ARTICLE

The neuropsychiatric effects of nitrous oxide and low vitamin B_{12}

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SUMMARY

This narrative review article aims to update knowledge on the neuropsychiatric complications of nitrous oxide use and low vitamin B_{12}. We consider common forms and uses of nitrous oxide (N_2O) and review its mechanism of action, and then explore the potential impacts of use. In particular, neuropsychiatric effects mediated by low vitamin B_{12} are considered and the correct interpretation of laboratory results explored. This is of particular importance as where vitamin B_{12} is inactivated by chronic nitrous oxide use, blood test levels of vitamin B_{12} may not reflect the quantity of functional B_{12} in patients.

LEARNING OBJECTIVES

After reading this article you will be able to:

- demonstrate up-to-date understanding of the current use of nitrous oxide
- demonstrate improved understanding of the neuropsychiatric effects of nitrous oxide use, low vitamin B_{12} and high homocysteine
- demonstrate increased understanding of the role of vitamin B_{12} and the impact of functionally inactive vitamin B_{12}.

KEYWORDS

Substance use disorders; drug interactions and side-effects; neuropsychiatry; vitamin B_{12}; nitrous oxide.

Medical use of nitrous oxide

Nitrous oxide (N_2O) can be commonly found as a component in prescribed medication. Alongside this, it is used as a recreational substance. In its prescribed form, N_2O is a gas indicated for use in combination with other anaesthetic agents and for short-term analgesia when used in a 50:50 combination with oxygen as Entonox®. This is beneficial where rapid onset and clearance are needed (Savage 2014; BOC 2015; Heads of Medicines Agencies 2019). The analgesic, sedative and cognitive effects of N_2O are dose-dependent (Heads of Medicines Agencies 2019). Analgesic effects are obtained at a 25% concentration of N_2O and so continuous inhalation is not needed; rather, it is given through a demand valve operated as the patient inhales (BOC 2015). Contraindications for medical use of N_2O include respiratory or cardiac risk factors such as pneumothorax and persistent signs of confusion; caution should also be used if there is a risk of exacerbating folate or vitamin B_{12} deficiency (Heads of Medicines Agencies 2019).

Interestingly, there is ongoing investigation into the potential role of N_2O in reducing withdrawal symptoms for those reducing use of other substances such as alcohol or cocaine. Furthermore, a role in depression treatment is being considered (Amsterdam 2015).

Recreational use of nitrous oxide

Randhawa & Bodenham (2016) described an increasing trend for use of N_2O as a recreational drug, in part because it is comparatively simple and inexpensive to obtain, owing for example to its common use in the catering events industry. N_2O is often bought for recreational use in the form of small pressurised canisters, which are then released into balloons for inhalation (Sheldon 2020). These cannisters contain variable percentages of N_2O (Randhawa 2016; Fidalgo 2019).

The effect of this is rapid onset (within 10 s) of euphoria, disinhibition, analgesia and in some cases a hallucinogenic effect which is short lasting without substantial hangover effects. Although this is usually well tolerated, in those with pre-existing risks such as epilepsy or cardiac disease, or if combined with centrally acting arrhythmogenic drugs, the potential for seizures, arrhythmias and respiratory or cardiac arrest is raised. Furthermore, any vomiting may present an aspiration risk (Amsterdam 2015; Randhawa 2016). In the event of N_2O overdose, potential consequences include hypoxaemia, unconsciousness and ischaemia (Heads of Medicines Agencies 2019). Treatment for N_2O overdose is focused on stopping administration and supportive oxygenation (BOC 2015).

When inhaled from a balloon there is a similar safety net as with the valve in medicinal use, as if the user becomes overly sedated, they will not be able to maintain administration. However, if administered as a continuous flow, this will not apply, significantly increasing the risk of adverse effects

and overdose (Randhawa 2016). Furthermore, in obtaining this drug for recreational use, there is an increased risk that manufacturing safeguards present for medical products will not be present and also that users might administer together with other drugs or alcohol (Randhawa 2016). When N_2O is used, it is often as multiple administrations over a few hours. In heavy use, balloons may be filled from larger tanks rather than small canisters (Amsterdam 2015).

Extent of recreational use

In 2016, 9% of schoolchildren in England reported being offered N_2O, with 2.4% of 16- to 59-year-olds reporting use in the previous year (2016–2017) and 2.3% in 2017–2018. In children and young adults, N_2O was found to be the second most used drug after cannabis, with 9% of 16- to 24-year-olds reporting use in 2016–2017 and 8.8% in 2017–2018, with this age group forming the majority of users (Home Office 2018). In 2016 there were 8 deaths recorded due to the use of N_2O in England and Wales, notably an increase from 0–5 deaths recorded each year between 2001 and 2015; however, whether the deaths resulted from recreational or medical use is not specified (Office for National Statistics 2018a, 2018b). After combining data from multiple studies, Sheldon et al (2020) concluded that use was highest amongst young males and in high income areas such as the UK.

The most recent Global Drug Survey was reported in 2020 and collects data from over 25 countries. In their population, use of N_2O was reported by 13.1% of respondents in 2020, with 11.9% reporting use in 2019 and 6.9% in 2018. Comparatively, lysergic acid diethylamide (LSD) use was reported by 21% and ketamine by 15.9% in 2020. Although this is a large data-set, the data are collected using non-probability sampling methods and so it is difficult to generalise from their findings (Winstock 2020).

