



The associations of exposure to combined hormonal contraceptive use on bone mineral content and areal bone mineral density accrual from adolescence to young adulthood: A longitudinal study



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ABSTRACT

Background: The association of long term combined hormone based contraceptives (CHC) use on bone mineral content (BMC) and areal bone mineral density (aBMD) development remains controversial, as it appears that the relationship may be age-dependent. The purpose of this study was to investigate the long-term associations of CHC exposure on the accrual of bone parameters from adolescence into young-adulthood.

Methods: 110 women (67 exposed to CHC) were drawn from the Pediatric Bone Mineral Accrual Study (PBMAS). Serial measures of total body (TB), lumbar spine (LS) and femoral neck (FN) BMC and aBMD were assessed by DXA (a total of 950 scans) and aligned by biological age (BA, years from peak height velocity [PHV]). Multilevel random effects models were constructed to assess the time dependent associations between annual CHC exposure and the development of bone parameters.

Results: After BA, height, lean tissue mass, fat mass, calcium and vitamin D intake, and physical activity were controlled, it was observed that those individuals exposed to CHC 6-years post PHV developed significantly less (-0.00986 ± 0.00422 g/cm²) TB aBMD than their non CHC exposed peers. Additionally, there were significant BA by CHC exposure interactions, where CHC exposure 6-years or more post PHV resulted in developing less TB BMC (-4.94 ± 2.41 g), LS BMC (-0.29 ± 0.11 g) and LS aBMD (-0.00307 ± 0.00109 g/cm²). One year after the attainment of PHV, CHC users were predicted to have 1.2% more TB BMC, 3.8% more LS BMC and 1.7% more LS aBMD than non-users. At 9-years post PHV the predicted differences showed that CHC users had 0.9% less TB BMC and 2.7% less LS BMC and 1.6% less LS BMD than those not exposed to CHC.

Conclusions: CHC may not hinder the development of BMC or aBMD during adolescence; however, exposure 6-years or more after PHV may be detrimental.

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1. Introduction

Osteoporosis is a skeletal disease that affects millions of individuals worldwide and is characterized by low areal bone mineral density (aBMD) and microarchitecture deterioration, leading to increased bone fragility. Although osteoporosis is a disease associated with fractures in old age, its antecedents are found in adolescence, with the accrual of adolescent bone mass proposed to influence fracture risk in adulthood (Bonjour et al., 2009; Baxter-Jones et al., 2011). Estrogen is a major regulator of bone growth, influencing the sexual dimorphism of the skeleton and the maintenance of bone mineral homeostasis, which in turn alters both the development of bone strength and the acquisition of peak bone mass (Berger et al., 2010). Estrogens act on bone

by regulating bone tissue metabolism, primarily through associations with the promotion of osteoblastic activity and the suppression of osteoclastic resorption (Dempster, 2006; Almeida, 2010; Chen et al., 2009; Zallone, 2006; Tremollieres, 2013). In women, the effects of estrogen on the skeleton appear most notably during adolescence and after menopause. During adolescence there is a surge in estrogen levels associated with adolescent growth and sexual development, and during menopause, estrogen levels rapidly decline, exposing women to a hypoestrogenic state (Tremollieres, 2013). Thus, the exposure to estrogen during these key periods may dramatically influence a woman's bone health.

Combined hormonal contraceptives (CHC) are one of the most commonly used methods of contraception (Black et al., 2009). In addition to contraception though, 88% of CHC users report taking CHC for non-contraceptive purposes, such as menstrual regulation, menstrual pain and treating acne (Jones, 2011) thus, it is not unusual to see CHC use beginning as early as 10 years of age and continuing well into adulthood.

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In Canada alone, nearly 67% of adolescents between 15–19 years of age report using CHCs (Black et al., 2009). The associations between CHC use and bone health remains controversial, with recent evidence suggesting that the association of CHCs may be age dependent (Tremollieres, 2013; Nappi et al., 2012; Ziglar and Hunter, 2012; Martins et al., 2006). Prospective observations show that compared with non-using age similar adolescent or young adult controls, CHC use is associated with negative changes in areal bone mineral density (BMD) and content (BMC) (Polatti et al., 1995; Scholes et al., 2010, 2011; Berenson et al., 2008). Other prospective studies show no BMD differences with CHC use (Bonny et al., 2011; Rome et al., 2004). The observed negative effects during adolescence are thought to be the result of CHCs reducing the physiological levels of systemic estrodiol, inhibiting the development of peak bone mass (Ziglar and Hunter, 2012; Martins et al., 2006). In adulthood, CHCs appear to provide no benefit to total body, lumbar spine or femoral neck aBMD in premenopausal women. Associations during the third and fourth decades of life are unknown. These conflicting results from adolescence to adulthood, highlight the potential age dependent associations that the exposure to CHCs may have on bone health throughout life; however previous studies results are often limited to 1 to 3 years in duration, with a limited number exceeding 5 years of follow-up (Beksinska et al., 2009; Lloyd et al., 2000), and often fail to control for the duration of CHC use, which may mediate the relationship between CHCs and bone health (Scholes et al., 2010, 2011). Additionally, bone undergoes vast changes during the adolescent period, and has been previously identified as a critical window for bone mineral accrual, with nearly 50% of adult bone mineral mass accrued during the 4 years around peak linear growth (Bailey, 1997; Baxter-Jones et al., 2003, 2010a). Therefore, the initiation of CHC use may have dramatically different effects on the bone if consumed during this key period of bone development. However, there remains a paucity of longitudinal studies that span the entire adolescent growth period into adulthood that address whether the exposure to CHCs is associated with the accrual of bone mass.

The advantage of using longitudinally gathered data is that it is possible to determine an individual's bone parameter accrual trajectories, while adjusting for the associations associated with other known confounders. The uniqueness of this longitudinal approach to data analysis is that it accounts for the wide variation shown among women's growth parameters at any given age, and in the velocity of these parameters from one age to the next. Growth curves for bone mineral accrual can be constructed by aligning all subjects on a comparable biological age index (age from peak height velocity [PHV]) to account for the known maturational impact on bone growth (Baxter-Jones et al., 2003). The introduction of multilevel statistical models (Goldstein et al., 2002) has assisted researchers in fitting growth curves to longitudinal measurements over time. In the multilevel framework each individual has their own straight line growth trajectory, with intercepts and slope coefficients varying between individuals. Using this technique the independent time dependent effects of growth, maturation, environmental effects, and CHC usage on BMC and aBMD accrual can be identified. Therefore, the purpose of this longitudinal study was to investigate the time dependent associations of exposure to CHCs on the accrual of bone mass from adolescence to young adulthood.

