CEDARVILLE UNIVERSITY

The Idea of an Essay

Volume 6 *Dads, The Humanities, and The Electoral College*

Article 16

September 2019

Nitrates: Death or Deliverance

Ryan K. Lokkesmoe
Cedarville University, rlokkesmoe@cedarville.edu

Ryan Lokkesmoe

Hailing from Eau Claire, WI, Ryan Lokkesmoe attended Cedarville University from 2015-2018, obtaining a BS in Molecular and Cellular Biology. After graduating in December he spent the Spring semester of 2019 teaching Anatomy & Physiology and Principles of Biology labs at Cedarville University. He is now an M.D. candidate at the Medical College of Wisconsin in Milwaukee in the class of 2023. In his free time, Ryan enjoys reading biographies, running, and playing rugby.

Nitrates: Death or Deliverance

In the past several decades, there has been a significant rise in consumption of organically grown produce (Bourn & Prescott, 2002). While this sizable trend may indicate a rising concern with health among the general population, it is worth considering the motivations behind purchasing organic food and comparing them with the benefits. This will provide a cost/benefit analysis that will allow consumers to make an educated decision about whether or not the cost of organic food is worth the benefits.

A study in 2002 by Magnusson, Arvola, Hursti, Aberg, and Sjoden,. indicates concerns for one's health and the environment are the most frequent justifications given for purchasing organic foods. However, when examining the practice of the individuals making these claims, they found that health concerns were better predictors of behavior compared to environmental concerns. They concluded that egoistic motives are stronger than altruistic motives, and consumers purchase organic foods to be healthy.

Without question, organic foods are perceived as a healthier and safer alternative to conventionally produced foods (Magkos, Arvaniti, & Zampelas, 2006), but what are the actual benefits? Typically three areas are considered: nutritional value, sensory quality, and food safety (Bourn & Prescott, 2002). While sensory quality certainly plays an important role in the experience of eating food and affects which foods consumers will purchase, it is not the focus of this discussion.

There are primarily three battlefields in the arguments about nutritional value and safety: pesticide residue levels, microbiological contamination, and nutrient content. Pesticide residue danger has not been well researched (Farha, et. al., 2018), and although they are observed more often in conventional foods, the levels seen are below health standards (Bourn & Prescott, 2002), and microbiological contamination from a lack of pesticides may be a larger safety issue (Magkos et al., 2006). Given these limitations, the intersection of the discussion with nutritional value and food safety lies in nitrates.

Inorganic nitrates are chemicals that occur naturally in many fruits and vegetables and are used in the preservation of various products (Badahoran et al., 2016; Chetty & Prasad, 2015). They consist of an oxygen atom bound to three nitrogen atoms, leaving a negative charge. Associated with several serious health concerns, nitrate levels are often higher in conventional food (Magkos et al., 2006), and likely are responsible for some of the trend toward organics. This review will examine recent research conducted on dietary nitrates in three areas: their role in methaemeglobinaemia and the formation of N-nitroso

carcinogens, their biochemical and physiological aspects, and the therapeutic or supplementation applications of nitrates.

Methaemeglobinaemia is simply an overabundance of methemoglobin in the blood, which results when the iron in hemoglobin is oxidized by nitrite to Fe3+, forming methemoglobin and preventing oxygen from binding (Lundberg, Weitzberg, & Gladwin, 2008, p. 165). Naturally, if a significant portion of hemoglobin cannot carry oxygen it will lead to hypoxia. However, there has been little or no evidence of meaningful increases in methaemeglobinaemia after nitrite levels rise (Mensinga Speijers, & Seulenbelt, 2003; Pluta, Dejam, Grimes, Gladwin, & Oldfield, 2005; Velmurugan et al., 2016). It is reasonable to conclude, therefore, that methaemeglobinaemia does not present a serious concern when nitrates are consumed in moderation.

