NUTRITION AND BONE DENSITY IN CHILDREN WITH CYSTIC FIBROSIS

by

Joanna K. Davidson

A thesis submitted in partial fulfillment
of the requirements for the degree
of
MASTER OF SCIENCE
in
Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah
2004
ABSTRACT

Nutrition and Bone Density in Children with Cystic Fibrosis

by

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Utah State University, 2004

Major Professor: Dr. Nedra C. Christensen
Department: Nutrition and Food Sciences

The purpose of these studies was to further research on bone density in children with cystic fibrosis, particularly as it pertains to nutritional parameters and care. The first paper presented a comparison of a group of 50 children with cystic fibrosis to a group of 32 control children. There were no significant differences between the groups in any of the pertinent bone density measurements. Serum 25(OH) vitamin D was positively correlated with spine density z score in the cystic fibrosis group.

The second paper, incorporating all of the information obtained from the first paper, describes an intervention study with the implementation of a fortified milk to determine the effects of additional calcium and vitamin D on bone density in the cystic fibrosis group. A follow-up bone scan was done. The fortified milk did not significantly improve bone density, but the fortified milk group did have significantly higher lung function scores on follow-up.

(122 pages)
I would like to acknowledge and thank the Utah/Nevada Dairy Commission for funding the development of the fortified milk product used in this study. I would also like to recognize the Primary Children’s Medical Center Foundation for their contributions (grant # Spring98/McDonald/$25,338/152) and thank them for their assistance in completing this research. I would like to thank Katie McDonald for all of her help and expertise. It would not have been possible to conduct this study without the assistance and generosity of Dr. Gary M. Chan and his lab assistant, Gurmail Gill. I also owe a debt of gratitude to Roxane Pfister for all of her help with statistics. Thanks to Nedra for all her encouragement and all of my family for the many ways they have supported and helped me throughout this project.

Joanna K. Davidson
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AMA</td>
<td>Arm muscle area</td>
</tr>
<tr>
<td>AMC</td>
<td>Arm muscle circumference</td>
</tr>
<tr>
<td>BMAD</td>
<td>Bone mineral apparent density</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone mineral content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CC</td>
<td>Chest circumference</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
</tr>
<tr>
<td>CFF</td>
<td>Cystic Fibrosis Foundation</td>
</tr>
<tr>
<td>CT</td>
<td>Computer tomography</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual energy x-ray absorptiometry</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Reference Intakes</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;%</td>
<td>Percent Predicted Forced expiratory volume in 1 second</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>IU</td>
<td>International Units</td>
</tr>
<tr>
<td>L2-L4</td>
<td>Lumbar 2 through lumbar 4 (spine)</td>
</tr>
<tr>
<td>LQM</td>
<td>Lactoval QM calcium complex</td>
</tr>
<tr>
<td>MAC</td>
<td>Mid-arm circumference</td>
</tr>
<tr>
<td>PCMC</td>
<td>Primary Children’s Medical Center</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>QCT</td>
<td>Quantitative computed tomography</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Dietary Allowance</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SS</td>
<td>Subscapular skinfold</td>
</tr>
<tr>
<td>TSF</td>
<td>Triceps skinfold</td>
</tr>
<tr>
<td>UHT</td>
<td>Ultra high temperature</td>
</tr>
<tr>
<td>USU</td>
<td>Utah State University</td>
</tr>
<tr>
<td>1,25(OH)2D</td>
<td>1,25 dihydroxyvitamin D</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>25 hydroxyvitamin</td>
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CHAPTER 1
GENERAL BACKGROUND AND INTRODUCTION

Definition of Cystic Fibrosis

Cystic fibrosis (CF) is a genetic disease that affects approximately 30,000 children and adults in the United States. In CF, the transport of sodium and chloride within cells lining organs (such as the lungs and pancreas) to their outer surfaces is faulty. This causes the body to produce an abnormally thick, sticky mucus. Nearly all exocrine glands are affected to varying degrees, but it is the lungs and pancreas that are most notably affected in most patients. Fifty percent of all patients presented with pulmonary symptoms including chronic cough and wheezing due to recurrent or chronic pulmonary infections. Pancreatic insufficiency is present early in life but may be progressive. Seven to 10% of CF patients have meconium ileus at birth. There are clinical manifestations of pancreatic insufficiency in 85 to 90% of patients.

The most common symptoms of CF include very salty-tasting skin; persistent coughing, wheezing, or pneumonia; excessive appetite but poor weight gain; and bulky stools. The standard diagnostic test for CF is the “sweat test” which measures the amount of salt in sweat. Sweat chloride concentrations less than 40 mmol/L are normal; concentrations greater than 60 mmol/L are consistent with the diagnosis of CF. Diagnosis can be confirmed by DNA analysis in a patient with clinical correlates by finding two known disease-causing mutations.
CF is an autosomal recessive disease, and thus, to have CF an individual must inherit a defective copy of the CF gene from both parents. More than 10 million individuals in the U.S. (one in 31) is a symptom-less carrier of the defective gene. Ninety percent of patients are diagnosed in infancy or early childhood. From 1985 through 2001, predicted survival increased from about 25 years to about 33 years. This is evidence of the great progress made in CF research and consequent treatments in recent years. Current common treatments include chest physical therapy (vigorous percussion on the back and chest to dislodge the thick mucus from the lungs), antibiotics to treat lung infections (administered intravenously, via pills, and/or medicated vapors which are inhaled to open up clogged airways), an enriched diet, replacement vitamins, and pancreatic enzymes.

The recent increase in life span of patients over a 16-year period has evidenced a new set of long-term complications. These include diabetes, liver disease, malnutrition, suboptimal growth, infertility, and osteopenia. Osteopenia in CF patients can lead to osteoporotic fractures prematurely, which decreases quality of life and increases medical costs. Now that length of life is increasing, it is equally important that quality of life increase with it.

Evidence of Osteopenia in Cystic Fibrosis

Bone mineral density (BMD) deficiencies in patients with CF were first published in 1979 by Mischler et al. They used photon absorptiometry to measure the radius and ulna in 27 patients with CF (range of ages not reported) and compared them with 968 age-matched controls. They found significant demineralization in 44% of the CF patients.
Aris et al. increased awareness of this problem with their reports of rib and vertebral fractures and high fracture rates in a study of 70 adult patients referred for lung transplantation. They found that vertebral compression and rib fractures were 100- and 10-fold (respectively) more common than expected and that the extent of kyphosis, and osteoporosis were higher than those of age-matched normal controls. A growing number of researchers have turned their attention to this problem in recent years.

Donovan et al., Grey et al., Bachrach et al., and Laursen et al. looked at adult CF patients and found significantly decreased BMD. Once it became apparent that osteopenia was a significant problem in the adult CF population, the question of when bone mineral deficiencies began became the subject of several research studies. Many researchers decided to include children and/or adolescents in their study populations. Gibbens et al., Haworth et al., two separate studies by Henderson and Madsen, and two separate studies by Bhudkhikanok et al. still found decreased bone density in these combined age group study populations.

Other researchers have found little or no evidence of bone mineral deficiencies in CF groups. It should be noted that these studies were all done on young CF populations with relatively mild disease. It is generally accepted that significant BMD deficiencies are problematic in adult CF populations, but when and why they begin is still not clearly understood.

Osteoporosis is usually the result of bone loss, which commonly occurs as adults age, but this is not always the case. Osteoporosis can also occur without accelerated bone loss when peak bone mass is not attained during childhood and adolescence. Factors affecting bone metabolism in children with CF may be especially complicated because
this population may risk both not reaching peak bone mass and early bone loss. Therefore, the periods of childhood and adolescence may be an especially important time to study bone density in CF. Much of the research done on bone density in CF is carried out with adult groups or combined youth and adult groups, in which children/prepubescent youth are not considered separately from adult/pubescent youth. Sex steroids secreted during puberty substantially increase BMD and because the age at onset of puberty can greatly differ in individuals, it is difficult to interpret and compare BMD/bone mineral content (BMC) results within a group of combined pubescent/prepubescent youths.

Only one published study was found that reported on a significantly sized group of prepubescent CF patients (and this study was published after our study was under way). Haslam et al.\textsuperscript{20} studied 22 prepubertal children with CF and found only a non-significant mild reduction in mean total body BMC. More research on prepubertal children with CF was needed. This research could be especially important because the prepubertal period may be an ideal time for preventive measures to be implemented.

Possible Causes of Osteopenia in Cystic Fibrosis

There are several known risk factors associated with low bone density. These include female gender, estrogen deficiency, increasing age, family history of osteoporosis, caucasian race, low body weight/body mass index (BMI), physical activity and smoking. Alcohol and caffeine intake and age at menarche and menopause may also be associated with decreased bone density. Although some of these risk factors may be
applicable in the case of CF patients, osteopenia in CF is definitely different than osteopenia in the general population.

There are several pathogenic factors of CF that could contribute to risk for low bone density. Many of them are related to poor nutritional status. Eighty-five to 90% of children with CF have exocrine pancreatic insufficiency which occurs when 98% of pancreatic exocrine function is lost. This leads to malabsorption of dietary nutrients (including calcium, magnesium, and vitamins D and K).

Although pancreatic enzyme replacement therapy is used to treat malabsorption, even optimal use of enzymes cannot fully compensate for pathogenic deficiencies. Benabdeslam et al. demonstrated this when they conducted biochemical assessment on 65 CF patients who were being treated with pancreatic enzyme replacement therapy and found that despite treatment, malnutrition was not completely corrected. Ninety-three percent of CF patients take pancreatic enzyme supplements. Published nutritional recommendations for both energy intake and specific nutrients for CF patients are higher than those for healthy populations, but are nearly impossible to precisely determine for individuals in various stages of growth and disease progression.

Malabsorption can lead to undernutrition resulting in suboptimal growth. It could also theoretically decrease peak bone mass (due to both suboptimal growth and malabsorption of bone metabolism-related nutrients, specifically calcium and vitamin D). Fat malabsorption, specifically, can additionally contribute to risk because not only is absorption of fat-soluble vitamins (like vitamin D) reduced, but it also produces excess fat in the stool to bind with dietary calcium. Eighteen percent of children with CF fall below the 5th percentile for weight and 16% fall below the 5th percentile for height. BMI
has been well documented as a predictor of BMD. No published studies were found on the effect of calcium fortification on bone density in CF.

Chronic diseases can alter body composition and delay sexual maturation. Delayed puberty (in adolescents) and hypogonadism (in adults) may affect bone metabolism. Delay in pubertal development is usually related to poor nutritional status and growth failure, rather than to a rare primary endocrine disorder. Delayed puberty may interfere with peak bone mass accretion, as shown by Henderson and Madsen in their study of 62 CF patients under 18 years of age. They found that although absolute BMD increased with age, when converted to a z score, it actually declined relative to normal values at a rate of approximately 1 standard deviation (SD) every 6 to 8 years. To further the problem, adults with hypogonadism may have accelerated bone loss. Better research in pre-pubescent CF populations may help determine the importance of these possible risk factors in the etiology of low bone density.

It is generally accepted that patients who chronically use corticosteroid medications for lung disease are at increased risk for poor bone health. In addition to possibly decreasing calcium absorption and suppressing linear growth, corticosteroid therapy may suppress osteoblastogenesis and promote apoptosis of osteoblasts and osteocytes. Steroid use alone is not responsible for the decreased bone density seen in CF, and in fact, low bone density is seen in patients who do not use corticosteroids. Research results like those from Bachrach et al. further confuse the issue. They found in their study that glucocorticoid therapy was not predictive of BMD. Therefore, the extent of its effect on bone density in CF is not fully understood.
Weight-bearing physical activity also affects bone density. The acquisition of maximal bone density is dependent upon getting adequate weight-bearing activity. Bass et al., Khan et al., and Jones and Dwyer all concluded from their research results that the prepubertal period may be vitally important in terms of the effects of physical activity on bone mass. Historically, children with CF were believed to be less active than healthy children due to decreased lung function and, consequently, decreased energy levels. However, the activity level differences between CF and non-CF children are now minimal. Conway et al., Bhudhikanok et al., and Haworth et al. found a significant positive association between BMD and physical activity. However, others, like Grey et al., have found no association.

Finally, disease severity may be an important factor for predicting BMD status in CF. Although several different factors can be taken into account when determining disease severity, chronic pulmonary infection and chronic respiratory acidosis specifically can negatively affect bone metabolism. In more severe cases of CF, the ongoing catabolic status and circulating inflammatory mediators (TNF-α, IL-1 and IL-6) are characteristic and can lead to an imbalance between bone formation and resorption. Henderson and Madsen and Haworth et al. found a relationship between BMD z scores and percent predicted forced expiratory volume in 1 second (FEV1%). Bachrach et al. and Haslem et al. did not find a relationship between BMD and disease severity.

This study is unique in that only pre-pubescent children with CF (and matched healthy controls) were recruited for the study in significant numbers. The purposes of this study are to evaluate the differences in bone mineralization between children with CF
and healthy control children. The results of this study will hopefully help health professionals to identify children in the CF population at risk for osteopenia.

**Hypotheses**

The hypotheses of this study are: (1) children with CF are smaller than healthy children without CF; (2) children with CF have less dense bones than healthy children without CF; (3) calcium intake is correlated with bone density in both children with CF and healthy children without CF.

**References**


CHAPTER 2
BONE DENSITY IN CHILDREN WITH CYSTIC FIBROSIS

Abstract

Aim—To compare bone density in children with cystic fibrosis (CF) and healthy children without CF, and to determine clinical correlates of bone density in both groups.

Methods—In 51 children with mild CF and 32 controls (ages 3-13), bone mineral density (BMD) of the lumbar spine (L2-L4) and proximal and distal radius and ulna were measured using dual energy x-ray absorptiometry (DEXA). The relation between BMD and anthropometrical measurements, dietary intake, biochemical indices, lung function and activity level were studied.

Results—Although the CF group was significantly lower in weight-for-age and height-for-age than the controls, they did not significantly differ from the controls in any measures of bone density. The CF group had significantly higher dietary intake of calories, protein, calcium and vitamin D than the control group. In the control group, there was a significant correlation between calcium intake and spine density z score. In the CF group, serum 25 hydroxyvitamin D (25(OH)D) levels were positively correlated with spine density z scores.

Conclusions—There were no bone deficits in this pre-pubescent CF population. Even though no dietary correlates for BMD were observed in the CF children, sup-optimal intakes or malabsorption, as well as worsening disease severity with age, may contribute to deficits over time.
Great advances in CF care have occurred in recent years. From 1985 through 2001, predicted survival for CF patients increased from about 25 years of age to about 33 years of age.\(^1\) This increase in life span has led to the development of a new set of long-term complications. These include diabetes, liver disease, malnutrition, suboptimal growth, infertility, and osteopenia.\(^2\) Osteopenia in CF patients can lead to premature osteoporotic fractures, which decrease quality of life and increase medical costs.

There are several pathogenic factors of CF that could contribute to the risk for low bone density. Many of them, like pancreatic insufficiency and suboptimal growth, are related to poor nutritional status. Other potential risk factors include delayed puberty in adolescents, hypogonadism in adults, chronic use of corticosteroid medications for lung disease, inadequate amounts of weight-bearing physical activity, and disease severity.

Much of the bone density research in CF is conducted with adult groups or combined youth and adult groups in which children/prepubescent youth have not been considered separately from adult/pubescent youth. This study is unique in that only prepubescent children with CF (and matched healthy controls) were recruited for the study. This research could be especially important because the prepubertal period may be an ideal time for preventive measures to be implemented.

The purposes of this study are to evaluate the differences in bone mineralization between children with CF and healthy control children and to determine which clinical variables can be used as predictors of risk for BMD deficits in children with CF. The
results of this study may be beneficial in the development of clinical screening tools and preventive measures for CF patients at risk for osteopenia.

Methods

Subjects

This study was approved by the University of Utah Medical Center’s Institutional Review Board (IRB). Study participants were recruited from the Outpatient Intermountain Cystic Fibrosis Center’s clinic at Primary Children’s Medical Center (PCMC) in Salt Lake City, Utah. This center serves children with CF from Utah, Idaho, Wyoming, and Nevada. At the beginning of this study, the CF clinic roster showed approximately 150 patients aged 2 to 16 years enrolled at the clinic. A letter describing the study, its purpose, and qualifications was sent to all qualified patients, inviting them to participate in the study (see Appendices A and C). Additionally, this letter included a request for referrals of healthy children without CF to serve as controls in the study (see Appendices B and C). Healthy controls were also recruited through advertisement within PCMC.

Fifty-one children with CF and 32 healthy controls ages 3 through 13 enrolled (18 of these were siblings of CF participants). A signed informed parental consent (see Appendices D and E), and when appropriate per patient’s age, assent (see Appendix F) form was obtained for each participant. All CF participants were to continue all treatments prescribed by their physician, including pancreatic enzymes, medications, and routine vitamin and mineral supplements per CF clinic protocol.
Data Collection

Most recent percent predicted forced expiratory volume in 1 minute (FEV$_1$%) score using Knudson equations$^4$ was obtained for each CF participant. The Knudsen equations are categorized as severe (<40%), moderate (40-69%), mild (70-89%), or normal (≥ 90%). Because several families had to travel from either another city or state to attend CF clinic, volunteer participants began the study on their next scheduled routine follow-up appointment. The range of dates for participation in the study were 11/09/1998 through 06/10/1999.

On the day that volunteers began the study, the study was explained in-depth by the graduate student, and any questions the participants or their parents had were answered before the consent/assent forms were signed. Preliminary data obtained on each subject included history of bone fractures (number, location[s] and cause[s] of) and biological parents’ self-reported heights (see Appendices G and H).

