Nitrate Handbook

Environmental, Agricultural, and Health Effects

Edited by
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First edition published 2022

ISBN: 978-0-367-33822-0 (hbk)
ISBN: 978-1-032-11805-5 (pbk)
ISBN: 978-0-429-32680-6 (ebk)

15 Nitrates and Methemoglobinemia

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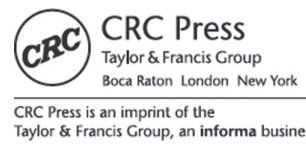
DOI: 10.1201/9780429326806-20

The funder for this chapter is Sarah Fossen Johnson.



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Sarah Fossen Johnson

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15.1 INTRODUCTION

Red blood cells contain a tetramer protein called hemoglobin. Hemoglobin binds iron and delivers oxygen to the tissues of the body. Methemoglobin is created when oxidizing chemicals or pharmaceuticals oxidize the iron in hemoglobin, leading to a conformational change. The hemoglobin can no longer bind and deliver oxygen easily, so the tissues of the body do not receive enough oxygen. If methemoglobin concentration in the blood reaches above 1–2 percent, it is considered to be methemoglobinemia, although most people with methemoglobin of 3 percent or below are asymptomatic (Fossen Johnson 2019).

Because there are also endogenous sources of oxidizing chemicals such as oxygen free radicals (Kuiper-Prins et al. 2016), the body has developed several pathways to reduce methemoglobin back to hemoglobin. The dominant pathway utilizes the NAD cytochrome b5 reductase enzyme to reduce methemoglobin to hemoglobin. In addition to its role in methemoglobinemia, NAD cytochrome b5 reductase plays a role in maintaining CoQ10 and ascorbic acid in their reduced states, and the prevention of lipid peroxidation in membranes, at both the cellular and mitochondrial levels (Fusco et al. 2011).

There is a lesser pathway that utilizes the enzyme NADPH methemoglobin reductase. This enzyme is likely only responsible for about 5 percent of all reductions (Mansouri and Lurie 1993). This path is minor; the enzyme is more of a non-specific reductase that can work on dyes that are usually found exogenously. Methylene blue is an example of the type of dye that NADPH methemoglobin reductase can work on. Interestingly, NADPH methemoglobin reductase reduces the dye which, in

DOI: 10.1201/9780429326806-20

turn, reduces methemoglobin, making it a good treatment for methemoglobinemia (Kuiper-Prins et al. 2016). This is a very important pathway for people who have a deficiency in NAD cytochrome b5 reductase because it may be the only path functioning to reduce methemoglobin. These two pathways work to maintain homeostasis (Ash-Bernal, Wise, and Wright 2004).

When more than 1 percent of a person's blood is methemoglobin, they have methemoglobinemia (Ash-Bernal, Wise, and Wright 2004). In babies, it may present as nonspecific symptoms such as fussiness and lethargy. In adults, it may present as shortness of breath, weakness, headache, hypoxia that cannot be improved with additional oxygen, a blue color to their skin – also described as a gray or dusky color, and chocolate-colored blood (Bayat and Kosinski 2011, Taleb et al. 2013). When methemoglobin levels reach 70 percent and higher, it can be fatal (Taleb et al. 2013).

Often, mild cases of methemoglobinemia will spontaneously resolve without treatment. In more serious cases, methylene blue will be administered intravenously, or ascorbic acid, N-adenylcystein (NAC), and/or Q10 will be administered. Methylene blue works by NADPH methemoglobin reductase reducing it to leukomethylene blue, which then reduces methemoglobin to hemoglobin. Interestingly, methylene blue at high concentrations can be an oxidizing agent that can create methemoglobin. Ascorbic acid can also be used to treat methemoglobinemia. It works directly on the methemoglobin to reduce it to hemoglobin (Kang et al. 2018). N-adenylcysteine acts as a cofactor in reducing the methemoglobin, or by intracellular glutathione cysteine, both of which are products of NAC metabolism (Tanen, LoVecchio, and Curry 2000).

Compared to adults, infants have different physiology that makes them more susceptible to methemoglobinemia than older children and adults. Adults reduce methemoglobinemia almost twice as fast as infants can and have higher levels of NAD cytochrome b5 reductase, the enzyme associated with a reduction of methemoglobin (Yip and Spyker 2018). Infants also have a higher percentage of fetal hemoglobin, as compared to adults. It is easier to oxidize fetal hemoglobin than adult hemoglobin (Kuiper-Prins et al. 2016). When infants are exposed to nitrate in water, those under six months drink more water for their weight as compared to adults, and their exposure will be higher (U.S. Environmental Protection Agency – Office of Research and Development 2011).