More concerning for long term health is the connection between nitrites and the formation of potentially carcinogenic N-nitroso compounds. When nitrite is mixed with secondary amines or N-alkylamides, nitrosamines and niros-amides can be formed (Mensinga et al., 2003, p. 46). However, "the extent to which endogenous N-nitroso formation occurs is not entirely clear" and "although a considerable number of studies have been conducted, firm incontrovertible evidence that exposure to N-nitroso compounds formed in the gastrointestinal tract from a nitrate rich diet, can induce human cancers is still lacking" (p. 46). Therefore, it is unclear N-nitroso compounds form in the body as a result of dietary nitrate consumption and actually lead to cancer.

Studies have not shown a consistent connection between nitrates and any forms of cancer (Ward, et. al., 2005), and diets

containing high amounts of vegetables, which contain many times the recommended dose of nitrates, have been linked with a generally lower cancer risk (Mensinga et al., 2003; Lee & Chan, 2011; Bedale, Sindelar, & Milkowski, 2016). In light of this, no conclusions can be made, but avoiding the nitrates found in vegetables for the sake of lowering the chance of cancer may accomplish exactly the opposite.

Prior to Lundberg, Weizberg, and Gladwin's influential analysis from 2008, nitrate and nitrite were "known predominately as undesired residues in the food chain with potentially carcinogenic effects, or as inert oxidative end products of endogenous nitric oxide (NO) metabolism" (p. 156). However, with the elucidation of nitrate's biochemical role in the mammalian nitrogen cycle, these authors call for nitrate and nitrite to be "viewed as storage pools for NO-like bioactivity, thereby complementing the NO synthase (NOS)-dependent pathway" (p. 156).

The NOS-dependent pathway, referred to above, consists of NOS enzymes acting on L-arginine and oxygen to produce nitric oxide, a radical, highly involved in autocrine and paracrine vasodilation. This means NO only affects tissues very near its generation point. Nitric oxide is extremely important for vascular function, influencing blood vessel diameter, controlling inflammation, and acting as an antiplatelet (Velmurugan, et al., 2016, p. 26).

In contrast to the NOS-dependent pathway, the nitrate-nitrite-NO pathway increases activity as oxygen levels decline and, "in this sense, NOS-independent NO formation can be viewed as a

back-up system to ensure that there is sufficient NO formation when oxygen supply is limited, which is analogous to the complementary role of anaerobic glycolysis in energetics" (Lundberg et al., 2008, p. 156). This means when strains are put on the cardiovascular system, such as during exercise, the body can generate the required nitric oxide from circulating nitrate and nitrite.

These molecules enter the body in two main ways. Either they are formed by the oxidation of NO previously in the body, or they are obtained through diet (Lundberg et al., 2008, p. 157). Nitrate is biologically inactive until it is reduced to nitrite by facultative anaerobic bacteria in the oral microbiome (Bryan, Tribble, & Angelov, 2017). This nitrite is then further reduced with the help of vitamin C and polyphenols to NO (Lundberg et al., 2008).

Deficiency in dietary nitrate studied in mouse models led to very serious health conditions including visceral adiposity, dyslipidemia, and glucose intolerance after only three months. By 18 months without nitrate, the mice displayed increased body weight, hypertension, insulin resistance, and impaired endothelium-dependent reactions to acetylcholine; after 22 months the low nitrate diet significantly led to death, usually due to cardiovascular disease (CVD). These effects were reversed by administering sodium nitrate (Kina-Tanada, et al., 2017, p. 1138).

In 2017, a prospective cohort study of 1226 women without arteriosclerotic vascular disease (ASVD) over the course of 15 years examined the connection between vegetable nitrate intake and ASVD mortality. Independently of lifestyle and cardiovascular

disease risk factors, nitrate intake from vegetables was inversely associated with ASVD mortality and all-cause mortality (Blekkenhorst, et al., 2017). Thus, dietary nitrate and nitrite appear to play an important biochemical role in physiological homeostasis in regards to vascular function (they are necessary for healthy arteries and veins).

