

# Nitrous Oxide and Long-Term Morbidity and Mortality in the ENIGMA Trial

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**BACKGROUND:** There is a plausible pathophysiologic rationale for increased long-term cardiovascular morbidity and mortality in patients receiving significant exposure to nitrous oxide. However, this relationship has not been established clinically. The ENIGMA trial randomized 2050 patients having noncardiac surgery lasting more than 2 hours to nitrous oxide–based or nitrous oxide–free anesthesia. We conducted a follow-up study of the ENIGMA patients to evaluate the risk of cardiovascular events in the longer term.

**METHODS:** The trial case report forms and medical records of all study patients were reviewed. The date and cause of death and occurrence of myocardial infarction or stroke were recorded. A telephone interview was then conducted with all surviving patients. The primary endpoint of the study was survival.

**RESULTS:** The median follow-up time was 3.5 (range: 0 to 5.7) years. Three hundred eighty patients (19%) had died since the index surgery, 91 (4.5%) were recorded as having myocardial infarction, and 44 (2.2%) had a stroke during the entire follow-up period. Nitrous oxide did not significantly increase the risk of death [hazard ratio = 0.98 (95% confidence interval, CI: 0.80 to 1.20;  $P = 0.82$ )]. The adjusted odds ratio for myocardial infarction in patients administered nitrous oxide was 1.59 (95% CI: 1.01 to 2.51;  $P = 0.04$ ) and for stroke was 1.01 (95% CI: 0.55 to 1.87;  $P = 0.97$ ).

**CONCLUSIONS:** The administration of nitrous oxide was associated with increased long-term risk of myocardial infarction, but not of death or stroke in patients enrolled in the ENIGMA trial. The exact relationship between nitrous oxide administration and serious long-term adverse outcomes will require confirmation by an appropriately designed large randomized controlled trial. (Anesth Analg 2011;112:387–93)

There is a plausible pathophysiologic rationale for increased long-term cardiovascular morbidity and mortality in patients receiving significant exposure to nitrous oxide.<sup>1,2</sup> Nitrous oxide oxidizes the cobalt atom on vitamin B<sub>12</sub>, inactivating methionine synthase and causing a dose-dependent increase in plasma homocysteine concentrations for days after surgery.<sup>3–5</sup> Acutely increased plasma homocysteine concentrations impair endothelial function,<sup>6</sup> induce oxidative stress<sup>7</sup> and potentially destabilize coronary artery plaques.<sup>8</sup> Furthermore, several studies reported increased incidences of myocardial ischemia within 48 hours<sup>9</sup> and cardiovascular events within 30 days<sup>5</sup> in patients receiving nitrous oxide. However, a relationship between nitrous oxide administration and long-term cardiovascular morbidity and mortality has not been

established. As the importance of anesthetic management to long-term outcomes has been questioned,<sup>10</sup> further exploration of this issue is warranted.

The ENIGMA trial randomized 2050 patients having noncardiac surgery lasting more than 2 hours to nitrous oxide–based or nitrous oxide–free anesthesia.<sup>11</sup> Within 30 days of surgery, 16 patients had died (9 in the nitrous oxide group and 3 in the nitrous oxide–free group;  $P = 0.10$ ). Myocardial infarction (MI) was confirmed by electrocardiographic changes and cardiac enzyme increase in 13 patients in the nitrous oxide group, and in 7 patients in the nitrous oxide–free group ( $P = 0.20$ ). Furthermore, 30 patients in the nitrous oxide group and 10 patients in the nitrous oxide–free group recorded electrocardiographic changes or cardiac enzyme increase suggestive of MI ( $P = 0.002$ ).<sup>12</sup> Finally, postoperative plasma homocysteine concentrations were increased in patients receiving nitrous oxide, emphasizing the pathophysiologic rationale for a relationship between nitrous oxide and MI.<sup>5,13</sup> Together these findings support further study of this issue.

We therefore conducted a follow-up study of the ENIGMA patients. We tested the hypothesis that patients exposed to nitrous oxide during noncardiac surgery would be at greater risk of death, MI, and stroke in subsequent years than would patients whose indexed anesthetic did not include nitrous oxide.