2. Methods

2.1. Participants

Women participants were drawn from the University of Saskatchewan's mixed-longitudinal designed Pediatric Bone Mineral Accrual Study (PBMAS). The PBMAS has been described in detail elsewhere (Bailey et al., 1999; Baxter-Jones et al., 2008). In brief, the PBMAS cohort consists of 259 individuals (aged 8 to 15 years) recruited from two elementary schools in the city of Saskatoon between 1991 and 1993. The PBMAS began with eight chronological age clusters (8 to

15 years) in 1991, with additional recruitment of 8 and 9 year olds in 1992 and 1993. In the initial study phase, data were collected annually until 1997; after a five-year break data collection resumed and were collected annually between 2002 and 2011. In 1991 a total of 228 students (115 girls; 8 to 15 years of age) provided written informed consent to participate, and 220 children (113 girls) were DXA scanned. By 1997, 197 individuals (aged 12 to 21 years) had been assessed on more than one occasion (median 6 occasions). From 2003 to 2007 data were collected on 169 returning participants (84 women aged 18 to 31 years) (median 4 occasions). From 2009 to 2011, 107 participants returned for another follow-up measurement (65 women aged 23 to 33 years of age; Table 1). This mixed-longitudinal study design allowed for the assessment of a 26 year developmental pattern between the ages of 8–33 years, within a 20 year data collection period. At each measurement occasion the same anthropometric, body composition, physical activity, dietary intakes and bone measures were recorded using the same instruments. From 2003 onwards CHC usages was recorded as an additional measure both retrospectively (1991–2001) and prospectively (2002–2011). To be included in the present study participants were required to be: (i) women; (ii) have a valid assessment of peak height velocity (PHV); (iii) have at least two DXA assessments (one during childhood/adolescence and one in young adulthood); (iv) have a record of CHC use; and (v) have no history of diseases known to affect growth or bone development. This resulted in the inclusion of 110 young women, with 67 women reporting to have used a CHC at least once between 1991 and 2011. Table 1 shows the number of women scanned each year by age group and CHC exposure; note that not all individuals were assessed at all measurement occasions hence the change in numbers from year to year within age groups. From 1991 to 2011 the median number of scans was 10 (range 1 to 14). A total of 950 scans were performed over a 20 year period, of these 205 scans were of women exposed to CHC in the previous 12 months. Written informed consent was obtained from all participants (parental assent for minors). All procedures were approved by the University of Saskatchewan's biomedical review committee.

2.2. Anthropometry

Anthropometric measures included height and weight, assessed following the anthropometric standards outlined by Ross and Marfell-Jones (Ross and Marfell-Jones, 1991). Stretch stature was recorded without shoes to the nearest 0.1 cm against a wall mounted stadiometer (Holtain Limited, Crymch, UK). Weight was measured on a calibrated digital scale to the nearest 0.5 kg (Model 1631, Tanita Corp, Tokyo, Japan).

2.3. Chronological age

A decimal chronologic age (CA, years) was determined by identifying the numbers of days between an individual's date of birth and the date at the assessment occasion.

2.4. Biological age (peak height velocity) assessments

Biological age (BA, years), a measure of somatic maturation, was defined by identifying the CA of attainment of peak linear growth during adolescence (peak height velocity [PHV]). To determine the CA at PHV, whole year height velocities were calculated for each participant. A cubic spline fitting procedure was applied to each individual's whole year velocity values and the CA at the highest point was estimated (GraphPad Prism 5, GraphPad Software, San Diego, CA, USA). A BA was then calculated by subtracting the CA at PHV from the CA at time of measurement for each individual (e.g. CA at time of measurement = 10.5 years, CA of PHV = 13.4 years, BA at measurement = 10.5 – 13.4 = –2.9 years).

Table 1

Number of women measured in each test year by age group and combined hormone contraceptive (CHC) exposure.

Age	1991	1992	1993	1994	1995	1996	1997	2002	2003	2004	2005	2009–11
8	4 (0)	9 (0)	2 (0)									
9	15 (0)	7 (0)	8 (0)	2 (0)								
10	10 (0)	16 (0)	8 (0)	10 (0)	2 (0)							
11	12 (0)	12 (0)	17 (0)	6 (0)	10 (0)	2 (0)						
12	15 (0)	11 (0)	11 (0)	13 (0)	6 (0)	10 (0)	2 (0)					
13	16 (0)	16 (0)	11 (0)	11 (0)	13 (0)	6 (0)	7 (0)					
14	14 (0)	14 (1)	14 (0)	10 (0)	11 (0)	11 (0)	7 (0)					
15	8 (0)	13 (0)	16 (1)	14 (0)	10 (0)	10 (1)	11 (0)					
16		8 (0)	13 (1)	9 (1)	10 (3)	5 (1)	6 (1)					
17			7 (0)	10 (1)	11 (1)	8 (4)	4 (1)	1 (1)				
18				6 (0)	7 (1)	9 (1)	5 (4)	2 (1)	1 (1)			
19					5 (0)	6 (0)	5 (2)	6 (4)	1 (1)	1 (1)		
20						4 (0)	3 (0)	7 (3)	5 (3)	3 (1)		
21							2 (0)	3 (3)	3 (3)	0 (5)	0 (2)	
22								3 (6)	3 (4)	6 (3)	3 (4)	
23								2 (6)	1 (7)	1 (4)	0 (2)	
24								4 (7)	3 (8)	1 (6)	6 (5)	0 (1)
25								4 (1)	5 (5)	5 (8)	2 (6)	1 (0)
26								5 (0)	1 (5)	2 (6)	3 (4)	1 (4)
27									5 (0)	1 (4)	6 (5)	2 (2)
28										3 (0)	5 (6)	3 (2)
29											2 (3)	2 (8)
30											0 (2)	3 (1)
31												0 (9)
32												3 (3)
33												2 (2)
	94 (0)	116 (1)	107 (2)	102 (2)	71 (7)	71 (7)	52 (8)	37 (32)	28 (37)	23 (38)	27 (39)	17 (32)

Numbers represent numbers for non-exposed (exposed) to CHC. Note that not all individuals returned for assessment at all measurement occasions hence the change in numbers from year to year in each age group.

