GRH WIRB Protocol

Title: 25-hydroxyvitamin D [25(OH)D] serum levels and associated health outcomes in the population resulting from a program of education and testing

Number: GRH D-1

Date: revised June 7, 2018

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Address and telephone of the research sites: This is a population study, not a clinical trial. There is open enrollment throughout the USA and internationally through the web.

Purpose of the study

To raise public awareness about vitamin D through education and vitamin D testing programs and to collect data on compliance and health outcomes.

Specific outcomes that will be tracked will be
Compliance

1. number of respondents to each educational piece/method (lecture, online courses, online materials)
2. number of subjects who take action based on the information from their responses to the questionnaire and the vitamin D test results (with other nutrient and lab test results when applicable)

Health Outcomes

1. relationship between vitamin D intake and serum 25(OH)D concentration
2. relationship between the intake of co-factor nutrients and their corresponding lab test results (for a sub-set of this group)
3. incidence and progression of diseases listed on the questionnaire (with tracking of specific lab markers for study sub-set(s)) as they relate to any of the nutrients studied and their corresponding lab test results
4. relationship between vitamin D intake and/or serum 25(OH)D concentration and that of other studied nutrient co-factors (for study sub-sets)

Study Sub-Sets

Several sub-sets of this study will have a targeted focus on specific health outcomes (such as pre-term birth, Type 1 Diabetes (T1D), and cancer) and/or nutrients thought to be co-factors of vitamin D, (such as omega-3 fatty acids, magnesium and vitamin K). Supplemental education, testing (when applicable), data collection, and analysis of co-factors' test results and intake levels will complement that currently done for vitamin D. Below are two examples of study sub-sets.

1. Omega-3 Fatty Acids

Omega-3s are often found in foods that are also naturally high in vitamin D (such as fatty fish). A number of health conditions related to low vitamin D have also been correlated to low levels of omega-3 fatty acids (1-10). There is very little research available on the combined effect of vitamin D and omega-3 fatty acid intake and resulting blood levels on specific health outcomes. Incorporating omega-3 education and testing (using omega-3 status testing such as the Omega-3 Index test (11) and the ratio of Arachidonic Acid (AA) to DHA/EPA (AA:EPA) (12,13)) into the current vitamin D protocol will help shed light on such a combined/co-nutrient effect. It will also allow us to study levels of omega-3 fatty acids in red blood cells, independent of consumption. As with vitamin D, the effect of intake of omega-3 fatty acids will vary from person to person, as it may take different doses to reduce inflammation if a person has a diet high in AA with a high AA:EPA ratio. he ratio of n-6 (pro-inflammatory) to n-3 (anti-inflammatory) fatty acids for humans has changed over time from 1:1 to approximately 20-30:1 (14,15,16).

2. Type 1 Diabetes (T1D)

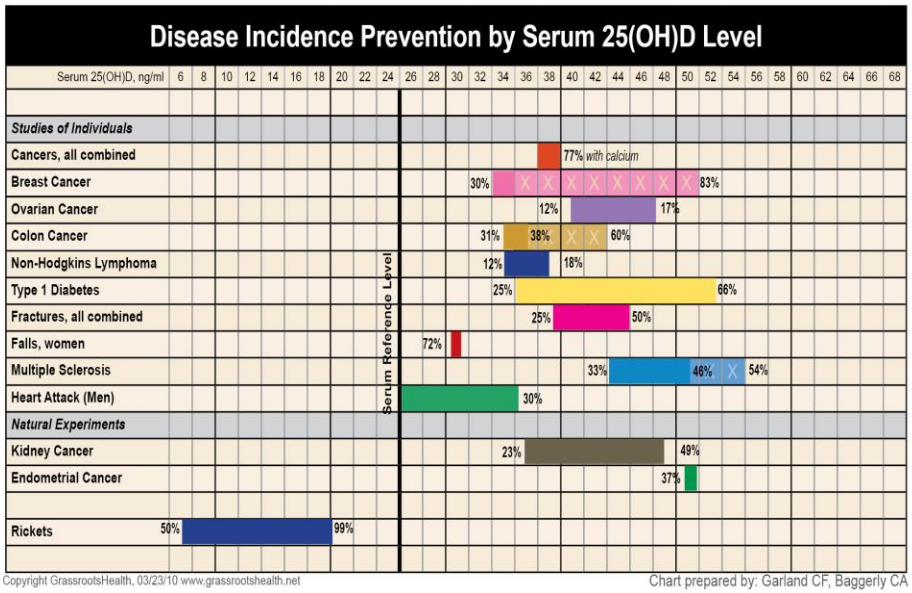
Multiple studies have published the observed link between lower 25(OH)D concentration and the increased incidence and development of T1D (17,18,19). Recently, the Juvenile Diabetes Research Foundation, the American Diabetes Association and the Endocrine Society have recognized that T1D should be diagnosed when a person develops multiple islet autoantibodies (stage 1), possibly irreversibly leading to dysglycemia (stage 2) and symptomatic hyperglycemia (stage 3). However, anecdotal evidence suggests vitamin D and omega-3 fatty acids could possibly reverse or stop this progression. It is known that progression to many forms of diabetes, including T1D, can be predicted by measuring levels of inflammation. Numerous studies show that intake of vitamin D and omega-3 fatty acids reduces inflammation (20-26). In the past, it was common to give Cod Liver Oil (CLO) as a source of vitamin D and omega-3 fatty acids to infants and toddlers, particularly in Scandinavia. Epidemiologic studies show that children given Cod Liver Oil have significantly reduced rates of diagnosis of T1D. (25,27).