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## METHODS

In the original ENIGMA trial, 2050 surgical patients aged 18 years or older were randomized to 70% nitrous oxide in 30% oxygen or 80% oxygen in 20% nitrogen. In all other respects, perioperative care was at the discretion of the anesthesiologists. The primary end point was duration of hospital stay. Major complications were assessed in hospital and at a 30-day medical record review and telephone interview.<sup>11</sup> A subset of patients consented to blood sampling for plasma homocysteine and folate assays preoperatively and on the first postoperative day.<sup>5</sup>

## Protocol

For the current study, ethics committee approval was obtained at each participating site. Patient consent had been obtained for the original study, and patients could refuse participation in the present study at the time of follow-up, so a second written consent process was waived by all centers. The trial case report forms and medical records of all study patients were reviewed for the study endpoints. The date and cause of death or the occurrence of MI or stroke were recorded. This was followed by a structured telephone interview with all surviving patients. Verbal consent was obtained before patients were questioned about the occurrence of MI and stroke since the indexed surgery. If the patient had died, the patient's relatives or doctors were interviewed, after verbal consent had been obtained, using the same questionnaire. At least 3 attempts were made to contact patients with the contact details on the original case report form and the hospitals' information management systems. If these attempts failed, at least 3 attempts were made to contact the patients' relatives, doctors, or both.

The primary endpoint of the study was survival, which was recorded as the time to the last confirmed contact with the patient or the time of death. Secondary endpoints were MI and stroke, defined by (1) a verbal report by the patient or his or her relatives or doctors or (2) a note in the patient's medical record. MI was defined as a typical increase and decline in cardiac enzymes (troponin or creatine kinase-MB fraction) 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 MI. Stroke was defined as new neurologic deficit persisting for 24 hours or longer, confirmed by assessment by a neurologist or computed tomography or magnetic resonance imaging.

## Data Analysis

The following preoperative and intraoperative characteristics were chosen prospectively as predictors in the models: age, gender, weight, ASA physical status, history of coronary artery disease, anemia, emergency surgery, abdominal surgery, propofol maintenance, volatile anesthetic administration, nitrous oxide use, bispectral index monitoring, and duration of anesthesia. Median volatile anesthetic concentrations for the case were recorded by the anesthesiologist on the case report form and were converted to minimum alveolar concentration (MAC) equivalents before analysis.

Survival rates were computed for each category of each predictor and are expressed as deaths per 1000 person-years. Univariate Cox proportional hazard models were used to define hazard ratios and 95% confidence intervals (CIs). The multivariable Cox proportional hazard models were constructed as follows: preoperative variables (age, gender, weight, ASA physical status, history of coronary artery disease, anemia, emergency surgery, and abdominal surgery) were adjusted for each other. Nitrous oxide, propofol maintenance, and bispectral index monitoring were adjusted for each other and preoperative variables. Volatile anesthetic administration <0.75 MAC equivalents (the median MAC value in patients receiving volatile anesthetic maintenance) and duration of anesthesia were adjusted for each other, preoperative variables, nitrous oxide, and bispectral index monitoring (propofol maintenance was not included because the 267 patients who received propofol for maintenance had missing data for volatile anesthetic administration, causing this variable to be dropped from the model). This approach was taken to minimize bias.<sup>14</sup> Assessment of proportionality of hazard functions was performed.

In many cases, the date of MI or stroke was imprecise (i.e., just the year of the event) or was missing, so logistic regression was used to compute odds ratios and 95% CIs for MI and stroke during the follow-up period. The multivariable models were constructed as above. The preplanned assessment of the interaction of each variable with nitrous oxide was performed using interaction terms in the regression models. We used the 90th percentile of the preoperative homocysteine concentration of patients in whom preoperative homocysteine concentration was measured (single measurement per patient) to define postoperative hyperhomocysteinemia.<sup>15</sup> No further blood sampling was undertaken as part of this follow-up study. Data were compared using paired *t*-tests or  $\chi^2$  tests as appropriate.

