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ORIGINAL ARTICLE: CYSTIC FIBROSIS

TITLE: BONE MINERAL DENSITY IS RELATED TO LUNG FUNCTION OUTCOMES IN YOUNG PEOPLE WITH CYSTIC FIBROSIS – A RETROSPECTIVE STUDY[1]

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Abstract:

Introduction: Improvements in the medical management of cystic fibrosis (CF) in recent years have resulted in increased prevalence of long-term sequelae of the condition, such as low bone mineral density (BMD) and hence an increased risk of fractures in later life. **Aim:** To explore the interaction between BMD and lung function, nutrition and genotype. **Methods:** This study was a retrospective audit of 202 children with CF from August 2000 to January 2016 to investigate associations between bone mineral density (BMD) Z-scores with clinical status, nutrition and genetics using dual-energy absorptiometry x-ray data from the Royal Children's Hospital Melbourne, Australia. **Results:** Severity of both lung disease ($p<0.0001$) and nutritional status ($p<0.05$) were found to be strongly associated with BMD Z-scores. **Conclusions:** This is the biggest study to date to provide further evidence that the severity of pulmonary disease is related to BMD in CF patients and therefore screening guidelines for bone health in children with CF should target individuals with the poorest clinical status.

Main text:

Introduction:

Cystic fibrosis (CF) is the most common autosomal recessive disorder prevalent within the Caucasian population. In Australia, it has a carrier rate of 4%, and approximately 1 in 2500 newborns are diagnosed with the disease.[1] Over the last 30 years, improved clinical care in CF has resulted in a near doubling of the mean age of death,[2] and people born today have a median predicted survival age of 39.3 years.[3] With increased survival, greater medical attention is required for potential long term sequelae, including bone disease. Poor bone health outcomes have been well documented in adults, with the pooled prevalence of osteoporosis and osteopaenia in adults with CF reported as 23.5% and 38.0% respectively [4].

This has been linked to an increase in other complications, such as kyphosis and fractures of the rib and vertebrae, that have the potential to further compromise respiratory function, increasing the risk of developing respiratory infections and reducing mobility.[1,5]

Although low bone mineral density (BMD) and its complications arise in adults, bone disease in CF most likely originates during childhood or adolescence, when bone mass is accrued.[4] The mechanism behind CF-related bone disease is incompletely understood; however, several potential risk factors for developing CF-related bone disease have been suggested, including poor nutritional status, malabsorption, and clinical status.[6-9] It has also been suggested that there may be a direct effect on bones, as the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutation has been found to have a role in lowering BMD - a recent study found that patients who are homozygous or heterozygous for the delta F508 mutation have lower BMD scores than those with other genetic mutations.[8]

In reality, the development of CF-related bone disease is likely to be a multifactorial process.[10] The limited research that has been conducted in this area thus far has hypothesised that targeting modifiable risk factors could be highly beneficial in preventing low BMD and fractures in CF patients.[8,11] The current gold standard tool for measuring BMD is the use of dual-energy X-ray absorptiometry (DXA).[12] The aim of this retrospective study was to determine the prevalence of bone disease within a large, unbiased population of children and adolescents with CF, and to compare BMD measured by DXA with clinical measurements including lung function (as a marker for disease severity), body mass index (BMI) and total fat composition (as markers for nutritional status), and genotype. There are no specific guidelines for monitoring BMD in children with CF; they follow the standard

recommendations that children with BMD Z-scores >-1 should have a bone density scan every 5 years.[13] The aim of this project was to explore the interaction between BMD and lung function, nutrition and genotype using retrospective analysis, which could provide information to inform screening programs that identify patients at most risk of developing bone disease. These patients may be eligible for more aggressive therapies in the future.

Materials and methods:

Study design

A retrospective audit of all CF patients who had at least one DXA scan at the Royal Children's Hospital, Melbourne, Australia, between August 2000 and January 2016. To identify potential participants with CF, the hospital's electronic scanned medical record (ESMR®) and Respiratory Medicine Department CF clinical database was searched for eligible patients. DXA scans were performed using the Hologic QDR 4500 or Horizon DXA scanner (Hologic Inc, Bedford, MA, USA). Raw BMD Z-scores for lumbar spine (LS) were calculated. For participants with more than one DXA scan, the latest scan was utilised.

