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Reduction in Blood Pressure from Consumption of Dietary Nitrate

ABSTRACT

The purpose of this meta-analysis is to examine the relationship between consumption of dietary nitrate and changes in blood pressure. Blood pressure is an important characteristic that indicates overall cardiovascular health; therefore, consumption of products that lower blood pressure are likely to be of interest to hypertensive individuals. Some research has indicated that ingestion of nitrate leads to a decrease in blood pressure while other research has not substantiated this claim. The results of a variety of research were examined in order to determine whether consumption of beetroot juice and leafy green vegetables, which tend to contain a high concentration of nitrates, lead to reduction in blood pressure. Analyses of the results were performed using t-tests along with several other linear regressions to determine whether reduction in blood pressure from nitrate is statistically significant from controls. Our results found that beetroot juice did not significantly decrease blood pressure in comparison to nitrate-deprived beverages, however, consumption of vegetable supplements did lead to significant decreases in blood pressure. It was also found that dosage of beetroot juice and vegetable supplements is positively correlated with blood pressure reduction.

INTRODUCTION

The National Health and Human Nutrition Examination Survey has found that over 80 million Americans over the age of twenty suffer from hypertension as of 2012 (Mozaffarian *et. al*, 2015). Furthermore, estimates have indicated that over 7 million deaths per year may be

credited to hypertension with as many as 1 billion people in the world suffering from this condition (Chobanian *et. al*, 2003). As humans age, the possibility of affliction with high blood pressure rises dramatically; reports indicate that the risk is over ninety percent in both men and women by the age of eighty-five (Pickering, 2007). Hypertension itself is not a condition that may kill people; however, it is a major risk factor for cardiovascular disease and stroke (Mozaffarian *et. al*, 2015). In fact, it has been shown that the risk of heart disease and stroke increase linearly with blood pressure (Lewington *et. al*, 2002). The reduction of systolic hypertension has been shown to be correlated with reductions in cardiovascular diseases.

The large prevalence of hypertension worldwide along with the staggering death toll of its associated complications make blood pressure control essential. Some clinicians have prescribed antihypertensive drugs that have found increasing market-share; however, these have had only moderately positive to mixed results (Pickering, 2007). One major reason for why antihypertensive drugs have not yielded positive benefits for patients is that as many as 35% of patients discontinue drug use within six months. While there are a variety of classes of antihypertensive drugs including diuretics, angiotensin-converting (ACE) inhibitors, and calcium-channel blockers to name a few, a common theme throughout all are their adverse side-effects. Patients on antihypertensive pharmaceutical drugs commonly encounter problems such as hypokalaemia, erectile dysfunction, dry irritating coughs, and peripheral oedema (Trinder, 2012). Consequently, patients with hypertension are encouraged to find blood pressure control treatment options with less interference in daily life.

In direct contrast to pharmaceutical approaches to hypertension, current health protocol involves lifestyle interventions for hypertensive patients aimed at combating some of the causes of hypertension. Specific interventions emphasize proactive and personal choice to reduce

hypertension and include healthy weight control, dietary sodium reduction via the DASH plan (Dietary Approaches to Stop Hypertension), increased physical activity, and decreased alcohol consumption (Whelton *et. al*, 2002). While such interventions have proven to be moderately effective at decreasing blood pressure in some individuals, these alternative proscriptive strategies should be researched further to justify their application.

One potential proposed treatment for decreasing blood pressure in a variety of subjects is the supplementation of dietary nitrate which may be found in leafy green vegetables such as lettuce, spinach and arugula, and beetroot juice (Hord *et. al*, 2009). [See Table 1.] This solution stems from hypertensive treatment that involves the consumption of fruits and vegetables which have a high concentration of inorganic nitrate (Ysart *et. al*, 1999). The proposed mechanism for nitrate reduction in blood pressure involves the conversion of nitrate to nitric oxide. When nitrate is ingested, bacteria within the mouth quickly reduce it to nitrite. The nitrite anions are further reduced to nitric oxide and other nitrogen intermediates when they are transported to the stomach via the esophagus (McDonagh *et. al*, 2018). Nitric oxide can cause vasodilation within blood vessels by reduction of intracellular calcium cations (Ca^{2+}) within smooth muscle cells. Constriction of vessels is a response to increased levels of Ca^{2+} ions as a result of their interaction with the messenger protein calmodulin. Calmodulin phosphorylates myosin within smooth muscle cells causing muscle contraction and thus vasoconstriction (Van Hove *et. al*, 2009). Since Ca^{2+} is limited by nitric oxide, muscle contraction is decreased and therefore vasodilation occurs, increasing the ease of blood flow and directly causing a reduction in blood pressure. Hypertension and prehypertension are associated with decreased nitric oxide bioactivity, and therefore it is reasonable to suggest that an increase in nitric oxide availability

may lead to opposite effects such as decreases in systolic and diastolic blood pressure (Webb *et. al*, 2008).

Studies examining whether the consumption of dietary nitrate reduces blood pressure have yielded mixed results. One recent study conducted by Kapil *et. al* (2014) reports that supplementation of beetroot juice in hypertensive patients over a period of four weeks led to a mean reduction of 8.1 mmHg systolic and 3.8 mmHg diastolic blood pressure when measured in the patients' home. However, another study conducted by Bondonno *et. al* (2015) also carried out on hypertensive individuals failed to find a decrease in blood pressure after a week of beetroot juice supplementation.

Similar discrepancies in results have been found when examining research administered on normotensive individuals. In studies of leafy green vegetables high in nitrates and their effects on blood pressure, it was rarer to see absolutely no effect on blood pressure. However, there are cases in which decrease in blood pressure was considered statistically insignificant (Bondonno *et. al* 2014). In addition, there are inconsistencies in the data found between studies. Two studies found that systolic blood pressure decreased by 3.55 mmHg in response to eating green leafy vegetables (Ashworth *et al*, 2015; Jovanovski *et al.* 2015) yet some cases showed no decrease (Sobko *et. al*, 2009; Larsen *et. al*, 2006). Effects on diastolic blood pressure were similarly varied, although some studies excluded it entirely.

