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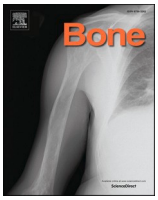

Full Length Article

Development of musculoskeletal deficits in children with cystic fibrosis in later childhood

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ABSTRACT

Cystic fibrosis (CF) is a genetic condition primarily affecting the respiratory system, with the associated progressive lung damage and loss of function resulting in reduced lifespan. Bone health is also impaired in individuals with CF, leading to much higher fracture risk even in adolescence. However, the development of these deficits during growth and the relative contributions of puberty, body size and muscular loading remain somewhat unexplored.

We therefore recruited 25 children with CF (10 girls, mean age 11.3 ± 2.9y) and 147 children without CF (75 girls, mean age 12.4 ± 2.6y). Bone characteristics were assessed using peripheral quantitative computed tomography (pQCT) at 4 % and 66 % distal-proximal tibia. Muscle cross-sectional area (CSA) and density (an indicator of muscle quality) were also assessed at the latter site. Tibial bone microstructure was assessed using high-resolution pQCT (HR-pQCT) at 8 % distal-proximal tibial length. In addition, peak jump power and hop force were measured using jumping mechanography. Group-by-age interactions and group differences in bone and muscle characteristics were examined using multiple linear regression, adjusted for age, sex and pubertal status and in additional models, height and muscle force.

In initial models group-by-age interactions were evident for distal tibial total bone mineral content (BMC) and trabecular volumetric bone mineral density (vBMD), with a lower rate of age-related accrual evident in children with CF. In assessments of distal tibial microstructure, similar patterns were observed for trabecular number and thickness, and cortical CSA. In the tibial shaft, group-by-age interactions indicating slower growth in CF were evident for total BMC and cortical CSA, whilst age-independent deficits in CF were observed for several other variables. Peak jump power and hop force also exhibited similar interactions. Group-by-age interactions for bone were partially attenuated at the distal tibia and fully attenuated at the tibial shaft by adjustment for muscle force.

These results suggest that bone and muscle deficits in children with CF develop throughout later childhood, independent of differences in pubertal stage and body size. These diverging growth patterns appear to be mediated by differences in muscle function, particularly for bone characteristics in the tibial shaft. Given the high fracture risk in this population from childhood onwards, development of interventions to improve bone health would be of substantial clinical value.

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1. Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic condition with a prevalence from 0.1 to 2.9/10,000 people in European countries [1]. CF affects the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which is in involved in production of mucus, digestive fluids and sweat. Mucus build-up in the respiratory system causes inflammation, infections and progressive lung damage and loss of function. CF also leads to thicker pancreatic fluids resulting in damage and fibrosis of pancreatic tissues. The resulting pancreatic dysfunction impairs absorption of nutrients such as fat and fat-soluble vitamins in the intestines. Life expectancy in individuals with CF is much lower than in the general population at around 45 years. Although low, this represents a substantial increase in survival over recent years due to new treatments and improvements in multidisciplinary care, such that the CF adult population is predicted to increase by 75 % from 2010 to 2025 [1].

Bone health is also impaired in individuals with CF, with high rates of osteopenia, osteoporosis and fractures [2]. The causes of bone deficits are multifactorial, including factors such as intestinal malabsorption, impaired vitamin D and calcium metabolism, lower sun exposure, systemic inflammation resulting from lung infections, physical inactivity, impaired growth and delayed puberty [3]. In a recent German study of adolescents with CF, fracture incidence was 9-fold that of age-matched controls, with 60 % of individuals with CF having a fracture by 25 years compared to 15 % in those without CF [4]. Therefore bone health in CF represents an important secondary health problem.

Previous studies have characterised the effects of CF on bone health. In studies which examined upper limb (radius) and lower limb (tibia) sites, deficits in the latter were more pronounced [5,6]. Peripheral quantitative computed tomography (pQCT) studies suggest that bone deficits in the trabecular-rich epiphysis derive from lower trabecular bone mineral density (BMD) [7]. In contrast, those in the diaphysis result from reduced cortical area due to smaller periosteal circumference, although the contribution of cortical BMD is disputed [7,8]. Previous work identified radius bone deficits in children with CF post- but not pre-puberty, suggesting that deficits may develop as children mature [5]. Whilst previous studies have identified group-by-age interactions, this work has been limited by low numbers of participants and lack of information on pubertal status [8] or consideration of a only a limited number of pQCT variables [5]. Therefore, the relative contributions of mass, size and density to development of greater fracture risk remains unexplored.

High-resolution pQCT (HR-pQCT) is a newer, higher-resolution technique which allows assessment of bone microarchitecture. HR-pQCT assessment of older children with CF found minimal differences in tibial or radial cortical or trabecular bone microarchitecture [9], but group-by-age interactions were not examined. Given that substantial lower values for bone microarchitecture have been observed in young adults [6,10], it may be that these deficits develop during growth.

Bone loading by muscular forces during physical activity and exercise is a key determinant of bone strength throughout life [11]. A number of studies have identified smaller muscle size and impaired function in individuals with CF [12], which may contribute to observed bone deficits. Indeed, previous work has identified strong relationships between muscle CSA and bone strength which predict bone weakness in individuals with CF [5]. However, assessment of peak muscle force and power relevant to skeletal development and mobility/falls risk respectively have not been assessed in CF. In addition, it is unknown to what extent deficits in muscle quality as well as quantity contribute to function deficits, and in turn to impaired bone health.

Therefore, we used pQCT, HR-pQCT and jumping mechanography in order to obtain detailed information on musculoskeletal development in children and adolescents with CF.

2. Methods

2.1. Recruitment

This observational study was conducted at the Medical Research Council Elsie Widdowson Laboratory (formerly MRC Human Nutrition Research (HNR)) in Cambridge, UK. Children with CF were recruited through outpatient clinics at Addenbrooke's Hospital in Cambridge. Control children were recruited using posters and leaflets placed in the local community and via the internet through emails and online adverts on the MRC HNR website. The study was approved by the National Research Ethics Service (REC) Cambridge South Committee (REC Number: 13/EE/0078), and informed parental consent and assent of children under 16 years of age was obtained from all participants prior to participation. Children between 8 and 16 years were recruited. Only children of white ethnicity were recruited, due to higher incidence of CF within this group. Exclusion criteria for control children were any chronic childhood disorders or use of medications affecting musculoskeletal health, immobilisation in the previous year, previous involvement in research studies involving radiation, or nervous system conditions or learning difficulties which would making remaining still difficult or painful.

