The association between breastfeeding, maternal smoking in utero and birth weight with bone mass and fractures in adolescents: A 16 year longitudinal study.

Running Title: Breastfeeding predicts bone mass and fractures in adolescents.

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ABSTRACT

Summary The aim of this birth cohort study was to determine whether early life factors (birth-weight, breast-feeding and maternal smoking) were associated with bone mass and fractures in 16-year old adolescents. The results suggest that breastfeeding is associated with higher bone mass and lower fracture risk at age 16 but not in utero smoking or birthweight.

Introduction There is limited data on early life influences on bone mass in adolescence but we have previously reported in utero smoking, breastfeeding and birth weight were associated with bone mass at age 8.

Methods Birth-weight, breastfeeding intention and habit, and maternal smoking during pregnancy were assessed at phase one in 1988-9 and by recall during phase two in 1996-7. Bone mineral density (BMD) was measured by dual-energy X-ray densitometry. Fractures were assessed by questionnaire. Subjects included 415 male and female adolescents from Southern Tasmania representing 29% of those who originally took part in a birth cohort study in 1988 and 1989.

Results Breastfeeding (assessed in a number of ways) was associated with a 2-3% increase in BMD at all sites apart from the radius and around a one third reduction in fracture risk which persisted after adjustment for confounders. In univariate analysis, birth weight was associated with BMD at the hip, radius and total body but this did not persist in multivariate analysis and there was no association with fracture. Smoking in utero had no association with BMD at any site or fracture.

Conclusions Breastfeeding,, , is associated with a beneficial increase in bone mass at age sixteen and a reduction in fracture risk during adolescence. The association previously observed at age 8 years is no longer present for birth weight or smoking in utero.

INTRODUCTION

Fractures are a major public health problem in males as well as females [1]. Bone density is one of the major predictors of these osteoporotic fractures [2,3] in the elderly and is the result of the amount of bone gained in early life (i.e peak bone mass) and subsequent bone loss [4]. Physical activity and, to a lesser extent, diet (particularly calcium intake) during adolescence and early adulthood have been implicated as determinants of peak bone mass [5,6]. However, the vast majority of adult bone mass is attained before age 14 [7]. In recent years, evidence has accumulated in support of the Barker hypothesis [8] for bone development for breastfeeding, smoking in utero and birthweight.

There are limited data for mode of feeding in early postnatal life. There is controversy about short term effects with most studies showing a deficit in bone mass in breast milk versus formula fed infants [9-11] and one showing no effect [12] with evidence suggestive of a catch up phase by 2 years in one of these cohorts [9]. These studies have been restricted to pre-term infants and cannot be generalised to term infants as unsupplemented breast milk may not fully meet the mineralisation requirements of pre-term infants [13]. In a long term study of pre-term infants, those supplemented with banked donor breast milk for the first four weeks of life (regardless of type of infant feeding), had improved bone mineralisation at the radius up to age 5 years [14] but this did not persist at age 20 although there was an association between the percentage of breast milk in the diet and whole body BMD [15].

There is less data in healthy children. Harvey et al reported no association at age 4 [16] and we have previously reported a beneficial association of breastfeeding for both bone mass [17 and fractures [18] up to age 8 in children especially in those born at term. More recent studies on breastfeeding and bone mass have had expanded the evidence base. In Copenhagen, duration of breastfeeding was associated with a number of bone variables at age 17 [19] while in Finland, breastfeeding was associated with bone mass at age 32 in males but not females [20].

Smoking in utero has consistently been associated with lower neonatal bone mass [21], and prepubertal bone mass at age 8 [22,23] but not fractures [24]. Similarly, birthweight has been consistently associated with bone mass at different ages [25,26] but again not fracture suggesting this may be through increased bone size rather than density. In the studies quoted above from our centre, breastfeeding, smoking in utero and birth weight were all associated with bone mass at age 8 years. Whether these associations persist through adolescence in healthy children is uncertain.

The aim of this study, therefore, was to use a birth cohort study to study associations between breastfeeding, birth-weight and smoking during pregnancy with bone mass and incident fracture in 16 year old male and female adolescents.

