Published in final edited form as:

Pediatr Blood Cancer. 2014 August ; 61(8): 1451–1456. doi:10.1002/pbc.25025.

Bone Turnover in Long-term Survivors of Childhood Acute Lymphoblastic Leukemia

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Abstract

Background—We investigated the effects of demographic, lifestyle (self-reported smoking status and physical activity levels), cancer-related treatment factors (radiation and chemotherapy), and diet (calcium and vitamin D intake) on bone turnover and the relationship of bone turnover to lumbar spine bone mineral density (BMD) Z-scores (LS-BMD Z-scores) determined by Quantitative Computed Tomography (QCT) in 418 5-year survivors of childhood acute lymphoblastic leukemia (ALL).

Procedure—Bone turnover was assessed by biomarkers including serum bone-specific alkaline phosphatase (BALP), Osteocalcin (OC), and urinary N-telopeptide of type I collagen indexed to creatinine (NTX/Cr). The 215 males ranged in age from 9 to 36 years (median age 17 years).

Results—Age and Tanner score were inversely associated with all biomarkers (BALP, OC, NTX/Cr) ($P < 0.001$). Males had higher BALP and OC than females ($P < 0.001$). Body mass index (BMI) was inversely associated with OC and NTX/Cr ($P < 0.001$). There was no significant association of biomarkers with lifestyle related factors, ALL treatment-related factors, dietary calcium, vitamin D or LS-BMD Z-score.

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CONFLICT OF INTEREST STATEMENT

No authors have any conflicts of interest relevant to this work.

Conclusions—In this population of long-term ALL survivors, bone turnover was significantly associated with age, gender, Tanner stage, and BMI. ALL-related treatments did not influence bone turnover and bone turnover was not predictive of volumetric LS-BMD Z-score.

Keywords

Acute lymphoblastic leukemia survivors; bone biomarkers; bone mineral density; QCT

INTRODUCTION

Significantly improved cure rates over the last two decades [1,2] have produced a large cohort of long-term acute lymphoblastic leukemia (ALL) survivors who are at risk for health complications related to cancer treatment [3]. We [4–6] and others [7–12] have documented impairments of bone mineral density (BMD) in these survivors. In adults, high bone turnover predicts low BMD [13]. However, the relationship of bone turnover to skeletal health outcomes in children is not fully understood. Importantly, bone turnover markers in children reflect not only bone remodeling but also modeling with new endochondral bone formation, longitudinal increases in growth of the bone and increases in the diameter of the bone [14]. The pubertal growth spurt is associated with marked increases in bone turnover markers which parallel growth velocity [15,16]. Gender, nutritional status, and pubertal stage are key physiologic factors regulating bone turnover in healthy children [15]. Vitamin D deficiency, premature birth, and malnutrition are common pathologies which adversely influence bone turnover [15]. Biochemical markers of bone turnover may provide insight into the impact of disease states, including cancer, on bone acquisition [17]. Yet, there have been limited reports on bone turnover markers in ALL survivors [9,18–23], despite that both disease-related and treatment-related associations with low BMD have been reported in this population [24]. No comprehensive studies exist on the impact of anthropometric, demographic, lifestyle, and treatment (cranial irradiation and chemotherapy) on these markers in long-term ALL survivors (those alive ≥ 5 years after remission) or the relationship of these markers to lumbar spine bone mineral density (BMD) Z-scores (LS-BMD Z-score). Thus we sought to determine the impact of anthropometric, demographic, and lifestyle factors on bone turnover markers in patients previously treated for ALL, the relationship of ALL treatment on these markers, and the relationship of bone turnover markers to volumetric LS-BMD Z-score as determined by quantitative computed tomography (QCT).

Methods

Eligibility and recruitment

These analyses included baseline data from 418 patients (215 males) of 424 patients age 9–36 enrolled in a double-blind, randomized, placebo-controlled trial (NCT00186901) investigating the effects of calcium and vitamin D supplementation on BMD in survivors of childhood ALL [25]. There were six patients who were not of White or Black race who were not included in this substudy. All patients were in continuous complete remission for at least 5 years. None had a secondary tumor or underwent bone marrow transplant. They had not taken supplemental calcium or vitamin D within 3 months of entering the study.

