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Study Protocol:

A Pilot Study Evaluating the Bioavailability and Absorption Rates of Two Vitamin B-12 Preparations in Normal Human Subjects.

Preliminary Study
Study #HPI-NF-B12-1
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Sponsor:

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- **Summary:**

The objective of this pre-clinical study is to determine the absorption rate across the buccal mucosa of vitamin B-12 prepared as nanosuspensions through a patented Nanofluidization™ (NF) technique in normal healthy subjects, when given by a spray applicator compared to vitamin B-12 in a tablet form, in normal healthy subjects. Health Plus International has developed a patented nanosuspension process for producing Nanodroplets™ of aqueous and oil based solutions for use in nutraceutical and pharmaceutical delivery systems. This process allows molecules to be embedded into micro and Nanodroplets™ at an average of 0.188 nm and 5.421µm in size, which are used to create stable, uniform and highly soluble emulsions and dispersions. Theoretically, such dispersions should allow molecules to be delivered across tissue barriers at a faster and more even rate than non-processed or "normal" solutions. The purpose of this type of delivery is to introduce vitamins, herbs and minerals into the circulatory system in a manner which allows, over time, rapid, uniform and complete absorption than pills, capsules or liquids which are absorbed through the gastrointestinal tract. Other key advantages are avoiding both contact with the GI tract and hepatic first-pass effects which is nutraceutically more acceptable to many patients. Another object of the study is to provide a patient-friendly mode of delivery to such effective amounts of vitamin B12 analogues without the inconvenience and discomfort of subcutaneous and intramuscular injections.

This study is designed to determine the effectiveness of the spray application of its proprietary and processed vitamin (Vitamin B-12) nanosuspension preparation .Vitamin B-12 has been chosen for this study because it is a commonly used over-the-counter nutritional supplement, both alone and combined with other vitamins in multi-vitamin preparations and is safe when given in supplemental doses. It is also relatively easy to assay in the laboratory, compared to other vitamins or supplements, which should allow consistent data analysis.

Key Words: Bioavailability, absorption rate, stable, uniform and highly soluble emulsions, Vitamin B-12, nutritional supplement.

Introduction:

Vitamin B12, also called cobalamin, is important to good health. It helps maintain healthy nerve cells and red blood cells, and is also needed to make DNA, the genetic material in all cells (1-4). Vitamin B12 is bound to the protein in food. Hydrochloric acid in the stomach releases B12 from protein during digestion. Once released, B12 combines with a substance called intrinsic factor (IF) before it is absorbed into the bloodstream.

Vitamin B12 is naturally found in animal foods including fish, milk and milk products, eggs, meat, and poultry. Fortified breakfast cereals are an excellent source of vitamin B12 and a particularly valuable source for vegetarians (5, 6, 7, 8).

Diets of most adult Americans provide recommended intakes of vitamin B12, but deficiency may still occur as a result of an inability to absorb B12 from food. It can also occur in individuals with dietary patterns that exclude animal or fortified foods (9). As a general rule, most individuals who develop a vitamin B12 deficiency have an underlying stomach or intestinal disorder that limits the absorption of vitamin B12 (10). Sometimes the only symptom of these intestinal disorders is anemia resulting from B12 deficiency.

Characteristic signs of B12 deficiency include fatigue, weakness, nausea, constipation, flatulence (gas), loss of appetite, and weight loss (1, 3, 11). Deficiency also can lead to neurological changes such as numbness and tingling in the hands and feet (7, 12). Additional symptoms of B12 deficiency are difficulty in maintaining balance, depression, confusion, poor memory, and soreness of the mouth or tongue (13). Some of these symptoms can also result from a variety of medical conditions other than vitamin B12 deficiency. It is important to have a physician evaluate these symptoms so that appropriate medical care can be given.

Pernicious anemia is a form of anemia that occurs when there is an absence of intrinsic factor, a substance normally present in the stomach. Vitamin B12 binds with intrinsic factor before it is absorbed and used by your body (7,14,15). An absence of intrinsic factor prevents normal absorption of B12 and results in pernicious anemia.

