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The Effects of Vitamin D Supplementation on Influenza

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DEFINING THE PROBLEM

Research Question

Vitamin D is an essential vitamin that is crucial for executing regular bodily functions. Vitamin D is most widely known for its role in assisting with calcium and phosphate homeostasis. Recently the role of vitamin D in other non-classical capacities has been evaluated. One of these non-classical vitamin D roles is assisting in maintaining proper immune system health. Vitamin D aids the immune system by helping to promote monocyte activity as well as helping to modulate the activity of dendritic cells and T-cells (Hewison, 2012). One disease that has a great effect on the immune system is influenza. Influenza is one of the most prominent diseases in the world and remains a problem in the United States (Center for Disease Control and Prevention, 2015). It causes an increase in inflammatory cytokines, which is detrimental to the tissues of the body (Cannell, et al., 2006). Vitamin D helps to prevent and limit influenza by moderating the inflammatory cytokines as well as promoting the production of monocytes that help destroy the invading materials. However, individuals might not be receiving an adequate amount of vitamin D daily, which results in detrimental health effects. One way this could be changed is through vitamin D supplementation. Through the use of vitamin D supplementation the recommended daily amount of vitamin D can be assured and optimal health could be achieved. Therefore, this study aims to answer the following question: Does vitamin D supplementation decrease the rate of out-patient doctor visits due to influenza-like illnesses in the general population?

Experimental Hypothesis

1. Vitamin D supplementation will significantly decrease the number of out-patient doctor visits due to influenza-like illnesses in the general population.
Assumptions

1. All subjects will accurately report their general medical history.
2. All subjects will accurately report their consumption of the supplements throughout the study.
3. All subjects will accurately report every out-patient doctor visit due to influenza-like illnesses.
4. All subjects will continue with a consistent diet.
5. All subjects will not consume any other supplementation.
6. All subjects will maintain their current BMI.
7. All subjects will retain a permanent residence within a one hundred mile radius of Akron, Ohio.

Delimitations

1. Healthy adults with no previous history of auto-immune disorders, diabetes, or cancer.
2. Individuals who will maintain a regular diet.
3. Individuals who will retain a permanent residence within a one hundred mile radius of Akron, Ohio.
4. D₃ 1000 IU will be the only vitamin D supplements used during the duration of the study period.

Operational Definitions

1. **Vitamin D**: Vitamin D is a fat soluble vitamin that cannot be found in most foods, but is added to others and can be taken as a supplement.
2. **Calcitriol**: The physiologically active form of vitamin D.
3. **Calcidiol**: The precursor to Calcitriol. This mineral is measured to determine vitamin D status.

4. **Immune System**: The immune system is a complex, multi-faceted gatekeeper that works to prevent, deter, and limit disease and infection. It uses numerous different cell types to destroy invading pathogens and dangerous mutated cells.

5. **Monocyte**: A cell of the innate immune system that attacks foreign invaders.

6. **T-cells**: A cell of the adaptive immune system. They respond to specific invaders based on previous encounters.

7. **Influenza**: A respiratory illness that is highly contagious and can most often spread through physical contact or contact with the surrounding air after an individual coughs or sneezes. The most commonly recognized form of influenza is the seasonal influenza.

8. **D₃ 1000 IU**: A vitamin D supplement (Nature Made®, Mission Hills, California).

9. **Necta Sweet Saccharin Sugar Substitute**: A sugar placebo tablet (Necta Sweet, Lincolnshire, IL).

10. **Out-patient Doctor Visit**: A consultation with a primary care or family doctor that does not require admittance to the hospital.

**Limitations**

No known limitations exist at this time.

**Significance of Study**

Influenza continues to be a prominent disease in the modern world and individuals need every possible tool to fight against it. Low vitamin D levels also remain prevalent and could result in a weakened immune system. This study will provide insight and significance to the battle against influenza and influenza-like illnesses and its association to vitamin D. It will
provide individuals education about the importance of vitamin D for maintaining optimal health and preventing and deterring influenza. This study will also provide clinicians and individuals with another option to use when other measures fail or are not effective in restoring the health of the patient. This will allow individuals to remain healthy through an arduous time of year as well as keep their bodies functioning at peak performance throughout this time.
LITERATURE REVIEW

The effects of vitamin D have been studied for many years and a great deal of research on this vitamin has come to light within the past couple of years. While vitamin D serves many functions in the body one of the most important is its function in assisting the immune system. Some of the specific mechanisms vitamin D exerts on the immune system have already been outlined in previous research and can theoretically be used to help explain the role of vitamin D in fighting off disease.