2.2. Anthropometry and clinical information

Body mass was measured to the nearest 0.1 kg using an electronic digital scale (Seca, Hamburg, Germany). Participants were asked to remove shoes and heavy clothing. Height was measured to the nearest 0.1 cm using a permanent wall-mounted stadiometer (Seca, Hamburg, Germany) in the vast majority (>95 %) of participants. Those who were very small were measured using a portable stadiometer (Leicester Height Measure Mk II, Seca, Hamburg, Germany) designed to measure children. All participants were asked to complete a self-assessment of pubertal staging with or without their parent/guardian in a private room. The assessment was against a series of drawings: for females this was breast development and pubic hair growth; for males this was pubic hair and mean testicular volume, measured by comparison to an orchidometer (Prader, ESP Ltd., UK) [13]. Participants assessed as Tanner stage 1 were classed as pre-pubertal, participants in stages 2 and 3 were classed as early pubertal, and participants in TS 4 and TS 5 were classed as late pubertal. Permission was obtained during consent from participants with CF and their parent/guardian to access their medical records. Most recent information on forced expiratory volume in the first second of expiration (FEV1, as a percentage of forced vital capacity), vitamin D (25(OH)D) status, comorbidities (pancreatic insufficiency, CF-related diabetes and liver disease), history of fracture and genotype were recorded.

Whole body dual-energy X-ray absorptiometry (DXA) scans were performed using a Lunar Prodigy Advance DXA (Prodigy enCORE 2006 software, version 10.51.006, GE Healthcare Lunar, Belgium) according to standard procedures. Participants wore light clothing during scanning, and all metal objects including clothing with metal buttons or zips were removed. Due to bed size, some larger participants had to hold their arms in a neutral position to remain within the scanning field. Daily quality assurance checks were performed prior to scanning to monitor the performance of the scanner, and all scans were reviewed and analysed by the same investigator. From these scans, total bone mineral content (BMC) and total bone area, as well as lean and fat mass were measured. Short-term error for 35 duplicate scans of adults using this scanner and procedures were 1.65 %, 2.05 %, 1.89 %, and 0.10 % for BMC, bone area, lean and fat mass respectively.

pQCT scans were conducted on an XCT 2000L scanner (Stratec, Pforzheim, Germany) using software version 6.20C supplied with the machine. The non-dominant leg was scanned, and images were taken at 4 % and 66 % distal-proximal tibial length (measured from the medial malleolus to the medial knee joint cleft). The reference line was placed

Table 1
Participant characteristics, split by group. FEV1 – forced expiratory volume in the first second of expiration.

Variable		Control		CF		P
n		147		25		
Sex (F/M)		75/72		10/15		0.509
Pubertal Stage	Pre-pubertal	33		5		0.714
	Early pubertal	67		14		
	Late pubertal	47		6		
		Mean	SD	Mean	SD	
Age (years)		12.4	2.6	11.3	2.9	0.151
Height (m)		1.53	0.15	1.46	0.15	
Body mass (kg)		46.4	14.6	38.5	11.9	
Height *Z*-score		0.46	1.01	−0.07	1.05	0.021
Body mass Z-score		0.47	1.02	−0.18	0.93	0.004
Whole-body DXA	Bone mineral content (kg)	1.41	0.58	1.03	0.43	<0.001
	Whole-body bone area (cm^2)	1532	428	1244	373	0.002
	Lean mass (kg)	32.8	9.8	28.5	8.6	0.038
	Fat mass (kg)	11.4	7.5	8	5.4	0.02
Jumping mechanography	Peak vertical jump power (kW)	1.93	0.86	1.43	0.61	0.003
	Peak hop force (kN)	1.34	0.43	1	0.33	<0.0.001
Most recent FEV1 (%)				85.9	19	
25(OH)D (nmol/l)				78.8	26.6	
Pancreatic insufficiency				22 (88 %)		
CF-related diabetes				2 (8 %)		
Liver disease				2 (16 %)		
History of fracture	All			6 (24 %)		
	Long bone			5 (20 %)		
Genotype	ΔF508/ΔF508			15 (60 %)		
	ΔF508/other			10 (40 %)		
	Other/other			0		

at the distal tibial endplate, except where a growth plate was visible in which case the reference line was positioned to bisect the medial border of the distal metaphysis. The researcher placed a reference line at the endplate of the tibia. If the growth plate was visible, the reference line was repositioned to bisect the medial border of the distal metaphysis. Regions of interest were drawn manually around the tibia and fibula bones, and around the whole leg cross-section. For both slices, a threshold of 180 $mg.mm^{-3}$ was used to separate the outer bone surface from soft tissue using peeling mode 1 in the software. For the 4 % tibial site total bone mineral content (BMC), total cross-sectional area (CSA) and trabecular volumetric bone mineral density (vBMD) were assessed. The inner 45 % of bone area was assessed as trabecular bone to measure vBMD, whereas for the fibula only total vBMD was assessed due to the thick cortical shell at this site. At the 66 % site, a threshold of 710 $mg.mm^{-3}$ was used to identify cortical bone, from which cortical CSA, cortical vBMD, endocortical circumference, cortical thickness and polar moment of inertia were calculated in addition to total BMC and total CSA. For soft tissue, a threshold of -50 $mg.mm^{-3}$ was used to delineate the outer edge of the limb using contour mode 3 and peel mode 1, with a threshold of 50 $mg.mm^{-3}$ to separate fat from muscle tissue using contour mode 1 and peeling mode 2. From these measurements, fat and muscle CSAs and muscle density were calculated.