This role in the cardiovascular system opens up the potential for therapeutic effects from nitrate supplementation. Organic nitrates, such as nitroglycerin, have been used historically to treat hypertension; could inorganic dietary nitrates have similar uses? There is some call for nitrate supplementation to reduce the effects of "lifestyle related diseases" (Kobayashi et al., 2015, p. 4926), due to the potentially causal relationship between nitric oxide levels and diseases such as insulin resistance, ischemic heart disease, and hypertension (p. 4911).

According to Bryan and Ivy (2015) "there are clear and delineated doses of both nitrite and nitrate that provide indisputable evidence of promoting health and even treating serious medical conditions" (p. 650). Most significantly, nitrate supplementation may lower blood pressure (Bryan et al., 2017; Lovegrove et al., 2017), potentially with sulfate (Kuhnle, Luben, Khaw, & Feelisch, 2017), which would contribute to many health benefits. Lowering systolic pressure by 5mmHg reduces the prevalence of strokes by 35% and ischemic heart disease by 21% (Bryan & Ivy, 2015, p. 650). The authors claim "the benefits. . . . well established by published literature" include improved vascular function, lower inflammation, improved exercise capacity,

improved mitochondrial function, lower triglyceride levels, and less heart attacks (p.650).

Indeed, literature regarding the therapeutic effects of nitrate was not difficult to find, and many reviews were vocal in their support for the exploration of new nitrate-based cardiovascular disease preventative measures. There are relatively few effective primary prevention options for reducing the risk of CVD (Velmurugan, et al., 2016, p. 25), and thus new pharmaceutical methods may prove extremely helpful. Cardiovascular disease in its many forms is largely due to improper vascular function, which is defined functionally by a reduced availability of nitric oxide, typically by dysfunction in the NOS-dependent pathway discussed previously (p. 26).

Not surprisingly, nitrate supplementation in individuals with hypercholesterolemia was shown to result in an increase in flow-mediated dilation, which indicates improved vascular function (Velmurugan, et al., 2016, p. 35). As further confirmation of the role of nitrates in this improvement, the changes were associated with adjustment in the oral microbiome in favor of organisms capable of nitrate reduction (p.35). The study "supports the use of dietary nitrate as a safe, well-tolerated, and potentially powerful prevention strategy in CVD in individuals with early vascular dysfunction" (p. 36). As one out of every three Americans has hypertension (Bryan et al., 2017, p 32), such a dietary prevention strategy could have widespread effects.

The benefits of nitrates as supplements extend also to otherwise healthy individuals, as they have been associated with muscle oxygenation increases during exercise (Masschelein et al.,

2012, p. 736) as well as tolerance for high intensity exercise (Bailey, et al., 2009, p. 1153). A single application of nitrate-rich beetroot-based gels has additionally been shown to increase muscle strength recovery after exercise (de Oliveira, Margado, Conte-Junior, & Alvares, 2017). As many have begun to use nitrates when exercising, an interesting phenomenon has developed, in which consumers will avoid nitrates in non-organic foods and preserved meats, while simultaneously using nitrate supplementation from beetroot juice.

Bedale, Sindelar, and Milkowski (2016) attempt to explain this dissonance, claiming the source of the nitrate (meat, vegetables, or water) may matter. They reference Bastide et al., Bouvard et al., and Van Hecke et al., each published in 2015, to suggest meat may contain components that facilitate the formation of N-nitroso compounds (p. 90), while antioxidants found in vegetables may help reduce any harmful effects (p. 90). They conclude it is likely that nitrate is helpful at certain levels and poisonous at others (p. 90).

This conclusion is reasonable, given the extraordinary number of studies that have surfaced in the debate on nitrates. Although "epidemiological studies have not demonstrated consistent relationships between nitrate and nitrite exposure and risk of any type of cancer" (Bedale et al., 2016, p. 88), and concerns about Methaemeglobinaemia seem to be misplaced (Mensinga et al., 2003), it still is wise to use caution in dealing with these compounds. At the same time, in light of their important role in vascular health and function (Lundberg et al., 2008) and the pyric

results of their absence (Kina-Tanada, et al., 2017), avoiding them completely may be a poor choice.