Age at menarche was asked at each measurement, prospectively in adolescence and retrospectively in young adulthood.

2.5. Body composition assessments (bone mass, lean tissue mass and fat mass)

At each measurement occasion participants underwent a dual energy X-ray absorptiometry (DXA, Hologic QDR-2000/4500, array mode) scan of the total body (TB), lumbar spine (LS) and proximal femur (FN) following the procedures outlined in the Hologic operator's manual to assess bone mineral content (BMC, in grams, g), areal bone mineral density (aBMD, grams per centimeter squared, g/cm²), lean tissue mass (LTM, kilograms, kg) and fat mass (FM, kilograms, kg). TB BMC, TB aBMD, LTM and FM were analyzed using software 5.67A. BMC and aBMD at the lumbar spine and femoral neck were analyzed using software version 4.66A. Scanner drift was assessed prior to each scan using the manufactures lumbar spine phantom and all assessments were performed by a qualified licensed technician. The inter-assay precision (CV%) in vivo for BMC and aBMD measures are 0.60–0.91% and 0.51–0.90%, respectively (Bailey et al., 1996). LTM and FM in our lab have been also previously reported as CV's of 0.5% and 2.95%, respectively (Bailey et al., 1996, 1999). Between 1991 and 2007 scans were obtained using the Hologic 2000 scanner, after 2007 scans were obtained using a Hologic 4500 scanner. To correct for differences between instruments in vivo determined precision across different Hologic instruments was undertaken using results obtained from 9 men and 15 women. From these data we developed conversion factors so that all outcomes used for analysis represented Hologic 2000 equivalent values (Baxter-Jones et al., 2010b).

2.6. Dietary assessment

Calcium and vitamin D intake was assessed annually using a 24 hour food recall questionnaire. Dietary data were analyzed using the Food Processor and Nutritional Software (ESHA Research Software, Salem, OR, USA, Version 8.5). The use of 24-hour food recall has been documented in this cohort previously and been suggested as an appropriate

method to assess nutrient intake in children and adults (Whiting and Shrestha, 1993).

2.7. Physical activity assessments

Physical activity (PA) was serially assessed using self-report questionnaires. Details of the physical activity questionnaires used have been reported elsewhere (Crocker et al., 1997; Kowalski et al., 1997, 2004; Copeland et al., 2005). In brief, during childhood and adolescence physical activity was assessed using the Physical Activity Questionnaire for Children (PAQ-C) and the Physical Activity Questionnaire for Adolescence (PAQ-A). The PAQ-C/A were designed to assess general PA levels over the past seven days, scoring nine items on a five point Likert-type scale. Final PA scores range from one to five, with higher scores indicating higher levels of PA. In adulthood, the Physical Activity Questionnaire for Adults (PAQ-AD), a 7 item version of the PAQ-C/A, was used; with individuals' PA scored on a five point scale. The PAQ family of questionnaires has been previously reported to be a valid and reliable measure of PA levels in children, adolescents and adults (Crocker et al., 1997; Kowalski et al., 1997; Copeland et al., 2005).

2.8. Combined hormonal contraceptive (CHC) measure

Between 2002 and 2011 participants filled out a lifestyle questionnaire that assessed contraceptive use. Contraceptive use was assessed prospectively from 2002 onwards and retrospectively between 1991 and 2001. The questionnaire consisted of contraceptive specific questions such as: Are you currently using oral contraceptives? What type of oral contraceptives do you use? How long have you been using oral contraceptives? What were the age of first oral contraceptive use and the duration of use? The reason for CHC use, however, was not ascertained at any time point. This questionnaire was used to categorize each testing occasions as a period of contraceptive use or non-use.

2.9. Statistical analysis

All variables were assessed for normality and violations were adjusted using logarithmic transformations. For the longitudinal analyses, multilevel (hierarchical) random effects models were constructed using a multilevel modeling approach (MlwiN version 2.26, Multilevel Models Project; Institute of Education, University of London, UK (Ross and Marfell-Jones, 1991)). A detailed description of the multilevel modeling procedures is presented elsewhere (Baxter-Jones and Mirwald, 2004). In brief, bone parameters (BMC and aBMD) were measured repeatedly in individuals (level 1 of the hierarchy) and between individuals (level 2 of the hierarchy). Analysis models that contain linked variables measured at different levels of a hierarchy are known as multilevel random effect regression models. Specifically, the following additive random effects multilevel regression models were adopted to describe the developmental changes in bone parameters with age.

$$y_{ij} = (\alpha + \mu_j) + (\beta + \nu_j)x_{ij} + (z_{-1ij} + z_{2ij} + \dots + z_{nij}) + \varepsilon_{ij} \quad (1)$$

which can be reorganized to

$$y_{ij} = (\alpha + \beta_j x_{ij}) + (z_{-1ij} + z_{2ij} + \dots + z_{nij}) + (\mu_j + \nu_j x_{ij} + \varepsilon_{ij}). \quad (2)$$

where: y is the bone parameter (e.g. BMC or aBMD) on measurement occasion i in the j -th individual; α is a constant; $\beta_j x_{ij}$ is the slope of the bone parameter over time (in the models presented BA is centered around +5 years from PHV) for the j -th individual; z_{-1} to z_n are the coefficients of various time dependent explanatory variables (i.e. height, LTM, FM, calcium intake, vitamin D intake, PA, duration of CHC use, CHC use (yes = 1 or no = 0) and CHC use by BA interaction) at assessment occasion i in the j -th individual. These are the fixed time dependent parameters in the model.

μ_j , $\nu_j x_{ij}$ and ε_{ij} are random quantities (see Tables 3 to 5), whose means are equal to zero; they form the random parameters in the model. They are assumed to be uncorrelated and follow a normal distribution and thus their variances can be estimated; $\varepsilon_{ij} \sim N[0, \text{var}(\varepsilon_{ij})]$ is the level-1 residual (within individual) variance for the i -th assessment of the bone parameter in the j -th individual; $\mu_j \sim N[0, \text{var}(\mu)]$ is the between individual intercept variance and $\nu_j x_{ij} \sim N[0, \text{var}(\nu_j x_{ij})]$ the between individuals slope variance; these are the level-2 residuals (between subjects) variances for the j -th individual. $\mu_j * \nu_j x_{ij} \sim N[0, \text{var}(\mu_j * \nu_j x_{ij})]$ explains the intercept-slope covariance relationship among the intercepts and slopes in the model.