HR-pQCT was performed using an XtremeCT scanner (Scanco Medical AG, Bassersdorf, Switzerland) and scans were assessed using the manufacturer's image processing language software (μCT Evaluation Program v6.0; Scanco Medical AG, Brüttisellen, Switzerland). All scans were performed using the following scan settings: an X-ray potential of 60 kVp, X-ray tube current of 900 μA, integration time of 100 ms, matrix size of 1536 × 1536 and voxel size of 82 μm. The non-dominant distal tibia was scanned at 8 % distal-proximal tibial length [14], with the reference line placed on the tibial plafond. For each scan, the periosteal perimeter was identified by using a semi-automated edge-finding algorithm which produced a closed contour around the periosteal surface. All contours were examined manually and modified as necessary to delineate the periosteal boundary. In addition to the standard analysis, a cortical porosity script was used to assess cortical parameters. This script identified the endosteal perimeter by using a semi-automated edge-finding algorithm which produced a closed contour around the endosteal surface. All contours were examined manually and modified as necessary. The precision of HR-pQCT measurements was 0.7–1.5 % for total, trabecular, and cortical densities and 2.5–4.4 % for trabecular architecture, <1 % for cortical vBMD, <1.5 % for cortical CSA and 6.0–13.5 % for cortical porosity [15].

Dynamic muscle function was assessed using a Leonardo Mechanography® Ground Reaction Force Plate (Novotec Medical, Pforzheim, Germany). The manufacturer's software (Leonardo Mechanography GRFP version 4.2, Novotec, Pforzheim, Germany) was used to store, and calculate the data. Participants were asked to complete a counter-movement jump with freely moving arms, being instructed to jump as high as possible. Three jumps were performed with 30 s break in-between jumps, and the highest jump was selected from which peak power was recorded. Participants were also asked to complete a series of one-legged hops on the forefoot of their non-dominant leg, keeping their knee straight and ankle stiff. Participants were instructed to place their whole body weight through the forefoot and not to touch the platform with their heel. Two trials were completed; the software automatically selects the valid hop with the greatest peak force, which was recorded. The CV of muscle power measurement in children is 3.7 %; peak jumping force in children was 8.9 % [16].

2.3. Statistical analysis

Unadjusted group differences in characteristics including muscle and bone were assessed using independent *t*-tests, with the alpha level set as $P = 0.05$. Where required, data were log or reciprocal transformed before *t*-test to ensure that assumptions of normality were met. Associations between group (control/CF) and bone and muscle outcomes were then assessed with multiple linear regression models using the R statistical environment (version 3.6.2, www.rproject.org). Model 1 was adjusted for sex and age, and also for pubertal status which did not substantially influence group differences. In order to examine whether body size or muscle function differences contributed to any observed differences in bone characteristics, Model 2 was further adjusted for height and Model 3 further for muscle force, Group-by-age interactions were assessed in order to examine whether age-related bone changes differed between the two groups. In addition, we explored two-way and

Table 2
Bone, muscle and fat characteristics assessed by peripheral quantitative computed tomography (pQCT) and high resolution pQCT (HR-pQCT). BMC – bone mineral content, CSA – cross-sectional area, BMD – bone mineral density, CSMI – cross-sectional moment of inertia. For pQCT variables, $n = 24$ for CF group.

Technique	Region	Site	Variable	Group				P
				Control		CF		
				Mean	SD	Mean	SD	
pQCT	Tibia	4 %	Total BMC (mg.mm^{-1})	233	63	185	46	<0.001
			Total CSA (mm^2)	750	175	645	173	0.01
			Trabecular BMD (mg.mm^{-3})	210	29	193	30	0.013
		66 %	Total BMC (mg.mm^{-1})	265	69	223	59	0.004
			Total CSA (mm^2)	342	82	291	77	0.005
			Cortical CSA (mm^2)	220	56	183	49	0.002
			Cortical BMD (mg.mm^{-3})	1067	52	1077	51	0.404
			Cortical thickness (mm)	4.19	0.67	3.76	0.57	0.002
			Periosteal circumference (mm)	65.1	7.8	60	7.8	0.005
			Endocortical circumference (mm)	38.8	5.4	36.4	5.5	0.043
			Polar CSMI (mm^4)	15,299	7401	10,861	6094	0.003
	Fibula	4 %	Total BMC (mg.mm^{-1})	58	17	45	14	>0.001
			Total CSA (mm^2)	108	27	94	25	0.16
			Total BMD (mg.mm^{-3})	310	39	290	31	>0.001
		66 %	Total BMC (mg.mm^{-1})	75	20	60	20	<0.001
			Total CSA (mm^2)	103	28	81	25	<0.001
			Cortical CSA (mm^2)	59	15	47	16	<0.001
			Cortical BMD (mg.mm^{-3})	1064	45	1054	51	0.326
			Cortical thickness (mm)	1.99	0.33	1.76	0.34	0.004
			Periosteal circumference (mm)	35.6	4.8	31.6	4.7	<0.001
			Endocortical circumference (mm)	23.2	4	20.6	3.2	0.002
			Polar CSMI (mm^4)	1089	551	722	536	0.003
	Calf	66 %	Muscle CSA (mm^2)	2623	740	2227	553	0.003
			Muscle density (mg.mm^{-3})	78.4	2.4	77.9	2	0.201
			Fat CSA (mm^2)	2003	921	1591	633	0.005
HR-pQCT	Tibia	8 %	Trabecular number (1/mm)	2.19	0.27	2.14	0.31	0.46
			Trabecular thickness (mm)	0.068	0.009	0.061	0.011	0.006
			Cortical CSA (mm^2)	101	29	83	19	<0.001
			Cortical BMD (mg.mm^{-3})	768	70	758	76	0.543
			Cortical porosity (%)	53.8	28.1	44.5	26.8	0.122

three-way group-by-age-by-sex interactions which were simplified by removal of interaction terms if interactions were not observed ($P > 0.1$). There was no evidence of group-by-age-by-sex interactions. Whilst group-by-sex interactions were observed for 4 % total CSA and 66 % periosteal circumference in the tibia (and also for a number of fibula variables) in initial models, these associations were fully attenuated by adjustment for height in Model 2 in all cases. Importantly, consideration of additional interactions did not substantially affect the group-by-age interactions which were the primary focus of the analyses. Therefore sex-adjusted analyses were performed with sexes combined. Where there was no evidence of an interaction ($P > 0.1$) the interaction term was removed from models and main effect of group was assessed. In sensitivity analyses, adjustment for hop force was replaced by lean and fat mass, and alternatively by jump power; group-by-pubertal status interactions were also assessed.

