

Effect of Nitrous Oxide Anesthesia on Plasma Homocysteine and Endothelial Function

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Background: Endothelial function is impaired with hyperhomocysteinemia. Plasma homocysteine is increased by nitrous oxide anesthesia. The current study was designed to determine whether endothelial function is impaired after surgery and whether this is made worse by exposure to nitrous oxide.

Methods: The authors studied 59 patients with cardiovascular disease undergoing noncardiac surgery. Patients were randomly allocated to nitrous oxide–based anesthesia (n = 25) or nitrous oxide–free anesthesia (control, n = 34). Endothelial function was measured by flow-mediated dilation of the brachial artery before and 24 h after surgery. In addition, blood was drawn at both time points for the measurements of plasma homocysteine, folate, L-arginine, L-citrulline, asymmetric dimethylarginine, and nitrate concentrations.

Results: The median duration of general anesthesia was 4.5 h. Patients had significantly lower flow-mediated dilation after surgery (5.1 ± 3.3 to $3.0 \pm 4.1\%$; $P = 0.001$). Duration of anesthesia affected endothelial function. In the nitrous oxide group, there was an inverse correlation with flow-mediated dilation ($r = -0.60$, $P = 0.004$), but in the control group, there was a positive correlation ($r = 0.61$, $P < 0.001$). When compared with control, nitrous oxide exposure was associated with a significant increase in postoperative homocysteine (mean

difference, $4.9 \mu\text{M}$; 95% confidence interval, 2.8 – $7.0 \mu\text{M}$; $P < 0.0005$) and decrease in flow-mediated dilation (3.2% ; 95% confidence interval, 0.1 – 5.3% ; $P = 0.001$). Nitrous oxide exposure was not associated with change in nitric oxide substrates.

Conclusions: Nitrous oxide–based anesthesia increased plasma homocysteine and significantly impaired endothelial function in patients undergoing noncardiac surgery. Nitrous oxide–based anesthesia could be a risk factor for postoperative cardiovascular morbidity.

PATIENTS with coronary artery disease are at increased risk of perioperative cardiac complications and death.^{1–3} Current guidelines emphasize the evaluation of cardiac risk,³ but there are few proven therapies to reduce perioperative cardiac events. Preliminary data suggest that exposure to nitrous oxide during general anesthesia may be an additional risk factor for these complications.^{4–6}

Nitrous oxide irreversibly inactivates the enzyme methionine synthase by oxidizing the cobalt atom in vitamin B₁₂.^{5,7,8} This impairs production of methionine, with a resultant increase in plasma homocysteine concentration that can persist for at least 1 week after surgery.^{8,9} A 2-h exposure to nitrous oxide is associated with a 50% reduction in methionine synthase activity.⁷

Long-term elevation of plasma homocysteine concentration is an independent risk factor for cardiovascular disease,¹⁰ and an acute increase in plasma homocysteine causes endothelial dysfunction^{11,12} and hypercoagulability.¹⁰ It has been shown that plasma homocysteine concentration is acutely increased after oral methionine and is reliably associated with endothelial dysfunction as measured by flow-mediated vasodilation.^{11,12} Nitrous oxide–induced elevation of homocysteine concentration may mirror that produced by oral methionine, but currently no data are available about the nitrous oxide effects on nitric oxide synthesis or endothelial function. Despite 10% of the population (more than 20 million people in the United States) undergoing general anesthesia each year, and millions of these being exposed to nitrous oxide, the endothelial effects and role of nitrous oxide in perioperative cardiac events are unknown. The purpose of the current study was to determine the effects of nitrous oxide on endothelial function in patients undergoing major noncardiac surgery.

Materials and Methods

Patient Population

Patients undergoing elective noncardiac surgery between March 2005 and January 2008 at Alfred Hospital, Melbourne, Australia, and Prince of Wales Hospital,

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Hong Kong, with risk factors or a known history of coronary artery disease were eligible for participation in the study. Eligibility criteria included any of: hypertension, diabetes, age older than 60 yr, or preexisting history of coronary artery disease.

Patients expected to require a high inspired oxygen concentration intraoperatively or with any relative contraindication to nitrous oxide (volvulus, pulmonary hypertension, increased intracranial pressure) were excluded. The study protocol was approved by the ethics committees at both participating institutions. All patients were approached before surgery and provided written, informed consent.

Study Protocol

On the day of surgery, patients fasted for at least 6 h before measurement of flow-mediated dilation (FMD) of the brachial artery using an established method.¹¹⁻¹³ Briefly, measurements were performed with a 10-12 MHz linear array transducer (Titan L38 [SonoSite, Bothell, WA] or HDI-5000 [Philips Medical Systems, Eindhoven, The Netherlands]). The subjects lay at rest in the supine position, in a quiet, temperature-controlled room throughout the study. The brachial artery was scanned in a longitudinal section at the elbow, and the center of the artery was identified when the clearest picture of the anterior and posterior wall layers was obtained. A resting scan was first recorded. FMD was then induced by inflation of a pneumatic tourniquet placed around the upper arm proximal to the segment of artery being scanned, to a pressure of 250-300 mmHg for 5 min, followed by release. A second scan was performed 60 s after cuff deflation, and a repeat baseline scan was performed after a further 15 min was allowed for vessel recovery. Usual cardiac medications were continued up until the morning of surgery; no caffeine or tobacco use was allowed within 6 h of the study procedures. Measurement of FMD was repeated on the day after surgery. FMD was calculated as the maximal percentage change in brachial artery diameter during hyperemia; FMD in young healthy adults is approximately 10%.^{11,13}