This study sub-set will help identify potential changes in the progression towards a T1D diagnosis that may result from nutrient status testing and education, as well as provide additional education about islet autoantibodies, inflammation and anti- and pro-inflammatory foods, and other T1D related information.

Background Material

The causal link between severe vitamin D deficiency and rickets or the bone disease of osteomalacia is overwhelming, while the link between vitamin D insufficiency and osteoporosis with associated decreased muscle strength and increased risk of falls in osteoporotic humans is well documented by evidence-based intervention studies. There are newly appreciated associations between vitamin D insufficiency and many other diseases, which are believed to be linked to the non-calcemic actions of the parent vitamin D and its daughter steroid hormone. Studies indicate that serum levels of 25(OH)D in the range of 30-60 ng/ml (based on the specific disease) may reduce or prevent serious health conditions including all cancers combined (28), breast cancer (29), ovarian cancer (30), colon cancer (31), non-Hodgkin's lymphoma (32), Type 1 diabetes (33), fractures (34), falls (35), multiple sclerosis (36), heart attack (myocardial infarction) (37), kidney cancer (38) and endometrial cancer (39). See Figure 1 below.

FIGURE 1. Disease Incidence Prevention by Serum 25(OH)D Level



A Scientists' Call to Action has been issued by 48 international vitamin D scientists to alert the public to the importance of having vitamin D serum levels between 40 and 60 nanograms/milliliter (100-150 nanomoles/liter) to prevent these diseases. Implementing this level is safe and inexpensive.

Almost all individuals are unaware of their vitamin D status and many may be deficient. Offering testing will allow individuals to become informed of their vitamin D status.

Currently, there is no way to tell a person's serum level without administering a blood test. Even if a person is taking vitamin D supplements, there is a 3 fold variation in the 25(OH)D concentration (40).

Number of Subjects

This is an ongoing population study that will recruit as many people as possible, with an overall goal of over 100,000 individuals. For each specific sub-study, as many individuals will be enrolled as possible, depending on whether inclusion criteria are met. T1D affects 1.3 million people in the U.S. and their expected lifetime medical expenses and income loss due to diabetes total $643 billion (2016). The incidence continues to increase 3-5% annually.

Gender of Subjects

This is a 'self-enrollment' study and based on other such studies, there will be a higher proportion of women than men who choose to enroll. There will be specific initiatives that may attract genders differentially (e.g., there is a group proposed in Arkansas that would attract women over 60 years of age, and other sub-sets that will enroll pregnant women), while other parts of the enrollment will attract both genders equally.

Pregnant women will be included, as the need for adequate vitamin D and omega-3 status is known to be essential for both mother and fetus (22-27). Merewood et al. demonstrated a 50% reduction in caesarians as a result of increasing the pregnant woman's serum concentration from 20 ng/ml to 40 ng/ml (41). Omega-3 (in the form of algal DHA) and vitamin D are commonly prescribed in pregnancy. Algal DHA was recently used in the Nutritional Intervention to Prevent (NIP) T1D pilot study (23). Pregnant women and infants were enrolled using an algal DHA which is commonly used in maternal vitamins, infant formulas and foods for infants and toddlers. DHA and vitamin D have been shown to help prevent post-partum depression and pre-term birth (41,42,43). Positive health outcomes from DHA are numerous and include improvements with Attention Deficit Disorder (44).