Models were built in a stepwise procedure, i.e. predictor variables (z-fixed effects) were added one at a time, and the log likelihood ratio statistic was used to judge the effects of including further variables on the fit of the model. BA center ($\beta_j x_{ij}$ -centered around +5 years from PHV) was added as both a random (level 2) and a fixed variable (Level 1). This permits individuals to have independent intercepts and slopes and a calculation of the intercept-slope covariance relationship. A significant BA centered coefficients (β) at level 1 of the models indicates that a bone measure is increasing significantly at each measurement occasion within individuals. Significant coefficients at the individuals variance matrix (level 2) in each model indicates that individuals have significantly different growth curves for bone measures, both in terms of their intercepts and the slopes and that there is a relationship between intercepts and slopes in the model. Predictor variables (z) were accepted as significant if the estimated mean coefficient was greater than twice the standard error of the estimate (SEE, i.e. $p < 0.05$). If both these retention criteria were not met the predictor variable was discarded. The power functions BA centered² and BA centered³ were introduced into the linear models to allow for the non-linearity of growth and were retained whether or not they were significant so as to shape the bone developmental curves. The predictor variable coefficients in the final models were used to predict

BMC and aBMD development at the total body, lumbar spine and femoral neck with BA, height, LTM, FM, calcium intake, vitamin D intake, PA, CHC duration of use, CHC use and CHC use by BA centered interaction controlled in the prediction equations using population averages at each BA category. A total of six independent multilevel (hierarchical) random effects models were constructed; one for each bone parameter (BMC and aBMD) at each assessment location (TB, LS and FN).

3. Results

Of the sixty-seven participants who reported using at least one form of hormone based contraception at a minimum of one time point, 92% of these individuals reported using a CHC as their method of contraception (i.e. oral pill), 7% used progestin only methods and 1% using other hormone based forms of contraceptives (i.e. IUD). Results are presented only for participants who reported use of CHC. All other users were excluded from the subsequent analyses. Table 2 displays the general characteristics of individuals exposed and not exposed to CHC usage. Those individuals exposed to CHC were older, consumed more calcium and vitamin D than individuals not exposed to CHC use. Only measurement occasions where CHC use was recorded was an individual considered being exposed to CHC in the longitudinal analyses. Average age of menarche was 12.7 years ranging from 9.8 to 15.0 years. The average age at which participants reported starting CHC use was 19.1 ± 3.4 years (range 14 to 31 years; Table 1). No individuals in this cohort reported CHC use prior to menarche.

3.1. Multilevel models and longitudinal analyses

Tables 3–5 summarize the results from the multilevel models for total body, lumbar spine and femoral neck BMC and aBMD development respectively by biological age (BA) centered around +5 years post PHV. For all models (Tables 3–5), the significant BA center random coefficients (β) at level 1 of the models indicates that bone measures were increasing significantly at each measurement occasion within individuals. The between individuals variance matrix (level 2 — μ_j and $\nu_j x_{ij}$) for each model indicates that individuals had significantly different growth curves for bone measures, both in terms of their intercepts (constant (μ_j)), and the slopes of their lines (BA center ($\nu_j x_{ij}$)). The positive BA centered * constant ($\mu_j * \nu_j x_{ij}$) coefficient indicates that in the model there was a relationship between intercepts and slopes, the higher the intercept the greater the slope.

In the fixed effects it was shown that BA centered, height, LTM, FM and physical activity significantly contributed to the prediction of TB BMC development (Table 3). Additionally, it was observed that there was a significant CHC user by BA interaction, identifying that those exposed to CHC use had significantly less TB BMC (-13.03 ± 6.09 g) for each year of exposure 6 years or more post PHV attainment (Fig. 1A)

Table 2

General descriptive characteristics of those individuals exposed and not exposed to combined hormonal contraceptive (CHC) use.

Variable	Not exposed to CHC (n = 43)	Exposed to CHC (n = 67)	Total (n = 110)
Age (years)	16.3 ± 5.6	18.0 ± 6.1 ^a	17.3 ± 5.9
APHV (years)	12.0 ± 1.0	11.8 ± 0.8	11.9 ± 0.9
Age of menarche	12.7 ± 1.1	12.8 ± 1.0	12.7 ± 1.1
Height (cm)	160.0 ± 11.4	162.0 ± 10.6	161.0 ± 11.0
Weight (kg)	58.6 ± 20.5	59.4 ± 18.7	59.0 ± 19.5
LTM (kg)	35.8 ± 8.4	36.7 ± 8.4	36.3 ± 8.4
FM (kg)	31.4 ± 9.9	31.7 ± 8.5	31.6 ± 9.1
Vit D (UI)	217.0 ± 151.0	242.0 ± 173.0 ^a	231.0 ± 164.0
Calcium (mg)	893.0 ± 395.0	983.0 ± 430.0 ^a	944.0 ± 415.0
Physical activity	2.6 ± 0.8	2.5 ± 0.7	2.6 ± 0.7
Years of CHC use	NA	4.9 ± 3.9	4.9 ± 3.9

APHV = age of peak height velocity; physical activity = physical activity score from subjective questionnaire (score from 1–5).

^a Indicates a significant difference from the non-users ($p < 0.05$).

Table 3

Multilevel regression models for total body bone mineral content (TB BMC) and areal bone mineral density (TB aBMD).