The study of methemoglobinemia and nitrate can be traced back more than seventy years in the United States. It started as a hunch about the well water from the father of an infant that had cyanosis and associated methemoglobinemia. It led to the first discovery that well water contaminated with nitrate could cause methemoglobinemia. Over the years there has been debate about the role of diarrheal illness in methemoglobinemia development in infants exposed to nitrate in well water. Regardless of the source, methemoglobinemia has been documented in many age groups including infants, older children, adults, and older adults.

15.2 NITRATE TOXICOKINETICS

When an adult ingests nitrate, it is rapidly absorbed by the small intestine and widely distributed into all body fluids (Dusdieker et al. 1996, Hord et al. 2011). Once it has been absorbed, some of the nitrate in the blood concentrates into the salivary glands

(Dusdieker et al. 1996, Qu et al. 2016). Within ten minutes, it is secreted into the mouth, where it interacts with commensal facultative anaerobes and is reduced to nitrite (Kanady et al. 2012, Dusdieker et al. 1996). Excretion of nitrate occurs mainly through the urinary route; however, there is some excretion through feces (Health Canada 2017). Interestingly, nitrate concentrations in serum are not a good indicator of the concentration of nitrate secreted in breastmilk (Dusdieker et al. 1996).

There are differences between infants and older children and adults. Infants have a very low level of the enzyme responsible for reducing nitrate to nitrite in their mouths (Kanady et al. 2012). In adults, the mouth is the major site for nitrate reduction to nitrite. This reduction happens in infants' stomachs, where the pH is high enough to allow for the growth of bacteria that converts nitrate to nitrite.

Infants also differ in that they have a lower expression of the enzyme that converts methemoglobin to hemoglobin (Yip and Spyker 2018, Mensinga, Speijers, and Meulenbelt 2003), so the half-life of nitrite in the body is likely longer in infants than in adults and children. This can cause an elevated level of methemoglobin. Finally, nitrate is excreted by infants only through the urinary route (Health Canada 2017).

There are two types of methemoglobinemia: Congenital and acquired. Congenital methemoglobinemia is due to a deficiency in the enzyme CYB5R3 (Kedar et al. 2018) or a mutation in the CYB5R gene (Lorenzo et al. 2011). Congenital methemoglobinemia can be a very severe disorder that can lead to death (DomBourian et al. 2015). It can also be a less severe, more treatable disease. The type of methemoglobinemia associated with nitrate exposure is acquired. Acquired methemoglobinemia can be caused by a wide variety of agents, including the topical anesthetics Lidocaine and Prilocaine (Bloom 2001), gastrointestinal infections, and nitrate/nitrite (Ash-Bernal, Wise, and Wright 2004). Unlike congenital methemoglobinemia, which is due to genetic factors, acquired methemoglobinemia is caused by chemicals that have an oxidizing effect on the iron center in the hemoglobin protein. For example, nitrate must first be reduced to nitrite by bacteria in the mouth, or stomach in the case of infants, and the nitrite can be absorbed into the bloodstream where it can oxidize hemoglobin directly.

15.3 CONGENITAL METHEMOGLOBINEMIA

Methemoglobinemia is an illness that can be acquired from an array of different things – for example, oxidizing chemicals, food with naturally occurring nitrate or nitrite, food that has been intentionally preserved with nitrite, pharmaceuticals, and water from nitrate-contaminated water wells. It is also a congenital disease. There are three types of congenital methemoglobinemia: Type I, Type II, and Hemoglobin M Disease (HbM).

Acquired methemoglobinemia occurs more frequently than congenital methemoglobinemia. Mannino and coworkers (2017) published the first case study in the United States of Type II methemoglobinemia that had been genetically confirmed. In contrast, Ash-Bernal (2004) noted 138 cases of acquired methemoglobinemia in just 28 months in two hospitals. There has been no actual incidence or prevalence of Type II congenital methemoglobinemia calculated due to its rarity (Da-Silva, Sajan, and Underwood 2003).

HbM is a congenital methemoglobinemia characterized by a mutation in the gene that codes for the globin chain of the hemoglobin protein. This results in hemoglobin that is resistant to reduction. This makes treatment for methemoglobinemia more difficult, but treatment is rarely needed, as most people with the mutation live a normal life (Bayat and Kosinski 2011, DomBourian et al. 2015). Treatment for methemoglobinemia for these patients is usually only cosmetic, as there are no other symptoms beyond slightly blue skin. There are variant types of HbM: Boston, Iwate, Hyde Park, Saskatoon, Milwaukee, and Osaka. Each variant has a different amino acid substitution (Mansouri and Lurie 1993).

Type I is the most common type of congenital methemoglobinemia and is characterized by a deficiency in the CYB5R3 gene that causes the loss of the erythrocyte form of the enzyme CYB5R. This loss has autosomal recessive inheritance and causes only minor symptoms, such as cyanosis, similar to HbM (DomBourian et al. 2015, Nicolas-Jilwan 2019, Fusco et al. 2011).

