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CLINICAL PRACTICE

Effect of nitrous oxide on plasma homocysteine and folate in patients undergoing major surgery

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Background. Nitrous oxide (N_2O) inhibits methionine synthetase resulting in elevated plasma homocysteine (Hcy) concentration after surgery. In epidemiological studies, hyperhomocysteinaemia is associated with increased risk of cardiovascular disease and dementia.

Methods. Blood samples were obtained to measure plasma folate and Hcy concentrations from two centres participating in a multicentre randomized trial investigating the effects of N_2O on the outcome after major surgery. The effect of N_2O and duration of anaesthesia on plasma Hcy, and the relationship between hyperhomocysteinaemia and outcomes were assessed.

Results. We enrolled 394 patients. The N_2O Group had an increase in plasma Hcy concentration after surgery when compared with the N_2O-free Group: 11.1 (3.8) *vs* 8.5 (4.0) μmol litre^{-1}, $P<0.0005$. Postoperative hyperhomocysteinaemia was associated with an increased risk of major complications: risk ratio (RR) 2.8 (95% CI: 1.4–5.4), $P=0.002$ and cardiovascular events, RR 5.1 (95% CI: 3.1–8.5), $P<0.0005$. There was a significant association between duration of anaesthesia and the relative change in plasma Hcy concentration, particularly in the N_2O Group: $r=0.42$, $P<0.001$.

Conclusions. N_2O increases plasma Hcy concentration; this effect is greater with a longer duration of anaesthesia. Hyperhomocysteinaemia is a risk factor for major postoperative complications. N_2O-induced increases in plasma Hcy concentration may be a cause of postoperative cardiovascular morbidity.

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Nitrous oxide (N_2O) oxidizes the cobalt atom on vitamin B_{12}, leading to inhibition of the enzyme methionine synthetase.[1–5] Because methionine synthetase is required for folate and DNA synthesis, prolonged or repeated exposure to N_2O can lead to megaloblastic anaemia and subacute degeneration of the spinal cord.[6–11] These adverse effects of N_2O exposure are not limited to intensive care patients and those undergoing exceedingly prolonged surgery. A 2-h exposure to N_2O is associated with a 50% reduction in methionine synthetase activity.[3]

Methionine synthetase catalyses the transfer of a methyl group from N5-methyltetrahydrofolate to homocysteine (Hcy), producing tetrahydrofolate and methionine.[1–5] Hcy has gained importance in cardiovascular medicine because

it induces endothelial dysfunction and has atherogenic properties,[12–17] both of which are associated with increased risk of myocardial infarction (MI), stroke, and dementia in the general population and those with other risk factors for cardiovascular disease.[12–18]

A few small studies have found that N_2O exposure during surgery leads to increased plasma Hcy concentrations after operation,[4 19–21] but the extent and clinical implications of this are unclear. In a recently completed study,[22] we took the opportunity to collect blood samples in patients from two centres to measure the effect of N_2O on postoperative plasma folate and Hcy concentrations. This enabled us to identify factors associated with elevated Hcy concentration and its relationship with postoperative complications.

Methods

The ENIGMA Trial evaluated outcomes associated with N_2O exposure in 2050 adult patients undergoing major non-cardiac surgery.[22] In brief, adult patients undergoing general anaesthesia and in whom surgery was anticipated to exceed 2 h duration were recruited in the study. Patients were randomly assigned to receive either N_2O-free or N_2O-containing general anaesthesia. The N_2O-free Group could receive higher inspired concentration of oxygen, such that most of them received 80% oxygen with air.[22] The patient and all surgical and research staff were blinded to group identity. Postoperative complications occurring in the first 30 days after surgery were recorded. Outcomes included wound infection, pneumonia, severe nausea and vomiting, MI, and death.[22] We defined major postoperative complication as one or more of: wound infection, MI, stroke, pneumonia, thromboembolism, awareness, and death; adverse cardiovascular events included thromboembolism, MI, and stroke; respiratory complications included atelectasis, pneumonia, pneumothorax, and pulmonary embolism.

In the present study, a sequential cohort of patients enrolled in the ENIGMA trial from two of the participating centres (Alfred Hospital, Melbourne; Prince of Wales Hospital, Hong Kong) were included in a sub-study of folate and Hcy flux after surgery. The study protocol was approved by the ethics committee of both the participating institutions. Patients were approached before surgery and provided written, informed consent.