Patients also had preoperative and postoperative fasting venous blood samples taken for measurement of plasma concentrations of homocysteine and folate. In a subgroup of patients ($n = 19$) at the Alfred Hospital, we also measured plasma concentrations of L-citrulline, L-arginine, asymmetric dimethylarginine (ADMA), and nitrate as biomarkers of nitric oxide production and transport. Folate and homocysteine were measured by chemiluminescence microparticle immunoassay and fluorescence polarization immunoassay using Abbott autoanalyzers (Abbott Laboratories, Abbott Park, IL), as previously described, respectively.¹⁴ L-Arginine, L-citrulline, and ADMA were measured by reverse-phase liquid chromatography with time-controlled or-

thophthaldialdehyde precolumn derivatization, as previously described.¹⁵

After enrollment, on the day of surgery, patients in an initial cohort ($n = 43$) were randomly allocated (1:1) to a control group with inspired oxygen fraction (F_{IO_2}) of 0.80 ($n = 21$) or a nitrous oxide group with an F_{IO_2} of 0.30 ($n = 22$) via a computer-generated random list, concealed in opaque, sealed envelopes. After submission of an initial manuscript reporting on this study, the journal editor advised that a further control group with F_{IO_2} 0.30 was required to discount a confounding effect on the difference in F_{IO_2} between groups. We therefore recruited another 16 patients to the study, randomly allocated in a 10:3:3 weighting to a control group with F_{IO_2} 0.30 (final $n = 10$), a control group with F_{IO_2} 0.80 (final $n = 24$), or a nitrous oxide group with F_{IO_2} 0.30 (final $n = 25$), respectively. Patients allocated to the nitrous oxide group had maintenance of general anesthesia with nitrous oxide and F_{IO_2} 0.30, and one of three other hypnotic agents (isoflurane, sevoflurane, or propofol) at the discretion of the anesthesiologist. Patients allocated to the control (nitrous oxide-free) group had their anesthesia maintained with one of the study hypnotic agents, but without nitrous oxide, using random allocation of F_{IO_2} 0.8 or F_{IO_2} 0.30.

Anesthesiologists had knowledge of nitrous oxide exposure, but administration and group identity were concealed from the surgeon and research staff. All other perioperative clinical care was according to routine practice. Patient and operative characteristics, including type of surgery, anesthetic drug administrations, duration of anesthesia, and adverse events, were recorded.

Patients were monitored daily after surgery until discharge. Blood was collected on the first postoperative day after surgery for measurement of serum troponin concentration. Any other additional laboratory tests or investigations were ordered when clinically indicated (e.g., chest pain, dyspnea). Finally, patients were contacted by phone at 30 days, and laboratory reports and the hospital record were reviewed to ascertain whether they had experienced any delayed adverse events. Myocardial infarction was defined as a typical increase and decrease in serum troponin concentration and with at least one of the following: typical ischemic symptoms, new Q-wave or ST-segment electrocardiographic changes, or coronary intervention; or pathologic findings of myocardial infarction.

Statistical Analysis

The primary endpoint was endothelial dysfunction, as measured by FMD. Secondary endpoints included perioperative changes in plasma concentrations of homocysteine and other biomarkers of endothelial function, cardiac events, and hospital duration of stay. Our sample size calculation was based on an absolute percent change in FMD of 3, with SD 3, and a mean FMD in the

control group of 10.¹³ This required 21 patients per group for a type I error of 0.05 and a type II error of 0.2.

All patients who were enrolled, were randomly assigned to treatment groups, and underwent induction of general anesthesia were considered as comprising the intention-to-treat population for analysis. Group comparisons were performed with *t* tests, after initial testing for normality with the Kolmogorov-Smirnov test. Inter-group postoperative comparisons were adjusted for preoperative (baseline) values using general linear models, with and without adjusting for F_{iO_2} . Associations were measured using the Pearson correlation coefficient (*r*). The analyses were performed with SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL). Data are presented as number (percent), mean \pm SD, median (interquartile range), or mean difference (95% confidence interval). All *P* values are two-sided, and *P* < 0.05 was considered statistically significant.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the article as written.

Results

Study Patients

A total of 59 patients were enrolled in the study and underwent anesthesia and surgery (nitrous oxide group, *n* = 25; control group with F_{iO_2} 0.80, *n* = 24; control group with F_{iO_2} 0.30, *n* = 10). In view of the lack of significant differences between an F_{iO_2} of 0.30 and 0.80, these were combined for subsequent analyses comparing nitrous oxide with control. Two of the 59 patients received a propofol infusion for maintenance of anesthesia; the remainder received an inhalational agent. The median duration (exposure) to general anesthesia was 4.5 h. Thirty-day follow-up was obtained in all patients. Patient and clinical characteristics are displayed in table 1. There were a similar number of male and female patients in each group, with a mean age of 66 yr. Patients in the nitrous oxide group received a similar amount of inhalational anesthesia compared with the controls (minimum alveolar concentration equivalents: nitrous oxide group 0.74 ± 0.13 vs. control group 0.77 ± 0.15 ; *P* = 0.44).