METHODS

Subjects

A total of 415 adolescents (150 girls and 265 boys), with a mean age 16.3 years, were examined. These subjects were part of an original cohort of 1443 individuals, born in 1988-89 in southern Tasmania, Australia, who participated in an investigation into Sudden Infant Death Syndrome and were selected based on a risk score for this outcome [27]. In brief, children were selected on the basis of a cutoff level in a composite risk score comprised of their birth weight, season of birth, gender, maternal age, duration of the second stage of labor and maternal intention to breastfeed. They represented 96.3% (1443/1498) of babies eligible to participate. Participants in the original cohort were eligible to be part of this follow-up study if they had participated in one of two earlier follow-up studies conducted in 1996 (1988 birth cohort) and in 1997 (1989 birth cohort), when they were aged 8 years. A total of 889 children participated in the 1996 (n=442) and 1997 (n=447) follow-ups, this being 61.6% (889/1443) of those eligible from the birth cohort. Of these, 415 went on to take part in the current study (29% of the original birth cohort). Informed consent was obtained from all participants and a parent/guardian. Ethical approval was obtained from the University of Tasmania Human Research Ethics Committee.

Relevant Early Life Measurements

Birth-weight, Gestation and other birth measures.

Measurements routinely collected by hospital staff, including birth-weight, length of gestation, duration of each stage of labour, placental weight, head circumference, length, and sex, were obtained from the child's medical record.

Breastfeeding

Intention to breastfed was measured by an administered post-natal questionnaire. Actual breastfeeding was assessed by questionnaire administered face-to-face when the children were approximately 1 month of age and by telephone questionnaire when the children were approximately 3 months of age. In addition, when the children were aged 8 years, a questionnaire administered to the mothers asked them to recall whether and how long they had breastfed their children.

Maternal Smoking

Maternal smoking was measured by an administered post-natal questionnaire while mother and baby were in hospital. Mothers were asked to recall for each trimester (1st trimester 0-13 weeks; 2nd trimester 14-27 weeks, and; 3rd trimester 28-40 weeks) "How much did you smoke during your pregnancy?" The five categories for each trimester were: nil, 1-10, 11-20, 21-40 and 41+ per day. Smoking for each trimester and for the whole pregnancy (ie trimesters 1,2 and 3 combined) was further classified as either yes or no.

Measurements at age 16 years (2004-05)

Bone Density

Dual X-ray Absorptiometry (DXA) measures were performed at hip, spine, radius and total body using a Hologic Delphi densitometer or array setting (Hologic, Waltham, MA). The CV over time in our hands (assessed by daily phantom measurements during the course of this study) was under 1%.

Fracture

A questionnaire provided self-report of previous fracture history with details provided by parents where required with confirmation from medical records. Age at fracture, location of fracture, and circumstances were recorded.

Other Measures

Height was recorded using a stadiometer (Leicester Height measure) following a standard protocol and recorded to the nearest 1mm. Weight was measured using electronic scales in subjects with shoes removed and recorded to the nearest 0.1 kg.

Data analysis

A combination of univariable and multivariable linear modeling techniques were used to assess the relationship between the early life factors, bone density and fracture. Each variable was examined in three ways. Firstly, in a univariate model, then in a model adjusted for age, sex, height and weight at age 16 and selection factors (except where the selection factor was the variable of interest) then in a further model adjusting for all these factors and socioeconomic factors. A p value less than 0.05 (two tailed) was regarded as statistically significant. The statistical program Stata (Intercooled Stata 9.2, StataCorp, Texas, USA) was used for all analyses.

RESULTS

A total of 415 subjects (150 girls and 265 boys) participated in the study. Study factors of interest stratified by breastfeeding status are presented in Table 1. As expected due to the selection factors, breastfed children had a lower birthweight (with subsequent greater weight gain), higher maternal age, lower smoking and higher maternal education. They also had higher bone mass at the spine, total body and hip but not radius and a trend to lower sunlight hours and fractures.

Bone Mineral Density

Birth-weight and Bone Mineral Density

In correlation analysis birth-weight was positively associated with BMD at the hip (ρ = 0.15, p=0.002), spine (ρ = 0.13, p=0.010), radius (ρ = 0.11, p=0.033), and total body (ρ = 0.13, p=0.010). However, no significant associations between birth-weight and BMD at any site remained after adjustment for sex, current height, weight, and age, and selection factors in multivariable analysis (Table 2).

