.4–7.3]
	Wild-type/heterozygous	2.1 [–0.2 to 5.5]	3.8 [0.4–6.7]
Peak hs-cTnT, ng/l	Homozygous	19.1 [13.3–34.2]	14.5 [10.0–24.0]
	Wild-type/heterozygous	14.1 [10.1–22.9]	16.5 [10.6–29.6]
Change hs-cTnT, ng/l	Homozygous	3.6 [1.3–13.4]	2.9 [1.3–6.2]
	Wild-type/heterozygous	2.4 [0.3–5.8]	2.5 [0.5–7.1]

Values are median and interquartile range. Homozygous = *MTHFR* 677TT or 1298CC patients; wild-type and het. (=heterozygous)= *MTHFR* 677CC and TT; and 1298 AA and AC.

hs-cTnT = high-sensitivity cardiac troponin T; *MTHFR* = methylenetetrahydrofolate reductase gene; tHcy = plasma total homocysteine.

Table 3. Primary and Secondary Study Outcomes Grouped by *MTHFR* Genotype and Intervention Arm

		Nitrous Oxide/ B-Vitamins (n = 250)	Nitrous Oxide/ Placebo (n = 250)	Subtotal
Cardiac troponin increase	Homozygous (n = 98)	6 (11.3%)	5 (11.1%)	11 (11.2%)
	Wild-type/heterozygous (n = 389)	27 (13.8%)	29 (14.1%)	56 (14.0%)
	Subtotal	33 (13.2%)	34 (13.6%)	67 (13.4%)
Myocardial infarction	Homozygous	2 (3.8%)	1 (2.3%)	3 (3.1%)
	Wild-type/heterozygous	5 (2.5%)	14 (6.9%)	19 (4.7%)
	Subtotal	7 (2.8%)	15 (6.0%)	22 (4.4%)
Length of stay, d	Median [IQR]	4 [2–6]	3 [2–5]	

Homozygous = *MTHFR* 677TT or 1298CC patients; wild-type and heterozygous = *MTHFR* 677CC and TT; and 1298 AA and AC.
IQR = interquartile range; *MTHFR* = methylenetetrahydrofolate reductase gene.

therefore, question the notion that acute nitrous oxide-induced hyperhomocysteinemia has a causal effect on perioperative myocardial ischemia and infarction. There is now an emerging consensus that homocysteine may be a marker, rather than a cause of atherosclerotic disease and increased cardiovascular risk,[23,28,29] despite impressive associations between chronically increased homocysteine and coronary artery disease.[30,31]