Table 3
Associations between group (CF/control, in addition to group-by-age interaction) and bone characteristics assessed by peripheral quantitative computed tomography (pQCT), high-resolution pQCT (HR-pQCT) and dual-energy X-ray absorptiometry (DXA). BMC – bone mineral content, CSA – cross-sectional area, BMD – bone mineral density. Adjustments: Model 1 – Sex, age, pubertal status, Model 2 – Model 1 + height, Model 3 – Model 2 plus muscle force. Covariates column indicates where positive associations between bone outcome and covariates were observed in fully-adjusted models as follows; 1 – Age, 2 – Sex, 3 – Pubertal status, 4 – height, 5 – maximal hop force. RC – regression coefficient.

Technique	Region	Site	Variable	Model 1								Model 2								Model 3								Covariates
				Group				Group-by-age				Group				Group-by-age				Group				Group-by-age				
				RC	95% CI		P	RC	95% CI		P	RC	95% CI		P	RC	95% CI		P	RC	95% CI		P	RC	95% CI		P	
pQCT	Tibia	4%	Total BMC (mg.mm^{-1})					**-10.7**	**-16.2**	**-5.1**	**<0.001**					**-10.0**	**-14.9**	**-5.0**	**<0.001**					**-7.1**	**-10.9**	**-3.2**	**0.000**	1,2,4,5
			Total CSA (mm^2)	**-74**	**-127**	**-20**	**0.007**				0.372	-34	-80	13	0.158				0.481	1	-43	45	0.957				0.957	1,2,4,5
			Trabecular vBMD (mg.mm^{-3})					**-7.6**	**-11.8**	**-3.3**	**0.001**					**-7.6**	**-11.9**	**-3.4**	**0.001**					**-6.3**	**-10.4**	**-2.3**	**0.002**	4,5
		66%	Total BMC (mg.mm^{-1})					**-7.1**	**-13.1**	**-1.0**	**0.023**					**-5.9**	**-11.0**	**-0.8**	**0.024**	4	-8	15	0.525				0.103	1,2,4,5
			Total CSA (mm^2)	**-34**	**-59**	**-10**	**0.006**				0.117	-16	-37	5	0.141				0.154	5	-13	22	0.608				0.528	1,2,4,5
			Cortical CSA (mm^2)					**-5.1**	**-10.4**	**0.3**	**0.065**					**-4.0**	**-8.5**	**0.4**	**0.079**	0	-11	11	0.996				0.315	1,2,4,5
			Cortical vBMD (mg.mm^{-3})	**24**	**9**	**38**	**0.002**				0.220	**22**	**7**	**37**	**0.004**				0.200	**22**	**7**	**38**	**0.006**				0.190	1,2,3
			Cortical Thickness (mm)	**0**	**0**	**0**	**0.002**				0.136	**0**	**0**	**0**	**0.036**				0.186	0	0	0	0.440				0.436	1,2,4,5
			Periosteal Circumference (mm)	**-3**	**-6**	**-1**	**0.004**				0.131	-2	-4	0	0.103				0.174	0	-1	2	0.815				0.563	1,2,4,5
			Endocortical Circumference (mm)	-1.6	-3.6	0.5	0.134				0.406	-0.4	-2.3	1.5	0.672				0.545	0.6	-1.2	2.5	0.499				0.963	4,5
			Polar CSMI (mm^4)	**-2968**	**-5133**	**-803**	**0.008**				0.112	-1393	-3283	497	0.151				0.149	582	-975	2139	0.465				0.540	1,2,4,5
HR-pQCT	Tibia	8%	Trabecular Number (mm^{-1})					**-0.06**	**-0.10**	**-0.02**	**0.005**					**-0.06**	**-0.10**	**-0.02**	**0.007**					**-0.05**	**-0.09**	**-0.01**	**0.020**	1,5
			Trabecular thickness (mm)					**-0.002**	**-0.003**	**0.000**	**0.030**					**-0.002**	**-0.003**	**0.000**	**0.025**					**-0.002**	**-0.003**	**0.000**	**0.031**	2
			Cortical CSA (mm^2)					**-4.1**	**-6.7**	**-1.6**	**0.002**					**-3.9**	**-6.4**	**-1.4**	**0.003**					**-2.9**	**-5.0**	**-0.7**	**0.011**	1,2,5
			Cortical vBMD (mg.mm^{-3})	11	-8	29	0.275				0.390	6	-13	26	0.511				0.328	6	-14	27	0.531				0.316	2,3,5
			Cortical porosity (%)	-10	-20	0	0.056				0.264	-7	-17	3	0.176				0.319	1	-8	10	0.822				0.756	1,2,3
Whole-body DXA			Bone mineral content (kg)					**-67**	**-112**	**-22**	**<0.001**					**-58**	**-94**	**-21**	**<0.001**					**-37**	**-61**	**-12**	**<0.001**	1,4,5
			Whole-body bone area (cm^2)					-30	-63	3	0.07					**-22**	**-45**	**1**	**0.06**					-9	-24	6	0.26	1,4,5

Table 4
Associations between group (CF/control, in addition to group-by-age interaction) and muscle and fat characteristics assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT), dual-energy X-ray absorptiometry (DXA) and jumping mechanography. BMC – bone mineral content, CSA – cross-sectional area, BMD – bone mineral density. Adjustments: Model 1 – Sex, age, pubertal status, Model 2 – Model 1 + height. Covariates column indicates where positive associations between bone outcome and covariates were observed in fully-adjusted models as follows; 1 – Age, 2 – Sex, 3 – Pubertal status, 4 – height, 5 – maximal hop force. RC – regression coefficient.

Variable	Model 1						Model 2						Covariates
	Group			Group-by-age			Group			Group-by-age			
	RC	95 % CI		P	RC	95 % CI	P	RC	95 % CI	P	RC	95 % CI	P	
Peak vertical jump power (kW)					−0.10	−0.17	−0.03	0.008			−0.09	−0.16	−0.02	0.011	1,2,3
Peak hop force (kN)					−0.03	−0.07	0.01	0.098	−0.16	−0.26	−0.05	0.004			0.142	1,2,3,4
Muscle CSA (mm^2)	−262	−505	−19	0.04				0.289	−172	−412	68	0.16			0.363	1,4
Muscle density ($mg.mm^{-3}$)					−0.45	−0.74	−0.15	0.004					−0.43	−0.72	−0.13	0.005	1,4
Fat CSA (mm^2)	−385	−745	−25	0.04				0.585	−291	−654	71	0.12				0.494	4
Lean mass (kg)					−0.0007	−0.0015	0.0001	0.071					−0.0005	−0.0012	0.0001	0.083	1,2,4
Fat mass (kg)	−0.0024	−0.0050	0.0002	0.077				0.948	−0.0014	−0.0040	0.0012	0.289				0.798	2,3,4

3. Results

Participant characteristics are shown in Table 1, along with bone and muscle characteristics obtained from DXA and jumping mechanography. Of 64 potentially eligible individuals with CF who were eligible, 41 agreed to discuss the study and 29 agreed to take part and were screened. After screening, four of those were ineligible for the study. 267 control participants contacted the research team, of which 254 were eligible and 147 agreed to attend an appointment and completed data collection. Unfortunately, data are not available to the group to compare the characteristics of eligible individuals with respect to those ultimately included in the study. For one participant with CF, movement artefacts meant that distal tibia and fibula scans could not be assessed.