Age of Subjects

All ages are appropriate for the study. There are diseases at every stage of life (rickets in infants to falls and fractures in elderly) that are strongly influenced by serum 25(OH)D concentrations (27-38, 45). Consent of the child's guardian will be required for children under 18 years of age.

There are reports that children of all ages are at high risk for vitamin D deficiency and insufficiency (46-48). Children are especially vulnerable to vitamin D deficiency due to the fact that children spend more time indoors now and often wear sun protection outdoors that limits their ability to make vitamin D in their skin. Studies also report that children and adolescents are also drinking less vitamin D-fortified milk (49-56).

Recent published clinical guidelines for vitamin D deficiency established by the Endocrine Society recommend serum level screening with 25(OH)D testing for all at risk individuals, which includes infants and children of all ages (57).

In young children who have little mineral in their skeleton, vitamin D deficiency results in a variety of skeletal deformities classically known as rickets (58). Vitamin D deficiency also causes muscle weakness and studies found that affected children have difficulty standing and walking (59,60) Studies of children with preventive serum levels, obtained with vitamin D supplementation, have found that negative health outcomes can be reduced or eliminated. One study in Finland found that infants who received at least 2000 IU/d of vitamin D during the first year of life reduced their risk of developing T1D in the ensuing 31 yr by 88% (32). The average age of T1D diagnosis is 11 years old, but autoantibodies may be present several years prior to diagnosis. Another study examined the relationship of vitamin D status and asthma exacerbations. The researchers found that insufficient vitamin D status was associated with higher odds of any hospitalization or emergency department visit and concluded that vitamin D status might play a role in preventing asthma exacerbations (61).

The clinical guidelines established by the Endocrine Society, along with the emerging data on health outcomes associated with vitamin D, support vitamin D testing and repletion for children of all ages and support inclusion of children in the current study.

US Census data 2010

> 0-14 years: 20.2% (male 31,639,127/female 30,305,704)
> 15–64 years: 67% (male 102,665,043/female 103,129,321)
> 65 years and over: 12.8% (male 16,901,232/female 22,571,696) (2010 est.)
> *The jurisdiction of the study is the entire US. The age of majority is 18 years in general in the US. NO restrictions are planned regarding exclusion of minors*

Racial and Ethnic Origin

Persons of all ethnicities are able to enroll. The US distribution is shown below and the enrollment is expected to reflect the US population.

US Census Data 2010

White alone	72.4%
Black or African American alone	12.6%
Some other race alone (Mestizo, Mulatto...)	6.2%
Asian alone	4.8%
Two or more races	2.9%
American Indian or Alaska Native alone	0.9%
Native Hawaiian or other Pacific Islander alone	0.2%
Total	**100.0%**

Inclusion Criteria

Anyone of any age or ethnicity is able to enroll in the study for vitamin D testing and education. There will be some targeted groups and study sub-sets where recruitment will be more intensive, such as women over 60 and those at risk of T1D. Individuals will be enrolled into specific sub-

studies depending on their qualifications. For example, the T1D sub-study will recruit as many people as possible who are at risk of developing T1D, identified as having tested positive for one or more of the known islet autoantibodies for T1D.

Exclusion Criteria

No one will be excluded except the 'vulnerable subjects' below other than children and pregnant women. Children and pregnant women will be included.

Vulnerable Subjects

The study will only be enrolling individuals who are of appropriate cognitive capability to consent to the testing process and are able to answer the health related questions. The study will not be enrolling any of the listed vulnerable populations except children (see rational for including children above with parental consent) and pregnant women.

Methods and Procedures

The specific aims of the project are to provide education and testing of serum 25(OH)D concentrations, with or without education and testing of specific co-nutrients. This is a population study (not a clinical trial) which is aimed at providing education about vitamin D and serum 25(OH)D testing to individuals so that they can decide what actions to take. It is a study to help transfer the findings of vitamin D research into public health practice.