The majority of nitrous oxide users take less than 10 balloons during one episode and 80% of users have less than 10 episodes of use per year. Around 1% of users take more than 50 cannisters in a session, with a small subgroup of heavy users requiring the equivalent of 75–125 cannisters to remain under the influence. Overall, quantity of use is highly variable (Amsterdam 2015; Garakani 2016).

Nitrous oxide and UK law

In the UK, nitrous oxide is a psychoactive drug covered by the Psychoactive Substances Act 2016, meaning that it is illegal to supply and produce for its psychoactive effect, with those convicted facing up to 7 years in prison and an unlimited fine. There is currently no penalty for possession (unless already in prison) but you could receive a fine, driving ban or prison sentence if found using N_2O while driving (Crown Prosecution Service 2017; FRANK 2020). Within the first 12 months following the introduction of the 2016 Act, there were 202 records of police across 32 forces in England and Wales seizing N_2O (Home Office 2018).

Addictive potential

Addiction is a potential risk where there has been repeated administration of N_2O, with a frequency that is not yet clear in the drug safety data (BOC 2015; Heads of Medicines Agencies 2019). Alhough there is not felt to be a physiological addiction, use may be habit forming, with associated financial and social implications (Randhawa 2016).

Fidalgo et al (2019) recently conducted a systematic review considering misuse or dependence on N_2O and EMONO (equimolar mixture of oxygen and nitrous oxide – a premixed combination of 50% oxygen and 50% N_2O) defined using DSM-5 criteria. They reported higher misuse in men, adolescents and young adults. Interestingly, they reported that alongside recreational use, there were cases in which people taking N_2O combinations for initially medical purposes later developed a substance use disorder. In some cases, this occurred when EMONO was given as a medical intervention; when it was stopped, withdrawal symptoms and tension with the medical team were reported. Overall, they concluded that there was the potential for substance use disorder to develop.

Mechanism of action

Nitrous oxide modulates central nervous system neurotransmitters, including noradrenergic transmission, gamma-aminobutyric acid (GABA) receptor systems, *N*-methyl-D-aspartate (NMDA) antagonism and endogenous opioid systems, leading to anxiolytic and anaesthetic effects (Savage 2014; Heads of Medicines Agencies 2019; Sheldon 2020). The TREK-1 potassium channel has also been implicated in the anaesthetic effects of N_2O and in pain perception (Savage 2014). Furthermore, there is evidence of influence on dopaminergic transmission in the nucleus accumbens reward system and of cross-tolerance with morphine (Fidalgo 2019; Sheldon 2020).

N_2O reportedly causes functional vitamin B_{12} deficiency (Sheldon 2020), where vitamin B_{12} remains present, but in an inactivated form and thus unable to function via its normal role. N_2O inactivation of B_{12} is via oxidation of the cobalt ion, preventing the conversion of homocysteine to methionine (which plays a role in myelination),

TABLE 1 Manifestations of nitrous oxide misuse

Neurological signs and symptoms	Psychiatric signs and symptoms	Medical signs and symptoms
Confusion	Hallucinations	Low/inactivated vitamin B_{12}
Disorientation	Delusions	High homocysteine
Cognitive difficulties	Psychosis	High methylmalonic acid
Weakness	Delirium	Anaemia
Dizziness	Depersonalisation	Hypothermic skin trauma
Loss of balance and falls	Depression	Pneumomediastinum
Gait changes	Self-harm	Acute respiratory distress syndrome
Impaired proprioception	Suicidal thoughts and attempts	Pneumothorax
Analgesia	Agitation	Cardiac arrythmia
Hyperalgesia	Anxiety	Hypoxia
Paraesthesia	Violent behaviours	Death
Lhermitte's sign		
Sensory loss or numbness		
Sound distortion		
Myeloneuropathy		
Subacute combined degeneration		
Peripheral neuropathy		
Bladder dysfunction		
Bowel dysfunction		
Sexual dysfunction		
Seizures		

Sources: Amsterdam et al (2015); Garakani et al (2016); Kaar et al (2016); Shoults (2016); Heads of Medicines Agencies (2019); Chien et al (2020); Samia et al (2020).

along with the conversion of 5-methyltetrahydrofolate to tetrahydrofolate (role in DNA synthesis), and finally, helping convert L-methylmalonyl-Co-A to succinyl-Co-A (involved in the citric acid cycle) (Sheldon 2020).

N_2O is excreted unchanged primarily through alveolar ventilation and exhalation (BOC 2015; Heads of Medicines Agencies 2019). Following a brief inhalation, adverse psychomotor effects are rarely evident after 20 min although neuropsychiatric effects such as those mediated through vitamin B_{12} inactivation may persist, particularly with prolonged use (Heads of Medicines Agencies 2019).

Manifestations of persistent nitrous oxide use

Persistent nitrous oxide misuse can lead to a range of neuropsychiatric and medical presentations. These are summarised in Table 1.

A systematic review by Garakani et al (2016) investigated the neuropsychiatric and medical consequences of nitrous oxide misuse. Their data searches combined information from 91 cases of misuse resulting in neuropsychiatric or medical problems across 77 publications. Of the 91 cases reported, 72 recorded neurological symptoms, including myeloneuropathy and subacute combined degeneration of the spinal cord; 11 cases reported psychiatric symptoms such as psychosis and 8 included medical effects such as pneumomediastinum and frostbite. Across these 91 cases, 52 of the patients had low or low-normal vitamin B_{12} levels and some had normal B_{12} levels even in the presence of significant symptoms. Where the data were available, the reviewers commented that quantity and duration of use was linked to sequelae. More often, when use was daily and multiple whippets/cartridges were used (they defined the N_2O content of one whippet/cartridge as 8 g of 100% N_2O) more sequelae were observed. Alongside this they recorded 11 publications reporting 29 deaths resulting from nitrous oxide misuse. Death was often associated with significantly increased self-administration directly from a larger tank and was as a result of cardiac arrhythmia and hypoxia.