Demographic and Anthropometric Characteristics of Study Population

Age at the time of diagnosis of ALL and treatment history were abstracted from clinical records. Gender and age at study enrollment were recorded. Facilitated by the study research nurse, the patient or parent/guardian completed a questionnaire to assess self-report of race, and current cigarette smoking use. The type and frequency of strenuous physical activity was collected [26]. Questionnaires were completed by the patient if older than 18 and by the parent/guardian if age 18 or younger.

Assessment of Growth and Pubertal Status

Body weight was measured to the nearest 0.1 kg on a digital scale (Stowaway Scales, Model 5202, Scale-tronix or In Floor Scales Model 6102, Scale-tronix), and height to the nearest 0.1 cm using a wall-mounted stadiometer (Model 242, Seca). BMI was calculated as weight (kg) divided by height². Tanner staging was determined by trained physicians or nurse practitioners.

Treatment Regimens

All patients were treated on one of three IRB-approved protocols – Total Therapy TXI – XIII [27] at a single institution between 1984 and 1997. ALL treatment was obtained from medical records by trained abstractors and included cranial irradiation dose and chemotherapeutic doses of glucocorticoids (in prednisone equivalent units assuming 0.75 mg of dexamethasone = 5 mg prednisone), methotrexate and cyclophosphamide.

Bone Mineral Density Determination

Trabecular LS-BMD Z-score of L1 and L2 vertebral bodies was determined using GE Lightspeed VCT CT scanner (GE Healthcare, Milwaukee, WI) performed with dose modulation techniques and Mindways QCT calibration phantoms and software (Mindways Software, Inc., Austin, TX), as previously reported [28–31]. LS-BMD Z-score for each patient was calculated based upon the manufacturer's normative database. QCT of the lumbar spine provides direct volumetric determination of BMD and obviates the need to adjust for (calculate) changes in vertebral body morphology as needs to be done when using areal DXA in pediatric patients to determine (apparent) BMD.

Nutritional Intakes

Patients completed a food-frequency questionnaire (Block '98 FFQ, Nutrition-Quest, Berkeley, CA. www.nutritionquest.com) that contains 110 food items, which was reviewed by registered dietitians. Only those that were completed and validated by the registered dietitians were included for analysis. Information abstracted from this questionnaire was used to estimate usual dietary intakes of calcium and vitamin D.

Biomarkers of Bone Metabolism

Following an overnight fast, serum was drawn at the baseline visit for bone-specific alkaline phosphatase (BALP) (U/L) and osteocalcin (OC) (µg/L) as markers of bone formation. Fasting, second void, morning urine was collected at baseline to assess N-telopeptide indexed to creatinine (NTX/Cr) (nMBCE/mmol), as the marker of bone resorption. BALP,

OC and NTX/Cr were measured by ELISA using kits manufactured by Quidel (San Diego, CA) for the bone formation markers and Inverness Medical Innovations, Inc. (Osteomark; Princeton, NJ), for the bone resorption marker. The intra-assay coefficients of variation (CV) were 3.83% for BALP, 4.18% for OC, and 1.35% for NTX/Cr. The respective inter-assay CVs were 5–7.6%, 9.35% and 1.48%.

Statistical Analysis

Descriptive statistics were calculated for demographic, treatment and lifestyle factors, as well as the bone turnover biomarkers to describe the baseline population. Multivariate linear regressions were used to examine the association between the biomarkers: BALP, OC, NTX/Cr and other clinical variables: life style variables, chemotherapy and radiation therapy treatment variables, and bone mineral density (QCT Z-score), adjusted for patient demographics (age, gender, tanner stage, and BMI). A log2 transformation was applied on the biomarker variables for the sake of normality. In a sensitivity analysis, patients with self-report of smoking and physical activity levels were analyzed separately from those with parent/guardian report of these descriptors. Statistical significance level was set at $p = 0.05$. All analyses were conducted using R 2.13.2.

RESULTS

Baseline characteristics of the 418 ALL survivors are shown in Table 1. The median age of diagnosis of ALL was 4.6 years (range, 0.24–18.8 years), the median time from completion of treatment of ALL was 8.5 years (range, 4.5 to 19.1 years) and the median age at time of study was 17.0 years (range, 9.0 to 36.1) years. Smoking status and physical activity levels were self-reported by 185 (88 females and 97 males) patients; the remainder were obtained by parental or guardian report.

