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CALCIUM AND VITAMIN D INTAKE IN ADULTS WITH SICKLE CELL ANEMIA

by

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A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

Major: Clinical Nutrition

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## **ABSTRACT**

**Burgmon, Taylor Marie. M.S. The University of Memphis. 12/2012. Calcium and Vitamin D Intake in Adults with Sickle Cell Anemia. Major Professor: Dr. Ruth Williams.**

Sickle cell anemia (SCA) is a genetic blood disorder that affects the transportation of oxygen through the blood. It causes episodes of pain, which may affect dietary intake. The purpose of this study was to look at the intake of calcium and vitamin D in adults with SCA, compared to the Recommended Dietary Allowance (RDA) for these nutrients. This was a retrospective study of 12 patients with SCA using food diaries collected from a parent study. There were 6 males and 6 females included and their ages ranged from 21 to 52 years (median=31.75 years). A one-sample t-test was performed and it was determined that the mean intake of both calcium and vitamin D were significantly less than the RDA. Calcium and vitamin D are important nutrients and patients with SCA may need education on good sources of these nutrients or supplementation because of their low intake.

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# CHAPTER I

## REVIEW OF LITERATURE

### Introduction

Hemoglobin disorders, such as sickle cell disease (SCD) have an effect on the transportation of oxygen throughout the body (1). These disorders are genetic and according to the World Health Organization (WHO), traits for hemoglobin disorders are carried by approximately 5% of the world's population. Sickle cell disease is a genetic disease caused by a substitution of the amino acid glutamic acid for valine on the beta-chain of hemoglobin (1). It is a disease of the blood in which the red blood cells (RBC) to become deformed (sickle shaped) and due to this the RBCs have difficulty passing through the blood vessels. Sickle beta<sup>0</sup> thalassemia (SB<sup>0</sup> Thal) and sickle cell anemia (SCA) are the most serious forms of SCD. This disease results when two people who have the SC trait have a child; there is a 25% chance with each pregnancy that a child will be born with some type of SCD. Beta thalassemia is caused by a decrease in the amount of hemoglobin that is produced by the RBCs. When patients have SCA or SB<sup>0</sup> Thal, they are unable to produce healthy red blood cells (RBC). For example a healthy RBC lives for approximately 120 days, while sickled RBCs only last for 10 to 20 days. This inability to have a sufficient supply of RBCs at all times is what leads to anemia (1). In patients with SCA, sickle hemoglobin (HbS) is formed instead of normal hemoglobin (HbA). This change causes RBCs to become misshapen, leading to an inability to pass through blood vessels. This inability of RBCs to travel through blood vessels causes a blockage or occlusion,

which leads to patients experiencing severe pain and discomfort (1,2). Sickle cell disease can also lead to other complications such as, leg ulcers, stroke, organ dysfunction, and general poor nutrition (3). Sickle cell anemia affects millions of people around the world and approximately 72,000 people in the United States. This disease is more prevalent in people of African-American descent and is seen in 1 in every 500 births of African-American children and in 1 in every 1000 to 1400 births of Hispanic American children (4).

Patients who are affected by this disorder often have decreased appetites, especially during pain crises, which can lead to nutrient deficiencies. Additionally these patients have other nutrition problems. A study done by Hyacinth, Gee, and Hibbert demonstrated that nutritional intervention could lead to improvements in growth in children who have SCA (3). SCA has been shown to cause both macronutrient (energy and protein) and micronutrient deficiencies (vitamins and minerals) in children (3). Micronutrient deficiencies such as iron, zinc, copper, calcium, vitamin D and vitamin E have been studied often in children because of their importance in growth and the immune system (3,5,6,7,8). The deficiencies that are often found in patients with SCA may be due to decreased appetite, malabsorption, or increased catabolism (3). Pell and colleagues looked at the association between the pain level of patients with SCA and their dietary intake (9). They found that 87% of patients reported eating less during a pain crisis; 13% said there was no change during pain, and none of them said they had an increased dietary intake during a pain episode. Their study also found that the adult patients were more likely to eat less fat and

protein during a pain crisis, but their intake of sugary and salty foods did not decrease (9). This is probably due to the fact that during episodes of pain, patients may not feel like eating meals, but instead relied on snacks, which primarily consisted of sugary and salty foods throughout the day (such as soda and chips). Pain crises in patients with SCA can often last for months at a time and because patients tend to consume less during these times, nutrient deficiencies and weight loss can easily occur (9).