As shown in Table 1 both groups were of similar age and pubertal stage, with a similar proportion of males and females. Controls were taller and heavier than children with CF (both $P < 0.05$). pQCT and HR-pQCT-derived bone and muscle variables are shown in Table 2. In un-adjusted analysis, 66 % tibial and fibular cortical vBMD, 66 % fibular total CSA, muscle density, 8 % tibia trabecular number, cortical BMD and cortical porosity did not differ between individuals with CF and controls (all $P > 0.05$). For all other bone and muscle variables, values were greater in controls than participants with CF (all $P < 0.05$). The vast majority of bone outcomes were positively associated with age and muscle force, and to a lesser extent with sex and height in fully-adjusted models (Table 3 and Supplementary Table 1). Similar associations were observed for most muscle and fat outcomes and age and height covariates (Table 4).

We refer to age-related increases and decreases throughout this section, indicating a positive or negative association between age and bone and muscle outcomes. At the 4 % tibial site, group-by-age interactions were observed, such that age-related differences in total BMC increased at a greater rate with age in control children whilst trabecular vBMD increased in controls but decreased in children with CF with increasing age (Table 3 and Fig. 1). The group-by-age interaction for total BMC was partially attenuated by adjustment for muscle force in Model 3, and less strongly for trabecular vBMD. Whilst children with CF also had lower total bone CSA at this site (no interaction) in Model 1, this was completely attenuated by adjustment for body size in Model 2.

In more detailed assessment of tibial trabecular bone using HR-pQCT, group-by-age interactions showed development of lower trabecular number and thickness in older children with CF (Table 3 and Fig. 4) whereas values were maintained in control participants across all models. Group-by-age interactions also showed a lower rate of cortical CSA accrual in children with CF across Models 1 and 2, with partial attenuation in Model 3. In contrast, there was little evidence of group or group-by-age interactions for cortical vBMD or porosity in any model.

At the 66 % tibial site, group-by age interactions were observed for total BMC and cortical CSA across Models 1 and 2 (Table 3 and Fig. 2). In both cases, children with CF had a lower rate of increase in these variables than controls, but these interactions were fully attenuated by adjustment for muscle force in Model 3. Total CSA, cortical vBMD, cortical thickness, periosteal circumference and polar CSMI were lower in children with CF than controls in Model 1 (Table 3 and Fig. 2). Whilst associations with cortical vBMD were robust to further adjustment in Models 2 and 3, deficits in total area, periosteal circumference and polar CSMI in children with CF were fully attenuated by adjustment for height in Model 2, and deficits in cortical thickness by adjustment for muscle force in Model 3.

For muscle density, peak jump power and peak hop force (Table 4 and Fig. 3), group-by-age interactions were observed such that children with CF displayed a slower rate of increase in Model 1. This interaction was attenuated in Model 2 for peak hop force, although a main effect of group remained evident with lower values in children with CF. In additional exploratory analyses, further adjustment for muscle density but not size partially attenuated group-by-age interactions for jump power whereas there was little effect of this adjustment on associations

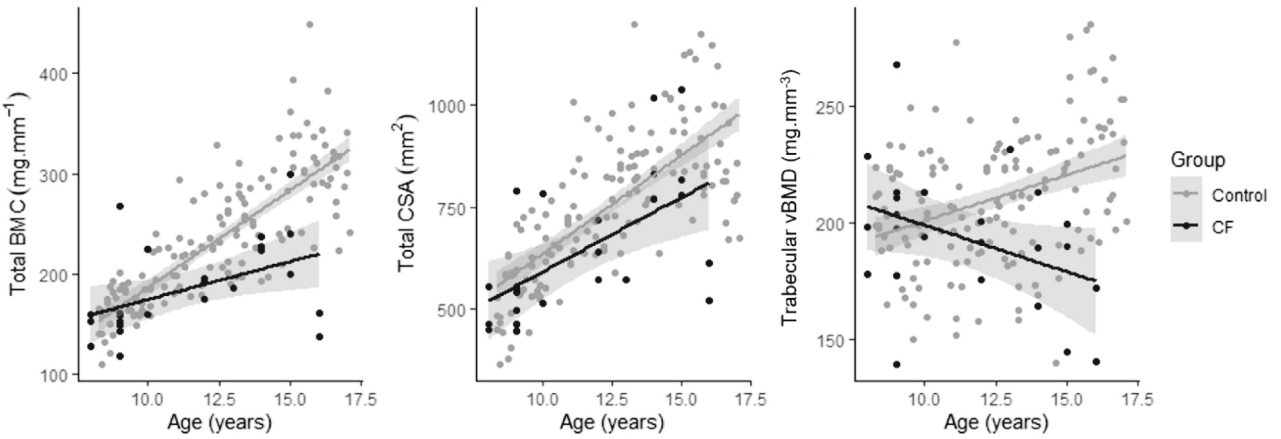

Fig. 1. Tibia bone characteristics at the 4 % distal-proximal site, split by group. Grey region indicates 95 % confidence interval of linear regression line. BMC – bone mineral content, CSA cross-sectional area, vBMD – volumetric bone mineral density.