For females, 10 participants had LS-BMD Z-score below -2 , 39 had scores of -2 to -1 , 70 had scores of -1 to 0 , and 84 had scores ≥ 0 . For males, 19 participants had LS-BMD Z-score below -2 SD, 61 had scores of -2 to -1 , 76 had scores of -1 to 0 , and 59 had scores ≥ 0 . The median NTX/Cr ratio was 53.9 (range, 5.5–2113.1) for females and 76.1 (range, 3.7–632.4) for males. For females, the respective BALP and OC median values were 34.1 (range, 11.1–316) and 15.1 (range, 2.1–348.8). For males, the respective BALP and OC median values were 60.8 (range, 13.6–313) and 22.4 (range, 4.7–879.6) (Table 1).

Age and Tanner stage were inversely associated with all biomarkers (BALP, OC, NTX/Cr) ($P < 0.001$). Males had higher BALP and OC than females ($P < 0.001$). BMI was inversely associated with OC and NTX/Cr ($P < 0.001$) (Table 2).

After adjustment for age, gender, Tanner Stage and BMI, we found no significant association of any biomarker with lifestyle related factors, ALL treatment related characteristics or dietary intake of calcium or vitamin D ($P = 0.09$) (Table 3). In sensitivity analyses stratified by patient (> 18 years) vs. parent/guardian self-report (< 18 years) of smoking status and physical activity levels, again there was no significant association of smoking status ($p = 0.38$ for < 18 years and $p = 0.70$ for ≥ 18 years) and physical activity levels ($p = 0.38$ for < 18 years and $p = 0.16$ for ≥ 18 years) with biomarkers of bone turnover.

After adjustment for age, gender, Tanner Stage and BMI, there was no significant association of any biomarker with LS-BMD Z-scores ($p = 0.47$) (Table 4). Subset stratification by Tanner Stage revealed no significant association between any of the biomarkers and LS-BMD Z-score, after accounting for multiple testings (Table 5).

DISCUSSION

In survivors of childhood ALL, similar to healthy children, bone turnover markers varied with age [32,33], Tanner stage [32], and gender [32]. ALL-related treatment regimens did not significantly impact on bone turnover, and bone turnover was not significantly associated with volumetric BMD of the lumbar spine.

We found a number of similarities between the relationship of bone turnover markers and demographic factors in our study population as reported for healthy children. Bone turnover was significantly inversely related to both age [34] and Tanner Stage [35]. Also, in agreement with reports in healthy children [32,36], male gender was associated with higher levels of bone formation [32] in this survivor cohort. However, in contrast to the positive association of male gender with bone resorption that has been reported in healthy children [36], we found that in ALL survivors, NTX/Cr, a marker of bone resorption, was not significantly associated with gender. In contrast to healthy children, in which a weak correlation between BALP and BMI [37] has been reported, in ALL survivors we found an inverse correlation between BMI and OC and NTX/Cr, and no significant relationship with BALP.

Among lifestyle factors, in healthy young individuals, physical activity has substantial effects on bone turnover [38–40]. A prior report found bone turnover was associated with higher measured physical performance but not with self-report of physical activity [23]. Although we also found no association between bone turnover and self-report of physical activity, we did not directly measure physical performance. Additionally, we found current tobacco use, did not significantly affect bone turnover.

Our findings that calcium and vitamin D intake were unrelated to bone turnover in ALL survivors were surprising. However, the relationship of diet to bone turnover even in healthy children is complex and depends partially on age and pubertal state. In one study, calcium supplementation lowered bone turnover in healthy 12-year-old girls [41], whereas inadequate dietary intakes of calcium and vitamin D in a separate study were associated with lower bone turnover in pre-pubertal children [42].

Bone turnover has been reported to increase in children with ALL during treatment with glucocorticoids and reportedly, high dose intravenous methotrexate enhances osteoclastic activity [43]. Our study, in which ALL survivors had been treated with chemotherapeutic protocols that included both agents, and in agreement with another report [23], suggests that these immediate post-chemotherapeutic effects on bone turnover in ALL [43] are temporary. While we previously showed that cranial irradiation adversely affected BMD, this treatment modality did not influence long-term bone turnover, a finding similar to that of another report [23].

Among these ALL survivors, 7% had a QCT BMD Z-score of less than -2 and 24% had a Z-score of between -1 and $-2SD$. These low BMD values are in excess of the 2.2% and 13.6% with this distribution of scores which would be expected with a normal distribution of BMD (as would be anticipated in normal healthy children). However, similar to another study which included 72 survivors of ALL, [44], there was no association of these LS-BMD Z-scores with bone turnover across all Tanner Stages. Our study extends the findings of this prior study, [44], to include that other bone turnover markers (BALP and NTX/Cr) are also not significantly associated with LS-BMD Z-scores across all Tanner Stages.