The CF participants brought a urine sample (first of that morning in a sterilized container, kept on ice) to clinic with them, which was frozen for later measurement of calcium, sodium, creatinine, and hydroxyproline. A 5-ml blood sample was obtained and frozen for later measurement of calcium, magnesium, phosphorus, 25(OH)D, 1,25 dihydroxyvitamin D(1,25(OH)$_2$D), parathyroid hormone (PTH), vitamin A, vitamin E, and alkaline phosphatase. Urine and serum samples were not obtained from the controls due to budget constraints, so normal reference values were used for comparison instead.

Participants and their parents were instructed on how to keep a 3-day diet record (see Appendices I and J) which was to be completed the following 3 days and then
returned by mail to the graduate student. Diet records were analyzed using Nutritionist IV (Nutritionist IV Diet Analysis, 1995 version 4.1, First Data Bank 1111 Bayhill Drive San Bruno, CA 94066) to evaluate intake of calories, protein, thiamin, vitamin D, vitamin K, calcium, phosphorus, and magnesium.

Anthropometric measurements were taken by the clinic’s registered dietitian. Weight was measured on a calibrated platform scale and height with the use of a stadiometer. Body mass index (BMI) was calculated using height and weight (kg/m²). Mid-arm circumference (MAC) and chest circumference (CC) were measured using a standard tape measure. Triceps (TSF) and subscapular skinfold (SS) measurements were determined with a Lange caliper, accurate to +/−1mm. De Meer et al. found skinfold measurements to be an appropriate tool in monitoring fat-free mass in all children with CF. Arm muscle circumference (AMC) was calculated using MAC and TSF [MAC – (TSF mm)π]. Arm muscle area (AMA) was calculated using MAC and TSF [(MAC−π(TSF mm))²/12.56]. Most recent percent predicted forced expiratory volume in 1 second (FEV₁,%) was obtained from each patient’s medical record.

Patients were then sent to the University of Utah Medical Center to have bone scans completed. The dominant arm’s radius and ulna bone mineral contents (BMC), BMD, and bone areas were measured with the Norland p-DEXA portable machine (Norland Corp., Fort Atkins, WI). The lumbar spine (L2-L4) BMC, BMD and bone area were measured using the Norland XR-26 DEXA bone absorptiometer. The accuracy error for determining bone mineral mass is <1% and the long-term precision error for both machines is <0.5%.6
BMCs were reported in grams, BMDs in grams per cm$^2$, and bone area in cm$^2$.

Currently, there are no evidence-based guidelines for classification of bone health in children. T scores are used for classification in adults, but because these patients have not reached their peak bone mass, T scores (standard deviations (SDs) in relation to sex matched peak BMD) were not appropriate for this young group. Therefore, z scores (the number of SDs that a BMD measurement is from the mean of an age and sex matched control population) were the best alternative. Spine BMD results were expressed as z scores to minimize the effects of age and sex. Appropriate reference data for these age groups could not be found for the other BMD measurement sites. This is probably due to the fact that technologic improvements in bone health assessment have out-paced pediatric research in this area.

SPSS version 10.0 was used to analyze the data (SPSS Inc. 1998, version 10, 444 N. Michigan Avenue Chicago, IL 60611). When t-tests were utilized, Levene’s Test was used for equality of variances. Pearson Correlations were used to examine associations between variables. For all tests, significance was based on a two-tailed test at the 0.05 $\alpha$ level. Trends were based on a two-tailed test at the 0.10 $\alpha$ level.

Results

Demographics and Anthropometrics

The anthropometric and demographic characteristics of the participants are reported in Table 1. The average age of the CF and control participants was 8.7 years (+/-2.8 years) and 8.4 years (+/-2.9 years), respectively. The healthy non-CF controls
had significantly higher means for height for age percentile (p=0.000) and weight for age percentile (p=0.000). The mean values for the control group were also significantly higher for measures of arm muscle and fat in terms of percentile for age, including mid-arm circumference (p=0.000), arm muscle circumference (p=0.000), arm muscle area (p=0.000), triceps skinfold (p=0.010), and subscapular skinfold (p=0.028) as shown in Table 1. The two groups did not significantly differ in their mean values for weight for height percentile, BMI or chest circumference. The CF participants’ mean FEV₁% value was 98.4 (SD+/-15.8), which is in the normal range.

Table 1
Anthropometric and demographic characteristics

<table>
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<tr>
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<th>CF</th>
<th>Controls</th>
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<tr>
<td>Age, yrs</td>
<td>8.7 +/- 2.8</td>
<td>8.3 +/- 2.7</td>
<td></td>
</tr>
<tr>
<td>FEV1%</td>
<td>16.4 +/- 1.5</td>
<td>16.9 +/- 2.2</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>3.3 +/- 1.8</td>
<td>4.7 +/- 1.8</td>
<td></td>
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<tr>
<td>Ht %ile group*</td>
<td>51</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Target ht %ile group*</td>
<td>51</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Wt %ile group*</td>
<td>51</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Wt/ht %ile group*</td>
<td>44</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>MAC %ile group**</td>
<td>51</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>AMC %ile group**</td>
<td>51</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>AMA %ile group**</td>
<td>51</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>TSF %ile group**</td>
<td>50</td>
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<td></td>
</tr>
<tr>
<td>SS (mm)</td>
<td>46</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

*1=<=5%, 2=5-<10%, 3=10-<25%, 4=25-<50%, 5=50-<75%, 6=75-<90%, 7=90-<95%, 8=>95%
**1=<=5%, 2=5-<10%, 3=10-<15%, 4=15-<20%, 5=20-<25%, 6=25-<30%, 7=30-<35%, 8=35-<40%, 9=40-<45%, 10=45-<50%, 11=50-<55%, 12=55-<60%, 13=60-<65%, 14=65-<70%, 15=70-<75%, 16=75-<80%, 17=80-<85%, 18=85-<90%, 19=90-<95%, 20=>95%
'p<0.03
'p<0.01

Anthropometric variables that positively correlated with spine BMD z scores in the CF group were height and weight for age percentiles (r=.325, p=.021 and r=.366, p=.009 respectively) as well as MAC (r=.404, p=.004), AMC (r=.379, p=.007), and AMA.
(r=.386, p=.006) percentiles. The control group showed a significant correlation for weight for age percentile and weight for height percentile with spine BMD z score (r=.379, p=.033 and r=.431, p=.040, respectively). There was also a correlation between BMD z score and MAC (r=.485, p=.006), AMC (r=.482, p=.006), and AMA (r=.507, p=.005) percentiles.

Dietary Intake

See Table 2. Although the CF group in this study easily exceeded general dietary intake recommendations for healthy individuals for the nutrients of interest in this study (protein, calcium, vitamin D, phosphorus, and magnesium), they just barely exceeded the recommended dietary allowance (RDA) for calories and did not meet the Cystic Fibrosis Foundation’s (CFF) recommendations for caloric intake. The CFF generally recommends 120-150% of the RDA for energy needs, while this CF group had a mean caloric intake of 105% of the RDA (SD 28). The controls’ mean was 60% of the RDA with an SD of 18.

In the control group (also in Table 2), there was a significant correlation (r=0.420, p=0.033) between percent dietary reference intake (DRI) calcium intake and spine density z score. There were no significant dietary correlates with spine BMD z score in the CF group. The controls had only a slightly higher mean self-reported activity level (3.1 vs. 2.8 on a scale of 0-5), and the difference was not significant.

Bone Measurements

Six CF participants and two controls had a history of previous fracture. None of these fractures were diagnosed as atraumatic, and none of these participants had spine
Table 2
Usual daily intake (per 3-day diet record) and activity

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Mean +/-SD</td>
<td>N</td>
</tr>
<tr>
<td>%RDA for Calories</td>
<td>39</td>
<td>60 +/-18**</td>
</tr>
<tr>
<td>kcals/kg</td>
<td>39</td>
<td>92 +/-27</td>
</tr>
<tr>
<td>%RDA for protein</td>
<td>39</td>
<td>224 +/-59**</td>
</tr>
<tr>
<td>grams protein/kg</td>
<td>39</td>
<td>2.1 +/-0.5**</td>
</tr>
<tr>
<td>%DRI for calcium</td>
<td>42</td>
<td>94 +/-30**</td>
</tr>
<tr>
<td>%RDA for vitamin D</td>
<td>39</td>
<td>111 +/-52**</td>
</tr>
<tr>
<td>%DRI for phosphorus</td>
<td>42</td>
<td>143 +/-53*</td>
</tr>
<tr>
<td>%DRI for magnesium</td>
<td>40</td>
<td>108 +/-35</td>
</tr>
<tr>
<td>Activity***</td>
<td>40</td>
<td>3.1 +/-0.8</td>
</tr>
</tbody>
</table>

*p<0.02
**p<0.01
*** on a scale of 0-5 (0=bed rest, 5=strenuous, sustained exercise)

BMD z scores less than or equal to -1.0. Table 3 shows the means for BMD, BMC, and spine area. There were no significant differences between the means for the control and CF groups for any of these raw values. When broken down by gender and age groups, there were still no significant differences. The CF patients’ mean BMDs were slightly higher for all three of the values noted, as well as the BMCs for both arm measurements. The control group had slightly higher means for the spine BMC and spine area. The spine z scores showed a slightly different picture than the raw BMD scores. The CF group had a negative mean z score (-0.001); the control group had a positive score (0.079), with the difference nearing significance (p=0.55).

Within the CF group, the difference between the males’ and females’ spine BMD z scores neared significance (p=0.062), with females having a positive mean spine density z score and males had a negative mean score. When broken down into age and
Table 3

Bone mineral density (g/cm²), bone mineral content (g), spine area (cm²) and spine z score

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>Controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean +/- SD</td>
</tr>
<tr>
<td>Distal radius+ulna BMD</td>
<td>50</td>
<td>0.217 +/- 0.045</td>
</tr>
<tr>
<td>Proximal radius+ulna BMD</td>
<td>50</td>
<td>0.449 +/- 0.098</td>
</tr>
<tr>
<td>Lumbar spine (L2-L4) BMD</td>
<td>50</td>
<td>0.588 +/- 0.107</td>
</tr>
<tr>
<td>Distal radius+ulna BMC</td>
<td>50</td>
<td>0.583 +/- 0.186</td>
</tr>
<tr>
<td>Proximal radius+ulna BMC</td>
<td>50</td>
<td>0.966 +/- 0.296</td>
</tr>
<tr>
<td>Lumbar spine (L2-L4) BMC</td>
<td>50</td>
<td>16.62 +/- 6.07</td>
</tr>
<tr>
<td>Spine area (L2-L4)</td>
<td>50</td>
<td>27.54 +/- 6.00</td>
</tr>
<tr>
<td>Lumbar spine (L2-L4) z score</td>
<td>50</td>
<td>-.012 +/- .594</td>
</tr>
</tbody>
</table>

*no significant differences between means

gender groups, both the control and CF groups had a negative spine BMD z score for females ages 10 through 13 and males ages 7 through 9.

Lab Values

Serum and urine samples were obtained from 40 of the CF participants (see Table 4). Serum 25(OH)D, the inactive form of vitamin D, was positively correlated (r=0.432, p=0.005) with spine density z score, BMI (r=0.417, p=0.007), height percentile group (r=0.334, p=0.035), and weight percentile group (r=0.426, p=0.006). Surprisingly, serum 25(OH)D was negatively correlated with serum vitamin A, another fat-soluble vitamin (r=-0.466, p=0.002). Serum 1,25(OH)₂D significantly correlated with serum PTH (r=0.358, p=0.029), as would be expected, but not with anything else.

Fat-soluble vitamin levels were generally low to normal (see Table 4) in the CF participants. More than half had low serum vitamin A levels and almost one third had low vitamin E levels. Very few participants had low levels of 25(OH)D. Vitamin A levels were negatively correlated with BMI (r=-0.339, p=0.033) and PTH (r=-0.336,
Table 4
Serum and urine laboratory values (CF patients)

<table>
<thead>
<tr>
<th></th>
<th>Below norm</th>
<th>Within norm</th>
<th>Above norm</th>
</tr>
</thead>
<tbody>
<tr>
<td>S Calcium</td>
<td>1</td>
<td>38</td>
<td>1</td>
</tr>
<tr>
<td>S Magnesium</td>
<td>5</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>S Phosphorus</td>
<td>7</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>S 25(OH) vitamin D</td>
<td>2</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>S 1,25 (OH)(_2) vitamin D</td>
<td>5</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>S Parathyroid hormone</td>
<td>2</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>S vitamin A</td>
<td>26</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>S vitamin E</td>
<td>12</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>S Alkaline phosphatase</td>
<td>13</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>U Calcium</td>
<td>4</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>U Sodium</td>
<td>(N/A)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U Creatinine</td>
<td>(N/A)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U Hydroxyproline</td>
<td>(N/A)*</td>
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</tr>
</tbody>
</table>

*Normal reference values not available/applicable

*p=0.039) and positively correlated with vitamin E levels (r=0.338, p=0.033). Alkaline phosphatase, a nonspecific measure of bone formation, was below normal levels in more than one third of CF participants. Alkaline phosphatase levels were negatively correlated (r=-0.405, p=0.012) with serum PTH levels.

Fourteen (37%) of the participants had elevated serum PTH, although only one had low serum calcium. As already mentioned, PTH was positively correlated with serum 1,25(OH)\(_2\)D. There was a weak positive correlation (r=0.305, p=0.063) between PTH and spine density average z score. For further analysis, the CF participants were divided into quartiles based on their PTH values. The top quartile had significantly higher (-0.3824 vs. 0.4099, p=0.017) spine density z scores than the bottom quartile. PTH was also positively correlated with AMC percentile (r=0.439, p=0.006) and AMA percentile (r=0.443, p=0.005).
Demographics and Anthropometrics

The CF population used in this study had mild disease severity compared to national averages and groups used in comparative studies. The CFF reported a range mean FEV₁% of approximately 95% to 80%, respectively, for children ages 6 up to 13.5 years. Our study included children ages 3-13.5 years and their mean FEV₁% score was 98% (SD 16), which is considered normal, and is a relatively high value, even for a young CF group like this one. Unlike Henderson and Madsen and Haworth et al., we did not find a relationship between BMD z scores and FEV₁%. Perhaps this was because most of our population had normal FEV₁% scores and BMD z scores. Haslam et al. also did not find a relationship between FEV₁% and BMD z scores in their children with CF.

It has often been hypothesized/stated that CF patients are less active than individuals without CF and that this may be a factor in decreased bone density. Although the control group had a slightly higher mean activity level than the CF group, the difference was not significant. This agrees with the findings of Grey et al. The only variable that activity level correlated with in either group was serum PTH in the CF group (r=0.394, p=0.028).

There was no significant difference between the groups’ mean target height percentiles. As expected, height and weight for age percentile groups were lower in the CF group, but not weight for height percentile groups. There was also no significant difference between the groups’ mean BMIs. McNaughton et al. found the same results in their study of 226 children with CF. Generally, a decreased height for age along with a
decreased weight for age is indicative of chronic malnutrition, and decreased weight for height indicates acute malnutrition. This may indicate that this CF population is mildly stunted and underweight, but not wasted.

**Dietary Intake**

The CF group had significantly higher nutrient intakes than the control group, as was seen in Mortensen and colleagues’ study,\textsuperscript{12} which was done in the same geographical area as our study. The higher intakes in the CF group as opposed to the control group (per percent of recommendations) of calories (75% higher in CF group, \(p=0.000\)), calcium (95% higher in CF group, \(p=0.003\)), protein (23% higher in CF group, \(p=0.007\)) and vitamin D (41% higher in CF group, \(p=0.005\)) may have had a protective effect on bone density as well as preventing nutritional wasting and severe growth stunting, which would additionally promote bone growth and strength.

It has been recommended\textsuperscript{13} that CF children’s and adolescents’ calcium intake levels should at minimum meet levels specified by the 1997 Institute of Medicine National Academy of Science, Food and Nutrition Board.\textsuperscript{14} The standard used for this study (DRI) meets or exceeds those recommended intake levels for each of the age groups represented in this study.

It is widely accepted that the CF population has greater nutrient needs, including that of calcium, than does the general population. While the CF group generously exceeded the DRI for calcium (183%), we do not know, at this time, a lot about the CF population’s or CF individuals’ actual calcium needs. Long-term studies that follow
calcium intake, pertinent lab values and bone growth may be necessary to better assess needs and judge intake levels for this population.

**Lab Values**

Serum alkaline phosphatase levels were low in more than one third of the CF patients. Alkaline phosphatase is an indicator of bone remodeling activity and turnover, although it is only a crude indicator because it is also associated with the liver. For future research, newer tests, like bone specific alkaline phosphatase, are becoming more affordable and give a better indication of what is going on specifically in bone.

It was interesting that, although it was weak and not significant, we found a positive correlation between PTH and spine density average z score. Other researchers, like Haworth et al., have reported a negative relationship between BMD z score and PTH. One other group of researchers, Bhudhikanok et al. also found a positive correlation between serum PTH and lumbar spine BMD. There is currently a great deal of research being done on the use of PTH as an anabolic agent in bone metabolism. Although there is evidence of PTH having net anabolic effects on bone, it is achieved through short-term intermittent injections once or twice daily, not sustained high levels produced in the body over a long period of time as would most likely be the case in secondary hyperparathyroidism. Secondary hyperparathyroidism is believed to lead to net bone deficits over time. Checking PTH levels periodically may be helpful in identifying individuals at risk for vitamin D deficiencies. Depending on PTH and vitamin D levels, a bone scan may be indicated as well.
Serum calcium levels are strictly controlled by the body's efficient homeostasis mechanisms, so it may not be surprising that serum calcium levels did not correlate with BMD. Therefore, serum Vitamin D metabolites should give us better information about possible nutrient deficiencies and bone metabolism in general. The two different measures of vitamin D used were 25(OH)D and 1,25(OH)$_2$D. Inactive vitamin D is first metabolized by the liver to 25(OH)D and then by the kidneys to the active form, 1,25(OH)$_2$D (calcitriol). When there is vitamin D deficiency, PTH levels rise and subsequently cause the production of more calcitriol. Nine out of 38 of our CF participants had elevated serum 1,25(OH)$_2$D, while 14 had elevated PTH.