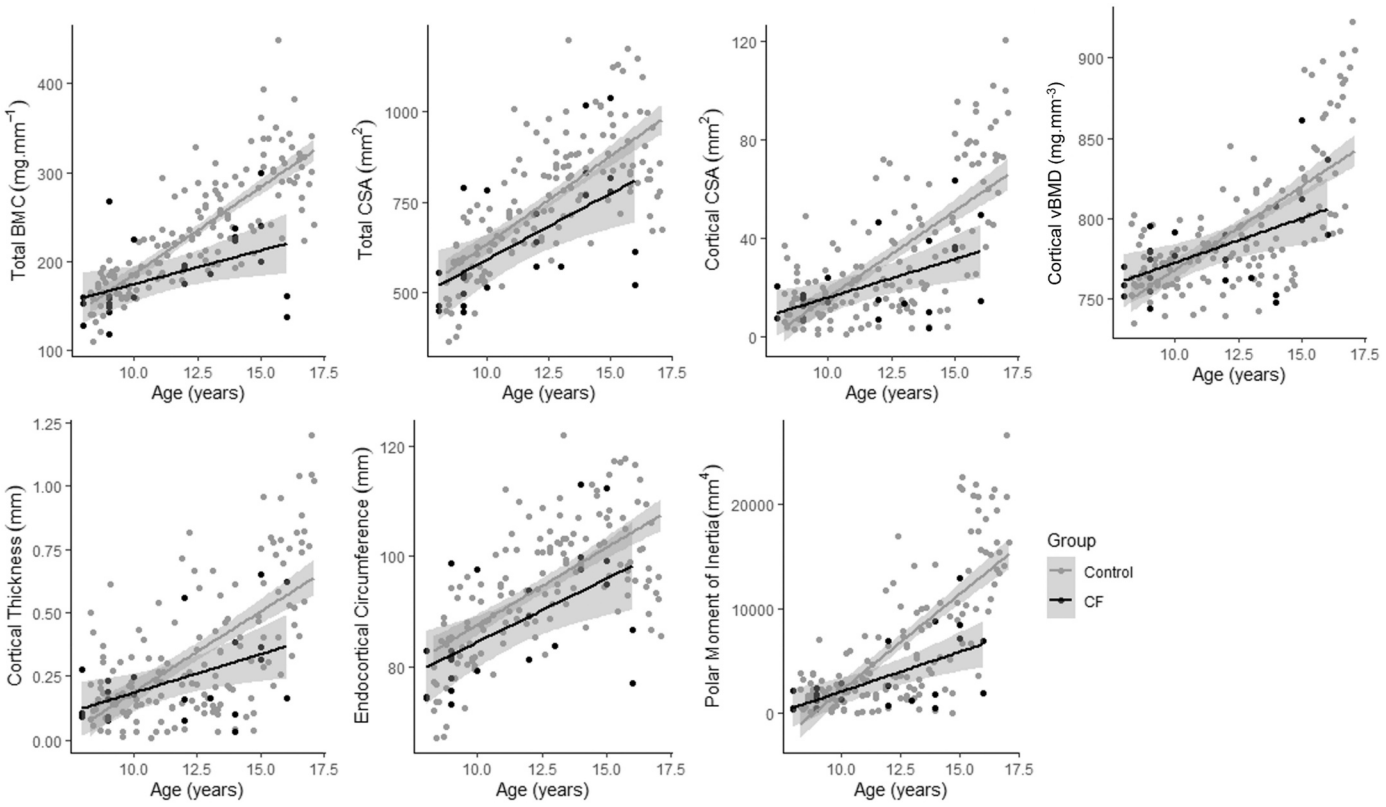

Fig. 2. Tibia bone characteristics at the 66 % distal-proximal site, split by group. Grey region indicates 95 % confidence interval of linear regression line. BMC – bone mineral content, CSA cross-sectional area, vBMD – volumetric bone mineral density.

with hop force. In unadjusted models there was a lower level of muscle and fat CSA in children with CF but this was fully attenuated following adjustment for height in Model 2.

For the fibula (Supplementary Table 1), similar group-by-age interactions to those observed in the tibia were evident for 4 % site total BMC and vBMD in all models. Lower total CSA in Model 1 in children with CF was fully attenuated by adjustment for height in Model 2. At the 66 % group-by-age interactions in Model 1 showed a lower rate of accrual of total BMC, total CSA, cortical CSA, periosteal and endocortical circumferences, and polar CSMI. Interactions for total BMC, total CSA, periosteal circumference were partially attenuated and cortical CSA, endocortical circumference and polar CSMI fully attenuated by adjustment for muscle force in Model 3.

In sensitivity analyses, adjustment for hop force was replaced by lean and fat mass, and alternatively by jump power but observed group and group-by-age interactions were similar (Supplementary Table 2). Group-by-pubertal status interactions were also assessed (data not shown). In all cases, the associations were weaker than those observed for group-by-age interactions and (with the exception of 4 % total BMC) fully attenuated by adjustment for age and body size.