Therefore, the choice to eat organic food is a complex and multifaceted issue. While some authors, such as Crinnion (2010), argue that organic food is more healthy, others claim "no hard evidence currently exists to support or refute claims that organic food is healthier and safer than conventional food, or *vice versa*, and assertions of such kind are inappropriate and not justified" (Magkos et al., 2003, p. 219). Concerned about fear-mongering about food, these authors sarcastically remark "complete fasting seems to be the only solution" (p. 219) when all potentially unsafe foods are avoided. The poison seems to indeed be in the dose.

Annotated Bibliography

Badahoran, Z., Mirmiran, P., Jeddi, S., Fereidoun, A., Ghasemi, A., Hadaegh, F. (2016). Nitrate and nitrite

content of vegetables, fruits, grains, legumes, dairy products, meats and processed meats. *Journal of Food Composition and Analysis, 51*, 93-105.

In this article, the authors evaluate inorganic nitrate/nitrite levels in order to begin to develop a database for a variety of foods. They claim that their data provides a reasonable estimation of nitrate/nitrite exposure in Iran, but the levels were comparable to other countries. I will use this source to state the ubiquitous nature of nitrates in food.

Bailey, S. J., Winyard, P., Vanhatalo, A., Blackwell, J. R., Dimenna, F. J., Wilkerson, D. P., . . . Jones, A.M. (2009). Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985), 107*(4), 1144-1155. 10.1152/japplphysiol.00722.2009 [doi]

In this study, a group of young adult males were given short term supplementation of nitrates, and saw blood pressure decrease and plasma nitrite concentration rise. When the participants were exercised, the researchers saw "improvements in NIRS-derived indexes of muscle oxygenation and a significant reduction in pulmonary $\dot{V}O_2$." The authors claim that these improvements are not

observed even from long-term endurance training. I will use this study to mention the exercise supplementation potentials of nitrates.

Bedale, W., Sindelar, J. J., & Milkowski, A. L. (2016). Dietary nitrate and nitrite: Benefits, risks, and evolving perceptions. *Meat Science, 120*, 85-92.

This study attempted to understand the odd consumer trend of embracing nitrate as a dietary supplement while fearing its addition in cured meats. They found that nitrate, nitrite, and nitric oxides are important for homeostasis, and are beneficial at certain levels, while harmful at others. They conclude that public education is necessary to assuage the fears and reduce the disconnect among consumers. I will use this source when discussing perceptions of nitrates to reveal some of the cognitive disconnect among the public.

Blekkenhorst, L. C., Bondonno, C. P., Lewis, J. R., Devine, A., Woodman, R. J., Croft, K. D., . . .Hodgson, J. M. (2017). Association of dietary nitrate with atherosclerotic vascular disease mortality: A prospective cohort study of older adult women. *The American Journal of Clinical Nutrition, 106*(1), 207-216.

This study followed 1226 women aged 70-85 over the course of 15 years, beginning in 1998. Nitrate intake was calculated for each participant, and atherosclerotic vascular disease (ASVD) factors were assessed. They found that each

SD higher of nitrate intake was associated with a lower risk of ASVD mortality in both unadjusted and multivariable-adjusted analyses. They also found that higher vegetable nitrate intake was associated with a lower risk of all-cause mortality. They found their results to support the concept that nitrate-rich vegetables may reduce the risk of age-related ASD mortality. I will use this article to bring light to the nitrate argument.

Bourn, D., & Prescott, J. (2002). A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Critical Reviews in Food Science and Nutrition, 42*(1), 1-34.

