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Nitrous Oxide Anesthesia and Plasma Homocysteine in Adolescents

Peter Nagele, MD, MSc, Danielle Tallchief, BS, RN, Jane Blood, BS, RN, Anshuman Sharma, MD, and Evan D. Kharasch, MD, PhD

Department of Anesthesiology, Washington University, St. Louis, MO

Abstract

Background—Nitrous oxide inactivates vitamin B₁₂, inhibits methionine synthase and consequently increases plasma total homocysteine (tHcy). Prolonged exposure to nitrous oxide can lead to neuropathy, spinal cord degeneration and even death in children. We tested the hypothesis that nitrous oxide anesthesia causes a significant increase in plasma tHcy in children.

Methods—Twenty-seven children (age 10-18 years) undergoing elective major spine surgery were enrolled and serial plasma samples from 0 – 96 hours after induction were obtained. The anesthetic regimen, including the use of nitrous oxide, was at the discretion of the anesthesiologist. Plasma tHcy was measured using standard enzymatic assays.

Results—The median baseline plasma tHcy concentration was 5.1 µmol/L (3.9 – 8.0 µmol/L, interquartile range) and increased in all patients exposed to nitrous oxide (n=26) by an average of +9.4 µmol/L (geometric mean; 95% CI 7.1 – 12.5 µmol/L) or +228% (mean; 95% CI 178% - 279%). Plasma tHcy peaked between 6-8 hours after induction of anesthesia. One patient who did not receive nitrous oxide had no increase in plasma tHcy. Several patients experienced a several-

Corresponding Author: Peter Nagele, MD, MSc, Washington University, Dept of Anesthesiology, Washington University School of Medicine 660 S. Euclid Ave, Box 8054, St. Louis, MO 63110, Phone: 314-362-5129, FAX: 314-362-1185, nagelep@wustl.edu.

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Name: Peter Nagele, MD, MSc

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Name: Danielle Tallchief, BS, RN

Contribution: This author helped conduct the study.

Attestation: Danielle Tallchief has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

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Name: Jane Blood, BS, RN

Contribution: This author helped conduct the study.

Attestation: Jane Blood has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

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Name: Anshuman Sharma, MD

Contribution: This author helped design the study, conduct the study, and write the manuscript.

Attestation: Anshuman Sharma has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

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Name: Evan D. Kharasch, MD, PhD

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fold increase in plasma tHcy (max. +567%). The increase in plasma tHcy was strongly correlated with the duration and average concentration of nitrous oxide anesthesia ($r = 0.80$; $p < 0.001$).

Conclusions—Pediatric patients undergoing nitrous oxide anesthesia develop significantly increased plasma tHcy concentrations. The magnitude of this effect appears to be greater compared to adults; however, the clinical relevance is unknown.

Introduction

Nitrous oxide (N_2O) has an unusual toxic side effect, unrelated to its anesthetic action: it inactivates vitamin B_{12} (cobalamin).^{1,2} The inactivation of cobalamin and cobalamin-dependent enzymes, such as methionine synthase, is chemically irreversible and can last clinically up to one week until new enzyme is synthesized.³ In patients, the inactivating effects of N_2O on cobalamin can be observed by an increase in plasma total homocysteine (tHcy) because methionine synthase, the enzyme responsible for the conversion of homocysteine to methionine, requires cobalamin as co-enzyme.^{4,5}

Clinically, cobalamin inactivation by N_2O and the subsequent increase in plasma tHcy and methionine deficiency can lead to megaloblastic bone marrow changes,⁶ neuropathy,⁷ severe spinal cord degeneration,⁸ and even death, particularly in patients with preexisting inborn or acquired disorders of folate metabolism. In 2003, the death of a 4 month-old infant with a previously unknown severe deficiency in a core enzyme of the folate cycle was attributed to the use of N_2O .⁹

The safety of N_2O has been questioned, both in adult¹⁰ and pediatric patients¹¹. However, the inactivating effects of N_2O on cobalamin and subsequent increase in plasma tHcy have never been systematically investigated in children. To our knowledge, this is the first study to test the hypothesis that N_2O anesthesia causes a significant increase in plasma tHcy in children.

