

Effect of Nitrous Oxide Exposure during Surgery on the Homocysteine Concentrations of Children

Dubraiicka Pichardo, M.Sc.,* Igor A. Luginbuehl, M.D.,† Yaseer Shakur, M.Sc., Ph.D.,* Paul W. Wales, M.D., M.Sc., F.R.C.S.(C), F.A.C.S.,‡ Ahmed El-Sohemy, Ph.D.,§ Deborah L. O'Connor, R.D., Ph.D.||

ABSTRACT

Background: Nitrous oxide converts vitamin B12 to its nonmetabolically active form, inhibits methionine synthase, and results in an elevation of plasma total homocysteine (tHcy). The authors investigated the effect of nitrous oxide anesthesia on the plasma tHcy concentrations in children the morning after surgery and whether blood concentrations of folate and vitamins B12 and B6 were associated with any potential increase.

Methods: The authors measured plasma tHcy concentrations in 32 children before and 24 h after initial exposure to nitrous oxide (≥ 2 h). Genotype for methylenetetrahydrofolate reductase C677T and blood concentrations of folate, vitamins B12 and B6, and methylmalonic acid were measured before surgery.

* Research Trainee, Physiology and Experimental Medicine Program, The Hospital for Sick Children, Toronto, Ontario, Canada, and Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada. † Associate Professor, Department of Anesthesia and Pain Medicine, University of Toronto, and Department of Anesthesia, The Hospital for Sick Children. ‡ Associate Professor, Department of Surgery, The Hospital for Sick Children, and Department of Surgery, University of Toronto. § Associate Professor, Department of Nutritional Sciences, University of Toronto. || Professor, Department of Nutritional Sciences, University of Toronto, and Physiology and Experimental Medicine Program, The Hospital for Sick Children.

Received from the Physiology and Experimental Medicine Program and the Departments of Anesthesia, Surgery and Clinical Dietetics, The Hospital for Sick Children, Toronto, Ontario, Canada. Submitted for publication May 6, 2011. Accepted for publication March 16, 2012. Supported by Mead Johnson/Heinz/Weston Endowment for Nutrition/Metabolism Research, Consejo Nacional de Ciencia y Tecnologia, Mexico City, Mexico. Presented as a poster at the American Societies of Experimental Biology Meeting, Anaheim, California, April 27, 2010.

Address correspondence to Dr. O'Connor: 555 University Avenue, Room 8511C, Toronto, Ontario, Canada M5G 1X8. deborah_l.oconnor@sickkids.ca. This article may be accessed for personal use at no charge through the Journal Web site, www.anesthesiology.org.

Copyright © 2012, the American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins. Anesthesiology 2012; 117:15–21

What We Already Know about This Topic

- In adults, nitrous oxide inhalation increases plasma total homocysteine, which possibly has cardiovascular consequences, but there are no studies in small children

What This Article Tells Us That Is New

- Nitrous oxide anesthesia in children increased plasma total homocysteine concentrations the morning after surgery
- The duration of exposure, initial plasma total homocysteine, and serum vitamin B12 concentrations were associated with this increase

Results: The median age of participants was 11 months (3–126 months). The median (first, third quartile) postoperative plasma tHcy concentration was significantly higher than the preoperative concentration (6.4 [4.7, 8.9] *vs.* 5.1[4.1, 6.4] μM , $P < 0.0001$), a 25% (2%, 42%) relative increase. Six of 28 (21%) children with normal, age-appropriate, preexposure plasma tHcy concentrations had postoperative plasma tHcy concentrations greater than the cutoff values. The duration of nitrous oxide exposure was associated positively with the rise in plasma tHcy concentration ($R^2 = 0.696$, $P = < 0.001$).

Conclusion: Exposure to ≥ 2 h nitrous oxide is associated with a small, albeit statistically significant, increase in postoperative plasma tHcy concentrations the morning after surgery in young children. The clinical significance of this increase is unknown.

