

Dietary Calcium Intake, Fractional Calcium Absorption, Urinary Calcium Excretion, And Levels Of Calcitropic Hormones And Bone Markers In Young, Healthy Chinese-American And Caucasian Women

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A. Study Purpose and Rationale

Osteoporosis is a major public health issue affecting over 10 million Americans. Many risk factors for osteoporosis and subsequent hip fracture have been identified. These include female sex, age, post-menopausal status, low calcium intake, certain medications, sedentary lifestyle, heavy alcohol or tobacco use, certain ethnic backgrounds, and some chronic diseases. Recently, inefficient calcium absorption has been identified as an independent risk factor for hip fracture (Ensrud, Duong et al. 2000; Fleet2001). Some clinicians question the need to identify risk factors for osteoporosis. Bone densitometry, they argue, provides an integrated measure of most of the risk factors. If a patient's BMD is known, the presence or absence of risk factors for low BMD is no longer pertinent. The cost of bone densitometry, however, precludes indiscriminant osteoporosis screening. Thus, clinicians can determine which patients should have bone densitometry by assessing the presence of risk factors for osteoporosis.

The current knowledge of risk factors for osteoporosis is derived predominantly from studies of Caucasian women. Many of the risk factors for osteoporosis in Caucasian women are also risk factors for Asian women(Lau, Suriwongpaisal et al. 2001). However, there are some inconsistencies, and few studies have investigated the situation for hybrid cultures such as people of Asian descent living in the United States. One contentious issue is whether the calcium intake recommended by the NIH Consensus Conference is appropriate for Asian-American women(anonymous 1994). The typical Asian diet is lower in calcium than the average North American diet, and the sources of calcium are different(Haines, Chung et al. 1994{Pan, 1992 #7}). Asians consume fewer dairy products, while deriving more dietary calcium from dark green leafy vegetables. As might be expected Asians have lower BMD than Caucasians. However, their hip fracture incidence is paradoxically lower than the incidence in Caucasians. There is no conclusive evidence as to why this might be the case.

Some have suggested that Asians absorb calcium more efficiently(Kung, Luk et al. 1998). Of course, low calcium intake would increase the efficiency of calcium absorption in any population(Dawson-Hughes, Harris et al. 1993). Low calcium intake lowers serum ionized calcium, increasing parathyroid hormone levels, and thereby instigating production of active Vitamin D. Vitamin D increases intestinal calcium absorption. However, in a study of Hong Kong women, Kung found that when these women consumed a diet rich in calcium, their fractional calcium absorption was higher than reported values for white women. On A low calcium diet, the fractional calcium absorption of these women was -70%, two to three times more efficient than values published for Caucasian women. To explain the etiology of this increased efficiency, Kung hypothesized it might reflect genetic adaptation to many generations of low calcium intake. If this were the case, we should expect nutritionally Americanized second generation Asian-Americans to have higher calcium absorption than Caucasian-Americans. Alternatively, Kung suggested that Asian's highly efficient calcium absorption could be due to adaptation to low calcium intake during childhood, early weaning, or to a diet with vegetables as the main source of calcium. In this case, we might expect the fractional calcium absorption of second generation Asian-Americans raised in an Americanized environment to be equivalent to that of Caucasian Americans(Kung, Luk et al. 1998).

While intriguing, Kung's results were forcefully challenged by Robert P Heaney, one of the fathers of mineral absorption research, in a letter to the American Journal of Clinical Nutrition (Heaney 1999). He questioned her conclusions regarding the differential efficiency of calcium absorption in Asians and Caucasians on three grounds. First, he argued that the Asian diet was not truly significantly lower in calcium than the Caucasian diet. Further, when adjusted for body size, the diet of Kung's subjects was actually higher in calcium per kilogram than the diet of the American women who participated in NHANES II (National Health and Nutrition Examination Survey). Second, he stated that results from Kung's method to measure fractional calcium absorption do not reflect true fractional calcium absorption of calcium from a meal. In her study, subjects ingested the labeled calcium in an aqueous solution. To accurately reflect true calcium absorption with a meal, the isotope must be ingested with a normal physiologic calcium load (Eastell, Vieira et al. 1989). Finally, he argued that the calcium absorption figures for Caucasians Kung referred to in her paper were derived using a method known to produce lower values than the method she used. He argued that using Kung's methods, calcium absorption in Caucasians would not be significantly different than her findings in Asians (50-60%). Overall, Heaney denied that Kung's study showed any evidence of a difference in dietary calcium intake or in calcium absorption between Asians and Caucasians.

