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Undercarboxylated osteocalcin and bone mass in 8–12 year old children with cystic fibrosis ☆

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Abstract

Young adults with cystic fibrosis (CF) frequently develop bone disease. One suggested aetiological factor is suboptimal vitamin K status with impaired carboxylation of osteocalcin and abnormal bone formation.

Methods: We measured bone mineralization and turnover in thirty-two 8–12 year old CF patients (14 boys) using Dual Energy X-ray absorptiometry (whole body (WB) and lumbar spine (LS)), 25-OH Vitamin D, PTH and markers of bone formation (plasma osteocalcin, N-terminal pro-peptide of type 1 collagen (P1NP)), plus an indirect measure of vitamin K status, undercarboxylated osteocalcin (uc-OC).

Results: LS bone mineral density (BMD) standard deviation (SD) scores were < -1.0 in 20% of subjects. Size-adjusted LS and WB bone mass was normal. Compared to reference data, % uc-OC was high and P1NP low. LS bone mass was predicted by % uc-OC but not other markers (0.4% decrease in size-adjusted LSBMC ($p=0.05$); 0.04 SD decrease in LSBMAD ($p=0.04$) per 1% increase in uc-OC).

Conclusion: Markers suggestive of sub-optimal vitamin K status and low bone formation were present despite normal size-adjusted bone mass. The association between LSBMC and % uc-OC is consistent with the hypothesis that sub-optimal vitamin K status is a risk factor for CF bone disease. This should ideally be investigated in an intervention trial.

Keywords: Cystic fibrosis; Bone mineralization; Bone turnover; Vitamin K; Undercarboxylated osteocalcin

1. Introduction

With earlier diagnosis and improved treatment, the life expectancy of patients with cystic fibrosis (CF) has steadily improved over the last few decades, and is likely to improve further in the future. However, a number of longer-term health problems have become apparent, including CF bone disease. There are now many reports of bone pain and spontaneous fractures in young adults with CF [1]. Histomorphometry in individuals with low bone mass measured using Dual Energy X-ray Absorptiometry (DXA) shows significantly reduced cancellous bone volume with low bone formation at both tissue and cellular level [2]. A recent study reported normal bone mass in 5–10 year old children with CF but significantly reduced whole body and wrist bone mass in older children and adolescents aged 11–20 years [3], consistent with the hypothesis that CF bone disease develops or becomes apparent during adolescence and young adulthood.

As highlighted and discussed in detail in recent consensus documents [1,4], the aetiology of CF bone disease is likely to be multifactorial, with poor nutritional intake, malabsorption, chronic inflammation and infection, reduced levels of physical

Abbreviations: CF, Cystic fibrosis; DXA, Dual energy X-ray absorptiometry; BMD, bone mineral density; BMC, bone mineral content; BMAD, bone mineral apparent density; OC, Osteocalcin; uc-OC, undercarboxylated osteocalcin.

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activity, corticosteroid use and pubertal delay all contributing to varying degrees. Recently, the role of sub-optimal vitamin K status has also been debated. This fat-soluble vitamin is vital as a cofactor for the post-translational conversion of glutamyl residues to γ-carboxyglutamyl residues in vitamin K-dependent proteins such as prothrombin and osteocalcin (OC). OC is produced by osteoblasts and is the main non-collagenous protein in bone. In its carboxylated form it binds to hydroxyapatite in bone and is believed to play a regulatory role in bone formation, mineralization and resorption, positively affecting bone quality [5,6]. In contrast, undercarboxylated OC (uc-OC) binds less effectively to hydroxyapatite and a significant association has been found between fracture incidence and uc-OC in elderly subjects [7,8]. The liver is more efficient than bone in using available vitamin K. Hence uc-OC is the first functional marker to appear in vitamin K deficiency and the last to respond to supplements. Furthermore, it is regarded as a better marker of vitamin K status than serum vitamin K concentrations which vary markedly according to recent dietary intake [9–11].

