Impaired vitamin B12 metabolic status in healthcare workers occupationally exposed to nitrous oxide

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Background. Previous studies demonstrated inactivation of vitamin B12 by nitrous oxide (N2O). The intraoperative exposure to N2O was shown to induce megaloblastic anaemia and myelopathy in subjects with subclinical vitamin B12 deficiency. In contrast, no data concerning the influence of occupational exposure to N2O on vitamin B12 metabolic status are available to date. In the present study, the vitamin B12 status in operating theatre personnel was assessed in relation to the extent of exposure.

Methods. Ninety-five operating theatre nurses with the history of exposure to N2O and 90 unexposed counterparts were examined. Vitamin B12 and folic acid were measured by immunoassay. Total homocysteine (tHcy), an indicator of impaired vitamin B12 metabolism, was determined by high performance liquid chromatography. N2O concentration was monitored by adsorption gas chromatography and mass spectrometry.

Results. No significant differences were found between both groups with respect to haematological parameters and folic acid. However, subjects exposed to N2O presented with lower vitamin B12 [372.8 (12.1) vs 436.8 (13.2) pmol litre⁻¹, P<0.001] and higher tHcy [11.2 (0.5) vs 8.9 (0.5) μmol litre⁻¹, P=0.006]. The changes in vitamin B12 status were aggravated in subjects exposed to N2O in concentrations substantially exceeding occupational exposure limit (180 mg m⁻³) [vitamin B12: 341.9 (17.7) vs 436.8 (13.2) pmol litre⁻¹, P=0.006; tHcy: 12.9 (0.7) vs 8.9 (0.5) μmol litre⁻¹, P=0.047].

Conclusions. Exposure to N2O in healthcare workers is associated with alterations of vitamin B12 metabolic status, the extent of which depends on the level of exposure.

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Nitrous oxide (N2O) is an inhaled anaesthetic commonly used in surgery. Whereas N2O is generally regarded as a safe agent, there is considerable evidence that the intraoperative exposure to N2O and the habitual N2O abuse cause adverse effects on haematopoietic and nervous systems. Complications ranging from subtle haematological changes to agranulocytosis, spinal cord degeneration, and polyneuropathy were reported in subjects anesthetized with N2O1–6 or inhaling N2O for recreational purposes.7–9 Clinical symptoms after the exposure to N2O are highly reminiscent of those encountered in vitamin B12 (cobalamin) deficit. Accordingly, adverse effects accompanying N2O anaesthesia were aggravated in subjects with subclinical cobalamin deficiency,10–13 either due to low dietary consumption or due to genetic predisposition.

N2O acts by oxidizing vitamin B12 from the active cob(I)alamin to the inactive cob(III)alamin.14 This renders methylcobalamin, a metabolite of cobalamin, inactive as a cofactor of methionine synthase and impairs the conversion of homocysteine to methionine, which is essential for
myelin synthesis. Increased level of total homocysteine (tHcy) in serum is, therefore, an indicator of cobalamin deficiency and a biomarker of N₂O exposure.

Healthcare workers active in operating theatres are repeatedly exposed to N₂O in the ambient air. Whereas deleterious effects of N₂O in patients undergoing anaesthesia and in habitual N₂O abuse were reported, little effort has been directed towards evaluation of the influence of N₂O on vitamin B₁₂ metabolism in operating personnel. This prompted us to investigate biochemical indices of vitamin B₁₂ metabolic status among surgical nurses working under various levels of exposure to N₂O. Our results for the first time document alteration of vitamin B₁₂ metabolism in subjects occupationally exposed to N₂O and relate them to the degree of exposure.

Methods

Study subjects and design

This study included 185 female nurses aged between 25 and 56 yr and with 5–26 yr employment history in 10 hospitals in the city area of Lodz. The exposed group was made up of 95 operating theatre nurses (surgical nurses), who routinely provide full-time assistance during operations on a day-to-day basis. They usually stayed in the polluted area of the operating room not less than 5 h and worked for the whole occupational activity in the environment polluted with N₂O and volatile anaesthetics such as isoflurane, sevoflurane, or halothane. Previous studies revealed that this group is the most exposed to N₂O among operating theatre staff. The control group consisted of 90 nurses from other departments of the same hospitals, who have never been occupationally exposed to N₂O or volatile anaesthetics in the course of their professional career. Excluded were subjects periodically active in polluted areas. All examined subjects received information on the purpose of the study and signed the participation consent. The protocol was approved by the local ethical committee.