Kaar et al (2016) reported in large a cohort of 4883 nitrous oxide users that common experiences included hallucinations (27.8%), confusion (23.9%) and persistent numbness (4.3%), alongside accidental injury (1.2%). Accidental injury in particular was shown to be a dose-dependent effect. Amsterdam et al (2015) reported that accidental injury in nitrous oxide use was linked with dizziness, disorientation, loss of balance and cognitive changes following use, meaning falls were more likely to occur. They further report presentations of hypothermic skin trauma when N_2O is used in larger quantities while being dispensed directly from a larger tank tap.

Chien et al (2020) combined data from seven cases of people with a history of severe nitrous oxide use disorder presenting for treatment of psychiatric manifestations. Five of these patients described daily use of nitrous oxide and the other two respectively reported use 2–3 times a week and weekly. Duration of use was between 4 months and 10 years. Primary symptoms included suicide attempts or suicidal thoughts, violent behaviours or

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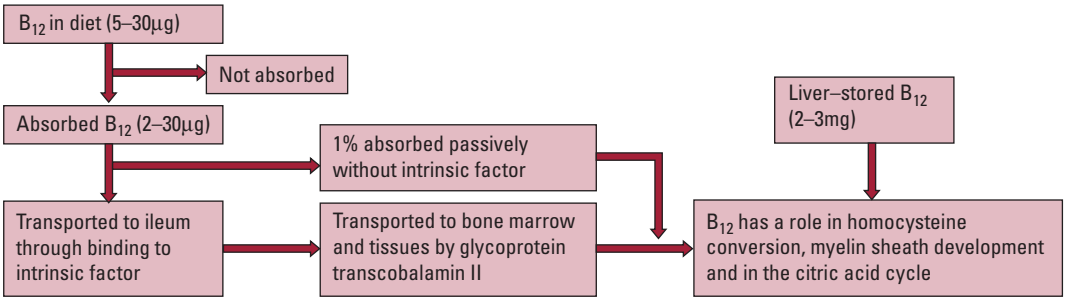

FIG 1 Summary of B_{12} metabolism (Kumar 2020).

irritability and psychosis symptoms such as hallucinations and delusions. Three of the seven people required a period of in-patient treatment in view of symptom severity. However, six of the seven had a history of mood disorders, including major depressive disorder and bipolar disorder. Furthermore, all were reported to have a history of substance misuse often including ketamine and 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), making it more difficult to establish the role of nitrous oxide use alone.

Indeed, combining the available evidence there appears to be a common pattern linking severity of symptoms to heavy and prolonged use or 'abuse' of nitrous oxide.

Vitamin B_{12} metabolism

Vitamin B_{12} is found in meat, fish, eggs and milk and the average daily diet contains approximately 5–30 µg, of which 2–3 µg is absorbed. The average adult will store 2–3 mg in the liver and so it may take up to 2 years before a deficiency becomes evident, as daily losses are small (Kumar 2020; Mukku 2018). Vitamin B_{12} is liberated from proteins by gastric enzymes, where it then becomes attached to a vitamin B_{12} binding protein (R-binder) and later is released by pancreatic enzymes, to instead become bound to intrinsic factor. When bound to intrinsic factor it is then transported to specific mucosal cells in the ileum. Here the intrinsic factor remains in the lumen of the ileum and vitamin B_{12} is transported to the bone marrow and other tissues by glycoprotein transcobalamin II (TC II). Approximately 1% of an oral dose of vitamin B_{12} is absorbed passively through the duodenum and ileum without the need for intrinsic factor (Kumar 2020). This process is summarised in Fig. 1.

Vitamin B_{12} deficiency or inactivation

The most common cause of vitamin B_{12} deficiency is pernicious anaemia. This is an autoimmune disorder with loss of parietal cells and subsequent reduction in intrinsic factor production, which leads to vitamin B_{12} malabsorption. Comparatively, nitrous oxide use is a recognised cause of vitamin B_{12} inactivation (Kumar 2020). Those at increased risk of vitamin B_{12} deficiency also include older adults and those who are pregnant or follow strict vegetarian diets (Mukku 2018).

Vitamin B_{12} ordinarily plays a role in converting homocysteine to methionine in order to support myelin sheath development and has further roles within the citric acid cycle. In view of this, when levels are low or it is inactivated by compounds such as N_2O, homocysteine and methylmalonic acid accumulate. It is possible to measure both homocysteine and methylmalonic acid levels to assess the extent of inactivation, as measurement of vitamin B_{12} level alone will not demonstrate the proportion of vitamin B_{12} able to complete its intended functions (Sheldon 2020). The lowest bracket of the vitamin B_{12} normal range in blood plasma is 160 ng/L and significant neurological changes can occur when levels drop below 60 ng/L (Kumar 2020). More recently, the National Institute for Health and Care Excellence (NICE) guidance stated that a serum level of under 200 ng/L (148 picomol/L) will diagnose 97% of people with a vitamin B_{12} deficiency (NICE 2019).

High or prolonged Entonox use has the potential to result in neurological and haematological effects similar to those of other causes of low vitamin B_{12} such as impaired dietary intake or pernicious anaemia (BOC 2015). However, neurological toxicity may occur without anaemia or macrocytosis and with vitamin B_{12} laboratory levels appearing to be in the normal range, as Entonox can cause existing B_{12} to become inactive (GOV.UK 2014; BOC 2015; Heads of Medicines Agencies 2019; Sheldon 2020). Therefore, clinicians should interpret B_{12} levels with caution where there has been nitrous oxide use.