Recruitment and Enrollment

Individuals will be recruited through outreach and educational efforts at churches, community centers, libraries, social service agencies, the GrassrootsHealth website and through referrals made by service providers. For the T1D study sub-set, individuals will be solicited to participate if they are already enrolled in one of the T1D screening studies and may be solicited at health exhibits and other places.

People will be enrolled when they logon to the website and create their enrollment and enter data on a health questionnaire. They will then receive a 25(OH)D test (with a co-nutrient test if they opt for that), generally in the mail, or from a GrassrootsHealth approved provider.

The vitamin D test, Omega-3 Index test, and any additional tests available by blood spot are sent by mail and require that the participant use a self-loaded lancet to prick a finger tip to get blood to drop on a card. Enclosed in the kit are an alcohol swab, gauze, band aid, the bloodspot card(s), a return envelope and instructions.

After the participant has dropped their blood onto the sample card(s), they are directed to let it dry (for 30 minutes or more) and mail the card(s) in the sealed envelope to GrassrootsHealth where the information is processed for shipment to the laboratory for analysis.

The laboratory sends the lab results electronically via secure password and encrypted electronic link to GrassrootsHealth. Access is through an https site with additional password security.

There are minimal expected hazards to doing the blood spot test. Hazards will be minimized by the use of the instructions to wash hands and swab the area where the blood spot will be obtained with an alcohol swab. These are routine procedures in common use by millions of individuals who obtain blood for testing glucose, often several times/day. Due to the widespread use of this procedure, no complications are anticipated based on existing experience.

The participant will receive the results of their test via the internet at their own account with a secure password. In addition to their test results, they are provided with data on vitamin D such as appropriate information regarding possible strategies for improving vitamin D status.

At regular intervals (generally every 6 months) for a total of up to five years (or longer if the participant chooses to remain in the study beyond the first 5 years), the participant will be notified to update the health survey and will receive another test and subsequent educational information. Different testing intervals and study duration may be requested or required for certain study sub-sets, specifically the T1D and pregnancy sub-sets. All participants will be informed of the intended duration of the study.

Similar education, reporting, and testing procedures will be followed for each of the study sub-sets.

Data Analysis and Data Monitoring

A statistical analysis of electronic file data will include vitamin D intake, serum 25(OH)D concentration, demographic and biometric characteristics and various health and illness outcome indicators. An evaluation of associations, if any, of health status will be made with different 25(OH)D concentrations. Additional analysis will be done for cofactor intake and measurement when available.

Information is provided with each set of test results indicating to the participant what the recommended levels are, and, if the participant's 25(OH)D concentration is 200 ng/ml or higher, they will be advised that this is an area where toxicity may be observed and will be referred to their personal physician (62).

DHA and EPA are found in foods and do not have an upper level for toxicity. However, EPA can have a blood thinning effect at very high levels. DHA is available in an algal form for those who do not desire to use the DHA and EPA combination. Pregnant mothers and infants use DHA rather than a DHA/EPA blend because the algal blend is not murine, and so there is no potential risk of mercury to the pregnant mother or infant (23).

Data Storage and Confidentiality

The accumulated data will be stored in a secure, encrypted database operating behind a firewall, and password protected. The only person that will have access to the identified data will be our primary data biostatistician. This will be for purposes of accessing hospital records as indicated and released by participants. The Principal Investigator and other researchers will access the data in a de-identified form. For all analyses only an arbitrary number will identify the participant. It will not include name, social security number, date of birth, street address or other personally identifying information.

In order to protect participant's personal information, trial results, and privacy to the best of our ability, GrassrootsHealth has implemented a three-step security methodology: isolation, encryption, and disconnection.

Isolation: GRH databases are maintained on private virtual servers with state of the art network firewalls, physical security, and administrative safeguards to limit access and to avoid the cross contamination, side door access, or targeted attacks that may occur in a shared server environment.

Encryption: Participant identification information is encrypted using the AES (Advanced Encryption Standard); a level that is recognized and required by many governmental institutions to protect the loss, misuse and alteration of the information under our control.

Disconnection: Participant information and trial results are stored in separate databases linked via a double blind series of encrypted locks preventing any stored data record from containing links that could directly connect participant and trial data.

A list of data elements seen by the customer service function vs that seen by the analyst is attached.

Transition from Research Participation

No medications are being given. Therefore no transition is necessary when individuals leave the study. Individuals will be advised to contact their personal physician for future 25(OH)D testing.