Each subject underwent general medical examination. Information concerning alcohol and coffee consumption and medication within the past 12 months was gathered using a questionnaire. To avoid inclusion of additional confounding factors, subjects with overt haematological diseases (three people), serious symptoms of neurological deterioration (one person), or heart failure (one person) were excluded. Except for minor illnesses nurses in both examined groups were in good health and presented with no clinical signs of vitamin B₁₂ deficiency. No apparent deterioration (one person), or heart failure (one person) were excluded. Except for minor illnesses nurses in both examined groups were in good health and presented with no clinical signs of vitamin B₁₂ deficiency. No apparent deterioration (one person), or heart failure (one person) were excluded. Except for minor illnesses nurses in both examined groups were in good health and presented with no clinical signs of vitamin B₁₂ deficiency. No apparent deterioration (one person), or heart failure (one person) were excluded. Except for minor illnesses nurses in both examined groups were in good health and presented with no clinical signs of vitamin B₁₂ deficiency.
than for 75% of the working shift, which allows expressing the N₂O air concentration in terms of occupational exposure limit (OEL). Static monitoring was carried out for N₂O measurement as previously described and individual dosimeters were applied for volatile anaesthetics. Quantification of N₂O and halogenated anaesthetics was done using, respectively, adsorption gas chromatography and partition gas chromatography followed by mass spectrometry.

**Statistical analysis**

An exploratory statistics was performed using the Statistical Package for the Social Sciences (SPSS-X). The results were expressed as mean (SEM) or as frequencies. Analysis of covariance (ANCOVA) with age and smoking as covariances were used for comparison of means. Pearson’s χ² or Fisher’s exact test were used to compare frequency distributions. Spearman’s rank correlation test was used to examine serial correlations. P-values <0.05 were considered significant.

**Results**

**Characteristics of examined populations**

The characteristics of the exposed group and the control group are shown in Table 1. Both groups did not differ significantly with respect to age, smoking, and alcohol and coffee consumption. The employment period was comparable in both groups.

**Long-term effects of exposure to anaesthetics on haematological parameters and indices of vitamin B12 metabolic status**

The values of haematological parameters and biochemical indices reflecting vitamin B12 status in exposed subjects and controls are shown in Table 2. No differences were observed in erythrocyte count, haemoglobin concentration, haematocrit, MCH, MCV, and MCHC. In contrast, serum concentrations of vitamin B12 were significantly lower in exposed subjects. As levels of vitamin B12 may not adequately reflect disturbances in cobalamin metabolism, plasma concentrations of tHcy were additionally determined. As shown in Table 2, the exposed group presented with increased mean baseline levels of tHcy. Figure 1 demonstrates the distribution (%) of subjects with tHcy concentrations exceeding the cut-off value (>12.8 µmol litre⁻¹) in groups with low, borderline low, medium, and high vitamin B12 concentrations. Owing to an inverse relationship between vitamin B12 and tHcy, the tendency towards over- and under-representation of subjects with increased plasma tHcy levels in groups with low and high vitamin B12 concentrations, respectively, was evident in the control population (χ²=10.5, df=3, P=0.015). This tendency was, however, attenuated in the exposed population (χ²=1.9, df=3, P=0.52).

**Levels of vitamin B12, folic acid, and tHcy in relation to the extent of exposure to N₂O**

Monitoring of anaesthetics in operating theatres revealed that N₂O most severely contributed to ambient air pollution.
Table 3 N2O and volatile anaesthetics concentrations measured in the ambient air of operating theatres, as described under Methods and are expressed as time-weighted average (TWA) in mg m\(^{-3}\) over an 8 h working shift. Concentrations were below, equal, or exceeded the OEL values for TWA over an 8 h working shift (source: occupational Safety and Health Administration www.osha.gov/dts/osta/anestheticgases/index.html). 1National Institute of Occupational Safety and Health (NIOSH), Cincinnati, USA. Recommended exposure limits—ceiling concentrations over a sampling period of 1 h for combined exposure to N2O and halogenated anaesthetics [source: NIOSH 1977 Criteria for a Recommended Standard: Occupational Exposure to Waste Anesthetic Gases and Vapors, Cincinnati, OH: US DH EW (NIOSH) Publication Nr 77–140 cited in: Horeua f and colleagues. Occupational exposure to sevoflurane and nitrous oxide in operating room personnel. Int Arch Occup Environ Health 1997, 69: 134–8]  