Good-quality preoperative and postoperative measurements of FMD could not be obtained in 7 patients (4 nitrous oxide group, 3 control group). In the remaining patients (*n* = 52), hyperemia was demonstrated during FMD measurements. Peak flow was markedly increased after tourniquet release, as reflected by the Doppler velocity, preoperatively 61 ± 15 versus 98 ± 27 cm/s (*P* < 0.001) and postoperatively 61 ± 16 versus 100 ± 25 cm/s (*P* < 0.001).

Table 1. Clinical Characteristics

| Characteristic | N ₂ O Group, n = 25 | Control Group | | |
|---------------------------|-----------------------------------|-------------------|---|---|
| | | Pooled, n = 34 | F _{iO₂} 0.30, n = 10 | F _{iO₂} 0.80, n = 24 |
| Age, yr | 65 \pm 8.0 | 67 \pm 9.1 | 68 \pm 7.0 | 67 \pm 10 |
| Male sex | 12 (55) | 14 (41) | 4 (40) | 10 (42) |
| Body weight, kg | 69 \pm 21 | 65 \pm 11 | 62 \pm 9.3 | 66 \pm 12 |
| ASA physical status | | | | |
| II | 15 (60) | 19 (56) | 7 (70) | 12 (50) |
| III | 8 (32) | 14 (41) | 3 (30) | 11 (46) |
| IV | 2 (8) | 1 (3) | 0 (0) | 1 (4) |
| Coronary artery disease | 1 (4) | 5 (15) | 2 (20) | 3 (13) |
| Hypertension | 7 (28) | 15 (44) | 9 (90) | 6 (25) |
| Heart failure | 10 (40) | 8 (24) | 0 (0) | 8 (33) |
| Diabetes | 8 (32) | 16 (47) | 7 (70) | 9 (38) |
| Pernicious anemia | 3 (12) | 2 (6) | 0 (0) | 2 (8) |
| Current smoker | 1 (4) | 2 (6) | 0 (0) | 2 (8) |
| Surgery | | | | |
| General | 10 (40) | 16 (47) | 4 (40) | 12 (50) |
| Urology | 6 (24) | 6 (18) | 2 (20) | 4 (17) |
| Neurosurgery | 3 (12) | 2 (6) | 0 (0) | 2 (8) |
| Orthopedic | 2 (8) | 3 (9) | 0 (0) | 3 (13) |
| Vascular | 3 (12) | 5 (15) | 3 (30) | 2 (8) |
| Other | 1 (4) | 2 (6) | 1 (10) | 1 (4) |
| Duration of anesthesia, h | 4.8 \pm 2.3 | 5.4 \pm 2.4 | 5.2 \pm 1.4 | 5.5 \pm 2.8 |

Data are n (%) or mean \pm SD.

ASA = American Society of Anesthesiologists; F_{iO_2} = inspired oxygen fraction; N₂O = nitrous oxide.

Changes with Surgery and Nitrous Oxide

Nitrous oxide exposure was associated with a significant increase in postoperative homocysteine and a decrease in FMD when compared with the control group (table 2 and fig. 1). However, there was no independent effect of F_{iO_2} on plasma homocysteine concentration or FMD; additional information regarding this is available on the ANESTHESIOLOGY Web site at <http://www.anesthesiology.org>. There was no association between end-tidal inhalational agent and FMD (*r* = 0.03, *P* = 0.84). Markers of endothelial function before and after surgery are displayed in table 3. Patients had significantly lower FMD 24 h after surgery.

The duration of anesthesia had a significant effect on FMD. In this regard, there was an inverse correlation between FMD and duration of nitrous oxide exposure (*r* = -0.60, *P* = 0.004). In contrast, a positive correlation with FMD was observed in the control group (*r* = 0.61, *P* < 0.001; fig. 2), and this was unaffected when adjusting for F_{iO_2} . In the control group, there was no relation between F_{iO_2} and FMD (*r* = -0.16, *P* = 0.40). Plasma concentrations of L-citrulline, L-arginine, and L-arginine:ADMA ratio were reduced after surgery, but the changes were similar between groups.