Influenza is one of the diseases that vitamin D helps prevent. Influenza is a widespread problem that reaches throughout every state in the United States. Even though there are vaccinations available for influenza each year the problem still persists due to mutation of the viruses and inability of some individuals to receive the vaccination. It would benefit the public greatly to have other tools to use to help deter influenza. Through its effects on the immune system, vitamin D could be one of those tools.

Vitamin D is an essential vitamin that is needed every day for regular bodily functions to occur. Vitamin D is a fat soluble vitamin that cannot be found in most foods, but is added to others and can be taken as a supplement (National Institutes of Health Office of Dietary Supplements, 2014). The most abundant way vitamin D is produced is by ultraviolet rays striking the skin and causing a photolysis of 7-dehydrocholesterol to previtamin D (Chen, et al., 2007). The previtamin D then undergoes an hydroxylation in the liver to form 25-hydroxyvitamin D [25(OH)D], otherwise known as calcidiol or calcifediol. This is mediated by the cytochrome P450 2R1 [CYP2R1]. One more hydroxylation must occur in the kidneys to form the physiologically active form of vitamin D 1, 25-dihydroxyvitamin D [1,25(OH)₂D], otherwise known as calcitriol. This hydroxylation is mediated by 1α-hydroxylase (CYP27B1). Calcidiol is
the major circulating form of vitamin D and it is bound to a specific plasma carrier protein called vitamin D binding protein (DBP) (Institute of Medicine & Food and Nutrition Board, 2010).

Vitamin D has been found to have many functions in the body. Two of the most important functions that vitamin D possesses are to assist in calcium absorption in the intestine and assist in bone health. When dietary calcium is decreased, increased intestinal calcium absorption is the main mechanism to prevent negative calcium balance. This is accomplished by the parathyroid gland secreting parathyroid hormone (PTH). The rise in serum PTH causes an increased activity of transcription of the cytochrome P450, CYP2R1. This results in an increase in calcidiol and calcitriol synthesis. The calcitriol is then allowed to leave the kidneys and bind to intracellular vitamin D receptors (VDR), which causes activation of vitamin D response elements (VDRE). VDRE are DNA motifs within gene promoters. The most widely-recognized genes that the VDRE attack are genes that are associated with calcium and phosphate uptake in the gastrointestinal tract. The VDRE also attack many genes that are associated with the regulation of bone turnover. These functions of vitamin D are recognized as the classical functions of vitamin D (Hewison, 2012).

The other less prominent functions of vitamin D are known as the non-classical roles of vitamin D. These can include anti-proliferative/anticancer effects, hypertension effects, and most importantly to this review immunomodulation. In these non-classical roles it is believed that the synthesis of calcitriol occurs in a cell-specific manner with the catalytic enzyme that is used to create calcitriol being expressed in many extra-renal tissues. One of the first extra-renal tissues that were discovered that displayed this synthesis was the placenta. In the discussion of vitamin D synthesis, the placenta is a part of a group in the body called barrier sites. These sites feature a
localized synthesis of vitamin D that is most likely not linked to classical vitamin D functions. These sites include the skin, lungs, and colon (Hewison, 2012).

Vitamin D status is determined through measuring the levels of 25(OH)D or calcidiol. This is a good indicator of vitamin D status for two main reasons. The first reason is that calcidiol represents the amount of vitamin D that is taken in by exposure and food intake (National Institutes of Health Office of Dietary Supplements, 2014). The second reason is that calcidiol has a circulating half-life of 15 days, which allows for a great representation of recent vitamin D levels (Jones, 2008). Although calcidiol is a good representative of the vitamin D that is available, there is no evidence that it does a good job of demonstrating the effect of vitamin D on the tissues. Calcitriol is a poor indicator for vitamin D levels because it has a very short half-life of 15 hours and its levels are closely monitored by PTH, calcium, and phosphate (National Institutes of Health Office of Dietary Supplements, 2014).