Patients' dietary folate and vitamin B_{12} status were assessed by a brief screening questionnaire, with yes or no responses to the following items:[23] (i) vegan or vegetarian status; (ii) regular breakfast cereal intake; (iii) daily fresh fruit and/or vegetables intake; (iv) regular vitamin B or folate tablets; (v) vitamin B_{12} injection in the previous 3 months.

On the day of surgery and in the morning of the first postoperative day, a 10 ml fasting venous blood sample was obtained from each of the study participants. The blood samples were stored at 4°C, and then centrifuged within 30 min at 3500 rpm for 10 min. Plasma was separated and stored at −70°C until analysis. Plasma folate and Hcy concentrations were measured by immunoassay, as previously described.[23]

Statistical analysis

We initially planned our sample size based on previous data,[21] using a mean increase in plasma Hcy concentration from 11 to 15 μmol litre^{-1}, with a standard deviation (SD) of 6 μmol litre^{-1}, and this required 47 patients per group (type I error of 0.05 and a type II error of 0.2). We subsequently increased the sample size to 394 patients in this study to allow meaningful exploratory analyses of a number of potential variables that may affect plasma Hcy concentration. The analyses were based on intention-to-treat.

We used the 90th centile of the preoperative Hcy concentration to define postoperative hyperhomocysteinaemia.[15] Group patient characteristics and perioperative data were tabulated using mean (SD), median [interquartile range (IQR)], or number (%). Numerical data were first tested for normality and then analysed using *t*-tests. Categorical data were analysed using χ^2; risk ratio (RR) and 95% confidence intervals (CI) were used as an estimate of risk. Associations between numerical variables were measured using Pearson correlation coefficients (*r*). We used logistic regression analyses to identify the risk of postoperative complications, adjusting for age, ASA physical status, and duration of anaesthesia, and then explored the effect of plasma Hcy and folate flux. We also did exploratory stepwise linear regression analysis to identify significant factors associated with plasma Hcy and folate flux. The effect of N_2O exposure and duration of anaesthesia on the postoperative change in plasma Hcy was analysed with generalized linear models. All analyses were done using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). All reported *P*-values were two-sided and not adjusted for multiple comparisons.

Results

We enrolled 394 patients undergoing a broad range of surgical procedures (Table 1). There were no dropouts or exclusions. The median (IQR) duration of anaesthesia was 4.5 (3.1–6.0) h, and after operation 80 patients (20%) were admitted to the intensive care unit directly from the operation theatre. A total of 67 patients (17%) had at least one major postoperative complication. There were only six patients with confirmed MI (N_2O Group, $n=2$; N_2O-free Group, $n=4$), and three deaths (all in the N_2O Group).

The N_2O-free Group received a higher inspired oxygen concentration and slightly more inhalation agent during maintenance of anaesthesia, both $P<0.001$ (Table 2). The N_2O Group had a significantly increased plasma Hcy concentration after surgery compared with the N_2O-free Group (Table 3).

The 90th centile of the preoperative plasma Hcy concentration was 13.5 μmol litre^{-1}. N_2O increased the risk of postoperative hyperhomocysteinaemia, N_2O Group 13 (7.7%) *vs* N_2O-free Group 38 (19%), RR 1.9 (95% CI: 1.2–3.1), P=0.002. This adverse effect was apparent across all sub-groups (Fig. 1). There was an increased risk of major complications in those with hyperhomocysteinaemia, RR 2.8 (95% CI: 1.4–5.4), P=0.002; but this was modified by N_2O exposure (interaction term P=0.06), and for those in the N_2O Group, the risk of major complications in those with hyperhomocysteinaemia was less, RR 1.44 (95% CI: 0.76–2.71), P=0.31; N_2O-free Group 6.02 (95% CI: 2.24–16.1), P<0.0005. There was also an increased risk of adverse cardiovascular events in those with hyperhomocysteinaemia, RR 5.1 (95% CI: 3.1–8.5), P<0.0005. There was no evidence that preoperative folate or vitamin B supplementation protected against postoperative hyperhomocysteinaemia (OR 1.4, P=0.32). N_2O was associated with more complications, but most were not affected by postoperative plasma folate concentration or hyperhomocysteinaemia (Table 4). The exception was major complications, for which hyperhomocysteinaemia had an independent additional effect on the risk of major complications (Table 4). This suggests that N_2O-associated complications cannot be entirely attributed to elevated plasma Hcy. Regular ingestion of cereals (P=0.17) or preoperative folate or vitamin B supplementation (P=0.97) had no significant effect on the risk of major complications.