Unlike HbM and Type I, which are mostly mild, Type II is associated with serious illness in infancy, with many affected infants dying before their first birthday (DomBourian et al. 2015). It is a more rare form of methemoglobinemia than Type I or HbM. Type II is caused by full stops or deletions in the CYB5R3 gene that results in an inactive Cytochrome B5 Reductase enzyme, or loss of expression altogether. This has severe consequences, especially for the neurological system. It has been hypothesized that this is due to altered fatty acid desaturation which can affect demyelination in the brain (Fusco et al. 2011, Mansouri and Lurie 1993). Infants present with a variety of different symptoms: mild cyanosis, progressive microcephaly, severe encephalopathy, generalized dystonia, seizures, strabismus, severe hypotonia, developmental delay, and white matter changes on brain imaging (Nicolas-Jilwan 2019, Fusco et al. 2011, Mannino et al. 2018). To help address symptoms, infants can be treated with ascorbic acid and Q10, or methylene blue if the methemoglobin level is above 20 percent (Nicolas-Jilwan 2019). There is no cure for Methemoglobinemia II. Any treatment is palliative in nature.

15.4 ACQUIRED METHEMOGLOBINEMIA

Acquired methemoglobinemia occurs when a person comes into contact with chemicals, pharmaceuticals, some vegetables, prepackaged food, and/or preserved meats that can oxidize hemoglobin into methemoglobin. There are two main ways in which this happens – with direct action to the hemoglobin protein, or by indirect means, where the compound itself is not able to oxidize hemoglobin, but can through other pathways create an oxidizing molecule. Nitrate is of concern for infants eating vegetables such as spinach and beets. Older children and adults can come into contact with preserved meat and prepackaged foods, which can be a significant source of nitrite.

Studies have been done looking at factors that cause infants to develop methemoglobinemia after eating vegetables. In one study, one- and two-year-old infants had developed constipation. The treating doctor for both infants recommended to the parents that they make zucchini soup, and use that to reconstitute formula. In both cases, the infant developed methemoglobinemia. The researcher found that in

addition to zucchini being high in nitrate, both soups had been allowed to sit for a period of time, which increased the conversion from nitrate to nitrite in the soup. Both infants were treated with 1 percent methylene blue and recovered (Savino et al. 2006).

Another study centered around seven infants who had developed methemoglobinemia after eating green vegetables. The infants' ages ranged from seven to thirteen months. The researchers analyzed data surrounding the methemoglobinemia and found that all of the cases had eaten baby food with beets. In order of highest nitrate content to lowest nitrate content, beets are the highest, followed by spinach, lettuces, leeks, cabbages, pumpkins, and green beans. Carrots show no, or very little, nitrate. The researchers note that these cases are unusual due to the infants being over 6 months of age, and so not having the usual physiological challenges associated with methemoglobinemia and infants (Sanchez-Echaniz, Benito-Fernandez, and Mintegui-Raso 2001). The amount of nitrogen in particular foods can vary due to different practices of farmers using nitrate fertilizer. Baby food prepared in the home imparts the largest chance for methemoglobinemia, as the nitrate cannot be controlled like with commercial baby foods.

An example of a pharmaceutical that can cause acquired methemoglobinemia is silver nitrate. It can be used to prevent secondary infections in people with severe burns. It is a topical agent that can easily pass into the blood stream where it becomes nitrite. Methemoglobinemia can be difficult to treat in these patients as the skin may not be intact, silver nitrate causes changes in skin color, there may be extensive bandaging, and burn patients already exhibit cardiovascular changes as a result of the burns. The best diagnostic test is looking for chocolate-colored blood (Geffner, Powars, and Choctaw 1981).

Although there is an emphasis on infants and their unique physiology in much of the methemoglobinemia literature, older children and adults can also develop it. The section below gives several examples of the types of acquired methemoglobinemia that have been published. Many of the situations described show classic symptoms of methemoglobinemia.

15.5 EXAMPLES OF METHEMOGLOBINEMIA CAUSED BY NITRATE OR NITRITE

In 1953, a story called "Eleven Blue Men" appeared in *New Yorker* magazine (Roueché 1953). It chronicled the appearance of eleven blue men one morning in New York City, and the subsequent investigation of what was causing the blue color. In this case, the men had used salt cellars that had accidentally been partially filled with sodium nitrite instead of sodium chloride at a diner. They received high doses of sodium nitrite. The nitrite acted quickly on their hemoglobin to convert it into methemoglobin in their bodies, and they developed very serious cases of methemoglobinemia. One of the men died.