Vitamin D is measured in the blood as serum of calcidiol, which is displayed in different units. The optimal level for vitamin D levels is between 30 ng/mL and 80 ng/mL. The level of vitamin D that is defined as vitamin D insufficiency is between 20 ng/mL and 30 ng/mL. The level that is defined as vitamin D deficiency is any level that is below 20 ng/mL. These values are not steadfast, but they do provide the general consensus on the proper definition of vitamin D level (Haines & Park, 2012).

The recommended daily allowance (RDA) of vitamin D is mostly dependent on age. The RDA was determined by the Food and Nutrition Board at the Institute of Medicine of The National Academies. It was determined that between 0 and 12 months 400 IU (10 mcg) is sufficient to meet the nutrient requirement. It was also determined that for individuals who are
between the ages of 1 and 70 600 IU (15 mcg) is sufficient to meet the nutrient requirement. Finally, for individuals who are 70 years or older 800 IU (20 mcg) is sufficient to meet the nutrient requirement. These values take into account minimal sun exposure, which can be a major source for vitamin D for some individuals. The minimal amount of vitamin D to ensure nutritional adequacy is the adequate intake (AI) of vitamin D. The AI for individuals between the ages of 0 and 50 years old is 5.0 mcg/d. The AI for individuals between the ages of 51-70 is 10.0 mcg/d. Finally, the AI for individuals over the age of 70 years old is 15 mcg/d (National Institutes of Health Office of Dietary Supplements, 2014).

In order to set these RDA values a determination of the status of vitamin D levels in the general population must be found. This was recently accomplished by the American Institute of Nutrition in a study that estimated calcium and vitamin D levels in US citizens who were at least 1 year old. The researchers from the American Institute of Nutrition determined the average vitamin D levels that were received from dietary sources and also the total amount of vitamin D intake. The information needed to estimate these values was taken from the NHANES 2003-2006 data and the Dietary Reference Intake panel age groupings (Bailey, et al., 2010).

It was determined that for males between the ages of 4 and 18 the average vitamin D received from the diet alone was 6.06 mcg/d. In males between the ages of 19 and 50 the average vitamin D received from the diet alone was 5.25 mcg/d. In males that were 51 years old or older the average vitamin D received from the diet alone was 5.35 mcg/d. In females between the ages of 4 and 18 the average vitamin D received from the diet alone was 4.87 mcg/d. In females between the ages of 19 and 50 years old the average vitamin D received from the diet alone was 4.0 mcg/d. Finally, in females that were 51 years old or older the average vitamin D received from the diet alone was 4.20 mcg/d. The age group that had the highest levels of dietary vitamin
D were 1-3 year olds in both the male and female areas posting levels of 7.2 mcg/d and 6.9 respectively (Bailey, et al., 2010).

The total vitamin D intake was also determined for these age and gender groups. Total vitamin D intake levels combine dietary vitamin D with vitamin D obtained from the sun. In males between the ages of 4 and 18 years old the average total vitamin D intake was 7.9 mcg/d. In males between the ages of 19 and 50 years old the average total vitamin D intake was 7.25 mcg/d. In males that were 51 years old or older the average total vitamin D intake was 9.75 mcg/d. In females between the ages of 4 and 18 years old the average total vitamin D intake was 6.87 mcg/d. In females between the ages of 19 and 50 years old the average total vitamin D intake was 6.75 mcg/d. In females between the ages of 51 years old and older the average total vitamin D intake was 10.05 mcg/d (Bailey, et al., 2010).

These values demonstrate two important points. The first is that all the values for dietary vitamin D intake were either above or within 1.0 mcg/d of the AI of vitamin D. This shows that most individuals can get the minimal amount of vitamin D needed from the diet and sunshine exposure does not play a huge role in achieving that AI value. However, the other important point that must be recognized is that no group reached the RDA value of vitamin D for their total vitamin D intake. This demonstrates that sunshine exposure might be almost necessary to reach that RDA value and vitamin D supplementation could be helpful in reaching the RDA and help to make individuals lead healthier lives.

While sunshine is a fantastic way to obtain the RDA of vitamin D, it is not the only way that one can receive that RDA. Most foods that contain vitamin D are fortified with vitamin D. The only food that is required to be fortified by law is infant formula, but milk is voluntarily
fortified as well as some cereals, yogurt, margarine, and some brands of orange juice. Foods that include vitamin D naturally include flesh of fatty fish and fish liver oils. Other foods that contain minimal amounts of naturally occurring vitamin D are cheese, egg yolk, and beef liver (National Institutes of Health Office of Dietary Supplements, 2014).