Calcium and vitamin D, which are important in bone health, are two particular nutrients that have been studied in patients with SCA, by Buison and colleagues (5,10). Having an adequate intake of these nutrients is particularly important in children because they are growing and developing at this time in their life (11). One study demonstrated that the status (blood concentration) of both of these nutrients was low in children with SCA (5). These researchers also found that dietary intake of vitamin D and calcium was low in these children. All of the children who participated in this study were of African, Afro-Caribbean, or African-American descent. Vitamin D is absorbed through the skin and metabolized in the body, but people with darker skin do not absorb as much vitamin D as people with lighter skin (5). This, coupled with a low intake leads to a vitamin D deficiency. Additionally, calcium needs vitamin D to be absorbed and these two nutrients are often found in the same foods. Children with vitamin D and calcium deficiencies have a lower whole body bone mineral content than children with normal vitamin D intakes and normal hemoglobin (3). Because of the importance of calcium and vitamin D to proper growth in children, more



studies need to be done looking at these nutrients. One study done with adults, by Goodman and colleagues, found that 98% of patients in their study had suboptimal vitamin D levels, and over half of them were considered to be severely deficient in vitamin D (12).

In addition to bone health, calcium is required for proper functioning of the heart, muscles, nerves, and blood clotting (10). For these reasons, calcium intake is also important to the adult population. Vitamin D is needed to absorb calcium from the diet; without vitamin D, the body cannot make enough of the hormone, calcitriol, which leads to inadequate calcium absorption (10). When this happens, the body uses the calcium that is stored in the bones and causes the bones to be weakened and unable to form new stronger bone (10). Calcium and vitamin D are both vital nutrients for the body, but the decreased dietary intake of patients with SCA may cause them to have a deficiency of these nutrients.

Because of the importance of vitamin D and calcium for the growth and development of children and other functions in children and adults, more research needs to be done with both populations. Therefore, the purpose of this study was to evaluate the dietary intake of calcium and vitamin D in adult patients with SCA and compare it to standard recommendations for these nutrients.

### **Dietary Intake Measurements**

The Institute of Medicine committee is responsible for reviewing the Dietary Reference Intakes (DRI) for various nutrients. The DRIs include various types of recommendations, including the Adequate Intake (AI), Estimated Average Requirement (EAR) and the Recommended Dietary Allowance (RDA).

The AI is defined as an intake by a healthy population who has an adequate nutritional status. This value is used for nutrients that do not have a RDA. The EAR describes an amount that meets nutrient needs for half of the healthy people of a particular age or gender group. The RDA is derived from the EAR. The calculation of the RDA considers the variance around the EAR for a particular nutrient. The calculated RDA meets or exceeds the needs for majority (97-98%) of the population. The RDA was used as a reference point in this study because of its increased probability of adequacy (13). For calcium, the Estimated Average Requirement (EAR) ranges from 500 to 1,100 mg per day, depending on age. The RDA ranges from 700 to 1,300 mg per day (14). For the age group included in this study, the EAR for calcium is 800 mg per day and the RDA is 1000 mg per day. The EAR for vitamin D is 400 International Units (IU) per day and the RDA is either 600 IU per day or 800 IU per day depending on age (14). The RDA for vitamin D is 600 IU per day for the age group of the participants included in this study. Calcium and vitamin D are both critical for bone health and this was the basis for the 2011 DRI recommendations.