Variable	TB BMC	TB aBMD
<i>Fixed effects</i>		
Constant	-1633.51 ± 209.59	0.74 ± 0.07
BA centered	29.55 ± 2.19	7.86 ± 0.72 ^a
BA centered ²	-1.26 ± 0.34	0.58 ± 0.11 ^a
BA centered ³	-0.04 ± 0.02	0.03 ± 0.01 ^a
Height	14.86 ± 1.38	0.35 ± 0.04 ^a
LTM	26.48 ± 1.37 ^a	0.07 ± 0.01 ^a
FM	6.75 ± 0.82	-1.03 ± 0.27 ^a
Calcium	NS	NS
Vitamin D	NS	NS
PA	-13.03 ± 6.09	-7.74 ± 1.91 ^a
CHC duration of use	NS	NS
CHC usage	NS	-9.86 ± 4.22 ^a
CHC usage * BA	-4.94 ± 2.41	NS
<i>Random effects</i>		
Level 1		
Constant (ϵ_{ij})	6422.33 ± 333.56	0.61 ± 0.03 ^a
Level 2		
Constant (μ_j)	22.78 ± 3.26 ^b	2.48 ± 0.35 ^a
BA centered ($\nu_j X_{ij}$)	72.1 ± 14.7 ^a	0.01 ± 0.002 ^a
Constant * BA centered ($\mu_j * \nu_j X_{ij}$)	672.9 ± 175.8 ^a	0.05 ± 0.01 ^a

Fixed effect values are estimated mean coefficients ± SEE (standard error estimate) of BMC (g) and aBMD (g/cm²).Random effects values estimated mean variance ± SEE [BMC (g) and aBMD (g/cm²)]².

BA (biological age) centered is BA in years centered on 5 years post peak height velocity (PHV, years).

Height (cm); total body lean mass (LTM) (g); total body fat mass (FM) (kg); calcium (mg); vitamin D (UI); PA (physical activity – score from 1–5); CHC usage (CHC use = 1, non-use = 0).

Numerical values are all significant, $p < 0.05$ (mean > 2 * SEE). Non-significant variables are indicated as 'NS' and removed from the final model.^a Indicates that numerical values are multiplied by 10⁻³.^b Indicates that numerical values are multiplied by 10³.

and significantly more TB BMC is added up until 5 years post PHV. Similarly, BA centered, height, LTM, FM and physical activity significantly contributed to the prediction of TB aBMD development (Table 3), with those who were exposed to CHC identified to have significantly less TB aBMD development (-0.00986 ± 0.00422 g/cm², Fig. 1B). There was no CHC use by BA centered interaction.

At the lumbar spine, BA centered, height, LTM, FM and PA were significant predictors of LS BMC, but only BA centered, height, LTM, and PA were significant predictors of LS aBMD (Table 4); however for both LS BMC and LS aBMD, there was a significant CHC user by BA interaction, with those exposed to CHC having significantly less LS BMC (-0.29 ± 0.11 g) and LS aBMD (-0.00307 ± 0.00109 g/cm²) for each year of exposure 6 years or more after the attainment of PHV (Fig. 2) and have significantly more BMC and BMD in the years prior to +5 years post PHV.

At the femoral neck, it was observed that BA centered, height, LTM and FM significantly predicted the development of FN BMC (Table 5), while only BA centered, height and LTM significantly predicted the development of FN aBMD (Table 4). Neither CHC usage nor CHC user by BA interactions were observed to be significant predictors of BMC and aBMD at the femoral neck (Table 3, Fig. 3).

4. Discussion

The aim of this study was to investigate the long-term associations of exposure to CHC on the accrual of total body, lumbar spine and femoral neck bone mass from adolescence to young adulthood. It was observed that an exposure to CHC 5 years after the attainment of peak height velocity (PHV; ~17–19 years of age) was associated with detrimental development of TB BMC, LS BMC and LS aBMD; but that exposure from PHV to 5 years post PHV was beneficial, in this cohort of healthy

Table 4

Multilevel regression models for lumbar spine bone mineral content (LS BMC) and areal bone mineral density (LS aBMD).

Variable	LS BMC	LS aBMD
<i>Fixed effects</i>		
Constant	-46.38 ± 9.94	NS
BA centered	1.19 ± 0.1	12.73 ± 1.06
BA centered ²	-0.07 ± 0.02	-0.70 ± 0.20 ^a
BA centered ^{3b}	0.38 ± 0.09 ^a	NS
Height	0.54 ± 0.04	4.70 ± 0.67
LTM	4.28 ± 0.61	0.06 ± 0.01 ^a
FM	-0.09 ± 0.04	NS
Calcium	NS	NS
Vitamin D	NS	NS
PA	-0.58 ± 0.28	-5.62 ± 2.76 ^a
CHC duration of use	NS	NS
CHC usage	NS	NS
CHC usage * BA	-0.29 ± 0.11	-3.07 ± 1.09 ^a
<i>Random effects</i>		
Level 1		
Constant (ϵ_{ij})	11.7 ± 0.65	1.12 ± 0.62 ^a
Level 2		
Constant (μ_j)	39.74 ± 5.75	6.46 ± 0.91 ^a
BA centered ($\nu_j X_{ij}$)	0.16 ± 0.03	0.02 ± 0.01 ^a
Constant * BA centered ($\mu_j * \nu_j X_{ij}$)	0.92 ± 0.33	0.09 ± 0.04 ^a

Fixed effect values are estimated mean coefficients ± SEE (standard error estimate) of BMC (g) and aBMD (g/cm²).Random effects values estimated mean variance ± SEE [BMC (g) and aBMD (g/cm²)]².

BA (biological age) centered is BA in years centered on 5 years post peak height velocity (PHV, years).

Height (cm); total body lean mass (LTM) (g); total body fat mass (FM) (kg); calcium (mg); vitamin D (UI); PA (physical activity – score from 1–5); CHC usage (CHC use = 1, non-use = 0).

Numerical values are all significant, $p < 0.05$ (mean > 2 * SEE). Non-significant variables are indicated as 'NS' and removed from the final model.^a Indicates that numerical values are multiplied by 10⁻³.^b Indicates that numerical values are multiplied by 10³.

women. Additionally, it was noted that those individuals exposed to CHCs, throughout adolescence and young adulthood, developed significantly lower TB aBMD. This is the first study, to our knowledge, to longitudinally investigate the time dependent associations of exposure to CHC on the accrual of bone mass measures in healthy young women, followed from early adolescence into young adulthood.