The immune system is a complex, multi-faceted gatekeeper that works to prevent, deter, and limit disease and infection. It uses numerous different cell types to destroy invading pathogens and dangerous mutated cells. The immune system can be activated when trouble arises, like an infection, or it can be activated when there is no trouble, which results in different issues such as auto-immune diseases or allergic reactions (National Institute of Allergy and Infectious Diseases, 2013).

The immune system is made up of various organs, tissues, and systems throughout the body. The first line of defense for the immune system is the skin. The skin secretes certain antimicrobial proteins and immune cells that deter microbes from entering the body. Another component of the immune system is the bone marrow. This is the location of the stem cells, which can be used to synthesize many different types of immune cells. The myeloid progenitor stem cells lead to neutrophils, eosinophils, basophils, mast cells, monocytes, dendritic cells, and macrophages. These cells are the first cell types to attack a foreign infiltrator and are commonly referred to as the innate immune system. The lymphoid progenitor stem cells lead to B cells, T cells, and natural killer cells. These cells are commonly referred to as the adaptive immune system because that can perform responses to specific invaders based on previous encounters. These cells are also commonly referred to as lymphocytes because they circulate through the lymphatic system (National Institute of Allergy and Infectious Diseases, 2013).
The lymphatic system is the next major part of the immune system. The lymphatic system is made up of a couple components including lymphoid organs, a network of vessels and tissues, and an extracellular fluid. The lymph nodes are lymphoid organs that act as communication centers for the lymphocytes. A microbe can be brought into the lymph nodes and the lymphocytes can recognize the microbe, replicate, and disperse through the network of vessels to eliminate it. Other components of the immune system include the bloodstream, thymus, spleen, and mucosal tissues (National Institute of Allergy and Infectious Diseases, 2013).

There are two different pathways that can be taken when dealing with an invader to the body. The fastest mechanism that is used is the innate immune system. The cells of the innate immune system are continually circulating through the bloodstream and contain toll-like receptors (TLR) that can recognize dangerous materials such as viruses, bacteria, and fungi. Many of the cells of the innate immune system have specialized functions, but their most important function is their ability to respond quickly (National Institute of Allergy and Infectious Diseases, 2013).

Although the innate immune system can act quickly, it cannot distinguish between specific pathogens. This is where the adaptive immune system must come in. The cells of the adaptive immune system can recognize specific antigens and attack those specific antigens. Usually the antigens are presented to the B and T cells by cells of the innate immune system, which causes the receptors of the B and T cells to recognize those specific antigens. They disperse and eliminate the antigen and once it is eliminated a memory cell is created so that if the antigen presents again, the action of eliminating it can be faster. The function of B cells is to make antibodies, which attack the antigen rendering it useless and harmless. The function of T
There are several ways in which the immune system can be slowed down or disordered. The first way this can occur is through an immune deficiency. This most often occurs when the immune cells are the target of the infection. Allergies are another disorder of the immune system. Allergies occur due to a hypersensitivity to a normally harmless antigen or food. Overproduction of antibodies results in an increased release of inflammatory chemicals. Another disorder of the immune system is autoimmune diseases. These diseases occur because adaptive immune cells recognize normal cells as antigens. Normally, these immune cells would be destroyed, but in these conditions they are allowed to continue unchecked. Autoimmune diseases can affect the whole body or just one organ of the body. One last major contributor to disorder in the immune system is cancer. Three types of cancer affect the immune system cells directly including leukemia, lymphoma, and myeloma (National Institute of Allergy and Infectious Diseases, 2013).

Vitamin D can have various effects on the immune system. The first mechanism that vitamin D employs is its effects on the innate immune system. Vitamin D works on the innate immune system by promoting the activity of the monocyte CYP27B1. Vitamin D is also able to promote immune system strength by causing an expression of the gene cathelicidin (LL-37). This gene encodes for a protein that is involved in causing an increase in intracellular killing of bacteria. This increase in bacterial killing can be caused specifically by increasing the amount of Calcidiol. This was shown in a study where monocytes that were cultured in a medium containing serum from vitamin D insufficient donors resulted in a decreased amount of LL-37 following activation the TLR (Liu, et al., 2006). In another study, serum from vitamin D
insufficient patients induced higher amounts of LL-37 following in vivo supplementation of vitamin D (Adams, et al., 2009).