Methods

This was an ancillary study, performed on residual plasma samples, from a pharmacokinetic study of approved anesthetic drugs in children, which was approved by the Washington University IRB. Written informed consent was obtained from parents and assent from children. The ancillary study also received Institutional Review Board approval.

Study Design

The parent study included pediatric patients scheduled for elective major spine surgery. Most patients suffered from idiopathic scoliosis. Patients could be included if they met the following inclusion criteria: age 5-18 years; undergoing general anesthesia and surgery with anticipated postoperative inpatient stay of ≥ 3 days ; and signed, written informed consent from legal guardians and assent from the patient. Patients were excluded if they had a history of liver or kidney disease, or were pregnant or nursing females. An additional inclusion criterion for the ancillary study was that sufficient residual plasma samples were available.

The patients received standard general anesthesia and monitoring. The protocol did not specify the choice of anesthetic drugs, including the use of N_2O , which were left at the sole discretion of the anesthesia team.

Measurements

Plasma samples obtained at baseline, and 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours after induction of anesthesia were analyzed. Baseline samples were obtained immediately after an IV line was placed, most commonly after inhaled induction of anesthesia. Blood samples were immediately put on ice and spun down within 2 hours of collection. Samples were then frozen at -80°C until assayed.

Plasma tHcy was measured on a Roche Hitachi 917 analyzer using the Diazyme homocysteine enzymatic assay (Diazyme Laboratories, Poway, CA) with reagents from Genzyme (Genzyme, Cambridge, MA). This assay has excellent correlation with high performance liquid chromatography and immunochemical methods with a linear range of 3-50 $\mu\text{mol/L}$ and inter %CV (coefficient of variation) values of <5%.¹²

Statistical analysis

Peak plasma tHcy concentrations were compared to baseline tHcy and absolute and relative changes calculated. Cumulative N₂O exposure was calculated as the product of average N₂O concentration used (as fraction of 1) and the duration of N₂O exposure (N₂O*min). As an example, if a patient had a N₂O anesthesia duration of 300 minutes at 50% concentration (=0.5), the resultant product would be 150 N₂O*min.

This calculation was done by hand from paper anesthesia records with measurements every 5-minute interval.

To model the effects of N₂O anesthesia on plasma tHcy in this longitudinal dataset, we used a linear mixed regression model (random slopes and random intercepts) with log-transformed tHcy as a dependent variable and a first-order autoregressive covariance structure.¹³ Several models were compared and the most parsimonious chosen based on -2LL (negative log-likelihood) or Akaike Information Criterion (AIC). A linear correlation was examined between N₂O*min and peak tHcy using the Pearson correlation coefficient and a simple linear regression performed; results include the 95% confidence intervals. All reported tests are two-sided and a p-value of <0.05 was considered statistically significant.

Results

In this study, 27 pediatric patients from the parent study cohort (n= 30) were included (Table 1). Most patients underwent idiopathic scoliosis repair.

At baseline, the median plasma tHcy concentration was 5.1 $\mu\text{mol/L}$ (0.9 – 9.8 $\mu\text{mol/L}$; min – max) and several patients (5/18 female, 28%; 0/9 male, 0%) met the diagnostic criteria for mild hyperhomocysteinemia (normal range for plasma tHcy at 12-19 years of age: female 3.3 – 7.2 $\mu\text{mol/L}$; male 4.3 – 9.9 $\mu\text{mol/L}$) (Table 1).

All but one pediatric patient received N₂O intraoperatively. Four children were only briefly (less than 30 minutes) exposed to N₂O during induction of or emergence from general anesthesia. The remaining 23 patients received N₂O (average concentration 55%) for the full duration of the spine surgery which in some patients lasted up to 10 hours.