In teen women, peak linear growth occurs, on average, at 12 years of age, and is landmarked by the milestone PHV. In the 4 years surrounding the attainment of PHV, nearly 50% of an individual's BMC is accrued (Bailey, 1997; Baxter-Jones et al., 2010a) making it a critical period towards adult bone development. Similarly, during adolescence there is a natural surge in circulating estrogen levels, which mediates the initiation of puberty and is necessary for linear growth, sexual dimorphism of the skeleton and epiphyseal closure (Almeida, 2010; Malina et al., 2004). Given that normal adolescent women have circulating estrogen concentrations ranging from 40–110 pg/ml and that elevated levels occur in the morning with the highest concentrations observed following the occurrence of menarche (Norjavaara et al., 1996; Albin et al., 2012), combined hormonal contraceptives that maintain these naturally occurring estrogen levels and follow a consumption schedule similar to the naturally occurring ebb and flow should provide an environment favorable to normal, healthy adolescent bone growth and development. In the present study, of the women reporting contraceptive use, 92% reported using CHC pills, consisting of both progestin and ethinyl estradiol, 7% reported using progesterin only methods (e.g. depoprovera) and, 1% reported using other combined hormonal contraceptive methods (e.g. IUD). Of the CHC brands reported, they are cited to delivery between 55–100 pg/ml of ethinyl estradiol¹ with normal and regular

¹ Concentrations derived from pharmacokinetic and bioavailability product information provided by pharmaceutical companies for brands reported by participants.

Table 5
Multilevel regression models for femoral neck bone mineral content (FN BMC) and areal bone mineral density (FN aBMD).

Variable	FN BMC	FN aBMD
<i>Fixed effects</i>		
Constant	-2.95 ± 0.58	NS
BA centered	NS	1.83 ± 0.96 ^a
BA centered ²	-1.88 ± 0.92 ^a	-0.42 ± 0.02 ^a
BA centered ³	NS	NS
Height	32.67 ± 3.75 ^a	2.97 ± 0.59 ^a
LTM	0.42 ± 0.03 ^a	0.07 ± 0.01 ^a
FM	5.81 ± 2.23 ^a	NS
Calcium	NS	NS
Vitamin D	NS	NS
PA	NS	NS
CHC duration of use	NS	NS
CHC usage	NS	NS
CHC usage * BA	NS	NS
<i>Random effects</i>		
Level 1		
Constant (ϵ_{ij})	52.18 ± 27.12 ^a	10.00 ± 0.05 ^a
Level 2		
Constant (μ_j)	0.16 ± 0.02	6.89 ± 0.96 ^a
BA centered ($\nu_j X_{ij}$)	0.04 ± 0.01 ^a	0.02 ± 0.003 ^a
Constant * BA centered ($\mu_j * \nu_j X_{ij}$)	4.52 ± 1.18 ^a	0.15 ± 0.04 ^a

Fixed effect values are estimated mean coefficients ± SEE (standard error estimate) of BMC (g) and aBMD (g/cm²).

Random effects values estimated mean variance ± SEE [BMC (g) and aBMD (g/cm²)²].

BA (biological age) centered is BA in years centered on 5 years post peak height velocity (PHV, years).

Height (cm); total body lean mass (LTM) (g); total body fat mass (FM) (kg); calcium (mg); vitamin D (UI); PA (physical activity score from 1–5); CHC usage (CHC use = 1, non-use = 0).

Numerical values are all significant, $p < 0.05$ (mean $> 2 * SEE$). Non-significant variables are indicated as 'NS' and removed from the final model.

^a Indicates that numerical values are multiplied by 10⁻³.

^b Indicates that numerical values are multiplied by 10³.

use. Since the ethinyl estradiol delivered in CHC is of a higher estrogenic potency compared to physiological estradiol, these serum levels meet, and may even exceed, normal circulating levels in healthy pubertal females. Though there are complex hormonal interactions taking place

during the pubertal period that may further complicate the impact of CHC use on bone development during this period, it can be reasonably argued that the CHCs, used by the majority of the current cohort, may serve to provide a hyperestrogenic environment, conducive to normal or improved adolescent bone growth and development. The findings of the current study support this supposition as it was observed that there were no significant detriments to the development of TB BMC, LS BMC, LS aBMD, FN BMC and FN aBMD during adolescence in individuals exposed to CHC. In fact there was a positive association with bone accrual between PHV and 5 years post PHV, with the detrimental effects not observed to occur until after 5 years post PHV. These observations support previous literature where combined oral contraceptive use was investigated during adolescence (Cromer et al., 1996, 2004, 2008) but dispute other finding [15,16]. It is important to note that the findings of Scholes et al. (Scholes et al., 2010, 2011) may differ from those presented here because of the different analytical approaches taken. Whereas the Scholes group reported % change in aBMD, we report adjusted raw values. Scholes et al. did not control for changes in maturation between CHC user and controls. As their CHC group was maturationally older it was possible that what was being observed was a natural increase in aBMD associated with normal growth. The advantage of the analytical technique presented in the present study was that maturational differences were controlled, at all ages suggesting perhaps the positive effects of CHC use observed after 20 years of age by the Scholes data could have been due to masking effects related to maturational differences.

Cromer and colleagues (Cromer et al., 2004) reported that adolescent women taking CHC had significant increases in aBMD at the lumbar spine and femoral neck after one year of combined oral contraceptive use, suggesting that significant bone growth may continue to occur during adolescence with the regular use of combined oral contraceptives. However, they also report that controls had significantly higher rates of change to aBMD compared to CHC users. These conflicting results may be a result of differences in oral contraceptive dosage. In the Cromer et al. study, participants were given a dose of 20 µg/100 µg ethinyl estradiol to progestin while in the current study participants report brands consisting of formulation of 30 µg/150 µg or higher. The higher levels associated with these brands may be more conducive to delivering higher levels of circulating ethinyl estradiol which may be more

