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Serum phospholipid fatty acid pattern is associated with bone mineral density in children, but not adults, with cystic fibrosis

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Essential fatty acids (EFA) have proved to be important for normal bone mineral density (BMD) and bone growth in animal studies. Patients with cystic fibrosis often have low serum EFA levels, and low BMD has also been reported in patients with normal anthropometry. The aim of the present study was to analyse if BMD during a 2-year period was related to fatty acid status in patients with cystic fibrosis. Fifty-four patients, aged 6–33 years, were studied prospectively. BMD was measured with dual X-ray absorptiometry, and fatty acid concentrations in serum phospholipids were determined with capillary GLC. The cystic fibrosis patients showed low linoleic acid concentration and a high arachidonic acid (AA):DHA ratio in serum. The high eicosatrienoic acid:AA ratio, an indicator of EFA deficiency, increased further over 2 years, as did the total concentration of saturated fatty acids. In the adults there were no significant changes in fatty acids during the study. In the children, positive correlations were found between palmitic acid and bone mineral content in the lumbar spine and femoral neck. The lumbar spine BMD *Z* score correlated negatively with the AA:DHA ratio. No correlation was seen in adults except for a positive correlation between EFA deficiency index and the areas of lumbar spine and femoral neck. The present results imply that fatty-acid status influenced BMD in cystic fibrosis children, but not in adults, indicating that fatty-acid status would be important for bone growth.

Arachidonic acid: Docosahexaenoic acid: Eicosatrienoic acid: Linoleic acid

Bone mineral density (BMD) has been reported to be low in patients with cystic fibrosis (CF), mainly related to malnutrition, pulmonary function and steroid treatment (Haworth *et al.* 1999; Conway *et al.* 2000; Gronowitz *et al.* 2003; Buntain *et al.* 2004). Some authors report normal BMD in CF patients, compared with other patients with similar growth (Salamoni *et al.* 1996; Hardin *et al.* 2001; Sood *et al.* 2001), suggesting that a common denominator for growth might be involved.

Essential fatty acids (EFA) are associated with normal growth (Holman, 1968) and in animals also with normal bone mass (Watkins *et al.* 2001*c*). Arachidonic acid (AA; 20 : 4*n*-6) is the main long-chain PUFA synthesised from linoleic acid (LA; 18 : 2*n*-6), the EFA that has been claimed as the more important of the two EFA (Holman, 1968). AA is the substrate for the synthesis of eicosanoids, an important group of mediators of metabolism and inflammation, for example, prostaglandin (PG) E_2 and leucotrienes. PGE_2 is a modulator of bone growth (Marks & Miller, 1993; Watkins *et al.* 2001*c*), but leucotrienes also influence bone metabolism (Garcia *et al.* 1996).

Increasing documentation is now available on the influence of the *n*-3 EFA series on bone metabolism (Watkins *et al.* 2001*a*). There is a balance in the synthesis of long-chain PUFA from the EFA, LA and α-linolenic acid (18 : 3*n*-3), which is determined by the competition of enzymes for elongation and desaturation of those fatty acids and the endogenous fatty acids of the *n*-7 and *n*-9 series. The influence of this integrated system, together with heredity, systemic hormones, cytokines, diet and other local factors constitute a complicated network controlling bone homeostasis (Marks & Miller, 1993; Gunnes & Lehmann, 1995; Garcia *et al.* 1996; Karsenty, 1999; Watkins *et al.* 2001*a*,*c*).

Abnormal AA metabolism has been documented in different systems in CF patients (Carlstedt-Duke *et al.* 1986; Levistre *et al.* 1993; Berguerand *et al.* 1997; Miele *et al.* 1997), and the metabolism of the precursor LA has also been found to be altered, resulting in low concentrations of LA in most analysed tissues (Underwood *et al.* 1972; Lai *et al.* 2000; Strandvik *et al.* 2001*b*). An abnormal ratio between AA and the long-chain PUFA product of α-linolenic acid, DHA (22 : 6*n*-3), has been reported in pancreatic tissue of CF transmembrane conductance regulator –/– mice and in serum of patients with CF (Freedman *et al.* 1999, 2004; Strandvik *et al.* 2001*a*).