All children who were exposed to N₂O (n=26) had a subsequent increase in their plasma tHcy concentration by an average of +9.4 $\mu\text{mol/L}$ (geometric mean; 95% CI 7.1 – 12.5 $\mu\text{mol/L}$) or +228% (mean; 95% CI 178% - 279%) (Figure 1 and Table 2). The increase in plasma tHcy was highly significant and peaked between 6-8 hours (Figure 2), most commonly just after the cessation of general anesthesia. Most children experienced a severalfold increase of their plasma tHcy concentration (maximum: +567%) and the

maximum observed peak concentration was 38.6 $\mu\text{mol/L}$. The highest tHcy levels were observed in children with high baseline plasma tHcy levels. No difference was observed between female and male patients. A linear mixed model was used to model the effects of N_2O exposure on plasma tHcy within each individual patient and among all patients. The results of the mixed model show a statistically highly significant difference among all patients ($p < 0.001$), the time-points ($p < 0.001$) and also a significant effect of the cumulative N_2O dose ($\text{N}_2\text{O} \cdot \text{min}$, $p = 0.02$) and time-point. Twenty-four hours after anesthesia start time plasma tHcy concentrations largely reverted to their baseline levels.

The magnitude of the increase in plasma tHcy caused by N_2O was strongly correlated with the product of duration of N_2O anesthesia and average N_2O concentration used ($\text{N}_2\text{O} \cdot \text{min}$) (Pearson's $r = 0.80$, 95% CI 0.61 – 0.91; $p < 0.0001$; Figure 3). More than 64% of the observed variation in plasma tHcy increase could be explained by the $\text{N}_2\text{O} \cdot \text{min}$ variable, making it a strong and significant predictor.

Discussion

This study showed that pediatric patients develop a significant increase in plasma tHcy when receiving N_2O during prolonged general anesthesia. Some children in our study developed a 400 to 600% increase from their baseline plasma tHcy levels, signifying a magnitude of an inhibitory effect of N_2O on folate and methionine metabolism that is larger than commonly observed in adult patients. Even though our patients had very long N_2O anesthesia durations, comparable studies in adults report increases in plasma tHcy of only between 50 – 80%. Badner et al. report a 74% increase;⁴ Myles et al. only 50% even after > 8 hours of anesthesia;¹⁴ and our own previous study shows an increase of 80% for > 4hrs of N_2O anesthesia.¹⁵ Only Ermens et al. show comparable results, although only reported as correlation.³ Our findings suggest that children experience an at least similar, if not greater, inhibition of methionine synthase and folate metabolism by N_2O but the lack of comparable pediatric studies make it difficult to generalize the results from a single study.

Baseline plasma tHcy levels varied widely within our study population and several children had mild hyperhomocysteinemia (normal range for plasma tHcy at 12-19 years of age: female 3.3 – 7.2 $\mu\text{mol/L}$; male 4.3 – 9.9 $\mu\text{mol/L}$). Baseline plasma tHcy levels vary within the population and are influenced by environmental (nutrition, folic acid and vitamin B₁₂ intake) as well as genetic factors, most importantly the MTHFR C677T polymorphism, considered the most important genetic predictor.¹⁶

A surprising finding of this study was that nearly all plasma tHcy levels reverted into the normal range after 24 hours, irrespective of the magnitude of the increase caused by N_2O . Until now, it was commonly believed and reported from adult patients that N_2O -induced hyperhomocysteinemia extends well beyond the immediate postoperative period and can last up to 1 week after exposure.³ If children have a better ability to recover from N_2O -induced inhibition of methionine synthase compared to adults or this observation was specific for our study population is unclear. A possible explanation may be that our patients experienced large shifts in their fluid balance including intravascular volume resuscitation and blood transfusions which may have decreased plasma homocysteine concentrations.

What is the clinical relevance of these findings? Two related but separate issues need to be addressed.

First, the question of adverse drug reactions caused by N_2O 's inhibition of methionine synthase. Over the last 60 years, a multitude of case reports and series have been published showing the following adverse clinical outcomes unequivocally and directly attributed to N_2O 's inactivation of vitamin B₁₂ and subsequent inhibition of methionine synthase: fatal⁹

and nonfatal¹⁷ neurologic degeneration, myelopathy,^{8,18-21} peripheral neuropathy,^{7,22-25} bone marrow depression and megaloblastic bone marrow changes^{6,26-31} and an increased risk for infection.^{32,33} Mostly these toxic side effects were only observed after prolonged or repeated exposure to N₂O (>24 hrs) or among N₂O drug abusers. While being apparently fairly uncommon, N₂O clearly can cause hematological and neurological side effects that may be irreversible and/or fatal. The true incidence of N₂O's adverse neurological and hematological effects when used in a clinically appropriate manner and dose has never been systematically investigated, neither in adults nor children.