While the issue may seem academic, it has significant clinical implications. The results could affect how Asian-American women are counseled regarding calcium intake, and how they are treated for osteoporosis. In addition, if Asian-Americans do have more efficient calcium absorption, the next reasonable question is why. The answer to this question, whether due to as yet undiscovered genetic differences or due to environmental factors, could serve as a clue to provide a greater understanding of the factors regulating calcium absorption. The more we know about the regulation of calcium absorption and metabolism, the more effectively we can treat various metabolic diseases affecting bone, most notably osteoporosis.

Currently, 1,25 dihydroxyvitamin D (1,25(OH)₂D) is the only known hormonal stimulus for active intestinal calcium absorption. Parathyroid hormone (PTH) secretion, increased as the consequence of low ionized serum calcium, stimulates the renal production of 1,25(OH)₂D. PTH, by direct upregulation of a renal enzyme, and indirectly via renal phosphorus wasting, increases the conversion of the inactive, storage form of Vitamin D (25(OH)D) to the active form in the kidney. There is some evidence that elderly people, and especially osteoporotic patients, may have impaired ability to produce active Vitamin D in response to low dietary calcium intake. Poor Vitamin D production in response to such a challenge could lead to inefficient intestinal calcium absorption. Of note, Kung's Hong Kong study found that despite impaired 1,25(OH)₂D response to a low calcium diet, the Asian osteoporotic women in her study increased their intestinal fractional calcium absorption as effectively as the age-matched and young controls. This raises the possibility that Asian women may have a Vitamin D independent mechanism by which intestinal calcium absorption can be increased in the face of low calcium intake.

There will be two parts to our study. The first will be a cross-sectional comparison of dietary calcium intake, fractional calcium absorption, urinary calcium excretion, and levels of calcitropic hormones and bone markers in young, healthy Chinese-American and Caucasian women. The second part will be an unblinded, unrandomized clinical intervention trial. For the first month of the trial, each participant will consume a high calcium diet as a result of calcium supplementation. Metabolic response to this intervention will be evaluated after that month. For the second month, women will be advised to avoid foods high in calcium, and thus they will consume a diet low in calcium. After this one month trial of low calcium intake, the metabolic response of the two groups will be evaluated.

Objectives

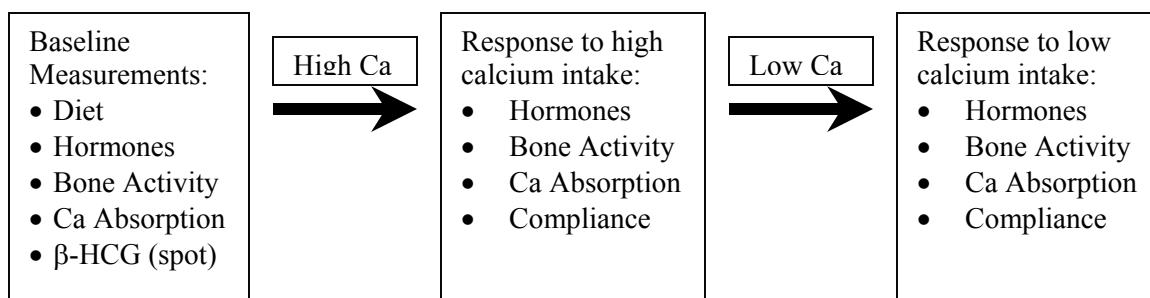
1. Evaluate true fractional calcium absorption with a meal for Asian-American and Caucasian women consuming their usual diets.
2. Describe the metabolic response to high and low calcium intake in Asian-American and Caucasian women with special reference to true fractional calcium absorption and Vitamin D.

3. Compare the metabolic responses of the two groups to these calcium challenges in terms of fractional calcium absorption, calcitropic hormone levels, urinary calcium loss, and markers of bone resorption and formation.

B. Study Design and Statistical Analysis

a. Power Calculation

(All calculations $\alpha=0.05$, two-sided, for a power of 80%) Using Kung's finding that young control Hong Kong women had a calcium absorption of 68% \pm 11%, and Heaney's assertion that using the same technique Caucasian women would have an absorption rate of 50-60%, we determined our expected difference would be 68%-55%, or 13%. We would need 12 subjects in each group to show such a difference. Since not all individuals will be able to complete the protocol, and there will be incomplete data for others, we will include 16 subjects in each group. There may be concern that because we will be using a different method than Kung, the standard deviation for our population may be different. Studies using the method we propose to use have reported standard deviations lower than Kung's. For example, Wolf found that mean fractional calcium absorption for 46-54 year-olds was 0.35, with a standard deviation of 0.08. Our study would be adequately powered to show a difference if Caucasians had a fractional absorption of calcium of 0.35, and Asian-Americans had a fractional absorption greater than 0.45.