Serum 25(OH)D may be a better indicator of vitamin D stores than 1,25(OH)$_2$D, because serum 1,25(OH)$_2$D levels decline only in severe deficiency when 25(OH)D levels are already depleted. Although only two participants had low 25(OH)D levels according the normal reference range used, several more patients may have had suboptimal levels, as indicated by elevated PTH and 1,25(OH)$_2$D. Surprisingly, neither of the CF participants with low 25(OH)D levels had elevated PTH levels, but both did have negative spine density z scores (-0.73 and -0.47).

We found a positive correlation between 25(OH)D and spine z score, quite possibly indicating the need to keep 25(OH)D levels at desirable levels. Poor absorption and/or utilization of oral intake of vitamin D may play a role in the development of osteoporosis in CF. Lark and colleagues' study$^{25}$ of 10 subjects with CF and 10 healthy controls showed that even with the use of supplemental pancreatic enzymes, CF subjects absorbed less than half of the vitamin the controls did. Additionally, rises in 25(OH)D levels were significantly lower in the CF group over time, which may suggest that
vitamin D conversion rates are reduced in CF subjects. This study also showed a high variability in CF subjects’ absorption rates, with two subjects showing virtually no absorption. All CF patients at this clinic were prescribed daily supplements of vitamins A, D, E and K at age-specific levels suggested for children with CF by the CFF, but this may not be adequate. Vitamin D levels should be checked at least once or twice per year. If oral supplementation of vitamin D is inadequate to sustain serum levels, parenteral administration of vitamin D may be warranted. It may be helpful to check alkaline phosphatase (or bone specific alkaline phosphatase, if available) levels periodically, as well.

Bone Measurements

It is difficult to compare studies on bone density because there is no general consensus in the research community on the most appropriate equipment and technique to assess bone. DEXA is currently the method of choice for measuring bone density because it is noninvasive, painless, relatively inexpensive, and gives high precision with low radiation dose. However, it has been argued that DEXA is potentially problematic when measuring and assessing patients with smaller than average size for age, as is the case with cystic fibrosis.

DEXA gives an areal BMD (gm/cm²), and not truly a volumetric (gm/cm³) measurement; therefore, it may underestimate bone mineral status in small bones. DEXA BMD is derived by dividing BMC within a defined region, in grams, by the projected area of the bone in cm². This means that bone thickness is not factored into these estimates; therefore, the bone densities of short people are inherently underestimated.
Researchers have tried to correct this in various ways. For example, they adjust DEXA BMD results for height and/or weight or use an estimated total vertebral volume to calculate “bone mineral apparent density” (BMAD). The effectiveness/accuracy of these methods has not been adequately proven or agreed upon. In addition, DEXA machines cannot distinguish between cortical and trabecular bone or give any information on bone architecture.

This pre-pubescent CF population did not significantly differ from controls in any of the measures of bone size or density used in this study. This agrees with the results of at least four other published studies,9,12,26,27 which also used children that were “well nourished” or had “mild” disease. Two other studies28,29 were found that reported a decrease in bone density in pediatric CF patients.

It is interesting to note the methods used to measure bone density in these comparative studies. Bhudhikanok et al.15 used DEXA to measure density of the lumbar spine (L2-L4) just as we did. They also measured whole body and hip bone density. Because they were worried about the bone densities of the smaller CF patients being underestimated, they calculated BMAD z scores as well. The disease severity of their pediatric CF patients was difficult to determine because the pediatric and adult patients were combined when reporting many of the characteristics and results of subjects. Although they did not separate results based on age or adult versus pediatric groups, they did state that bone mineral was reduced with increasing age in the majority of CF patients at all sites measured—most notably at the femoral neck (hip). After adjusting for smaller bones with BMAD scores, they still found significant differences between the CF group and controls (again, no differentiation made between children/adolescents/adults).
Our CF patients were smaller than the controls. This means we could theoretically expect the bone densities of our patients to be under-estimated with DEXA, leading to a finding of decreased bone density in the CF patients compared to controls. However, we did not see a difference in spine BMD z scores between the groups. Therefore, calculating BMAD would have been redundant in our case.

We cannot draw any conclusions about the disease severity of the children in the Bhudhikanok et al.\textsuperscript{15} study, which may in large part account for the differences in our results. In addition, they studied 49 patients ages 8.4 to 48.5, with a mean age of 20.6 years. Their actual pediatric population in the study could have been relatively small, as well as older than this study’s pediatric group.

The Gibbens et al.\textsuperscript{29} study is noteworthy because they used computer tomography (CT) scanning to determine authentic volumetric BMD and to distinguish between cortical and trabecular bone. Trabecular bone is relatively prominent in the vertebrae (at approximately 70\%) and hip (specifically, the intertrochanteric area), while the midshaft of long bones consists entirely of cortical bone.\textsuperscript{30} Because trabecular and cortical bone respond to stress and change differently, it may be especially important in disease states to study them separately. Cortical bone metabolism is controlled largely by hormones such as PTH and 1,25(OH)\textsubscript{2}D.

Differences in skeletal sites studied may also account for some of the seemingly wide variances in the research on bone density in CF. There is no general consensus on which bone sites are best to measure in children with CF. Because different sites have different proportions of cortical versus trabecular bone, variances in exact site(s) measured could produce drastically different results. We measured the radius, ulna and
lumbar spine in our study. Whereas the radius and ulna are comprised predominantly of cortical bone, the vertebrae are prominently trabecular bone. Unfortunately, we were unable to find sufficient reference data on the radius and ulna sites that we measured.

Although our data on the radius and ulna were not standardized, there were no significant differences in raw data averages for both BMD and BMC between the CF and control groups on these bones. None of the measurements done on the spine produced any significant differences between the groups, either (including the z score for L2-L4 spine density). When broken down by gender or age group, the only significant difference found was a higher mean value for the area of lumbar two in the 10-13 year old controls versus the 10-13 year old CF participants. When the groups were broken down further by gender and age, the only significant differences found were between the males ages 3-6 groups (BMC of L2-L4 and spine area of L2-L4) and the males ages 7-9 groups (spine area of L2). These differences could be explained by the difference in height and weight for age between the two groups.

In a review done on pediatric bone disease, Leonard and Zemel\textsuperscript{30} reported that in their study of others' research, they found no gender effect on bone density (cortical or trabecular) during childhood and adolescence, but they did in cross-sectional trabecular bone area (specifically, in the spine). Boys' vertebral bodies were wider than girls', even when adjusted for height and weight. We found no significant differences between males and females within the CF or control group on any of the bone measurements. Nor were there any significant differences between males and females in raw height, weight or BMI averages in the CF or control groups.
Although not significant, in our study females in both groups had higher mean spine density z scores than males (nearing significance in the CF group with a p value of 0.062). It is interesting to note that Bhudhikanok et al.\textsuperscript{15} also found a significant difference between males and females in bone density of the lumbar spine, with female patients having significantly higher values. (Although, in their 1998 study, Bhudhikanok et al.\textsuperscript{28} found no gender differences in BMD z scores in CF youth.) In addition, Jones and Dwyer\textsuperscript{31} found in their study of 330 8-year-old children that males had higher size-adjusted BMD at the hip; females had higher size-adjusted BMD at the spine. Therefore, our results may not be unexpected or unusual.

**Notable Individual Results**

In terms of individuals in this study, there were some noteworthy results. Low bone mass is generally defined as a BMD between 1 and 2.5 SD below a mean value for a given population. Goulding et al.\textsuperscript{32} reported from their findings that each SD decline below the mean total body BMD for youth doubles the risk of fracture. In our study, there were two children in both the CF and control groups than had BMD z scores less than \(-1.0\).

The control group had the two lowest BMD z scores (see Table 5). Both of them were 6-year old females with low height and weight. One of them did not complete a 3-day diet record or anthropometric measurements, but the other showed low dietary intake levels of calcium (61% DRI) and energy (66% RDA), and very low fat stores per TSF measurement (less than the 5\textsuperscript{th} percentile).
Both of the CF subjects with low spine bone density were males. One was 3 years old, the other 8. Both exceeded calcium intake levels, but the 3-year old exceeded CF recommendations for caloric needs while the 8-year old fell short. The 3-year old had close to average height and weight while the eight-year old was below the 5th percentile on height for age and below the 25th percentile on weight for age.

Laboratory values varied between these two subjects. The only noteworthy labs on the 3-year old subject were low PTH and 25(OH)D levels (per CFF proposed...
recommendations). Typically, one would expect to see elevated PTH if vitamin D levels were low. The 8-year old had several abnormal lab values, including low 1,25(OH)\(_2\)D, 25(OH)D (per CFF proposed recommendations), vitamin A, vitamin E, and alkaline phosphatase. PTH was elevated. These laboratory values could indicate malabsorption and consequent mild secondary hyperparathyroidism, which could be contributing to decreased bone density. He had good lung function, as evidenced by a high FEV\(_1\)% score (112%).

**Conclusion**

Several other studies have shown differences in young adult/adult groups, and Bhudhikanok et al.\(^{15}\) concluded from their research that “Osteopenia is common at all ages in cystic fibrosis…” But this is still a point of debate and was the focus of our research.

Pubertal development is often delayed in patients with CF, most likely due to growth failure and poor nutritional status. We studied prepubertal children to help determine exactly when bone loss, or slowed/inadequate bone gain, begins. It is fairly well established that osteopenia is prevalent in adults with CF, but there is more debate regarding younger patients. Because the pubertal period is so important in achieving peak bone mass, delayed puberty may decrease the final peak bone mass attained, and therefore, be at least one of the factors that leads to decreased bone density in adults with CF. Our results could support this theory.

Disease severity is another factor of interest based on our results. Because our CF group was young and had mild disease, another contributing factor to bone deficits could
be the advancing disease severity as patients age. Several researchers\textsuperscript{8,10,15,29} have shown a correlation between FEV\textsubscript{1} % and bone density, but these were seen in older groups with more advanced disease. We may not have seen this relationship because our CF group was generally healthy with good FEV\textsubscript{1} % scores and bone densities. Again, different bone sites may react differently to the stress of disease (and diet, exercise, biochemical serum levels, etc).

Perhaps different sites in the body where bone is a higher proportion of cortical bone would have shown more of a difference. Future studies should obtain multiple sites, possibly including spine, arm, hip, and femur. Although not as practical as DEXA in most circumstances, a whole body QCT (quantitative computed tomography) scan may give us the best information in future research. QCT is costly and high radiation exposure is a concern with this technique, but new technology is finding safer alternatives. Peripheral QCT, which uses high resolution scanners for the peripheral skeleton may be an option.\textsuperscript{30} As more knowledge and data are added to this body of research, we will be better able to standardize and compare results.

REFERENCES


CHAPTER 3
THE EFFECT OF FORTIFIED MILK ON BONE DENSITY
IN CHILDREN WITH CYSTIC FIBROSIS

Abstract

**Aim**—To compare bone density in children with Cystic Fibrosis (CF) and healthy children without CF, and to determine clinical correlates of bone density in both groups.

**Methods**—In 51 children with mild CF and 32 controls (ages 3-13), bone mineral density (BMD) of the lumbar spine (L2-L4) and proximal and distal radius and ulna were measured at baseline and at least three months later using dual energy x-ray absorptiometry (DEXA). The CF group was divided into two groups, one of which drank a milk product fortified with double the normal amount of calcium and vitamin D three times daily for three months. The other CF group drank a non-fortified milk product. The relation between BMD and anthropometrical measurements, dietary intake, biochemical indices, lung function, activity level, enzyme and vitamin usage, and malabsorption symptoms were studied.

**Results**—Although the CF group was significantly lower in weight-for-age and height-for-age than the controls, they did not significantly differ from the controls in any measures of bone density. Both the CF and control groups had significant increases in spine BMD z score over the course of the study. The CF group had significantly higher dietary intake of calories, protein, calcium and vitamin D than the control group at baseline. In the control group, there was a significant correlation between calcium intake and spine density z score. There were no significant differences between the regular and
fortified CF milk groups in any bone measurements. In the CF group, serum 25 hydroxyvitamin D (25(OH)D) levels were positively correlated with spine density z scores. Proper enzyme usage was correlated with malabsorption symptoms. 

Conclusions—There were no bone deficits in this pre-pubescent CF population. While no dietary correlates for BMD were observed in the CF children, sup-optimal intakes or malabsorption, as well as worsening disease severity with age, may contribute to deficits over time. While increasing calcium intake did not appear to increase bone density in this group of children with CF in the given length of time, the protective effect of calcium cannot be ruled out and should be studied in other age groups at varying levels of intake.
Great advancements in CF care have occurred in recent years. From 1985 through 2001, predicted survival for CF patients increased from about 25 years of age to about 33 years of age.\(^1\) This increase in life span has led to the development of a new set of long-term complications. These include diabetes, liver disease, malnutrition, suboptimal growth, infertility, and osteopenia.\(^2\) Osteopenia in CF patients can lead to premature osteoporotic fractures which decrease quality of life and increase medical costs.

There are several pathogenic factors of CF that could contribute to the risk for low bone density. Potential risk factors include delayed puberty in adolescents, hypogonadism in adults, chronic use of corticosteroid medications for lung disease, inadequate amounts of weight-bearing physical activity and disease severity.

Many correlated risk factors, like pancreatic insufficiency and suboptimal growth, are related to poor nutritional status. Eighty-five to 90% of children with CF have exocrine pancreatic insufficiency,\(^3\) which leads to malabsorption of dietary nutrients (including calcium, magnesium, and vitamins D and K). Although pancreatic enzyme replacement therapy is used to treat this, even optimal use of enzymes cannot fully compensate for pathogenic deficiencies. Standard nutritional recommendations (for both energy intake and specific nutrients) for CF patients are higher than that of healthy populations, but it is nearly impossible to precisely determine nutrient needs for individuals in various stages of growth and disease progression. Malabsorption can lead to undernutrition and resulting suboptimal growth. It could also theoretically decrease
peak bone mass (due to both suboptimal growth and malabsorption of bone metabolism-related nutrients, specifically calcium and vitamin D).

Much of the research done on bone density in CF is carried out with adult groups or combined youth and adult groups, in which children/prepubescent youth are not considered separately from adult/pubescent youth. This study is unique in that only prepubescent children with CF (and matched healthy controls) were recruited for the study in significant numbers. This research could be especially important because the prepubertal period may be an ideal time for preventive measures to be implemented.

This study is also unique in that it is an intervention study. No other published study was found at the time this study was done that implemented any kind of treatment or preventive measure in the study of bone density in CF. The intervention used in this study was a fortified milk product.

The purposes of this study are to evaluate the differences in bone mineralization between children with CF and healthy control children over a 3-month period, and to determine which clinical variables can be used as predictors of risk for BMD deficits in CF. The effect of calcium and vitamin D-fortified milk on bone density in children with CF will be evaluated. The results of this study may be beneficial in the development of clinical screening tools and preventive measures for CF patients at risk for osteopenia.
Methods

Sample Selection

This study was approved by the University of Utah Medical Center’s Institutional Review Board (IRB) and Utah State University’s (USU) IRB before participants were enrolled. Study participants were recruited from the Intermountain Cystic Fibrosis Center’s clinic at Primary Children’s Medical Center (PCMC) in Salt Lake City, Utah. This center serves children with Cystic Fibrosis (CF) from Utah, Idaho, Wyoming, and Nevada. At the beginning of this study, the CF clinic roster showed approximately 150 patients aged 2 to 16 years enrolled at the clinic. A letter describing the study, its purpose, qualifications, and possible benefits of enrollment (see Appendices A and C) was sent to all qualified patients, inviting them to participate in the study. Additionally, this letter included a request for referrals of healthy children without CF to serve as controls in the study (see Appendices B and C). Healthy controls were also recruited through advertisement within PCMC.

Fifty-one children with CF and 32 healthy controls (20 of these were siblings of CF participants) enrolled in the study. A signed informed consent (see Appendices D and E) and, when appropriate per patient’s age, assent (see Appendix F) form was obtained for each participant. All CF participants were to continue all treatments prescribed by their physician, including pancreatic enzymes, medications, and routine vitamin and mineral supplements per CF clinic protocol.
Data Collection

Participants with CF were stratified by age (ages 3-6, 7-10, and 11-16) and sex and paired with a control participant. Most recent percent predicted forced expiratory volume in 1 second (FEV$_1$%) score using Knudson equations$^5$ was obtained for each CF participant. The Knudsen equations are categorized as severe (<40%), moderate (40-69%), mild (70-89%), or normal (≥ 90%).$^5$ The participants in each CF pair were randomly assigned to either the treatment (fortified milk) or control (regular milk) group. Because several families had to travel from either another city or state to attend CF clinic, volunteer participants began the study on their next scheduled routine follow-up appointment. Follow-up appointments were routinely scheduled every 3 months. Per Dr. Gary Chan at the University of Utah Medical Center, a change in bone mineral accretion can be detected in that time frame, so subjects were to participate in the study for 3 months. The range of dates for beginning the study were 11/09/1998 through 06/10/1999, and the range of dates for completing the study was 01/26/1999 through 09/15/1999.

The CF participants began the study on the next regularly scheduled follow-up appointment. Control subjects made an appointment with the graduate student, a registered dietitian, and bone scan lab. On day 1 of the study, it was explained in-depth by the graduate student, and any questions the participants or their parents had were answered before the consent/assent forms were signed (see Appendices D, E, and F). Preliminary data was obtained on the patients, including history of bone fractures (number, location[s] and cause[s] of) and biological parents’ self-reported heights (see Appendices G and H).
The CF participants brought a urine sample (first of that morning) in a sterilized container kept on ice. It was frozen for later measurement of calcium, sodium, creatinine, and hydroxyproline. A serum sample was also obtained and frozen for later measurement of calcium, magnesium, phosphorus, 25(OH)D, 1,25 dihydroxyvitamin D (1,25(OH)₂), parathyroid hormone (PTH), vitamin A, vitamin E, and alkaline phosphatase. Urine and serum samples were not obtained from the controls, due to budget constraints, so normal reference values were used for comparison instead.