ESMR® was also used to collect information on the patient's health status and past medical history. Lung function test results were searched for patients who had completed a DXA scan at the RCH, and data within a 3 month period of the DXA scan were used in this study. In instances where multiple spirometry results were eligible, the result closest to the DXA scan date was used. Forced vital capacity (FVC) and Forced expiratory volume in one second (FEV_1) and their Z-scores were recorded, as well as the ratio FEV_1/FVC. A Global Lungs Initiative (GLI) calculator[14] was utilised in order to calculate Z-scores for lung function data. BMI Z-scores were calculated using an online paediatric calculator provided by The

Children's Hospital of Philadelphia.[15] Site specific CF genotype data were provided by the Australian CF data registry (ACFDR).[16] For the purposes of this study, subjects were reported as either having a genotype that was homozygous or heterozygous for the delta F508 mutation.

Participants

Patients of the Royal Children's Hospital (4-18 years of age) were selected if they had a confirmed diagnosis of CF by sweat test and / or genotype, and had undergone a DXA scan during their clinical care from August 2000 to January 2016. No exclusion criteria were specified for the study; participants were only excluded if DXA data for the lumbar spine regions were unavailable.

Primary outcome

The primary outcome of this study was to investigate the correlation between lung function and associated CF related risk factors of nutritional status, BMI and genotype, and bone density in CF patients at RCH.

Ethics approval

This project was approved by The Royal Children's Hospital Human Research Ethics Committee; HREC #35056.

Statistical analysis:

All data were analysed using IBM SPSS Statistics version 22 (IBM, Armonk, NY, USA, 2013). Independent samples t-tests were carried out as well as multiple linear regression. The significance level was fixed at $p<0.05$.

Results:

From the 15 year search, 453 patients with CF were listed on the RCH database, of which 202 (44.6%) had results available for analysis. The ethnicity of the entire sample was Caucasian, the mean age was similar between males and females, although we note that the median age for males was 15.6 years (equal to the mean) while for females it was 16.6 years, indicating a more negatively skewed distribution. Demographics and clinical characteristics of the sample split by gender are displayed in Table 1. In terms of the Z-scores (which account for differences in age and gender), the only significant difference between means of male and female groups was the BMI Z-score, higher for girls but below 0 in both populations. 175 patients of the 202 patients in the sample (86.6%) had available lung function results.

Thirty-two children (15.8%) had BMD Z-scores of -2 or below, which means they are classified as having very low bone mineral density or CF-related bone disease.[12] Furthermore, sixty-two individuals (30.7%) had a BMD Z-score above -2 but below or equal to -1, which characterises them as having low bone density. Fifty patients (24.8%) had BMD Z-scores at or above 0.

Low to moderate correlations were found to exist between the BMD Z-score of patients and their age, height Z-score, BMI Z-score, total fat percentage, FVC Z-score and FEV_1 Z-score (Table 2). As would be expected, the age, height and total fat percentage are all themselves

correlated with the BMI Z-scores, while FVC and FEV_1 are highly correlated. The age of the patients held a low negative correlation with both BMD Z-score and BMI Z-score (-0.140 (p=0.046) and -0.196 (p=0.005) respectively).

Multiple linear regression was performed on the 175 cases that had lung function data available using lumbar spine BMD Z-score as the target variable. When building regression models to identify relationships between variables, we can pay attention to the regression coefficients of each variable as well as the overall correlation coefficient of the model, which indicates how well it fits the data. In some cases, variables may not be associated with statistically significant regression coefficients, however their inclusion in the model can still lead to higher accuracy suggesting that they do have some predictive value and may be related to the target variable. We looked at all combinations of the six variables, observing the change in accuracy and regression coefficient significance when variables or subsets of variables were removed. We summarise the models that had the highest accuracy (overall correlation coefficient) and only included variables that were associated with statistically significant regression coefficients.