Breastfeeding and Bone Mineral Density

Breastfeeding was examined in a number of ways (table 2). In multivariable analysis, after adjustment for current height, weight, and age, and all selection factors (except intention to breastfeed), recall of breastfeeding was significantly associated with BMD at the spine, hip and total body but not radius; breastfeeding at one month was associated with spine and total body while breastfeeding at 3 months was not significantly associated at any site. Of note, intention to breastfeed had lower coefficients than the one month and ever breastfed variables and was significant only at the hip. Examination of the duration of breast-feeding for total body BMD revealed that the association was most consistent in subjects breast-fed for 25 days or more at the spine but was also significant at the total body in those breastfed for less than 25 days (Figure 1). In contrast to our earlier report where these associations were only observed in children born at or after 37 weeks, the length of gestation was not found to be an effect modifier at any site. Further adjustment for paternal unemployment and maternal education at the time of the child's birth for the two main breastfeeding variables led to a general weakening of associations but three remained significant (Table 2).

Maternal smoking and Bone Mineral Density

The effect of smoking on bone mineral density was examined by trimester and across the whole pregnancy. No associations between smoking, at any stage during pregnancy, were found with BMD at any site (Table 2) although female children whose mothers smoked in utero were shorter (Table 3).

Fractures (Table 4)

The mean age of fracture was 10.4 years (SD 4). The relative risk of fracture among adolescents who had low birth weight defined according to standards when this study commenced (<=2500g) was not significantly different from those with a normal birth weight (>2500g). Length of gestation was found not to be an effect modifier (data not shown).

Breastfeeding (intention, one month and recall but not 3 months) was associated with a significant reduction in fracture which persisted in multivariate analysis. No consistent associations were observed for fracture location but all had an OR less than one. Further adjustment for spine BMD, paternal unemployment and maternal education led to little change (eg breastfeeding at one month OR 0.67 95% CI 0.47, 0.95).

None of the measures of smoking (ie smoking in 1st trimester, smoking in 2nd trimester, smoking in 3rd trimester, or smoking in any trimester) predicted fracture when the subjects were aged 16 years.

DISCUSSION

In 2000 our research group demonstrated an association between breastfeeding in early life and bone mass in a cohort of 8-year old children [17]. Children who were breastfed had significantly higher BMD at the femoral neck, lumbar spine and total body compared with children who were not breastfed. This association was found in children born at term, but not in those born before 37 weeks gestation.

This 16-year longitudinal study reports a beneficial association between breastfeeding in early life and bone mass in adolescents. This effect was most marked in those breast fed for 25 days or more. These associations have persisted into adolescence and seem to be larger than at age 8 when they were only significant in children born at term although the optimal duration at age 8 appeared to be around 3 months. A number of breastfeeding variables were examined. The most consistent associations were for breastfeeding at one month and maternal recall but not 3 months. The lack of latter association is perplexing but may reflect the incomplete data at the 3 month time point and the data from the figure are based on recall of duration in the whole cohort which would seem more robust given there was strong agreement between breastfeeding recall at age 8 and actual breastfeeding [19]. Intention to breastfeed was associated with a consistently smaller effect than actual breastfeeding supporting a biological effect. However, there wasn't a significant association with duration which supports a critical period of exposure rather than duration of exposure. Furthermore, adjustment for paternal unemployment and maternal education weakened these associations even though neither variable was itself associated with bone mass. This suggests these factors are directly associated with the decision to breastfeed rather than acting as confounders. We cant comment on whether it is exclusive breastfeeding as all were exclusive at this timepoint but further adjustment for timing of introduction of solids did not change these associations. This association extended to a reduction in fractures which was independent of all factors including bone mass (either spine or total body). The implication is that breastfeeding in early life, even for a short period, may program bone development and thus increase peak bone mass and protect against fracture. These results are broadly consistent with the other long term studies all of which suggest breastfeeding has a long term influence on bone mass in both preterm and healthy children. However, there is limited other data on fractures apart from our earlier report in 8 year old children [18]. The fracture data were only significant for all fractures. However, all ORs for upper limb fractures were less than one which is consistent with the overall results and most likely reflects sample size issues (106 upper limb fractures versus a weakly significant result when all 160 fractures were included). There were smaller numbers of fractures by specific type in the upper limb and none of these were significant thus larger studies will be needed to further examine fracture subtypes given that BMD is most strongly associated with wrist and forearm fractures [29,30].