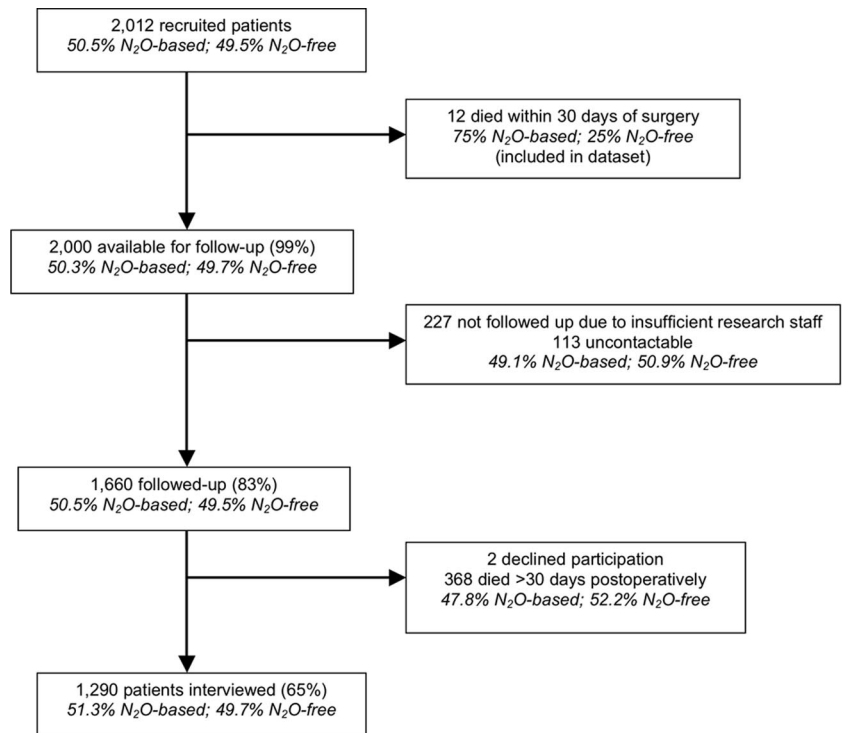
To assess the effects of missing follow-up data, we undertook two sets of sensitivity analyses. Multiple imputation based on the multivariate normal distribution was performed,<sup>16</sup> assuming that the missing stroke and MI outcomes were missing at random. For the survival analyses, inverse probability-of-censoring weights were estimated using logistic models, and the Cox models were refitted with these weights.<sup>14</sup> Results were unchanged under both sensitivity analyses.

Analyses were conducted using Stata 10.0 (Stata Corporation, College Station, TX, USA). All *P* values are two-sided, and *P* < 0.05 was considered statistically significant.

## RESULTS

Recruitment to the ENIGMA trial occurred between April 2003 and November 2004, and 1212 patients were included in the intention-to-treat analysis. Long-term follow-up occurred between January 2007 and November 2008, with a median follow-up of 3.5 (range: 0 to 5.7) years. No attempt was made to follow-up 227 of the patients who had survived 30 days because of a lack of resources at the recruiting centers; 113 patients who had survived 30 days could not be contacted when follow-up was attempted; and 2 patients declined further participation. The follow-up time of all of these patients was recorded as 30 days; they

**Figure 1.** Flow chart for inclusion of ENIGMA Trial patients in this long-term follow-up study (N<sub>2</sub>O = nitrous oxide).



were coded as alive, and the occurrence of MI and stroke by 30 days was used in the analyses. Follow-up data were obtained for 1660 (83%) of study patients (Fig. 1).

Three hundred eighty patients (19%) had died since the indexed surgery (12 before 30 days and 368 subsequently). The causes of death were cancer (76%), MI (5%), stroke (1%), other cardiovascular death (2%), respiratory failure (1%), sepsis (6%), other causes (6%), and unknown (3%). Interviews therefore were completed in 1290 (65%) patients. Ninety-one patients (4.5%) were recorded as having an MI, and 44 patients (2.2%) were recorded as having a stroke during the entire follow-up period.