Individuals with stomach and small intestinal disorders may not absorb enough vitamin B12 from food to maintain healthy body stores (16). Sprue and celiac disease are intestinal disorders caused by intolerance to protein in wheat and wheat products.

Regional enteritis, localized inflammation of the stomach or small intestine, also results in generalized malabsorption of vitamin B12 (7). Excess bacteria in the stomach and small intestine also can decrease vitamin B12 absorption.

Surgical procedures of the gastrointestinal tract such as surgery to remove all or part of the stomach often result in a loss of cells that secrete stomach acid and intrinsic factor (7, 17, 18). Surgical removal of the distal ileum, a section of the intestines, also can result in the inability to absorb B12.

Vitamin B12 must be separated from protein in food before it can bind with intrinsic factor and be absorbed by your body. Bacterial overgrowth in the stomach and/or atrophic gastritis, an inflammation of the stomach, contribute to vitamin B12 deficiency in adults by limiting secretions of stomach acid needed to separate vitamin B12 from protein in food (10, 20-24). Adults 50 years of age and older with these conditions are able to absorb the B12 in fortified foods and dietary supplements. Health care professionals may advise adults over the age of 50 to get their vitamin B12 from a dietary supplement or from foods fortified with vitamin B12 because 10 to 30 percent of older people may be unable to absorb vitamin B12 in food (7, 19).

Vegetarians who do not eat meats, fish, eggs, milk or milk products, or B12 fortified foods consume no vitamin B12 and are at high risk of developing a deficiency of vitamin B12 (9, 25). When adults adopt a vegetarian diet, deficiency symptoms can be slow to appear because it usually takes years to deplete normal body stores of B12. However, severe symptoms of B12 deficiency, most often featuring poor neurological development, can show up quickly in children and breast-fed infants of women who follow a strict vegetarian diet (26).

Fortified cereals are one of the few plant food sources of vitamin B12, and are an important dietary source of B12 for vegetarians who consume no eggs, milk or milk products. Vegetarian adults who do not consume plant foods fortified with vitamin B12 need to consider taking a B12-containing supplement. Vegetarian mothers should consult with a pediatrician regarding appropriate vitamin B12.

Folic acid can correct the anemia that is caused by vitamin B12 deficiency. Unfortunately, folic acid will not correct the underlying B12 deficiency (1, 27, 28). Permanent nerve damage can occur if vitamin B12 deficiency is not treated. Folic acid intake from food and supplements should not exceed 1,000 micrograms (mcg) daily because large amounts of folic acid can hide the damaging effects of vitamin B12 deficiency (7). Adults older than 50 years are advised to consult with their physician about the advisability of taking folic acid without also taking a vitamin B12 supplement..

A deficiency of vitamin B12, folate, or vitamin B6 may increase your blood level of homocysteine, an amino acid normally found in your blood. There is evidence that an elevated blood level of homocysteine is an independent risk factor for heart disease and stroke (29-38). The evidence suggests that high levels of homocysteine may damage coronary arteries (34) or make it easier for blood clotting cells called platelets to clump together and form a clot. However, there is currently no evidence available to suggest that lowering homocysteine level with vitamins will actually reduce your risk of heart disease. Clinical intervention trials are needed to determine whether supplementation with vitamin B12, folic acid, or vitamin B6 can help protect you against developing coronary heart disease.

Vitamin B12 has a very low potential for toxicity.

Study Design:

A. Subject:

A Pilot Study Evaluating the Bioavailability and Absorption Rates of Two Vitamin B-12 Preparations in Normal Human Subjects.

B. Inclusion Criteria

Normal healthy subjects 18 years of age or older, b. Able to understand and sign the informed consent, c. Able to be available for the full study time, d. Normal physical examination findings, e. Non-critical laboratory findings.

C. Exclusion Criteria

Younger than 18, b. Unable or unwilling to sign informed consent, c. Clinically significant illness within 6 months of the study, d. Current throat or mouth disorders, e. Current respiratory allergies, f. Current respiratory infections g. On prescription medications, h. Subjects who have taken supplements of Vitamin B-12 or multivitamins containing Vitamin B-12 within seven days, i. Critical laboratory values, j. Abnormal physical examination, k. Allergic to Vitamin B-12 or any Vitamin B-12 containing vitamin mixture, l. Peptic ulcer, m. History of Pernicious Anemia or other Vitamin B-12 deficiency disorder, n. Pregnant, o. Any other condition, illness or history of illness which; in the principle investigators judgement would exclude the volunteer from the study.