There was a negative correlation between plasma folate and Hcy concentrations before operation, r=−0.27 (P<0.001), but not after operation, r=0.02 (P=0.65). However, this lack of association in the postoperative period was confounded by N_2O exposure: for patients in the N_2O-free Group there was a negative correlation between postoperative folate and postoperative Hcy, r=−0.24 (P=0.002); for patients in the N_2O Group there was no correlation between postoperative folate and postoperative Hcy, r=0.04 (P=0.57).

There was a significant effect of duration of anaesthesia on the relative change in plasma Hcy concentration, and this effect differed between the groups (P<0.001): N_2O Group r=0.42 (P<0.001); N_2O-free Group r=0.26 (P=0.001) (Fig. 2). An exploratory logistic regression analysis identified several significant predictors of hyperhomocysteinaemia (Table 5). Preoperative folate or vitamin B supplementation was protective in this regard.

An exploratory stepwise linear regression analysis identified N_2O exposure (increase), smoking (increase), female gender (increase), regular breakfast cereal (decrease),

Table 1 Patient and surgical characteristics expressed as number (%), unless otherwise stated. ASA, American Society of Anesthesiologists; ENT, ear, nose and, throat

Factors	N_2O Group n=215	N_2O-free Group n=179
Males	118 (55)	108 (60)
Age (yr), mean (SD)	59 (15)	58 (16)
Weight (kg), mean (SD)	63 (16)	63 (17)
ASA physical status		
I	29 (14)	30 (17)
II	129 (60)	105 (59)
III or IV	57 (27)	44 (25)
Dietary factors		
Vegan	2 (0.9)	3 (1.7)
Regular breakfast cereals	66 (31)	63 (35)
Regular fruit/vegetables	124 (58)	98 (55)
Folate/vitamin B supplementation	61 (28)	43 (24)
Medical conditions		
Smoker	51 (24)	27 (15)
Diabetes	29 (14)	33 (18)
Hypertension	90 (42)	66 (37)
Coronary artery disease	28 (13)	16 (8.9)
Heart failure	8 (3.7)	2 (1.1)
Previous thromboembolism	5 (2.3)	5 (2.8)
Previous stroke	16 (7.4)	8 (4.5)
Type of surgery		
General	104 (48)	92 (51)
Urology	35 (16)	33 (18)
Neurosurgery	19 (8.8)	17 (9.5)
Orthopaedic	13 (6.0)	11 (6.1)
Vascular	17 (7.9)	7 (3.9)
Gynaecology	14 (6.5)	7 (3.9)
ENT or faciomaxillary	10 (4.7)	9 (5.0)
Plastics	3 (1.4)	3 (1.7)
Duration of anaesthesia (h), mean (SD)	4.9 (2.4)	4.9 (2.5)

Table 3 Changes in plasma folate and homocysteine (Hcy) concentration according to nitrous oxide (N_2O) exposure during surgery, expressed as mean (SD). *Adjusted for preoperative value

Factors	N_2O Group n=215	N_2O-free Group n=179	P-value*
Preoperative			
Hcy (μmol litre^{-1})	9.2 (3.0)	9.7 (5.3)	–
Folate (mmol litre^{-1})	23.5 (5.1)	23.9 (5.4)	–
Postoperative			
Hcy (μmol litre^{-1})	11.1 (3.8)	8.5 (4.0)	<0.0005
Folate (mmol litre^{-1})	26.5 (5.7)	22.4 (6.0)	<0.0005

Table 2 Anaesthetic factors expressed as mean (SD). MAC, minimum alveolar concentration. MAC values used for sevoflurane, isoflurane, and desflurane as 1.80, 1.15, and 6.0, respectively

Factors	N_2O Group n=215	N_2O-free Group n=179	P-value
Inspired oxygen concentration (%)	32 (5.8)	73 (19.3)	<0.001
End-tidal volatile concentration (MAC equivalents)	n=162 0.63 (0.02)	n=130 0.76 (0.02)	<0.001
Target propofol plasma concentration (μg ml^{-1})	n=48 3.28 (0.50)	n=46 3.30 (0.58)	0.85
Intraoperative fentanyl (μg)	n=154 106 (50)	n=114 117 (72)	0.12
Intraoperative morphine (mg)	n=169 11.0 (5.2)	n=142 11.0 (7.2)	0.99