Neuropsychiatric presentations of low functional vitamin B_{12} levels

Neuropsychiatric changes linked to low vitamin B_{12} include neuropathy, myelopathy and psychiatric disturbances such as symptoms of depression,

psychosis or cognitive dysfunction, and these may occur prior to haematological changes such as anaemia (Hutto 1997; Lerner 2002; Kumar 2020; Bar-Shai 2011; Mukku 2018; NICE 2019; NHS 2020). It is not always clear whether the vitamin deficiency caused the psychiatric presentation or was a consequence of it, given the potential for psychiatric illness to disrupt appetite and dietary intake, making analysis of case reports challenging (Hutto 1997).

A systematic review and meta-analysis by Petridou et al (2016) identified 11 studies that explored the relationship between folate and depression in older adults, of which 9 also considered vitamin B_{12}. The 9 studies with data on vitamin B_{12} included 6308 participants across multiple countries. Depression was linked to low vitamin B_{12}, with an overall odds ratio of 1.20 (95% CI 1.02–1.42).

A recent cross-sectional study of 303 participants within the Bhil indigenous population of India reported that those with depression and anxiety did not have a different distribution of folate and B_{12} status when compared with those without these disorders. However, it did demonstrate that high homocysteine, mediated by vitamin B_{12} deficiency, was linked to an increased risk of depression and anxiety disorders. In this study the cut-off used for vitamin B_{12} deficiency was higher than the NICE guidance levels above as they used <220 picomol/L and in this case the cause of the vitamin deficiencies was felt to be linked to difficulties in obtaining adequate nutrition linked to the lower economic resources of the studied group (Saraswathy 2019). Similarly, in a smaller study of 66 individuals in a Greek population who were aged 60 years or more Dimopoulos et al (2007) reported a correlation between depression and low folate and/or B_{12} as well as higher plasma homocysteine. They noted that this is important as there is the potential for homocysteine accumulation to cause neurotoxic effects.

Sun et al (2019) described a patient who presented with slowed reactions, apathy and communication difficulties who was initially treated for depression without success. He later developed weakness in his lower limbs, urinary incontinence and cognitive impairment, alongside difficulty in movements and gait and was demonstrated to have changes in his cerebellum, dorsal and lateral columns of the spinal cord and hydrocephalus. Further blood test analysis identified anaemia, low vitamin B_{12}, elevated homocysteine and he was positive for anti-intrinsic factor antibodies. Once treatment for his hydrocephalus and low B_{12} had taken place, both his neurological and psychiatric symptoms improved. This highlights the importance of assessing for B_{12} levels in those presenting with depression, although in this case the cause of low B_{12} was linked to autoimmune abnormalities in relation to intrinsic factor rather than nitrous oxide use. Similarly, a further case report by Bram et al (2015) focused on B_{12} deficiency caused by pernicious anaemia reported that the patient's first presentation was with symptoms of insomnia, general slowing and anxiety, with later examination demonstrating symptoms of catatonia. In this case they were successfully treated with increased cyanocobalamin (vitamin B_{12}) administration and lorazepam (Bram 2015).

A presentation of psychosis and depression without other neurological signs was reported by Bar-Shai et al (2011). In this case the 64-year-old male patient experienced auditory hallucinations, delusional content, core depressive symptoms such as low mood and anhedonia, and difficulties with short-term memory. Delusional content included thoughts of being terminally ill, fear of being watched by police and the tax department, and a fear of being poisoned. Importantly, this person had a history of chemotherapy for lymphoma and so was extensively investigated to ensure there was no recurrence of malignancy. He had also had a resection of the terminal ileum during this period, the primary site of B_{12} absorption, and had not received prophylactic supplements of vitamin B_{12}. He was not found to be anaemic, but he did have low B_{12} levels. He was started on antipsychotics and antidepressants for 2 weeks without success and then on hydroxocobalamin once the B_{12} level was available. Within 1 week of commencing hydroxocobalamin, there was a complete resolution of symptoms. Although it is possible that this could be linked to the effects of antipsychotics and antidepressants becoming more visible, these were subsequently reduced with a goal of complete discontinuation and there was not an associated relapse in symptoms (Bar-Shai 2011).

A similar rapid symptom resolution was described in a case reported by Lerner & Kanevsky (2002). This 52-year-old man was admitted to a psychiatric ward for disturbance in memory, depressive mood, apathy and disorientation and later developed distressing hallucinations and delirium. He had experienced poor nutrition prior to this episode and had lost 10 kg in 6 months; this was thought to be linked to significant life stressors. Laboratory blood test analysis demonstrated low B_{12} and raised homocysteine levels, and treatment with vitamin B_{12} replacement resulted in symptom resolution within 7 days.

Sheldon et al (2020) described the case of a patient developing tactile, auditory and visual hallucinations alongside delusional content. This later developed into myeloneuropathy and symptoms were

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thought to be resulting from inactivated B_{12} following inhalation of a high quantity of N_2O. Overall, they suggested that for weakness, numbness, paraesthesia or psychiatric effects in the context of known nitrous oxide use, tests should be conducted to measure not only B_{12} but also homocysteine and methylmalonic acid, which accumulate when there is B_{12} inactivation.