Participants and their parents were instructed on how to keep a 3-day diet record (see Appendices I and J), which was to be completed the following 3 days before they began drinking the study milk. Diet records were returned by mail to the graduate student and analyzed using Nutritionist IV (Nutritionist IV Diet Analysis, 1995 version 4.1, First Data Bank 1111 Bayhill Drive San Bruno, CA 94066) to evaluate intake of calories, protein, thiamin, vitamin D, vitamin K, calcium, phosphorus, and magnesium. They were also instructed on how to complete log sheets (see Appendices K and L). Log sheets were to be completed daily for the duration of the study and mailed weekly to the graduate student. Malabsorption symptoms, intake of high calcium foods, proper enzyme usage, vitamin/mineral supplementation, and activity level for each day were tracked via these forms. An information sheet on malabsorption was also given to participants to encourage use of prescribed enzymes (see Appendix M).

During the course of their regular follow-up visit for clinic that day, anthropometric measurements were taken by the clinic’s registered dietitian. Weight was measured on a calibrated platform scale and height with the use of a stadiometer. Body mass index (BMI) was calculated using height and weight (kg/m²). Mid-arm
circumference (MAC) and chest circumference (CC) were measured using a standard
tape measure. Triceps skinfold (TSF) and subscapular skinfold (SS) measurements were
determined with a Lange caliper, accurate to +/-1mm. De Meer et al.⁶ found skinfold
measurements to be an appropriate tool in monitoring fat-free mass in all children with
CF. Arm muscle circumference (AMC) was calculated using MAC and TSF [MAC –
(TSF mm)π]. Arm muscle area (AMA) was calculated using MAC and TSF [[MAC–
π(TSF mm)]²/12.56].

Patients were then sent to the University of Utah Medical Center to have bone
scans completed. The dominant arm’s radius and ulna bone mineral content (BMC),
BMD, and bone areas were measured with the Norland p-DEXA portable machine
(Norland Corp., Fort Atkins, WI). The lumbar spine (L2-L4) BMC, BMD and bone area
were measured using the Norland XR-26 DEXA bone absorptiometer. The accuracy
error for determining bone mineral mass is <1%, and the long-term precision error for
both machines is <0.5%.⁷

BMCs were reported in grams, BMDs in grams per cm², and bone area in cm².
Currently, there are no evidence-based guidelines for classification of bone health in
children. T scores are used for classification in adults, but because these patients have
not reached their peak bone mass, T scores (standard deviations (SD) in relation to sex
matched peak BMD) were not appropriate for this young group. Therefore, z scores (the
number of SDs that a BMD measurement is from the mean of an age and sex matched
control population) were the best alternative. Spine BMD results were expressed as z
scores to minimize the effects of age and sex. Appropriate reference data for these age
groups could not be found for the other BMD measurement sites. This is probably due to
the fact that technologic improvements in bone health assessment have out-paced pediatric research in this area.

Once all of the baseline data was obtained, the CF participants were given 3 months' worth of milk product (see “Description of Milk Product”, below). The study milk product was described and additional information on the milk was given to patients and their parents (see Appendix N). CF participants were to drink 8 oz of this milk three times each day for the duration of the study. Control subjects did not drink this milk product, nor were they to make any dietary changes during the period of the study.

There were several reasons that a whole milk product was used to administer the calcium to participants. First, it has been advised that 60% of the recommended daily allowance (RDA) of calcium be dairy calcium.\textsuperscript{8,9,10} Second, in CF clinic, the patients are strongly encouraged to drink whole milk, as it is a good source of calories, protein, calcium, vitamin D and other vitamins and minerals. We wanted to reinforce this, as well as provide an “intervention” that many of the participants were already using. Because the study population is a juvenile one, it was predicted that flavored whole milk would be more palatable and accepted and more easily taken by children (especially the very young children) than a pill. A third reason to use whole milk as the source of calcium was that many of these children are already taking several pills/medications as part of their CF treatment, and any additional pill may have decreased compliance for this study or made the study burdensome for some children. There is no conclusive data that there is better absorption of calcium from milk versus other sources, but milk does contain lactose and casein phosphopeptides, which may enhance calcium absorption and mineral retention.\textsuperscript{11}
Follow-up letters (see Appendix O) were sent to participants as needed through the duration of the study to ensure diet records and daily logs were kept and sent to the graduate student. Upon completion of the study, participants returned to PCMC to repeat the same anthropometrical and bone density measurements made at the beginning of the study.

Description of Milk Product

Two flavors (chocolate and vanilla) of ultra high temperature (UHT) whole milk (manufactured by Gossner's in Logan, Utah) were used for this study. The milk was packaged in sterile 8-oz boxes with drinking straws attached to them. The boxes were white and unmarked, except for a letter code stamp, to blind the participants from knowing the type of milk they were assigned to consume. This type of milk was chosen because it does not require refrigeration and has a long shelf life (11 months), making it more practical for participants to take 3 months' worth of milk home with them the day they started the study. It also allowed for better compliance by making it more convenient for participants to drink three boxes each day, whether at home, school, or on vacation.

Two popular flavors (as determined by a taste panel in the CF clinic) were used to help increase acceptance of milk product by children in the study. Vanilla and chocolate flavoring were used and produced milks of very similar nutritional content. Participants were given six cases (with 27 boxes of milk per case or 162 cartons) of each flavor unless otherwise requested.
The milk was fortified to double the amounts of both calcium and vitamin D normally found in 8 oz of Gossner’s UHT milk (see Table 6). The calcium fortifier used was Lactoval QM (LQM) Calcium Complex (manufactured by DMV International Nutritionals, The Netherlands). LQM is 24.5% calcium and also includes phosphate (36.2%) citric acid (18.6%), sodium (2.7%) and trace amounts of other minerals. It also contains small amounts of protein (1.0%), moisture (5.5%), lactose (1.0%) and fat (0.4%). This calcium fortifier was chosen due to its compatibility with milk. It has a high solubility, bland flavor, neutral odor, smooth mouth feel, slow sedimentation in liquids, and a particle size of 95%<9 um (DMV International Nutritionals, The Netherlands). LQM was added to the whole milk at a ratio of 5.225 gm LQM powder per kg milk. Each box of finished milk was approximately 240 gm or 8 oz.

The formulation and testing of the milk product was done at USU. Eight ounces of Gossner UHT milk regularly contains 100 International Units (IU) (equal to 2.5 micrograms) of vitamin D, which was doubled to 200 IU (5 micrograms). This totaled 600 IU per day of vitamin D for the children on the fortified milk. The PCMC CF clinic recommends 400-800 IU per day.

Because some settling of the calcium fortifier was possible, calcium analysis was done periodically (by USU Analytical Laboratories, Logan, UT) throughout the duration of the study. Through these analyses, it was found that a significant amount of the calcium settled out of the fortified vanilla milk throughout the duration of the study (see Table 6).
Table 6  
Calcium content of study milk

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
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<tr>
<td>E</td>
<td>Fortified chocolate</td>
<td>552</td>
<td>504</td>
<td>528</td>
<td>528</td>
<td>528</td>
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<tr>
<td>WC</td>
<td>Regular chocolate</td>
<td>264</td>
<td>240</td>
<td>240</td>
<td>**</td>
<td>248</td>
</tr>
<tr>
<td>D</td>
<td>Fortified vanilla</td>
<td>408</td>
<td>264</td>
<td>360</td>
<td>240</td>
<td>318</td>
</tr>
<tr>
<td>C</td>
<td>Regular vanilla</td>
<td>240</td>
<td>240</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
</tbody>
</table>

*Amount of calcium in mg per 240 gm milk (amount in each 8 oz box)  
**Unable to complete analysis

The amount of each flavor and the time period in which it was consumed were taken into account when possible for each participant. This was done by noting the time period in which the patients on fortified milk participated in the study (and using the corresponding values for calcium found in the fortified vanilla milk during that time period, using an average of multiple time periods if needed) and also taking into account what proportion of chocolate versus vanilla fortified milk the participant drank. In addition, not all participants drank the full amount of milk that they were instructed to drink. If the study milk was not consumed, participants were to report how much they did drink and when they drank it. Phone calls to remind participants to complete and mail in daily logs (enzyme use and calcium intake) were made monthly.

Statistical Analyses

SPSS version 10.0 was used to analyze the data (SPSS Inc. 1998, version 10, 444 N. Michigan Avenue Chicago, IL 60611). When t-tests were utilized, Levene’s Test was used for equality of variances. Pearson Correlations were used to examine associations between variables. For all tests, significance was based on a two-tailed test at the 0.05 α level. Trends were based on a two-tailed test at the 0.10 α level.
Results

Demographics and Anthropometrics

The anthropometric and demographic characteristics of the participants are reported in Table 7. The average age of the CF and control participants was 8.7 years (+/-2.8 years) and 8.4 years (+/-2.9 years), respectively. The mean length of participation (time between initial bone density scans/anthropometrical measurements and final ones) in the study was 20 weeks (+/-9.5 weeks) for the CF participants and 17 weeks (+/-5.0 weeks) for the controls. For a report of baseline data, see Chapter 2 Results (page 19).

Over the course of the study period, the CF and control groups' anthropometrical means changed differently (see Table 7). Although the changes weren't significant, the CF group’s mean height percentile group slightly declined, while the control’s increased slightly. The CF group’s weight for height percentile mean value went slightly up (p=0.059) while the control group’s went slightly down (p=0.835). The means for the CF group’s TSF and MAC percentile groups and SS measurements went up significantly (p=0.035, 0.005 and 0.042, respectively), but the control group’s did not change significantly. While the weight percentile group means remained virtually unchanged for both groups, the control group saw a greater increase in height, although not significant.

Relationships between changes in arm fat and muscle stores differed between the CF and control groups. Change in the CF group’s TSF percentile group negatively correlated with change in AMA (r=-0.381, p=0.017) and AMC (r=-0.373, p=0.019) percentile groups. In the control group, change in TSF percentile group positively
Table 7
Initial and final anthropometric and demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean +/-SD</td>
<td>N</td>
</tr>
<tr>
<td>Start age, yrs</td>
<td>51</td>
<td>8.7 +/-2.8</td>
<td>32</td>
</tr>
<tr>
<td>End age, yrs</td>
<td>44</td>
<td>9.0 +/-2.8</td>
<td>29</td>
</tr>
<tr>
<td>Weeks in study</td>
<td>44</td>
<td>20 +/-9.5</td>
<td>29</td>
</tr>
<tr>
<td>Start FEV,%</td>
<td>46</td>
<td>98.4 +/-15.8</td>
<td>(N/A)</td>
</tr>
<tr>
<td>End FEV,%</td>
<td>27</td>
<td>98.1 +/-17.4</td>
<td>(N/A)</td>
</tr>
<tr>
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<td>51</td>
<td>5.1 +/-1.2</td>
<td>30</td>
</tr>
<tr>
<td>Start ht %ile group*</td>
<td>51</td>
<td>3.3 +/-1.8</td>
<td>32</td>
</tr>
<tr>
<td>End ht %ile group*</td>
<td>39</td>
<td>3.0 +/-1.7</td>
<td>27</td>
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<td>Start wt %ile group*</td>
<td>51</td>
<td>3.8 +/-1.3</td>
<td>32</td>
</tr>
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<td>3.7 +/-1.3</td>
<td>27</td>
</tr>
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<td>44</td>
<td>4.7 +/-1.2</td>
<td>23</td>
</tr>
<tr>
<td>End wt/ht %ile group*</td>
<td>32</td>
<td>4.9 +/-0.9</td>
<td>16</td>
</tr>
<tr>
<td>Start MAC %ile group**</td>
<td>51</td>
<td>7.6 +/-4.6</td>
<td>31</td>
</tr>
<tr>
<td>End MAC %ile group**</td>
<td>40</td>
<td>8.3 +/-4.9</td>
<td>29</td>
</tr>
<tr>
<td>Start AMC %ile group**</td>
<td>51</td>
<td>9.6 +/-5.0</td>
<td>31</td>
</tr>
<tr>
<td>End AMC %ile group**</td>
<td>40</td>
<td>10.2 +/-5.1</td>
<td>29</td>
</tr>
<tr>
<td>Start AMA %ile group**</td>
<td>51</td>
<td>9.5 +/-5.0</td>
<td>29</td>
</tr>
<tr>
<td>End AMA %ile group**</td>
<td>40</td>
<td>10.0 +/-5.2</td>
<td>29</td>
</tr>
<tr>
<td>Start TSF %ile group**</td>
<td>50</td>
<td>6.5 +/-4.3</td>
<td>31</td>
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<td>End TSF %ile group**</td>
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<tr>
<td>Start SS (mm)</td>
<td>46</td>
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</tr>
<tr>
<td>End SS (mm)</td>
<td>40</td>
<td>7.8 +/-10.6</td>
<td>28</td>
</tr>
</tbody>
</table>

*1= <5%, 2=5-<10%, 3=10-<25%, 4=25-<50%, 5=50-<75%, 6=75-<90%, 7=90-<95%, 8=>1=95%
**1=<5%, 2=5-<10%, 3=10-<15%, 18=85-<90%, 19=90-<95%, 20=>1=95%
'p<0.03
'p<0.01

correlated with change in MAC percentile group (r=0.453, p=0.014), and change in weight percentile group positively correlated with change in AMA and AMC percentile groups (r=0.608, p=0.001 and r=0.550 and p=0.003, respectively). None of the measures of change in arm fat and muscle stores correlated with change in spine BMD z score in either group. In the control group, change in TSF percentile group did correlate with change in distal arm BMD (r=0.466, p=0.011).
Initial Dietary Intake

For a report on the initial 3-day diet intakes, see Chapter 2 Results (page 20).

Daily Logs and Study Milk

The mean length of participation in the study for the CF group was 20 weeks. The mean length of time that the daily logs were kept was 6.6 weeks (range of 0 to 17.7 weeks). The daily vitamin consumption rate was 92% for this group. While the CF participants were instructed to drink three boxes of study milk/day during the study, the mean consumption was only 1.7 boxes of milk/day. In addition to the study milk, they averaged 2.5 servings of high calcium foods/day. When the calcium contents of the study milk and high calcium foods were added together, the mean percent Dietary Reference Intake (DRI) of calcium from these sources during the study was 151% (+/-70) for the CF group.

The CF participants who received fortified milk were compared with the CF participants who received regular milk. The fortified milk group averaged 1.8 (SD 1.1) boxes of study milk per day, which was 917 mg calcium per day, while the regular milk group averaged 1.7 (SD 1.1) boxes per day, which was 442 mg calcium per day. The groups did not significantly differ in the number of additional servings of high calcium foods eaten per day (2.6 +/- 1.2 in the fortified group versus 2.4 +/- 1.3 in the regular group). When the calcium contents for the study milk and high calcium foods were added together, the fortified group had a mean daily calcium intake of 1486 mg (SD 517) and the regular group’s was 978 mg (SD 485).

There were no significant differences between the fortified milk CF group and the
regular milk CF group in any measures of bone density or changes in bone density (Table 8). However, there were a couple of differences found between the regular milk CF group and the control group that were not evident in the fortified milk CF group versus the control group. The regular milk CF group had less change in proximal arm BMD per week (p=0.073) as well as significantly lower final TSF percentile group values

<table>
<thead>
<tr>
<th>CF fortified milk</th>
<th>CF regular milk</th>
<th>No milk/controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Mean +/- SD</td>
<td>N</td>
</tr>
<tr>
<td>Start age</td>
<td>29   8.5 +/- 2.9</td>
<td>21   9.0 +/- 2.6</td>
</tr>
<tr>
<td># of weeks in study</td>
<td>27   18.9 +/- 8.0</td>
<td>17   21.8 +/- 11.6</td>
</tr>
<tr>
<td># of weeks daily log kept</td>
<td>29   7.8 +/- 5.5</td>
<td>21   5.2 +/- 5.5</td>
</tr>
<tr>
<td>Avg # of box milk/day*</td>
<td>18   1.8 +/- 1.1</td>
<td>9     1.7 +/- 1.1</td>
</tr>
<tr>
<td>Avg # servgs high Ca foods/day*</td>
<td>24   2.6 +/- 1.2</td>
<td>16   2.4 +/- 1.3</td>
</tr>
<tr>
<td>Avg mg Ca intake from box milk + high Ca foods/day*</td>
<td>17   1486 +/- 517</td>
<td>9    978 +/- 485**</td>
</tr>
<tr>
<td>%DRI for Ca from daily logs*</td>
<td>17   164 +/- 67</td>
<td>8     121 +/- 74</td>
</tr>
<tr>
<td>%DRI for calcium from 3DDR</td>
<td>25   195 +/- 185</td>
<td>17   165 +/- 171</td>
</tr>
<tr>
<td>S 25(OH) vitamin D*</td>
<td>20   34 +/- 17</td>
<td>19   35 +/- 18</td>
</tr>
<tr>
<td>S 1,25(OH)2 vitamin D**</td>
<td>18   47 +/- 25</td>
<td>19   36 +/- 31</td>
</tr>
<tr>
<td>S PTH**</td>
<td>18   66 +/- 41</td>
<td>19   44 +/- 28**</td>
</tr>
<tr>
<td>Initial FEV₁,%</td>
<td>25   97 +/- 17</td>
<td>20   99 +/- 15</td>
</tr>
<tr>
<td>Final FEV₁,%</td>
<td>18   103 +/- 15</td>
<td>8     87 +/- 20*</td>
</tr>
<tr>
<td>Initial spine (L2-L4) z score</td>
<td>28   -0.018 +/- 0.558</td>
<td>21   0.031 +/- 0.646</td>
</tr>
<tr>
<td>Change in L2-L4 z score/wk</td>
<td>26   0.008 +/- 0.013</td>
<td>17   0.013 +/- 0.027</td>
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<tr>
<td>Initial spine (L2-L4) BMD</td>
<td>28   0.585 +/- 0.116</td>
<td>21   0.595 +/- 0.098</td>
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<tr>
<td>Change in spine (L2-L4) BMD/wk</td>
<td>26   9.92 +/- 1.39-3</td>
<td>17   1.20-3 +/- 1.53-3</td>
</tr>
<tr>
<td>Initial distal radius+ulna BMD</td>
<td>28   0.218 +/- 0.046</td>
<td>21   0.216 +/- 0.045</td>
</tr>
<tr>
<td>Change in distal r+u BMD/wk</td>
<td>24   9.63-5 +/- 2.21-3</td>
<td>17   7.90-4 +/- 1.49-3</td>
</tr>
<tr>
<td>Initial proximal r+u BMD</td>
<td>28   0.448 +/- 0.103</td>
<td>21   0.451 +/- 0.095</td>
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</tbody>
</table>
| Change in proximal r+u BMD/wk | 24   6.68-4 +/- 1.13-3 | 17   (2.93-4 +/- 9.56-4 | 29   9.23-4 +/- 1.22-3)**

*per daily log sheets

*does not include total dietary intake

*in ng/ml, normal reference range used = 12-60, CFF proposed minimum = 30

*in pg/ml, normal reference range used = 18-62

*in pg/ml, normal reference range used = 11-54

*p<0.10

**p<0.04
The fortified milk group had a mean serum PTH value that was above normal and higher than the regular milk group’s (p=0.064), which was within the normal range.