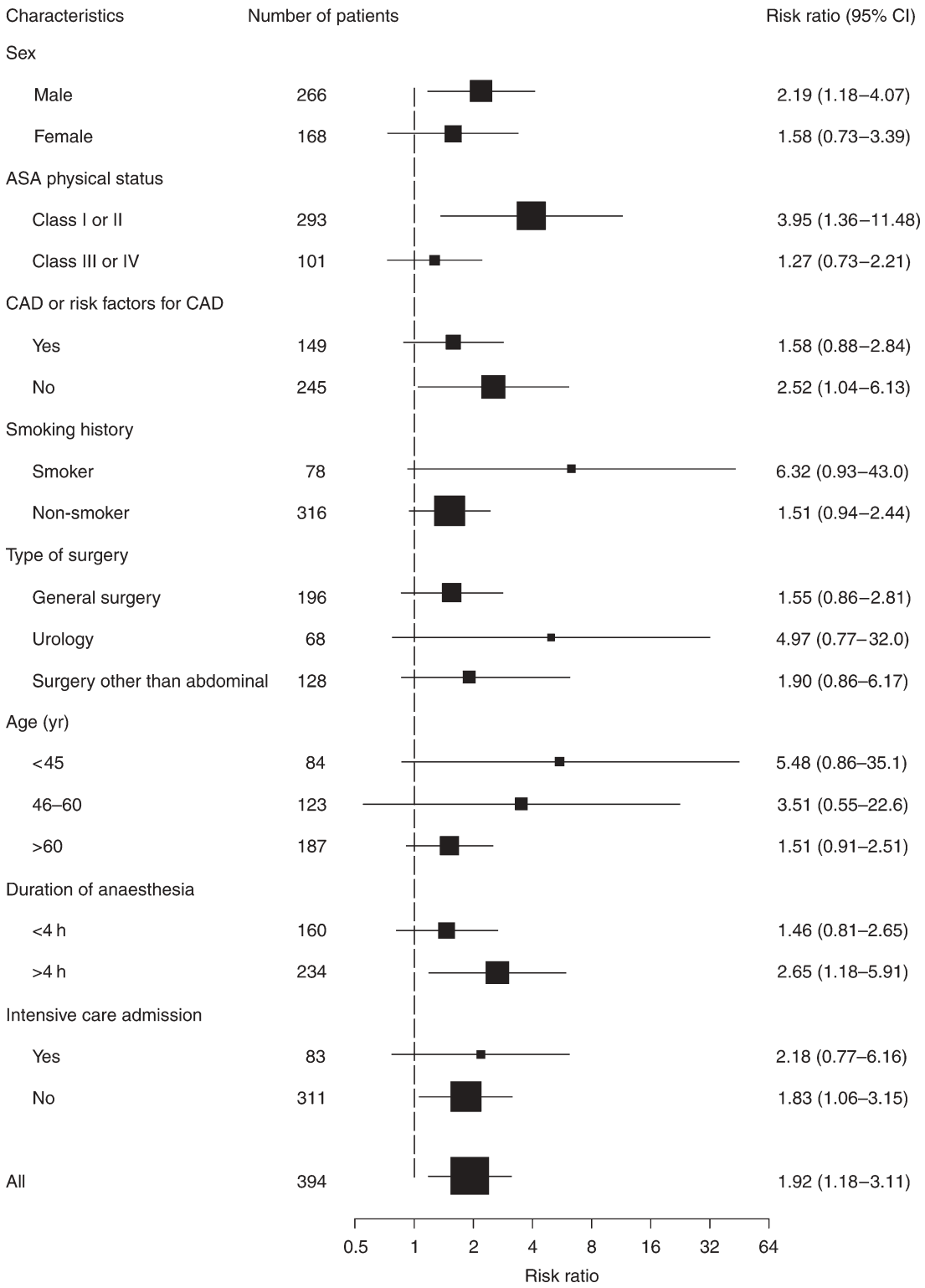

Fig 1 The risk of hyperhomocysteinaemia with nitrous oxide exposure in selected sub-groups, expressed as risk ratio (95% confidence interval). The vertical interrupted line represents a risk ratio of 1.0 (no effect). Sub-group differences existed for ASA ($P<0.0005$) and CAD ($P<0.0005$), but not for others (all $P>0.05$). ASA, American Society of Anesthesiologists physical status; CAD, coronary artery disease.

folate or vitamin B supplementation (decrease), and duration of anaesthesia (decrease) as the only significant predictors of a relative change in plasma folate concentration after surgery (all $P<0.001$).