Seizures

M.K. reports a case of recurrent seizures in the context of B_{12} deficiency (Kumar 2004). This person responded well to B_{12} replacement. He reports that the exact mechanism by which seizure phenomena are caused is less established compared with other neurological effects; however, he felt it was likely to be linked to the potential for low B_{12} to cause myelin sheath damage to cerebral neurons and the altered susceptibility to glutamate effects. More recently Silva et al (2019) described functional deterioration, psychosis and seizures in the context of B_{12} deficiency in pernicious anaemia. With B_{12} correction their patient was able to make a significant recovery.

Similarly, Lee et al (2005) report the case of a patient who presented with generalised tonic–clonic seizures who was given a diagnosis of epilepsy of unknown etiology and anti-epileptic treatment. This person later developed some loss of sensation, cognitive impairment and altered reflexes and was found to have a very low B_{12} level and high homocysteine, with normocytic anaemia. Following B_{12} treatment, neuropsychiatric symptoms normalised with no further seizures reported and gait normalising after 6 months. They concluded that B_{12} deficiency should be considered when attempting to identify the cause of seizures.

Alongside the risk of seizures linked to low B_{12}, seizures are listed as a side-effect in the summary of product characteristics data for N_2O, although the frequency of this effect is as yet unclear (Heads of Medicines Agencies 2019). This ambiguity is noted by Zier & Doescher (2010), who reported three cases of clinical seizure activity in children which were temporally related to procedural sedation with N_2O, but they were unable to fully establish causality.

Mechanisms of neurotoxicity

There are multiple mechanisms proposed to account for the neurotoxicity associated with both nitrous oxide use and low vitamin B_{12}. Savage & Ma (2014) reported that the brain is most vulnerable to N_2O-associated neurotoxicity during early development (specifically, the perinatal period) and in older adulthood; the majority of this evidence came from studies in rat populations. Neurotoxicity is linked to NMDA antagonism, altered cerebral blood flow and enzyme inhibition, with each process being modulated by existing brain conditions (Savage 2014).

NMDA receptors are excitatory receptors that respond to endogenous glutamate. NMDA antagonists can have both protective and harmful effects, depending on their activation. Prolonged exposure can result in neuronal cell death and some NDMA antagonists have demonstrated the ability to alter hippocampal synapses and pathways involved in learning and memory (Savage 2014).

N_2O binds to the cobalt atom in vitamin B_{12} and causes its inactivation. B_{12} is a cofactor for methionine synthase and so there is disruption in the metabolic pathways converting homocysteine to methionine. Homocysteine itself does not appear to have a role outside of this process, although accumulation can lead to neurotoxicity, atherosclerosis and cardiovascular dysfunction, with its primary mechanisms of cellular damage being oxidative stress, mitochondrial dysfunction and depletion of the myelin layer (Lerner 2002; Savage 2014; Mukku 2018).

Homocysteine levels can take up to a week to normalise after N_2O administration despite the N_2O itself being rapidly cleared. Notably, methionine synthase inactivation can begin as early as 40 min into administration of 50% N_2O compounds (Savage 2014). Folate functioning is also altered when B_{12} is low or inactive, and this in itself can have neuropsychiatric complications, with both folate and B_{12} implicated in monoamine synthesis alongside methionine cycles (Hutto 1997; Lerner 2002; Bar-Shai 2011; Mukku 2018; Allott 2019). Increased synthesis of tetrahydrobiopterin (BH_4) has further been proposed as a potential mechanism through which nitrous oxide use can be linked to experiences of psychosis, as this is also involved in synthesis of monoamines such as dopamine (Garakani 2016).

Risk factors for high homocysteine notably include Alzheimer's disease, vitamin B_{12} deficiency and mutations in the methylenetetrahydrofolate reductase (*MTHFR*) gene (Savage 2014; Allott 2019). Interestingly, high maternal homocysteine in the perinatal period is linked to increased risk for later development of schizophrenia in the offspring. Furthermore, single nucleotide polymorphisms (SNPs) altering homocysteine levels are one of many genetic factors linked to increased psychosis risk (Allott 2019; Romain 2019). High homocysteine has been observed from illness onset in first-episode psychosis and has been linked to higher negative symptom scores (Allott 2019). The *MTHFR* gene is involved in the regulation of folate, and polymorphisms here have been linked

to schizophrenia, particularly negative symptoms (Allott 2019).

In view of the concerns regarding N_2O and functional vitamin B_{12} deficiency, a drug safety update from the UK's Medicines and Healthcare products Regulatory Agency (MHRA) reports that vitamin B_{12} levels should be considered prior to administration of N_2O where possible, particularly for those with risk factors for vitamin B_{12} deficiency. It suggests that N_2O should not be given continuously for longer than 24 h or more frequently than every 4 days without close supervision and blood test monitoring, although it recognises that symptoms may occur in the absence of haematological changes (GOV.UK 2014).

Use in pregnancy

Nitrous oxide has been used as a simple inhalant anaesthetic agent during obstetric care of women in labour and is present on most UK labour wards as part of normal care. From available research, there appears little in the way of evidence showing negative effects when used as a short-term anaesthetic option during labour. More research, however, exists into the effects of repeated environmental exposure to N_2O of healthcare workers involved in the obstetric teams caring for these patients (Rowland 1992; Ahlborg 1996). Ahlborg et al suggest that there is an increased rate of infertility and association with longer time to conception among these cohorts, thought to be linked to increased exposure to N_2O.

Utility of vitamin supplementation

When discussing the neuropsychiatric presentations of low vitamin B_{12}, many case reports described a positive symptom response to supplementation (Lerner 2002; Bar-Shai 2011; Bram 2015; Sun 2019).