Just prior to "Eleven Blue Men" being published, Dr. Hunter Comely published a case report on nitrate in well water causing methemoglobinemia in infants, also known as Blue Baby Syndrome (Comley 1945). He described two cases of methemoglobinemia in infants younger than 6 months old. The infants had been drinking formula that had been reconstituted with well water. Once the nitrate-containing

well water was replaced with water from another source, the methemoglobinemia resolved.

In 1948 and 1950 two epidemiology studies were published that described more than a hundred methemoglobinemia cases in southwestern Minnesota, and cases all over the nation (Rosenfield and Huston 1950, Cornblath and Hartmann 1948). The same pattern was repeated as Comely described; the cases were infants under the age of 6 months, and they had been drinking well water with high concentrations of nitrate in it. Many of the wells were shallow and were close to animal enclosures, two attributes that make it easy for nitrate to migrate into well water.

There have also been cases of nitrate-induced methemoglobinemia documented more recently in adults, children, and infants (Knobeloch and Proctor 2001, Knobeloch et al. 2000, Centers for Disease Control 1997, Funke et al. 2018). The infant cases follow the same pattern as described above. The adult and child cases have various toxic exposures associated with them. For example, in New Jersey in the 1990s there were two incidents involving nitrite being introduced into hot tap water by a failing backflow prevention valve on boilers. In the first case, 29 students were sickened after eating soup that had been reconstituted with the nitrite-containing boiler water. The majority of the students exhibited symptoms such as cyanosis, nausea, abdominal pain, vomiting, and dizziness. Fourteen of the children were hospitalized and treated with methylene blue; the methemoglobinemia resolved in the remaining 15 students within 36 hours after ingestion (Centers for Disease Control 1997).

The second case occurred in adults who had also been subjected to boiler water entering their hot water tap. In this case, the people who were sickened all drank coffee prepared with water from the hot water tap. Physicians determined they had methemoglobinemia due to the presence of elevated levels of methemoglobin in their blood. For those who were most seriously affected, oxygen and intravenous methylene blue was administered. All of the affected people recovered within 24 hours (Centers for Disease Control 1997).

Another example of a person developing methemoglobinemia induced by nitrate they had ingested is the case of a 71-year-old-man who opened and drank the fluid in a lava lamp. He had a history of alcohol abuse and had wrongly assumed the fluid inside the lamp was alcohol. It actually contained 76 percent calcium nitrate. When he was taken to the emergency room, he at first appeared normal, but after six hours in the emergency room his skin turned a grayish color; his methemoglobin was 45.6 percent, his oxygen saturation decreased, and additional oxygen did not bring his saturation level up. Based on these and other symptoms he was diagnosed with methemoglobinemia. He was treated with methylene blue and survived (Funke et al. 2018).

15.6 CONCLUSION

Although methemoglobinemia is not very frequently seen outside medical situations, it is still an illness that is worth understanding. Whether it is Type II congenital methemoglobinemia or acquired methemoglobinemia, it can pose significant problems for people who are affected. In congenital methemoglobinemia,

the Cy5b3r enzyme is either only partially functional or not functional at all and that causes the illness. Acquired methemoglobinemia is characterized by the action of an oxidizing agent on the tetrameric hemoglobin protein. Both acquired and congenital methemoglobinemia share some symptoms, such a blueish color to the person's skin, shortness of breath, chocolate-colored blood, and a low dissolved oxygen concentration that cannot be improved with the addition of accessory oxygen.

Some forms of congenital methemoglobinemia are so rare their incidence and prevalence are unknown. Acquired methemoglobin, in contrast, happens in medical facilities with some frequency. It is a known side effect of many topical anesthetic medications although, when used properly, this will rarely be a side-effect. Additionally, there have been well-documented cases of accidental poisonings that have occurred in adults and children that resulted in acquired methemoglobinemia.

Infants have an increased risk of methemoglobinemia due to physiological factors. They have a lower concentration of enzyme necessary to convert methemoglobin to hemoglobin than adults, and they have a higher concentration of fetal hemoglobin, which is easier to convert to methemoglobin. Infants are often harder to diagnose as they present with nonspecific symptoms such as fussiness and lethargy. Because the speed of converting from methemoglobin to hemoglobin is slower in infants, this also results in a longer half-life of nitrate in the body.

In closing, nitrate is an important chemical when it comes to methemoglobinemia. It is an exposure factor that can come both from food and contaminated water, and it is tasteless, so people are unaware when they have been exposed. It is well-known that in the world, nitrate in drinking water is a major risk factor for methemoglobinemia. Although this is usually associated with an environmental exposure that pertains to babies, it is also true that older children and adults can develop methemoglobinemia. In most acquired cases, the methemoglobinemia is reversible, but in a small percentage of cases, methemoglobin can build up in the bloodstream to high concentrations that can result in death.

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