A regression model including only height Z-score, BMI Z-score and FEV_1 Z-score had an overall correlation coefficient of 0.656 ($p < 0.001$) with all variables significant and regression coefficients of 0.403 ($p < 0.001$), 0.229 ($p = 0.001$) and 0.216 ($p < 0.001$) respectively. These variables were the only ones to be associated with statistically significant regression coefficients in a model including all six variables, and they also had the highest correlation coefficients in the bivariate analysis. Although FVC Z-scores had a moderate correlation with BMD Z-scores, since they are also correlated with FEV_1 Z-scores they did not result in improvements in accuracy. Removing any of these three variables resulted in

moderate decreases in accuracy, with results of r = 0.537, r = 0.629 and r = 0.597 when either height, BMI or FEV_1 were respectively removed. Including FVC Z-scores instead of FEV_1 Z-scores also produced worse accuracy. Overall this suggests that the most important variable in predicting BMD Z-scores is the height Z-score, however both BMI Z-score and FEV_1 also contribute significant information, and so poor lung function does appear to bear a correlation with low BMD even when controlled for other factors.

The same analysis was performed on males and females separately. The bivariate correlation coefficients with BMD z-values are shown in Table 3. Age was not significantly correlated with BMD for females, while total fat percentage was not significant for males, however the remaining variables all showed significant correlations for both genders.

Multiple regression models built for each gender separately both showed height Z-scores to have significant regression coefficients. For the males, BMI Z-score did not show a significant relationship nor did its inclusion in regression models result in meaningful improvements to overall accuracy. The best-fitting regression model using only variables with significantly associated regression coefficients included the variables of age (-0.86, $p = 0.027$), height Z-score (0.436, $p < 0.001$) and FEV_1 Z-score (0.305, $p < 0.001$). It had an overall correlation coefficient of 0.719 ($p < 0.001$). Removing any of these variables resulted in decreases to accuracy and inclusion of additional variables failed to see improvements. On the other hand for females, a regression model including height Z-score (0.417, $p < 0.001$) and BMI Z-score (0.544, $p < 0.001$) showed these variables to have significant regression coefficients and an overall coefficient of 0.622 ($p < 0.001$). When FEV_1 Z-score was included in the model, although it had a significant regression coefficient (0.139, $p = 0.029$), it did not increase the overall accuracy a great deal (r = 0.627, $p < 0.001$). Including fat percentage did

increase the overall accuracy ($r = 0.693$, $p < 0.001$), however its regression coefficient was not statistically significant ($p = 0.402$).

Overall this suggests that while nutritional status and lung function may both be moderately correlated with severity in reduction of bone mass density and CF, the risk factors may affect males and females differently. While FEV_1 Z-scores were shown to be a significant indicator for males, other health factors such as BMI and fat percentage were more important for females. Some relationships between contributing factors and BMD are depicted as scatter plots in figure 1.

Genotype information was available for 189 individuals. A higher percentage of the homozygous patients had pancreatic insufficiency (89% compared to 77%), and so to avoid bias, we performed analysis on these 158 individuals, (91 homozygous and 67 non-homozygous) with the results summarised in Table 4. Some differences in mean values were observed between homozygous and non-homozygous groups in terms of BMD Z-scores with homozygous patients being slightly lower for males and females, however none of these were statistically significant. No significant or apparent differences were detected between homozygous and non-homozygous groups in any of the remaining indicators, either when males and females were grouped together or grouped separately.

Out of 12 patients who started Ivacaftor® (a new drug available on the market for Australia in 2014 to treat cystic fibrosis by acting on the cystic fibrosis transmembrane conductance regulator (CFTR) gene), only 6 participants were on using the drug. The six participants did have BMD z-scores dissimilar to that of the other patients not taking Ivacaftor®. This result

is limited by the sample size. All patients who were eligible for government funding for Ivacaftor® were on Ivacaftor® (at the time of data collection, this was those aged six and above but is approval is now for children two and above). Given the short-time period these children were exposed to Ivacaftor® at the time of data collection, it is unlikely that there would be a big difference even though a recent study has shown the drug to improve bone physiology.[17]