Our study has a number of unique strengths. The study population comprised a large, well-defined cohort treated at a single institution, for which detailed treatment history was available. This study cohort was almost equally divided between males and females, with a wide range of ages and Tanner stages. We addressed values and significance of biomarkers that were measured on average more than 10 years following treatment for ALL. The breadth of variables analyzed exceeds those in previously published reports and include results investigating the relationship of OC, NTX/Cr, BALP, dietary intake of calcium and vitamin D, and tobacco smoking. Our use of QCT provided a direct volumetric assessment of vertebral body BMD without the need to adjust for vertebral body size as when DXA is used.

Despite these strengths, there are several limitations to this study. As sample size of races other than Black or White from the parent study [45] was very small ($n=6$), these were not included in the current analysis. Thus, we were unable to determine the relationship of other races to bone markers.

Second, though the physical activity questionnaire used in this study [26] has not been validated for use in children but most of the participants were late adolescent or young adults (median age @ enrollment = 17y) (range 9.0 – 36/y). Additionally, parent/guardian report of cigarette smoking and physical activity levels in the child may not be reliable. We determined BMD by QCT, not DXA, which limits comparison of our results with those of other reported cohorts. We categorized the patients by radiation exposures as ≥ 24 Gy and 1–23 Gy or none as per the treatment protocols. Moreover, we previously reported that we found no difference in BMD in ALL survivors who received radiation vs. those receiving 18 Gy [5]. However, others have reported a dose-related association with BMD in survivors of childhood cancer [46].

We did not have information on bone turnover at diagnosis of ALL, and it has been reported that the disease itself causes a low bone turnover state [43]. We analyzed cross-sectional and not longitudinal data, precluding our ability to ascertain change over time and to assess changes as may occur with increasing maturity and time from ALL therapy. We did not have validated fracture data to determine the association of bone turnover with fractures. Finally, we did not have a control population of healthy children in this study, and thus we were unable to determine whether bone turnover in ALL survivors differs from healthy children with similar characteristics.

In conclusion, more than a decade following completion of treatment for ALL, factors that influence bone turnover are similar to those of healthy children. Long term bone turnover is not associated with prior treatment for ALL or with LS-BMD Z-scores. The BMD deficits we have previously reported in this cohort (6) appear to be independent of bone turnover.

ACKNOWLEDGEMENTS

Supported in part by grant P30 CA-21765 from the National Institutes of Health, a Center of Excellence grant from the State of Tennessee, and the American Lebanese Syrian Associated Charities (ALSAC). The authors thank Sandra Gaither for manuscript preparation.

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Table 1

Descriptive Anthropometric and Demographic Characteristics of Study Population (N=418)

		GENDER	
		Female (N=203)	Male (N=215)
Variables	Categories	Freq (%)	
Race	Black	25 (12.3)	28 (13.0)
	White	178 (87.7)	187 (87.0)
Tanner Stage	I	6 (3.0)	19 (8.8)
	II	11 (5.4)	15 (7.0)
	III	22 (10.8)	13 (6.0)
	IV	37 (18.2)	37 (17.2)
	V	127 (62.6)	131 (60.9)
Age at diagnosis	Years	4.6 (0.6, 18.7)	4.6 (0.2, 18.8)
Age at study entry	Years	17.0 (9.4, 36.1)	17.2 (9.0, 35.4)
Height	cm	159.6 (127.5, 180.1)	171.0 (124.6, 192.6)
Weight	kg	60.4 (31.8, 155.3)	67.9 (28.7, 172.6)
Body mass index	kg/m ²	24.0 (15.2, 53.8)	24.1 (14.2, 54.1)
Lifestyle			
Smoking	No	184 (90.6)	174 (80.9)
	Yes	19 (9.4)	41 (19.1)
Moderate/vigorous physical activity	Minutes/week	68.3 (10.0, 1 257.3)	170.8 (10.0, 768.2)
ALL Treatment Factors			
Radiation Group	24 Gy	13 (6.4)	22 (10.2)
	1-23 Gy or none	190 (93.6)	193 (89.8)
Chemotherapeutic Group	Intermediate to High risk	118 (58.1)	152 (70.7)
	Low risk	85 (41.9)	63 (29.3)
Dietary Intake			
Dietary calcium	mg	786.5 (129.4, 2 276.2)	832.0 (159.8, 2420.0)
Dietary Vitamin D	Mg	128.6 (12.1, 554.8)	157.2 (16.5, 630.5)
LS-BMD Z-scores			
QCT Z-score	Below -2	10 (4.9)	19 (8.8)
	-2 to -1	39 (19.2)	61 (28.4)
	-1 to 0	70 (34.5)	76 (35.3)
	Above 0	84 (41.4)	59 (27.4)
Biomarkers			
BALP	U/L	34.1 (11.1, 316.0)	60.8 (13.6, 313.0)
N-telopeptide	nMBCE/mmol	53.9 (5.5, 2 113.1)	76.1 (3.7, 632.4)
Osteocalcin	ug/L	15.1 (2.1, 348.8)	22.4 (4.6, 879.6)