Abbreviations: AA, arachidonic acid; BMD, bone mineral density; CF, cystic fibrosis; EFA, essential fatty acid; ETA, eicosatrienoic acid; FN, femoral neck; LA, linoleic acid; LS, lumbar spine; PG, prostaglandin.

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We have previously reported low BMD in patients with CF despite normal growth and good lung function (Gronowitz *et al.* 2003, 2004). The aim of the present study was to investigate, during a 2-year prospective study (Gronowitz *et al.* 2004), if the abnormal fatty acid metabolism in CF, as reflected in serum phospholipid fatty acids, was associated with low bone mass in the lumbar spine (LS) and femoral neck (FN) in children and adult patients.

Subjects and methods

Subjects

Fifty-four CF patients (twenty-five males and twenty-nine females), consisting of thirty-five children and nineteen adults regularly attending the West Swedish CF Centre (Gothenburg, Sweden), with a median age of 16·0 (range 6–33) years, were investigated. Patients were consecutively included in the study except for pregnant or transplanted patients and investigated in stable condition without acute infections. All patients had pathological sweat tests (Cl $>$ 60 mmol/l) and typical clinical symptoms. Fifty patients had pancreatic insufficiency, ten patients had slight biochemical liver involvement and five patients had clinical liver disease. Four patients had CF-related diabetes mellitus. Thirty-three patients were homozygotes for dF508, and seventeen were compound heterozygotes for dF508. In two patients only one mutation was identified. A total of twelve mutations were identified in the patients and no patient had two unidentified mutations (Strandvik *et al.* 2001*a*). The average height, weight (Table 1) and lung function were within the normal range and clinical and nutritional data and biochemical markers in relation to BMD during the 2-year study have been reported previously (Gronowitz *et al.* 2004). Most children had a normal annual accretion of BMD. No dietary changes were performed during the study period. All patients were physically active as part of our treatment policy (Blomquist *et al.* 1986; Lannefors *et al.* 2004).

Fatty acids in serum phospholipids

Serum samples were taken in the morning at the annual check up of the patients after an overnight fast in relation to investigation of BMD (Gronowitz *et al.* 2004). The serum was frozen at -70°C until analysis within 2 weeks. Total lipids from the serum were extracted, fractionated on a single SEP-PAK aminopropyl cartridge (Waters Corp., Milford, MA, USA), transmethylated and separated by capillary GLC in a Hewlett-Packard 6890 gas chromatograph as described previously (Korotkova *et al.* 2001). The fatty acid 21 : 0 was used as internal standard and the fatty acid methyl esters identified by comparison with retention times of pure reference substances (Sigma Aldrich Sweden AB, Stockholm, Sweden). The CV for inter-assays were 1·7 % for palmitic acid (16 : 0) and 0·8 % for LA and 1·5 % for AA (*n* 25). The ratio of eicosatrienoic acid (ETA; Mead acid; 20 : 3*n*-9):AA was used to define the deficiency state; a ratio of $>$0·025 was used as the biochemical criterion of EFA deficiency (Siguel *et al.* 1987).

Reference values for the fatty acid pattern were obtained from 152 healthy individuals (100 subjects 6–18 years and fifty-four 18–50 years).

Dual X-ray absorptiometry

Dual X-ray absorptiometry was performed with a Hologic QDR 2000 (Hologic Inc., Bedford, MA, USA) at baseline and after 2 years. The CV with spine phantom measurements in the Hologic 2000 during the period 1996 to 2000 was 0·48 %. Reference values were obtained from the manufacturer and expressed in age- and sex-matched *Z* scores. Reference values for FN for children aged 9–19 years of age were obtained from the literature (Bachrach *et al.* 1999). Findings in a longitudinal community-based cohort of 234 healthy 16-year-old individuals in Gothenburg followed up annually until age 21 years concurred well with the manufacturer's values, the deviation being 0–2 % in the females and -4 to 2 % in the males.