The inconsistency in results between individual studies leads to the necessity for a meta-analysis investigating the potential for dietary nitrate supplementation to lower blood pressure. The objective for this meta-analysis is to quantitatively determine if dietary nitrate consumption affects systolic and diastolic blood pressure. Nitrate supplements will be analyzed according to two categorical variables: nitrate supplements that are beet root derivatives and supplements that

are of other vegetable origin. We hypothesize that decreases in both systolic and diastolic blood pressure will be associated with ingestion of dietary nitrate supplements within both categories. In order to determine these associations, mean differences of change in blood pressure between treatment and control groups within the categories of supplements will be statistically analyzed by a two-tailed t-test for each subcategory. Results of these tests will allow us to determine whether supplementation of nitrate through either beetroot juice or other vegetable sources are viable methods for lowering individuals' systolic and diastolic blood pressure as hypothesized.

METHODS

The research question of how beetroot juice and other vegetable nitrate sources affect systolic and diastolic blood pressures was divided into two subcategories. The first involved consideration of studies regarding beetroot juice supplementation and the second examined studies regarding non-beetroot derived vegetables supplements.

Beetroot Juice Supplementation

A literature search was conducted on the databases PubMed and Web of Science with key terms "beetroot", "blood pressure" and "nitrate" to find relevant studies for consideration in our meta-analysis. The initial search yielded 308 pertinent studies. Search results were then refined to include only randomized, placebo-controlled, double-blind studies. This led to consideration of 78 studies for meta-analysis. From these works, six studies were selected with the criteria that they reported on a change between baseline systolic and diastolic blood pressures and post-treatment blood pressures.

After it was determined that a particular study met the requirements for inclusion in this meta-analysis, it underwent data extraction. Information about authors, year of publication, study

design, duration, participants, methods, results and funding sources were recorded. Extracted information was used to determine the dosage of beetroot juice (or control), length of treatment, time between treatment and measurement and change in systolic and diastolic blood pressures between baseline and post-treatment measurements. Change in blood pressure was defined as the subtraction of baseline blood pressure from post-treatment blood pressure.

Change in systolic and diastolic blood pressure between treatment and control groups was analyzed by finding mean changes in blood pressure between participants ingesting beetroot juice or nitrate-deprived substitute. A two-tailed t-test was carried out in order to determine whether there was a significant difference between interventional and placebo groups. An additional t-test was run excluding studies with normotensive or hypotensive participants to determine if a significant reduction in systolic and diastolic blood pressures could be found in hypertensive participants. Furthermore, regression analysis was conducted to determine whether there was a relationship between dosage of beetroot beverage and change in systolic and diastolic blood pressures.

Vegetable Derived Nitrate Supplementation

Searches were conducted on the databases Google Scholar and PubMed Science in order to recover articles relevant to leafy green vegetables and their effects on hypertension. The key terms “[green leafy] vegetable”, “hypertension” or “blood pressure” and “dietary nitrates” were used to search literature. Most studies involving dietary nitrates used beetroot or its juice in order to draw conclusions concerning the effect of nitrates on systolic and diastolic blood pressure. Therefore, it was important to exclude the word “beetroot” while searching databases for articles related to effects of vegetable consumption on blood pressure. The initial search found 128 studies matching search criteria prior to dismissing results that did not satisfy requirements. In

order to be considered for inclusion in meta-analysis, it was required that studies compared effects of vegetable derived nitrates on both a treatment group and control group so that they could be compared in statistical analysis. After analyzing studies, six studies were found that met requirements for inclusion in our meta-analysis.

Data was compiled in a Microsoft Excel spreadsheet comparing metrics, experiment type, subjects, methods, and results in order to reveal similarities and differences. The mean change in blood pressure (pre- vs. post-treatment) for non-beetroot nitrate supplements were calculated for the combined data set. Mean changes were then analyzed by a two-tailed t-test to determine whether there is a significant difference between treatment and control group decreases in systolic and diastolic blood pressures. A linear regression between blood pressure reduction and dosage was also made to analyze the effects of nitrate dosage on systolic and diastolic blood pressure reduction.

RESULTS

A total of 12 studies were found that met inclusion criteria with 6 examining the association between beetroot juice, and 6 addressing the association between other vegetable nitrate supplements and blood pressure. Of 12 total studies, 3 specifically examined the effects of dietary nitrates on individuals with hypertension.

Effects of consumption of beetroots and their derivatives

Out of the studies that examined association between beetroot juice consumption and decrease in systolic and diastolic blood pressure, 33.3% found that a significant decrease in both blood pressures could be attributed to beetroot ingestion. After pooling blood pressure reduction between studies, it was found that mean systolic blood pressure decreased by 4.83 mmHg in

individuals that had received the beetroot juice treatment. Participants that consumed the control (placebo) beverage also experienced a decrease in their systolic blood pressure by a mean of 3.6 mmHg (Figure 1). Diastolic blood pressure decreased by 3.45 mmHg from drinking beetroot juice and 2.95 mmHg from placebo consumption (Figure 2). Two-tailed t-tests found that there were no significant differences in mean reduction between the treatment and control groups with p-values of 0.45 and 0.49 for systolic and diastolic blood pressure respectively.

Further analysis that included only studies with hypertensive participants found that beetroot juice was associated with a decrease in systolic blood pressure by 6 mmHg compared to placebo beverage that showed a decrease of 3.2 mmHg (Figure 3). Two-tailed t-tests analysis found that there was not a significant difference between mean reductions for either systolic or diastolic blood pressures with p-values of 0.39 and 0.53 respectively.

Additional analysis was done to determine whether there was an association between beverage dosage (mL) and reduction in systolic and diastolic blood pressures (Figure 5, Figure 6). Pearson's correlation coefficient was calculated for each regression to determine the strength of association. It was found that increased beetroot juice dosage was weakly positively associated with a reduction in systolic blood pressure ($r = 0.26$) and had no association with reduction in diastolic blood pressure ($r = -0.07$). No association was found for reduction in either systolic or diastolic blood pressure with increased dosage with $r = -0.08$ and $r = -0.11$ respectively.