Table 2

Association of Demographic Variables with Bone Turnover

Outcome variable: log2 transformed BALP				
<i>Parameter</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>C.I.</i>	<i>P-value</i>
(Intercept)	8.52	0.19	(8.149, 8.901)	<0.001
Age	−0.097	0.0084	(−0.114, −0.081)	<0.001
Gender (Male vs. Female)	0.52	0.079	(0.367, 0.676)	<0.001
Tanner	−0.31	0.043	(−0.39, −0.221)	<0.001
BMI	0.0015	0.0065	(−0.011, 0.014)	0.82
Outcome variable: log2 transformed: N-telopeptide				
<i>Parameter</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>C.I.</i>	<i>P-value</i>
(Intercept)	10.99	0.26	(10.487, 11.498)	<0.001
Age	−0.13	0.012	(−0.152, −0.106)	<0.001
Gender (Male vs. Female)	0.15	0.11	(−0.067, 0.361)	0.18
Tanner	−0.39	0.059	(−0.509, −0.277)	<0.001
BMI	−0.03	0.009	(−0.048, −0.013)	0.0009
Outcome variable: log2 transformed Osteocalcin				
<i>Parameter</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>C.I.</i>	<i>P-value</i>
(Intercept)	6.6	0.21	(6.187, 7.007)	<0.001
Age	−0.07	0.0091	(−0.087, −0.051)	<0.001
Gender (Male vs. Female)	0.42	0.086	(0.25, 0.586)	<0.001
Tanner	−0.17	0.047	(−0.264, −0.079)	0.0003
BMI	−0.019	0.007	(−0.033, −0.005)	0.009

Table 3

Association of Biomarkers with Clinical Variables: Multivariable Analysis Adjusted for Age, Gender, Tanner Stage, and BMI

Outcome variable: log2 transformed BALP				
<i>Clinical Variable</i>	<i>Coefficient</i>	<i>SE</i>	<i>95% C.I.</i>	<i>P-value</i>
Smoking (Yes vs. No)	−0.11	0.11	(−0.332, 0.107)	0.31
Dietary Calcium Intake	0.00003	0.00015	(−0.00026, 0.00031)	0.84
Dietary Vitamin D Intake	0.0002	0.0005	(−0.001, 0.001)	0.68
Moderate/Vigorous Physical Activity	0.0002	0.0003	(0, 0.001)	0.51
Risk Group (Low risk vs. Intermediate/High risk)	0.14	0.085	(−0.03, 0.301)	0.11
Radiation Level (Low/none vs. High)	0.2	0.14	(−0.068, 0.473)	0.14
Outcome variable: log2 transformed: N-telopeptide				
<i>Clinical Variable</i>	<i>Coefficient</i>	<i>SE</i>	<i>95% C.I.</i>	<i>P-value</i>
Smoking (Yes vs. No)	−0.11	0.16	(−0.415, 0.197)	0.49
Dietary Calcium Intake	0.00007	0.00014	(−2e-04, 0.00034)	0.61
Dietary Vitamin D Intake	0.0001	0.0005	(−0.001, 0.001)	0.84
Moderate/Vigorous Physical Activity	0.0003	0.0004	(−0.001, 0.001)	0.47
Risk Group (Low risk vs. Intermediate/High risk)	0.12	0.12	(−0.104, 0.352)	0.29
Radiation Level (Low/none vs. High)	0.12	0.2	(−0.26, 0.504)	0.53
Outcome variable: log2 transformed Osteocalcin				
<i>Clinical Variable</i>	<i>Coefficient</i>	<i>SE</i>	<i>95% C.I.</i>	<i>P-value</i>
Smoking (Yes vs. No)	−0.045	0.12	(−0.285, 0.195)	0.71
Dietary Calcium Intake	0.0001	0.00014	(−0.00016, 0.00039)	0.41
Dietary Vitamin D Intake	0.0002	0.0005	(−0.001, 0.001)	0.64
Moderate/Vigorous Physical Activity	0.0003	0.0003	(−0.00039, 0.001)	0.43
Risk Group (Low risk vs. Intermediate/High risk)	0.16	0.09	(−0.024, 0.336)	0.09
Radiation Level (Low/none vs. High)	0.19	0.15	(−0.101, 0.483)	0.2