Statistical analysis

Statistical analysis was performed with Student's *t* test, one-sample *t* test and paired *t* test when data were normally distributed, otherwise with non-parametric tests such as the Mann–Whitney *U* test and Wilcoxon paired signed rank test. Univariant correlation was made with linear regression and with Spearman rank correlation. Mean values and SD were used, if not otherwise indicated. *Z* score was used both for BMD and anthropometric

Table 1. Anthropometric data in fifty-four patients with cystic fibrosis at baseline and at 2-year follow-up
(Mean values and standard deviations)

	Age (years)				Height (*Z* score)				Weight (*Z* score)				BMI (kg/m^2)			
	Male		Female		Male		Female		Male		Female		Male		Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Children (*n* 35)*																
At baseline	13·2	3·8	11·6	3·4	−0·16	1·2	0·24	1·0	−0·17	1·4	0·13	1·1	18·3	3·0	17·7	3·3
After 2 years	15·3	3·7	13·9	3·2	−0·18	1·0	0·34	1·1	−0·26	1·1	0·32	1·6	18·3	2·8	18·9	4·1
Adults (*n* 19)†																
At baseline	24·8	4·6	25·5	3·8	−0·63	1·0	−0·53	0·8	−0·57	1·1	−0·51	1·0	20·9	2·5	20·7	2·5
After 2 years	27·0	4·7	27·6	3·9	−0·61	1·0	−0·48	0·9	−0·44	1·0	−0·36	1·1	21·7	2·3	20·6	2·4

* Sixteen males and nineteen females aged less than 20 years.
† Nine males and ten females aged more than 20 years.
For details of subjects and procedures, see this page.

data as both children and adults were included in the study. Statistical significance was set at $P<0{\cdot}05$.

Results

The young patients grew normally during the 2 years, as evidenced by unchanged *Z* score for height and weight (Gronowitz *et al.* 2004). The adult patients had a lower *Z* score for height than the children ($P<0{\cdot}05$), but still not significantly different from normal; their status was stable (Table 1).

The serum concentration of fatty acids in phospholipids differed significantly in most fatty acids compared with those in controls in both adults and children. In the children, LA and all long-chain PUFA were decreased except ETA and the ETA:AA ratio, which were increased, indicating EFA deficiency. The AA:DHA ratio was high (Table 2). During the study period, oleic acid, LA and total MUFA concentration decreased in the CF children (Table 2). The ETA concentration and the ETA:DHA ratio and total saturated fatty acids concentration increased significantly (Table 2). In the adult CF patients there were no changes during the study except for a marginal increase of total saturated fatty acids concentration.

There was no correlation between the change in weight or height and serum concentration of EFA.

The mean BMD *Z* score in children and adults was slightly lower in the LS ($-0{\cdot}7$ and $-0{\cdot}5$, respectively) and in the FN ($-0{\cdot}4$ and $-1{\cdot}2$, respectively) and did not change over the 2 years (Gronowitz *et al.* 2004). The bone growth was significant in the children and, as expected, no change was seen in the adults (Table 3).

Data of bone growth showed similar correlation with phospholipid fatty acids at baseline and follow-up. Changes in bone growth were reported when relevant, but otherwise only associations at 2-year follow-up are given.

Table 2. Major fatty acids (molar percentage) in serum phospholipids in fifty-four patients with cystic fibrosis at baseline and at 2-year follow-up compared with 152 healthy controls

(Mean values and standard deviations)