HOMOCYSTEINE is an intermediary metabolite in the body produced by demethylation of the essential amino acid methionine. Homocysteine may be remethylated to methionine by the enzyme methionine synthetase, requiring the presence of the reduced form of vitamin B12

- ◆ This article is accompanied by an Editorial View. Please see: Nagele P: Notorious oxide. ANESTHESIOLOGY 2012; 117:3–5.

and methyltetrahydrofolate as a methyl donor. Homocysteine can be converted to the conditionally essential amino acid cysteine by the enzymes cystathionine β -synthase and cystathionine γ -lyase with vitamin B6 serving as a cofactor.¹ Alternatively, homocysteine can be converted to methionine by the enzyme betaine-homocysteine methyltransferase.²

It has been shown that nitrous oxide increases plasma total homocysteine (tHcy) concentrations in adolescents³ and adults^{4–11} as a result of the conversion of vitamin B12 to its metabolically inactive form.^{12–14} Similarly, nitrous oxide exposure has been shown to be associated with an increased risk of postoperative cardiovascular events in adults.¹⁵ This has led to speculation that the elevation in plasma tHcy concentration after nitrous oxide exposure may play a specific role in these complications; however, this remains unclear. Indeed, increased postoperative plasma tHcy concentrations after nitrous oxide exposure have been shown to be associated with myocardial infarction and stroke,⁵ myocardial ischemia⁹ and impaired endothelial function⁶ in adults. No evidence exists to suggest an association between nitrous oxide exposure and postoperative cardiovascular events in children. At the population level, both nutritional (folate and vitamins B12 and B6) and genetic factors have been associated with changes in plasma tHcy concentrations.¹⁶ Synthesis of 5-methyltetrahydrofolate is regulated by the enzyme methylenetetrahydrofolate reductase (MTHFR).¹⁷ The common single nucleotide polymorphism (C677T) yields an enzyme with reduced activity, often an important genetic determinant of plasma tHcy concentration.^{17–19}

Although there is a large body of literature investigating postoperative plasma tHcy concentrations in adults after exposure to nitrous oxide, limited data exist on children.³ Therefore, the purpose of this study was to evaluate if young children exposed to nitrous oxide demonstrate an increase in plasma tHcy concentrations the morning after surgery and whether blood concentrations of folate and vitamins B12 and B6 were associated with the increase in plasma tHcy concentrations.

Materials and Methods

Patients

The study was approved by The Human Research Ethics Committee at The Hospital for Sick Children, Toronto, Ontario, Canada. Informed written consent from parents and assent from the child, where appropriate, were obtained before participation. Children were recruited from preoperative clinics in the Plastics Surgery Division at The Hospital for Sick Children from February to July 2009. Eligible children needed to weigh ≥ 3 kg, be younger than 11 yr, and be scheduled for surgery with general anesthesia expected to last ≥ 2 h using nitrous oxide at an inspired concentration $\geq 50\%$. Children were not eligible if they were 11 yr of age or older because puberty is known to lead to sex differences in plasma tHcy concentrations.²⁰ Children parenterally fed, ex-

posed to nitrous oxide 30 days before surgery, diagnosed with active disease, and/or using medication(s) known to affect plasma tHcy concentrations (e.g., kidney disease, methotrexate treatment) were excluded.

Study Protocol

A blood sample (after more than 8 h of fasting) was obtained 14 ± 7 min after induction of anesthesia (preoperative), and a second specimen was collected 24 ± 3 h thereafter (next morning, after surgery). Children were not fasted before collection of the postoperative blood draw. Given the age and size of the children, a limited volume of blood could be drawn. In the event that there was insufficient plasma or serum to perform all planned biochemical analyses, they were prioritized as follows: (1) plasma tHcy, (2) plasma folate, (3) plasma methylmalonic acid (MMA), (4) serum vitamin B12, (5) MTHFR C677T genotype, and (6) plasma vitamin B6. Plasma tHcy concentrations were determined before and after surgery. Total plasma folate, serum vitamin B12, plasma MMA (for cobalamin status), and plasma pyridoxal-5-phosphate (for vitamin B6 status) were measured in blood collected before surgery. The normative cutoff values for the vitamin biomarkers and citations used to support them can be found in table 1. A whole blood sample was obtained for MTHFR C677T genotyping. Intraoperative data, such as type of surgery and duration of exposure to nitrous oxide, were collected from anesthesia records. Demographic, history of vitamin(s) supplement use, and postoperative dietary intake data were obtained by interviewing parents.