Many Americans fail to meet the recommendations for vitamin D based on the RDA. According to a National Health and Nutrition Examination Survey (NHANES) (15), the mean amount of vitamin D consumed by males older than 20 was 200 IU and females of the same age consumed 152 IU of vitamin D. Both of these intakes are far below the recommended intake, RDA, of vitamin D. The same survey showed that males had an adequate intake of calcium (1038 mg) when compared to the recommended amounts, RDA of 1000 mg. On the other

hand, females were found to have an intake of only 833 mg, which is below the recommended 1000 mg per day (15). The results of the NHANES survey show that in general, Americans do not meet the RDA for nutrients such as calcium and vitamin D. Therefore, it is likely much more difficult for a person with a sickness and decreased dietary intake to consume an adequate amount of these nutrients in their diet.

### **Nutrient Intake in SCA**

Patients with SCA spend much time in hospitals, which may be associated with a lack of appetite and therefore lead to an inadequate intake of nutrients (16). They may have an inadequate intake of energy, macronutrients such as protein, and micronutrients such as zinc, iron, copper, folic acid, and vitamin E (3). Patients with SCA have reported that during times of pain crises, they eat less than they eat during times when they are not experiencing pain (9). Having frequent hospitalizations and decreased intake puts SCA patients at greater risk for malnutrition. When patients with SCA have a decreased intake for an extended amount of time, their metabolism can also be affected. For example, by not eating sufficient food, they can develop catabolism and begin to lose weight (9). In 2007, a study was reported that looked at the adequacy of dietary intake in children with SCA (6). This prospective study evaluated the dietary intake in 97 children and adolescents, aged 1.5 to 18.7 years (mean=  $8.7 \pm 4.7$  years). Intake was recorded by conducting 24-hour recalls during four annual visits. The study looked at macronutrient and micronutrient intakes compared to the DRIs. The researchers found that the intake of vitamin E, folate, fiber, vitamin D, and

calcium was low for all children involved in the study. For these nutrients, 63% to 85% of the children had intakes below the EAR or AI (6). In general, the dietary intake of adolescent subjects was found to be lower than that of the younger children. The intake of protein, vitamin C, riboflavin, vitamin B-12, and magnesium was lower in the older children than the younger children by 28% or more, (28%, 80%, 40%, 57%, and 73% decrease in %RDA, respectively) (6). The intake of vitamin A, magnesium, and phosphorus also decreased with age. The intake was less in children older than 9 years than the intake in children younger than 9 years. The study found that 50% to 75% of the children in the older category had an intake of these nutrients that was below the EAR (6). The results of this study show that poor dietary intake has a great impact on nutritional status. In contrast, other studies suggest that the increased nutrient need of patients with SCA is the reason why they often have suboptimal nutritional status (7).

### **Vitamin D and SCA**

Studies have been conducted to examine the vitamin D intake and status in children with SCA (5,8). The intake of vitamin D is especially important in this population because of its role in calcium absorption. Decreased vitamin D intake can lead to decreased calcium in the blood, which can increase synthesis and secretion of parathyroid hormone (PTH) (5). In the study by Buisson and colleagues, vitamin D status of children and the relation to the season of the year and dietary intake was evaluated. African-American children with SCA (n=65), aged 5 to 18 years were included in this 5-year study. The growth and stage of

development were assessed and compared to reference standards. Serum 25-hydroxyvitamin D (25-OHD) and PTH was measured and the results were compared to those of healthy children, aged 7 to 10 years. To assess the dietary intake of the children involved in the study, a 24-hour recall was conducted and nutrients were analyzed (5). The nutrients of interest that were analyzed were calcium and vitamin D. The intake of these nutrients was compared to the AI by calculating a percentage of the AI that was consumed. Sixty-five percent of the children with SCA had low concentrations of 25-OHD (defined as  $<27.5$  nmol/L). The mean 25-OHD concentration of the children with low vitamin D was  $18.4 \pm 5.9$  nmol/L and the concentration for the children with normal vitamin D status was  $38.6 \pm 11.4$  nmol/L. This study also found that vitamin D status decreased as age increased. Children with low vitamin D also had elevated concentrations of PTH. All of the children with SCA in this study had a low intake of calcium ( $66 \pm 39\%$  of AI) and vitamin D ( $78 \pm 78\%$  of AI). The concentration of vitamin D in the blood was not correlated to intake of vitamin D. However, the children who were classified as having low vitamin D status also consumed less calcium and vitamin D than the children with normal vitamin D status (5). This shows that having an adequate intake vitamin D in patients with SCA is important, particularly in children because they are constantly growing and developing.