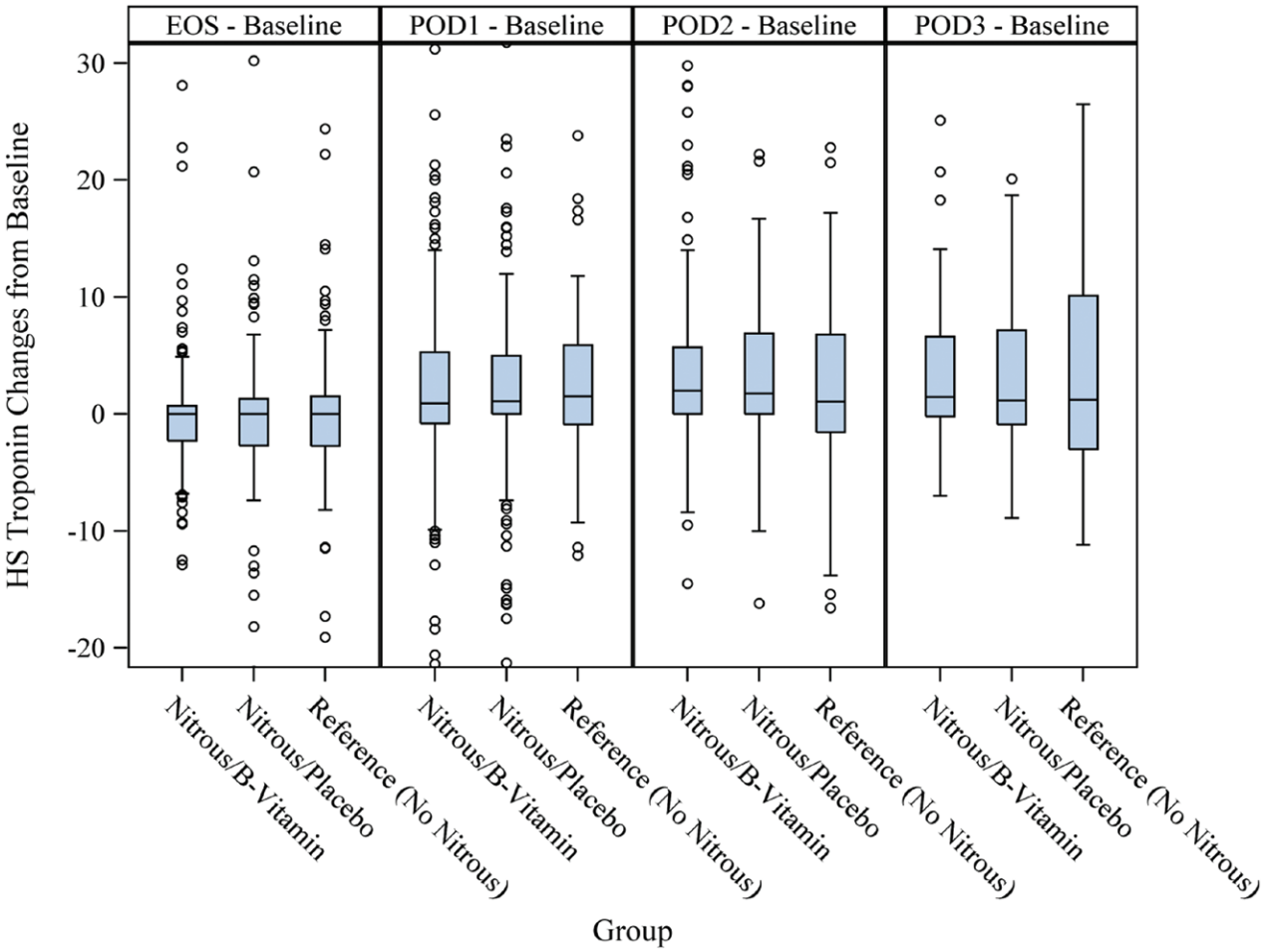

Fig. 3. Boxplots of high-sensitivity troponin T changes after baseline per group over time. Results are shown per group: nitrous oxide/B-vitamins; nitrous oxide/placebo, and reference group (no nitrous oxide) at four time points: end of surgery (EOS), postoperative days (POD) 1, 2, and 3 (POD 1, POD2, and POD 3). No statistically significant difference between any group at any time point were observed. *Boxes* represent the interquartile range (25 and 75% percentile); the *line* represents the median and the *whiskers* the Tukey distance (1.5 times the interquartile range).

Table 4. Adverse Outcomes

	Nitrous Oxide/B-Vitamins (n = 250)	Nitrous Oxide/Placebo (n = 250)
Within 3 d after surgery		
Nausea and vomiting, no. (%)	3 (1.2)	13 (5.2)
Wound infection, no. (%)	3 (1.2)	4 (1.6)
Any pulmonary, no. (%)*	5 (2.0)	4 (1.6)
Any cardiovascular, no. (%)†	16 (6.4)	25 (10.0)
Any renal, no. (%)‡	5 (2.0)	4 (1.6)
Bleeding, no. (%)	20 (8.0)	14 (5.6)
Ileus, no. (%)	4 (1.6)	4 (1.6)
Within 30 d after surgery		
Readmission, no. (%)	9 (3.6)	23 (9.2)
Wound infection, no. (%)	15 (6)	18 (7.2)
Any pulmonary, no. (%)*	3 (1.2)	2 (0.8)
Any cardiovascular, no. (%)†	5 (2.0)	11 (4.4)
Any renal, no. (%)‡	0	0
Bleeding, no. (%)	4 (1.6)	2 (0.8)
Ileus, no. (%)	1 (0.4)	0
Deep venous thrombosis, no. (%)	2 (0.8)	5 (2.0)
Death, no. (%)	0	3 (1.2)

* Pulmonary complications included respiratory distress, pulmonary edema, pneumothorax, reintubation, pneumonia, pleural effusion.
† Cardiovascular complications included new onset atrial fibrillation, ventricular tachycardia, myocardial infarction, cardiac arrest.
‡ Renal complications included creatinine increase, acute kidney failure, kidney injury during surgery.