	Baseline		2 years		Difference over 2 years		Reference values‡	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Children (*n* 35)								
Age (years)	12·4	3·6	14·6	3·1	2·2	0·5		
Fatty acids								
Palmitic acid (16 : 0)	32·0	1·8	32·8***	2·2	0·8	2·4	30·3	0·7
Palmitoleic acid (16 : 1)	1·1	0·4	1·2***	0·4	0·05	0·5	0·4	0·1
Oleic acid (18 : 1)	15·5	1·9	13·9***	2·0	−1·6†††	2·2	9·2	1·2
Linoleic acid (18 : 2*n*-6)	20·4	2·7	19·2***	3·2	−1·2†††	3·9	22·0	2·3
α-Linolenic acid (18 : 3*n*-3)	0·6	0·2	0·5***	0·2	−0·09†	0·24	0·3	0·09
ETA (20 : 3*n*-9)	0·26	0·2	0·33***	0·2	0·07††	0·2	0·2	0·1
AA (20 : 4*n*-6)	7·3	1·2	6·9***	1·5	−0·4	1·4	8·7	1·2
DHA (22 : 6*n*-3)	2·2	0·7	2·3**	0·8	0·05	0·8	4·3	1·2
ETA : AA	0·04	0·2	0·05***	0·03	0·01†††	0·02	0·02	0·01
AA : DHA	3·5	1·0	3·4***	1·3	−0·1	1·1	2·1	0·5
Σ SFA	50·0	2·2	52·8***	2·0	2·8†††	3·3	47·5	0·9
Σ MUFA	18·9	2·1	17·6***	2·4	−1·3††	2·4	12·6	1·3
Σ PUFA	31·1	2·6	29·6***	3·2	−1·5†	4·2	39·9	1·7
Σ *n*-6	28·0	2·6	26·5***	3·0	−1·5†	4·0	34·0	1·9
Σ *n*-3	2·8	0·7	2·8***	0·8	−0·04	0·9	5·8	1·6
Adults								
Age (years)	25·2	4·1	27·3	4·0	2·1	0·5		
Fatty acids								
Palmitic acid (16 : 0)	34·7	2·3	34·1***	2·9	−0·6	2·4	31·8	1·0
Palmitoleic acid (16 : 1)	1·8	0·8	1·5***	1·1	−0·3	1·1	0·4	0·2
Oleic acid (18 : 1)	16·0	1·8	14·9***	3·0	−1·2	3·3	9·5	1·1
Linoleic acid (18 : 2*n*-6)	18·2	2·9	18·1***	3·0	−0·04	3·2	22·9	2·1
α-Linolenic acid (18 : 3*n*-3)	0·6	0·1	0·6***	0·4	−0·02	0·4	0·3	0·1
ETA (20 : 3*n*-9)	0·4	0·4	0·5***	0·4	0·06	0·2	0·1	0·1
AA (20 : 4*n*-6)	7·4	1·6	7·0	2·0	−0·4	1·5	7·6	1·2
DHA (22 : 6*n*-3)	2·5	1·1	2·4***	0·9	−0·04	1·1	4·3	1·2
ETA : AA	0·06	0·1	0·08***	0·08	0·03	0·06	0·02	0·01
AA : DHA	3·2	1·0	3·1*	1·1	0·1	0·9	2·1	0·5
Σ SFA	50·0	2·3	51·8***	3·1	1·8†	3·5	47·3	1·1
Σ MUFA	20·5	2·3	19·1***	3·7	−1·4	3·9	12·6	1·1
Σ PUFA	29·4	3·5	28·9***	3·7	−0·5	3·6	40·1	1·7
Σ *n*-6	25·9	3·5	25·4***	3·6	−0·4	3·5	33·8	2·0
Σ *n*-3	3·1	1·0	2·8***	0·8	−0·06	1·1	6·1	1·8

ETA, eicosatrienoic acid; AA, arachidonic acid; SFA, saturated fatty acids.
Mean value was significantly different from that of the reference group (controls): * $P\leq0{\cdot}05$, ** $P\leq0{\cdot}01$, *** $P\leq0{\cdot}001$.
The change over the 2 years was significant: † $P\leq0{\cdot}05$, †† $P\leq0{\cdot}01$, ††† $P\leq0{\cdot}001$.
‡ Reference (control) values are for 100 children aged less than 18 years and for fifty-two adults aged more than 18 years.
blood sedimentation rate serum
For details of subjects and procedures, see p. 1160.

Table 3. Bone mineral variables measured by dual X-ray absorptiometry in fifty-four patients with cystic fibrosis

(Mean values and standard deviations)

	Baseline		2 years follow-up	
	Mean	SD	Mean	SD
Children (*n* 35)				
Lumbar spine				
BMD (*Z* score)	−0·7	1·1	−0·7	1·3
BMC (g)	36·2***	17·6	44·0***†	18·0
Area (cm^2)	46·6***	11·9	51·9**†	10·3
Femoral neck				
BMD (*Z* score)	−0·3**	0·9	−0·4**	1·0
BMC (g)	3·4**	1·1	3·9†	1·1
Area (cm^2)	4·4***	0·7	4·7**†	0·7
Adults (*n* 19)				
Lumbar spine				
BMD (*Z* score)	−0·5	1·1	−0·5	1·1
BMC (g)	60·7	12·6	60·0	12·9
Area (cm^2)	59·4	5·9	59·0	6·1
Femoral neck				
BMD (*Z* score)	−1·2	1·1	−1·2	1·1
BMC (g)	4·1	0·8	4·1	0·9
Area (cm^2)	5·2	0·5	5·3	0·6

BMD, bone mineral density; BMC, bone mineral content.
Mean value was significantly different to that of the adult group: ** $P \leq 0.01$, *** $P \leq 0.001$.
† Mean value was significantly different to that at baseline ($P \leq 0.001$).
For details of subjects and procedures, see p. 1160.

