Can mineral content in diet influence mineralization in Pseudoxanthoma Elasticum (PXE)?

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Abstract

Pseudoxanthoma Elasticum (PXE) is a rare genetic disorder with no effective treatment. The usual signs and symptoms of the disease are skin lesions, vision loss, and/or cardiovascular complications as a result of mineralization. Environment and lifestyle, including diet, influence health; this report examines potential effects of mineral content (calcium, vitamin K, magnesium, or zinc plus antioxidants) in diet on the signs and symptoms of PXE in animal models and a few human studies between 1984 to 2016.

Calcium:
A paper published long ago wrongly asserted that reducing calcium intake in people affected by PXE lessened the severity of PXE. Abcc6−/− mouse models (mice that have been given the same genetic mutations as people with PXE) show no correlation between calcium intake and the severity of the condition.

Vitamin K:
No correlation has been found between vitamin K intake and mineralization in mouse models. However, in zebrafish an increase of vitamin K has completely eliminated mineralization on the skeleton.

Magnesium:
Diets with high amounts of magnesium result in a reduction or elimination of mineralization in Abcc6−/− mice and a decrease in magnesium has the opposite effect. A clinical trial was completed in 2015 examining the effects of magnesium content in people affected by PXE. The results are not yet published.

Other minerals:
Similarities between Age-Related Macular Degeneration (AMD) and PXE related vision loss might offer some insight into PXE. AMD progression slows with an increase of zinc plus antioxidants. Clinical trials and Abcc6−/− mouse models suggest mineral content in diet modifies PXE symptoms, however, there are no conclusive results.
Introduction

Pseudoxanthoma Elasticum (PXE) is a rare heritable disorder affecting as many as 1 in 25,000 people. PXE is caused by a mutation in the ABCC6 gene located on chromosome 16; more than 300 mutations are known. Even when people have the same mutation, their clinical signs and symptoms are often different. Common signs of PXE include [1] mineralization of the middle layer of the skin, leading to bumps and loose skin, [2] deterioration of the membrane beneath the retinal Bruch’s membrane from mineralizing tissues lead to loss of central vision, and [3] obstruction of small and medium sized arteries resulting in cardiovascular complications such as claudication or cramping in calves or early manifestations of heart disease. PXE patients demonstrate a variety of symptoms and severity all caused by mineralization.

The different signs symptoms from person to person suggests other factors contribute to PXE manifestations, including environment, lifestyle, or diet. Mouse models, zebrafish, PXE clinical trials, and Age-Related Macular Degeneration (AMD) clinical trials suggests mineral content can modify the severity of PXE. This report examines research publications on calcium, vitamin K, magnesium, and zinc plus antioxidants on the severity of PXE symptoms.

PXE advancements have been made through knock out mouse models. These Abcc6−/− mice replicate genetic and structural features of humans with PXE. The mice demonstrate mineralization in connective tissues of the skin, eyes, and arterial blood vessels, as seen in people affected by PXE. Unlike humans, mice have a biomarker in the area around their whiskers that show at 5-6 weeks of age, reflecting inactive Abcc6. The degree of mineralization at this location and in the blood vessels can be measured quantitatively. This data reflects how influential a factor is, such as mineral content in diet. Humans do not have a clear biomarker to measure influential factors, which challenges PXE research.

Studies on AMD give insight to PXE related vision loss. AMD is the leading cause of blindness among people over the age of 50 and is similar to PXE. Drusens are deposits formed between two areas of the eye as AMD advances. These areas are the retinal pigmented epithelium (RPE) and Bruch’s Membrane. PXE related vision loss is due to cracks in Bruch’s membrane, known as angioid streaks. Central vision loss in AMD and PXE appears blurry, distorted, or dark and may worsen over time due to mineral build up. Many studies have been conducted to better understand the cause and progression of AMD because it’s a common eye condition. These studies provide insight on what’s happening with people’s vision who are affected by PXE.
Methods

Consumption of calcium, vitamin K, magnesium, and zinc plus antioxidants all play a role in the human body. This report focuses on published research from 1984-2016 on these four minerals to determine the effects on people with PXE.

The studies reviewed are electronic, published papers found on PubMed. Other information on mineral mechanisms was discovered in a video lecture on bone mineralization or the National Institute of Health’s website.

Results

Calcium

Major functions of calcium include cell signaling and binding with another substance called phosphate to form minerals needed in bone development. People affected by PXE lack a substance, called inorganic pyrophosphate (PPi), needed to stop a molecular pathway. As a result, an excess calcium and PPI will combine and form hard tissue in places it doesn’t belong. Calcium and PPI mainly deposit in the skin, retina, and cardiovascular tissues.