Discussion

We found that N_2O exposure increased plasma Hcy concentration and this was dose-related, in which a longer duration of anaesthesia (and surgery) had a greater impact

Table 4 The risk of postoperative complications in patients receiving nitrous oxide (N_2O) adjusted for age, ASAs' physical status, and duration of anaesthesia. Hyperhomocysteinaemia and plasma folate variables were then added to logistic regression models to determine whether they had an independent effect. *Respiratory complications included pneumonia, atelectasis, pneumothorax, or pulmonary embolism within 30 days of surgery. †Major complications included pneumonia, pulmonary embolism, wound infection, myocardial infarction, venous thromboembolism, stroke, awareness, or death within 30 days of surgery. ‡Hyperhomocysteinaemia had an independent effect on the risk of major complications, adjusted odds ratio (OR) 2.54 (95% CI: 1.28–5.01), modifying the N_2O effect, adjusted OR 1.56 (95% CI: 0.87–2.80)

Complication	Incidence (%)		Total N_2O effect, OR (95% CI)	Total N_2O effect, *P*-value	Independent Hcy effect, *P*-value	Independent folate effect, *P*-value
	N_2O Group (n=215)	N_2O-free Group (n=179)				
Severe nausea or vomiting	27	12	2.64 (1.54–4.55)	<0.0005	0.54	0.5
Wound infection	16	7.8	2.31 (1.18–4.53)	<0.0005	0.91	0.46
Any respiratory complication*	24	13	2.17 (1.26–3.74)	0.005	0.72	0.94
All major complications†	21	12	1.93 (1.10–3.41)	0.023	0.007‡	0.57

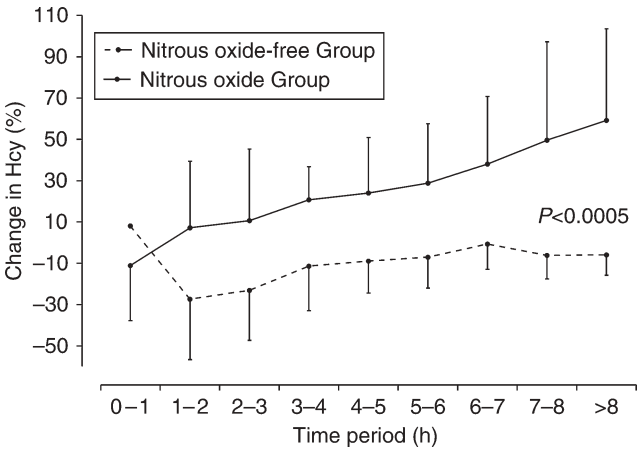

Fig 2 The effect of duration of anaesthesia on the postoperative change in plasma homocysteine (Hcy) according to nitrous oxide exposure, expressed as % change from the preoperative value. Values are mean (SD). There was a group–time interaction (P<0.0005).

Table 5 Patient and anaesthetic factors associated with hyperhomocysteinaemia after surgery. ASA, American Society of Anesthesiologists; ICU, intensive care unit; OR, odds ratio

Factors	Univariate OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Male sex	1.47 (0.79–2.72)	0.22	–	–
Age >65 yr	2.16 (1.19–3.92)	0.01	0.97 (0.39–2.40)	0.94
ASA III or IV	4.15 (2.25–7.66)	<0.0005	6.48 (2.95–14.2)	<0.0005
Coronary artery disease	2.99 (1.63–5.48)	<0.0005	1.86 (0.74–4.72)	0.19
Smoker	1.92 (0.98–3.73)	0.053	1.53 (0.71–4.72)	0.27
Folate/vitamin B supplementation	0.26 (0.10–0.67)	0.003	0.09 (0.03–0.28)	<0.0005
Abdominal surgery	0.68 (0.37–1.25)	0.21	–	–
Nitrous oxide exposure	2.80 (1.44–5.46)	0.002	3.91 (1.82–8.40)	<0.0005
Duration of anaesthesia >4 h	1.42 (0.78–2.56)	0.25	–	–
Admission to ICU	1.59 (0.80–3.08)	0.19	–	–

on postoperative Hcy concentration. Furthermore, this effect was apparent in a variety of patient categories, both with and without comorbidity, and undergoing a diverse range of surgical procedures. We identified hyperhomocysteinaemia as a risk factor for major postoperative complications in this cohort.