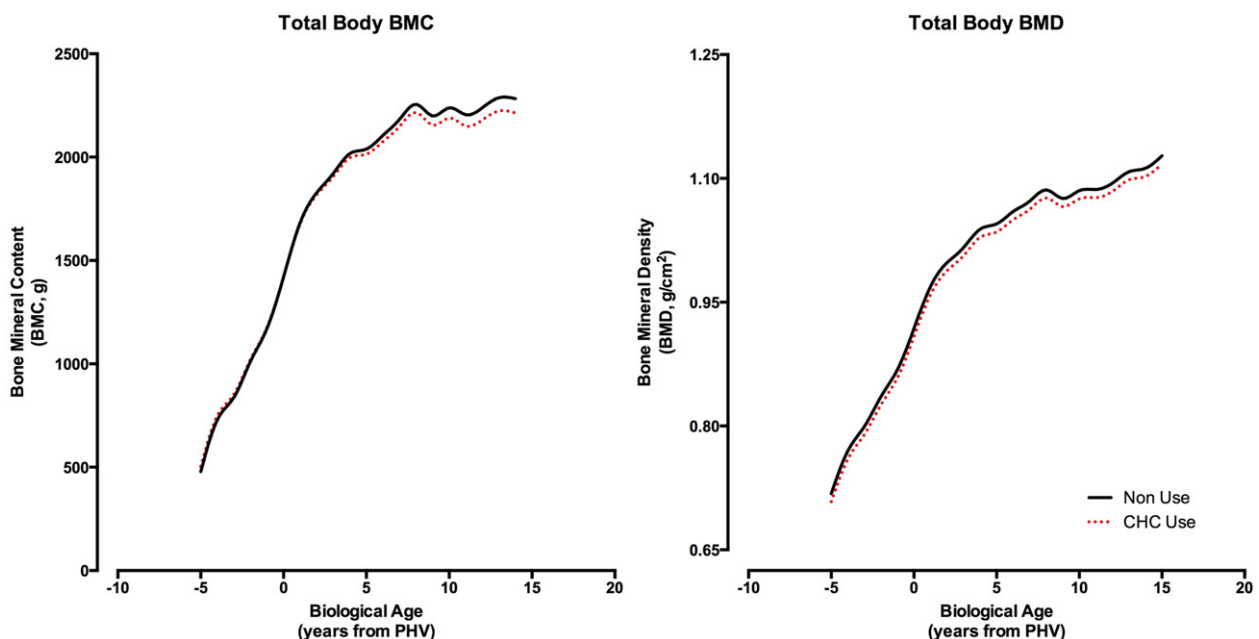


Fig. 1. Predicted total body bone mineral content (BMC) and areal bone mineral density (aBMD) between those exposed and not exposed to combined hormonal contraceptives (CHC). Predicted models adjusted for BA, height, LTM, FM, calcium intake, vitamin D intake, physical activity, duration of use, CHC use at measurement and CHC use by BA interaction.

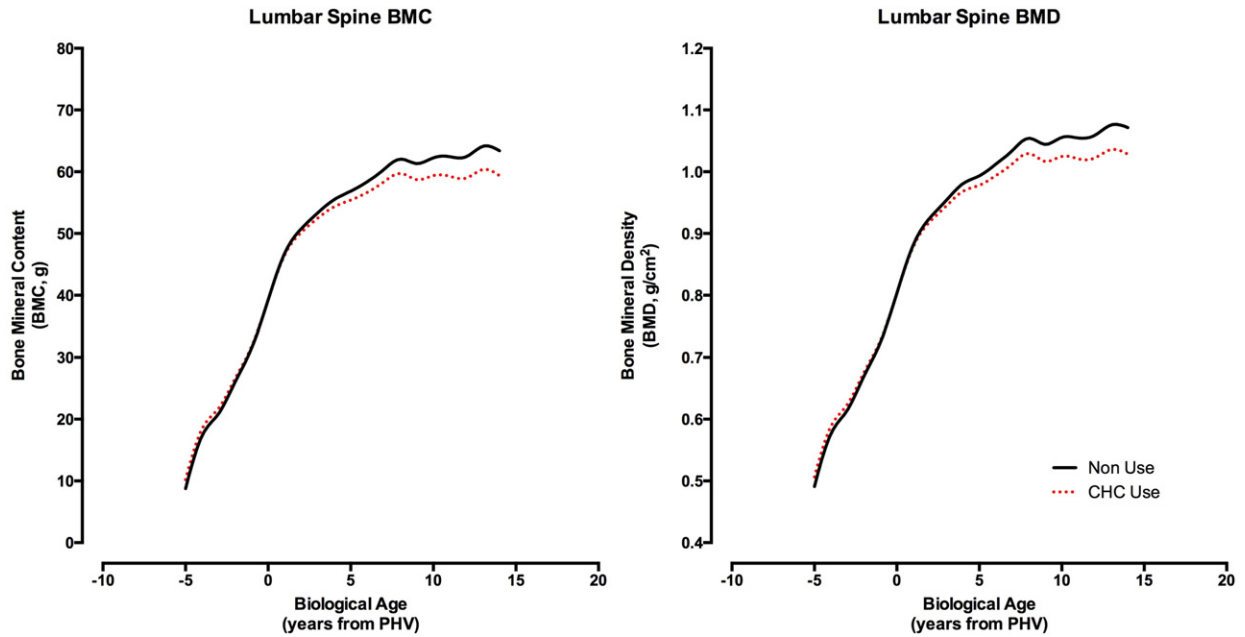


Fig. 2. Predicted lumbar spine bone mineral content (BMC) and areal bone mineral density (aBMD) between those exposed and not exposed to combined hormonal contraceptives (CHC). Predicted models adjusted for BA, height, LTM, FM, calcium intake, vitamin D intake, physical activity, duration of use, CHC use at measurement and CHC use by BA interaction.

favorable to adolescent bone development. This conjecture is supported by Cromer and colleagues' earlier studies which employed a higher dosage (30 µg/150 µg) and observed that aBMD at the lumbar spine significantly increased after 1 year, and that these increases were not significantly different between controls and CHC users (Cromer et al., 1996). It is important to recognize that CHC pill formulations have undergone a reduction in the level of synthetic ethinyl estradiol due to the documented associations with increased thromboembolic events. It appears that it is possible that these new lower dose formulations may reduce the risk of thromboembolisms, but may also lead to reduced bone mass accrual if taken during adolescence. Further research is

required to address the optimal dosage that may benefit future cancer risk, while maintaining optimal bone mass acquisition, given the adolescent reliance on CHC as a method of contraception.