An assessment of studies from three perspectives on the benefits of organic food over non organic food: nutritional value, sensory quality, and food safety. They find few studies are capable of comparing the two, and only nitrates were found to be in different concentrations (likely due to the use of lower amounts of nitrogen in an organic farming system). They found "inconsistent" findings from reports on varieties of sensory qualities. They found no conclusive difference in the microbiological contamination potentials between organic and non-organic food. I intend to use this to hone the conversation in specifically on nitrates, rather than assessing all of the variables.

Bryan, N. S., Tribble, G., & Angelov, N. (2017). Oral Microbiome and Nitric Oxide: The Missing Link in the Management of Blood Pressure. *Current Hypertension Reports, 19*(4), 32-33. doi:10.1007/s11906-017-0725-2

This review seeks to provide evidence of a connection between the oral microbiome and the production of NO/regulation of blood pressure. They find that eradication of Nitrate-reducing bacteria eliminates health benefits of nitrate-based diets. “in the absence of any dietary modifications, a 7-day period of antiseptic mouthwash treatment. . . was associated with a sustained increase in both systolic and diastolic blood pressure. Providing nitrite was able to overcome the absence of microbial nitrate reduction, as expected. I will use this article to link the oral microbiome to nitrate reduction.

Bryan, N. S., & Ivy, J. L. (2015). Inorganic nitrite and nitrate: Evidence to support consideration as dietary nutrients. *Nutrition Research (New York, N.Y.), 35*(8), 643-654.

This recent article argues strongly in support of a daily intake of nitrates, proposing that the benefits (Lower blood pressure, improved vascular function, lower inflammation, improved exercise capacity, improved mitochondrial function, lower triglycerides, less heart attacks, and less strokes) from a moderate consumption of nitrates far outweigh the risks (potential formation of carcinogenic low-molecular-weight N-nitrosamines). They come to this conclusion based on a large mortality rate of

cardiovascular diseases, and the fact that nitrite and nitrate have never actually been directly implicated in carcinogenesis. I will use this article to dive into the risk/benefit analysis of nitrates.

Chetty, A. A., & Prasad, S. (2016). Flow injection analysis of nitrate and nitrite in commercial baby foods. *Food Chemistry, 197*(Pt A), 503-508.10.1016/j.foodchem.2015.10.079 [doi]

This article demonstrates an attitude of distrust toward nitrates or nitrites that yet remains, even in 2016, in the scientific community of Fiji. The study attempted to quantify the levels of nitrates in baby food, under the assumption that such nitrates are bad. Without explaining their rational for such an assumption, they say: "it is imperative for manufacturing companies to ensure that the nitrate and nitite values are kept to as low as reasonable possible." Neglecting the poor grammar, such an unsubstantiated claim has no place in an academic journal, although they do call for research to evaluate the risk of nitrates. I will use this article to help establish the other side of the nitrate debate.

Crinnion, W. J. (2010). Organic foods contain higher levels of certain nutrients, lower levels of pesticides, and may provide health benefits for the consumer. *Alternative Medicine Review : A Journal of Clinical Therapeutic; Alternative Medicine Review, 15*(1), 4-12.

Published by an ND (naturopathic medicine), this article seeks to present the other side of the argument, claiming that organic foods are superior from a variety of perspectives. The authorship (1982 graduate of Bastyr University; practice since 1982 with a special focus on treating chronic diseases caused by environmental toxin burden) immediately throws this article into doubt, since the naturopathic approach is commonly regarded as unscientific and his entire field relies on seeing a benefits from "natural" products. This introduces significant bias. This article claims that organic foods provide increased levels of antioxidant phytochemicals (although he admits that these have not been shown to affect antioxidant status *in vivo*), as well as vitamin C, iron, magnesium, and phosphorus. I'm not sure if I'll use this article yet, since it does not address the issue of nitrates at all.

de Oliveira, G. V., Morgado, M., Conte-Junior, C. A., & Alvares, T. S. (2017). Acute effect of dietary nitrate on forearm muscle oxygenation, blood volume and strength in older adults: A randomized clinical trial. *PloS One, 12*(11), e0188893.