Maternal smoking during pregnancy was associated with decreased height especially in girls at age 16 but we found no association with maternal smoking and BMD at any site. When measured at age 8, smoking by the mother during pregnancy was associated with a deficit in bone mass in children born at term [22]. The total attenuation of these results in the same cohort of children suggests that the smoking effect on bone is transient and disappears possibly during puberty. Other studies to date have only reported on prepubertal children [21,23] so it will be important to see if this loss of association is confirmed in other studies.

The literature suggests that birth weight makes a modest contribution to bone mass (most commonly bone mineral content) and fracture in later life [25]. In this study, we report a much weaker association with bone mass than we did at age eight [26] which was completely abolished by further adjustment most notably for body size. A potential explanation is that the relative contribution of birth weight decreases over time especially as the relative magnitude of growth after birth increases. Further, there was no suggestion of any association with fractures during puberty consistent with New Zealand data [24].

This study has a number of potential limitations. The children who took part in this study are not representative of Tasmanian children. They were originally selected on the basis of having a higher risk of sudden infant death syndrome [27]. As a result, there was a higher proportion of males, premature babies, teenage mothers and smoking during pregnancy. These findings suggest that this cohort is of lower socio-economic status than the Tasmanian population as a whole. Over time, those who dropped out were less likely to have been breastfed and more likely to have mothers who smoked suggesting this bias may be decreasing over time. According to Miettinen [28], an analytical cohort study, to be generalisable to other populations does not have to be representative of the community from which it was selected provided it meets the following key criteria with regard to definition of eligible participants, sample size and a proper distribution of determinants, modifiers and confounders. This study fulfils all three criteria. The study population was explicitly defined and is of adequate sample size. Furthermore, it has considerable variability in bone mass. In addition, measurement error may have affected our results.

However, all bone measures were highly reproducible. Fractures may have been misreported but were confirmed by reference to medical records. Smoking may have been misreported and has not been validated against cotinine levels in this cohort but the same data were used for the analyses at both age 8 years and 16 years and the loss of subsequent association is the most noteworthy finding for smoking.

In conclusion, breastfeeding in early life, but not birth weight or smoking in utero, is associated with a beneficial increase in bone mass at age sixteen and a reduction in fracture risk during adolescence.

Disclosures

All authors have no conflict of interest.

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Authors' roles: Study design GJ, TD. Study conduct, data collection and analysis GJ, KH. Data interpretation, manuscript drafting, revision and final approval GJ, KH, TD. GJ takes responsibility for the integrity of the data analysis.

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Table 1. Characteristics of study participants

	Breastfeeding Status[1]		
	Never (n=172)	Ever (n=233)	P-value
EARLY LIFE MEASURES			
Birth-weight (g)	3191 (769)	2892 (812)	<0.01
Low birth weight (<=2500g)	18%	36%	<0.01
Weight at one month (g)	4328 (754)	4185 (816)	0.09
Weight gain in first month (g)	1146 (568)	1292 (671)	0.03
Preterm births (<37 weeks)	16%	30%	<0.01
Percentage male	61%	65%	0.39
Maternal age (years) at birth of child	24.1 (5.0)	26.2 (4.6)	<0.01
Maternal smoking (in any trimester)	59%	36%	<0.01
Maternal education to ≥ year 12	12%	37%	<0.01
Paternal unemployment	26%	5%	<0.01
Age solids introduced (weeks)	13.7 (5.9)	13.7 (5.9)	0.98
Maternal calcium intake during pregnancy (mg/day)	1843 (1482)	1704 (824)	0.28
AGE 16 MEASURES			
Age (years)	16.3 (0.5)	16.3 (0.4)	0.14
Height (cm)	169.4 (9.0)	170.8 (8.5)	0.12
Weight (kg)	68.5 (14.8)	66.4 (13.8)	0.14
Sports participation (%)	71%	73%	0.58
Sunlight exposure (hours)	2.5 (1.0)	2.3 (1.0)	0.07
Calcium intake (mg/day)	1087.2 (545.2)	1083.5 (446.0)	0.94
Bone mineral density (g/cm^2)			
Spine	0.95 (0.12)	0.97 (0.11)	0.02
Hip	0.99 (0.13)	1.02 (0.13)	0.06
Radius	0.54 (0.06)	0.54 (0.06)	0.52
Total Body	1.03 (0.09)	1.06 (0.09)	<0.01
Fractures (one or more)	44%	35%	0.09

*According to maternal recall (when child was aged 8 years) of having ever breastfed
(Results are mean ± SD unless stated)

Table 2. Multivariate associations between early life factors and bone mass [1].