Biochemical Methods

Blood samples were collected in EDTA and serum separator tubes. All samples were immediately cooled and kept on ice until centrifuged (1,250g, 4°C, 15 min). Blood samples were processed within 30 min of the blood draw, and aliquots were stored at -80°C until analyzed. With the exception of plasma folate, all blood samples were analyzed as a single batch for each analyte. Plasma tHcy concentration was determined by liquid chromatography-electrospray tandem mass spectrometry as described by Rafii *et al.*²¹ Total plasma folate concentrations were determined by microbiologic assay using the test organism *Lactobacillus rhamnosus* (ATCC 7649; American Type Tissue Culture Collection, Manassas, VA), as described by Molloy and Scott.²² Serum vitamin B12 was analyzed by solid-phase competitive chemiluminescent enzyme immunoassay and the IMMULITE 2500 Analyzer (Diagnostics Products Corporation, Los Angeles, CA). The concentration of plasma MMA was measured by chemical ionization gas chromatography mass spectrometry according to the method described by Yazdanpanah *et al.*²³ Plasma vitamin B6 (pyridoxal-5-phosphate) concentrations were measured by reversed-phase high-performance liquid chromatography with chlorite postcolumn derivatization, as described by Rybak and Pfeiffer.²⁴ Genotyping of MTHFR

Table 1. Preexposure Biomarker Values

Biomarker	n (%) [*]	Median (range)	Outside Normative Cutoff n (%)	Normative Cutoff†
Plasma tHcy (μM)	32 (100)	5.1 (2.9–10.3)	4 (13)	>8.0
Plasma folate (nM)	32 (100)	76.6 (31.3–250.3)	0 (0)	<6.8
Serum vitamin B12 (pM)	27 (84)	638 (226–997)	0 (0)	<221
Plasma MMA (μM)	27 (84)	0.10 (0.02–0.88)	5 (19)	>0.21
Plasma vitamin B6 (nM)	15 (41)	92 (18–187)	1 (7)	<20
Hemoglobin (g/l)	21 (66)	112 (92–135)	7 (33)	<110
Hematocrit (1/1)	32 (100)	0.34 (0.28–0.42)	10 (31)	<0.33

Vitamin B6 was measured as plasma pyridoxal-5-phosphate.

^{*} n = The number of subjects for whom sufficient plasma or serum was obtained to perform the biochemical analysis for the biomarker; percentage (%) with respect to total sample size (n = 32). † Normative cutoff values from the literature: folate,³¹ hemoglobin and hematocrit,³² homocysteine,¹⁶ MMA,³³ vitamin B6,³⁴ vitamin B12.³⁵

MMA = methylmalonic acid; tHcy = total homocysteine.

C677T (CC, CT, TT) was determined by allele-specific, real-time polymerase chain reaction,²⁵ using the TaqMan SNP Genotyping Assay for MTHFR (Applied Biosystems, Foster City, CA).

Sample Size Calculation

Because there were no available data in children at study initiation, we estimated the sample size from previous studies using adults. A thorough review of the literature pertaining to the use of nitrous oxide of any duration during anesthesia in adults suggested that on average a 0.55 SD increase in plasma tHcy concentration would be expected.^{4–7,9–11,26} We estimated that a final sample size of 28 children would enable us to observe a 0.55 SD difference in plasma tHcy concentrations before surgery *versus* after surgery with 80% power and an α level of 0.05.

Statistical Analyses

Distributions of continuous variables were assessed for normality using the Kolmogorov–Smirnov test, and skewed data were transformed where possible. Parametric data are reported as mean \pm SD and nonparametric data as median (first, third quartile). Student *t* tests or Wilcoxon signed-rank test were used for two-group comparisons of parametric and nonparametric data, respectively. Linear univariate regression analysis was performed to identify significant associations between various sociodemographic variables and blood biochemical indices and the main outcome variables: preoperative plasma tHcy concentration and increase in plasma tHcy concentration after anesthesia. Multivariate analyses (general linear model) were then performed, including variables from the univariate analyses shown to be associated with the increase in plasma tHcy after anesthesia ($P < 0.05$). All statistical analyses were performed with SAS software (version 9.1; SAS Institute Inc., Cary, NC) and a two-tailed $P < 0.05$ was considered statistically significant.

Results

Participant Characteristics and Preoperative Plasma tHcy Concentration

The sample consisted of 18 boys and 14 girls with a median (first, third quartile) age of 11 (5, 40.5) months and weight of 9.2 (7.1, 15.5) kg. Subjects underwent surgery to repair a birth defect but were otherwise healthy: oral cleft, 17; speech repair, 3; brachial plexus repair, 3; facial correction, 4; other, 5. Only three children consumed multivitamin supplements containing folic acid (260 or 400 μg) and vitamins B12 (6 μg) and B6 (2 mg). Results for the vitamin biomarkers can be found in table 1. The median preoperative plasma tHcy concentration of all children was 5.1 (4.1, 6.4) μM . The only measured variables associated with preoperative plasma tHcy were vitamin B12 and MMA concentrations (table 2).