Patients in the nitrous oxide group had a postoperative hospital stay of 16 ± 14 (median, 12) days, and those in the control group had a stay of 8.6 ± 3.7 (median, 8) days (U test, *P* = 0.043). Site (Hong Kong, Melbourne)

Table 2. The Effect of Nitrous Oxide on Endothelial Biomarkers and Flow-mediated Dilatation

| | N ₂ O Group | Control Group | Difference in Postoperative Mean Values (95% CI)* | P Value* |
|---|------------------------|---------------|---|----------|
| Folate, nM | n = 25 | n = 34 | | |
| Preoperative | 21.6 ± 7.6 | 24.6 ± 7.3 | | |
| Postoperative | 24.5 ± 8.7 | 22.9 ± 8.8 | 4.8 (0.3 to 9.2) | 0.037 |
| Homocysteine, μM | n = 25 | n = 34 | | |
| Preoperative | 9.7 ± 3.4 | 9.1 ± 3.6 | | |
| Postoperative | 14.1 ± 5.7 | 7.9 ± 4.2 | 5.9 (3.7 to 8.1) | < 0.0005 |
| L-Citrulline, μM | n = 10 | n = 9 | | |
| Preoperative | 95 ± 35 | 115 ± 43 | | |
| Postoperative | 78 ± 40 | 62 ± 21 | 20.0 (-12.2 to 52.3) | 0.21 |
| L-Arginine, μM | n = 10 | n = 9 | | |
| Preoperative | 147 ± 36 | 138 ± 16 | | |
| Postoperative | 90 ± 18 | 87 ± 28 | 1.5 (-22.1 to 25.0) | 0.90 |
| ADMA, μM | n = 10 | n = 9 | | |
| Preoperative | 1.5 ± 0.6 | 1.2 ± 0.7 | | |
| Postoperative | 1.5 ± 0.8 | 1.3 ± 0.6 | 0.13 (-0.59 to 0.85) | 0.70 |
| Nitrate, μM | n = 10 | n = 9 | | |
| Preoperative | 40 ± 11 | 40 ± 13 | | |
| Postoperative | 36 ± 14 | 34 ± 4.9 | 2.2 (-8.5 to 12.9) | 0.67 |
| L-Arginine:ADMA ratio, n = 19 | n = 10 | n = 9 | | |
| Preoperative | 121 ± 74 | 151 ± 75 | | |
| Postoperative | 82 ± 49 | 75 ± 23 | 6.6 (-33.1 to 46.3) | 0.73 |
| Endothelial function changes in vessel diameter, mm | n = 21 | n = 31 | | |
| Preoperative | 0.025 ± 0.013 | 0.019 ± 0.013 | | |
| Postoperative | 0.004 ± 0.015 | 0.017 ± 0.016 | 0.01 (0.00 to 0.03) | 0.003 |
| Flow-mediated dilatation, % | n = 21 | n = 31 | | |
| Preoperative | 6.3 ± 3.3 | 4.3 ± 3.1 | | |
| Postoperative | 1.1 ± 4.0 | 4.3 ± 3.6 | 3.2 (1.0 to 5.3) | 0.001 |

Data are mean ± SD unless specified.

* General linear model to adjust for baseline (preoperative) value.

ADMA = asymmetric dimethylarginine; CI = confidence interval; N₂O = nitrous oxide.

was included as a term in exploratory analyses for each of the above endpoints, and the results were essentially unchanged.

Relation between Flow-mediated Dilatation, Homocysteine, and Biomarkers of Endothelial Oxidative Stress

Preoperatively, we could not identify any significant associations between FMD and plasma homocysteine ($r = -0.15$, $P = 0.30$), L-citrulline ($r = -0.34$, $P = 0.25$), L-arginine ($r = -0.12$, $P = 0.70$), ADMA ($r = 0.44$, $P = 0.13$), and nitrate ($r = -0.12$, $P = 0.70$).

Postoperatively, there was a negative association between FMD and plasma homocysteine ($r = -0.49$, $P < 0.001$), but we could not identify a significant association between FMD and L-citrulline ($r = -0.33$, $P = 0.17$), L-arginine ($r = -0.10$, $P = 0.75$), ADMA ($r = 0.14$, $P = 0.64$), or nitrate ($r = 0.04$, $P = 0.90$).

Discussion

This prospective study demonstrated that endothelial function was impaired after surgery in patients with car-

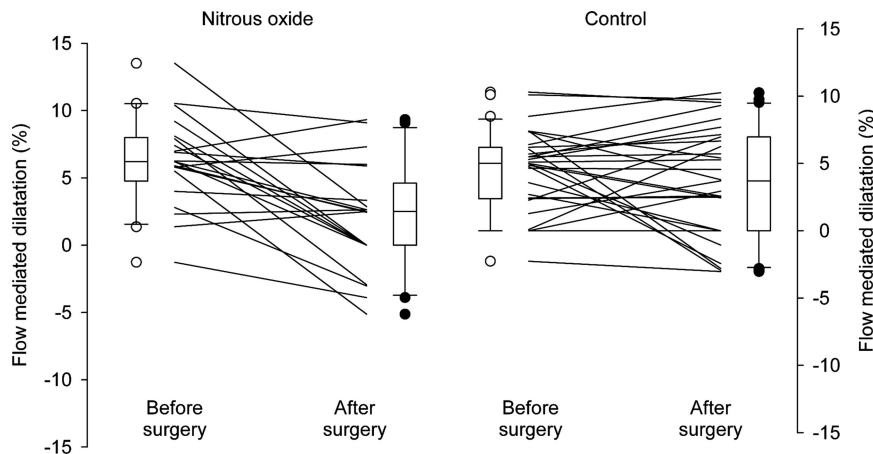


Fig. 1. Flow-mediated dilatation before and after surgery in patients with cardiovascular disease exposed to nitrous oxide anesthesia (n = 21) or nitrous-free anesthesia (control, n = 31). When compared with control, nitrous oxide exposure was associated with a reduction in endothelial function as assessed by flow-mediated dilatation ($P = 0.001$).