Another mechanism that vitamin D takes to assist the innate immune system is by the modulation of dendritic cells (DC). DC are antigen presenting cells that work with T-cells to promote tolerogenic effects. DC also express VDR, which most likely works in a paracrine mechanism. When DC mature, an expression of CYP27B1 is produced with a suppression of VDR levels. This suppression of VDR slows down the maturation thus limiting an exaggerated response of DC (Hewison, 2012).

One last mechanism that vitamin D uses to assist the immune system is through the adaptive immune system. Once again vitamin D plays the role of a modulator. It works to keep the balance between type 1 t-helper (Th1) and type 17 t-helper (Th17) cells, and type 2 t-helper (Th2) cells and regulatory T-cells (Treg). Th1 and Th17 cells promote a tolerogenic response and Th2 and Treg cells promote an inflammatory response. When vitamin D levels are normal the Th1 and Th17 cells are favored when vitamin D insufficiency occurs the Th2 and Treg cells are favored (Hewison, 2012). This is another demonstration of the tolerogenic effects of vitamin D on the immune system.

Influenza is one of the most prevalent diseases in the world and can cause death if left untreated. It is a respiratory illness that is highly contagious and can most often spread through physical contact or contact with the surrounding air after an individual coughs or sneezes. The most commonly recognized form of influenza is the seasonal Influenza. This type of influenza is most prevalent during “flu season,” which commonly lasts from October to May (Center for Disease Control and Prevention, 2015).
Influenza can be caused by many types of viruses. The most common viruses that cause seasonal influenza are influenza A and B viruses. Influenza A has many different sub-types associated with it including famous strains such as H1N1, or swine flu, and H5N1, or avian flu. Symptoms from seasonal influenza include fever, cough, sore throat, runny or stuffy nose, muscle or body aches, headaches, and fatigue. There are many complications that can occur from seasonal influenza including pneumonia, bronchitis, and sinus and ear infections. Individuals with congestive heart failure and asthma might also find worsening of their symptoms from those diseases when influenza is contracted (Center for Disease Control and Prevention, 2015).

Any individual can contract seasonal influenza, but there are some groups that are at a higher risk than others. This includes people that are on the edges of the age spectrum. Children that are younger than five years old are at a higher risk of getting seasonal influenza. They are at a higher risk because they display a great amount of hand and face contact. Adults that are 65 years or older are another group that are at a higher risk of contracting seasonal Influenza. They are more susceptible because of decreased immune system function due to old age (Center for Disease Control and Prevention, 2015).

The current prevention technique that is widely used is a yearly influenza vaccine. All individuals over 6 months old are eligible to receive the influenza vaccine. An influenza vaccine can be very successful or not at all successful depending on the year. There are many different strains of seasonal influenza, but the vaccine can only target a small number of those viruses. If an individual contracts a strain of influenza that is not covered by the vaccine, the vaccine will not be effective in protecting the individual. However, by receiving the vaccine it can help to prevent the spread of seasonal influenza thus helping to lower the prevalence in the population (Center for Disease Control and Prevention, 2015).
Even though a vaccine is available to most every person influenza is still a very prevalent disease in the United States. According to the Centers for Disease Control and Prevention (CDC) influenza activity during the 2013-2014 flu season increased in November and peaked in late December and the influenza A H1N1 and pH1N1 viruses predominated. The percentage of outpatient doctor visits for influenza-like illnesses peaked during the last week in December at 4.6% and it stayed above the national baseline of 2% for 15 consecutive weeks of the flu season. Between October 1, 2013 and April 30, 2014 there were 9,635 reported influenza hospitalizations and the cumulative incidence for all age groups for this period was 35.6 per 100,000 of the population. The mortality rate for deaths attributed to pneumonia and influenza were above the epidemic threshold for 8 consecutive weeks and peaked at 8.7% during the 4th week of the year (Centers for Disease Control and Prevention, 2014).