4. Discussion

This work offers the first detailed description of age-related

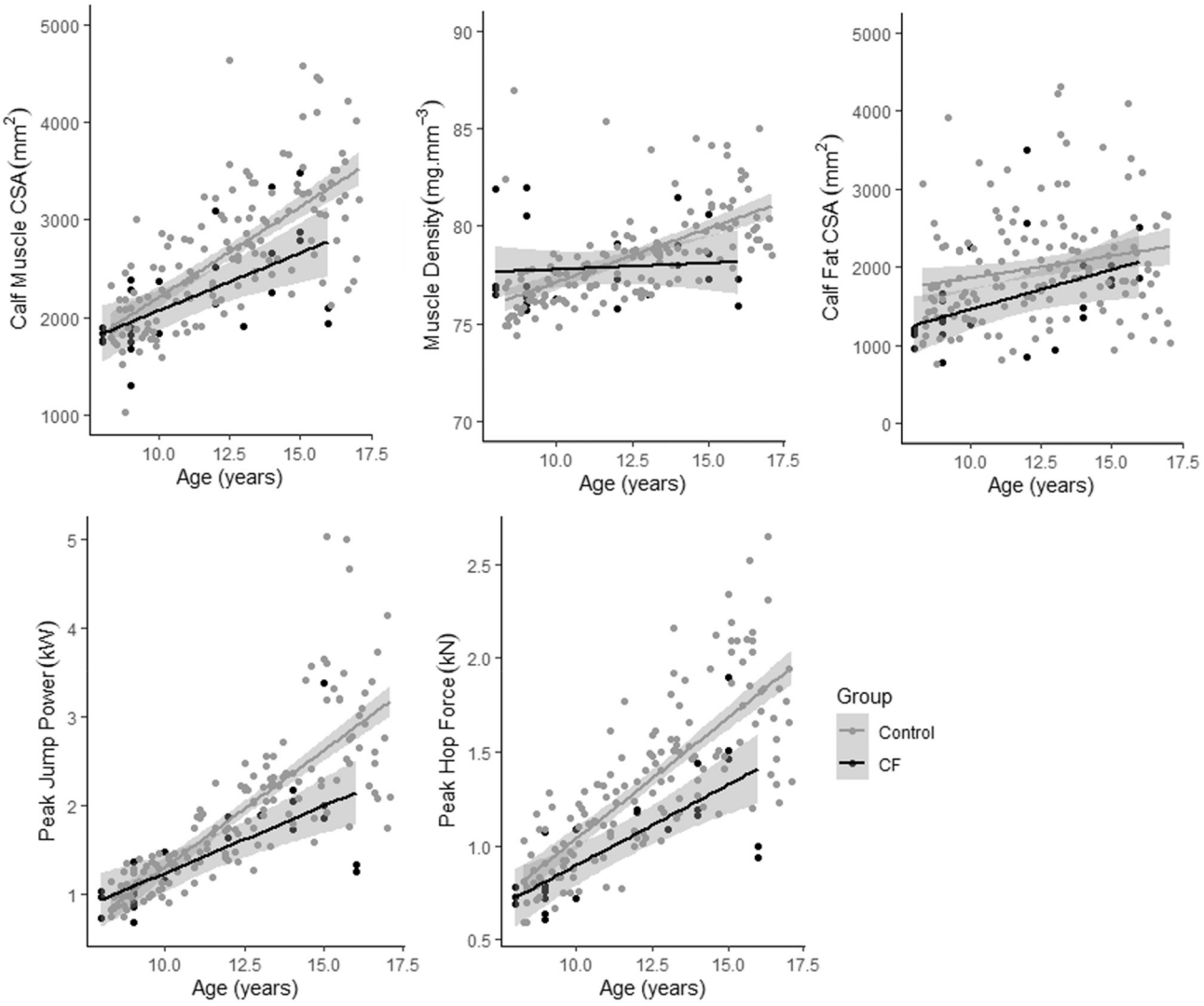

Fig. 3. Muscle imaging and functional characteristics assessed by peripheral quantitative computed tomography (pQCT) and jumping mechanography, split by group. Grey region indicates 95 % confidence interval of linear regression line. CSA - cross-sectional area.

musculoskeletal changes in children with CF. The current study described in detail age-related epiphyseal bone mass deficits developing during growth in children with CF, primarily resulting from decreasing trabecular BMD rather than impaired growth of bone CSA. In a first description of age-related changes in bone microstructure in CF, we observed decreases in trabecular number and thickness in CF in comparison to maintained values in control children. A reduced rate of distal tibial cortical shell thickening in children was also observed, whereas cortical BMD and porosity were similar across age in both groups. In the tibial shaft, diaphyseal bone mass deficits developing from impaired cortical CSA increases during growth in children with CF were evident. These observed associations were partially attenuated at epiphyseal sites and fully at diaphyseal sites following adjustment for muscle function. We observed group-by-age interactions for clinically-relevant jump power and hop force measurements important for mobility and skeletal development respectively, such that as with bone deficits developed through childhood in children with CF. In the case of jump power, this growing deficit was independent of diverging body size.

A previous study by Brookes and colleagues found similar group-by-age interactions in bone strength index (a combination of bone size and density measures related to bone strength) but not detailed bone geometry or density [5]. Whilst previous studies have described muscle weakness in children with CF, [12] this is the first study to assess age-related changes in muscle function and to examine the contribution of underlying muscle size and density (an indicator of quality). Bai and colleagues observed group-by-age interactions in radial cortical area and CSMI but not other variables including epiphyseal trabecular BMD, although this may be related to small study size (12 CF, 23 control) [8]. Our findings of impaired trabecular and cortical microstructure are qualitatively similar to deficits observed in young adults with CF [6,10] but not in younger children [9] which supports the idea of deficits developing with age. Similar patterns were observed for muscle density, and adjustments for muscle density attenuated the group-by-age interactions evident for jump power suggesting that it may contribute to impaired development of muscle function.

Whilst pubertal development has previously been suggested to contribute to bone deficits in children with CF [5] our observations were independent of adjustment for pubertal stage. In support of this, whilst pubertal stage-group associations were evident they were weaker than the age-by-group associations and largely fully attenuated by adjustment for body size and age. The lack of longitudinal assessment and a binary classification of puberty may have limited our ability to detect associations with puberty independent of these other factors. Loading by body weight and muscular forces during physical activity is an important determinant of childhood bone strength [11], adjustment for muscle force but not body size led to attenuation of observed group-by-