Study Factor	Spine β (95% CI)	Hip β (95% CI)	Radius β (95% CI)	Total Body β (95% CI)
Birth-weight (g)	-0.002 (-0.014, +0.018)	+0.010 (-0.007, +0.027)	-0.004 (-0.018, +0.011)	-0.002 (-0.009, +0.014)
Breastfeeding variables (y v no)				
Intention to breastfeed [2]	+2.41% (-0.08, +4.96)	**+2.69% (+0.15, +5.29)**	+0.47% (-1.61, +2.59)	+2.67% (+0.99, +4.38)
Breast-feeding at one month [3]	**+2.96% (+0.21, +5.78)**	+2.92% (-0.02, +5.93%)	+1.32% (-1.00, +3.64%)	**+3.30% (+1.35, +5.30%)**
Breast-feeding at one month SE model[4]	+2.33% (-0.22, +4.95%)	+2.21% (-0.41, +4.90%)	-0.12% (-2.27, +2.07%)	**+2.58% (+0.82, +4.37%)**
Breast-feeding at 3 months [5]	+1.83% (-0.71, +4.43%)	+0.97% (-1.61, +3.62%)	-0.55% (-2.67, +1.62%)	+1.21% (-0.48, +2.93%)
Maternal recall of breast-feeding[6]	**+3.42% (+0.10, +5.90)**	**+2.84% (+0.38, +5.36)**	+0.54% (-1.47, +2.59%)	**+2.85% (+1.22, +4.51%)**
Maternal recall of breast-feeding SE model[4]	+1.65% (-1.02, +4.39%)	**+2.78% (+0.01, +5.63%)**	+0.58% (-0.70, +2.90%)	**+2.93% (+1.11, +4.78%)**
Smoking in utero (y v n)[7]	+0.002 (-0.02, +0.03)	-0.005 (-0.03, +0.02)	+0.010 (-0.01, +0.03)	-0.005 (-0.01, +0.02)

1. Adjusted for current height, weight and age, sex and all selection factors except birth-weight.
2. Intention to breastfeed indicated during hospital interview after birth of child.
3. Breastfeeding assessment at home interview when children aged on average 43 days old
4. Adjusted for maternal education and paternal unemployment when child was born
5. Breastfeeding assessment at telephone interview when children aged on average 94 days old
6. Mother's recall of having breastfed when child was aged ~8 years old.
7. Smoking during any trimester.

Table 3: Changes in height (cm) and weight (kg) among adolescents at age 16 years whose mothers smoked (during any trimester).

	All subjects	Females	Males
Height (cm)			
Unadjusted	**-1.82 (-3.52, -0.13)**	**-2.40 (-4.41, -0.40)**	-0.48 (-1.99, +1.03)
Adjusted[1]	-1.38 (-2.98, 0.22)	**-2.25 (-4.22, -0.28)**	-0.10 (-1.59, 1.39)
Weight (kg)			
Unadjusted	+1.05 (-1.72, 3.82)	+3.56 (-4.58, 7.58)	+0.26 (-3.28, 3.79)
Adjusted[1]	+1.52 (-1.26, 4.29)	+3.57 (-0.55, 7.68)	+0.73 (-2.87, 4.34)