Table 3. Perioperative Endothelial Biomarkers and Flow-mediated Dilatation before and after Surgery

| | Preoperative | Postoperative | P Value |
|---|--------------|---------------|---------|
| Folate, n = 59, nM | 23 ± 7.3 | 23 ± 7.5 | 0.97 |
| Homocysteine, n = 59, μM | 9.4 ± 3.5 | 10.26 ± 5.3 | 0.026 |
| L-Citrulline, n = 19, μM | 105 ± 40 | 70.4 ± 32 | 0.005 |
| L-Arginine, n = 19, μM | 143 ± 28 | 88.6 ± 23 | <0.0005 |
| ADMA, n = 19, μM | 1.35 ± 0.69 | 1.37 ± 0.68 | 0.92 |
| Nitrate, n = 19, μM | 40.1 ± 12 | 35.2 ± 10 | 0.20 |
| L-Arginine:ADMA ratio, n = 19 | 135 ± 74 | 78 ± 38 | 0.009 |
| Flow-mediated vasodilatation, n = 52, % | 5.1 ± 3.3 | 3.0 ± 4.1 | 0.001 |

Data are mean ± SD. P values calculated with paired t tests. ADMA = asymmetric dimethylarginine.

diovascular disease, but seemingly only in those exposed to nitrous oxide. Furthermore, in marked contrast to patients in the control group, the duration of nitrous oxide exposure strongly correlated with the extent of endothelial dysfunction. In the current study, we identified two potential mechanisms for the nitrous oxide-mediated impairment of endothelial function: (1) We found that some or all of the endothelial dysfunction can probably be explained by the observed increase in homocysteine; (2) in addition, we identified an overall reduction in L-arginine and L-citrulline postoperatively, indicating an increase in endothelial oxidative stress after surgery and/or nitrous oxide exposure. Differences in FiO₂ between groups did not explain any of these adverse effects of nitrous oxide.

Several previous studies have suggested a link between endothelial dysfunction and cardiovascular risk.¹⁶⁻¹⁹ Given the increased incidence of myocardial ischemia in the hours and days after surgery in patients with coronary artery disease,¹ it is feasible that endothelial dysfunction is a significant contributory factor. We found that nitrous oxide is associated with impaired endothelial function after surgery, and so nitrous oxide-based anesthesia may aggravate myocardial ischemia, particularly in susceptible individuals. The endothelium plays a central role in the regulation of vascular tone.²⁰ A key component is endothelium-derived nitric oxide, metabolized from L-arginine by the enzyme endothelial nitric oxide synthase (eNOS), with L-citrulline as a byproduct. Human endothelial cells have some capacity to recycle L-citrulline to L-arginine, although the contribution of this process to nitric oxide generation is probably small.²⁰

It has been previously shown that nitrous oxide increases homocysteine,^{5,7-9} a known risk factor for cardiovascular disease.¹⁰ The inactivation of methionine synthase by nitrous oxide is dose dependent, with greater inactivation occurring with longer exposure, as might be experienced during complex surgery. The extent of enzyme inactivation is greater with higher inspired concentrations of nitrous oxide, and probably worse in patients with multiple comorbidities.^{6,7} Of relevance, oral methionine administration at a dose of 100 mg/kg increases homocysteine, and leads to endothelial dysfunction within hours.^{11,12} Homocysteine-induced