Although routinely supplemented with other fat-soluble vitamins (A, D and E), in many UK centres CF patients do not routinely receive vitamin K supplements [12]. Some degree of uncarboxylation of OC is present in healthy individuals [9,10], but several studies have reported high proportions of uc-OC as well as other indicators of sub-optimal vitamin K status in patients with CF [13–17]. Moreover, supplementation with vitamin K has been shown to reduce the concentration of Proteins Induced by Vitamin K Absence (PIVKA-II) [15], the proportion of uc-OC [14] and the absolute concentration of uc-OC [17]. However, there are few data from populations with simultaneous measurements of vitamin K status and bone mass, hence a relationship between sub-optimal vitamin K status and CF bone disease has not been established.

The aim of our study was to measure bone mass and markers of bone turnover in a population of 8–12 year old children with CF, and specifically to investigate the relationship between these parameters and the proportion of uc-OC as a measure of sub-optimal vitamin K status.

2. Methods

Children aged 8 to 12 years attending the CF clinic at Great Ormond Street Hospital for Children (London, UK) for annual review were recruited and studied between October 1st 2002 and September 30th 2004. The complete study protocol included measurement of body composition (not reported here) using whole body plethysmography, which requires the subject to remain still with a regular quiet breathing pattern for a few minutes at a time. Children who were considered to be too unwell to comply with this measurement due to their respiratory status were excluded from the study. Parents or guardians gave informed consent and assent was obtained from the child. The study was approved by the Great Ormond Street Hospital for Children National Health Service Trust and Institute of Child Health Research Ethics Committee.

All children had a DXA scan (Lunar Prodigy, GE Medical Systems, US; Encore 2002 software) of whole body (WB) and lumbar spine (LS) performed whilst wearing light indoor clothing. The radiation exposure was approximately 2.2 μSv.

Weight was recorded using digital scales and height measured using a stadiometer. Pubertal stage was self-assessed using line drawings showing the different Tanner stages for breast or genital development and for pubic hair. Children were classified as 'pre-pubertal' if they rated themselves stage 1 for both parameters, and as 'pubertal' if any parameter was rated as 2 or above.

Blood was obtained for measurement of markers of bone formation (plasma osteocalcin (N-Mid osteocalcin, Nordic Bioscience Diagnostics A/S, Herlev, Denmark) and N-terminal pro-peptide of type 1 collagen (P1NP; Orion Diagnostica, Finland) and for PTH and 25-OH vitamin D (DiaSorin LTD, Wokingham, UK). Samples were obtained in a non-fasting state at the time of routine sampling for clinical purposes. Plasma or serum were separated as soon as possible and stored at −80 °C prior to analysis. Samples were run in duplicate and repeated if the %CV was >10. With the exception of osteocalcin and undercarboxylated osteocalcin, all samples were analysed in one run. 25-OH vitamin D measurements were monitored by the vitamin D external quality assurance scheme (DEQUAS).

Plasma total OC (t-OC) and uc-OC were analysed by one-step ELISA. This assay measures intact osteocalcin and the large N-terminal-mid molecule fragment. Uc-OC was measured using the same assay following a hydroxyapatite binding stage in a modification of the method described by Gundberg [10]. Intra-assay CVs were 3.3% for t-OC and 5.1% for uc-OC. The percentage of uc-OC (% uc-OC) was calculated as (uncarboxylated/total) × 100.

Demographic data (sex, age, and genotype) were collected. Laboratory spirometry (percentage forced expiratory volume in 1 s, FEV_1; percentage forced vital capacity, FVC) was performed according to laboratory protocols, based on adult American Thoracic Society (ATS: [18]) and European Respiratory Society (ERS) standards for spirometry [19]. Computerised frontal chest radiographs were assessed for radiological features of CF lung disease using the modified Chrispin–Norman chest radiograph scoring system (CNS), which applies a maximum score of 38 for radiological signs of severe lung pathology [20,21].

2.1. Statistics

Weight, height and Body Mass Index (BMI SD; weight/height^2) scores were calculated using British reference data. WB and LS (L2-4) BMDSD scores for age and sex were obtained from the manufacturer's reference database. To adjust for the effects of bone size, bone mineral apparent density (BMAD) was calculated for the lumbar spine as $BMC/BA^{1.5}$ and BMAD SD scores were calculated using our local reference data for children aged 5–19 years [22]. These data were collected by our research group in a project establishing reference bone and body composition data using the same DXA machine over the same period. The power relationship between BMC and BA determined by log–log regression for our subjects was in fact 1.3 and did not differ significantly between patients