Total correlation of saturated fatty acids was only correlated with FN parameters, but the association disappeared when the children and adults were analysed separately. Palmitic acid (16 : 0) was only associated with bone growth in children; the relationship to LS is presented in Fig. 1 and the correlation with FN bone mineral content and FN area were 0·54 ($P=0.001$) and 0·40 ($P=0.02$), respectively.

The LS *Z* score and the change in LS *Z* score showed an inverse correlation in the CF children with AA:DHA ratio (Fig. 2). This was supported by the significant correlation between the change of LS BMD *Z* score and the phospholipid concentration of DHA (*r* 0·40; $P=0.03$).

No correlations were found with AA, LA or α-linolenic acid concentrations. The EFA deficiency ratio ETA:AA showed strong correlations with the LS ($P<0.01$) and FN ($P<0.001$) areas in both children and adults.

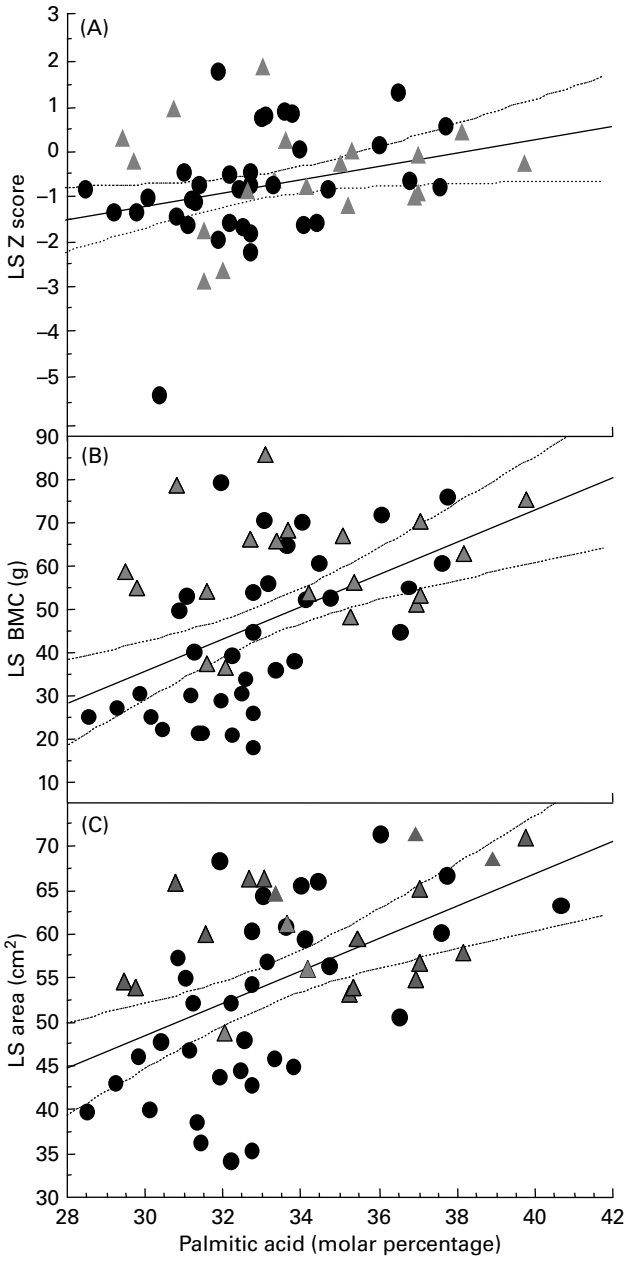

Fig. 1. Relationships between palmitic acid (16 : 0) in serum phospholipids and bone parameters at 2-year follow-up in thirty-four children (●) and nineteen adults (▲) with cystic fibrosis. (A) Relationship between palmitic acid and the *Z* score of bone mineral density of the lumbar spine (LS) (total patients *r* 0·3 ($P<0.04$), children *r* 0·4 ($P<0.02$), adults *r* 0·1 (NS)). (B) Relationship between palmitic acid and bone mineral content (BMC) of the LS (total patients *r* 0·5 ($P<0.0001$), children *r* 0·6 ($P<0.0001$), adults *r* 0·2 (NS)). (C) Relationship between palmitic acid and area of the LS (total patients *r* 0·5 ($P<0.0002$), children *r* 0·6 ($P<0.0005$), adults *r* 0·3 (NS)). The medians and 95 % CI are indicated.