The second question is, what are the clinical consequences of an acute increase in plasma homocysteine concentrations? This question is much more difficult to answer. As the substrate for methionine synthase, homocysteine accumulates due to the inhibition by N₂O. It is therefore possible that an acute increase in plasma tHcy in itself causes no additional adverse effects; alternatively, a direct toxic effect of elevated plasma tHcy may also be possible. While chronic plasma homocysteine increase at the level observed in our study (up to 40 µmol/L) is unequivocally associated with a substantially increased risk of atherosclerosis, coronary artery disease³⁴ and premature death in adults³⁵, as well as venous thromboembolism in children³⁶, the effects of an acute increase in plasma tHcy are largely unknown. The direct effects of an acute increase in blood homocysteine levels on the cardiovascular system have mostly been shown experimentally³⁷⁻³⁹ and not been unequivocally correlated with any clinical outcomes (separate from N₂O's inhibition of methionine synthase).

This study has several strengths and limitations. It must be emphasized that this study was exploratory in nature, taking advantage of a unique opportunity to collect serial blood samples in a pediatric population, and therefore was not designed to investigate any clinical outcomes.

The present observational study did not have a formal control group which did not receive N₂O. Two independent lines of evidence strongly suggest that in our study the exposure to N₂O was causally linked to the subsequent increase in plasma tHcy. First, it is commonly accepted that in non-experimental, observational studies the presence of a dose-response relationship infers, but naturally does not prove, causality. In our study, a very strong relationship between the cumulative N₂O dose and subsequent increase in homocysteine was found ($r=0.80$). Second, it has been repeatedly shown in more than 6 human studies (>1,000 patients) that in the absence of N₂O, patients will not develop an increase in postoperative plasma tHcy.^{3,4,14,40-42} Based on these preexisting data, it is a reasonable prediction that we would have observed a similar result (no increase in plasma tHcy without N₂O), if our study had a formal control group.

The study was limited in its generalizability because it enrolled mostly teenagers undergoing major spine surgery and no patient younger than 10 years of age. No study has investigated the interaction between N₂O, folate metabolism and homocysteine in younger children (0-10 years) thus far, so our findings should not be extrapolated to younger children. Because most of our patients suffered from scoliosis it is possible that the findings of this study were influenced by scoliosis-associated conditions or spine surgery (prone position, large fluid shifts). Our study did not determine any influence of gene variants, most notably the MTHFR C677T polymorphism, which is considered the most important genetic predictor for baseline blood tHcy concentrations and which also has a significant effect on the increase in plasma tHcy after N₂O anesthesia.¹⁵

Conclusions

This study showed that children undergoing N₂O anesthesia develop significantly increased plasma tHcy concentrations. The clinical relevance of this observation is unknown and would require well-designed randomized controlled trials.

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Figure 1.

Response of plasma total homocysteine concentrations among individual study patients (n=27). tHcy = total homocysteine. Plasma tHcy concentrations exceeding 10 $\mu\text{mol/L}$ are commonly considered abnormal. Red dotted line: patients who received nitrous oxide only during induction and emergence; red full line: no exposure to nitrous oxide.

Figure 2.

Box plot representation of pooled average response of plasma total homocysteine at different timepoints. Boxes represent the interquartile range, the line the median and the whiskers the 5 – 95 percentile. tHcy = total homocysteine. Asterisks refer to statistical significance (P value : ** 0.001 – 0.01; *** < 0.001)

Figure 3.
Linear correlation of the increase in plasma total homocysteine and the cumulative nitrous oxide dose (N₂O*min). tHcy = total homocysteine