A study in 2008 found that there is a high risk of vitamin D deficiency in children with SCA (8). The vitamin D status of 61 African-American children ages 5 to 18 with SCA was compared to that of 89 healthy African-American children ages 6 to 18. For this study, vitamin D deficiency was defined as a 25-OHD

concentration <11 ng/mL, vitamin D insufficiency was defined as <30 ng/mL to  $\geq$ 11 ng/mL, and vitamin D sufficiency was defined as  $\geq$ 30 ng/mL (8). The median 25-OHD concentration for children with SCA (15 ng/mL) was lower than that of healthy children (21 ng/mL). Ninety-three percent of children with SCA and 90% of healthy children were considered to be vitamin D insufficient. The researchers of this study found that 33% of the children with SCA were vitamin D deficient and 9% of the healthy children were vitamin D deficient. To analyze dietary intake, 24-hour recalls were conducted and entered into a nutrient analysis program to examine the intake of calcium and vitamin D. Both the healthy children and those with SCA consumed less than the AI for calcium and vitamin D. The children with SCA consumed 69% AI for vitamin D and 69% AI for calcium. The healthy controls consumed 74% and 57% of the AI for vitamin D and calcium, respectively. Rovner and colleagues found that children with SCA were 5.3 times more likely to be at risk for vitamin D deficiency than healthy children (8). The results of this study were consistent with the results found in previous studies (5,8). The differences seen in vitamin D status of children with SCA compared to healthy children may be due to several factors. The large amount of vitamin D insufficiency found in both groups of children in the study may be due to the darker pigment in the skin of African-Americans, which makes it more difficult for the body to synthesize vitamin D from sunlight. Children with SCA may have increased vitamin D requirements or may have improper metabolism of vitamin D, which would result in a lower concentration of the vitamin in the blood (5,8).

## **Calcium and SCA**

Intakes of calcium and blood levels of calcium have been evaluated less frequently than other nutrients in patients with SCA. A study of 25 children with severe SCA was conducted to evaluate bone mineral density (BMD) and risk factors for poor bone mineralization. The children included in the study were ages 9 to 19 years (median age 12.8 years) and all of them had SCA (HbSS) (17). Since the density of bones was being studied, the dietary intake of calcium was also evaluated by using a questionnaire. The calcium intake of the children ranged from 207-2,996 mg per day. Sixty percent of the participants had an inadequate intake of calcium (< the 1,300 mg per day RDA) and 48% of patients consumed <1000 mg per day. Lal and colleagues concluded that the BMD of children with severe SCA is low and that they have an inadequate intake of calcium and blood concentrations of vitamin D. Only 26% of the children in this study had 25-OHD concentration levels that were considered to be high enough for optimal bone mineralization. However, this study was not able to show a significant correlation between bone density and calcium intake or 25-OHD concentration (17).

Although a decreased calcium intake and vitamin D deficiency are often associated with decreased BMD, that is not always the case. A study of BMD in 53 children with SCD (45 HbSS, 4 HbSC, 4 SB<sup>0</sup> Thal) in Paris, France showed that there was a slight decrease in BMD in these children. For 27 of the children, calcium intake was also recorded by completing a 24-hour recall (18). Vitamin D levels and PTH were measured to assess vitamin D and calcium status. In this

study, 76% of the children were vitamin D deficient (<12 ng/mL). Chapelon and colleagues found that this decrease in BMD was not related to calcium intake or vitamin D status. They concluded that the main cause of the decrease in BMD was abnormal bone formation (18).

Much of the research on the intake of nutrients such as calcium and vitamin D in patients with SCA has been conducted with children. Both of these nutrients are vital to bone development, which is vital to the proper growth and development of children. However, calcium and vitamin D are both important to people of all ages (10) and a deficiency of these nutrients may cause problems. For this reason, this study aims to evaluate the intake of calcium and vitamin D in the adult population of patients with SCA, in comparison to the RDA. Identification of an inadequate intake of calcium and vitamin D can warrant further research to determine whether an increase in the intake or supplementation of these nutrients would be beneficial to these patients.