Effect of Nitrous Oxide Exposure

For all children, anesthesia was induced using nitrous oxide. The median (first, third quartile) duration of exposure to nitrous oxide was 180 (142.0, 217.5) min, and the median concentration was 60% (60%, 65%) in the inhaled gas mixture. Six of 28 (21%) children with normal, age-appropriate, preexposure plasma tHcy concentrations had postoperative plasma tHcy concentrations greater than cutoff values (fig. 1). The median increase in plasma tHcy concentration was 1.2 μM (0.1, 2.7 μM ; $P < 0.0001$), an increase of 25% (2%, 42%). This increase remained statistically significant after removing the results for three children whose plasma tHcy concentrations increased more than 2 SD (outliers) above the mean of the other children (6.7 ± 2.8 *vs.* 5.2 ± 1.5 μM , respectively; $P < 0.0003$). Two of the children whose tHcy values were outliers had been exposed to nitrous oxide for more than 700 min. The third child presented with a preoperative plasma MMA concentration indicative of vitamin B12 deficiency. All 32 subjects were included in all other statistical analyses. Nine children exposed to more than 3.5 h of nitrous oxide (median 246[225,667] min) experienced an approximate 80% increase in plasma tHcy concentration

Table 2. Results from Univariate Linear Regression Analyses Comparing Various Potential Prognostic Variables and Either Preoperative tHcy Concentration or the Increase in Postoperative tHcy Concentration

Predictor Variable	Preoperative tHcy Concentration			Increase in Postoperative tHcy Concentration		
	Preoperative tHcy R ²	Standardized Coefficient (β)	P Value	Increase in tHcy R ²	Standardized Coefficient (β)	P Value
Sex	0.034	-0.186	0.309	0.094	0.308	0.086
Age	0.004	-0.066	0.722	0.061	-0.246	0.174
Weight	0.003	-0.056	0.762	0.031	-0.176	0.335
Duration of nitrous oxide exposure	—	—	—	0.696	0.835	<0.0001
Preoperative tHcy	—	—	—	0.305	0.552	0.001
Folate	0.021	-0.145	0.427	0.001	0.036	0.843
Vitamin B6	0.048	-0.219	0.433	0.055	0.237	0.395
Vitamin B12	0.277	-0.526	0.004	0.149	-0.387	0.046
MMA (continuous variable)	0.257	0.507	0.007	0.065	0.254	0.201
MMA status ($\geq 0.21 \mu\text{M}$ deficient)	0.196	0.446	0.020	0.001	0.037	0.853
MTHFR C677T genotype	0.050	-0.226	0.230	0.038	-0.197	0.297

Standardized coefficient interpreted as the amount of change in either the preoperative tHcy concentration or increase in tHcy concentration as a result of a change (SD units) in each of the predictor variables.

MMA = methylmalonic acid; MTHFR = methylenetetrahydrofolate reductase; tHcy = total homocysteine.

(median 4.1 [4.0, 6.1] *vs.* 7.5 [4.7, 19.3] μM , respectively; $P = 0.0078$).

The duration of nitrous oxide exposure (minutes) was associated with the increase in plasma tHcy concentration before and after surgery ($R^2 = 0.696$, $P = <0.0001$; table 2, fig. 2). We observed a greater spread in tHcy concentrations at higher nitrous oxide exposures. To correct for heteroscedasticity of the data and reduce the skewness of both the dependent and independent variables and, consequently, the high leverage of the more extreme values, we reanalyzed our data using a fourth root transformation of both the dependent and independent variables after replacing some small

negative values with zero. Our conclusions were unchanged when this model was used.

When expressed as total nitrous oxide exposure (minutes \times nitrous oxide concentration), the association between duration of nitrous oxide exposure and the increase in tHcy concentration after surgery remained unchanged ($R^2 = 0.687$, $P = <0.0001$). In a similar manner, preoperative plasma tHcy concentrations were associated positively with the increase in plasma tHcy concentrations after nitrous oxide exposure (table 2). Serum vitamin B12 concentrations were associated inversely with the increase in plasma tHcy concentrations after nitrous oxide exposure ($R^2 = 0.149$;