Effects of consumption of leafy green vegetables

Of the studies that examined association between consumption of green leafy vegetables and decrease in systolic and diastolic blood pressure, 83% found a significant effect of green leafy vegetable consumption on decrease in systolic and diastolic blood pressure. Pooled results

demonstrated a mean decrease of 5.0 mmHg in systolic blood pressure compared to a 0.8 mmHg increase in blood pressure in the control group (Figure 6). Mean diastolic blood pressure decreased by 4.0 mmHg in comparison to no change seen in the control group (Figure 7). Two-tailed t-tests comparing mean blood pressure reduction in both systolic and diastolic between vegetable supplement and control groups found p-values of 0.002 and 0.006 respectively, indicating that there was a significant reduction in both systolic and diastolic blood pressures.

Regression analysis was conducted to determine whether there was an association between vegetable supplement dosage and change in blood pressure (Figure 9, Figure 10). Pearson's correlation coefficient was determined in order to analyze the strength of correlation between change in systolic and diastolic blood pressure as a function of dosage. It was found that there was a strong correlation between increased vegetable supplement dosage and reduction of both systolic and diastolic blood pressures with correlation coefficients of 0.77 and 0.85 respectively.

DISCUSSION

Our meta-analysis examining effects of nitrate consumption on systolic and diastolic blood pressure yielded mixed results. Contrary to our hypothesis, no significant difference was found in reduction of both systolic and diastolic blood pressure between beetroot consumption and nitrate deprived beverage consumption. Additionally, no significant difference between treatment and control groups was found when specifically examining effects of beetroot juice consumption on participants with elevated blood pressure. Although previous research found correlation between dietary nitrate consumption and reduced systolic and diastolic blood pressure, results of the meta-analysis indicate that the nitrate content in beetroot juice is not

sufficient to bring about a reduction in blood pressure. This is in conflict with literature that affirms nitrates as vasodilators that reduce blood pressure, and is also in conflict with studies such as that of Kapil *et. al* (2015) which found that both systolic and diastolic blood pressure drop significantly compared to a control group from consumption of beetroot supplements in individuals with hypertension. However, when comparing data, it was found that the supposed conflict may have stemmed from the low amount of dietary nitrate that was administered in beetroot studies.

Despite beetroot being a vegetable known for its nitrate content, it has only 110 mg of dietary nitrate per 100 g, whereas other leafy green vegetables such as basil have 183 mg, cilantro 247 mg, and arugula 480 mg of nitrates per 100 g respectively (Brkić *et al*, 2017). In addition, consumption of beetroot derived juice rather than the beetroot itself may have caused a further loss in nitrate content. Another likely contribution to a lack of significant decrease in blood pressure from beetroot juice consumption was the inclusion of studies with hypertensive participants. Hypertensive individuals are likely to be taking medications that would decrease nitric oxide production from dietary nitrate (Bondonno *et. al*, 2015). This means that increased nitric oxide would not be created from ingestion of beetroot juice, and therefore benefits of nitric oxide acting as a vasodilator would be limited. Furthermore, control/placebo beverages may have also reduced blood pressure. Several studies used beverages such as apple juice as a control beverage. Apple juice is known to contain a blood pressure reducing flavanol, quercetin (Edwards *et. al*, 2007). These study limitations may explain why there was little to no correlation between beetroot juice dosage and reduction in systolic and diastolic blood pressure upon analysis in linear regression.

Therefore, although our results indicate that there is no measurable benefit to consuming beetroot derivatives to achieve decreased blood pressure, there is not enough information to draw a conclusion that consuming beetroot (and its juice) will not lead to a reduction. In fact, if the amount of beetroot consumption was increased and controls properly accounted for, then it is possible that consuming beetroots would have a correlation with decreased blood pressure.

Statistically significant results were found when comparing blood pressure reductions of green leafy vegetable derived supplements to control groups. This is consistent with literature that explains that reduction of nitrate ions to nitric oxide leads to vasodilation and reduction of systolic and diastolic blood pressure. Regression analysis found that there was a strong positive correlation between vegetable supplement dosage and reduction in blood pressure. This supports the mechanistic explanation that increased amounts of nitric oxide lead to greater amounts of vasodilation through interaction with calcium cations, and therefore blood pressure is reduced.

Results in vegetable supplementation may have been modulated by a variety of factors; studies that used this method of lowering blood pressure were difficult to find as often the studies defaulted to using only beetroot juice as their source of nitrate. Because of the lack of choice in the studies, complete data sets for both systolic and diastolic blood pressure could not be found; several studies reported only one and not the other. Additionally, results were affected by availability of dosages. In the case of systolic blood pressure reduction, vegetable supplementation doses were inconsistent and consisted of three different dosages: 300 mg, 1800 mg, and 1900 mg. This range of dosages includes an extremely large jump, and it is imperative that future studies consider examining what happens to blood pressure from dosages of vegetable supplement that are somewhere in between 300 and 1800 mg.

Knowing that there is a statistically significant decrease in both the systolic and diastolic blood pressure resulting from consumption of vegetable derived supplements, it is evident that vegetables like spinach and arugula have a significant effect on blood pressure and are therefore a good short-term method to reduce both systolic and diastolic blood pressure in normotensive patients. However, linear regressions between dosage and blood pressure reduction provide a good reason to believe that it is necessary to eat a high dosage of nitrate to see significant reductions in blood pressure. Although extremely high doses of nitrate may prove harmful to the human body, doses that are too low can prevent visible effects on blood pressure. This is a similar finding to that of Sobko *et. al* (2009), in which participants ate a high nitrate diet of Asian vegetables. The high doses of nitrate consumption in vegetables resulted in a higher mean decrease in diastolic blood pressure measured. Further study in high doses of nitrate may result in better reduction in blood pressure and an experiment measuring the intervals of dosages of nitrates is a good starting point to pursue.