C. Study Procedure

Participants will visit the Irving Center for Clinical Research (ICCR) three times over the course of two months. Prior to the first visit, participants will pick up a container to collect a 24-hour urine sample. At the first visit, baseline characteristics of the participants will be evaluated. The following data will be collected:

- Diet: Each participant will complete a food frequency questionnaire. Average daily dietary fat, fiber, and protein, calcium intake, sodium intake, and phosphate intake over the previous year will be estimated.
- Physical Exam: Height and weight will be measured.
- Questionnaire: Age, immigrant status, alcohol consumption, caffeine intake, symptoms of lactose intolerance, recent history of constipation, physical activity, sun exposure, sexual and birth control history, and pertinent family, medical, surgical, and medication history will be addressed.
- Serum: Serum will be collected and evaluated for parathyroid hormone (PTT-1), calcium, phosphorus, albumin, creatinine, 1,25 (OH)₂ D, 25 (OH) D, osteocalcin, bone-specific alkaline phosphatase, and pyridinoline crosslinks.

- Urine: A 24-hour urine will be collected for calcium, creatinine, and B-HCG (spot). Prior to each visit, participants will be reminded to bring the 24-hour urine sample, and instructed on how to collect the urine.
- Calcium Absorption: Participants will be asked to fast overnight, and after baseline serum samples are taken they will drink 8 ounces of calcium fortified orange juice (approximately 300mg of calcium), labeled with 5gCi of 45 Calcium. Two ounces of 45 Ca labeled orange juice will be supplied by Creighton University, and will be mixed with 6 ounces of non-labeled fortified orange juice. Venipuncture will be performed five hours after the subject ingests the orange juice. These samples will be packed and shipped overnight to Creighton University, where they will be evaluated. This method was described as a valid (r=.905 to double tracer method taking into account serum specific activity, height and weight) alternative to the double tracer method by Heaney and Recker in 1985 (Heaney and Recker 1985).

After the baseline visit, participants will be provided with a bottle of sixty (60) calcium supplement pills (600mg calcium citrate each). They will be counseled to take one pill between meals two times a day, refrain from taking Vitamin D supplements, and not to alter their usual routine of physical activity, diet, alcohol and caffeine consumption, or tobacco use.

The participants will return for the second visit 30 days later. After the second visit, the participants will be counseled not to take calcium or Vitamin D supplements for the next month. They will also be instructed to avoid various calcium rich foods such as dairy products, dark green leafy vegetables, and calcium fortified foods. After 30 days, they will return for their third, and final, visit.

During the second and third visits, fractional calcium absorption will be evaluated, and serum and a 24-hour urine sample will be collected. Participants will also complete a questionnaire inquiring about their adherence to the protocol. They will be asked if they adjusted their diet as instructed and if there were any significant changes in their physical activity levels, intake of caffeine or alcohol, use of tobacco, or a change in bowel habits. They will also be asked whether they are taking any new medications, prescription, over the counter, or alternative.

All of the above tests and measurements are for research purposes only. They would not be part of the standard care for young healthy women. None of the procedures above involve any discomfort to the participants, except for venipuncture.

D. Study Drugs

For the first month, participants will be taking 1200mg per day of calcium citrate, a mineral salt supplement.

E. Medical Device

N/A

F. Study Questionnaire

See attached.

G. Study Subjects

- a. **To be included in the study, individuals must be:**
 - Between 18 and 36 years old,
 - Female,
 - Of either exclusively Chinese or European descent (by report),

- Nulliparous,
- Not pregnant,
- Not lactating,
- Willing to use adequate birth control during the study and for at least one year after the completion of the study,
- Able to understand the risks of participating in the study,

In addition, women with a history of certain diseases or on medications that might affect calcium absorption will not be included. Such diseases include thyroid disease, severe renal disease, rickets, malabsorption syndromes, hypo- or hyperparathyroidism, hypo- or hyperphosphatemia, premature ovarian failure, amenorrhea, or cancer. Excluded medications include greater than 600 mg/day of calcium supplements regularly for the past year, supplemental Vitamin D (greater than 1000/day), systemic or inhaled steroids in the past year, or diuretics within the past year.