The 2014-2015 flu season has already seen increases in influenza activity. The cumulative hospitalization rate for all age groups currently sits at 51.7 per 100,000 per population. The number of outpatient visits for influenza-like illnesses peaked at 6.0% during the last week in December and has been above the national baseline for 14 consecutive weeks, as of week 7 of 2015. In the estimated spread of influenza prepared by state and territorial epidemiologists there is widespread influenza activity in 20 states including many in the northeast region such as Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Rhode Island, and Vermont. (Centers for Disease Control and Prevention, 2015).

The expansive nature of seasonal influenza is a problem that cannot be fixed by just vaccination alone. The immune system plays a huge part in helping to fight off the viruses of the seasonal flu. As demonstrated earlier, vitamin D plays a role in helping to fight off infection and
viruses and may play a role in helping to defeat influenza viruses also. According to a review article on the effects of vitamin D on influenza vitamin D uses two of the main mechanisms described above to deter seasonal influenza. The first mechanism that it performs is to limit the macrophage release of inflammatory cytokines. Influenza causes such an increase in inflammatory cytokines that it can be overwhelming to the body’s own tissues. By limiting the activity of macrophages, vitamin D allows the body to keep a more tolerogenic environment. The other mechanism that vitamin D uses to assist the immune system is to promote monocytes and the expression of LL-37. This helps destroy the invading viruses and return the body to a healthier state (Cannell, et al., 2006).

While vitamin D can have very beneficial effects when it comes to disease, it was shown above that Americans might not be receiving the RDA they need to help them fight off these diseases. This can be especially true of those who live in areas where there is decreased UV radiation during the winter and influenza seasons. One solution that could help to solve this problem is vitamin D supplementation.

Supplementation can be an effective way to meet the RDA for vitamin D. Supplements of vitamin D usually come in either one of the two most common forms of the vitamin D2 and D3 and are normally taken orally. There is no significant difference between these two forms of vitamin D other than a chemical side chain. D2 and D3 both are able to raise calcidiol serum levels (National Institutes of Health Office of Dietary Supplements, 2014). Commonly vitamin D supplements will provide 1000 IU of vitamin D per serving. Toxicity can occur if an individual intakes an excessive amount of vitamin D, but those levels are not reached until intake is around 10,000-40,000 IU per day.
The effectiveness of Vitamin D supplementation has been studied many times before in relation to a variety of different illnesses. One of these diseases that have been studied in relation to vitamin D supplementation is Tuberculosis. In a study performed by Larcombe et al. it was found that in the Caucasian population that was evaluated there was an increase in TLR induced macrophage responses in the innate immune system (Larcombe, 2012). In another study focusing on the effect of vitamin D supplementation on tuberculosis it was discovered that vitamin D supplementation assisted in the resolution of inflammatory responses by slowing down the antigen-stimulated cytokine responses (Coussens, et al., 2012).

Another major disease that has been investigated greatly in regard to vitamin D supplementation is cancer. In a study evaluating the cancer incidence and mortality rate and vitamin D supplementation it was found that while there was little effect on cancer incidence there was a significant decrease in cancer mortality (Keum & Giovannucci, 2014). Another disease that is of high importance in the United States is diabetes. In another study looking at the effects of vitamin D supplementation on those with diabetes it was discovered that individuals with type two diabetes showed decreases in body mass index (BMI) score, blood glucose levels, and increases in liver health. Liver health was determined by measuring alanine transaminase and blood glucose was determined by measuring hemoglobin A1c (Nwosu & Maranda, 2014).

Overall, vitamin D supplementation has been shown to have beneficial effects on many diseases. One theory for this is at a genomic basis. It was determined in research that looked at vitamin D supplementation between two groups receiving different amounts of vitamin D supplementation that both groups demonstrated increased expression in 66 specific genes. These genes have many functions that include assisting in over 160 pathways that are associated with cancer, autoimmune disorders, and cardiovascular disease (Hossein-nezhad, Spira, & Holick,
2013). This study truly shows that vitamin D plays an important role in the immune system and that through supplementation optimal health effects will result.

Influenza is a disease that still affects a large amount of people. While it can affect individuals at all times during the year, it is largely widespread during flu season between the months of October and May. This is also the time of year that vitamin D can be at its lowest levels due to decreased sun exposure. Vitamin D has been shown to partake in assisting the immune system in fighting off harmful infections and viruses such as influenza. The correlation must then be drawn between low levels of vitamin D and increased rates of influenza. Perhaps one of the most effective and easiest ways to increase vitamin D levels can be to take an oral vitamin D supplement.