In this study, the researchers were concerned with discovering whether or not beetroot based gel would have positive effects on grip strength and O2 saturation in the forearms of older adults. In a randomized cross over double blind experiment, they found that a single dose of beetroot-based gels increases blood volume and improved handgrip recovery after handgrip exercise. I will use this to

contribute to the discussion on the pharmacological effects of nitrate.

Farha, W., Abd El-Aty, A. M., Rahman, M. M., Jeong, J. H., Shin, H. C., Wang, J., . . . Shim, J. H. (2018). Analytical approach, dissipation pattern and risk assessment of pesticide residue in green leafy vegetables: A comprehensive review. *Biomedical Chromatography : BMC, 32*(1), 10.1002/bmc.4134. 10.1002/bmc.4134 [doi]

This review focuses on pesticide residues in green leafy vegetables. They sought to discover patterns and evaluate risks in consuming such vegetables. They expected leafy vegetables to have high pesticide levels compared to other vegetables, due to their large surface area. They highlight the importance of such vegetables for a healthy diet and lifestyle, mentioning that they are a "good source of nitrates" which they say plays a significant role in healing wounds, reducing HTN, and reducing the chance of CVD. Their major conclusions are that pesticides, when consumed within acceptable limits, do not present any negative health consequences, but they call for additional research, especially high throughput screenings, on the quantification of residues in green leafy vegetables. I plan to use this both to steer the conversation towards nitrates, and to provide an example of how nitrates have been accepted by the medical community as an essential nutrient.

Kina-Tanada, M., Sakanashi, M., Tanimoto, A., Kaname, T., Matsuzaki, T., Noguchi, K., . . . Tsutsui, M. (2017). Long-term dietary nitrite and nitrate deficiency causes the metabolic syndrome, endothelial dysfunction and cardiovascular death in mice. *Diabetologia*, *60*(6), 1138-1151. 10.1007/s00125-017-4259-6 [doi]

This study looks at the results of a long-term dietary nitrite and nitrate deficiency. When receiving neither of these nutrients in their diet, mice began to experience some serious complications. At three months, they displayed visceral adiposity, dyslipidemia, and glucose intolerance. After 18 months, further results included: increased body weight, hypertension, insulin resistance, and impaired endothelium-dependent reactions to acetylcholine. Most seriously, at 22 months, the low nitrate diet significantly led to death, usually due to CVD. It is important to note that the normal diet mice and low nitrate mice received the same caloric content. I will use this to establish the importance of nitrate as a nutrient, apart from any other implications.

Kobayashi, J., Ohtake, K., & Uchida, H. (2015). NO-rich diet for lifestyle-related diseases. *Nutrients, 7*(6), 4911-4937. 10.3390/nu7064911 [doi]

In this review, the authors begin with the concept that decreased nitric oxide availability may be causally related to the development of diseases such as insulin resistance, ischemic heart disease, and hypertension. They overview

knowledge of NO generation through the nitrate-nitrite pathway, and discuss safety and effects of this method of NO formation. They conclude that "the intake of nitrate as a nutrient in vegetables might be beneficial to human health as a result of synergistic effects with other nutrients present in vegetables and would be recommended as a nutritional approach to the prevention and treatment of the lifestyle related diseases." I will use this source to discuss the potential therapeutic effects of nitrate.

Kuhnle, G. G., Luben, R., Khaw, K. T., & Feelisch, M. (2017). Sulfate, nitrate and blood pressure – an EPIC interaction between sulfur and nitrogen. *Pharmacological Research, 122*, 127-129.

This study looks at long term benefits of nitrates on 14,000 participants. They found no inverse association with blood pressure, unless the nitrate was combined with small amounts of sulfate, in which case they found an inverse association between nitrate and BP. They conclude that nitrate bioactivity may be more complicated than was previously assumed. I plan to use this to contribute to the nitrate conversation.

Lee, J. E., & Chan, A. T. (2011). Fruit, vegetables, and folate: Cultivating the evidence for cancer prevention. *Gastroenterology, 141*(1), 16-20.