A 1984 study evaluated 32 people affected by PXE and the amount of calcium they ate or drank during different stages of life. The evaluation was set up in two parts. One, a survey to estimate the amount of calcium and phosphate intake, through the consumption of milk and other dairy products, during childhood, adolescents, and adult life. Second, a physical examination scored the severity of each person’s PXE-related symptoms. In this group, people who reportedly consumed less calcium throughout their life also received a lower severity score of their signs and symptoms. An analysis concluded the more calcium consumed by a person affected with PXE, the worse their symptoms would be. This publication wrongfully advised children and adolescents to consume less calcium immediately after diagnoses of PXE.

In 2009 a high calcium diet was fed to mice with PXE to obtain more information on the different opinions regarding calcium intake and PXE. After Abcc6⁻/⁻ mice were given high calcium diets, the severity of PXE was measured by the amount of hardened tissues of blood vessels. Even after increasing the amount of calcium four times for one year, only a small increase of calcium concentrations was found in the blood. This study concluded, calcium did not affect the change in severity or speed of mineralization.
**Vitamin K**

People affected by PXE have low concentrations of vitamin K. Once vitamin K is consumed, typically through leafy green vegetables, the digestive system sends it to the liver. The liver is the location where the gene ABCC6 is mainly expressed. Researchers asked if the absence of ABCC6 in humans prevent the liver from providing sufficient vitamin K to other parts of the body. The lack of vitamin K suggests vitamin K may be the anti-mineralization factor that is lacking in people affected by PXE.

“Factor X” was an unknown substance that is pumped out of the cell by the Abcc6 protein. Vitamin K was a candidate for “factor X”, known as the PXE-K hypothesis, until a form of energy, called ATP, was discovered as the mystery substance in 2012. To test the PXE-K hypothesis, in 2010 mice with signs of PXE and normal mice were fed either a diet with vitamin K doses 100 times higher than a normal rodent diet or a minimum dose for three months. Abcc6\(^{-/-}\) mice fed high quantities experienced high levels of vitamin K in their liver cells, but the diet did not stop the mineralization. No study provides evidence for the PXE-K hypothesis.

In 2011, another mouse model was used to show vitamin K’s role in PXE. After four weeks of being fed 440 times the normal amount of vitamin K, mice showed no difference in the degree of mineralization. This study reported changes of vitamin K levels may not reflect the role of vitamin K and instead serve as a marker of the ongoing mineralization process.

A 2015 zebrafish model administered vitamin K intake with hyper-mineralization expression in the skeleton. This study found vitamin K completely counteracted mineralization in Abcc6\(^{-/-}\) zebrafish. This research proposed increasing vitamin K can prevent mineralization in zebrafish. These results contrast with the results seen in Abcc6\(^{-/-}\) mouse models.

**Magnesium**

Magnesium is an essential nutrient used for energy, muscles, nerves, and bones. Half the human body’s magnesium is found in bone and used for growth and maintenance of bone cells. Studies suggest an increase in magnesium content prevents mineralization, however, little is understood on magnesium’s role in mineralization.

In 2008 a mouse model study was conducted based on the suggestion that magnesium quantity influences mineralization. Abcc6\(^{-/-}\) mice on the standard diet developed tissue mineralization at five weeks old, compared to increasing magnesium by five times that completely prevented mineralization in
Abcc6<sup>−/−</sup> mice for the first twelve weeks of age. Complete prevention of ectopic mineralization after a high magnesium diet suggests magnesium is not influenced by other factors impacting mineralization.

Increasing magnesium by five times was included in the diet when the Abcc6<sup>−/−</sup> mice began to show signs of mineralization at 14 days old. This study was to determine if the diet can reverse effects of lacking the Abcc6 gene. High magnesium concentration did not noticeably reverse mineralization.

In 2012 another magnesium diet experiment was conducted. Abcc6<sup>−/−</sup> mice were fed either a regular rodent diet or an accelerated diet consisting of a 200% increase of phosphate and a 20% reduction of magnesium. After two months on the accelerated diet, Abcc6<sup>−/−</sup> mice showed a advancement of mineralization by 40 times. Reducing magnesium lead to an increase of mineralization severity in Abcc6<sup>−/−</sup> mice.

In 2015, the same accelerated diet was fed to Abcc6<sup>−/−</sup> pregnant mice to determine the effect of magnesium and phosphate content in the maternal diet on Abcc6<sup>−/−</sup> offspring. The offspring demonstrated mineralization at just four weeks of age compared to those who showed no mineralization until five or six weeks of age. Magnesium content of the maternal diet influences the severity of mineralization in the Abcc6<sup>−/−</sup> offspring.