Discussion

To the best of our knowledge, the present study is the first to show an association in human subjects between EFA and BMD and the first one to indicate an association between lipids and bone growth in children with CF.

The serum phospholipid EFA pattern differed as expected in the CF patients compared with controls (Rogiers *et al.* 1980, Strandvik *et al.* 2001*b*) in both children and adults. The anthropometric data were within the normal range, although the adult patients had a lower *Z* score for height compared with children. This might be due to the fact that they had not had centralised treatment from early childhood, since the West Swedish CF Centre was not established in Gothenburg before 1992. An association between BMD and saturated fatty acids in serum has previously been documented in healthy children (Gunnes & Lehmann, 1995). We also found a similar correlation in the children but not in adults with CF. This correlation was mainly referred to palmitic acid (Fig. 1), a correlation probably reflecting the children's good nutritional status since palmitic acid is endogenously synthesised or obtained by diet. A strong negative correlation between BMD and bone accretion and AA:DHA ratio was found at 2-year follow-up, and this was associated with DHA concentration and not with AA, indicating

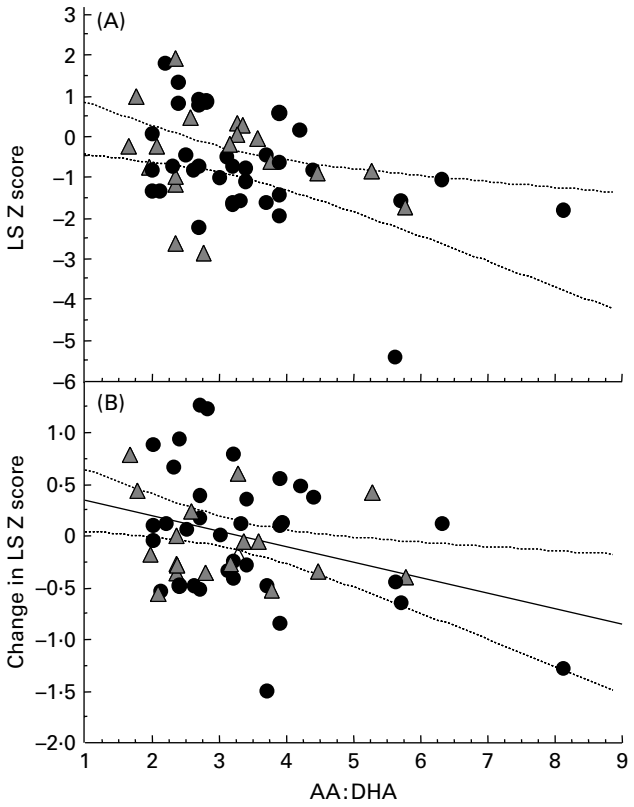

Fig. 2. Relationships between arachidonic acid (AA; 20:4*n*-6):DHA ratio in serum phospholipids and *Z* scores of bone mineral density of the lumbar spine (LS) in thirty-four children (●) and nineteen adult (▲) patients with cystic fibrosis. (A) Relationship between AA:DHA ratio and *Z* scores of bone mineral density of the LS at 2-year follow-up (total patients $r -0{\cdot}40$ ($P<0{\cdot}004$), children $r -0{\cdot}39$ ($P<0{\cdot}0035$), adults $r -0{\cdot}23$ (NS)). (B) Relationship between AA:DHA ratio and change in *Z* scores of bone mineral density of the LS over the 2 years (total patients $r -0{\cdot}34$ ($P<0{\cdot}01$), children $r -0{\cdot}38$ ($P<0{\cdot}02$), adults $r -0{\cdot}24$ (NS)). The medians and 95 % CI are indicated.