This editorial examines the evidence of the cancer-inhibiting properties of vegetables. They find that extremely

high levels of vegetables do not provide extra benefit, but low levels of vegetable consumption "pose an increase in risk that can be easily minimized with modestly increasing intake." Furthermore, they conclude that the cardiovascular benefits of vegetables outweigh even the anti-cancer benefits. I will use this, in juxtaposition with the high nitrate content of these vegetables, to provide evidence that nitrates do not appear to increase risk of cancer.

Lovegrove, J. A., Stainer, A., & Hobbs, D. A. (2017). Role of flavonoids and nitrates in cardiovascular health. *Proceedings of the Nutrition Society, 76*(02), 83-95. doi:10.1017/s0029665116002871

This article looks into the effects of both flavonoids and nitrates in cardio health, finding both to be beneficial in both chronic and acute situations. Among other things, they find that dietary and supplemental nitrate have been reported to significantly reduce platelet aggregation in healthy individuals, reduce systolic and/or diastolic BP in humans, and reverse features of metabolic syndrome in mice. They conclude that "diets rich in flavonoids and nitrates may be important in reducing CVD risk and promoting vascular benefit". I will use this source to provide more opinions on nitrates.

Lundberg, J. O., Weitzberg, E., & Gladwin, M. T. (2008). The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nature Reviews Drug Discovery, 7*(2), 156-67.

A review that describes the nitrate, nitrite, NO pathway, and reevaluates the commonly held belief that nitrates and nitrites are only harmful substances in the diet or inert metabolites of endogenous NO. They instead argue that nitrates and nitrites serve a critical physiological role, and can, in fact, be used therapeutically. I plan to use this article to shed light on the nitrate controversy from a physiologic perspective.

Magkos, F., Arvaniti, F., & Zampelas, A. (2006). Organic food: Buying more safety or just peace of mind? A critical review of the literature. *Critical Reviews in Food Science and Nutrition, 46*(1), 23-56.

Are-evaluation of the same authors' 2003 article, this review brings the discussion more up to date and reiterates many of the same claims made previously, often bringing more clarity and specificity to the discussion. Interestingly, they find that nitrate content among several organically grown foods was higher than their conventional counterparts, although the data was limited. They again find that microbiological concerns are far more worrisome than chemical contamination.

They attempt to explain the recent consumer perception that organically grown food is significantly more "healthy" with an emotional processing pathway, claiming that the scientists investigating the issue are much less concerned than the general public with the entire discussion. I intend

to use this to shed more light on the nitrate topic and introduce non-health-related factors into the discussion of whether or not organically grown food is a good idea (that is, monetary expense, psychological benefit, and economical strain).

Magkos, F., Arvaniti, F., & Zampelas, A. (2003). Putting the safety of organic food into perspective. *Nutrition Research Reviews, 16*(2), 211-22.

A review of literature that demonstrates that nutritionally speaking, nothing conclusive can be said as to whether organic foods or conventionally grown foods are better. They find that nitrate content in organically grown leaf, root, and tuber vegetables is lower, but other crops have no differences. They found no difference in microbiological contamination, and they found that microbiological contamination is the major source of food contamination, not chemical residues.

Additionally, they found no evidence of toxic hazards having a major impact on total cancer mortality. They conclude that there is no hard evidence to support the claim that organic food is healthier and safer than conventional food or *vice versa*. I will use this to discuss briefly the more broad nature of whether organically grown food is more healthy in any ways, and focus the conversation on the only quantifiably different factor: nitrates.

Magnusson, M. K., Arvola, A., Hursti, U. K., Aberg, L., & Sjoden, P. O. (2003). Choice of organic foods is related to perceived consequences for human health and to environmentally friendly behaviour. *Appetite; Appetite, 40*(2), 109-17.