Nitrous oxide did not increase the risk of death (hazard ratio = 0.98; 95% CI: 0.80 to 1.20;  $P = 0.82$ ) (Table 1). Increasing age, male gender, abdominal surgery, propofol maintenance, MAC equivalents  $<0.75$ , and longer duration of anesthesia were significant predictors of death. There was a significant interaction between nitrous oxide administration and abdominal surgery (overall  $P = 0.028$ ). The hazard ratio for death after the use of nitrous oxide in abdominal surgery was 1.02 (95% CI: 0.55 to 1.90;  $P = 0.95$ ), and in nonabdominal surgery, it was 0.64 (95% CI: 0.43 to 0.96;  $P = 0.03$ ) (i.e., there was a 36% reduction in the risk of mortality among nonabdominal surgery patients having nitrous oxide-free anesthesia, but no effect was observed in patients having abdominal surgery).

The adjusted odds ratio for MI in patients receiving nitrous oxide was 1.59 (95% CI: 1.01 to 2.51;  $P = 0.04$ ) (Table 2). In addition, increasing age, higher ASA physical status, known coronary artery disease, anemia, and increasing duration of anesthesia were significant predictors of MI. There were no significant interactions among predictors. The adjusted odds ratio was largely unaffected after multiple imputation for missing data (1.56 [95% CI: 0.99 to 2.46;  $P = 0.05$ ]).

Nitrous oxide did not increase the risk of stroke (odds ratio = 1.01 (95% CI: 0.55 to 1.87;  $P = 0.97$ ). Increasing age was the only significant predictor of stroke in a multivariable model that included the same predictors used in the survival and MI analyses (results not shown). The adjusted odds ratio was largely unaffected after multiple imputation for missing data (1.01 [95% CI: 0.55 to 1.87;  $P = 0.98$ ]).

Postoperative plasma homocysteine and folate concentrations were significantly increased in comparison with preoperative values in patients who had an MI (Table 3). In addition, a larger proportion of patients with MI recorded postoperative hyperhomocysteinemia. There were no differences found between surviving and deceased patients with respect to plasma homocysteine and folate concentrations.

## DISCUSSION

Nitrous oxide was associated with a marginal increase in the long-term risk of MI, but not of death or stroke in the ENIGMA patients. The ENIGMA trial recruited relatively unselected patients with low 30-day and long-term event rates, and therefore may have been underpowered to confidently confirm a “true” increased risk of MI in patients receiving nitrous oxide.<sup>17</sup> A clinically important increase or decrease in the risk of these major outcomes is still possible and therefore should be explored. Consequently, we are conducting a randomized trial of nitrous oxide-based versus nitrous oxide-free anesthesia in 7000 noncardiac surgery patients who have or are at risk of ischemic heart disease (the ENIGMA-II trial; [www.enigma2.org.au](http://www.enigma2.org.au)).<sup>12</sup>

Our long-term follow-up results are consistent with the 30-day incidences of MI (0.5%) and stroke (0.1%) in ENIGMA patients. These patients were not selected on the basis of cardiovascular risk factors: only 11% of the patients