<table>
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<th>Number of operating theatres</th>
<th>Concentration (mg m(^{-3}))</th>
<th>Range</th>
<th>OEL Germany(^*)</th>
<th>OEL ACGIH(^†)</th>
<th>REL NIOSH(^‡)</th>
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<td>Nitrous oxide 26</td>
<td>468.0 (350.3)</td>
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<td>180.0</td>
<td>90.0</td>
<td>45.0</td>
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<td>Sevoflurane 26</td>
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<td>0.35–23.0</td>
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<td>Not assigned</td>
<td>3.8</td>
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<tr>
<td>Halothane 4</td>
<td>2.2 (16.1)</td>
<td>0.41–42.0</td>
<td>40.0</td>
<td>400</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Table 4 N2O concentrations measured in the ambient air of operating rooms with low and high exposure levels, as described under Methods and are expressed as time-weighted average (TWA) in mg m\(^{-3}\) over an 8 h working shift. Concentrations were below, equal, or exceeded the (German) OEL value (‘high exposure’ group). In contrast, none of the operating theatre concentrations of volatile anaesthetics, including isoflurane and halothane, exceeded values recommended in Germany or by ACGIH (Table 3). The analysis of the distribution of N2O levels in polluted areas revealed that in operating theatres equipped with complete air condition or pressure/exhaust ventilation system N2O concentrations were below, equal, or exceeded the (German) OEL value less than twice (mean 185.2 mg m\(^{-3}\); range 35.8–320.4 mg m\(^{-3}\); Table 4). In contrast, N2O concentrations substantially higher than OEL (mean 752.5 mg m\(^{-3}\); range 447.4–1502 mg m\(^{-3}\)) were registered in operating rooms equipped only with the natural ventilation system. Therefore, to assess the impact of the N2O exposure on the magnitude of changes in the vitamin B12 metabolic status in relation to OEL value, the levels of vitamin B12, folic acid, and tHcy were evaluated in two arbitrary subsets of study subjects: individuals working in operating theatres with N2O exposure below 2×OEL (360 mg m\(^{-3}\)) or above 2×OEL were categorized as ‘low exposure’ or as ‘high exposure’ group, respectively.

The effect of the low- and high-exposure levels on biochemical indices reflecting vitamin B12 status is shown in Table 5. Serum concentrations of vitamin B12 were significantly decreased, whereas tHcy concentrations in plasma were considerably increased in the group of nurses exposed to N2O in concentrations substantially exceeding (German) OEL value (‘high exposure’ group). In contrast, the levels of vitamin B12 and tHcy were not significantly different in ‘low exposure’ group. Moreover, there was a significant negative correlation between N2O exposure level and vitamin B12 concentration (r=−0.22; P=0.038) and a significant positive correlation between N2O exposure level and tHcy concentration (r=0.51; P<0.001) (Fig. 2A and B). No significant correlations were observed between N2O exposure levels and haemoglobin (r=0.078, P=0.62), haematocrit (r=0.09, P=0.29), MCV (r=0.061, P=0.45), and folic acid (r=0.16, P=0.09). Moreover, no significant correlations were observed between duration of exposure to N2O and vitamin B12 (r=0.18, P=0.3) and tHcy (r=0.18, P=0.09). The distribution of subjects exposed against high and low N2O levels across vitamin B12 and tHcy quartiles in unexposed population is shown in Figure 2C and D. Subjects from high exposure group were significantly over- and under-represented in lower and higher vitamin B12 quartiles, respectively, and in higher and lower tHcy quartiles, respectively.

Discussion

Current knowledge regarding adverse effects of the occupational exposure to N2O on vitamin B12 metabolism is incomplete. In one study, Salo and colleagues examined...
Compared with hospital staff working outside operating theatres. This observation was the first indication that repeated occupational exposure to N\textsubscript{2}O may disturb vitamin B\textsubscript{12} metabolic status.

Decreased levels of vitamin B\textsubscript{12} were previously reported in habitual N\textsubscript{2}O abuse and sporadically during N\textsubscript{2}O anaesthesia.\textsuperscript{8,9,11,13} As N\textsubscript{2}O preferentially targets metabolically active cob(I)alamin, the suppressing effect of this anaesthetic on plasma vitamin B\textsubscript{12} levels is surprising. In this study, we excluded the possibility that N\textsubscript{2}O interferes with the assay used for vitamin B\textsubscript{12} determination. In our view, it is more likely that N\textsubscript{2}O perturbs tissue distribution or degradation of cobalamin and thereby affects its concentration in plasma. Actually, gradual development of vitamin B\textsubscript{12} deficiency due to depletion of cobalamin in the liver and its conversion to inactive cobalamin analogues was observed in rats exposed to N\textsubscript{2}O.\textsuperscript{31} Moreover, xenobiotics, similarly to N\textsubscript{2}O inactivate cob(I)alamin, were shown to decrease vitamin B\textsubscript{12} in plasma.\textsuperscript{32}