Our overall research question aimed to determine whether dietary nitrates reduced systolic and diastolic blood pressure. Our results found that treatment of high nitrate vegetable supplements led to significant decreases in blood pressure while beetroot juice supplementation did not. Although beetroot juice and its effects were not as strong as the effects of green leafy vegetables, the problems of varying blood pressure between participants (normotensive and hypertensive) as well as control groups that had other blood pressure lowering mechanisms may have altered results. Further experimentation is clearly needed for both treatment options to prevent previously discussed confounds and also determine effects of varying doses of nitrate. This would strengthen findings in a linear regression between nitrate dosage and blood pressure reduction that previously relied on extrapolation for intermediate doses.

From our analysis, there are tangible benefits of the lifestyle approach to reduce blood pressure through a change in diet; specifically, a diet that is rich in leafy green vegetables may result in a significant short-term reduction of systolic and diastolic blood pressure. Although there is a reduction in blood pressure resultant from beetroot juice in large doses as well as leafy green vegetables, it is yet to be proven that beetroot juice has a significant effect and only further experimentation will provide more data that will help guide patients in their effort to reduce blood pressure and treat hypertension through nutritional intervention. From our results we are able to recommend a diet with increased amounts of leafy green vegetables such as spinach, lettuce, and arugula rich in dietary nitrates in opposition to pharmaceutical nitrates. High dosages of nitrates alone are likely to have negative effects on the human body and increase risk of illness, while a similar dosage of nitrates taken by way of vegetables did not show negative effects in the studies found. A daily lifestyle diet of nitrate-rich vegetables will persistently lower blood pressure, and if it is started earlier in the human lifespan, the likelihood of hypertension occurring later in life will decrease. In individuals with hypertension, consuming dietary nitrates is a good alternative and supplement in addition to other treatment options to help reduce blood pressure; however, the effect is greatest when the diet is started by normotensive individuals. This preventative strategy, if followed, may prove to reduce the growing number of hypertensive individuals across the nation and the world.

REFERENCES

Ashworth A, Mitchell K, Blackwell J, Vanhatalo A, and Jones A. 2015. High-nitrate vegetable diet increases plasma nitrate and nitrite concentrations and reduces blood pressure in healthy women. *Public Health Nutr*. 18(14):2669–2678.

Bondonno C, Liu A, Croft K, Ward N, Yang X, Considine M, Puddey I, Woodman R, and Hodgson J. 2014. Short-term effects of nitrate-rich green leafy vegetables on blood pressure and arterial stiffness in individuals with high-normal blood pressure. *Free Radic Biol Med*. 77:353-62.

Bondonno C, Liu A, Croft K, Ward N, Shinde S, Moodley Y, Lundberg J, Puddey I, Woodman R, and Hodgson J. 2015. Absence of an effect of high nitrate intake from beetroot juice on blood pressure in treated hypertensive individuals: a randomized controlled trial. *Am J Clin Nutr*. 102(2):368-75.

Brkić, D., Bošnir, J., Bevardi, M., Bošković, A. G., Miloš, S., Lasić, D., Trstenjak, N. U. (2017). NITRATE IN LEAFY GREEN VEGETABLES AND ESTIMATED INTAKE. *Afric J Trad Comp Alt Med*, 14(3):31–41.

Coggan A, Leibowitz J, Spearie C, Kadkhodyan A, Thomas D, Ramamurthy S, Mahmood K, Park S, Waller S, Farmer M, and Peterson L. 2015. Acute Dietary Nitrate Intake Improves Muscle Contractile Function in Patients With Heart Failure. *Circulation*. 8:914-920.

Coles L and Clifton P. 2012. Effect of beetroot juice on lowering blood pressure in free-living, disease-free adults: a randomized, placebo-controlled trial. Nutr. J. 11:106.

Chobanian A, Bakris G, Black H, Cushman W, Green L, Izzo J, Jones D, Materson B, Oparil S, Wright J, and Roccella E. 2003. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 42(6):1206-52.

d'El-Rei J, Cunha A, Trindade M, Neves M. 2016. Beneficial Effects of Dietary Nitrate on Endothelial Function and Blood Pressure Levels. *Int J Hypertens*. 2016:6791519.

Edwards R, Lyon T, Litwin S, Rabovsky A, Symons J, and Jalili T. 2007. Quercatin Reduces Blood Pressure in Hypertensive Subjects. *J. Nutr.* 137(11):2405-2411.

Eglin C, Costello J, Bailey S, Gilchrist M, Massey H, and Shepherd A. 2017. Effects of dietary nitrate supplementation on the response to extremity cooling and endothelial function in individuals with cold sensitivity. A double blind, placebo controlled, crossover, randomized control trial. *Nitric Oxide*. 70:76-85.

Hord N, Tang Y, and Bryan N. 2009. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr*. 90(1):1-10.

Jonvik K, Nyakayiru J, Pinckaers P, Senden J, van Loon L, and Verdijk L. 2016. Nitrate-Rich Vegetables Increase Plasma Nitrate and Nitrite Concentrations and Lower Blood Pressure in Healthy Adults. *J Nutr.* 146(5):986-993.

Jovanovski E, Bosco L, Khan K, Au-Yeung F, Ho H, Zurbau A, Jenkins A, Vuksan V. 2015. Effect of Spinach, a High Dietary Nitrate Source, on Arterial Stiffness and Related Hemodynamic Measures: A Randomized, Controlled Trial in Healthy Adults. *Clin Nutr Res.* 4(3):160-167.

Kapil V, Khambata R, Robertson A, Caulfield M, and Ahluwalia A. 2015. Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: a randomized, phase 2, double-blind, placebo-controlled study. *Hypertension*. 65(2):320-7.

Larsen F, Ekblom B, Sahlin K, Lundberg J, and Weitsberg E, 2006. Effects of Dietary Nitrate on Blood Pressure in Healthy Volunteers. *New Eng J Med.* 355:2792-2793

Lewington S, Clarke R, Qizilbash N, Peto R, and Collins R. 2002. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 360(9349):1903-13.

McDonagh S, Wylie L, Webster J, Vanhatalo A, and Jones A. 2018. Influence of dietary nitrate food forms on nitrate metabolism and blood pressure in healthy normotensive adults. *Nitric Oxide*. 72:66-74.