Similarly, Lan et al (2019) identified nine patients presenting with symptoms of subacute combined degeneration of the spinal cord (SCD) in the context of nitrous oxide misuse. Of these, four patients were found to have low serum vitamin B_{12} and eight were shown to have raised homocysteine. When treated, they observed full recovery of muscle power, but persistence of sensory deficit symptoms was noted in five patients and sensory ataxia in one. They therefore suggested prompt treatment with vitamin replacement in patients with chronic nitrous oxide misuse to avoid permanent neurological damage. Samia et al (2020) followed a patient case of N_2O-induced SCD, identifying low vitamin B_{12} levels. Following a treatment regime of 7 days of daily vitamin B_{12} injections and subsequent once weekly injections, the patient reported overall symptomatic improvement. However, their results were less positive for some neurological symptoms, as ataxia and neuropathy associated with loss of vibratory sense and proprioception persisted.

Allott et al (2019) conducted a randomised controlled trial (RCT) in a first-episode psychosis out-patient cohort in which they randomised 120 individuals with psychosis to either the placebo control group or the group who were given adjunctive supplements of B_{12}, B_6 and B_9 (folate) for 12 weeks with the aim of assessing whether reducing homocysteine levels could be therapeutic. Only 14% of their total cohort had raised homocysteine at baseline. They measured change in homocysteine level, psychosis symptoms and neurocognition. During baseline testing they also assessed for genotypes, including the *MTHFR* gene, but did not find any interactive effects in this case. This study demonstrated reduced homocysteine in the supplemented group but did not demonstrate any benefit over placebo with regard to symptom management. Although there is the possibility that reducing homocysteine may be neuroprotective, they were not able to demonstrate symptom change in this study and concluded that vitamin supplementation should be considered on an individual needs basis. When they compared their results with studies showing more positive outcomes, it is apparent that higher supplement doses were used in the latter. This study notably had a heterogeneous participant group in that it included participants with a variety of different diagnoses, including schizophrenia, schizoaffective disorder, bipolar disorder with psychosis features and delusional disorder (Allott 2019).

Firth et al (2017, 2018) combined data from 18 RCTs involving a total of 832 patients for their systematic review and meta-analysis. This work focused on trials in which over 90% had a diagnosis of non-affective psychosis and demonstrated a moderate benefit in psychosis symptom reduction with supplements of B_6, B_9 and B_{12}, with no benefit over placebo shown for antioxidant vitamins or minerals. However, there was significant heterogeneity noted in the included trials and often vitamins were co-administered, making it difficult to determine which was responsible for outcomes or whether the combination was important. Trials using higher doses or combined vitamins were often more successful in reducing symptoms, as were trials in which participants were selected on the basis of nutritional deficiencies such as low folate or high homocysteine at baseline and those targeting participants at earlier stages of illness. They reported a potential role of genetic variance where outcomes differed, and this will be an important consideration in future research.

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When considering cognition, Behrens et al (2020) in their systematic review and meta-analysis of 20 studies found no benefit from supplementation of oral vitamin B compounds (B_1, B_6, folic acid and B_{12}) to prevent cognitive decline in the absence of existing cognitive impairment. On the other hand, they reported no evidence that vitamin B oral supplementation harmed cognitive function and suggested that further work could consider more specific cognitive domains rather than global function. Similarly, despite raised homocysteine being linked to cognitive impairment, no cognitive benefit was found when supplementing vitamin B with an aim of lowering homocysteine levels in the systematic review and meta-analysis completed by Ford & Almeida (2019) of 10 RCTs.

Health Quality Ontario (2013) combined data from 7 systematic reviews and 11 observational studies evaluating vitamin B_{12} and cognition and assessed the data quality and limitations. Key findings included that treatment with vitamin B_{12} and folate for those with mild cognitive impairment appeared to slow the rate of brain atrophy. However, this finding was based on low to moderate-quality evidence and it was not clear whether this would translate to clinical benefit. They also found an association between elevated plasma homocysteine levels and the onset of dementia but reported that this conclusion was on the basis of very low-quality evidence, limiting its utility and generalisability. Further data are therefore needed to investigate these two hypotheses.

Zhang et al (2020) also completed a systematic review and meta-analysis and identified 21 cross-sectional and longitudinal studies investigating whether B vitamins could help prevent cognitive decline. They reported that better cognition was linked with higher B_{12} levels in their cross-sectional studies, but this was not further demonstrated during stratified analysis or within the prospective studies that investigated this either on the basis of vitamin B_{12} intake or blood concentration. They therefore concluded that vitamin B_{12} may not be a modifiable risk factor when trying to slow cognitive decline.

Thus, overall there is little evidence for prophylactic supplementation at present. However, for cases where low vitamin B_{12} is present or vitamin B_{12} is inactivated through nitrous oxide use, correcting this deficiency has been shown to have significant benefits in reducing neuropsychiatric symptoms.

Conclusions

There is still knowledge to be gained in understanding the links between low vitamin B_{12}, high homocysteine, nitrous oxide use and their respective neuropsychiatric effects. It is important that clinicians consider assessment of vitamin B_{12} levels and nitrous oxide use for those presenting with neuropsychiatric symptoms (key practice points are summarised in Box 1).