N_2O is a potent inhibitor of methionine synthetase, with even low concentrations (10–50%) decreasing enzyme activity to undetectable concentrations within hours.[1 2] Hyperhomocysteinaemia may then ensue.[4 19–21] Hyperhomocysteinaemia is a recognized risk factor for MI, dementia, and stroke.[12–18] A meta-analysis of randomized trials found that reducing plasma Hcy concentration by 3 μmol litre^{-1} could reduce the risk of MI by 16% (95% CI: 11–20%), stroke by 24% (95% CI: 15–33%), and venous thromboembolism by 25% (95% CI: 8–38%).[17] A randomized trial of 90 patients undergoing carotid endarterectomy under general anaesthesia[21] found that N_2O exposure led to a significant increase in postoperative Hcy concentration and myocardial ischaemia. Acute increases in Hcy concentration lead to endothelial dysfunction[24] and thrombogenesis,[12 13 15 17] both of which could increase the risk of perioperative MI.[14 15]

As found in our clinical trial from which this cohort was selected,[22] avoidance of N_2O and replacement with additional inspired oxygen is associated with a reduction in severe nausea or vomiting, wound infection, respiratory complications, and major complications. The study design leaves open the possibility that supplemental oxygen could have had an independent effect on some of the results, but there are no pre-existing data suggesting an oxygen effect on methionine synthetase or plasma Hcy. In the present study, we found no apparent modification of the risk of any of these complications by preoperative vitamin supplementation or postoperative plasma folate concentrations. Similarly, postoperative hyperhomocysteinaemia had no effect on nausea or vomiting, wound infection, or respiratory complications. There was however an independent effect of hyperhomocysteinaemia on the risk of major complications, and this can be attributed to increased rates of cardiovascular complications.

Low plasma folate concentrations are a common problem in the elderly,[25] and there is an association between folate

status and total Hcy concentrations.[15 23] Preoperative vitamin B supplementation for 1 week before surgery can prevent N_2O-induced hyperhomocysteinaemia,[26] but this is dose-dependent. Folinic acid 30 mg given immediately before and 12 h after anaesthesia prevents the N_2O-induced increase in plasma Hcy concentration in most patients, whereas smaller amounts are ineffective.[27] We found that regular vitamin supplementation reduced the risk of hyperhomocysteinaemia but did not protect against complications associated with N_2O administration. However, we did not record the vitamin dosage or compliance.

As shown by other studies,[1 2 4] we found that N_2O exposure was associated with increased plasma folate concentrations after operation. This may be explained by a probable failure of the intracellular transport of folate,[3 5] and enhanced excretion,[1] such that a folate deficiency state may exist in the setting of normal plasma (total) folate concentrations. This is known as the 'methyl trap hypothesis',[1 5] whereby decreased amounts of 10-formyl- and 5,10-methylenetetrahydrofolate are available for purine and thymidine synthesis. In rats, N_2O exposure is followed by increased plasma folate concentrations but reduced tissue folate stores, this loss being most marked in the liver.[1] In humans, N_2O also results in diminished intracellular folate concentrations and a redistribution of folate derivatives.[5 28] Our study findings of no association between plasma folate and Hcy concentrations after operation in those exposed to N_2O, in contrast to those in the N_2O-free Group, supports a mechanism of N_2O-induced impairment of intracellular transport of folate in the early postoperative period.

Our results have implications for patients undergoing surgery and general anaesthesia. Elderly, vegan, and chronically ill patients may be deficient in dietary folate, methionine, or vitamin B_{12}.[8 10 13 25] N_2O can interfere with folate and Hcy metabolism and may increase the risk of postoperative cardiovascular and other adverse outcomes.[21 22] Such data provide some reasons to question the routine use of N_2O in contemporary practice.[22 29]

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