Mozaffarian D, Benjamin E, Go A, Arnett D, Blaha M, Cushman M, de Ferranti S, Després J, Fullerton H, Howard V, Huffman M, Judd S, Kissela B, Lackland D, Lichtman J, Lisabeth L, Liu S, Mackey R, Matchar D, McGuire D, Mohler E, Moy C, Muntner P, Mussolino M, Nasir

K, Neumar R, Nichol G, Palaniappan L, Pandey D, Reeves M, Rodriguez C, Sorlie P, Stein J, Towfighi A, Turan T, Virani S, Willey J, Woo D, Yeh R, and Turner M. 2015. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation*. 131(4):e29-322.

Pickering T. 2007. Why Don't We Use Nitrates to Treat Older Hypertensive Patients? *J Clin Hypertens*. 7(11):685-690.

Shepherd A, Wilkerson D, Dobson L, Kelly J, Winyard P, Jones A, Benjamin N, Shore A, and Gilchrist M. 2015. The effect of dietary nitrate supplementation on the oxygen cost of cycling, walking performance and resting blood pressure in individuals with chronic obstructive pulmonary disease: A double blind placebo controlled, randomised control trial. *Nitric Oxide*. 48:31-37.

Sobko T, Marcus C, Govoni M, and Kamiya S, 2010. Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. *Nitric Oxide.* 22(2):136-140.

Trinder Y. 2012. Common and less common adverse effects of antihypertensives: a general practitioner’s perspective. *S Afr Fam Pract*. 54(2):S31-S32.

Van Hove C, Van der Donckt C, Herman A, Bult H, and Fransen P. 2009. Vasodilator efficacy of nitric oxide depends on mechanisms of intracellular calcium mobilization in mouse aortic smooth muscle cells. *Br J Pharmacol*. 158(3):920-930.

Webb A, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R, Hobbs A, and Ahluwalia A. 2008. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension*. 51(3):784-90.

Whelton P, He J, Appel L, Cutler J, Havas S, Kotchen T, Roccella E, Stout R, Vallbona C, Winston M, and Karimbakas J. 2002. Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. *Jama*. 288(15):1882-8.

Ysart G, Miller P, Barrett G, Farrington D, Lawrance P, and Harrison N. 1999. Dietary exposures to nitrate in the UK. *Food Addit Contam*. 16(12):521-32.

APPENDIX

Tables and Figures

Table 1. List of vegetables according to the level of nitrate concentration. It should be noted that in the ‘very high’ category, beetroots have a significantly lower concentration of nitrates than all other vegetables but is most widely used in clinical trials (d’El-Rei, Jenifer, et al., 2016).

Nitrate content (mg/100g of fresh food)	**Vegetables**

Very low, <20 mg	Asparagus, garlic, onion, green bean, pepper, potato, sweet potato, tomato, and watermelon
Low, 20-<50 mg	Broccoli, carrot, cauliflower, and chicory
Regular, 50-<100 mg	Cabbage, turnip, and dill
High, 100-<250 mg	Endive, sweet leaf, parsley, and leek
Very high, >250 mg	Celery, chard, lettuce, beetroot, spinach, arugula, and watercress

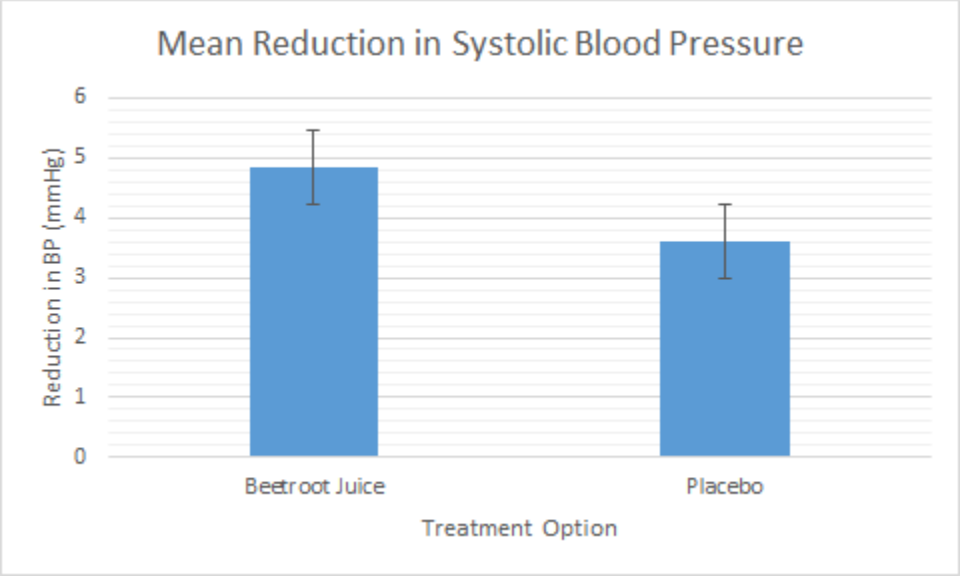

Figure 1. This bar graph shows the mean reduction in systolic blood pressure between beetroot juice and placebo treatments. Beetroot juice has a mean reduction of 4.83 mmHg while placebo nitrate-deprived beverage has a mean reduction of 3.6 mmHg.