### Table 9
**Daily logs (fortified and regular CF milk groups combined)**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td># of weeks log was kept</td>
<td>51</td>
<td>6.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Servings high Ca foods/day(^a)</td>
<td>40</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Boxes of study milk/day(^b)</td>
<td>27</td>
<td>1.7</td>
<td>1.1</td>
</tr>
<tr>
<td>High Ca foods + study milk (mg)/day</td>
<td>25</td>
<td>1323</td>
<td>553</td>
</tr>
<tr>
<td>Missed or late enzymes/day(^c)</td>
<td>40</td>
<td>0.42</td>
<td>0.86</td>
</tr>
<tr>
<td>Malabsorption symptoms/day(^d)</td>
<td>40</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Daily vitamins taken(^e)</td>
<td>40</td>
<td>0.92</td>
<td>0.13</td>
</tr>
</tbody>
</table>

\(^a\) 1 point each for 8 oz milk, 1 oz cheese, 5 oz yogurt; 1/2 point each for 4 oz pudding, 6 oz cottage cheese, 3/4 cup broccoli
\(^b\) A box of study milk is 8 oz
\(^c\) 1 point = no enzymes at meal, 1/2 point = no enzymes at snack, or enzymes taken after meal/snack instead of before eating
\(^d\) 1 point each for: more than 2 bowel movements/day, large amount of stool or diarrhea, stool floats in toilet, visible grease/oil with stool in toilet, gas/bloating
\(^e\) 'O'=vitamin not taken, 'I'=vitamin taken

The fortified milk group had significantly higher (p=0.037) final FEV\(_1\)\% scores than the regular milk group. Other than FEV\(_1\)\% scores, there were no significant differences between the regular and fortified milk CF groups, so they were combined to report daily log data (Table 9). Per the daily logs, the CF participants averaged 1.1 malabsorption symptoms per day. The mean missed enzymes/day score was 0.42, with the following scoring system: one point for no enzymes at a meal, or one half point for no enzymes for a snack or if enzymes were taken after a meal or snack instead of before. The missed enzymes scores were highly correlated (r=0.529, p=0.001) with malabsorption symptoms. Missed enzymes were negatively correlated with the final
AMC and AMA (r=-0.392, p=0.024 and r=-0.391, p=0.024, respectively) percentile groups, as well as the final FEV$_1$% score (r=-0.513, p=0.012). The change in FEV$_1$% score was negatively correlated with daily activity level (r=-0.582, p=0.004).

Bone Measurements

Six CF participants and two controls had a history of previous fracture. None of these fractures were diagnosed as atraumatic, and none of these participants had spine BMD z scores less than or equal to -1.0. Table 10 shows the group means for final and initial BMDs, BMCs, and spine areas. There were no significant differences between the means for the controls and CF group for any of these values. For a report on baseline bone measurements, see Chapter 2 Results.

Within the CF group, the difference between males’ and females’ initial mean z score neared significance (p=.062), with females having a positive initial mean z score while males had a negative score (see Table 10). The difference between CF males’ and females’ final mean spine BMD z scores (females’ mean z score was 0.5033, males’ was -0.0594) was significant with a p value of 0.007. Jones and Dwyer\textsuperscript{12} found in their study of 330 8-year olds that females had higher size-adjusted BMD at the spine, while males had higher size-adjusted BMD at the hip. No significant differences were found between males’ and females’ BMD z scores in the control group, although the males’ mean scores were slightly higher than the females’.

Although there was no significant difference in either the initial or final BMD, BMC, spine area, or spine BMD z scores for the CF and control groups, there was a
Table 10

Initial and final BMD (g/cm²), BMC (g), spine area (cm²) and spine z score

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>Controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean +/-SD</td>
</tr>
<tr>
<td>Start distal radius + ulna BMD</td>
<td>50</td>
<td>0.217+/-0.045</td>
</tr>
<tr>
<td>End distal radius + ulna BMD</td>
<td>41</td>
<td>0.226+/-0.056</td>
</tr>
<tr>
<td>Start proximal radius + ulna BMD</td>
<td>50</td>
<td>0.449+/-0.098</td>
</tr>
<tr>
<td>End proximal radius + ulna BMD</td>
<td>41</td>
<td>0.460+/-0.112</td>
</tr>
<tr>
<td>Start lumbar spine (L2-L4) BMD</td>
<td>50</td>
<td>0.588+/-0.107</td>
</tr>
<tr>
<td>End lumbar spine (L2-L4) BMD</td>
<td>43</td>
<td>0.610+/-0.121</td>
</tr>
<tr>
<td>Start distal radius + ulna BMC</td>
<td>50</td>
<td>0.583+/-0.186</td>
</tr>
<tr>
<td>End distal radius + ulna BMC</td>
<td>41</td>
<td>0.610+/-0.220</td>
</tr>
<tr>
<td>Start proximal radius + ulna BMC</td>
<td>50</td>
<td>0.966+/-0.296</td>
</tr>
<tr>
<td>End proximal radius + ulna BMC</td>
<td>41</td>
<td>0.982+/-0.314</td>
</tr>
<tr>
<td>Start lumbar spine (L2-L4) BMC</td>
<td>50</td>
<td>16.62+/-6.07</td>
</tr>
<tr>
<td>End lumbar spine (L2-L4) BMC</td>
<td>43</td>
<td>17.29+/-6.69</td>
</tr>
<tr>
<td>Start spine area (L2-L4)</td>
<td>50</td>
<td>27.54+/-6.00</td>
</tr>
<tr>
<td>End spine area (L2-L4)</td>
<td>43</td>
<td>27.60+/-5.83</td>
</tr>
<tr>
<td>Start lumbar spine (L2-L4) z score</td>
<td>50</td>
<td>-0.012+/-0.594</td>
</tr>
<tr>
<td>End lumbar spine (L2-L4) z score</td>
<td>43</td>
<td>0.189+/-0.699</td>
</tr>
<tr>
<td>Change in spine z score/wk</td>
<td>43</td>
<td>0.0096+/-0.0196</td>
</tr>
</tbody>
</table>

*no significant differences between means of two groups

difference in how they changed over the course of the study (Table 10). There was a significant difference in the change in spine BMD per week between the CF (mean=0.0028) and control (mean=0.0011) groups (p=0.000). However, when looked at in terms of change in spine BMD z score per week, the CF group mean was slightly higher that the control group’s mean, although not significant (p=0.827). When initial and final spine BMD z scores were compared within the groups, both the CF and control groups saw a significant increase over the period of the study (p=0.000).

When the CF and control groups were split by gender and compared again, the difference between the CF and control males’ mean change in spine BMD z score per week (0.0048 and 0.0116, respectively) neared significance (p=0.075), with the control males seeing a greater gain. When gender groups were compared within the CF and
control groups, the control males had a significantly higher increase (p=0.053) than the females (mean=0.0058). In the CF group, the difference neared significance (p=0.069) with the female group (mean=0.0157) having a greater increase in change in spine BMD z score per week than the males. Table 10 shows the group means for BMD, BMC, and spine area. There were no significant differences between the means for the controls and CF group for any of these values.

Arm BMD values could not be converted to z scores for this data group. However, it is interesting to note than change in L2-L4 BMD z score was positively correlated with change in distal radius plus ulna BMD in both the CF and control groups (r=0.424, p=0.06 and r=0.442, p=0.016, respectively), but not with change in proximal radius plus ulna BMD. Change in proximal and distal arm BMDs were not correlated with each other in either group.

Because individuals had varying lengths of enrollment in the study, change in mean spine BMD z score was figured per week. There was no significant difference between the two groups in change in mean spine BMD z score per week. When the groups were split by gender however, there was a significant (p=0.044) difference between the CF and control females, with the CF females having a greater increase in mean z score. The control males had a slightly higher mean change in spine BMD z score than the CF males, but it was not significant. There were no significant differences between the groups in change in arm BMD per week.

Regression analyses using the enter method were done for final spine BMD z score and change in spine BMD z score per week. However, even though the adjusted R square was high (0.455) for the final spine BMD z score model, the only significant
predictor was TSF percentile group (p=0.018). In the model with change in spine BMD z score per week as the dependent variable, the beta values were very low and no predictors were significant. A regression model may not be an appropriate assessment tool for this set of data because list-wise only 20 of the participants had all of the entered variables.

**Lab Values**

Serum and urine samples were obtained from 40 of the CF participants (see Table 4). For a report on lab values and correlations with baseline data, see Chapter 2 Results. Serum 25(OH)D, the inactive form of vitamin D, was positively correlated with the final MAC (r=0.442, p=0.009), AMC (r=0.371, p=0.031) and AMA (r=0.366, p=0.033) percentile groups.

Serum vitamin A levels were positively correlated with change in proximal arm BMD per week (r=0.346, p=0.049) and negatively correlated with final MAC percentile group (r=-0.359, p=0.037), final TSF percentile group (r=-0.456, p=0.007), and final subscapular skinfold value (r=-0.343, p=0.047). No other lab values significantly correlated with change in spine BMD z score per week, change in distal arm BMD per week or change in proximal arm BMD per week. Serum vitamin E levels negatively correlated with final MAC (r=-0.440, p=0.009), AMC (r=-0.436, p=0.010), and AMA (r=-0.433, p=0.010) percentile groups.

**Compliance**

Several participants in the CF group were noncompliant in meeting and/or reporting all of the requirements for the study. Therefore, compliance was looked at as a
factor in itself. The CF group was broken down into compliant (n=17), less compliant (n=8) and unknown groups (n=16). The compliant group was simply defined as those who drank at least 75% of the boxed study milk and had both sets of bone scans completed. The less compliant group was defined as those who drank less than 75% of the boxed milk and had both sets of bone scans completed. The unknowns were those who failed to record or report how much of the milk they drank, but still completed the bone scans. The only significant difference found between these groups was for change in distal radius plus ulna BMD per week. The compliant and unknown groups both had significantly greater increases than the noncompliant group (p=0.002 and 0.012, respectively). The difference between the compliant and noncompliant groups in change in weight per week neared significance (p=0.085) with the compliant group gaining more weight.

Discussion and Conclusions

Demographics and Anthropometrics

The CF population used in this study had mild disease severity compared to national averages and groups used in comparative studies. The Cystic Fibrosis Foundation\(^1\) (CFF) reported a range mean FEV\(_1\)% of approximately 95% to 80%, respectively, for children ages 6 up to 13.5 years. Our study included children ages 3 through 13.5 years and their mean FEV\(_1\)% predicted score was 98% (SD 16), which is considered normal, and is a relatively high value, even for a young CF group like this one. Unlike Henderson and Madsen\(^13\) and Haworth et al.,\(^14\) we did not find a relationship between BMD z scores and FEV\(_1\)%.

Perhaps this was because most of our population
had normal FEV₁% scores and BMD z scores. Haslam et al.¹⁵ also did not find a relationship between FEV₁% and BMD z scores in their children with CF.

The differences in how change in measures of arm muscle and fat correlated with each other and weight may reflect differences in how the groups gain weight. The fact that change in TSF percentile group negatively correlated with changes in AMA and AMC percentile groups may reflect the fact that when children with CF gain muscle, it is at the expense of fat tissue, or vice versa. Meanwhile, in the control group, change in TSF percentile group positively correlated with change in MAC percentile group, suggesting that gains in arm fat or muscle are additive, not replacing the other. Because adequate fat stores/nutrition are necessary for linear growth, this may be evidence of malnutrition associated with growth stunting in children with CF.

The control group also saw a correlation between change in TSF percentile group and change in distal arm BMD, while the CF group did not. Perhaps weight gain (in terms of fat) influences arm bone density to a greater extent and/or more quickly than spine bone density. (The control group had a [nonsignificant] greater increase in weight percentile group than the CF group.)

Changes in anthropometric percentile groups for age are difficult to interpret. This study was completed in less than a year for most participants. Meanwhile, age is used in increments of whole years to determine percentile groups. Therefore, the biggest determinant of change in percentile group may be whether a participant’s birthday happened to fall within the study period, thus putting them in the next age group. Likewise, small increases in percentile group for age may be attributed to participants growing while still remaining in the same age group.
For example, a participant may go from recently turning 7 years old at the beginning of the study to being almost 8 years old at the end of the study. They grow during this time period, but still remain in the same age group. They go from being one of the youngest, and usually smaller individuals in this age group to being one of the oldest, and usually larger individuals in this age group. Therefore, their percentile group value may increase while their exact percentile for age may not.

Another factor affecting change in percentile group is whether an individual was at the low or high end of their percentile group at the beginning of the study. Someone at the high end of their percentile group would be able to move up to the next percentile group much more easily than someone who started at the low end.

Fortified Milk Group vs. Regular Milk Group

On average, CF participants drank only about half of the required amount of study milk, and kept the daily logs for only about one third of the required amount of time. This made it difficult to analyze and interpret the results.

Even with the lack of compliance, the fortified and regular milk groups ended up consuming about the same amount of boxed milk and high calcium foods. Therefore, the difference in total calcium intake came from the fortification of milk. The fortified milk group ended up having double the calcium intake from boxed milk than the regular milk group did, and an average of approximately 50% more total calcium per day than the regular milk group.

Another factor to take into consideration is that the “total” calcium intake from the boxed milk and high calcium foods consumed over the course of the study is not truly
a “total” calcium intake. Because participants did not keep a complete diet record, this
“total” calcium intake is probably lower than actual calcium intake. The only foods
recorded on the daily logs were specific high calcium foods. However, other foods eaten
may still have had significant levels of calcium. For example, calcium fortified foods
(like some orange juices, breakfast cereals, or snack foods), ice creams and sherbets, and
cream soups or sauces could add significant amounts of calcium to the daily diet, not to
mention other foods eaten that could contain lesser or trace amounts of calcium. “Total”
calcium intake for the CF group over the course of the study was probably significantly
underestimated.

Having stated this, the CF group as a whole still had a significantly higher
(p=0.014) mean calcium intake than the control group when their boxed milk and high
calcium food calcium contents alone (mean daily “total” calcium intake between initial
and final bone scans) were compared to the control group’s mean daily calcium intake
from the 3-day diet record at the beginning of the study.

Although it probably would have been difficult to get this study group to comply,
another 3-day diet record completed while drinking the study milk would have been
informative. It could have shown if the study milk was being drunk in addition to the
usual diet or in place of food items (specifically high versus low calcium foods) usually
eaten. It was assumed that the controls’ 3-day diet records would be representative of
their typical intake throughout the study period.

Although there were no significant differences between the fortified and regular
milk CF groups in any measures of bone (Table 8), the fact that the CF regular milk
group had significantly less change in proximal radius plus ulna BMD per week than the
control group did may be significant. Meanwhile, the CF fortified milk group did not
differ significantly from the control group. In addition, although not significant, the
fortified milk group had double the change in proximal radius plus ulna BMD per week
than the regular milk group had (Table 8). So, while significant differences and changes
in spine BMD and spine BMD z scores were hard to find, maybe the proximal bones of
the arm better reflect short-term changes in calcium intake than does the spine.

One factor that may have made differences between the fortified and regular milk
groups difficult to find was initial serum PTH values (Table 8). The fortified group as a
whole had much higher PTH values than the regular milk group. As noted in the
previous section, the difference neared significance. Not only did the fortified milk
group have higher values than the regular milk group, but their mean value was even
above the normal range. Because serum samples were obtained at the start of the study,
nothing done in the study could have influenced PTH values. Individuals were randomly
assigned to milk groups, so it is interesting that there was such a difference between the
groups with regards to PTH value. It is a factor that may have significantly impacted the
results of the study, however. An elevated PTH value could be indicative of mild
secondary hyperparathyroidism, which is a risk factor for osteopenia. In future studies,
perhaps PTH values should be obtained prior to grouping individuals so that it can either
be controlled for or used as a grouping variable.

Daily Logs

As expected, the missed enzyme score highly correlated with malabsorption
symptoms. However, malabsorption symptoms did not correlate with any of the serum
fat-soluble vitamin levels, or any of the serum/urine lab values, as might have been expected. They did not correlate with spine BMD z scores, either. It would have been interesting to have had z scores for BMD values of other bone sites, like the arm or hip, to see if malabsorption symptoms correlated with other types/sites of bone.