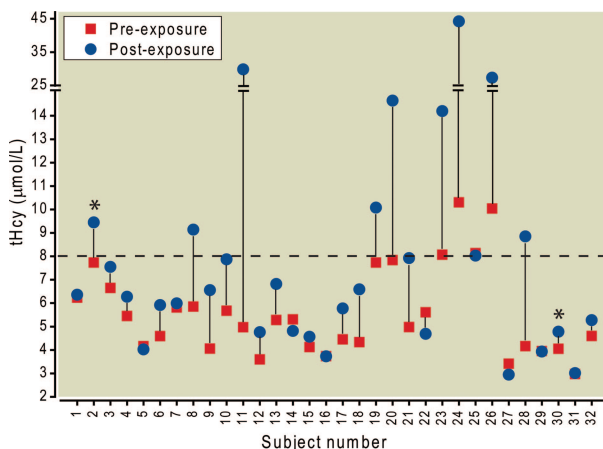


Fig. 1. The plasma total homocysteine concentration (tHcy) of children before and after exposure to nitrous oxide. Subjects TT homozygous for the methylenetetrahydrofolate reductase (MTHFR) C677T genotype are identified with asterisks. The horizontal line at 8 μM denotes the cutoff used to define normal plasma tHcy concentration in young children.

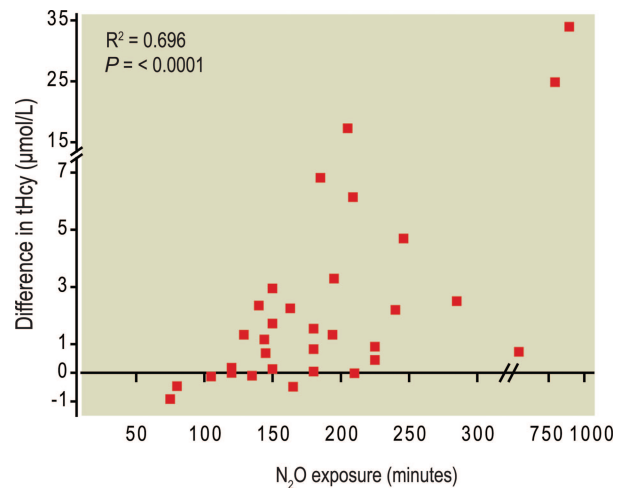


Fig. 2. Association between the duration of nitrous oxide exposure ($\geq 50\%$) and the increase in total plasma homocysteine (tHcy) concentration. The increase in plasma tHcy concentration was calculated as the difference between plasma tHcy concentration before and after surgery.

Table 3. Total Plasma Homocysteine Concentration of Children by MTHFR Genotype

MTHFR C677T Genotype	n	Preoperative tHcy (μM)	Postoperative tHcy (μM)	Nitrous Oxide Exposure Time (min)
All genotypes	32	5.1 (4.1, 6.4)	6.4 (4.7, 8.9)	180.0 (142.0, 217.5)
CC homozygous	15	5.3 (4.1, 7.8)	6.8 (5.9, 14.2)	180.0 (145.0, 225.0)
CT heterozygous	14	5.1 (4.0, 5.8)	5.8 (4.0, 8.5)	164.0 (120.0, 202.5)
TT homozygous	2	7.7, 4.0	9.5, 4.8	150, 540

n = the number of subjects per genotype. Values presented are median (first, third quartiles), except for the TT homozygous group, for which individual values are provided.

MTHFR = methylenetetrahydrofolate reductase; tHcy = total homocysteine.

$P = 0.046$). The impact of nitrous oxide exposure on the increase in Hcy concentration by MTHFR C677T genotype is summarized in table 3.

Multivariate Linear Regression Analyses

Multiple linear regression analyses were conducted to determine the influence of more than one associated variable (as determined by univariate analysis) on the change in plasma tHcy concentration from before to after surgery. Potential variables for inclusion were duration of exposure to nitrous oxide, preoperative plasma tHcy concentration, and serum vitamin B12 concentration. Because the serum vitamin B12 concentration was inversely associated with the preoperative plasma tHcy concentration, we ran the multiple linear regression analyses twice, first with the preoperative plasma tHcy concentration and duration of nitrous oxide exposure and then with the serum vitamin B12 concentration and the duration of nitrous oxide exposure. In the first model, the duration of nitrous oxide exposure and preoperative plasma tHcy concentration explained 82% of variance in the increase in postoperative plasma tHcy concentration ($R^2 = 0.821$; $P < 0.0001$). In the second model, the duration of nitrous oxide exposure and serum vitamin B12 concentration accounted for 72% of the increase in postoperative plasma tHcy concentration ($R^2 = 0.721$; $P < 0.0001$).