## CHAPTER II

### METHODS

#### Participants and Recruitment

This study included a convenience sample of 13 male and female participants between the ages of 21 and 52 years. Participants were from a parent study involving SCA and nutrition supplements. These participants were recruited by flyers posted at The University of Memphis, word of mouth, and advertisement at private doctors' offices and the University of Tennessee Hematology group, or by advertisement of the study through public media. Participants were adults that have been diagnosed with sickle cell anemia or SB<sup>0</sup> Thal and were considered medically stable by their doctor. Before participation in the study, participants were informed of procedures, potential risks and benefits of the study, and signed informed consent. One participant was not included in this study because of missing data. Therefore, the intake of 12 subjects was included in this study. One of the participants had SB<sup>0</sup> Thal and 11 had SCA.

#### Study Design

This was a retrospective study using the dietary recalls collected for the parent study, The Use of a Nutrition Supplement to Raise Nitric Oxide in Patients with SCA and B<sup>0</sup> Thalassemia. Approval for this study was obtained from the University of Memphis Institutional Review Board (Appendix A). Food records were collected during the first, middle, and last visits during the parent study. Participants were instructed to record intake for a period of 7 days prior to the visit and to bring it with them to each visit. Participants were given specific

instructions for recording portion sizes and pictures were used to help determine accurate portion sizes. Food records were reviewed for clarification by the study Principal Investigator or designee.

### **Data Analysis**

The diet records were entered into The Food Processor nutrient analysis program (Food Processor Pro Version 9 2006, ESHA Research, Salem, OR). An average of each participant's pre, mid, and post calcium and vitamin D intake (21-day average) was used and compared to the RDA for these nutrients. Although instructed to record diet records for 7 days before all 3 visits, many participants recorded diet records for less than 7 days instead. For these participants, their total calcium and vitamin D intake was averaged using the total number of days for which they kept food records instead of 21 days.

## **CHAPTER III**

### **RESULTS**

#### **Descriptive Statistics**

A total of 12 participants was included in the statistical analysis. There were 6 males and 6 females in the study with a median age of 31.75 years. All of the participants (100%) consumed less than the RDA for vitamin D and calcium intake from food (Table 1 and 2). The mean daily vitamin D intake from food for individual participants ranged from 0-268.66 IU. The mean daily calcium intake from food ranged from 22.23-872.23 mg. Of the 12 participants, 3 of them were taking vitamin D supplements (50,000 IU 1-2 times per week) during the time period of the parent study. Also, 3 participants were taking calcium supplements (1000-1200 mg/day) during the study. The numbers reported in these tables only include nutrient intake from food and beverages, not the supplements consumed.

**Table 1** Mean Daily Vitamin D Intake from Food and Percent of Recommendation

<b>Subject Number</b>	<b>Mean Vitamin D Intake from Food (IU)</b>	<b>% of RDA<sup>a</sup></b>
1	119.40	19.9
2	220.05	36.7
3	19.16	3.2
4	17.48	2.9
5	40.50	6.8
6	59.50	9.9
7	0.08	<1
8	0.00	0
9	268.66	44.8
10	26.47	4.4
11	0.00	0
12	151.58	25.3

<sup>a</sup>RDA=600 IU

**Table 2** Mean Daily Calcium Intake from Food and Percent of Recommendation

<b>Subject Number</b>	<b>Mean Calcium Intake from Food (mg)</b>	<b>% of RDA<sup>a</sup></b>
1	822.98	82.3
2	872.23	87.2
3	210.75	21.1
4	346.07	34.6
5	588.02	58.8
6	243.85	24.4
7	109.81	11.0
8	22.23	2.2
9	240.24	24.0
10	351.27	35.1
11	206.80	20.7
12	565.98	56.6

<sup>a</sup>RDA=1000 mg

## Statistical Analysis

Data was entered and analyzed using IBM SPSS Version 20, 2011. A one-sample t-test was performed to analyze calcium and vitamin D intake from food for the group of participants. The mean daily intake of calcium for the group was 381.69 mg, which is significantly lower ( $p < 0.05$ ) than the recommendation (Table 3). The mean daily vitamin D intake for the group was 76.91 IU, which is also significantly lower ( $p < 0.05$ ) than the recommended intake (Table 3).