Table 1

Characteristics of the study population

Pat #	Sex	Age (yr)	Height (cm)	Weight (kg)	Baseline tHcy (μmol/L)	Primary Diagnosis	Co-Morbidities	Chronic Medications
1	F	12	145	50	3.7	Idiopathic Scoliosis	Dextrocardia, Aortic root dilatation, Asthma	Fluticasone
2	F	17	156	55	7.6	Idiopathic Scoliosis	Kidney stones	Loratadine
3	M	15	156	54	8.0	Idiopathic Scoliosis		
4	F	12	172	66	4.5	Idiopathic Scoliosis		
5	F	11	150	39	5.4	Thoracic Scoliosis	Seasonal allergies	Montelukast Sodium
6	F	14	165	80	4.9	Idiopathic Scoliosis		
7	F	13	160	67	8.6	Idiopathic Scoliosis		Fexofenadine
8	F	13	158	51	4.4	Idiopathic Scoliosis	Seasonal allergies, Migraine	Cetirizine
9	M	14	177	77	5.1	L4 Compression Fracture		Cyclobenzaprine, Propoxyphene, Acetaminophen
10	F	13	150	56	9.0	Idiopathic Scoliosis		
11	F	14	156	54	6.7	Idiopathic Scoliosis	Anxiety, Depression	Bupropion
12	F	11	163	41	3.8	Idiopathic Scoliosis	Febrile seizure	Diphenhydramine, Loratadine, Multivitamin
13	F	10	147	41	3.2	Idiopathic Scoliosis		
14	M	11	152	38	4.7	Idiopathic Scoliosis		Multivitamin
15	M	14	166	56	6.8	Idiopathic Scoliosis	ADHD	Atomoxetine
16	M	16	161	55	7.9	Congenital Kyphosis	Asthma	Albuterol
17	M	16	174	57	4.7	Idiopathic Scoliosis		
18	M	12	152	48	3.1	Idiopathic Scoliosis		
19	F	11	154	69	4.0	Idiopathic Scoliosis	Asthma	
20	M	15	161	50	5.8	Idiopathic Scoliosis		Acetaminophen
21	M	14	168	52	6.6	Idiopathic Scoliosis	Marfan's Syndrome, Asthma, Mitral Valve Prolapse, GERD	Methylphenidate
22	F	13	155	54	8.6	Idiopathic Scoliosis	OCD/ADHD	Lisdexamfetamine
23	F	18	163	68	3.8	Idiopathic Scoliosis		
24	F	13	173	59	6.7	Idiopathic Scoliosis		
25	F	14	156	60	9.8	Idiopathic Scoliosis		
26	F	14	170	96	3.6	Idiopathic Scoliosis	Asthma	Acetaminophen
27	F	11	150	52	0.9	Idiopathic Scoliosis		Loratadine, Multivitamin

tHcy = plasma total homocysteine; ADHD = Attention deficit hyperactivity disorder; GERD = Gastroesophageal reflux disease; OCD = Obsessive compulsive disorder

Table 2
Absolute and relative change in plasma total homocysteine in individual patients

Pat #	Baseline tHcy (μmol/L)	Peak tHcy (μmol/L)	Change tHcy (μmol/L)	Relative change	N ₂ O*min
1	3.7	20.7	17.0	459%	310
2	7.6	18.6	11.0	145%	125
3	8.0	25.1	17.1	214%	182
4	4.5	16.2	11.7	260%	135
5	5.4	15.8	10.4	193%	195
6	4.9	11.5	6.6	135%	142
7	8.6	16.2	7.6	88%	20*
8	4.4	14.4	10.0	227%	150
9	5.1	5.8	0.7	14%	20*
10	9.0	9.0	0.0	0%	0
11	6.7	20.5	13.8	206%	150
12	3.8	11.1	7.3	192%	113
13	3.2	11.0	7.8	244%	123
14	4.7	16.0	11.3	240%	158
15	6.8	21.2	14.4	212%	196
16	7.9	31.7	23.8	301%	234
17	4.7	17.8	13.1	279%	143
18	3.1	17.6	14.5	468%	110
19	4.0	13.6	9.6	240%	165
20	5.8	9.8	4.0	69%	83
21	6.6	18.0	11.4	173%	173
22	8.6	13.1	4.5	52%	25*
23	3.8	14.6	10.8	284%	210
24	6.7	18.1	11.4	170%	173
25	9.8	38.6	28.8	294%	263
26	3.6	11.3	7.7	214%	128
27	0.9	6.0	5.1	567%	105

tHcy = plasma total homocysteine; N₂O*min = product of average nitrous oxide concentration used (as fraction of 1) and duration of nitrous oxide exposure in minutes. A * in the last column indicates patients who received nitrous oxide only during induction of and emergence from anesthesia.