Discussion

In this study, we investigated the effects of exposure to nitrous oxide anesthesia on the plasma tHcy concentrations in young children the morning after surgery. In our sample of children exposed to nitrous oxide (≥ 2 h) at an inspired concentration of $\geq 50\%$, postoperative plasma tHcy concentrations increased by 25% ($1.2 \mu\text{M}$), a small, albeit statistically significant, increase. This magnitude of increase is consistent with reports of an overall average 20–26% increase in plasma tHcy concentrations after exposure to nitrous oxide in adults (~ 3 h at $\sim 70\%$ inspired concentration)^{5,7} but less than the 228% ($9.4 \mu\text{M}$) increase newly reported for adolescents (0.5–10 h at $\sim 55\%$ inspired concentration) 6–8 h after the induction of anesthesia.³ By 24 h after anesthesia induction in the latter study, plasma tHcy concentrations had returned to baseline.

The duration of nitrous oxide exposure was the main factor associated with the increase in postoperative plasma tHcy concentrations among young children in our study, a relationship first reported in adults by Ermens *et al.*⁴ and subsequently confirmed by others.^{5–7,9,10} In the current study, children exposed to more than 3.5 h of nitrous oxide ($n = 9$) presented with postoperative plasma tHcy concentrations approximately 80% greater than initial concentrations. Similarly, Nagele *et al.* reported an 80% increase in (median [first, third quartile]) plasma tHcy concentration from baseline ($8.1 [7.3, 10.0] \mu\text{M}$) to the end of surgery ($14 [13.1, 22.8] \mu\text{M}$) among 16 adults exposed to nitrous oxide for more than 4 h.⁷

Preoperative plasma tHcy concentration was also significantly associated with the increase in plasma tHcy concentration after anesthesia among children in the current study ($R^2 = 0.305$, $P = 0.001$), a finding consistent with that reported for adults.²⁷ In the current study, both serum vitamin B12 and plasma MMA concentrations were significantly associated with baseline tHcy, and serum vitamin B12 was associated with the increase in tHcy after nitrous oxide exposure. These findings are consistent with the known metabolic role of vitamin B12 as a cofactor in the methyl transfer reactions involved in the conversion of homocysteine to methionine.²⁸ At a population level, vitamin B12 has been identified as a determinant of plasma tHcy concentrations in children.^{29,30} Whether supplementation of patients with vitamin B12 before nitrous oxide anesthesia would prevent the postoperative increase in tHcy remains to be determined.^{8,26}

The clinical significance of an acute increase in plasma tHcy concentrations induced by nitrous oxide is unclear. Five children in our study presented with postoperative plasma tHcy concentrations greater than $13.5 \mu\text{M}$. Published studies of adults treated with nitrous oxide found that postoperative plasma tHcy concentrations $\geq 13.5 \mu\text{M}$ were associated with myocardial infarction and stroke,⁵ concentrations of $14.1 \pm 5.7 \mu\text{M}$ were associated with endothelial dysfunction,⁶ and concentrations greater than $17 \mu\text{M}$ were associated with a 2-fold increased risk of myocardial ischemia.⁹ Whether increased plasma tHcy concentrations play a causal role in these adverse cardiovascular outcomes or merely reflect nitrous oxide exposure will need to be clarified in future studies.

We acknowledge a number of limitations in our study. To minimize the need to collect blood samples from small children, we did not include a randomized control group for which an anesthetic without nitrous oxide was used. Rather, we obtained a blood sample before and after surgery from each child. We do not think surgery alone was responsible for the increase in plasma tHcy concentrations in the current investigation because the ENIGMA Trial and other studies in adults have shown that plasma tHcy concentrations do not increase after anesthesia during surgery using other anesthetic agents.^{5,8} All children in this study were recruited from the Plastics Surgery Clinic at The Hospital for Sick Children, where surgery was being arranged for treatment of a birth defect. We can not rule out the possibility that some of the children in our study had underlying genetic defects that could have affected their response to nitrous oxide. Likewise, we are uncertain whether our results in this otherwise healthy cohort of children can be generalized to children exposed to nitrous oxide who are of poor health or have major organ dysfunction. We acknowledge that our sample size was too small to explore the effect of MTHFR C677T genotype. In small children nitrous oxide often is used to induce anesthesia, and this was the case for all subjects in the current study. Therefore, the nitrous oxide used for induction inadvertently may have increased the baseline plasma tHcy concentration, resulting in a lower than expected postoperative increase. We acknowledge several statistical inferences were made in this study without adjustment of the *P* value to account for multiple comparisons. Although most comparisons were planned, it does raise the possibility of a type I error. Finally, it is possible that the peak in plasma tHcy concentration after nitrous oxide exposure occurred before collection of the second blood sample in our study. If this was the case, the increase in plasma tHcy concentrations reported in the current study likely is a very conservative estimate of the increase.