D. Study Preparations

Spray applicator containing processed B-12 (cyanacobolomin) at a concentration of 15000 mcg/dose Test Solution. Other ingredients in the study solutions are citric acid, potassium sorbate, purified water.

E. Visit One-Pre-Enrollment Interview

Volunteers will have the study explained and, if acceptable, sign the informed consent. 2. A history and physical will be obtained and recorded on case report form. 3. Two tubes of blood will be drawn for chemistries, hematocrit, hemoglobin, and cbc. The reference laboratory will be Quest Laboratories. 4. Only volunteers who meet the inclusion and exclusion criteria will be entered into the study. 5. Volunteers will be instructed not to take any form of vitamin preparation containing Vitamin B-12 for 7 days prior to the second visit.

F. Visit Two-Initiation

Consented volunteers who have met the inclusion criteria, including acceptable laboratory values will be entered into the study. 2. All participants will undergo venipuncture for baseline Vitamin B-12 levels.

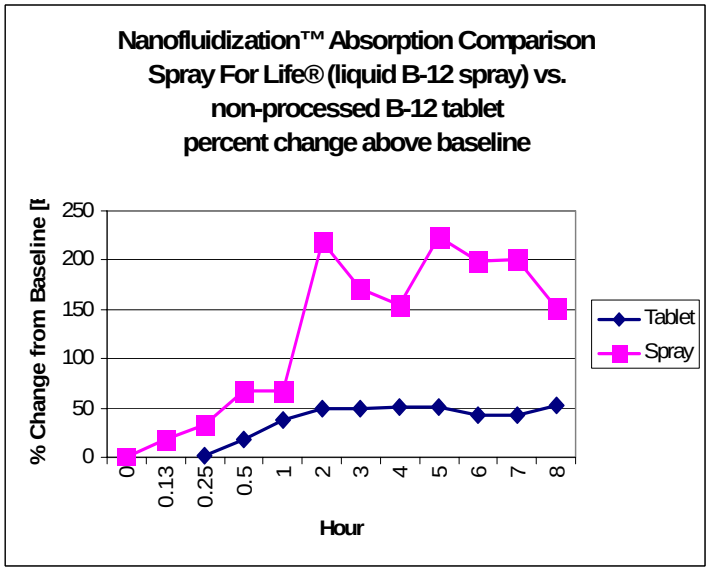
Participants will self-adminster the nano-processed study preparation by applying five complete sprays to the inside of each cheek (buccal mucosa) for a total of 10 sprays. This is accomplished by depressing the trigger of the spray applicator down to a complete stop. Ten sprays deliver a dosage of 15 mg's of Vitamin B-12. The administration of the drug will be supervised and the participants will return the spray applicator to the study coordinator immediately after spraying. 4. Blood (1 tube, approximately 5 ml) will be drawn for Vitamin B-12 levels at the following times after administration. Alternate arms after each draw: 0 minutes, 2 minutes, 5 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 5. Volunteers will be allowed to drink water, non-carbonated beverages other than tea or coffee and to eat snacks after the first hour.

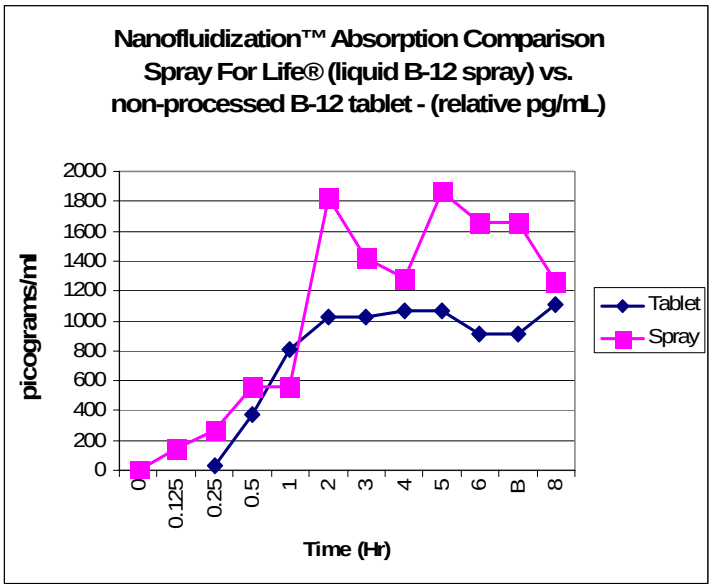
Materials and Methods:

At the first visit, the test subject was administered 30 tablets (x500 mcg per tablet) equivalent to 15 mgs of Vitamin B-12. Approximately 5 ml of blood (SST tubes) were drawn at baseline (pre-dosing), and at 7.5, 15 and 30 minutes, and 1, 2, 3, 4, 5, 6, and 8 hours post-dosing. Samples were then shipped, on ice to a nationally recognized analytical laboratory. Approximately, one week after the first visit, the subject was administered 10 sprays of the NF test solution (for a total dose of 15 mg of Vitamin B-12). The solution was sprayed on to the buccal mucosa (5 sprays on the inside of each cheek). Blood was drawn in the same manner as during the first visit. The blood was assayed using the Access Immunoassay system (Beckman Coulter, Inc.). The assays were performed at Kronos Science Laboratories (Phoenix, AZ). The test subject did not take any vitamin B-12 supplements for one month prior to the study or between the visits and avoided all dairy and meat products.

Results:

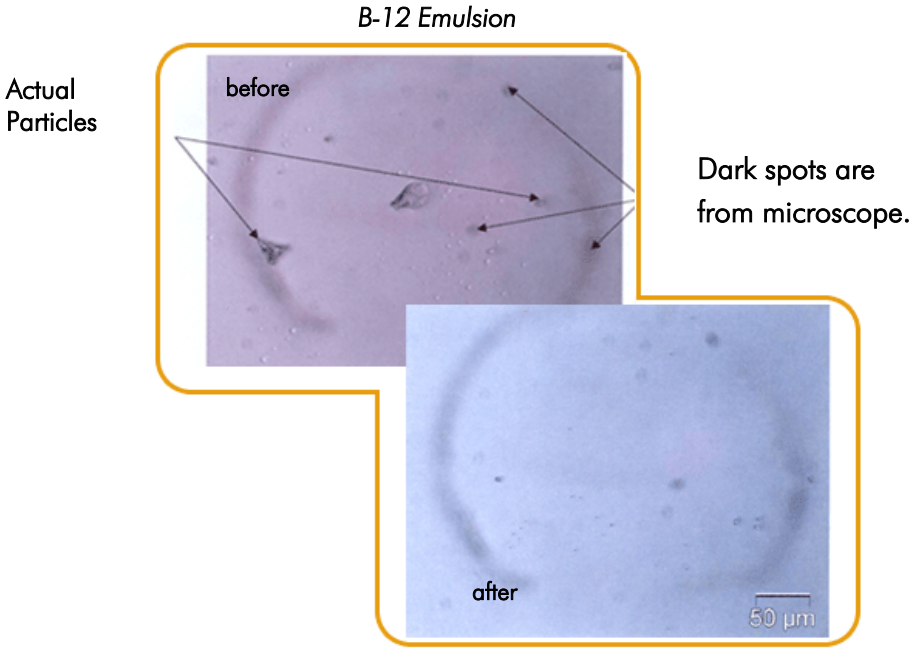
The graphs below shows the plasma concentration curves for vitamin B-12. The results show that the vitamin B-12 was more rapidly absorbed when administered using the NF solution. In addition, the Area-Under-the-Curve (AUC) for the NF solution was 47% greater than that of the tablet. This suggested that vitamin B-12 is more completely absorbed by an individual when administered sublingually using the NF processing technique.





Conclusion:

We believe these results demonstrate that the NF technology has a potentially significant advantage over the conventional dosing form for vitamin B-12 and other nutritional products without swallowing difficulties and digestibility issues. This processing approach would also have a major impact on the delivery of pharmaceuticals as well. Additional clinical studies are required to confirm these findings.



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