Adequate methods of birth control include, but are not limited to, regular use of oral contraceptive pills, condoms, diaphragm, cervical cap, abstention from sexual intercourse, or use of an intrauterine device. Each participant will be required to describe their method of birth control, and to sign a form confirming that they will adhere strictly to the regimen chosen throughout the study and for one year after the study is completed. Because the risk to a fetus from the small dose of radioactivity administered is so minimal, participants will not be required to use two different types of birth control. However, that option will be mentioned.

H. Recruitment of Subjects

Participants will be recruited using several methods. Flyers will be posted around Columbia-Presbyterian Medical Center. Email messages will be sent to medical and graduate students through their class email lists, and through specific organizations such as the American Medical Women's Association. The physicians at the Columbia Health Sciences Student Health program will be asked to refer patients they think are suitable for the study and who are willing to discuss the study with the research team. Advertisements in the Columbia Spectator may be placed, and Columbia undergraduates 18 years old or older may be recruited, if recruitment by these other means is inadequate.

I. Confidentiality of Study Data

All study data will be coded with a unique participant identification number, and data will be stored securely in at least two locations. The identity of the participants will be confidential.

J. Potential Conflict of Interest

None

K. Location of Study

This study will be conducted at the Irving Center for Clinical Research, on the I& Floor of the Presbyterian Hospital Building.

L. Potential Risks

Discomfort for the participants will be limited to approximately six needle sticks.

The major risk to participants in this study is exposure to a radioactive substance. Each participant will orally ingest orange juice containing 5 gCi of ^{45}Ca on three occasions, for a total dose of 15gCi. Previous studies using this method have found that women absorb approximately 35% of the

calcium thus administered, so the average absorbed dose of 45 Ca should be approximately 5.25gCi. However, absorption can vary, and some women may absorb up to 70% of the calcium, or 10.5gCi. To be conservative in our calculation of the radiation dose to which participants will be exposed, we will assume that participants in this trial will absorb this higher quantity of 41 Ca. Dose of radiation is calculated using the following formula:

$$\text{Dose} = 5 \cdot 1.1 \cdot \text{concentration} (\mu\text{Ci}/\text{gram}) \cdot E\beta \cdot \text{Mean Residence Time}$$

For calcium, the volume of distribution is ~10L or 10,000 grams. The mean residence time equals one divided by the natural logarithm of two divided by the half-life (162.7 days).

$$=51.1 \cdot 10.5\mu\text{Ci}/10,000\text{g} \cdot 0.3\text{MeVs} \cdot 234.7$$

$$=3.78 \text{ mrad}$$

$$=3.78 \text{ mrem (since the quality factor for } \beta \text{ emitters is 1)}$$

According to PerkinElmer Life Sciences, the Annual Limit for oral Intake (ALI) for 4'Ca is 2 mCi (74MBq), one hundred and thirty-three times more than these women will be ingesting (www.lifesciences.perkinelmer.com/downloads/calcium45.pdf). A round-trip airplane flight from New York to San Francisco would expose a women to more radiation (~5mrem), and the occupational exposure limit to a fetus is 500 mrems, over 100 times higher than the exposure sustained in this study. Pelvic x-rays submit the body to 44 mrems, and a CT of the head and body expose an individual to over 500 mrems. (www.Dhysic.s.isu.edu/.rad.inf/~isk.htm) Both of these radiologic tests are commonly used in research studies. Thus, the risk to participants from their exposure to radiation is minimal. It must nevertheless be stressed to possible participants that the risk of small doses of radiation is unknown, and should be taken into account when considering participation in this trial.

M. Potential Benefits

This study is strictly for research purposes, and is not intended to benefit the participants directly. The participants in this trial may or may not benefit from this study. Participants will be educated about high calcium foods, and participation will undoubtedly increase their awareness of the importance of dietary calcium in prevention of osteoporosis. In addition, participants will receive a printed evaluation of the macroand micro-nutrient content of their baseline diet, and nutritional counseling will be available for those who request it. The benefits to scientific knowledge, and possibly to society, will be significant. This study will directly address the question of whether calcium is absorbed more efficiently by Asian women. A clear understanding of this issue is important for the prevention of osteoporosis in Asian people, and may possibly provide a stepping stone towards a deeper understanding of hormone dependent calcium absorption in-general.

N. Alternative Therapies

N/A

O. Compensation to Subjects

Subjects will not be compensated for their participation.

P. Costs to Subjects

Subjects will not be expected to incur any additional costs.

Q. Minors

N/A

R. Radiation or Radioactive Substances:

S. References

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