Discussion:

This is the largest study exploring the effects of CF and disease severity on bone health outcomes in children with cystic fibrosis.[8,9,18] In a large population of young Australian patients with CF, we found that the prevalence of severely low BMD (z score less than –2) was 15.8%. The prevalence of low BMD in this study was in line with a similar, recent study conducted on Polish children and adolescents,[8] which found 17% of their CF patients had very low BMD (Z-score <-2). Results also confirm conclusions from smaller, international BMD studies with combined adult data,[19-24] and smaller, national BMD studies.[7,25]

The clinical risk factors that correlate most with low BMD included lung function and nutritional status (determined by BMI and total fat percentage). Results are in line with the case-control study conducted by Hardin *et al.*[26] which found patients with poor clinical status were at most risk of developing osteoporosis. A longitudinal study by Bianchi *et al.*[5] in 136 children, adolescents and young adults also found that lung function had the greatest influence on bone density, with 45% of the variability in BMD being attributed to the patient's FEV_1. While other studies have also looked at these outcomes, all have been smaller than our cohort, and many have mixed populations of both young people and adults.[27]

An association between BMI and BMD has been noted in the literature previously.[7,28] This would suggest that increased focus on improving nutritional status in patients with CF would cause bone density to improve accordingly. However, a study comparing two cohorts of CF patients, one cohort studied 15 years ago and one cohort in the present day period found that BMI was not significantly different between the two groups.[29] Furthermore, despite increased awareness of the importance of nutrition in CF, BMD levels have also been shown to have remained consistent over the years.[29] Perhaps this suggests that, while a clear and consistent association has been shown between BMI and BMD, it is merely only one of a number of factors responsible for bone mineral accrual. However, a study conducted in France [10] did not detect an association between poor nutritional status and BMD, although their sample size of 117 was considerably below the one obtained in this study. As both lung function and nutritional status are representations of disease severity it is unsurprising that both might be positively associated with bone health. Total fat percentage showed positive association and is a more reliable measure of nutrition for growing children.

Once some confounding factors were accounted for, the current study did not show genotype to be associated significantly with low BMD, although the mean for homozygous patients was lower in our sample. Previous studies have identified mutation as a potential cause of developing CF-related bone disease. The case-control study conducted in Italy by Bianchi *et al*.[5] found that BMD levels were reduced in individuals who were homozygous for the delta F508 mutation, while heterozygous patients did not have such significant abnormalities in their bones; however, the authors did concede that for definitive conclusions to be reached in this area a larger number of individuals should be studied than their sample of 136 children

and young adults. Our study was also limited by small numbers to investigate genotype. Sands *et al*.[8] found that individuals who were either homozygous or heterozygous for the delta F508 mutation had poorer bone density when compared to patients with other genetic mutations. Unfortunately, it was not feasible to analyse the BMD of individuals with mutations other than delta F508 separately in this study due to the small numbers of such patients available (n= 17) and therefore these individuals were grouped together with heterozygous patients and labelled non-homozygous collectively.

This study's sample size is the largest known to date, but had major limitations due to its retrospective nature and absence of important data. Prospective longitudinal studies are warranted; although this may need several sites to recruit a sizeable sample size in an adequate amount of time. Unfortunately, we were unable to comment on pubertal stage in our subjects. Children with CF often have delayed puberty which confounds DXA measures by both relatively short stature and difficulty of age matching with discordant exposure to sex steroids.[12] Another limitation is that our institute's policy for DXA scan referral may mean our sample has a population with poor health outcomes than the general population (our sample having a mean FEV1 Z-score of -2.2 (males) and -2.5 (females). CF patients are generally referred for a DXA scan when the child turns 15 years of age (at the family and doctor's discretion) or if there was a clinical suspicion related to severity of disease. There are many other potential confounders that may affect bone health in this population e.g. Vitamin D and corticosteroid treatment. This paper has focused on the relationship between bone health and lung function, with further research planned to explore the effect of other variables on the observed DXA outcomes.