1. Adjusted for birth length and birth weight.

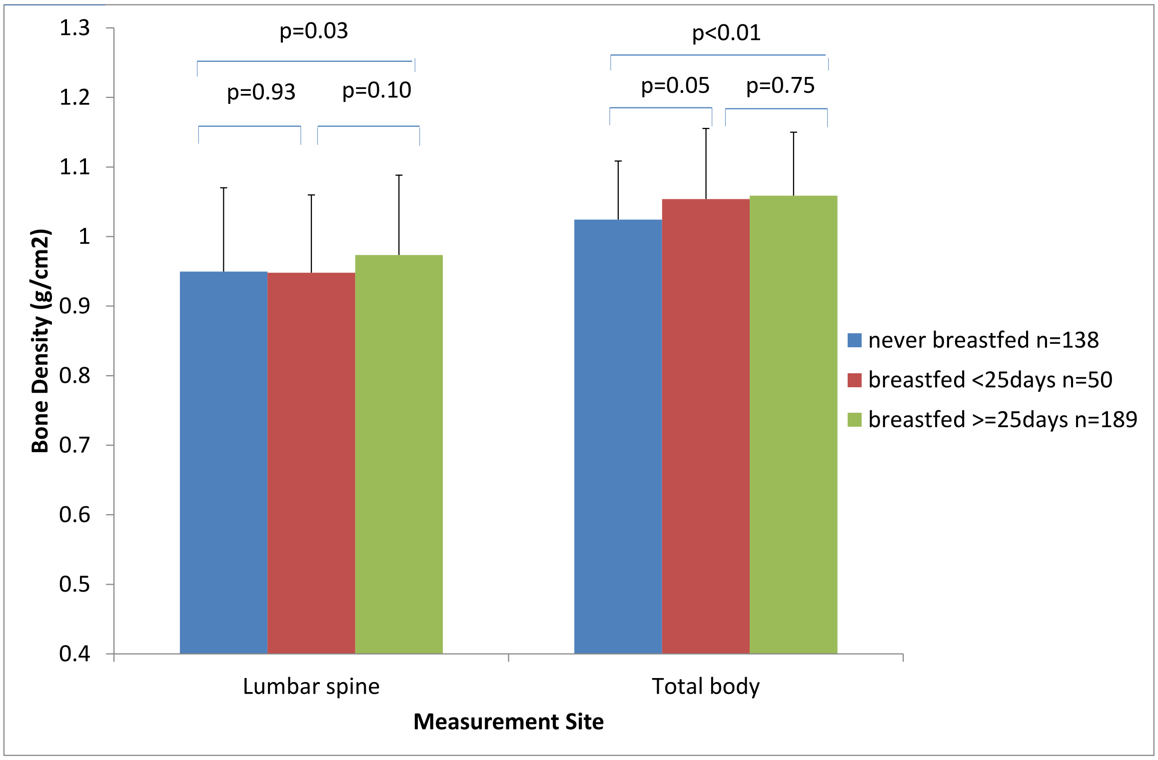
Table 4. Associations between early life factors and subsequent fractures[1].

Study Factor	Any fracture (n=160) OR (95% CI)	Upper limb fracture (n=106) OR (95% CI)	Lower limb fracture (n=52) OR (95% CI)
Birth-weight (<=2500g v>2500g)	1.05 (0.75, 1.46)	0.87 (0.55, 1.37)	1.23 (0.62, 2.44)
Breastfeeding variables			
Intention to breastfeed (y v n)	**0.71 (0.55, 0.94)**	0.80 (0.55, 1.16)	0.63 (0.36, 1.13)
Breast-feeding at 1 month (y v n)	**0.65 (0.48, 0.87)**	0.84 (0.54, 1.73)	**0.49 (0.26, 0.93)**
Breast-feeding at 3 months (y v n)	0.80 (0.60, 1.09)	0.87 (0.58, 1.31)	0.82 (0.45, 1.48)
Maternal recall of BF (y v n)	**0.69 (0.54, 0.89)**	0.73 (0.51, 1.39)	0.75 (0.42, 1.34)
Smoking in utero (y v n)	0.89 (0.67, 1.17)	0.92 (0.64, 1.34)	1.36 (0.75, 2.47)

1. Adjusted for current height, weight and age, sex and all selection factors, except birth-weight (when birth-weight is the study factor) and intention to breastfeed (when breast feeding is the study factor). Bold indicates statistical significance.

Figure legend

Figure 1: The association between duration of breastfeeding and bone mass at the spine and total body (date are mean plus SD).



1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
Bone Density (g/cm2)
p=0.03
p=0.93
p=0.10
p<0.01
p=0.05
p=0.75
never breastfed n=138
breastfed <25days n=50
breastfed >=25days n=189
Lumbar spine
Total body
Measurement Site