To our surprise, and contrary to our previous work, the *MTHFR* C677T and A1298C genotype had no influence on nitrous oxide-induced homocysteine increase.[20] A likely explanation for this lack of influence lies in the mandatory dietary folate fortification of all grain products in the United States, which has increased folate levels throughout the population.[32] Dietary folate fortification also reduces the effects of the *MTHFR* polymorphisms.[19,33] Our previous study was conducted in Austria, a country without mandatory folate fortification, which may explain why we previously observed a significant effect of *MTHFR* polymorphisms on nitrous oxide-induced homocysteine concentrations.

Previous research provides conflicting evidence regarding nitrous oxide and perioperative myocardial ischemia and infarction. Several smaller studies in high-risk patients observed no effect of nitrous oxide on myocardial ischemia,[4–6] whereas, a recent study showed an increased risk for ischemic electrocardiogram changes;[1] however, in this latter study, no cardiac biomarkers or clinical outcomes were reported. The only large clinical trial investigating nitrous oxide and cardiovascular outcomes, the ENIGMA trial, reported an inconclusive, statistically nonsignificant increase in the incidence of myocardial infarction from 0.7% (n = 7 of 997) to 1.3% (n = 13 of 1,015; odds ratio, 0.54; 95% CI, 0.22–1.37; P = 0.2), in patients receiving nitrous oxide.[2] Nevertheless, since the publication of these studies, many practitioners have abandoned the use of nitrous oxide for patients with cardiac risk factors. Our study suggests that there is no increased cardiac risk from acute nitrous oxide-induced hyperhomocysteinemia. The ongoing ENIGMA-II trial, a large-scale multicenter clinical trial, will provide robust and definitive evidence to the question of the association between nitrous oxide and perioperative myocardial infarction.[34]

In addition to a standard Food and Drug Administration-cleared troponin I assay, our study used a novel high-sensitivity troponin T assay, to detect myocardial necrosis and infarction. High-sensitivity troponin assays allow the detection of circulating cardiac troponins at extremely low levels (nanogram/liter), allowing for the calculation of a relative change Δhs-cTnT compared with baseline within each patient.[35] In our study, more than 80% of patients had a measurable rise in postoperative high-sensitivity troponin T compared with baseline. Our data suggest that high-sensitivity troponin assays may be the most sensitive method for the detection of perioperative myocardial injury and infarction.

The study has several limitations. First, the observed event rate of cardiac troponin I increases was substantially lower than expected. In hindsight, expecting an event rate of 47% among patients homozygous for either of the *MTHFR* variant who received nitrous oxide, was probably overly optimistic and resulted in a lower than expected study power. Given the remarkably negative results, both in the incidence rates between the groups, and the extent of myocardial injury as indicated by the nearly identical change in high-sensitivity troponin T, we doubt that a larger sample size would have resulted in a different study outcome.

Second, because our study was conducted in a country with dietary folate fortification, it is possible that its findings may not be generalizable to regions that do not have dietary folate fortification. Third, in the United States, many adults

use over-the counter multivitamins, and we cannot rule out the possibility that some of the patients self-medicated multivitamins within the first days after surgery, which could have affected plasma homocysteine concentration and theoretically, study outcomes.

In summary, the findings of this trial suggest that neither nitrous oxide-induced acute hyperhomocysteinemia, nor *MTHFR* genotype is associated with perioperative cardiac troponin increase after nitrous oxide anesthesia. The prophylactic use of B-vitamins is efficacious in blunting nitrous oxide-induced homocysteine increase but has no effect on perioperative cardiac events. We therefore, believe that based on current evidence, practitioners who feel that nitrous oxide could be beneficial for their patients should not refrain from administering it because of concern for acute homocysteine increase or *MTHFR* gene variant.

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Appendix

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