This questionnaire study from 1154 random Swedes looked at attitudes and behavior towards organic foods, among other things, and found two major motivations for purchasing organic food: personal health (most significant) and environment and animal welfare. Their conclusion is that egoistic motives (personal health) are better predictors of purchase of organic food than altruistic motives (such as helping the environment). I intend to use this to shed light on motives for purchasing organic food, highlighting the common belief that organically grown food is more healthy.

Masschelein, E., Thienen, R. V., Wang, X., Schepdael, A. V., Thomis, M., & Hespel, P. (2012). Dietary nitrate improves muscle but not cerebral oxygenation status during exercise in hypoxia. *Journal of Applied Physiology, 113*(5), 736-745. doi:10.1152/japplphysiol.01253.2011

This study looked at the effects of oral nitrate supplementation during both maximal and submaximal exercise. They found that during acute hypoxia, muscle oxygenation status improved with the oral nitrate supplementation. Acute mountain sickness was not inhibited however. I will use this research to increase the background behind the benefits on nitrate on exercising muscle oxygenation status.

Mensinga, T. T., Speijers, G. J., & Meulenbelt, J. (2003). Health implications of exposure to environmental nitrogenous compounds. *Toxicological Reviews, 22*(1), 41-51.

The National Institute of Public Health from the Netherlands and the Utrecht University Medical Centre combine to produce an article exploring the benefits and hazards of dietary nitrates. They find two major toxicological concerns with nitrites (converted from nitrate by commensal bacteria in the mouth). First is the risk of methaemeglobinaemia, and second is the potential for the formation of N-nitroso carcinogens upon reaction with N-alkyl-amides. However, they do not find an unequivocal relationship between nitrate intake and risk of cancer. They then affirm the daily doses of nitrates recommended by JECFA. I plan to use this in the discussion of nitrates to cover some of the risks, and evaluate scientific attitudes to them.

Pluta, R. M., Dejam, A., Grimes, G., Gladwin, M., & Oldfield, E. (2005). Nitrite Infusions to Prevent Delayed Cerebral Vasospasm in a Primate Model of Subarachnoid Hemorrhage. *Jama,293*(12), 1477-1484. doi:10.1001/jama.293.12.1477

The purpose of this study was to investigate the effects of infusions of nitrate on delayed cerebral vasospasm. They found that sodium nitrate protected against the development of vasospasm and there was even a negative correlation between nitrate concentration in CSF and

severity of vasospasm. I will use this study to explore some of the potential therapeutic effects of nitrate.

Velmurugan, S., Gan, J. M., Rathod, K. S., Khambata, R. S., Ghosh, S. M., Hartley, A., . . . Ahluwalia, A. (2016). Dietary nitrate improves vascular function in patients with hypercholesterolemia: A randomized, double-blind, placebo-controlled study. *The American Journal of Clinical Nutrition, 103*(1), 25-38. 10.3945/ajcn.115.116244 [doi]

This study looked at the effects of daily beetroot juice supplementation in patients with hypercholesterolemia over the course of six weeks. They saw no negative effects, either from methemoglobinemia or N-nitrosoamines. Instead, they saw significant improvement in vascular function and platelet-monocyte aggregations. Additionally they saw a change in the composition of the oral microbiome. They concluded that their study "supports the use of dietary nitrate as a safe, well-tolerated, and potentially powerful prevention strategy in CVD". I will use this article to bring vascular function and hypercholesterolemia into the conversation.

Ward, M. H., Dekok, T. M., Levallois, P., Brender, J., Gulis, G., Nolan, B. T., & Vanderslice, J. (2005). Workgroup Report: Drinking-Water Nitrate and Health—Recent Findings and Research Needs. *Environmental Health Perspectives, 113*(11), 1607-1614. doi:10.1289/ehp.8043

This review is concerned with the rising concentration of nitrate in drinking water, and seeks to understand the long-term health effects of this accumulation of nitrate. They conclude that negative effects can be attenuated by vitamin C and alpha-tocopherol, but can only call for more research to answer the question of whether or not the nitrate daily standard can safely be raised. I will use this review to gather information about safety concerns related to nitrates.