Nutrient	N	Range	Mean Intake	RDA	Difference	p-value
Calcium (mg)	12	22.23-872.23	381.69	1000	618.31	0.000*
Vitamin D (IU)	12	0-268.66	76.91	600	523.09	0.000*

\* $p < 0.05$

Supplement use was considered separately and the mean intake of calcium and vitamin D from food was compared between the participants who were taking a supplement and those who were not. Levene's test for equality of variances was done to assess the equality of variance between the 2 groups ("Supplement" and "No Supplement"). Equal variances were assumed for both calcium and vitamin D (Levene's test  $p > 0.05$ ). When looking at calcium intake and supplementation, the intake from food was greater in the group taking supplements when compared to those not taking supplements (Table 4). However, this difference was not statistically significant. Similar results were also seen with vitamin D, there was a greater intake from food in the participants taking a supplement, but it was not statistically significant (Table 5).

<b>Table 4</b> Calcium Intake from Food and Supplementation			
<b>Group</b>	<b>N</b>	<b>Mean Intake (mg)</b>	<b>Significance<sup>a</sup></b>
Supplement	3	421.11	0.787 <sup>b</sup>
No Supplement	9	368.55	

<sup>a</sup>With equal variances assumed; Levene's Test for Equality of Variances p=0.083  
<sup>b</sup>p>0.05

<b>Table 5</b> Vitamin D Intake from Food and Supplementation			
<b>Group</b>	<b>N</b>	<b>Mean Intake (IU)</b>	<b>Significance<sup>a</sup></b>
Supplement	3	96.15	0.697 <sup>b</sup>
No Supplement	9	70.49	

<sup>a</sup>With equal variances assumed; Levene's Test for Equality of Variances p=0.512  
<sup>b</sup>p>0.05

## **CHAPTER IV**

### **DISCUSSION**

There have been a number of studies looking at the nutritional status of patients with SCA (5,9,12,16). These studies demonstrated that these patients have a decreased intake especially during times of pain crises. During this study, 8 of the 12 participants experienced a pain crisis at some point. Whether crises affected intake of calcium and vitamin D or intake in general was not examined in this study. This is a question that future studies could address. As a result of poor intake, nutritional status is affected. The decreased intake of essential vitamins and minerals is one of the most important aspects of a decreased intake overall. When there is a decreased overall intake, there is also a decrease in the nutrients that the body needs in order to function properly. The intake of specific vitamins and minerals, including calcium and vitamin D, has been examined in studies involving children with SCA (3,7,8,16,18). There is limited focus on the intake of these particular nutrients in adult patients with SCA, which was the aim of this study.

The results found in this study were similar to results that have been found in previous studies involving vitamin and mineral intake in patients with SCA. In this study, all 12 participants consumed less than the RDA for both calcium and vitamin D. This indicates that when these patients are actually eating, they are not consuming many foods high in calcium or vitamin D. When compared to the general population of adults ages 20 and older, the calcium intake for the participants is low. In the general population, calcium intake is 1038 mg (104% of

RDA) and 833 mg (83% of RDA) for males and females respectively (15). The participants in this study had a mean calcium intake of 381.69 mg. This indicates that the participants did not consume many food sources that are high in calcium such as dairy products. This could be due to the fact that lactase deficiency is highly prevalent in African-Americans (20). Some may experience some discomfort when consuming dairy products, attribute this to lactose intolerance, and choose to avoid dairy completely. However, this problem can be solved by consuming only a small amount of dairy at one time or by consuming dairy products like yogurt or some types of cheeses instead of milk (20).