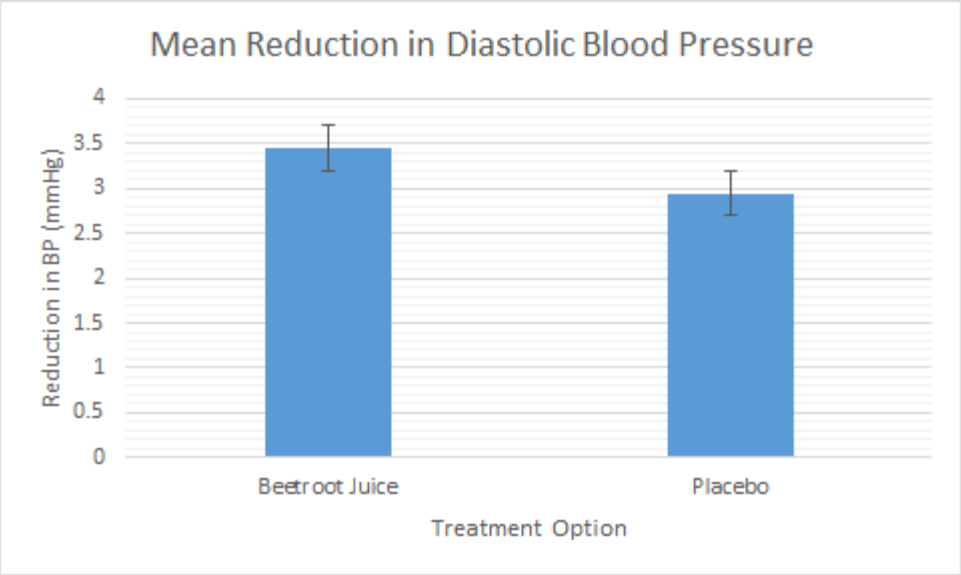

Figure 2. This bar graph shows the mean reduction in diastolic blood pressure between beetroot juice and placebo treatments. Beetroot juice has a mean reduction of 3.45 mmHg while placebo nitrate-deprived beverage has a mean reduction of 2.95 mmHg.

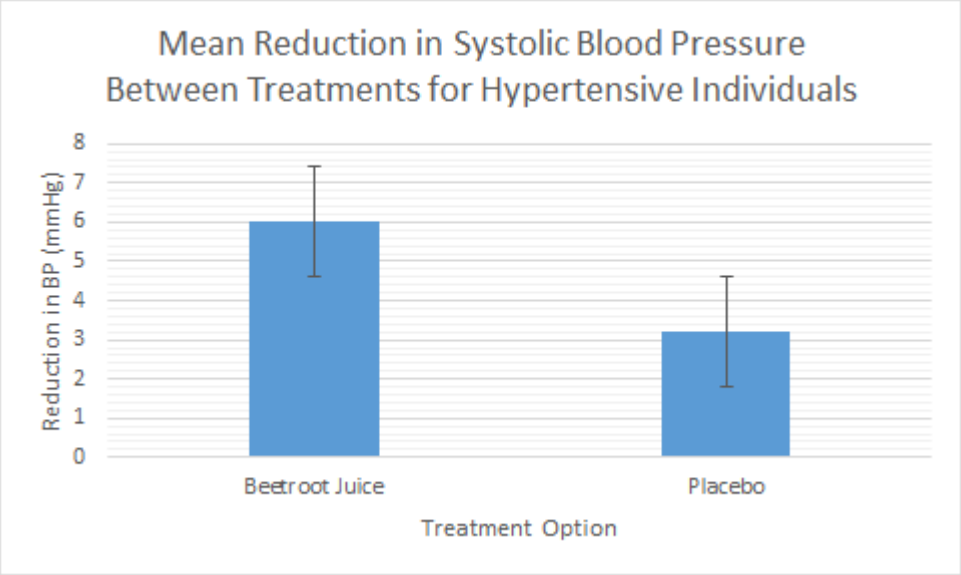

Figure 3. This bar graph shows the mean reduction in systolic blood pressure between beetroot juice and placebo treatments for individuals with hypertension. Beetroot juice has a mean reduction of 6 mmHg while placebo nitrate-deprived beverage has a decrease of 3.2 mmHg.

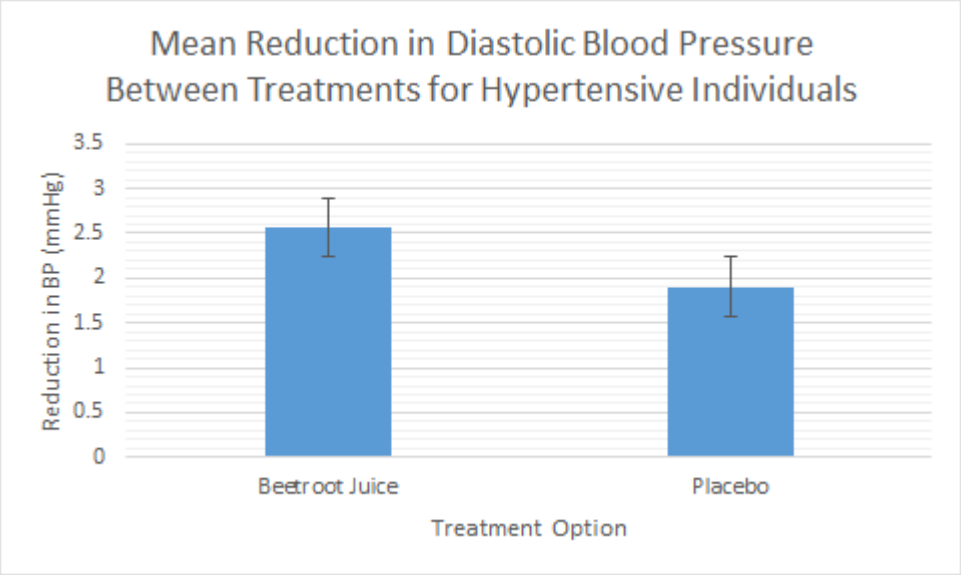

Figure 4. This bar graph shows the mean reduction in diastolic blood pressure between beetroot juice and placebo treatments for individuals with hypertension. Beetroot juice has a mean reduction of 2.57 mmHg while placebo nitrate-deprived beverage has a decrease of 1.9 mmHg.