**Table 1. Hazard Ratios for Death in All Patients (n = 2012)**

Predictor	Death rate (95% CI)	Univariate HR (95% CI)	P value	Multivariate HR (95% CI)	P value
Age (years) <sup>a</sup>					
<49	50 (40–62)				
49–64	63 (53–76)	1.26 (0.95–1.66)		1.06 (0.80–1.41)	
≥65	91 (78–105)	1.79 (1.39–2.32)	<0.0001	1.45 (1.09–1.91)	0.01
Sex <sup>a</sup>					
Male	76 (67–87)				
Female	60 (51–70)	0.78 (0.64–0.96)	0.02	0.77 (0.62–0.95)	0.02
Weight (kg) <sup>a</sup>					
<60	82 (70–96)				
61–74	66 (55–79)	0.80 (0.63–1.01)		0.83 (0.65–1.06)	
≥75	58 (48–70)	0.71 (0.55–0.90)	0.02	0.83 (0.64–1.08)	0.23
ASA physical status <sup>a</sup>					
1–2	63 (56–72)				
3–4	84 (70–100)	1.31 (1.06–1.63)	0.01	1.12 (0.87–1.45)	0.36
Coronary artery disease <sup>a</sup>					
No	66 (59–74)				
Yes	87 (67–113)	1.31 (0.99–1.74)	0.06	1.10 (0.80–1.53)	0.55
Anemia <sup>a</sup>					
No	64 (57–72)				
Yes	104 (82–133)	1.61 (1.23–2.10)	0.0005	1.22 (0.92–1.63)	0.16
Emergency surgery <sup>a</sup>					
No	67 (61–75)				
Yes	90 (58–140)	1.31 (0.83–2.05)	0.24	1.27 (0.80–2.01)	0.31
Abdominal surgery <sup>a</sup>					
No	41 (34–50)				
Yes	90 (80–102)	2.15 (1.71–2.70)	<0.0001	2.04 (1.60–2.59)	<0.0001
BIS monitoring <sup>b</sup>					
No	62 (55–70)				
Yes	93 (77–112)	1.47 (1.18–1.84)	0.001	1.20 (0.95–1.51)	0.13
Nitrous oxide <sup>b</sup>					
No	72 (63–83)				
Yes	65 (56–75)	0.91 (0.74–1.11)	0.34	0.98 (0.80–1.20)	0.82
Propofol maintenance <sup>b</sup>					
No	63 (56–71)				
Yes	102 (82–127)	1.59 (1.24–2.03)	<0.0001	1.60 (1.23–2.07)	0.0004
MAC equivalents <sup>c</sup>					
≥0.75	45 (37–55)				
<0.75	80 (70–92)	1.77 (1.39–2.24)	<0.0001	1.59 (1.22–2.08)	0.0007
Duration of anesthesia (hours) <sup>c</sup>					
<2.5	37 (28–47)				
2.5–3.9	63 (53–75)	1.73 (1.26–2.36)		1.43 (1.01–2.02)	
≥4.0	97 (86–114)	2.65 (1.97–3.57)	<0.0001	2.03 (1.44–2.87)	0.0001

CI = confidence interval; HR = hazard ratio (the hazard in the exposed group divided by the hazard in the unexposed group, in which hazard is the incidence rate of an event); ASA = American Society of Anesthesiologists; BIS = bispectral index; MAC = minimum alveolar concentration.

<sup>a</sup> Adjusted for each other.

<sup>b</sup> Adjusted for each other and all predictors identified by footnote a.

<sup>c</sup> Adjusted for each other and all predictors identified by footnotes a and b except for propofol maintenance (267 missing values for MAC equivalents).

reported a history of coronary artery disease, and only 4% reported a history of stroke. Our results are also consistent with the incidence of MI and stroke in unselected surgical patients,<sup>18,19</sup> rather than those incidences that are typical in patients at higher risk.<sup>20</sup>

Hyperhomocysteinemia was present in 11% of patients without MI and 46% with MI in this long-term follow-up study. Laboratory studies have suggested that homocysteine is both atherogenic and thrombogenic. Epidemiological, dietary, and genetic studies support higher incidences of cardiac events in patients with hyperhomocysteinemia.<sup>15</sup> However, folate supplementation (which normalizes

plasma homocysteine concentrations)<sup>21–23</sup> and omission of nitrous oxide<sup>11</sup> are not currently convincingly proven to reduce the risk of MI. The effects of both of these strategies await confirmation with large randomized trials.<sup>12,22</sup>

Nitrous oxide may adversely affect outcomes through mechanisms other than hyperhomocysteinemia. This includes immunosuppression, impairment of erythrocyte production, promotion of pulmonary atelectasis, and the need to use low inspired oxygen concentrations.<sup>1,2</sup> Indeed, ENIGMA patients had an increased risk of wound infection, fever, and pulmonary complications during the first 30 postoperative days.<sup>11</sup> However, our follow-up study did