Although FEV₁ % did not correlate with malabsorption symptoms, it did negatively correlate with missed enzymes. This means that the more enzymes missed, the lower the final FEV₁ % score was. This may be of interest because while patients’ observation and interpretation of malabsorption symptoms is a more subjective value, number of enzymes missed is an objective value. As may be expected, change in FEV₁ % score and activity level were negatively correlated. In CF, FEV₁ % scores generally are maintained in patients who are doing well, or decrease as patients age/decline in overall health. This means that as FEV₁ % scores declined, so did activity level. Although causation cannot be concluded from correlation, it would make sense that decline in lung function would lead to decreased activity tolerance.

It has often been hypothesized/stated that CF patients are less active than individuals without CF, and that this may be a factor in decreased bone density. Although the control group had a slightly higher mean activity level than the CF group, the difference was not significant. This agrees with the findings of Grey et al.¹⁶

**Bone Measurements**

Our study was unique not only in that it used an intervention (study milk), but also because a second bone scan was done at least 3 months after the initial one. This allowed us to see how CF patients’ bone measurements changed over time compared to
controls. We saw no significant differences between the CF and control groups in how their spine bone density z scores changed over the course of the study.

The only other longitudinal study found that was done on bone density in CF was the one published by Bhudhikanok et al. in 1998. They did include some children in their study, but the youngest was nine years old, and they were combined as a group with youth up to age 18 (n=20) in the results. Bhudhikanok et al. reported that their youth showed either static or decreasing BMD z scores (spine, hip, and whole body) over the 1.5 year period of their study.

In our study, spine BMD z scores significantly increased in both the control and CF groups. And, although the control group had significantly more improvement in change in spine BMD per week, there was virtually no difference between the groups when looked at in terms of change in spine BMD z score per week. Perhaps because z scores were determined categorically by age, it was more difficult to find significance in changes. For example, a participant who had a birthday while in the study could theoretically have the same change in bone density as another individual of the same initial age in years, but his/her z score may not reflect that change because their final z score would reflect the average of the next year of age group.

Although not significant, both we and Bhudhikanok et al. found greater improvement in the CF females’ mean spine BMD z score than in the males’. In our study, the difference neared significance with a p value of 0.069. There were no differences in the CF group between the genders in changes in arm BMDs per week. In the control group, the males did have significantly higher improvement in change in distal arm BMD per week (0.0012 versus 0.0002, p=0.045) than the females did.
It was notable that the CF females had significantly more improvement in spine BMD z scores than both the control females and the CF males. There were no significant differences between the genders in change in weight, height, or weight-for-height percentile groups. So while the gender differences in bone density cannot be attributed to differences in weight gained, there are likely other gender-specific variables that influence bone mineral accretion rates in various bone sites.

Lab Values

Other than vitamin A and change in proximal arm BMD per week, none of the lab values correlated with change in arm BMDs per week or change in spine BMD z score per week, as might be expected. There may be several reasons this relationship was not found. One might be that over the course of the study lab values improved if individuals took vitamins and enzymes more regularly/correctly than they did prior to the study because they had to report on it daily. Another possibility might be that the follow-up bone scan was too soon to show effects of high or low lab levels on bone formation.

The fact that two of the fat-soluble vitamins (A and E) were negatively correlated with final measures of arm muscle/mass may be noteworthy, particularly for nutrition monitoring. Those with low vitamin A and E levels may be at increased risk for nutritional wasting. Perhaps malabsorption is a factor in both low serum vitamin levels and less muscle/mass accumulation.

Compliance

Noncompliance on the part of participants was one of the biggest obstacles of this study. A large N value was an important objective to ensure that significance in results
would not be limited by a small study population. There could be several reasons for noncompliance in this population. One is that individuals with CF have highly regimented care. This includes treatments like daily home chest physical therapy, aerosol treatments, vitamin supplements, enzyme pills, high-calorie/protein diets, dietary supplements, regular check-ups, and hospital stays. In talking with care providers at the CF clinic as well as CF patients and their parents, we found that patients and their caregivers sometimes feel overwhelmed or discouraged by the demands of CF treatment.

A main consideration in the development of this study was the rigorous care for individuals with CF, and trying to design a study with which they would be able to comply with. So, while the ideal may have been more detailed information, for example in dietary and activity records, or more information, like follow-up labs, these requirements probably would not have been practical or successful with this population. Perhaps in future studies where more funding is available, a pilot study should be done to identify and select compliant participants, who would then be randomly assigned to treatment groups. Greater incentive for compliance (like prizes or monetary compensation) may also help obtain a more detailed and complete data set.

The fact that the compliant group in this study had a significantly greater increase in distal radius plus ulna BMD per week than the non-compliant group is noteworthy. The fact that these individuals were compliant with study requirements may indicate that they were also compliant with other aspects of their CF care. It would make sense that non-compliance itself may be a risk factor for osteopenia in CF. CF patients who do not correctly or regularly follow prescribed care like medications, vitamins, supplements, diets, therapies, check-ups, and routine hospital visits would be more likely to get
sick/infected more often (leading to deterioration of the lungs, lower activity levels, and/or declined overall health), and be malnourished, with consequent stunting of growth and delayed puberty. These are all likely risk factors for osteopenia.

**Notable Individual Results**

In terms of individuals in this study, there were some noteworthy results (Table 11). Low bone mass is generally defined as a BMD between one and 2.5 SD below a mean value for a given population. Goulding et al.\textsuperscript{18} reported from their findings that each SD decline below the mean total body BMD for youth doubles the risk of fracture. In our study, there were two children in both the CF and control groups than had BMD z scores less than –1.0.

The control group had the two lowest BMD z scores (see Table 11). Both of them were 6-year old females with low height and weight. One of them did not complete a 3-day diet record or anthropometric measurements, but the other showed low dietary intake levels of calcium (61% DRI) and energy (66% RDA), and very low fat stores per TSF measurement (less than the 5\textsuperscript{th} percentile).

Both of the CF subjects with low spine bone density were males. One was 3 years old, the other 8. Both exceeded calcium intake levels, but the 3-year old exceeded CF recommendations for caloric needs while the 8-year old fell short. The 3-year old had close to average height and weight while the 8-year old was below 5\textsuperscript{th} percentile on height for age and below the 25\textsuperscript{th} percentile on weight for age.

Labs varied between these two subjects. The only noteworthy labs on the 3-year old subject were low PTH and 25(OH)D levels (per CFF proposed recommendations).
## Table 11

Initial and final characteristics of individuals with low bone density

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M, age 3</td>
<td>M, age 8</td>
<td>Group Mean</td>
<td>F, age 6</td>
</tr>
<tr>
<td>ID#</td>
<td>34</td>
<td>20</td>
<td>-</td>
<td>73</td>
</tr>
<tr>
<td>Start L2-L4 BMD z score</td>
<td>-1.07</td>
<td>-1.10</td>
<td>-0.01</td>
<td>-1.17</td>
</tr>
<tr>
<td>End L2-L4 BMD z score</td>
<td>-1.10</td>
<td>-1.12</td>
<td>0.19</td>
<td>N/A</td>
</tr>
<tr>
<td>Change in L2-L4 BMD z score/wk</td>
<td>-0.0022</td>
<td>-0.0017</td>
<td>0.0097</td>
<td>N/A</td>
</tr>
<tr>
<td>Target ht %ile group*</td>
<td>6</td>
<td>4</td>
<td>5.1</td>
<td>5</td>
</tr>
<tr>
<td>Start ht %ile group*</td>
<td>5</td>
<td>1</td>
<td>3.3</td>
<td>2</td>
</tr>
<tr>
<td>End ht %ile group*</td>
<td>5</td>
<td>1</td>
<td>3.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Start wt %ile group*</td>
<td>4</td>
<td>3</td>
<td>3.8</td>
<td>2</td>
</tr>
<tr>
<td>End wt %ile group*</td>
<td>5</td>
<td>2</td>
<td>3.7</td>
<td>N/A</td>
</tr>
<tr>
<td>Wt ht %ile group*</td>
<td>4</td>
<td>4</td>
<td>4.7</td>
<td>4</td>
</tr>
<tr>
<td>Start AMC %ile group**</td>
<td>8</td>
<td>3</td>
<td>9.6</td>
<td>N/A</td>
</tr>
<tr>
<td>End AMC %ile group**</td>
<td>10</td>
<td>3</td>
<td>10.2</td>
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<tr>
<td>Start AMA %ile group**</td>
<td>8</td>
<td>3</td>
<td>9.5</td>
<td>N/A</td>
</tr>
<tr>
<td>End AMA %ile group**</td>
<td>10</td>
<td>3</td>
<td>10.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Start TSF %ile group**</td>
<td>4</td>
<td>8</td>
<td>6.5</td>
<td>N/A</td>
</tr>
<tr>
<td>End TSF %ile group**</td>
<td>4</td>
<td>13</td>
<td>7.1</td>
<td>N/A</td>
</tr>
<tr>
<td>FEV, %</td>
<td>(too young)</td>
<td>112</td>
<td>98.4</td>
<td>N/A</td>
</tr>
<tr>
<td>$1.25$(OH)$_2$ vitamin D$^*$</td>
<td>54</td>
<td>10</td>
<td>41.1</td>
<td>N/A</td>
</tr>
<tr>
<td>S 25(OH) vitamin D$^*$</td>
<td>20.5</td>
<td>25.0</td>
<td>33.6</td>
<td>N/A</td>
</tr>
<tr>
<td>S PTH$^*$</td>
<td>10</td>
<td>62</td>
<td>53.8</td>
<td>N/A</td>
</tr>
<tr>
<td>S vitamin A$^*$</td>
<td>56</td>
<td>39.5</td>
<td>51</td>
<td>N/A</td>
</tr>
<tr>
<td>S vitamin E$^*$</td>
<td>16.5</td>
<td>12.4</td>
<td>14.1</td>
<td>N/A</td>
</tr>
<tr>
<td>S alkaline phosphatase$^*$</td>
<td>93.5</td>
<td>28.4</td>
<td>64.6</td>
<td>N/A</td>
</tr>
<tr>
<td>%RDA for Calories$^*$</td>
<td>117</td>
<td>150</td>
<td>105</td>
<td>N/A</td>
</tr>
<tr>
<td>%DRI for calcium$^*$</td>
<td>238</td>
<td>274</td>
<td>183</td>
<td>N/A</td>
</tr>
<tr>
<td>Avg # enzyme doses missed/d</td>
<td>0</td>
<td>2.0</td>
<td>0.4</td>
<td>N/A</td>
</tr>
<tr>
<td>Avg # malabsorption symptoms/d</td>
<td>0.6</td>
<td>2.3</td>
<td>1.1</td>
<td>N/A</td>
</tr>
<tr>
<td>Avg activity level (on scale 0-5)</td>
<td>2.5</td>
<td>2.7</td>
<td>2.8</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*1=5%, 2=5-<10%, 3=10-<25%, 4=25-<50%, 5=50-<75%, 6=75-<90%, 7=90-<95%, 8=>95%  
**1=5%, 2=5-<10%, 3=10-<15%, 4=15-<20%, 5=20-<25%, 6=25-<30%, 7=30-<35%, 8=35-<40%, 9=40-<45%, 10=45-<50%, 11=50-<55%, 12=55-<60%, 13=60-<65%, 14=65-<70%, 15=70-<75%, 16=75-<80%, 17=80-<85%, 18=85-<90%, 19=90-<95%, 20=>95%  
$^a$in pg/ml, normal reference range used = 18-62  
$^b$in ng/ml, normal reference range used = 12-60, CFF proposed minimum = 30  
$^c$in pg/ml, normal reference range used = 11-54  
$^d$in mg/ml, normal reference range used = 30-100 for ages 1-5y, 60-100 for ages 5-16y  
$^e$in mmol/l, normal reference range used = 10-21 for ages 1-6y, 13-24 for >6y  
$^f$in IU/L, normal reference range used = 47-112

Typically, one would expect to see elevated PTH if vitamin D levels were low. The 8-
year old had several abnormal lab values, including low \(1,25(\text{OH})_2\text{D}, 25(\text{OH})\text{D}\) (per CFF proposed recommendations), vitamin A, vitamin E, and alkaline phosphatase. PTH was elevated. These labs could indicate malabsorption and consequent mild secondary hyperparathyroidism, which could be contributing to decreased bone density. He had good lung function, as evidenced by a high FEV\(_1\%\) score (112%).

Both of the CF subjects completed the study, but only one of the two controls did. Both of the CF subjects had a slightly negative change in spine BMD z score per week while the control had a slightly positive change (see Table 11). There were also differences in how their anthropometrics changed. The 8-year old CF male stayed in the same AMC and AMA percentile groups (group three for both) over the course of the study, but went from percentile group eight to 13 for TSF. He was catching up in fat stores, but remained malnourished in terms of muscle stores. The 3-year old CF male increased in both AMC and AMA percentile groups (from eight to 10 for both), but TSF percentile group stayed the same (group four). The 6-year old control female went down in both AMC and AMA percentile groups (from 12 to nine for both), but remained in group one for TSF percentile (less than the 5\(^{th}\) percentile for age).

Both CF children surpassed the RDA for caloric intake and more than doubled the DRI for calcium intake (Table 11). On average, the 3-year old CF male missed less than one half an enzyme dose each day and had less than one malabsorption symptom per day. On average, the 8-year old CF male missed two enzyme doses each day and had over two malabsorption symptoms per day. This evidence would support the above-mentioned theory that this child had significant malabsorption, contributing to low serum fat-soluble
vitamin levels, including vitamin D, leading to secondary hyperparathyroidism and consequent bone deficits.

References


CHAPTER 4
CONCLUSIONS

Our results were in full accordance with one of our three hypotheses. The hypotheses of this study were: (1) children with Cystic Fibrosis (CF) are smaller than healthy children without CF; (2) children with CF have less dense bones than healthy children without CF; (3) calcium intake is correlated with bone density in both children with CF and healthy children without CF. Children in this CF study population are smaller than healthy children without CF in the same population. It could not be proved that children with CF have less dense bones than healthy children without CF. Calcium intake was not correlated with bone density in children with CF, but there was a correlation in the healthy control children.

No major differences were found in how children with CF and healthy controls gain bone mass. The results of these two studies concur with at least four other published studies,\(^1\)\(^2\)\(^3\)\(^4\) as discussed in the individual papers, and provide further evidence for normal bone density in children with CF. However, the fact that there are at least two other published studies\(^5\)\(^6\) that have shown decreased bone density in youth with CF requires addressing.

Each of these studies was done on a different CF population, except for the Mortensen et al.\(^2\) study, which drew its participants from the same population (same geographical area and clinic) as this study and also found no bone deficiencies in CF youth. So, perhaps CF populations in different geographical areas differ in mean bone density. Factors such as continuity and quality of care at individual CF centers could be a
contributing factor to overall health of individuals. Where a CF population is geographically located may affect the amount of sunshine exposure patients receive in a given year, thus affecting vitamin D levels.

If deficits are due to a CF-related primary defect in bone mineral metabolism, they may only be evident in more severe disease/as disease progresses. Our CF population was young and had mild disease per percent predicted forced expiratory volume in 1 second (FEV$_1$%). Perhaps multi-center research involving larger child populations with varying degrees of disease severity would help to define if and what the role of disease severity is in the development of osteopenia.

Another important point is that our CF group was well-nourished and had mild disease relative to national CF averages and other studies. If the etiology of osteoporosis in CF is related to either of these factors, our population may be more protected than other groups studied. Perhaps deficits seen in other studies were related to more severe malnourishment or more advanced disease/poorer health and related factors (such as activity). Then, the question would be “Why does this population have relatively mild disease and better nutritional status?” These would be issues to address in future research.

The age of a study population could also significantly impact results. The range and mean of ages in a study group would impact mean bone density for a group of “children,” “youth,” “adolescents,” or “pre-pubescents” in each of these studies. The size of the study group is also important in determining if results/differences are significant. As mentioned in the individual papers, some of the studies referenced had small study groups. Ours was the largest pre-pubescent study group of those found on
bone density in CF. Our group was also younger overall than other study groups, which could have several implications. One may be that delayed puberty and insufficient catch-up are important in the etiology of osteopenia. Our study group was young enough that these factors should not have had an impact yet. Additional studies on separate pre-pubescent, pubescent, and post-pubescent groups would help to confirm if this were the case.

Another important factor in all studies done on bone density, and perhaps one of the greatest sources of variance between studies, is type of bone measurement – both bone site and equipment/technique used. There is no generally accepted standard at this time to allow direct comparison in study results. While dual energy x-ray absorptiometry (DEXA) is currently considered the “gold standard” by many in the field due to precision, accessibility, low radiation, and lower cost, computer tomography (CT) gives much more information, but at the cost of higher radiation exposure, less accessibility, and higher price. Until the field of bone density research agrees upon a standard, both in bone site measured and equipment/technique used, the puzzle of osteoporosis in CF may be a difficult one to solve.

There is need for better reference data and diagnostic criteria for bone disease in children. Until there is, it will be difficult to standardize and compare results within and between studies. Not only is more adequate data needed for bone density in children in general, but also in relation to body size and pubertal stage to make results more meaningful.

New markers of bone metabolism are being researched and discovered continually. As new tests become available that are more precise, affordable and
accessible, researchers will be able to better pinpoint which metabolic markers may indicate risk for net bone deficits. In our study, parathyroid hormone (PTH) was an interesting variable in relation to bone density and vitamin D levels. The results of our study would merit additional research on the relationship between PTH and bone density in CF. In the meantime, more focus should be placed on bone density within CF clinics by implementing screening tools, like monitoring serum PTH, vitamin D and alkaline phosphatase levels to identify those children who may be at risk for developing osteopenia. Then, additional precautions can be taken, like increased vitamin D/calcium supplementation or bone scans, or even just monitoring at-risk individuals more frequently.