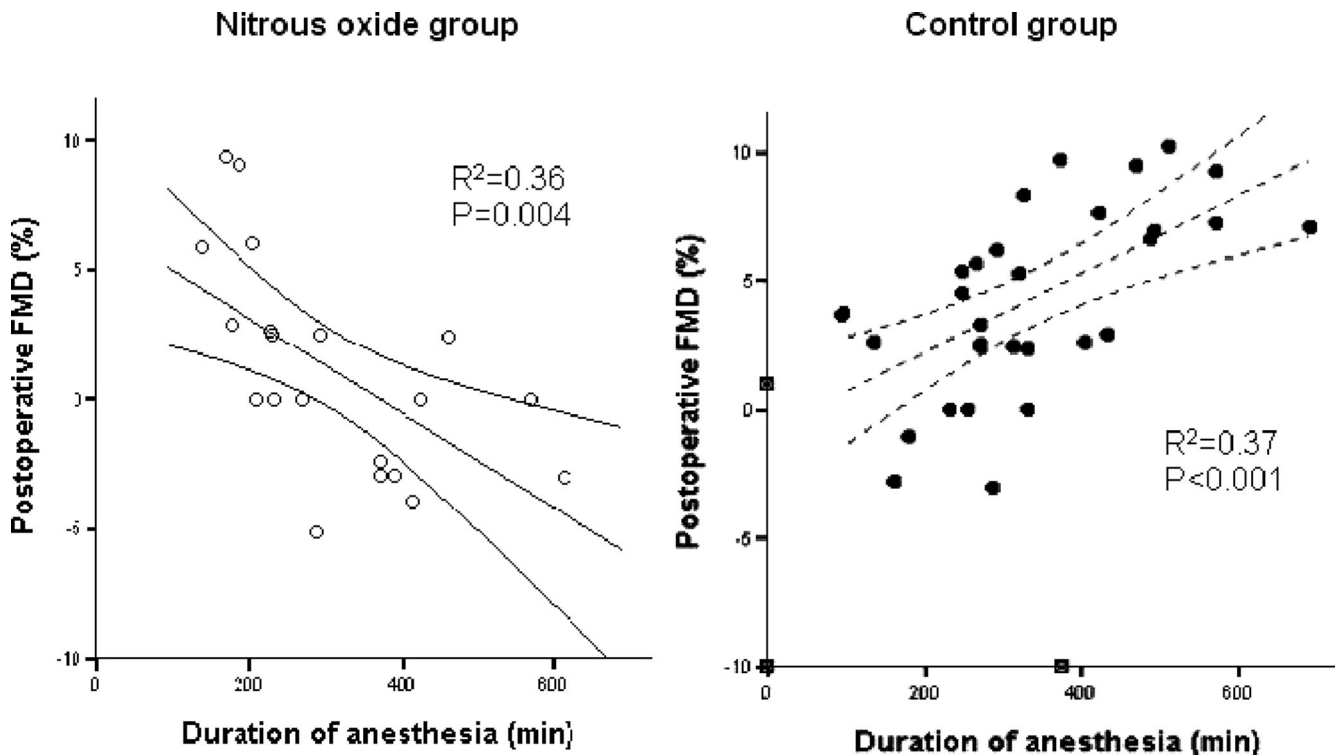


Fig. 2. The effect of duration of anesthesia on the percent change in flow-mediated dilation (FMD) of the brachial artery after surgery, according to nitrous oxide exposure. Regression lines with 95% confidence intervals.

endothelial dysfunction may in part be explained by inhibition of eNOS.^{16,17,20,21} Methionine administration also increases ADMA, which can directly compete with arginine and inhibit NOS, decreasing nitric oxide production and its resulting vasodilation and physiologic benefits on endothelial function.²¹ The known effects of nitrous oxide on the methionine-homocysteine pathway suggest that a similar mechanism could lead to nitrous oxide increasing ADMA. Others have suggested that the endothelial effects of homocysteine are due to oxidative stress, mediated by the formation of reactive oxygen species,²² or perhaps a defective L-arginine-nitric oxide pathway.¹⁷ It seems likely that, as others have suggested,²¹ there is individual variability in the effects of homocysteine on endothelial oxidative pathways.²⁰ We could not identify any substantial effect of nitrous oxide or surgery on ADMA concentration alone. Previous researchers have shown an inverse correlation between endothelial function and ADMA,^{17,21} suggesting that the adverse effects of homocysteine on the endothelium are mediated by ADMA. Our results do not support this hypothesis, but it must be emphasized that our sample size was small and may have been underpowered.

In an attempt to provide additional insights into the mechanism for the adverse effects of nitrous oxide on endothelial function, we measured preoperative and postoperative levels of the nitric oxide precursor, L-arginine. Impairment of L-arginine transport has been identified in patients with heart failure and hypertension,¹⁷ and uncoupling of eNOS, as seen in hypertension and diabetes, contributes to endothelial dysfunction.²⁰ These varied findings raise the possibility of eNOS-dependent and eNOS-independent mechanisms to explain our observed association between nitrous oxide and endothelial dysfunction. Irrespective of anesthetic treatment group, we found that L-arginine levels decreased significantly. Although we did not investigate the specific mechanism, this observation would be consistent with the previously recognized effect of stress on stimulating hepatic L-arginine transport.²³

L-Arginine availability may be a rate-limiting step in the production of nitric oxide,^{17,18} particularly in the setting of cardiovascular disease. In addition, endothelial arginases compete with eNOS for substrate,²¹ and thus, up-regulated arginase activity will lead to a relative L-arginine deficiency. These two processes may be an explanation for the beneficial effects of L-arginine supplementation in clinical practice.^{21,24} Although we did not find a relation between plasma L-arginine and endothelial function, it is possible that adverse effects might be observed in patients with more pronounced vascular disease. Consistent with the decrease in plasma L-arginine, we also observed a reduction in plasma L-citrulline, which would be consistent with some reduction in nitric oxide production. The decrease in L-citrulline, as a potential guide to eNOS activity, might also reflect the

effect of eNOS inhibition by ADMA, which we showed to be increased postoperatively.¹⁵

Two previous studies provide preliminary evidence that nitrous oxide-based anesthesia may be associated with perioperative cardiac events. Badner *et al.*⁹ assessed 90 patients undergoing carotid endarterectomy during general anesthesia. They randomly assigned patients to anesthesia with or without nitrous oxide. The nitrous oxide group had significantly increased homocysteine and cardiac ischemic events.⁹ In a large clinical trial in 2,050 surgical patients comparing nitrous oxide with a nitrous oxide-free technique, Myles *et al.*⁶ observed a possible increased risk of myocardial infarction with nitrous oxide exposure. When all reports (confirmed and unconfirmed by the endpoint committee) of cardiac events were included, there was an increased rate of myocardial infarction in the nitrous oxide group, 30 versus 10 cases ($P = 0.002$). Neither study was powered to detect a difference in major cardiac events with nitrous oxide.