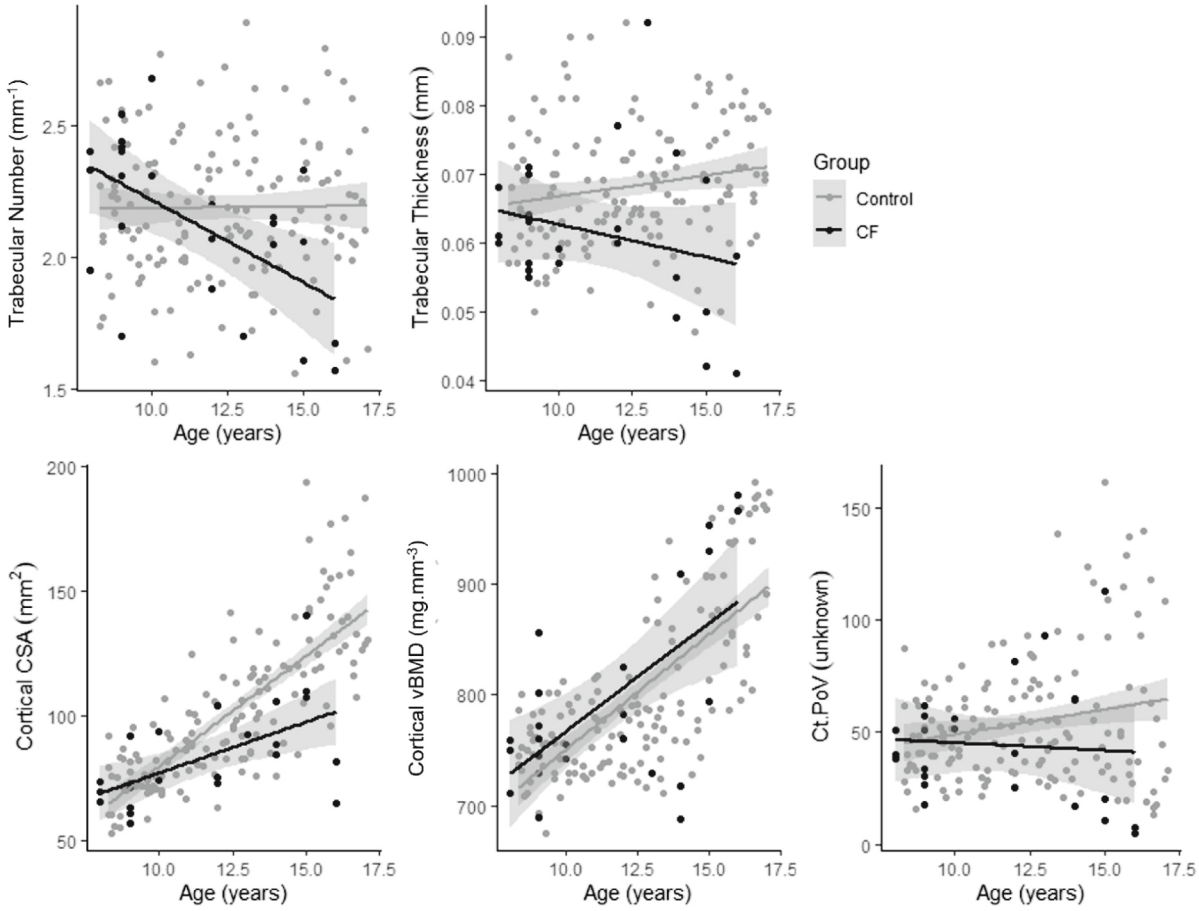

Fig. 4. Tibia bone characteristics assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT) at 8 % distal-proximal site. Grey region indicates 95 % confidence interval of linear regression line. CSA - cross-sectional area, vBMD – volumetric bone mineral density, Ct.PoV – cortical porosity.

age differences. Therefore it seems that differences in skeletal loading attributable to physical inactivity contribute to developing bone deficits in children with CF particularly in diaphyseal regions.

Similar muscle-bone relationships have been observed in a number of other pediatric populations with low bone mass [17]. This could be through common direct effects on both tissues such as the collagen defect in osteogenesis imperfecta or impaired phosphate metabolism in X-linked hypophosphatemic rickets (XLH). Alternatively through reduced mechanical loading by muscle during physical activity in neuromuscular disorders including cerebral palsy and Duchenne muscular dystrophy. Indeed, we have shown previously that impaired physical activity and lower lean mass in children with low motor competence in infancy make a sizeable contribution to observed bone deficits in adolescence in this population [18]. Finally, conditions such as CF or inflammatory bowel disease where indirect effects such as inflammation or impaired nutrion contribute to observed deficits.

A number of other contributing factors to bone deficits in CF have been identified, including ventilatory function and steroid treatment [19]. Ventilatory function is an indication of pulmonary disease, and as such greater disease burden may lead to reduced appetite and poorer nutritional status in addition to higher levels of inflammation. Given the progressive nature of ventilatory function decline in CF [20], it may contribute to the observed emergence of bone deficits with age. Similarly, increased corticosteroid use in older children [21] and the effect of cumulative corticosteroid dose on bone health [19] may also be an important factor. Future longitudinal studies should examine the contribution of these and other clinical factors to bone strength trajectories.

Given the high rate of osteopenia, osteoporosis and fractures [2] present an important clinical problem in CF across lifespan. Even in adolescence, individuals with CF have a 9-fold increase in fracture risk and more than half of individuals with CF will have had a fracture by 25 years [4]. That these deficits appear to develop in later childhood suggests that interventions targeted at this age may be highly beneficial. A one-year resistance and plyometrics training programme was ineffective in improving BMD in young children (mean age 10.5y) with CF [22], but detailed information on habitual physical activity was not reported so it is unknown to what extent this intervention represented a departure from typical activity. Bisphosphonates are effective in improving BMD in adults with CF [23] and have been used in other pediatric groups with bone problems but not CF. Vitamin D and calcium supplementation may be beneficial in attenuating bone deficits but this has only been tested in a small trial of adults with CF where differences in bone loss over 12 months were observed but were not significant [24]. This has not been applied in children, whereas fatty acid supplementation [25] failed to improve BMD.

This study used a variety of highly repeatable and detailed measures to characterise musculoskeletal health in a relatively large population of control and CF children of different ages. In addition, we examined the potential role of skeletal loading in development of bone deficits in this population. Whilst we characterised a number of clinical characteristics in this population, it may be that clinical treatments or other clinical characteristics contribute to the observed age-related trends. This was a cross-sectional study, and therefore we inferred age-related patterns from observation of different children rather than examining longitudinal trajectories. The study design was not aimed at examining group-by-sex interactions, as the division into two sexes would substantially reduce the statistical power of the sample. Whilst in sensitivity analyses

we observed some minor group-by-sex interactions, in all cases these were fully attenuated by adjustment for height. Future studies could examine whether sex-specific patterns of bone growth are evident in CF relative to children without CF, although the complex nature of these analyses would demand a large sample size.

Children with CF develop a characteristic pattern of bone deficits during later childhood. At joint sites, these deficits are attributable to both trabecular loss and impaired cortical bone accrual but not bone size. At diaphyseal sites, they primarily result from impaired accrual of cortical area and partly cortical BMD. These age-related trends are independent of pubertal development, and body size. However, it appears that differences in muscle function may mediate these developing deficits, particularly at diaphyseal sites. To date, there are no effective interventions to improve bone accrual in children with CF. Given the greatly increased fracture risk in this population across adult life, development of interventions would be of substantial clinical value.

CRediT authorship contribution statement

Alex Ireland: Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Amy Riddell:** Investigation, Methodology, Data curation, Project administration, Writing – review & editing. **Antony Colombo:** Formal analysis, Writing – review & editing. **Robert Ross-Russell:** Conceptualization, Investigation, Writing – review & editing. **Ann Prentice:** Resources, Data curation, Writing – review & editing, Funding acquisition. **Kate A. Ward:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors have nothing to disclose.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2022.116657.

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