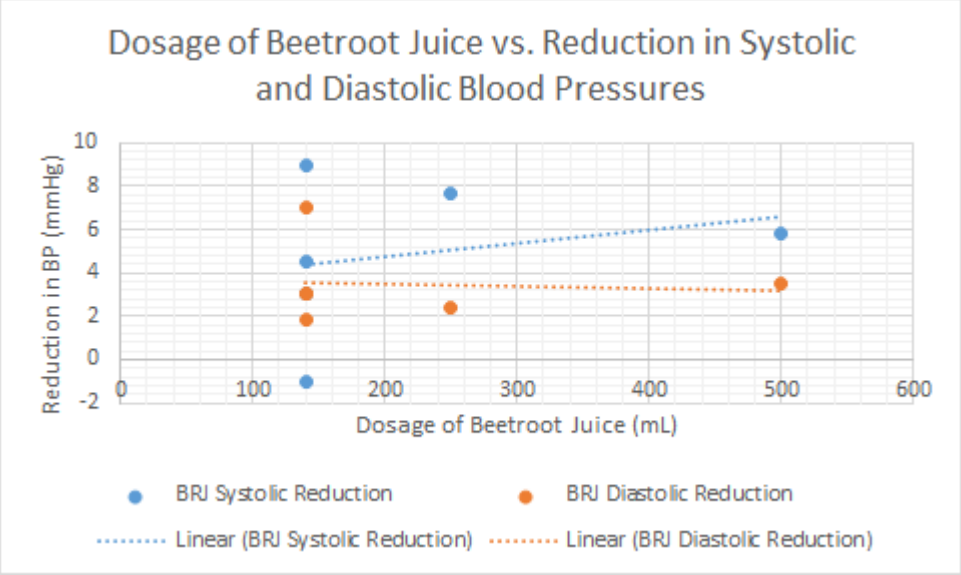

Figure 5. This is a scatterplot showing a linear regression between beetroot juice dosage and systolic and diastolic blood pressure reduction. It can be seen that there is a weak positive

correlation between increased dosage and systolic blood pressure reduction (r = 0.26) and there is no correlation between dosage and diastolic blood pressure reduction (r = -0.07).

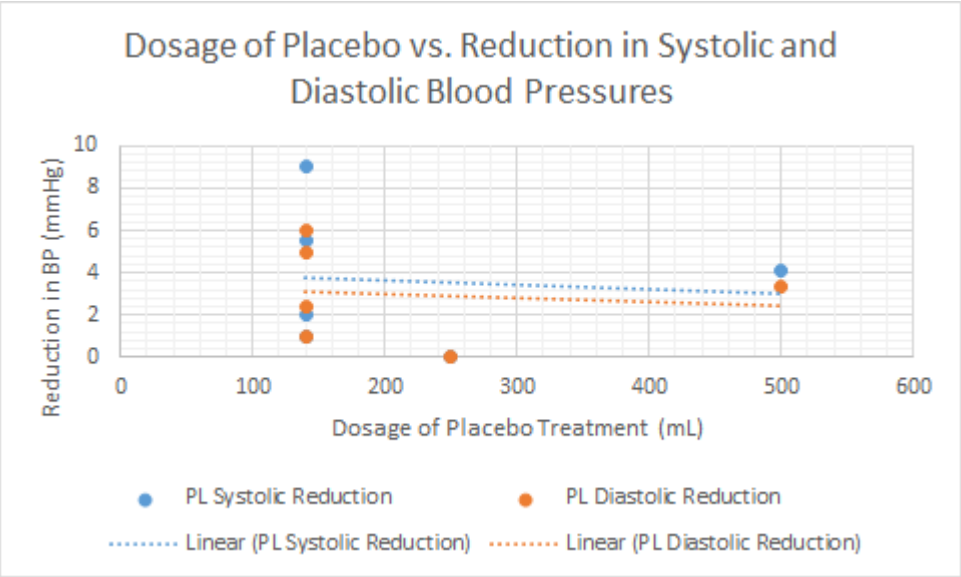

Figure 6. This is a scatterplot showing a linear regression between placebo dosage and systolic and diastolic blood pressure reduction. There is no correlation between dosage and systolic (r = -0.08) or diastolic (r = 0.11) blood pressure reduction .

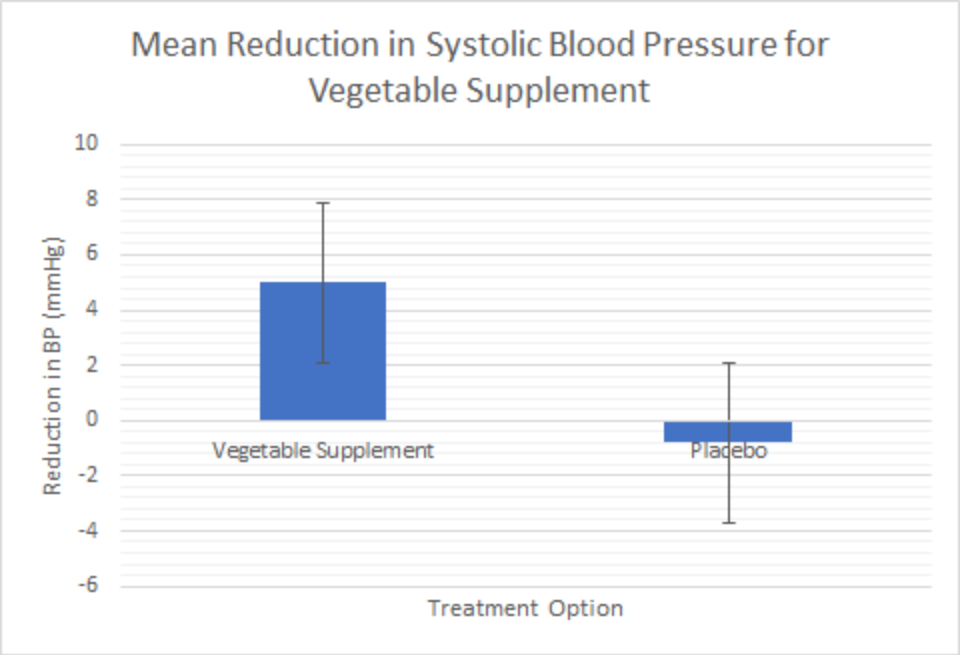

Figure 7. This is a bar graph depicting differences in blood pressure reduction between vegetable nitrate supplementation and the control group. The vegetable supplement have a mean reduction of 5.0 mmHg while the control has an increase of 0.8 mmHg (reduction of -0.8 mmHg).