From 2012-2015, a clinical study consisted of magnesium supplements to treat PXE. Mark Lebwohl at the Icahn School of Medicine at Mount Sinai conducted this trial. The goal was to evaluate how effective increasing magnesium is on reversing mineralization in the skin of people with PXE. During the first 12 months, 40 people with PXE were given a placebo, and 40 people with PXE were given 1000mg of magnesium daily. During the second 12 months, all participants were given 100mg of magnesium daily. This study was completed in March 2015; no results have been posted yet.

**Zinc and antioxidants**

In 2001, an Age-Related Eye Disease Study (AREDS) began. In this study, zinc supplements were assessed under the suggestions that elderly people with zinc deficiency have worsening AMD. The trial evaluated Age-Related Macular Degeneration (AMD) and visual acuity after the intake of a combination of antioxidants including:

- 500mg of vitamin C (five times the intake of the general population)
- 400-IU dose of vitamin E (13 times the recommended dietary allowance)
- 15mg of beta carotene
- 80mg of zinc (five times the recommended dietary allowance)
About 67% of participants chose to take Centrum, which increased daily intake of vitamin C, vitamin E, beta carotene, and zinc to approximately 100% of the recommended daily allowance. To measure regression or progression towards advanced AMD, the people were identified in one of four categories: category 1 is essentially free of AMD, category 2 is mild or borderline AMD in one or both eyes, category 3 requires an absence of advanced AMD in both eyes and one eye with visual acuity of 20/32 or better, and category 4 is one eye with advanced AMD or visual acuity less than 20/32.

The results were measured by photograph assessment and visual acuity loss over time. Participants who were randomly assigned to take only zinc, reduced risk of progression to advanced AMD by 21%. Those assigned to take only antioxidants reduced risk of progressing to advanced AMD by 17%. People assigned to take a combination of zinc plus antioxidants showed a reduction in visual acuity loss by 25%. These results were determined after being compared to the results of people assigned a placebo for the duration of the trial.

A follow up evaluation in 2011 of participants from this clinical trial determined 44% of participants in categories 3 and 4 of AMD randomly assigned to the placebo, had progressed to advanced stages of AMD. Of participants in categories 3 and 4 assigned to take zinc plus antioxidants, only 34% progressed to advanced AMD. The AREDS formula remains the preferred treatment for people with AMD categories 3 or 4 in at least one eye.

Discussion

Abcc6−/− mice replicate the genetic and structural features of humans with PXE. Comparing the biomarker of Abcc6−/− mice fed a different diet shows evidence of the effects of mineral and nutrient content on mineralization. The mouse models examined here suggest alterations of mineral content of vitamin K, magnesium, or zinc plus antioxidants influences ectopic mineralization in mice. However, differences between mouse models and humans make it difficult to compare the processes. Short term experiments with Abcc6−/− mice a reliable biomarker to determine the severity of mineralization. No reliable biomarkers exist to mineralization severity during the short term of clinical trials for people with PXE. Metabolism and side effects of Abcc6−/− mice likely differs from humans, such as those seen in vitamin K content in mice versus zebrafish. This complicates the understanding of mouse models and humans.

The 1984 clinical trial counseled PXE patients “to avoid indulging in foods and liquids of high calcium content”, with little discussion of potential negative side effects. Weaknesses in this study include the small sample size of 32 people affected by PXE and inaccurate survey results. The survey
required participants to recall calcium intake from as many as five or six decades earlier. A more reliable study would follow a larger group of people affected by PXE through childhood and adulthood to measure calcium intake to compare mineralization severity. The 2009 Abcc6−/− mouse model, concluded there is no correlation between reducing or increasing calcium intake on the severity of vascular calcification in Abcc6−/− mice.

Although people with PXE tend to have reduced levels of vitamin K, the 2010 and 2011 Abcc6−/− mouse models show no correlation between vitamin K intake and PXE manifestations. Alternatively, zebrafish model demonstrated an increase of vitamin K completed eliminated mineralization in the axial skeleton. Differences in zebrafish, mice, and humans complicate the comparison of vitamin K function in mineralization.

A 2008 Abcc6−/− mouse model demonstrated an increase of magnesium prevents but does not reverse mineralization in Abcc6−/− mice. In 2012, researchers found increasing phosphorus and decreasing magnesium content lead to increased severity of mineralization for both newborn Abcc6−/− mice and Abcc6−/− offspring of pregnant mothers fed this diet. A clinical trial using magnesium supplements to treat PXE was completed in March 2015. The results have not been published yet.

The results of the zinc plus antioxidant supplement 2001 clinical trial and revisited 2011 survey, appear to benefit AMD vision loss in categories 3 and 4. This research on AMD may provide insight on PXE related vision loss. However, differences between AMD and PXE complicates the direct effectiveness of zinc plus antioxidants to benefit people affected by PXE who experience vision loss. No study has been published to determine how zinc plus antioxidants affect vision loss in PXE patients.