Cardiovascular events such as myocardial infarction, unstable angina, and ischemic stroke are believed to be precipitated by plaque rupture and thrombus formation, as well as reactive vasospasm.^{19,25} Endothelial dysfunction is likely to contribute to each of these events.¹⁹ We have observed for the first time an impairment of endothelial function after noncardiac surgery. Others have identified a similar effect after cardiopulmonary bypass.^{26,27} We know that major surgery is associated with a neuroendocrine ("stress") response, and this might aggravate oxidative stress within the endothelium, but our study clearly identifies exposure to nitrous oxide as a major factor.

Nitrous oxide has other possible adverse effects on the cardiovascular system.⁵ It has previously been shown to produce epicardial coronary artery vasoconstriction in dogs,²⁸ and unlike other inhalational anesthetics, nitrous oxide does not protect the myocardium from ischemia and reperfusion injury.^{29,30} Nitrous oxide may also increase the risk of intraoperative and postoperative hypoxic episodes.⁵ Last, endothelial dysfunction may impair graft patency in peripheral vascular surgery by decreasing runoff.

There are some limitations to our study. We did not standardize the anesthetic or analgesic regimens. Our previous study highlighted a small (20%) dose reduction in inhalational anesthetic agents when nitrous oxide was used, but there was a minimal effect on propofol or opioid administration.⁶ Inhalational anesthetic agents and propofol may improve endothelial function,³¹ but it is unclear whether this would persist on the day after surgery; however, it is possible that any imbalance in their use or dose may have affected our results. There are other possible changes occurring perioperatively; therefore, hyperglycemia and a variety of inflammatory mediators may affect endothelial oxidative processes, and

these could contribute to postoperative endothelial dysfunction. We measured biochemical markers of nitric oxide production in a subset of patients in an attempt to identify associations between nitrous oxide exposure, nitric oxide production and transport, and endothelial function. These latter data were from a small subset of patients, and we may have missed important associations. No attempt was made to adjust the final *P* values for the interim analyses performed before the addition of the second control group (F_{IO_2} 0.30).

There is substantial biologic rationale and evidence from small studies to postulate a causal relation between nitrous oxide and increased homocysteine. We have further demonstrated nitrous oxide-induced endothelial dysfunction postoperatively in patients with cardiovascular disease undergoing noncardiac surgery. This provides mechanistic evidence that nitrous oxide may increase the risk of postoperative cardiac events and mortality. We are currently undertaking a large clinical trial to address this question.**