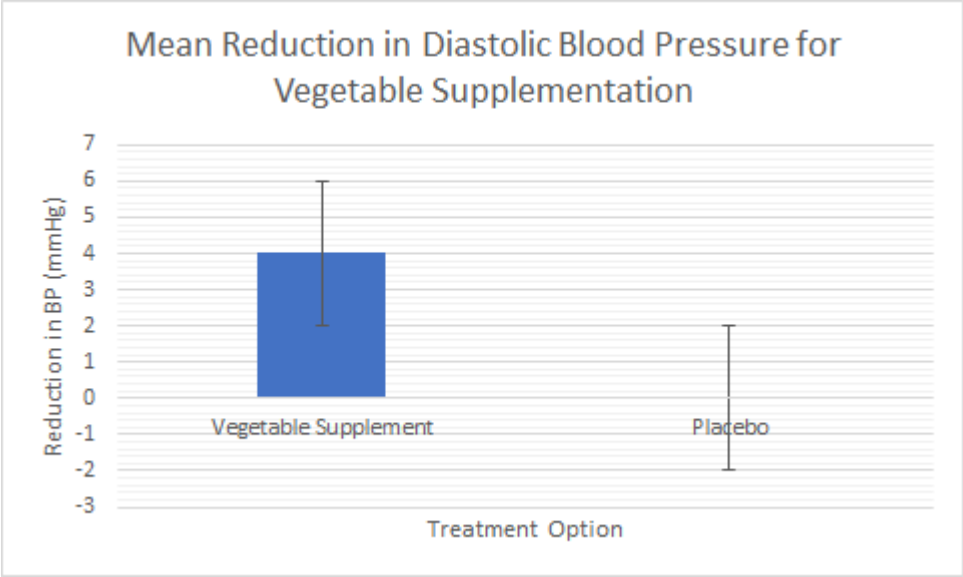

Figure 8. This is a bar graph depicting the differences in mean diastolic blood pressure between vegetable supplement group and control group. The vegetable supplement group has a mean decrease in 4.0 mmHg while the control group does not show any decrease in diastolic blood pressure.

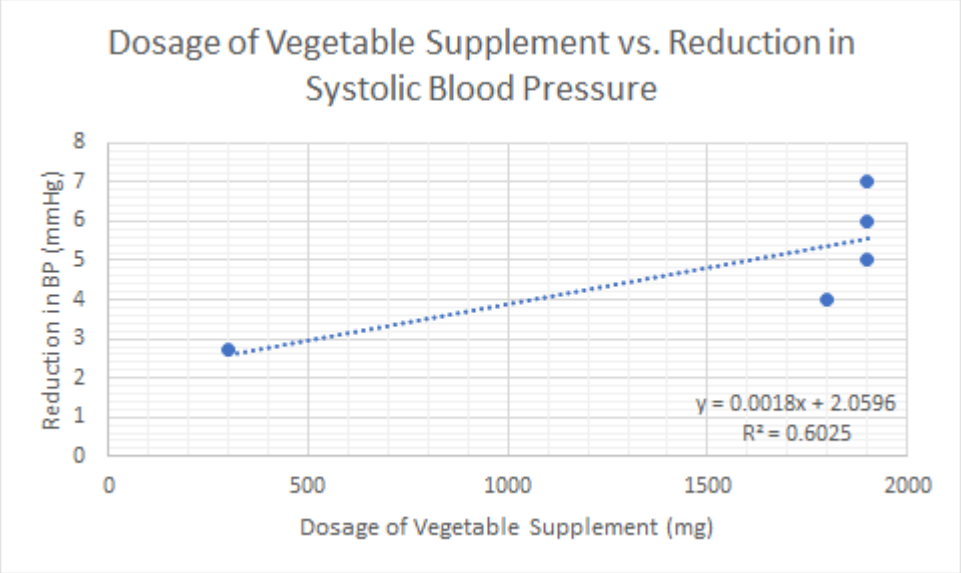

Figure 9. This scatterplot displays a strong positive correlation between vegetable supplement dosage and reduction in systolic blood pressure (r = 0.77).

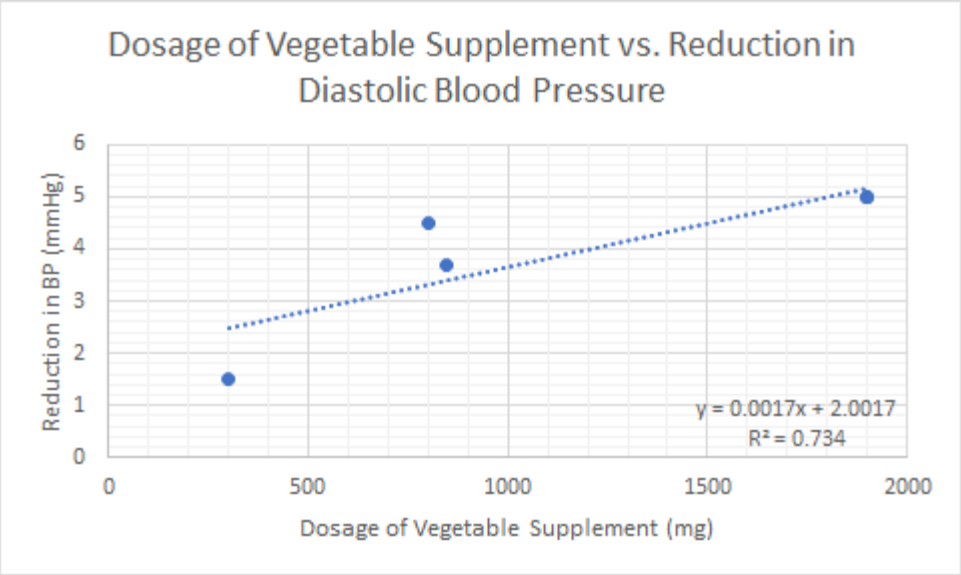

Figure 10. This scatterplot displays a strong positive correlation between vegetable supplement dosage and reduction in diastolic blood pressure (r = 0.85).