**Table 2. Odds Ratios for Myocardial Infarction in All Patients (n = 2012)**

Predictor	n (%)	Univariate OR (95% CI)	P value	Multivariate OR (95% CI)	P value
Age (years) <sup>a</sup>					
<49	7 (1)				
49–64	27 (4)	4.26 (1.84–9.84)		3.25 (1.38–7.67)	
≥65	57 (9)	9.41 (4.26–20.18)	<0.0001	4.47 (1.93–10.35)	0.002
Sex <sup>a</sup>					
Male	57 (5)				
Female	34 (4)	0.64 (0.42–0.99)	0.05	0.73 (0.46–1.16)	0.19
Weight (kg) <sup>a</sup>					
<60	36 (6)				
61–74	35 (5)	0.90 (0.56–1.45)		0.97 (0.58–1.60)	
≥75	20 (3)	0.50 (0.28–0.87)	0.04	0.56 (0.31–1.03)	0.13
ASA physical status <sup>a</sup>					
1–2	31 (2)				
3–4	60 (12)	6.67 (4.27–10.43)	<0.0001	3.50 (2.08–5.86)	<0.0001
Coronary artery disease <sup>a</sup>					
No	57 (3)				
Yes	34 (15)	5.47 (3.48–8.58)	<0.0001	1.71 (1.01–2.92)	0.048
Anemia <sup>a</sup>					
No	67 (4)				
Yes	24 (10)	2.98 (1.83–4.86)	<0.0001	1.85 (1.06–3.25)	0.03
Emergency surgery <sup>a</sup>					
No	84 (4)				
Yes	7 (9)	2.08 (0.93–4.65)	0.08	1.02 (0.42–2.50)	0.96
Abdominal surgery <sup>a</sup>					
No	41 (5)				
Yes	50 (4)	0.93 (0.61–1.42)	0.74	0.78 (0.49–1.26)	0.31
BIS monitoring <sup>b</sup>					
No	69 (4)				
Yes	22 (5)	1.22 (0.75–2.00)	0.42	1.16 (0.68–1.97)	0.58
Nitrous oxide <sup>b</sup>					
No	37 (4)				
Yes	54 (5)	1.46 (0.95–2.24)	0.08	1.59 (1.01–2.51)	0.04
Propofol maintenance <sup>b</sup>					
No	98 (4)				
Yes	13 (5)	1.09 (0.60–2.00)	0.77	1.36 (0.71–2.59)	0.35
MAC equivalents <sup>c</sup>					
≥0.75	29 (3)				
<0.75	49 (6)	1.75 (1.10–2.90)	0.02	0.84 (0.48–1.46)	0.53
Duration of anesthesia (hours) <sup>c</sup>					
<2.5	19 (3)				
2.5–3.9	23 (3)	1.05 (0.57–1.95)		0.81 (0.40–1.63)	
≥4.0	49 (7)	2.36 (1.38–4.06)	0.0006	1.93 (1.00–3.72)	0.009

OR = odds ratio (the odds of an event in the exposed group divided by the odds of an event in the unexposed group); CI = confidence interval; ASA = American Society of Anesthesiologists; BIS = bispectral index; MAC = minimum alveolar concentration.

<sup>a</sup> Adjusted for each other.

<sup>b</sup> Adjusted for each other and all predictors identified by footnote a.

<sup>c</sup> Adjusted for each other and all predictors identified by footnotes a and b except for propofol maintenance (267 missing values for MAC equivalents).

not provide any evidence to support a concern that these consequences of nitrous oxide use increase long-term mortality in noncardiac surgery patients.

In this follow-up study, various baseline characteristics were identified as independent predictors of adverse outcomes. Many of these have been reported, including increasing age,<sup>24–26</sup> increasing ASA physical status score,<sup>24,26,27</sup> and longer duration of surgery.<sup>25</sup> Increased mortality among abdominal surgery patients may reflect cancer treatment, although we cannot confirm this because we did not record malignancy status in this study. Similarly, the administration of lower volatile anesthetic concentrations to patients who subsequently died probably reflects the presence

of serious comorbidities and intolerance of the hemodynamic effects of volatile anesthetics in these patients.