Risk/Benefit Assessment

There is minimal risk to obtaining blood drops for 25(OH)D and other blood spot testing. The test generally will be done in the participant's home with the participant using a sterile self-loaded lancet to puncture the fingertip. This will be done after a thorough washing of hands with soap and warm water and the use of an alcohol swab where the blood drops will be obtained. We are aware of no reports of harm other than short term pain with using the test. Participants are advised that if any complications develop they should consult their own healthcare provider.

A small subset of participants may have their 25(OH)D test done under the direct care and supervision of their health care provider.

Potential Risk

The potential risks of taking the test are extremely minimal.

If a person chooses to use vitamin D supplements to raise their vitamin D levels, the IOM has indicated that 10,000 IU/day is currently considered the 'no observed adverse event level (63). A study by Hathcock et. al, indicated that potential toxicity (hypercalcemia) may occur if the serum level reaches 200 ng/ml (62). The participants will be informed of the potential toxicity and of the 'no observed adverse event level'. Based on the study in Anticancer Research, 2011, by Garland et al., the dose-response curve shows that no individuals in a population of over 3500 participants reached 200 ng/ml while taking less than 40,000 IU/day (39).

Participants will be advised that a serum 25(OH)D concentration that is considered too high may be reversed by stopping taking vitamin D supplements until the level is lowered. They will also be advised to work with their personal physician.

It should be noted that vitamin D levels with full-sun exposure for a summer (working outdoors) provide individuals with levels in the 40-60 ng/ml range (64).

Should participants in the omega-3 sub-set choose to raise their Omega-3 Index or other omega-3 measure through supplementation or diet, the American Heart Association has indicated that up to 3000 mg of supplemental fish oils per day is generally considered safe for most adults along with a general recommendation that all adults eat fish (particularly fatty fish) at least two times per week (65,66). The European Food Safety Authority has concluded that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for the adult population (67).

To date, there have been no documented harmful effects associated with a high Omega-3 Index, yet no known benefits or risks to having an Omega-3 Index above 12.

The only other risk is that of data privacy. That is addressed in the Data Storage and Confidentiality Section.

Protection Against Risks

Participants will be advised in writing to wash their hands fully with soap and water and swab with alcohol prior to testing in order to minimize infection.

Subjects will be notified of their serum results within two weeks of testing. If subjects' 25(OH)D serum levels are found to be above the 100 ng/ml range, researchers will contact the subject and advise them that the observed toxicity levels start at 200 ng/ml (500 nmol/L) per J. Hathcock, Am J. Clin Nutr. 2007;85:6-18 (62), and they may wish to consult with their health care provider to discuss their results. We do not currently have any data to support any additional health benefit of having a level of 100 ng/ml or above.

Potential Benefits to the Subjects

Education about 25(OH)D and testing of their own vitamin D levels will be helpful in providing information participants need to take personal action for their own health.

By using the education provided about vitamin D's influence on so many health outcomes (and those of vitamin D's co-factors), the benefits to the individual are expected to occur for several health outcomes, especially for those in the study sub-sets. As noted earlier, vitamin D status affects health outcomes from osteopenia to falls to cancer prevention and reduction in myocardial infarctions.

Alternatives to Participation

Participation in this study is voluntary. Subjects are free to decline or withdraw participation at any time. Subjects can use the free educational pieces on the website without participating in the study. They may also be able to request their own physician to order the 25(OH)D test for them.

Method of Subject Identification and Recruitment

Recruitment will occur via the internet and by various outreach and educational programs at local public venues such as health fairs, community centers, libraries, churches, health centers, and social service agencies. It will also occur through referrals from service providers. The study has no patients and therefore there is no opportunity for coercion to participate. The study will not attempt to enroll employees in the 25(OH)D testing process. No students are involved in this project but, if students are included in the future, they will not be required to participate in the 25(OH)D testing or other aspects of the study.

Process of Consent

Informed consent will be obtained through an interactive web site. The individual will be informed of everything that participation involves including the length and health content of the questionnaire. The individual may print out the questionnaire and review it in their own time. No enrollment is done until the participant checks a clearly marked 'check box' that they have given their consent to participate.

Please refer to the Waiver for the Documentation of Consent that is attached to this submission.

Subject Capacity N/A

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