Low intake of vitamin D is not surprising considering the fact that the average intake of vitamin D for the general population for the ages considered in this study is 200 IU (~33% of RDA) or less (15). This is mainly because the foods that are the highest sources of vitamin D include salmon, swordfish, and trout (19), which are generally not consumed often. Fortified milk, some fortified cereals, and tuna are more common, good sources of this nutrient but patients in this study did not consume these products often.

Another source of vitamin D comes from exposure to sunlight, which activates vitamin D in the skin. There are several factors that can affect the efficiency of this process, including the color of skin. Those with darker skin require more exposure to the sun in order to have the same results as those with skin of a lighter pigment (21). Sickle cell disease occurs mainly in African-Americans (4), who have darker skin than those of some other ethnicities. This



means that this group of people may need to rely more on vitamin D intake from food and supplements (22).

There were some limitations during this study. One of the major limitations was that only 12 participants were included in the study. There were several possible reasons for not having many participants. Difficulty recruiting participants, transportation issues, and lack of time may have affected the amount of participation. This small sample size makes it difficult to generalize the results of this study to larger populations. The use of a food diary may not be a reliable method to determine nutrient intake. However, the diaries were recorded for 7 days, which gives a more accurate estimate of intake than a 1 or 2 day record. Although participants were instructed to keep three 7-day food diaries (pre, mid, and post), not all of the participants recorded intake for 7 days each time. This was accounted for when analyzing data by calculating each individual's mean intake based on the number of days that specific person kept a food record. Not including information about pain crises and their affect on calcium and vitamin D intake was also a limitation to this study. However, intake was recorded for 3 weeks throughout the study, which gives a mean intake of calcium and vitamin D on any day whether experiencing a pain crisis or not.

In summary, these adults who had sickle cell anemia had a low intake of calcium and vitamin D. Due to decreased intake of these nutrients, this type of patient may need supplements or education on consuming foods that are good sources. Calcium and vitamin D are both essential parts of a healthy diet and are needed for specific functions of the body. The importance of these nutrients is

often stressed when dealing with the proper growth and development of children. However, calcium also plays a role in the functioning of the heart, muscles, nerves, and blood clotting. Additionally, vitamin D is needed to ensure proper absorption calcium so it can be utilized properly. For this reason, both nutrients are also important in adults and people of all ages.

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## **APPENDICES**

**APPENDIX A**  
**IRB APPROVAL FORM**

**THE UNIVERSITY OF MEMPHIS**

**Institutional Review Board**

To: Ruth Williams, R. Bloomer, T. Farley, B. Canale, K. Yeoman  
Health & Sport Sciences

From: Chair, Institutional Review Board  
for the Protection of Human Subjects

Subject: **A pilot study of the use of a nutrition supplement to increase  
blood nitric oxide levels in adults with sickle cell anemia  
(H11-02)**

Approval Date: **September 9, 2010**

This is to notify you of the board approval of the above referenced protocol. This project was reviewed in accordance with all applicable statutes and regulations as well as ethical principles.

Approval of this project is given with the following obligations:

1. At the end of one year from the approval date an approved renewal must be in effect to continue the project. If approval is not obtained, the human consent form is no longer valid and accrual of new subjects must stop.
2. When the project is finished or terminated, the attached form must be completed and sent to the board.
3. No change may be made in the approved protocol without board approval, except where necessary to eliminate apparent immediate hazards or threats to subjects. Such changes must be reported promptly to the board to obtain approval.
4. The stamped, approved human subjects consent form must be used. Photocopies of the form may be made.

This approval expires one year from the date above, and must be renewed prior to that date if the study is ongoing.