A potential limitation of the ENIGMA trial was that inspired oxygen concentrations were not the same in the 2 groups (30% in the nitrous oxide–based group and 80% in the nitrous oxide–free group). The study was designed that way because these gaseous combinations reflected routine practice and allowed patients to take advantage of all the reported benefits of the omission of nitrous oxide (including high inspired oxygen concentration).<sup>11</sup> In addition, the design allowed us to examine the data for an independent effect of oxygen, which we did not find.<sup>11</sup> A further limitation of the follow-up study was that surveillance for

**Table 3. Plasma Homocysteine and Folate Concentrations**

	Alive	Dead	P value
Preoperative			
Homocysteine ( $\mu\text{mol/L}$ ) ( <i>n</i> = 387)	9.3 $\pm$ 4.0	9.8 $\pm$ 4.0	—
Folate (mmol/L) ( <i>n</i> = 386)	23.6 $\pm$ 5.0	23.8 $\pm$ 5.0	—
Postoperative			
Homocysteine ( $\mu\text{mol/L}$ ) ( <i>n</i> = 370)	9.9 $\pm$ 6.0	10.6 $\pm$ 4.0	0.8831 <sup>a</sup>
Folate (mmol/L) ( <i>n</i> = 370)	24.3 $\pm$ 6.0	25.5 $\pm$ 6.0	0.2442 <sup>a</sup>
Homocysteine $\geq$ 13.5 ( $\mu\text{mol/L}$ ) ( <i>n</i> = 52)	32 (12)	20 (18)	0.151
	No myocardial infarction	Myocardial infarction	P value
Preoperative			
Homocysteine ( $\mu\text{mol/L}$ ) ( <i>n</i> = 387)	9.2 $\pm$ 4.1	11.9 $\pm$ 4.1	—
Folate (mmol/L) ( <i>n</i> = 386)	23.8 $\pm$ 5.2	22.8 $\pm$ 5.8	—
Postoperative			
Homocysteine ( $\mu\text{mol/L}$ ) ( <i>n</i> = 370)	9.7 $\pm$ 5.8	13.9 $\pm$ 5.1	0.0447 <sup>a</sup>
Folate (mmol/L) ( <i>n</i> = 370)	24.6 $\pm$ 6.2	25.0 $\pm$ 5.7	0.0286 <sup>a</sup>
Homocysteine $\geq$ 13.5 ( $\mu\text{mol/L}$ ) ( <i>n</i> = 52)	36 (11)	16 (46)	<0.0001

Data are presented as mean  $\pm$  standard deviation or number (percentage). Hyperhomocysteinaemia was defined by the 90th percentile of the preoperative homocysteine concentration (i.e., 13.5  $\mu\text{mol/L}$ ).

<sup>a</sup> Adjusted for preoperative value.

postoperative MI and stroke was at the discretion of the patients' doctors, and we relied on the review of medical records after patient interviews to confirm the occurrence of endpoints. For these reasons, the reported incidences of MI and stroke in this follow-up study are likely to be underestimated. Finally, we did not collect data on intraoperative hemodynamic treatment or anesthetic depth, both of which have been implicated in adverse postoperative outcomes.<sup>10</sup>

In conclusion, the administration of nitrous oxide was associated with increased long-term risk of MI but not of death or stroke in patients enrolled in the ENIGMA trial. The exact relationship between nitrous oxide administration and serious long-term adverse outcomes requires investigation in an appropriately designed large randomized controlled trial.<sup>12</sup> ■■

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KL helped design the study, conduct the study, analyze the data, and write the manuscript and was responsible for archiving the study files. PSM helped design the study, conduct the study, analyze the data, and write the manuscript. MTVC helped conduct the study and write the manuscript. AF helped analyze the data and write the manuscript. MJP helped conduct the study and write the manuscript. PP helped conduct the study and write the manuscript. BSS helped conduct the study and write the manuscript. EW helped analyze the data and write the manuscript.

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