BOX 1 Key practice points

- For patients using N_2O-containing compounds, consider the impact on B_{12} levels
- B_{12} and folate should be routinely checked when establishing a diagnosis in patients presenting with psychiatric symptoms
- Functional deficiency of B_{12} should be considered for patients with neurological or psychiatric symptoms and a history of nitrous oxide use
- Investigations for suspected functional deficiency of B_{12} should include blood tests for B_{12}; if there is diagnostic uncertainty, homocysteine and methylmalonic acid levels could be considered
- Where possible, do not give N_2O compounds continuously for longer than 24 h or more frequently than every 4 days without close monitoring

MCQ answers
1 c 2 c 3 e 4 b 5 b

Author contributions

All authors contributed significantly to this work, with J.F. leading the project, K.R. and M.I. contributing to the writing, and M.K. and W.Y.M. developing and editing the manuscript.

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Declaration of interest

None.

References

Ahlborg G, Axelsson G, Bodin L (1996) Shift work, nitrous oxide exposure and subfertility among Swedish midwives. *International Journal of Epidemiology*, **25**: 783–90.

Allott K, McGory PD, Yuen HP, et al (2019) The vitamins in psychosis study: a randomised, double-blind, placebo-controlled trial of the effects of vitamins B_{12}, B_6 and folic acid on symptoms and neurocognition in first-episode psychosis. *Biological Psychiatry*, **86**: 35–44.

Amsterdam J, Nabben T, van den Brink W (2015) Recreational nitrous oxide use: prevalence and risks. *Regulatory Toxicology and Pharmacology*, **73**: 790–6.

Bar-Shai M, Gott D, Marmor S (2011) Acute psychotic depression as a sole manifestation of vitamin B_{12} deficiency. *Psychosomatics*, **52**: 384–6.

Behrens A, Graessel E, Pendergrass A, et al (2020) Vitamin B – can it prevent cognitive decline? A systematic review and meta analysis. *Systems Review*, **9**: 111.

BOC (2015) *Entonox® Essential Safety Information (Medical Gas Data Sheet)*. BOC (https://safety365.sevron.co.uk/substances/accessSDS/SDS-113184-56a0f8c6e3f211.72010058 [cited 17 Jan 2020]).

Bram D, Bubrovsky M, Durand JP, et al (2015) Pernicious anaemia presenting as catatonia: correlating vitamin B12 levels and catatonic symptoms. *General Hospital Psychiatry*, **37**: 273.e5–7.

Chien W-H, Huang M-C, Chen L-Y (2020) Psychiatric and other medical manifestations of nitrous oxide abuse. implications from case series. *Journal of Clinical Psychopharmacology*, **40**: 80–3.

Crown Prosecution Service (2017) *Psychoactive Substances*. CPS (https://www.cps.gov.uk/legal-guidance/psychoactive-substances [cited 17 Jan 2020]).

Dimopoulos N, Piperi C, Salonicioti A, et al (2007) Correlation of folate, vitamin B12 and homocysteine plasma levels with depression in an elderly Greek population. *Clinical Biochemistry*, **40**: 604–8.

Fidalgo M, Prud'homme T, Allio A, et al (2019) Nitrous oxide: what do we know about its use disorder potential? Results of the French monitoring centre for addiction network survey and literature review. *Substance Abuse*, **40**: 33–42.

Firth J, Stubbs B, Rosenbaum S, et al (2017) The effects of vitamin and mineral supplementation on symptoms of schizophrenia: a systematic review and meta-analysis. *Psychological Medicine*, **47**: 1515–27.

Firth J, Stubbs B, Rosenbaum S, et al (2018) The effects of vitamin and mineral supplementation on symptoms of schizophrenia: a systematic review and meta-analysis – corrigendum. *Psychological Medicine*, **48**: 528.

Ford A, Almeida O (2019) Effect of vitamin B supplementation on cognitive function in the elderly: a systematic review and meta-analysis. *Drugs Aging*, **36**: 419–34.

FRANK (2020) *Nitrous Oxide*. GOV.UK (https://www.talktofrank.com/drug/nitrous-oxide#the-law [cited 17 Jan 2020]).

Garakani A, Jaffe R, Savla D, et al (2016) Neurologic, psychiatric, and other medical manifestations of nitrous oxide abuse: a systematic review of the case literature. *American Journal on Addictions*, **25**: 358–69.

GOV.UK (2014) *Nitrous Oxide: Neurological and Haematological Toxic Effects*. GOV.UK (https://www.gov.uk/drug-safety-update/nitrous-oxide-neurological-and-haematological-toxic-effects#risk-with-prolonged-use [cited 20 Jan 2020]).

Heads of Medicines Agencies (2019) *SPC A: Niontix 100% Medicinal Gas, Liquefied*, Linde Gas a.s.. (https://mri.cts-mrp.eu/Human/Downloads/CZ_H_0260_001_FinalSPC.pdf [cited 17 Jan 2020]).

Health Quality Ontario (2013) Vitamin B12 and cognitive function: an evidence-based analysis. *Ontario Health Technology Assessment Series*, **13** (23): 1–45.

Home Office (2018) *Review of the Psychoactive Substances Act 2016*. TSO (The Stationery Office).

Hutto BR (1997) Folate and cobalamin in psychiatric illness. *Comprehensive Psychiatry*, **38**: 305–14.

Kaar S, Ferris J, Waldron J, et al (2016) Up: the rise of nitrous oxide abuse. an international survey of contemporary nitrous oxide use. *J Psychopharmacol*, **30**: 395–401.

Kumar S (2004) Recurrent seizures: an unusual manifestation of B12 deficiency. *Neurol India*, **52**: 122–3.

Kumar P, Clark M (2020) Haematology. In *Clinical Medicine* (10th edn), (eds MF Murphy, KJ Pasi, N Roy): Ch. 10. *Elsevier Saunders*.