The study informs developing guidelines for bone health screening in CF, particularly targeting those with severe pulmonary disease. This study also confirms, in a larger cohort of patients, the relationship between lungs and bones clearly. When we assessed those with BMD Z-scores above -2 and below -2 significant differences in lung function were seen between the two groups. Due to the limitations with generating BMAD (height-adjusted BMD Z-scores), some BMD Z-scores may appear lower in those who are shorter. Delayed puberty was also not adjusted for and could affect BMD of CF patients. The DXA machine also reports US reference scores as per manufacturer's database. Perhaps this information could be used to enlighten further research aimed at determining a cut-off level of lung function severity that puts a child at greatest risk of developing very low BMD (BMD Z-score less than -2). Further research should also aim at investigating risk factors such as vitamin D level, transplant status, frequency/number of CF exacerbations, dietary intake, glucocorticoid use, pancreatic insufficiency, fractures and activity levels. Our findings found the cut-off Z-score levels for FEV_1 and FVC was -0.99 and -0.27 respectively for very low BMD. Other possible risk factors besides those studied in this audit should also be explored.

Conclusions:

This study has investigated the largest population of children and adolescents with CF in terms of their bone health, and found that lung function, BMI and total fat percentage were associated with bone health outcomes. It is recommended that a screening program in CF patients which incorporates either or both of these non-invasive measures as a surrogate predictor/marker of bone health would be beneficial, thus targeting high risk individuals in order to prevent progression of complications due to bone disease.

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Table 1: Demographics and clinical characteristics of the study participants (n=202)

	Males (n=103)		**Females (n=99)**		**Total (n=202)**		**Difference between groups p-value**
	mean	**(SD)**	**mean**	**(SD)**	**mean**	**(SD)**	
Age in years, mean (SD)	15.6	(2.3)	15.9	(2.3)	15.7	(2.3)	0.303
Height (cm)	165.5	(13.5)	158.4	(10.6)	162	(12.7)	
Height Z-score	-0.3	(1.2)	0.4	(1.0)	-0.3	(1.1)	0.899
Weight (kg)	52.8	(13.1)	50.4	(9.8)	51.62	(11.6)	
BMI	19.0	(2.7)	19.9	(2.5)	19.4	(2.6)	
BMI Z-score	-0.7	(1.1)	-0.3	(0.9)	-0.47	(1.0)	0.007

Total fat percentage (%) *	17.0	(7.5)	27.1	(5.1)	21.8	(8.2)	
Lumbar spine BMD ** (g/cm^2)	0.80	(0.2)	0.86	(0.1)	0.83	(0.2)	
Lumbar spine BMD Z-score **	-0.9	(1.2)	-0.8	(1.1)	-0.8	(1.1)	0.518
FVC (L) **	3.5	(1.1)	2.8	(0.9)	3.1	(1.0)	
FVC Z-score **	-1.5	(1.4)	-1.6	(1.8)	-1.6	(1.6)	0.536
FEV_1 (L) **	2.7	(0.9)	2.1	(0.8)	2.4	(0.9)	
FEV_1 Z-score **	-2.2	(1.5)	-2.5	(1.7)	-2.3	(1.6)	0.238
FEV_1/FVC **	0.8	(0.1)	0.8	(0.1)	0.8	(0.1)	0.555

BMD = Bone mineral Density; BMI = Body mass index; FEV_1 = Forced expiratory volume in one second; FVC = Forced vital capacity, SD = standard deviation. * Only 59 Males and 54 Females had data available for fat percentage. ** 85 Males and 90 Females had lung function data available.

Table 2: Correlations between BMD Z-score and age, weight, total fat percentage, BMI Z-score, FVC Z-score, FEV_1 Z-score