A vast body of evidence suggests that serum levels of vitamin B\textsubscript{12} are not the most sensitive indicators of cobalamin deficiency. For instance, neurological abnormalities related to vitamin B\textsubscript{12} deficiency were repeatedly seen in subjects with normal serum cobalamin concentrations.\textsuperscript{33,34} Therefore, in addition to determination of vitamin B\textsubscript{12}, the levels of tHcy were measured in the present study. tHcy excessively accumulates in various forms of vitamin B\textsubscript{12} deficiency and its increased concentrations precede the overt vitamin B\textsubscript{12} deficiency.\textsuperscript{17} Several studies demonstrated that intraoperative acute exposure to N\textsubscript{2}O is invariably associated with postoperative increases in plasma tHcy.\textsuperscript{18–20} In the present study, we extend these observations to show that increased plasma tHcy levels are found in operating theatre personnel under repeated occupational exposure to N\textsubscript{2}O. Moreover, abnormal tHcy concentrations were found to be roughly equally distributed among N\textsubscript{2}O-exposed subjects with various vitamin B\textsubscript{12} concentrations (Fig. 1), suggesting that disturbances of cobalamin metabolism may occur in this group in the absence of measurable changes in vitamin B\textsubscript{12} levels in plasma. Cumulatively, our data for the first time provide the evidence that occupational exposure to N\textsubscript{2}O leads to alterations of vitamin B\textsubscript{12} metabolic status.

Previous studies demonstrated that the air pollution with N\textsubscript{2}O during surgical procedures is critically dependent on
the waste gas removing systems installed in operating theatres. Only combination of exhaust ventilation or air conditioning with scavenging device was revealed to effectively reduce the level of N₂O contamination. The important novel aspect of the present study is the demonstration that the extent of disturbances in vitamin B12 status observed in operating theatre personnel is closely related to the N₂O exposure level. Only nurses working in the environment, in which OEL value of 180 mg m⁻³ was several fold exceeded, presented with decreased vitamin B12 and increased tHcy levels. In contrast, less pronounced changes in parameters reflecting cobalamin metabolism were noted among nurses exposed to N₂O in concentrations that were lower than or exceeded (German) OEL less than twice. In addition, we observed negative and positive correlations between N₂O exposure and, respectively, vitamin B12 and tHcy concentrations. To our knowledge, this is the first demonstration that maintaining N₂O concentrations in the ambient air close to OEL may prevent derangement of vitamin B12 metabolism in medical personnel occupationally exposed to this anaesthetic. Our data emphasize the pivotal role of effective gas waste removing systems for reducing N₂O levels below OEL, but at the same time shed new light on the validity of exposure thresholds used in various countries. Actually, in spite of substantial overstepping of threshold values for N₂O recommended by US agencies (ACGIH, NIOSH) in almost all operating theatres in this study, vitamin B12 metabolism alterations appeared only in operating personnel exposed to the highest N₂O levels.

The clinical relevance of the present results remains unclear. Theoretically, healthcare workers active under excessive occupational exposure to N₂O, by whom disturbances of vitamin B12 metabolism were evident, might be more susceptible to development of symptomatic vitamin B12 deficiency under certain permissive conditions such as dietary vitamin B12 restriction. Moreover, they would be likely to develop hyperhomocystinaemia, which is a well-recognized independent risk factor for arterial and venous thrombosis and coronary heart disease. tHcy levels similar to those observed here in subjects exposed to high N₂O concentrations were previously demonstrated to substantially increase cardiovascular risk. Unfortunately, no epidemiological data concerning the frequency of coronary or thrombotic events among subjects occupationally exposed to N₂O are available to date. Clearly, further prospective studies will be necessary to fully assess the clinical relevance of the long-term excessive exposure to N₂O.

The cross-sectional design of the present study is a source of further limitations. Plasma levels of vitamin B12 and tHcy were determined once in the course of the study. Whereas symptoms of acute N₂O intoxications were absent in the examined cohort, it cannot be entirely excluded that some short-term influence additionally compounded the effects of chronic N₂O exposure. This contention is supported by the absence of a clear-cut relationship between the duration of employment and the degree of abnormalities, albeit the tendency (P=0.09) towards higher tHcy levels has been noted among nurses with longest occupational exposure. A longitudinal assessment of vitamin B12 metabolic status will be necessary to fully discriminate between chronic and temporary effects of excessive N₂O exposure in operating theatre personnel.

In conclusion, the present study demonstrates that maintaining N₂O concentrations in operating theatres under OEL is sufficient for preventing disturbances of vitamin B12 metabolism. Conversely, excessive repeated occupational exposure to N₂O is associated with alterations of vitamin B12 metabolic status, the extent of which is critically dependent on the level of exposure.

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