\_\_\_\_\_  
Approved

Cc:



THE UNIVERSITY OF MEMPHIS  
*Institutional Review Board for the Protection of Human Subjects*

**CONSENT TO ACT AS A HUMAN SUBJECT**

Title of Investigation: A pilot study of the use of a nutrition supplement to increase blood nitric oxide levels in adults with sickle cell anemia

Principal Investigator: Ruth Williams  
Office phone: 901-678-3108  
Email: [mrwillia@memphis.edu](mailto:mrwillia@memphis.edu)

Co-Investigators/Research Assistants:  
Richard Bloomer, Tyler Farley, Bob Canale, Katie Yeoman

**A. Purpose and Procedures**

The purpose of this study is to determine if taking a nutrition supplement (Glycine-Propionyl-L-Carnitine (GPLC)) will increase blood levels of nitric oxide (NO) and other blood levels in adults with sickle cell anemia (SCA). There have been some studies done with athletes taking this supplement and it has raised their NO level in the blood. Sickle cell patients have low NO and this causes leg ulcers and an increase in the blood pressure in the arteries/veins in your lungs. You may or may not have these problems, however, we still want to see if taking the nutrition supplement will raise your NO level.

As part of this study, you will have your heart rate, blood pressure, height, weight, waist, and hip circumference, and skinfold thickness measured. A blood sample will also be collected, as described below. You will be assigned the nutrition supplement to take twice daily (with meals) for eight (8) weeks.

***Blood Sampling***

Blood will be collected from you on three different days throughout the course of this study: at the beginning, middle, and end of the study (day 1, in 4 weeks and 8 weeks. Blood samples (10mL; 2 teaspoons) will be taken using a needle by a trained phlebotomist (someone skilled in taking blood). Blood samples will be collected after a 10-minute rest period. We will use sanitary methods so you do not get an infection, however, infection and bruising are possible.

***Dietary Records and Physical Activity***

You will follow your normal diet during the 8 weeks and to fill out food records for seven day periods during weeks 1, 4, and 8 of the study. You will be shown how to do the food records.

**B. Risks and Discomforts**

As stated earlier, there is a risk of bruising and infection at the site where your blood is drawn. We will make every effort to assure that this does not happen. This dietary supplement has been shown to be safe when given at 4.5 grams a day. This is the dose we will be using.

**C. Benefits**

If desired, you will receive information regarding how you responded to the supplement.

**D. Alternative Procedures or Treatment**

If any abnormal signs or symptoms are present during your participation, the testing will be stopped and you will receive immediate attention. Otherwise, no treatment will be provided.

**E. Confidentiality**

Your privacy will be protected during the study to the extent allowed by law. Your name will not be used on the study documents, but you will be assigned a number. All documents from the study will be

kept in a file cabinet in a locked office. Following all analyses, all materials associated with individual subjects will be shredded.

**F. Compensation**

You will be paid \$60 for your participation. You will receive \$20 at each visit.

**G. Compensation for Injury**

None. The University of Memphis does not have any funds budgeted for compensation for injury, damages, or other expenses. However, if adverse circumstances arise as a direct result of your participation in this research, the investigators associated with this work will provide guidance in terms of seeking appropriate medical treatment. This may include a visit to your personal physician or to the student health center if appropriate. In extreme cases, this may involve a visit to the emergency room. The guidance provided by investigators will follow the standard procedures of the Human Performance Laboratories. If adverse events occur outside of the Human Performance Laboratories (e.g., at home), you should seek medical attention and notify the investigators as soon as possible concerning the event. The investigators may also be able to provide advice as to what actions to take if you are uncertain.

**H. Questions Regarding Research**

Questions regarding the research itself or research-related injuries can be directed to Ruth Williams at 901-678-3108 (office). You are encouraged to ask questions not only at the time of signing the informed consent form, but throughout your participation as a research subject. Any new information that develops during the research will be provided to you if the information might affect your willingness to continue participating.

**I. Voluntary Participation**

You are free to refuse to participate or to withdraw your consent to participate in this research at any time without prejudice. Participation is entirely voluntary.

**J. Questions Regarding Subjects' Rights**

Questions regarding research subjects' rights can be directed to the Chair of the Institutional Review Board for the Protection of Human Subjects at 678-2533.

You have been given the opportunity to ask questions regarding your participation, and all questions have been answered to your satisfaction. By signing this form, you are agreeing to participate in the research as described to you by Ruth Williams or research assistant.

_____	_____
Subject Signature	Date
_____	
Subject Printed Name	
_____	_____
Phone	Email
_____	_____
Witness to Signature	Date