	Height Z-score	**BMI Z-score**	**Total fat %**	**FVC Z-score**	**FEV_1 Z-score**	**Lumbar spine BMD Z-score**
Age in years	*r* = 0.034 *p* = -0.631	*r* = **-0.196** *p* = 0.005	*r* = **-0.202** *p* = 0.032	*r* = -0.148 *p* = 0.051	*r* = -0.125 *p* = 0.099	*r* = **-0.140** *p* = 0.046
Height Z-score		*r* = **0.211** *p* = 0.003	*r* = 0.182 *p* = 0.053	*r* = **0.151** *p* = 0.047	*r* = **0.216** *p* = 0.004	*r* = **0.486** *p* < 0.001
BMI Z-score			*r* = **0.572** *p* < 0.001	*r* = **0.429** *p* < 0.001	*r* = **0.419** *p* < 0.001	*r* = **0.442** *p* < 0.001
Total fat %				*r* = 0.093 *p* = 0.37	r = 0.129 *p* = 0.217	*r* = **0.211** *p* = 0.025
FVC Z-score					*r* = **0.862** *p* < 0.001	*r* = **0.418** *p* < 0.001
FEV_1 Z-score						*r* = **0.486** *p* < 0.001

Bold r values indicate statistical significance at the level of 0.05.

Table 3: Correlations between BMD Z-score and age, weight, total fat percentage, BMI, FVC, FEV_1 with gender analysis

Lumbar spine BMD Z-score	**Age in years (n = 202)**	**Height Z-score (n = 202)**	**Total Fat % (n=113)**	**BMI Z-score (n = 202)**	**FVC Z-score (n = 175)**	**FEV_1 Z-score (n = 175)**
Males	r = -0.241 p = 0.014	r = 0.518 p < 0.001	r = 0.087 p = 0.511	r = 0.386 p < 0.001	r = 0.499 p < 0.001	r = 0.550 p < 0.001
Females	r = -0.044 p = 0.667	r = 0.451 p < 0.001	r = 0.305 p = 0.025	r = 0.512 p < 0.001	r = 0.367 p < 0.001	r = 0.445 p < 0.001

Table 4. Comparison of BMD, BMI and lung function between groups of different genotype (n=158) and separated by gender

	Homozygous (n=91)		**Non-homozygous (n=67)**		**Significance**
	mean	(SD)	mean	(SD)	p-value
BMD Z-score	-0.849	(1.23)	-0.594	(1.09)	0.172
Males	-0.834	(1.13)	-0.689	(1.27)	0.610
Females	-0.865	(1.35)	-0.517	(0.94)	0.179
BMI Z-score	-0.470	(1.01)	-0.421	(1.01)	0.762
Males	-0.699	(1.01)	-0.544	(1.12)	0.540
Females	-0.215	(0.95)	-0.321	(0.92)	0.612
FVC Z-score*	-1.521	(1.45)	-1.592	(1.89)	0.808
Males	-1.430	(1.11)	-1.463	(1.79)	0.932
Females	-1.618	(1.74)	-1.688	(1.97)	0.872
FEV Z-score*	-2.357	(1.46)	-2.325	(1.91)	0.911
Males	-2.217	(1.32)	-2.053	(1.85)	0.694
Females	-2.504	(1.60)	-2.527	(1.95)	0.957

BMD = Bone mineral Density; BMI = Body mass index; FEV_1 = Forced expiratory volume in one second; FVC = Forced vital capacity, SD = standard deviation. * 84 homozygous and 61 non-homozygous had lung function data available.