that the balance between *n*-6 and *n*-3 fatty acids is of importance. This corroborates previous studies in animals (Watkins *et al.* 2001*b*). EFA, by modulating eicosanoids, leptin and IGF-1, are involved in the regulation of bone growth and bone status (Watkins *et al.* 2001*b*,*c*). IGF-1 is related to lean body mass and has been reported to be decreased in CF patients (Arumugam *et al.* 1998; Sermet-Gaudelus *et al.* 2003). Lean body mass was normal in our patients (Gronowitz *et al.* 2003), as were serum leptin levels (A Lindblad, personal communication). Leptin has previously been shown to be decreased in CF patients related to the decreased fat mass (Arumugam *et al.* 1998). On the other hand, leptin has also been reported to be increased despite low fat mass, probably related to the impact of cytokines in response to infection and inflammation (Ahme *et al.* 2004). A weakness in the present study is that we did not have the opportunity to analyse these hormones, but the normal anthropometry, good lung function, normal lean body mass and relatively normal bone mass suggest that the hormones probably would not be significantly affected in our group of patients. Nor did we find any correlations between EFA and clinical infection parameters such as blood sedimentation rate serum and IgG.

The serum concentration of LA was decreased in our patients as has generally been reported in CF patients (Kuo *et al.* 1962; Strandvik *et al.* 2001*a*), more marked during early infancy and during adolescence (Lai *et al.* 2000). In the rat we have recently shown that EFA deficiency during the perinatal period can programme for low trabecular BMD in the adult animal (Korotkova *et al.* 2005). It might be speculated that severe EFA deficiency in the perinatal period (Lai *et al.* 2000) could contribute to the low BMD often reported in CF. Such a hypothesis is partly supported by our previous finding of normal bone accretion during childhood, indicating that the low BMD must start early, either related directly to the CF transmembrane conductance regulator or to early environmental influences (Gronowitz *et al.* 2004).

The importance of EFA for bone metabolism has been well documented (Marks & Miller, 1993; Watkins *et al.* 2001*a*,*b*,*c*). PGE_2, one important product of AA, affects bone metabolism. In CF, AA release is increased (Carlstedt-Duke *et al.* 1986) and since this step is rate limiting for PG synthesis, that will explain the high levels of prostanoids in CF (Strandvik *et al.* 1996). Both bone formation and bone resorption are influenced by PGE_2 and its effect on bone is dose dependent. Low levels of PGE_2 enhance bone formation by osteoblasts, while, at higher concentrations, PGE_2 suppresses osteoblast differentiation and promotes bone resorption by osteoclasts (Marks & Miller, 1993; Watkins *et al.* 2001*c*). Moreover, leucotriene B_4, another product of AA, which stimulates osteoclastic bone resorption *in vivo* and *in vitro* (Watkins *et al.* 2001*c*), has been shown to be increased in CF (Shimizu *et al.* 1994). The low LA concentration in CF is supposed to be secondary to the abnormal AA release (Strandvik, 2004) and, if not compensated for, it will progress and thus the AA synthesis might decrease, which will eventually also decrease the eicosanoid synthesis. In the present study, the inverse relationship of BMD accretion to the AA:DHA ratio at 2 years might reflect an influence on eicosanoid metabolism favouring bone growth, i.e. that the decrease during the 2-year period of *n*-6 fatty acids and especially the decrease of AA might probably have decreased PGE_2 synthesis and decreased the *n*-6:*n*-3 ratio and consequently decreased bone resorption. Due to ethical reasons bone biopsies could not be performed and the specific EFA ratio in bone tissue could therefore not be analysed. Diet, including vitamin D and Ca intake, was not changed during the study (Gronowitz *et al.* 2004). There was no indication of increased intake of *n*-3 fatty acids during the study period either.

The present findings imply that changes in serum EFA can be significant during a 2-year period in stable patients and also that the balance between *n*-6 and *n*-3 fatty acids might be important for long-term bone growth as recently confirmed in experimental studies (Korotkova *et al.* 2004, 2005). The fatty-acid status seems to be especially important in growing children, but to evaluate the impact in adults, probably a larger study sample and longer observation period are needed. Furthermore, a slight EFA deficiency, as documented by the increasing ETA:AA ratio over 2 years, might not be detrimental for bone mass in CF, possibly due to modulations in PG metabolism. If this would be the case, PG metabolites in urine might be valuable indicators of bone metabolism in further studies. Prospective intervention studies must be performed in order to find which EFA levels would optimise bone growth in CF children. If our finding has relevance for healthy children, the ideal fat intake to obtain optimal peak bone mass would need special attention.

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