Lan S, Kuo C, Chou C, et al (2019) Recreational nitrous oxide abuse related subacute combined degeneration of the spinal cord in adolescents – a case series and literature review. *Brain and Development*, **41**: 428–35.

Lee M, Chang HS, Wu HT, et al (2005) Intractable epilepsy as the presentation of B_{12} deficiency in the absence of macrocytic anaemia. *Epilepsia*, **46**: 7.

Lerner V, Kanevsky M (2002) Acute dementia with delirium due to vitamin B_{12} deficiency: a case report. *International Journal of Psychiatry in Medicine*, **32**: 215–20.

Mukku SSR, Suhas S, Thippeswamy H, et al (2018) Mixed neuropsychiatric clinical manifestations associated with vitamin B_{12} deficiency. *Asian Journal of Psychiatry*, **36**: 25–7.

National Institute for Health and Care Excellence (2019) *Anaemia – B12 and Folate Deficiency*. NICE (https://cks.nice.org.uk/anaemia-b12-and-folate-deficiency#!diagnosisSub:3 [cited 21 Feb 2020]).

NHS (2020) *Symptoms: B12 or Folate Deficiency Anaemia*. NHS (https://www.nhs.uk/conditions/vitamin-b12-or-folate-deficiency-anaemia/symptoms/ [cited 21 Feb 2020]).

Office for National Statistics (2018a) *Nitrous Oxide Deaths in 2016 and 2017*. ONS (https://www.ons.gov.uk/aboutus/transparencyandgovernance/freedomofinformationfoi/nitrousoxidedeathsin2016and2017 [cited 17 Feb 2020]).

Office for National Statistics (2018b) *Deaths Involving Nitrogen and Nitrous Oxide in England and Wales, 2001 to 2016*. ONS (https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/adhocs/008203deathsinvolvingnitrogenandnitrousoxideinenglandandwales2001to2016 [cited 17 Jan 2020]).

Petridou E, Kousoulis A, Michelakos T, et al (2016) Folate and B12 serum levels in association with depression in the aged: a systematic review and meta-analysis. *Aging & Mental Health*, **20**: 965–73.

Randhawa G, Bodenham A (2016) The increasing recreational use of nitrous oxide: history revisited. *British Journal of Anaesthesia*, **116**: 321–4.

Romain K, Eriksson A, Onyon R, et al (2019) The psychosis risk timeline: can we improve our preventive strategies? Part 1: early life. *BJPsych Advances*, **25**: 299–308.

Rowland AS, Baird DD, Weinberg CR, et al (1992) Reduced fertility among women employed as dental nurses exposed to high levels of nitrous oxide. *New England Journal of Medicine*, **327**: 993–7.

Samia AM, Nenow J, Price D (2020) Subacute combined degeneration secondary to nitrous oxide abuse: quantification of use with patient follow-up. *Cureus*, **12**(10): e11041.

Saraswathy KN, Ansari SN, Kaur G, et al (2019) Association of vitamin B12 mediated hyperhomocysteinemia with depression and anxiety disorder: a cross-sectional study among Bhil indigenous population of India. *Clinical Nutrition ESPEN*, **30**: 199–203.

Savage S, Ma D (2014) The neurotoxicity of nitrous oxide: the facts and "putative" mechanisms. *Brain Sciences*, **4**: 73–90.

Sheldon RG, Reid M, Schon F, et al (2020) Just say N_2O – nitrous oxide misuse: essential information for psychiatrists. *BJPsych Advances*, **26**: 72–81.

Shoults K (2016) Case report: neurological complications of nitrous oxide abuse. *BC Medical Journal*, **58**: 192–4.

Silva B, Velosa A, Barahona-Corrêa JB (2019) Reversible dementia, psychotic symptoms and epilepsy in a patient with B_{12} deficiency. *BMJ Case Reports*, **12**: e229044.

Sun W, Li G, Lai Z, et al (2019) Subacute combined degeneration of the spinal cord and hydrocephalus associated with vitamin B_{12} deficiency. *World Neurosurgery*, **128**: 277–83.

Winstock AR, Timmerman C, Davies E, et al (2020) *The GDS 2020 Psychedelics Report*. Global Drug Survey.

Zhang C, Luo J, Yuan C, et al (2020) Vitamin B_{12}, B_6, or folate and cognitive function in community-dwelling older adults: a systematic review and meta-analysis. *Journal of Alzheimer's Disease*, **77**: 781–94.

Zier JL, Doescher JS (2010) Seizures temporally associated with nitrous oxide administration for paediatric procedural sedation. *Journal of Child Neurology*, **25**: 1517–20.

MCQs

Select the single best option for each question stem

1 Which of the following neurotransmitters does not have a key role in the mechanism of action of nitrous oxide?

a GABA
b NMDA
c serotonin
d noradrenaline
e endogenous opioid systems.

2 NICE defines vitamin B_{12} deficiency as lower than:

a 300 ng/L
b 250 ng/L
c 200 ng/L
d 150 ng/L
e 100 ng/L.

3 Entonox contains a mix of nitrous oxide and oxygen in the ratio:

a 10:90
b 20:80
c 30:70
d 40:60
e 50:50.

4 Close haematological monitoring should be carried out if N_2O-containing compounds are used more frequently than every:

a 2 days
b 4 days
c 6 days
d 8 days
e 10 days.

5 The immediate euphoric effects of N_2O usually wear off within:

a 10 min
b 20 min
c 30 min
d 45 min
e 60 min.

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