Table 4

Biomarkers and Bone Mineral Density: Multivariable Analysis Adjusted for Age, Gender, Turner Stage, and BMI

Outcome variable: log2 transformed BALP				
<i>Parameter</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>C.I.</i>	<i>P-value</i>
(Intercept)	8.502	0.202	(8.106, 8.898)	<0.001
Age	−0.098	0.009	(−0.115, −0.082)	<0.001
Gender (Male vs. Female)	0.517	0.080	(0.361, 0.673)	<0.001
Tanner	−0.303	0.043	(−0.388, −0.219)	<0.001
BMI	0.003	0.007	(−0.01, 0.016)	0.66
BMD (qctz)	−0.025	0.034	(−0.092, 0.043)	0.47
Outcome variable: log2 transformed: N-telopeptide				
<i>Parameter</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>C.I.</i>	<i>P-value</i>
(Intercept)	10.956	0.275	(10.418, 11.494)	<0.001
Age	−0.131	0.012	(−0.155, −0.108)	<0.001
Gender (Male vs. Female)	0.140	0.113	(−0.08, 0.36)	0.21
Tanner	−0.386	0.060	(−0.505, −0.268)	<0.001
BMI	−0.028	0.010	(−0.047, −0.01)	0.003
BMD (qctz)	−0.029	0.049	(−0.125, 0.066)	0.55
Outcome variable: log2 transformed Osteocalcin				
<i>Parameter</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>C.I.</i>	<i>P-value</i>
(Intercept)	6.569	0.219	(6.14, 6.998)	<0.001
Age	−0.070	0.009	(−0.088, −0.052)	<0.001
Gender (Male vs. Female)	0.418	0.086	(0.25, 0.587)	<0.001
Tanner	−0.173	0.047	(−0.265, −0.08)	<0.001
BMI	−0.017	0.007	(−0.032, −0.003)	0.02
BMD (qctz)	−0.024	0.037	(−0.096, 0.049)	0.52

Table 5

Biomarkers and BMD by Tanner Stage

Outcome variable: log2 transformed BALP					
Tanner stage	Parameter	Estimate	Std. Error	C.I.	P-value
1	BMD (qctz)	0.03	0.11	(-0.19, 0.249)	0.79
2	BMD (qctz)	-0.06	0.09	(-0.232, 0.11)	0.49
3	BMD (qctz)	-0.09	0.15	(-0.389, 0.217)	0.58
4	BMD (qctz)	-0.07	0.08	(-0.224, 0.095)	0.43
5	BMD (qctz)	-0.02	0.04	(-0.103, 0.059)	0.6
Outcome variable: log2 transformed: N-telopeptide					
Tanner stage	Parameter	Estimate	Std. Error	C.I.	P-value
1	BMD (qctz)	-0.02	0.08	(-0.177, 0.131)	0.77
2	BMD (qctz)	-0.22	0.22	(-0.655, 0.213)	0.33
3	BMD (qctz)	-0.76	0.27	(-1.282, -0.23)	0.01*
4	BMD (qctz)	-0.10	0.13	(-0.354, 0.154)	0.44
5	BMD (qctz)	0.00	0.06	(-0.111, 0.118)	0.95
Outcome variable: log2 transformed osteocalcin					
Tanner stage	Parameter	Estimate	Std. Error	C.I.	P-value
1	BMD (qctz)	-0.01	0.10	(-0.216, 0.187)	0.89
2	BMD (qctz)	-0.19	0.22	(-0.621, 0.235)	0.39
3	BMD (qctz)	-0.47	0.24	(-0.93, -0.006)	0.06
4	BMD (qctz)	-0.09	0.09	(-0.277, 0.089)	0.32
5	BMD (qctz)	0.03	0.05	(-0.061, 0.114)	0.56

* Because of multiple testing, p-value for significance = 0.0057 = .0007.