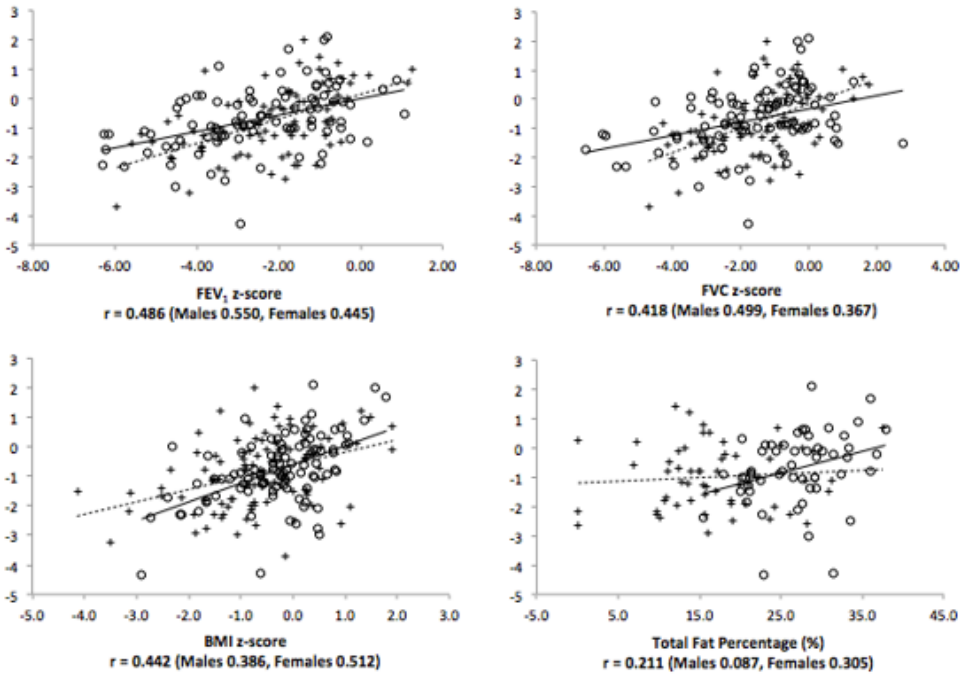

Figure 1. Positive correlations between BMD z-score and each of FEV1 (top left), FVC (top right), BMI (bottom left) and total fat percentage (n = 202). Trend lines shown for males (+, dashed) and females (o, solid).

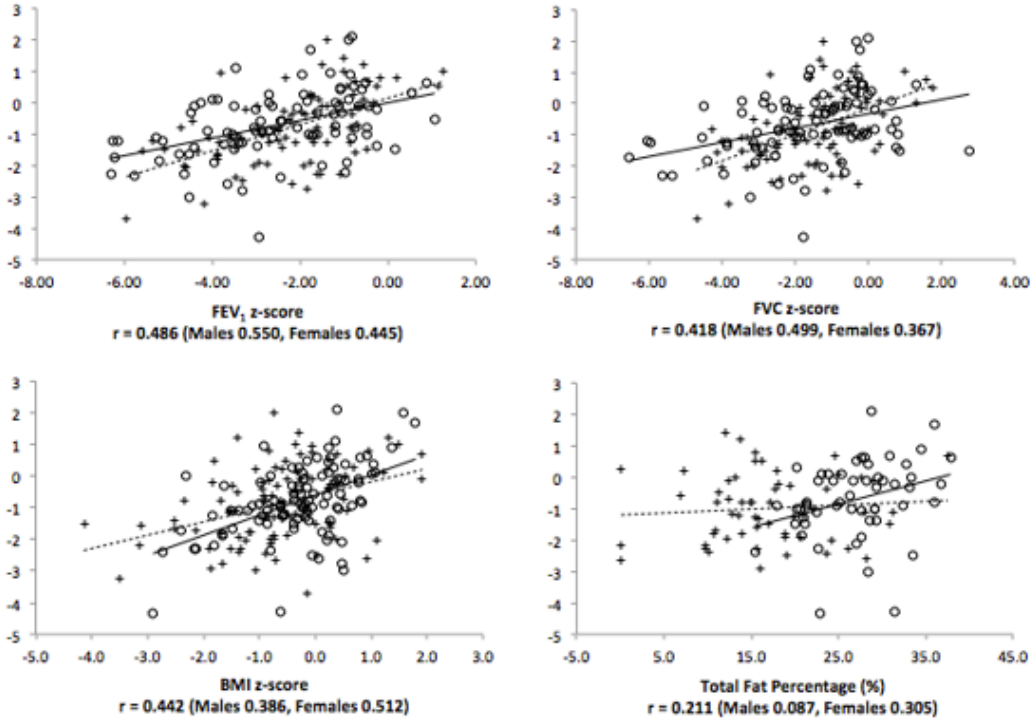

Figure 1. Positive correlations between BMD z-score and each of FEV1 (top left), FVC (top right), BMI (bottom left) and total fat percentage (n = 202). Trend lines shown for males (+, dashed) and females (o, solid).

Figure_1_revised (1) .