In conclusion, children exposed to ≥ 2 h nitrous oxide anesthesia showed a small but significant increase in plasma tHcy concentrations the morning after surgery (1.2 μM [0.1, 2.7 μM]). The clinical significance of this increase is unknown. The duration of exposure to nitrous oxide and initial plasma tHcy concentrations were associated significantly with the increase in plasma tHcy concentrations after nitrous oxide exposure.

The authors thank Christopher Forrest, M.D., M.Sc., F.R.C.S.(C), F.A.C.S., Division Head, Department of Plastic Surgery, The Hospital for Sick Children, Toronto, Ontario, Canada, and Associate Professor, Department of Surgery, University of Toronto, Toronto, Ontario, Canada.

References

- Finkelstein JD: Pathways and regulation of homocysteine metabolism in mammals. *Semin Thromb Hemost* 2000; 26: 219-25
- Zeisel SH, Blusztajn JK: Choline and human nutrition. *Annu Rev Nutr* 1994; 14:269-96
- Nagele P, Tallchief D, Blood J, Sharma A, Kharasch ED: Nitrous oxide anesthesia and plasma homocysteine in adolescents. *Anesth Analg* 2011; 113:843-8
- Ermens AA, Refsum H, Ruprecht J, Spijkers LJ, Guttormsen AB, Lindemans J, Ueland PM, Abels J: Monitoring cobalamin inactivation during nitrous oxide anesthesia by determination of homocysteine and folate in plasma and urine. *Clin Pharmacol Ther* 1991; 49:385-93
- Myles PS, Chan MT, Leslie K, Peyton P, Paech M, Forbes A: Effect of nitrous oxide on plasma homocysteine and folate in patients undergoing major surgery. *Br J Anaesth* 2008; 100: 780-6
- Myles PS, Chan MT, Kaye DM, McIlroy DR, Lau CW, Symons JA, Chen S: Effect of nitrous oxide anesthesia on plasma homocysteine and endothelial function. *ANESTHESIOLOGY* 2008; 109:657-63
- Nagele P, Zeugswetter B, Wiener C, Burger H, Hüpfel M, Mittlböck M, Födinger M: Influence of methylenetetrahydrofolate reductase gene polymorphisms on homocysteine concentrations after nitrous oxide anesthesia. *ANESTHESIOLOGY* 2008; 109:36-43
- Rao LK, Francis AM, Wilcox U, Miller JP, Nagele P: Preoperative vitamin B infusion and prevention of nitrous oxide-induced homocysteine increase. *Anaesthesia* 2010; 65:710-5
- Badner NH, Beattie WS, Freeman D, Spence JD: Nitrous oxide-induced increased homocysteine concentrations are associated with increased postoperative myocardial ischemia in patients undergoing carotid endarterectomy. *Anesth Analg* 2000; 91:1073-9
- Badner NH, Drader K, Freeman D, Spence JD: The use of intraoperative nitrous oxide leads to postoperative increases in plasma homocysteine. *Anesth Analg* 1998; 87:711-3
- Foschi D, Rizzi A, Zighetti ML, Bissi M, Corsi F, Trabucchi E, Mezzetti M, Cattaneo M: Effects of surgical stress and nitrous oxide anaesthesia on peri-operative plasma levels of total homocysteine. A randomised, controlled study in general surgery. *Anaesthesia* 2001; 56:676-9
- Banks RGS, Henderson RJ, Pratt JM: Reactions of gases in solution. Part III. Some reactions of nitrous oxide with transition-metal complexes. *J Chem Soc* 1968; A:2886-89
- Guttormsen AB, Refsum H, Ueland PM: The interaction between nitrous oxide and cobalamin. Biochemical effects and clinical consequences. *Acta Anaesthesiol Scand* 1994; 38: 753-6
- Chanarin I: Cobalamins and nitrous oxide: A review. *J Clin Pathol* 1980; 33:909-16
- Leslie K, Myles PS, Chan MT, Forbes A, Paech MJ, Peyton P, Silbert BS, Williamson E: Nitrous oxide and long-term morbidity and mortality in the ENIGMA trial. *Anesth Analg* 2011; 112:387-93
- Refsum H, Smith AD, Ueland PM, Nexø E, Clarke R, McPartlin J, Johnston C, Engbaek F, Schneede J, McPartlin C, Scott JM: Facts and recommendations about total homocysteine determinations: An expert opinion. *Clin Chem* 2004; 50:3-32
- Goyette P, Christensen B, Rosenblatt DS, Rozen R: Severe and mild mutations in cis for the methylenetetrahydrofolate reductase (MTHFR) gene, and description of five novel mutations in MTHFR. *Am J Hum Genet* 1996; 59:1268-75
- Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, Rozen R: A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; 10:111-3
- Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE: Biological and clinical implications of the MTHFR C677T polymorphism. *Trends Pharmacol Sci* 2001; 22:195-201
- Ganji V, Kafai MR: Population references for plasma total homocysteine concentrations for U.S. children and adolescents in the post-folic acid fortification era. *J Nutr* 2005; 135:2253-6