Malabsorption is an ever-present problem in CF. Our study group was no exception, as evidenced by abnormal lab values, malabsorption symptoms, and suboptimal enzyme usage. These indicators all point to risk for nutrient deficiencies which could increase risk for osteopenia in the future. Future longitudinal studies need to address and study the issue of malabsorption and bone density in CF more closely.

Osteoporosis in CF is a very complicated issue to research. CF and osteoporosis are very complex diseases independently, and to try and find individual relationships when looking at them together is very difficult. It is probably safe to assume that osteoporosis in CF is most likely multifactorial. The fact that the substantial amount of research that has been done points in so many directions, rather than at just one or two factors, would attest to this. The two individuals in this CF group with low bone density may also be evidence of this. Compared to each other, they had very different profiles. There may be various risk factors for osteopenia in CF present to different extents in
different individuals, and when external/environmental factors figure in also, individual variance in risk could be very high.

Although additional calcium and vitamin D did not significantly increase bone density in this study, they can not be ruled out as treatment options by any means. It may just be that levels were not right, or that the time period in which they were administered were not long enough to see results. Or, perhaps the high levels of calcium that this CF group was already consuming was at a level for maximum benefit in terms of bone building. Perhaps in individuals with CF who are consuming sub-optimal levels of calcium this type of fortification may be a very inexpensive and convenient way to help reduce risk for osteopenia. The additional calories, fat, protein, vitamin D, and other nutrients contained in the milk product would be of nutritional benefit, as well.

Additional intervention studies further examining calcium and vitamin D, as well as other osteoporosis treatment options (like those used in post-menopausal women), in CF populations that have or are at risk for osteoporosis would be informative and beneficial in developing preventative and treatment options.

References

APPENDICES
APPENDIX A

INVITATION FOR CHILDREN WITH CF TO PARTICIPATE IN STUDY
Dear Parent(s):

We invite your child to participate in a research study on bone mineralization in children with cystic fibrosis (CF). This study is being conducted by Primary Children’s Medical Center, the Intermountain Cystic Fibrosis Center, and Utah State University. There will be no additional cost to you for participation. The care your child receives at Primary Children’s Medical Center or the Intermountain CF Center will not be affected by whether you decide to have your child participate or not participate in this study.

**Purpose:**
The purposes of this study are to evaluate the differences in bone mineralization between children with CF and children without CF, and the effect of a specially fortified milk on bone mineralization. It is estimated that up to 20-50% of children with CF may have undermineralized or weak bones. Improving bone mineralization will promote better health.

**Qualifications:**
Your child must have CF, be between the ages of 3 and 16 years, and not reached puberty.

**Requirements:**
Your child will be asked to:
1) Drink three 8 ounce servings of either regular milk or fortified milk each day for at least three months. The milk will be provided for your child.
2) Have blood drawn at the beginning of the study. We will try to coordinate these blood draws with the routine or annual lab work done in CF clinic.
3) Give a urine sample.
4) Have a bone scan done (similar to an X-ray). Your child will need to lie still for about 10 minutes.
5) Keep a 3-day diet record at the beginning of the study.
6) Keep a simple daily log of enzyme use, activity level, malabsorption symptoms and dietary calcium intake. (Estimated time to complete this log will be less than 5 minutes per day.)

**Benefits:**
1) Assessment of bone mineralization (early detection if there are problems)
2) Three servings of milk per day for at least three months at no cost
3) Increased skills in monitoring signs and symptoms of malabsorption
4) Possibly stronger bones
5) Possibly improved nutritional status

We are enclosing a response card and postage-paid self-addressed envelope. If we do not hear back from you in the next week, we may call you to obtain your preference. We look forward to hearing from you.

Sincerely,

Joanna Kelley
USU Graduate Student

Katie McDonald, RD
Dietitian, CF Outpatient PCMC
(801) 588-3898
APPENDIX B

LETTER INSERT FOR RECRUITMENT OF HEALTHY CONTROLS
P.S. We are also looking for healthy children without CF to participate in this study. To be eligible, they must be between the ages of 3 and 16 and not have reached puberty yet. They will not need to drink any special milk or certain amount of milk -- they will consume their usual diet. They will not need to give a blood or urine sample or keep a daily log. They will be asked for the following:

1) a bone scan (like an x-ray) at the beginning of the study
2) anthropometric measurements (height, weight, etc.) at the beginning and end of the study (which will last 3-4 months)
3) a 3-day diet record at the beginning of the study

If you would be interested in having any of your children without CF involved in this study (you may have more than one child enrolled in the study - with or without CF), please write their name(s), sex and age below along with a phone number and return it with the enclosed response card in the provided envelope.

Name ____________________ Sex: F M Birthdate: ___________ Phone #: ( ) ____________

If you have any relatives, friends, or neighbors that may be interested in participating, please have them call Katie McDonald at (801) 588-3895 or e-mail Joanna Kelley at sly7b@cc.usu.edu.

P.S. We are also looking for healthy children without CF to participate in this study. To be eligible, they must be between the ages of 3 and 16 and not have reached puberty yet. They will not need to drink any special milk or certain amount of milk -- they will consume their usual diet. They will not need to give a blood or urine sample or keep a daily log. They will be asked for the following:

1) a bone scan (like an x-ray) at the beginning of the study
2) anthropometric measurements (height, weight, etc.) at the beginning and end of the study (which will last 3-4 months)
3) a 3-day diet record at the beginning of the study

If you would be interested in having any of your children without CF involved in this study (you may have more than one child enrolled in the study - with or without CF), please write their name(s), sex and age below along with a phone number and return it with the enclosed response card in the provided envelope.

Name ____________________ Sex: F M Birthdate: ___________ Phone #: ( ) ____________

If you have any relatives, friends, or neighbors that may be interested in participating, please have them call Katie McDonald at (801) 588-3895 or e-mail Joanna Kelley at sly7b@cc.usu.edu.
APPENDIX C

RESPONSE CARD
RESPONSE CARD

Please return this card within the next few days to let us know if you are interested in participating in this study.

☐ Thank you, but we are not interested in participating at this time.
☐ Yes, we are interested in participating. Please send us additional information about the study.

| Child’s Name: | 
| Parent’s Name: |
| Phone # to be reached at: | Day: | Evening: |

If we do not receive this in the next week, we may call you to obtain your preference. Thank you for your time!

RESPONSE CARD

Please return this card within the next few days to let us know if you are interested in participating in this study.

☐ Thank you, but we are not interested in participating at this time.
☐ Yes, we are interested in participating. Please send us additional information about the study.

| Child’s Name: | 
| Parent’s Name: |
| Phone # to be reached at: | Day: | Evening: |

If we do not receive this in the next week, we may call you to obtain your preference. Thank you for your time!

RESPONSE CARD

Please return this card within the next few days to let us know if you are interested in participating in this study.

☐ Thank you, but we are not interested in participating at this time.
☐ Yes, we are interested in participating. Please send us additional information about the study.

| Child’s Name: | 
| Parent’s Name: |
| Phone # to be reached at: | Day: | Evening: |

If we do not receive this in the next week, we may call you to obtain your preference. Thank you for your time!
APPENDIX D

INFORMED CONSENT FOR THE CHILD WITH CF
I 00 North Medical Drive  
Salt Lake City, Utah 84113  
(801) 588-2000  
(801) 588-2460 Fax

Informed consent for the child with cystic fibrosis

The Effect of Fortified Milk on Bone in Cystic Fibrosis

We invite your child to take part in a research study at Primary Children’s Medical Center and the Intermountain Cystic Fibrosis Center. It is important that you read and understand several general principles that apply to all who take part in our studies:

(a) taking part in the study is entirely voluntary;  
(b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others;  
(c) you may withdraw your child from the study at any time without penalty or loss of any benefits to which your child is otherwise entitled.

The nature of this study, risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you have about this study with the staff members who explain it to you.

Purposes:  
The purposes of this study are:  
1) to evaluate the differences in bone mineralization between children with cystic fibrosis (CF) and healthy children without cystic fibrosis.  
2) to determine the physiology (or body’s use) of calcium and vitamin D in children with CF.  
3) to evaluate the effects of calcium supplementation using specially fortified dairy products on bone mineralization in children with CF.

Basis for selection  
We are asking that your child participate in this study because she/he is between the ages of 2 and 16 years and has not reached puberty. Children with CF and healthy children without CF will be asked to participate in this study.

Explanation of procedure for the child with CF  
If you decide to participate in this study, your child will be asked to consume at least three 8 ounce servings of either regular milk or the fortified milk each day for a period of three months. Which dairy product (either regular milk or a fortified calcium and vitamin D milk) your child will use for a three month period will be decided based on chance. All children will continue to eat their regular diet in all other respects and receive their usual medical treatments. All CF children will continue on their prescribed pancreatic enzymes, medications and vitamin and mineral supplements during the study period.

A mutually agreeable time will be arranged for study participants to come in for the pre-study data collection. The study participants will return at three months for the post-study data collection. Both the pre-study and post-study data collections can be timed to occur on the
same days as regularly scheduled, quarterly CF Outpatient Clinic appointments at Primary Children's Medical Center. The pre- and post-study data collections for children with CF will include:

1. Weight, height, chest circumference, arm circumference and skinfold measurements.
2. Bone mineral analyses by DEXA (Dual energy X-ray absorptiometry, similar to an X-ray) of the lower spine and/or forearm will be completed at Dr. Gary Chan's lab at the University of Utah Medical Center.
3. A 5 ml (1 teaspoon) blood sample taken from a vein.*
4. An early morning urine sample.*

*Note: Any abnormal value in the blood or urine sample will be reported immediately to your doctor who will arrange appropriate medical follow up.

The CF child or a parent will complete and mail in a three day diet record at the beginning of the study. For the remainder of the three months study, the child and/or parent will keep simple daily logs to report changes in bowel movement, the amount of calcium in your child's diet, pancreatic enzyme usage, level of activity and vitamin mineral supplement usage. The child and/or parent will be instructed on using the diet records and the log. The logs will be submitted weekly. Prepaid, self addressed envelopes will be provided.

Procedures that are not part of routine CF care:

By agreeing to participate in this study, you should realize that the bone mineral analyses, the blood draws, urine collections and the daily logs are not part of ordinary CF care.

The bone mineral analyses take about five minutes and require walking from PCMC Outpatient CF clinic to the Dr. Chan's Lab at the University of Utah Medical Center (approximately 10 minutes each way).

In order to minimize the number of blood draws, entry into the study can be scheduled to coincide with annual or scheduled blood work.

The daily log will be completed every day for three months. It is estimated that the time to complete the log should be less than five minutes per day.

Listing of costs resulting from the research:

The costs of this research are being paid for by a grant from the Primary Children's Medical Center Foundation. The fortified milk was developed at Utah State University and donated by the Utah Dairy Commission. All investigators are donating their time to conduct this research. There is no cost above the usual for routine CF care to the study subjects or their parents.

Potential benefits

The potential benefits of this study include early identification of any bone demineralization, a procedure that is not currently a part of routine care for children with CF. Increased skill in monitoring signs and symptoms of poor intestinal absorption may result in
Improved control and better weight gain. It is possible that the use of the fortified dairy product may increase bone strength.

**Description of risks and discomforts:**

Any treatment has potential side effects. It is expected there should be minimal side effects from the substitution of fortified milk for regular milk in the diet of a child with CF. Lactose intolerance is a potential side effect for the child who does not normally consume milk. Gastrointestinal symptoms such as diarrhea, gas or possibly constipation could occur.

A small discomfort may be felt for less than five seconds with the compression of skinfolds or "pinch" in determining skinfold measurements. Children who attend the Outpatient CF clinic at PCMC have these measurements done as a routine part of care.

Although the risks of having blood taken from a vein are minimal, there may be a small amount of pain when the needle is inserted into the vein and there may be some residual bruising in the days after the blood is drawn.

A small radiation exposure occurs with the use of the DEXA bone analysis. This exposure is estimated to be approximately 40 times less than the radiation exposure of a chest or dental X-ray.

**Disclosure of alternatives:**

The alternative to this study is to decline enrollment. You should feel free to discuss with your child's doctor the possibility of increasing the amount of calcium your child consumes before you decide whether to participate in this study.

**Voluntary Participation:**

Participation in this study is entirely voluntary and you may choose to decline to have your child enrolled in this study. If you do participate, you can withdraw your child at any time without any effect on his/her medical care.

**Questions?**

If you have any questions about this study, they will be answered by Katie McDonald or Dee Anne Evans. You can contact Katie at (801) 588-3898 and Dee Anne at (801) 588-2716 or through the operator at Primary Children's Medical Center at (801) 588-2000. If you have any questions concerning your child's rights as a research subject, you may contact David P. Carlton, MD, Chairman of the Research and Human Subjects Committee at Primary Children's Medical Center at (801)581-4186.

**In case of injury:**

Realistically, neither Primary Children's Medical Center, the Intermountain Cystic Fibrosis Center, Utah State University nor the investigators can guarantee or assure that unknown consequences will not occur. If you believe that your child has suffered an injury as a result of participation in this research program, please contact Primary Children's Medical Center Risk Manager, Susan W. Adams, RN, BSN at (801)588-2281. You will not give up any of your child's legal rights by signing this form.
Informed consent for the child with CF

The Effect of Fortified Milk on Bone in CF

Upon consideration of the possible benefits and risks of this study outlined, I voluntarily agree to allow the participation of __________________ in the study.

I understand that effective medical care is my doctors’ and the investigators’ main concern and that they may stop the study and change my child’s treatment according to their best judgement. My questions regarding participation in this study have been answered and I understand the explanation.

Confidentiality assurance:

I give permission for the information gathered in this study and pertinent information from my child’s medical records to be released to the investigators with the understanding that they may be published for scientific purposes, but my child’s identity will not be publicly revealed without my written consent. By federal law, the medical information gathered in the course of this study may be reviewed by the United States Food and Drug Administration. I acknowledge receipt of a copy of this consent document.

Signature of Patient (if applicable) __________________________ Date

Signature of Parent/Guardian __________________________ Date

Signature of Witness __________________________ Date

Katie McDonald, MS, RD
Clinical Dietitian
Intermountain Cystic Fibrosis Center
Primary Children’s Medical Center

I have reviewed for “typos” and spelling and grammatical errors.

If any of the above items are not included, describe basis for exclusion:
APPENDIX E

INFORMED CONSENT FOR THE CHILD WITHOUT CF
Informed consent for the child who does not have cystic fibrosis

The Effect of Fortified Milk on Bone in Cystic Fibrosis

We invite your child to take part in a research study at Primary Children’s Medical Center and the Intermountain Cystic Fibrosis Center. It is important that you read and understand several general principles that apply to all who take part in our studies:

(a) taking part in the study is entirely voluntary;
(b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others;
(c) you may withdraw your child from the study at any time without penalty or loss of any benefits to which your child is otherwise entitled.

The nature of this study, risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you have about this study with the staff members who explain it to you.

Purposes:
The purposes of this study are:
1) to evaluate the differences in bone mineralization between children with cystic fibrosis (CF) and healthy children without cystic fibrosis.
2) to determine the physiology (or body’s use) of calcium and vitamin D in children with CF.
3) to evaluate the effects of calcium supplementation using specially fortified dairy products on bone mineralization in children with CF.

Basis for selection
We are asking that your child participate in this study because she/he is between the ages of 2 and 16 years and has not reached puberty. Children with CF and healthy children without CF will be asked to participate in this study.

An explanation of procedure for the child without CF:
If you decide to participate in this study and your child does not have CF, your child will continue to eat his or her usual diet and continue his or her usual consumption of milk for the three month study period. A mutually agreeable time will be arranged for study participants to come in for the pre-study data collection. The study participants will return at three months for the post-study data collection.

Data collection for the child without CF will be the following:
1. Pre- and post-study weight, height, chest circumference, arm circumference and skinfold measurements.
2. A bone mineral analysis by DEXA (Dual energy X-ray absorptiometry, similar to an X-ray) of the lower spine and/or forearm will be completed at Dr. Gary Chan’s lab.
Informed consent for child who does not have CF
The Effect of Fortified Milk on Bone in CF

at the University of Utah Medical Center.
3. A three day diet record will be completed and mailed in. The child and/or parent will be instructed on using the diet records. Prepaid, self-addressed envelopes will be provided.

Listing of costs resulting from the research:
The costs of this research are being paid for by a grant from the Primary Children's Medical Center Foundation. All investigators are donating their time to conduct this research. There is no cost to the study subjects or their parents.

Potential benefits
The potential benefits of this study include early identification of any bone demineralization, a procedure that is not currently a part of routine care for children.

Description of risks and discomforts:
A small discomfort may be felt for less than five seconds with the compression of skinfolds or "pinch" in determining skinfold measurements.
A small radiation exposure occurs with the use of the DEXA bone analysis. This exposure is estimated to be approximately 40 times less than the radiation exposure of a chest or dental X-ray.

Disclosure of alternatives:
The alternative to this study is to decline enrollment.

Voluntary Participation:
Participation in this study is entirely voluntary and you may choose to decline to have your child enrolled in this study. If you do participate, you can withdraw your child at any time without any effect on his/her medical care.

Questions?
If you have any questions about this study, they will be answered by Katie McDonald or Dee Anne Evans. You can contact Katie at (801) 588-3898 and Dee Anne at (801) 588-2716 or through the operator at Primary Children's Medical Center at (801) 588-2000. If you have any questions concerning your child's rights as a research subject, you may contact David P. Carlton, MD, Chairman of the Research and Human Subjects Committee at Primary Children's Medical Center at (801) 581-4186.