Therefore, the purpose of this research study is to determine the effect of vitamin D supplementation on the incidence and rate of influenza. Specifically, this study aims to answer the following research question: Does vitamin D supplementation decrease the rate of out-patient doctor visits due to influenza-like illnesses in the general population? The hypothesis of this study is that vitamin D will significantly decrease the number of out-patient doctor visits due to influenza-like illnesses in the general population. This study is significant as it may demonstrate that vitamin D can be a mechanism that can be used by individuals to protect themselves from contracting influenza.
METHODS

An experimental design will be used in this study. The independent variable is vitamin D supplementation or no supplementation. The dependent variable is the number of out-patient doctor visits due to influenza-like illnesses.

Subjects

Two hundred healthy adults will be recruited for this study. They will be between the ages of 18 and 50 years old and must be at a normal weight or overweight on the BMI scale. Subjects will have no previous history of auto-immune disorders, diabetes, or cancer. The subjects must have a permanent residence within a hundred mile radius of Akron, Ohio for the duration of the study. Subjects must have regular access to a computer or any device that support email. Subjects must be able to maintain a consistent diet that is normal for them. Informed consent to participate in this study will be obtained prior to subject participation. This study will be approved by the Institutional Review Board for Social and Behavioral Sciences at the University of Akron prior to beginning the study.

Instruments

The number of out-patient doctor visits due to influenza-like illnesses will be measured by using self-reported measures. The subjects will use email to report these illnesses. This can be done through any electronic device that can support email.

The vitamin D supplements product that will be used is called D₃ 1000 IU (Nature Made®, Mission Hills, California). They are in solid tablet form and do not contain any color, artificial flavors, preservatives, yeast, or gluten. The tablets do contain calcium carbonate, cellulose gel, Maltodextrin, Croscarmellose Sodium, Stearic Acid, Magnesium Stearate, Corn
Starch, and Gelatin. Each tablet contains 1000 IU of vitamin D, which is equivalent to 25 mcg.
This product has not been evaluated by the Food and Drug Administration.

The placebo tablets that will be used are called Necta Sweet Saccharin Sugar Substitute (Necta Sweet, Lincolnshire, IL). The tablets contain 64.8 mcg with a combination of sodium saccharin, sodium bicarbonate, silicon dioxide, modified cellulose gum, and povidone. The tablets will be cut to resemble the vitamin D supplements.

**Testing Procedures**

After signing the consent form, each subject will be assigned a number and randomly placed into one of two groups. One group will be the experimental group (group 1) and the other will be the control group (group 2). Group 1 will contain one hundred subjects and group 2 will contain one hundred subjects. Each group will receive seven tablets once a week with the instruction to take one each day with a meal. Group 1 will be given the vitamin D supplements and group 2 will be given the placebo tablets. Two groups of researchers will assist in distributing the tablets. The first group of researchers will prepare the tablets to be distributed and the second group of researchers will distribute the tablets without knowing the type of tablets.

The subjects will all be instructed to report any influenza or influenza-like illnesses by emailing the researchers after every out-patient doctor visit that resulted in a diagnosis of influenza or influenza-like illness. The email will contain their subject number and their diagnosis. The email will also have an attached note from the doctor verifying the illness with their signature. If any subjects have any out-patient doctor visits not due to influenza or influenza-like illnesses they will report these through the same procedures, but these reports will
not be included in the statistical analysis. The study will last the duration of “influenza season” from October 1st to April 30th.

The number of out-patient doctor visits for each subject in group 1 will be added together and the number of out-patient doctor visits for each subject in group 2 will be added together. These two values will be used in the statistical analysis. The number of visits for each group will also further be broken down by month and be statistically analyzed.

**Statistical Analysis**

A two-sample t-test will be used to determine the difference in number of out-patient doctor visits for influenza or influenza-like illnesses between subjects taking vitamin D supplements and subjects taking placebo tablets. The number of visits for the duration of the study will be analyzed using the two-sample t-test as well as the number visits broken down by month. The *a priori* alpha level will be set at P<.05.
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