21. Rafii M, Elango R, Courtney-Martin G, House JD, Fisher L, Pencharz PB: High-throughput and simultaneous measurement of homocysteine and cysteine in human plasma and urine by liquid chromatography-electrospray tandem mass spectrometry. *Anal Biochem* 2007; 371:71-81
22. Molloy AM, Scott JM: Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods Enzymol* 1997; 281:43-53
23. Yazdanpanah M, Chan PC, Evrovski J, Romaschin A, Cole DE: An improved assay for plasma methylmalonic acid using chemical ionization gas chromatography mass spectrometry. *Clin Biochem* 2003; 36:617-20
24. Rybak ME, Pfeiffer CM: Clinical analysis of vitamin B(6): Determination of pyridoxal 5'-phosphate and 4-pyridoxic acid in human serum by reversed-phase high-performance liquid chromatography with chlorite postcolumn derivatization. *Anal Biochem* 2004; 333:336-44
25. Eny KM, Wolever TM, Fontaine-Bisson B, El-Sohemy A: Genetic variant in the glucose transporter type 2 is associated with higher intakes of sugars in two distinct populations. *Physiol Genomics* 2008; 33:355-60
26. Badner NH, Freeman D, Spence JD: Preoperative oral B vitamins prevent nitrous oxide-induced postoperative plasma homocysteine increases. *Anesth Analg* 2001; 93:1507-10
27. Nagele P, Zeugswetter B, Eberle C, Hüpfel M, Mittlböck M, Födinger M: A common gene variant in methionine synthase reductase is not associated with peak homocysteine concentrations after nitrous oxide anesthesia. *Pharmacogenet Genomics* 2009; 19:325-9
28. Groff S, Gropper SAS. *Folic acid, Advanced Nutrition and Human Metabolism*, 3rd edition. Edited by Gropper SAS, Groff S. Belmont, California, Thomson/Wadsworth, 2004, pp 289-98
29. Monsen AL, Refsum H, Markestad T, Ueland PM: Cobalamin status and its biochemical markers methylmalonic acid and homocysteine in different age groups from 4 days to 19 years. *Clin Chem* 2003; 49:2067-75
30. Rogers LM, Boy E, Miller JW, Green R, Sabel JC, Allen LH: High prevalence of cobalamin deficiency in Guatemalan schoolchildren: Associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations. *Am J Clin Nutr* 2003; 77:433-40
31. Pfeiffer CM, Johnson CL, Jain RB, Yetley EA, Picciano MF, Rader JI, Fisher KD, Mulinare J, Osterloh JD: Trends in blood folate and vitamin B-12 concentrations in the United States, 1988-2004. *Am J Clin Nutr* 2007; 86:718-27
32. United Nations Children's Fund, United Nations University, World Health Organization: *Iron Deficiency Anemia Assessment, Prevention and Control: A Guide for Program Managers* (Report No.: WHO/NHD/01.3). Geneva, Switzerland, World Health Organization, 2001
33. Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ: Biochemical indicators of B vitamin status in the US population after folic acid fortification: Results from the National Health and Nutrition Examination Survey 1999-2000. *Am J Clin Nutr* 2005; 82:442-50
34. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Panel on Folate, Other B Vitamins, and Choline, Subcommittee on Upper Reference Levels of Nutrients, Food and Nutrition Board, Institute of Medicine: *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington, D.C., National Academy Press, 1998
35. Allen LH: How common is vitamin B-12 deficiency? *Am J Clin Nutr* 2009; 89:693S-6S