In case of injury:
Realistically, neither Primary Children's Medical Center, the Intermountain Cystic Fibrosis Center, Utah State University nor the investigators can guarantee or assure that unknown consequences will not occur. If you believe that your child has suffered an injury as a result of participation in this research program, please contact Primary Children's Medical Center Risk Manager, Susan W. Adams, RN, BSN at (801) 588-2281. You will not give up any of your child's legal rights by signing this form.
Informed consent for child who does not have CF
The Effect of Fortified Milk on Bone in CF

PRIMARY CHILDREN’S MEDICAL CENTER
A Service of Intermountain Health Care

Signatures:
Upon consideration of the possible benefits and risks of this study outlined, I voluntarily agree to allow the participation of ______________________ in the study.

I understand that effective medical care is my doctors’ and the investigators’ main concern and that they may stop the study and change my child’s treatment according to their best judgement. My questions regarding participation in this study have been answered and I understand the explanation.

Confidentiality assurance:
I give permission for the information gathered in this study and pertinent information from my child’s medical records to be released to the investigators with the understanding that they may be published for scientific purposes, but my child’s identity will not be publicly revealed without my written consent. By federal law, the medical information gathered in the course of this study may be reviewed by the United States Food and Drug Administration. I acknowledge receipt of a copy of this consent document.

Signature of Patient (if applicable) ______________________ Date ______________

Signature of Parent/ Guardian ______________________ Date ______________

Signature of Witness ______________________ Date ______________

Katie McDonald, MS, RD
Clinical Dietitian
Intermountain Cystic Fibrosis Center
Primary Children’s Medical Center

I have reviewed for “typos” and spelling and grammatical errors.

If any of the above items are not included, describe basis for exclusion:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________
APPENDIX F

YOUTH ASSENT FORM
Informed Consent

The Effect of Fortified Milk on Bone in Cystic Fibrosis

Youth Assent:

explained this research project to me. I understand what will happen during this research. I have asked the questions I want to ask and my questions have been answered. I understand that my parent(s) has/have given their permission for me to participate in this study. My signature below indicates that I am willing to participate. I know that I do not have to do this. I know I can stop being in this study at any time by telling my parents, Katie McDonald (the CF clinic dietitian), or Dee Anne Evans (the CF nurse practitioner) that I do not want to be in this research project.

Signature of child  Age of child  Date

This statement has been read to the above child and he or she seems to understand.

Signature of person obtaining assent
APPENDIX G

DATA SHEET FOR CHILD WITH CF
CF Bone Mineralization Study Data Sheet

<table>
<thead>
<tr>
<th>Date</th>
<th>Name</th>
<th>Group</th>
<th>ID#</th>
<th>Age</th>
<th>Sex</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Anthropometry</th>
<th>INITIAL Date:</th>
<th>FINAL Date:</th>
<th>Dad’s Ht</th>
<th>Mom’s Ht</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
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<td></td>
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<tr>
<td>Weight</td>
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<tr>
<td>AMC</td>
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<tr>
<td>Arm Area</td>
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<tr>
<td>Triceps</td>
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<tr>
<td>Subscapular</td>
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<td></td>
</tr>
<tr>
<td>Chest Circumf.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CF Severity (Schwachman-Kulczycki): ________

Kyphosis? (per x-ray) Y / N

Previous Fractures? Y / N

How Many? ________

Location(s): ________

Cause: ________

Consent Form

_ Explained/Qstns Answered
Signed? Y / N Date: ________

Starter Packet Given & Explained

_ 3-Day Diet Record
_ Portion Sizes
_ Daily Log (see reverse side)
_ SASE’s/Weekly Mail-in
_ Gossner UHT Milk Info (Remind to SHAKE before drinking!!)

Urine Sample Received

Date ________

Void # ________

Blood Sample Obtained

Date ________

Time ________

Bone Scan Done

INITIAL: Date ________

Time ________

Lumbar ________

Forearm ________

FINAL: Date ________

Time ________

Lumbar ________

Forearm ________

Milk Given

Date ________

Amount ________

Kind ________

How/Where: ________

Follow-Up/Final Data Gathering Visit Scheduled? Y / N Date Scheduled For: ________

Interviewer Initials: ________
APPENDIX H

DATA SHEET FOR CHILD WITHOUT CF
CF Bone Mineralization Study Data Sheet for NON-CF/CONTROLS

Date ____________________ Case-Match #________
Name ____________________
Group Control #________ Sibling/Relative ________
ID# ______________________
Birthdate ________________
Sex ______________________

<table>
<thead>
<tr>
<th>Anthropometry</th>
<th>INITIAL Date</th>
<th>FINAL Date</th>
<th>Dad's Ht</th>
<th>Mom's Ht</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
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<td>BMI</td>
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<td>AMC</td>
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<tr>
<td>Arm Area</td>
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<td>AMA</td>
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<td>Triceps</td>
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<tr>
<td>Subscapular</td>
<td>____________</td>
<td>__________</td>
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</tr>
<tr>
<td>Chest Circumf.</td>
<td>____________</td>
<td>__________</td>
<td>__________</td>
<td>__________</td>
</tr>
</tbody>
</table>

Consent Form
- Explained/Qstns Answered
  - Signed? Y / N Date: __________

Starter Packet Given & Explained
- 3-Day Diet Record Date 3-Day Record Returned: __________

Medical History
- Previous Disease or Surgery:
- Current Disease/Health Problems:
- Previous Fractures (#, when, where):
- Medications:
- Vitamin/Supplement Use:
- Dairy or Other Food Intolerances:

Usual Level of Activity: (Circle One)
0 = no activity (ie stay in bed/watch tv all day)
1 = light (less than other children of same age)
2 = moderate (normal activity @ school/home but no extra exercise )
3 = high (normal activity + 20 min active play/exercise)
4 = strenuous exercise for 20 min - 1 hr (ie basketball, soccer, dance)
5 = strenuous, sustained exercise for 1 hr or longer
  (ie long distance running, endurance swimming, weight lifting)

Bone Scan Done
- INITIAL: Date __________ Lumbar ________
- Time ________ Forearm ________
- FINAL: Date __________ Lumbar ________
- Time ________ Forearm ________

Follow-Up/Final Data Gathering Visit Scheduled? Y / N Date Scheduled For: __________
APPENDIX I

3-DAY DIET RECORD FORM
INSTRUCTIONS:

1. Write down **EVERYTHING** you eat and drink for at least 3 days (before you start drinking the boxed milks). Don't forget water, soda, or "little nibbles."

2. Record information immediately after eating, drinking, or exercising.

3. Include details about each food or beverage including the portion size, how the food was prepared, and any sauces or dressings on the food.

4. Measure your food portions before you eat. If measuring is not possible, estimate the serving size.

5. After you have recorded everything you eat and drink for 3 days on these sheets, please mail them back to Primary Children’s Hospital in one of the stamped envelopes provided for you.

**EXAMPLE OF FOOD/BEVERAGE DIARY:**

<table>
<thead>
<tr>
<th>TIME</th>
<th>FOOD/BEVERAGE</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00</td>
<td>SANDWICH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-WHITE BREAD</td>
<td>2 SLICES</td>
</tr>
<tr>
<td></td>
<td>-PEANUT BUTTER, SMOOTH</td>
<td>3 TABLESPOONS</td>
</tr>
<tr>
<td></td>
<td>-STRAWBERRY JAM</td>
<td>2 TABLESPOONS</td>
</tr>
<tr>
<td></td>
<td>DIET ROOT BEER</td>
<td>12 OUNCE CAN</td>
</tr>
<tr>
<td></td>
<td>POTATO CHIPS</td>
<td>15 CHIPS</td>
</tr>
<tr>
<td></td>
<td>CELERY STICKS</td>
<td>5 SMALL</td>
</tr>
<tr>
<td></td>
<td>CARROT STICKS</td>
<td>10 SMALL</td>
</tr>
<tr>
<td></td>
<td>RANCH DRESSING</td>
<td>2 TABLESPOONS</td>
</tr>
</tbody>
</table>

Please complete the following information:

NAME____________________________ AGE_____
ADDRESS_________________________________
PHONE__________________________________
## FOOD AND BEVERAGE DIARY

<table>
<thead>
<tr>
<th>Name</th>
<th>Date</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>TIME</th>
<th>FOOD / BEVERAGE</th>
<th>AMOUNT EATEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
APPENDIX J

GUIDE TO PORTION SIZES FOR 3-DAY DIET RECORD
This is a guide to help you figure out portion sizes for your 3-day diet record and daily calcium servings (on your daily log).

**SEVEN WAYS TO SIZE UP YOUR SERVINGS**

Measure food portions so you know exactly how much food you're eating. When a food scale or measuring cups aren't handy, you can still estimate your portion. Remember:

| **1** | 3 ounces of meat is about the size and thickness of a deck of playing cards or an audiotape cassette. |
| **2** | A medium apple or peach is about the size of a tennis ball. |
| **3** | 1 oz of cheese is about the size of 4 stacked dice. |
| **4** | ½ cup of ice cream is about the size of a racquetball or tennis ball. |
| **5** | 1 cup of mashed potatoes or broccoli is about the size of your fist. |
| **6** | 1 teaspoon of butter or peanut butter is about the size of the tip of your thumb. |
| **7** | 1 ounce of nuts or small candies equals one handful. |

**MOST IMPORTANT**

 Especially if you're cutting calories, remember to keep your diet nutritious.

- 2-4 servings from the Milk Group for calcium
- 2-3 servings from the Meat Group for iron
- 3-5 servings from the Vegetable Group for vitamin A
- 2-4 servings from the Fruit Group for vitamin C
- 6-11 servings from the Grain Group for fiber
APPENDIX K

DAILY LOG FORM
**The Effect of Fortified Milk on Bone in Cystic Fibrosis Study Log**

*Please fill this out every day. Refer to the scoring sheet on the back of the page.*

Name

From Date: ___________________________ To Date: ___________________________

<table>
<thead>
<tr>
<th></th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
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<tr>
<td>Calcium</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
</tr>
<tr>
<td>Enzymes</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Yes No</td>
<td>Yes No</td>
<td>Yes No</td>
<td>Yes No</td>
<td>Yes No</td>
<td>Yes No</td>
<td>Yes No</td>
</tr>
<tr>
<td>Activity</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
<td>0 1 2 3 4 5</td>
</tr>
</tbody>
</table>

**Comments**

---

105
APPENDIX L

SCORING GUIDE FOR DAILY LOG FORM
Scoring for Study Log

Absorption
How many of the following symptoms of malabsorption did you have? Add 1 point for each:
- More than 2 bowel movements in a day,
- Large amount of stool or diarrhea,
- Stool floats in toilet,
- Visible grease/oil with stool in toilet,
- Gas or bloating

Calcium
How many calcium-rich foods did you eat?
* Add one point for each:
  - 8 ounce milk
  - 1 ounce cheese
  - 5 ounce yogurt
* Add 1/2 point for each
  - 4 ounces pudding
  - 6 ounces cottage cheese
  - 3/4 cup broccoli

Enzymes
How many times did you miss taking enzymes or take enzymes after eating? Score according to the following:
1 point - No enzymes at a meal
1/2 point - No enzymes at snack
1/2 point - Enzymes taken after a meal or snack instead of before eating.

Vitamins
Did you take your vitamin supplement today?

Activity
Rate your level of activity for the day:
0 Stayed in bed or watched TV/played video games all day.
1 Light activity in school or at home but less than other children of same age.
2 Moderate activity - normal activity at school/home for age but no extra exercise.
3 High activity - normal activity at school/home plus active play/exercise for at least 20 minutes.
4 Strenuous exercise (elevated heart rate, breathing hard, sweating) sustained for at least 20 minutes but no longer than 1 hour. Examples, basketball or soccer game, dance class. Exercise is in addition to high levels of activity at home/school.
5 Strenuous, sustained exercise (elevated heart rate, breathing hard, sweating) - high activity at home and school plus strenuous exercise for one hour or longer. Examples, long distance running, endurance swimming.

Comments:
How are things going? Good day, bad day?
APPENDIX M

TIPS FOR IMPROVING DIGESTION
This is just meant to be a helpful reminder for you -- you don’t need to fill it out for the study.

---

**EASY IDEAS FOR IMPROVING ABSORPTION**

✅ **Check the ones you are doing:**

- Chew food well.
- Take enzymes with every calorie-containing food or beverage (especially milk).
- Take vitamins with a meal or snack, after taking enzymes.
- Eat 5-6 times a day, but avoid “grazing” or constant nibbling (this includes milk or other calorie-containing beverages).
- Limit juices to less than 4 ounces per day.
- Take enzymes right before eating.
- For a big meal or a meal that lasts longer than 20-30 minutes, take an enzyme in the middle of the meal.

*Enzymes are fragile!*

- Check expiration dates. Don’t use expired enzymes.
- Keep enzymes in a cool place. Avoid hot places like glove compartments in cars.

Other suggestions:
APPENDIX N

PATIENT HAND-OUT ON UHT MILK
Gossner Foods UHT Milk

1. **WHAT IS U.H.T. SHELF STABLE MILK?**
   Gossner's U.H.T. Shelf Stable Milk is a premium quality Grade A fluid milk. It has been specially processed and packaged so that no refrigeration is required until the package is opened.

2. **WHAT DOES U.H.T. STAND FOR?**
   The letters U.H.T. stand for Ultra High Temperature. These letters are used to tell the consumer the milk or milk product has been subjected to a thermo process much higher than normal pasteurizing temperatures.

3. **WHY DOESN'T U.H.T. SHELF STABLE MILK NEED REFRIGERATION?**
   Gassner's U.H.T. Shelf Stable Milk is heated to 282°F and held for several seconds, then cooled to 70°F in a continuous pressurized system. The milk is then packaged aseptically in a special Tetra Pak® container. The elevated temperatures are sufficient to destroy all pathogenic and spoilage bacteria, including spores. The special Tetra Pak® package is hermetically sealed to retain freshness.

4. **WHAT IS ASEPTIC PACKAGING?**
   A process by which a product is packaged free from bacteria. Our Tetra Pak® package is free from bacteria and hermetically sealed to keep out air and light. Bacteria, air and light are the basic ingredients that cause milk to sour, lose food value, and take on undesirable oxidized flavors.

5. **IS IT REAL MILK?**
   Yes, each package of Gossner foods U.H.T. Shelf Stable Milk carries the "Real" seal label, this guarantees that the milk is an authentic dairy product. Gossner Milk is Grade A liquid milk ready to use, just open the package and use, cool for best results and refrigerate after opening.

6. **IS IT JUST AS NUTRITIOUS AS CONVENTIONAL PASTEURIZED MILK?**
   Yes, U.H.T. processing and foil lined aseptic packaging of Gossner's Shelf Stable Dairy Products ensures safety and long shelf life without significantly altering the product's nutritive value over the recommended use by date printed on the top of each hermetically sealed carton.

7. **ARE PRESERVATIVES USED OR ADDED TO THE MILK?**
   No, preservatives are not added to Gossner Milk.

8. **HOW LONG DOES GOSSNER MILK LAST?**
   Gossner Milk can be stored on the shelf at room temperature for months before
being opened. It is recommended that it is used by the date stamped on the top of the carton. Once the package is opened it must remain refrigerated.

9. **IS AN ASEPTIC TETRA PAK® PACKAGE SIMILAR TO A CAN?**
   Yes, the package is a flexible can and should be treated like a can. If it is damaged, punctured or bloated it should be discarded and never used.

10. **WILL GOSSNER MILK SPOIL?**
    Yes, if the package is damaged or if the package has been opened. Once the package is opened the milk is exposed to the air and will spoil just like pasteurized milk. Always refrigerate after opening for maximum shelf life.

11. **HOW SHOULD I STORE GOSSNER U.H.T. SHELF STABLE MILK?**
    Store Gossner's Milk as you would store canned goods on the shelf at room temperature 70°F or below. At higher temperatures (80°F to 100°F) over a period of weeks, the milk develops an off-color and taste; although, the milk remains sterile and is safe to use.

12. **FOR BEST RESULTS HOW SHOULD I USE GOSSNER SHELF STABLE MILK?**
    For best taste results chill before drinking. Once the carton is opened, it must remain refrigerated as if it were pasteurized milk (use within approximately 5 days). If straw has been inserted and used, refrigerate remaining milk and use within a day. Gossner Foods Milk Products are best if used by the code date printed on the top of each carton.

13. **WHERE CAN I BUY GOSSNER SHELF STABLE MILK?**
    Availability of Gossner products varies with locations, however some stores that may carry Gossner UHT milk products are Macey's, Harmon's, Granato's, Winegar's, Dan's Food Stores, and Ream's (selection may vary).

😊 **SUGGESTIONS FOR USE OF U.H.T. MILK FOR CHILDREN WITH CF:**
   (Remember…it's important that children participating in the CF Bone Density Study consume the full 3 cartons of UHT milk each day!)

   * Add flavored syrups and powders for variety (i.e. strawberry or chocolate syrup, chocolate malt flavoring powder, etc.).
   * Pour over cold cereal or use to make hot cereal (i.e. oatmeal or creamed wheat).
   * Use to make nutrition shakes (i.e. Scandi-Shake, Carnation Instant Breakfast Powder, etc.)
   * Use to make pudding
   * Substitute for milk in any of your favorite recipes!
APPENDIX O

FOLLOW-UP LETTER FOR PATIENTS ENROLLED IN STUDY
1/25/99

Dear,

Hello! Just wanted to send a quick note to check in on how you're doing with the CF Bone Density Study. I hope that everything's going well. If not, please don't hesitate to call with any questions or concerns you might have. I also wanted to let you know what we've received from you so far and what we still need. (If you've already mailed the following, please disregard -- our mail is a little slow sometimes!) Thank you for your participation.

Sincerely,

Joanna Kelley, RD
(435) 753-3287

We have received:

Have not received:

P.S. Just a reminder to schedule and attend your 3-month follow-up appointment for clinic so that we can get the final bone scan and anthropometric measurements completed.

P.P.S. Please remember to shake the milk well before drinking it!