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References

- Mangano DT: Perioperative cardiac morbidity. *ANESTHESIOLOGY* 1990; 72:153-84
- Devereaux PJ, Goldman L, Cook DJ, Gilbert K, Leslie K, Guyatt GH: Perioperative cardiac events in patients undergoing noncardiac surgery: A review of the magnitude of the problem, the pathophysiology of the events and methods to estimate and communicate risk. *CMAJ* 2005; 173:627-34
- Fleisher LA, Beckman JA, Brown KA, Calkins H, Chaikof E, Fleischmann KE, Freeman WK, Froehlich JB, Kasper EK, Kersten JR, Riegel B, Robb JF, Smith SC Jr, Jacobs AK, Adams CD, Anderson JL, Antman EM, Buller CE, Creager MA, Ettinger SM, Faxon DP, Fuster V, Halperin JL, Hiratzka LF, Hunt SA, Lytle BW, Nishimura R, Ornato JP, Page RL, Riegel B, Tarkington LG, Yancy CW; American College of Cardiology; American Heart Association Task Force on Practice Guidelines (writing Committee to Revise the 2002 Guidelines on Perioperative Cardiovascular Evaluation for Noncardiac Surgery); American Society of Echocardiography; American Society of Nuclear Cardiology, Heart Rhythm Society; Society of Cardiovascular Anesthesiologists; Society for Cardiovascular Angiography and Interventions; Society for Vascular Medicine and Biology; Society for Vascular Surgery: ACC/AHA 2007 guidelines on perioperative cardiovascular evaluation and care for noncardiac surgery: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines on Perioperative Cardiovascular Evaluation for Noncardiac Surgery) developed in collaboration with the American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Rhythm Society, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, and Society for Vascular Surgery. *J Am Coll Cardiol* 2007; 50:159-241
- Badner NH, Beattie WS, Freeman D, Spence JD: Nitrous oxide-induced elevated homocysteine concentrations are associated with increased myocardial ischemia in patients undergoing carotid endarterectomy. *Anesth Analg* 2000; 91:1073-9
- Myles PS, Leslie K, Silbert B, Paech MJ, Peyton P: A review of the risks and benefits of nitrous oxide in current anaesthetic practice. *Anaesth Intensive Care* 2004; 32:165-72
- Myles PS, Leslie K, Chan MTV, Forbes A, Paech MJ, Peyton P, Silbert BS, Pascoe E; ENIGMA Trial Group: Avoidance of nitrous oxide for patients undergoing major surgery: A randomized controlled trial. *ANESTHESIOLOGY* 2007; 107:221-31
- Nunn JF: Clinical aspects of the interaction between nitrous oxide and vitamin B12. *Br J Anaesth* 1987; 59:3-13
- Ermens AA, Refsum H, Ruprecht J, Spijkers IJ, 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
- Badner NH, Drader K, Freeman D, Spence JD: The use of intraoperative nitrous oxide leads to postoperative elevations in plasma homocysteine. *Anesth Analg* 1998; 87:711-3
- Mayer EL, Jacobsen DW, Robinson K: Homocysteine and coronary atherosclerosis. *J Am Coll Cardiol* 1996; 27:517-27
- Bellamy MF, McDowell IF, Ramsey MW, Brownlee M, Bones C, Newcombe RG, Lewis MJ: Hyperhomocysteinemia after an oral methionine load acutely impairs endothelial function in healthy adults. *Circulation* 1998; 98:1848-52
- Chambers JC, McGregor A, Jean-Marie J, Obeid OA, Kooner JS: Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: An effect reversible with vitamin C therapy. *Circulation* 1999; 99:1156-60
- Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force: Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39:257-65
- Myles PS, Chan MT, Forbes A, Leslie K, Paech M, Peyton P: Preoperative folate and homocysteine status in patients undergoing major surgery. *Clin Nutr* 2006; 25:736-45
- Zhang WZ, Kaye DM: Simultaneous determination of arginine and seven metabolites in plasma by reversed-phase liquid chromatography with a time-controlled ortho-phthalaldehyde precolumn derivatization. *Anal Biochem* 2004; 326:87-92
- Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE: Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol* 1994; 24:1468-74
- Schlaich MP, Parnell MM, Ahlers BA, Finch S, Marshall T, Zhang WZ, Kaye DM: Impaired L-arginine transport and endothelial function in hypertensive and genetically predisposed normotensive subjects. *Circulation* 2004; 110:3680-6
- Suzuki T, Hayase M, Hibi K, Hosokawa H, Yokoya K, Fitzgerald PJ, Yock PG, Cooke JP, Suzuki T, Yeung AC: Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. *Circulation* 1996; 94:1298-303
- Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Menzoian JO, Vita JA: Risk stratification for postoperative cardiovascular events *via* noninvasive assessment of endothelial function: A prospective study. *Circulation* 2002; 105:1567-72
- Forstermann U, Munzel T: Endothelial nitric oxide synthase in vascular disease: From marvel to menace. *Circulation* 2006; 113:1708-14
- Stuhlinger MC, Oka RK, Graf EE, Schmolzer I, Upton BM, Kapoor O, Szuba A, Malinow MR, Wascher TC, Pachinger O, Cooke JP: Endothelial dysfunction induced by hyperhomocysteinemia: Role of asymmetric dimethylarginine. *Circulation* 2003; 108:933-8
- Weiss N, Zhang YY, Heydrick S, Bierl C, Loscalzo J: Overexpression of cellular glutathione peroxidase rescues homocyst(e)ine-induced endothelial dysfunction. *Proc Natl Acad Sci U S A* 2001; 98:12503-8
- Pan M, Choudry HA, Epler MJ, Meng Q, Karinch A, Lin C, Souba W: Arginine transport in catabolic disease states. *J Nutr* 2004; 134:2826S-9S
- Suzuki T, Hayase M, Hibi K, Hosokawa H, Yokoya K, Fitzgerald PJ, Yock PG, Cooke JP, Suzuki T, Yeung AC: Effect of local delivery of L-arginine on in-stent restenosis in humans. *Am J Cardiol* 2002; 89:363-7
- Fuster V, Badimon L, Badimon JJ, Chesebro JH: The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med* 1992; 326:242-50
- Morelos M, Amyot R, Picano E, Rodriguez O, Mazzone AM, Glauber M, Biagini A: Effect of coronary bypass and cardiac valve surgery on systemic endothelial function. *Am J Cardiol* 2001; 87:364-6
- Chello M, Goffredo C, Patti G, Candura D, Melfi R, Mastrobuoni S, Di Sciascio G, Covino E: Effects of atorvastatin on arterial endothelial function in coronary bypass surgery. *Eur J Cardiothorac Surg* 2005; 28:805-10
- Wilkowski DA, Sill JC, Bonta W, Owen R, Bove AA: Nitrous oxide constricts epicardial coronary arteries without effect on coronary arterioles. *ANESTHESIOLOGY* 1987; 66:659-65
- Siker D, Pagel PS, Pelc LR, Kampine JP, Schmeling WT, Warltier DC: Nitrous oxide impairs functional recovery of stunned myocardium in barbiturate-anesthetized, acutely instrumented dogs. *Anesth Analg* 1992; 75:539-48
- De Hert SG, Turani F, Mathur S, Stowe DF: Cardioprotection with volatile anesthetics: Mechanisms and clinical implications. *Anesth Analg* 2005; 100:1584-93
- Boillot A, Haddad E, Vallet B, Barale F: Effects of anesthetic agents on arterial reactivity. *Ann Fr Anesth Reanim* 1999; 18:415-29

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