In contrast to the adolescent findings, exposure to CHC resulted in lower levels of BMC and aBMD development when consumed 5 years after PHV is attained, with individuals exposed to CHC having 1–3% less TB BMC, 2–7% less LS BMC and 2–5% less LS aBMD compared to those not exposed to CHC. This 5 year PHV period coincides with the cessation of linear growth and the occurrence of peak bone mass (Bailey, 1997; Bailey et al., 1999) and may explain these unique age by exposure interactions. During growth, bone adaptation occurs primarily

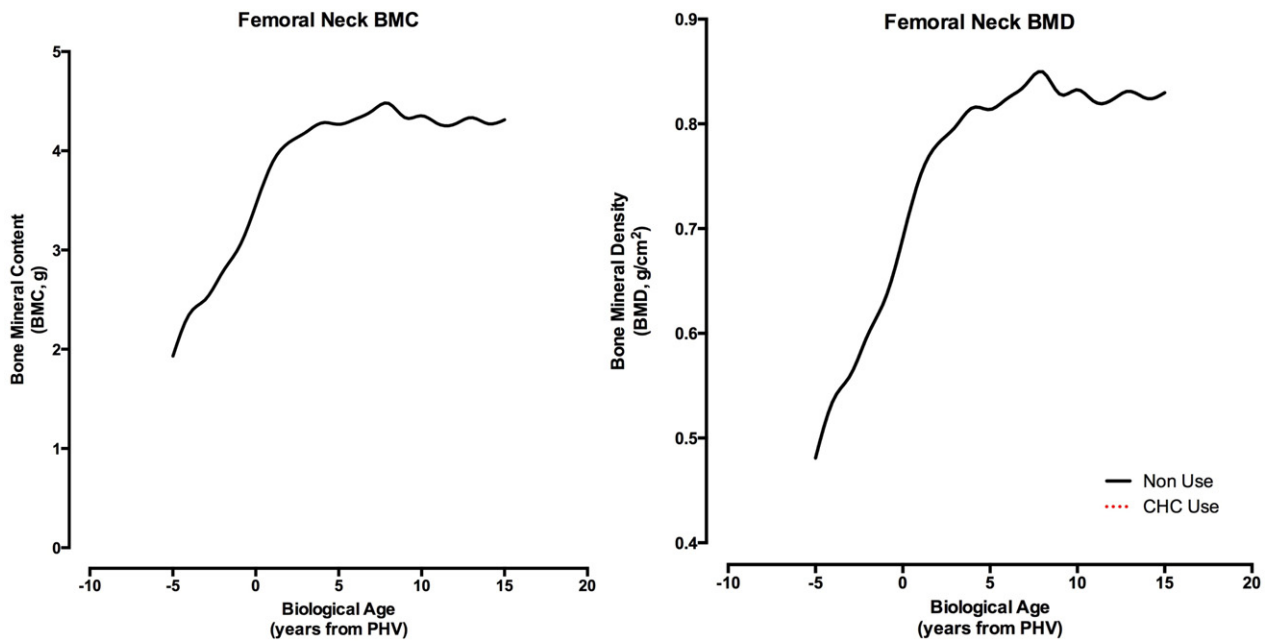


Fig. 3. Predicted femoral neck bone mineral content (BMC) and areal bone mineral density (aBMD) between those exposed and not exposed to combined hormonal contraceptives (CHC). Predicted models adjusted for BA, height, LTM, FM, calcium intake, vitamin D intake, physical activity, duration of use, CHC use at measurement and CHC use by BA interaction. Note: no difference between groups so lines overlap.

through the process of bone modeling, where there is an addition of bone through the independent actions of osteoblasts and osteoclasts. Here, elevated ovarian hormone levels may reduce osteoclastic bone resorption, but the bone acquisition is not hindered due to the independent actions of the osteoblasts. In adulthood, osteoblast and osteoclast activity are tightly coupled through the process of bone remodeling. During this period, the elevated ovarian hormone levels may continue to suppress osteoclastic activity, but because of the coupled process, there may also be a reduction in osteoblastic proliferation and bone formation. Thus, the exposure to CHC after the cessation of growth may continue to provide a hyperestrogenic environment, but this is now hindering bone mass acquisition as a result of a compromised bone remodeling process. Additionally, ethinyl estradiol may influence the binding affinity of ability of androgen receptors, further limiting bone mass acquisition during this period. These observations contradict previous studies that largely document no significant differences in bone mass measures in CHC users during young adulthood. Although discrepancies in dosage may be attributed to these conflicting findings, there are a number of studies that report ultra-low dose, low-dose and CHC doses equivalent to those reported by the present cohort to have no significant effect on total body, lumbar spine, femoral neck and heel aBMD (Polatti et al., 1995; Nappi et al., 2003; Nappi et al., 2005). Alternatively, the conflicting results may be due to the duration of use and the follow-up interval. Polatti et al. (Polatti et al., 1995) observed that the aBMD at the spine was significantly different between OC users and controls between 19–22 years of age after a 5 year follow-up, with non-users gaining 7.8% more aBMD. In the present study, the duration of use was not significant in the models, but individuals exposed to CHC had reported, on average, 5 years of CHC use (Table 2) and initiated use as early as 14 years of age (Table 1). This length of exposure is congruent with Polatti et al. (Polatti et al., 1995), though the earlier age of initiation may explain the reduced losses (2–5% vs. 7.8%) compared to those previously reported.

This study provides novel insights into the relationship between CHC exposure and the development of bone mass, but the conclusions are limited by a number of factors. First, this study was derived from observational data and therefore susceptible to uncontrolled variables, selection bias and reverse causality. The observational nature of this study is also limited by the assessment and control of CHC type and formulations used. Even though all types of CHC formulations were included in this study, all non-oral hormonal contraceptives were excluded, and most users switched brands and formulations throughout the duration of the study. Thus, the present study is unable to assess or speculate on any benefits associated with any particular type of hormone based contraceptive and/or formulation. Similarly, the reason for taking CHC was not identified and may have influenced the bone mineral accrual if CHC usage was prescribed for conditions known to alter bone mineral accrual (e.g. amenorrhea). Finally, CHC exposure was determined prospectively for the majority of the cohort, however, in some participants, initiation of CHC use and exposure was determined retrospectively, and then followed prospectively. Future studies should consider using medical records to confirm CHC initiation if assessed by retrospective questionnaires. Despite these limitations, the present findings provide novel insight into the development of BMC and aBMD from adolescence to adulthood for individuals exposed and not exposed to CHC use.

5. Conclusions

In conclusion, there is conflicting evidence of the associations between CHC exposure and bone mass development throughout life. Although the results of this study are not definitive, they do suggest that the exposure to CHC may be detrimental to the development of BMC and aBMD in females after the cessation of growth has occurred; however, they do not appear to be detrimental to bone

mass development during the adolescence period and may even have a positive effect.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.bonr.2015.06.001>.

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