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# The Shifting Trajectory of Growth in Femur Length During Gestation

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## ABSTRACT

Bone size is a determinant of bone strength and tracks in its percentile of origin during childhood and adolescence. We hypothesized that the ranking of an individual's femur length (FL) is established in early gestation and tracks thereafter. Fetal FL was measured serially using 2D ultrasound in 625 Norwegian fetuses. Tracking was assessed using Pearson correlation, a generalized estimating equation model, and by calculating the proportion of fetuses whose FL remained within the same quartile. Baseline FL *Z*-score (weeks 10 to 19) and later measurements correlated, but more weakly as gestation advanced: $r = 0.59$ (weeks 20 to 26); $r = 0.45$ (weeks 27 to 33); and $r = 0.32$ (weeks 34 to 39) ($p < 0.001$). Tracking within the same quartile throughout gestation occurred in 13% of fetuses. Of the 87% deviating, 21% returned to the quartile of origin, so 34% began and ended in the same quartile, 38% deviated by one quartile, and 28% deviated by two or more quartiles by the end of gestation. A standard deviation higher baseline FL *Z*-score, placental weight (150 g), maternal height (5 cm), and weight (10 kg), was associated with a 0.25, 0.15, 0.10, and 0.05 SD higher FL *Z*-score at the end of gestation, respectively ($p$ ranging from $<0.001$ to 0.02). Tracking within the same percentile throughout the whole of gestation, as suggest by growth charts, is uncommon. Deviation from tracking is more common and is the result of changes in growth velocity within and between fetuses and is partly influenced by maternal, fetal, and placental factors. © 2010 American Society for Bone and Mineral Research.

**KEY WORDS:** FETAL GROWTH; TRACKING; COHORT STUDY; OSTEOPOROSIS; ULTRASOUND

## Introduction

Bone strength in old age is the net result of the peak structural strength achieved during growth and the strength lost during aging by structural decay.[1] Since variance in bone size, mass, and architecture in young adulthood is an order of magnitude greater than the variance in rates of bone loss during aging, the position of an individual's bone size and mass in the population distribution in young adulthood is likely to influence fracture risk in old age.[2–6] Thus, understanding genetic and environmental factors influencing the position of an individual's bone trait relative to others in the population distribution during growth is likely to provide insights into the pathogenesis of bone fragility.

Bone size and mass are larger in adults than in children, but the variance or dispersion around the mean value in these traits is similar.[7,8] Thus trait variances in adulthood are likely to be established during growth and probably before puberty.[9,10] Variance in a trait (eg, bone size) may be established during the first weeks of organogenesis, by differences in growth rates during gestation such that individuals growing rapidly achieve bone size in the upper tertile of a population distribution, whereas those growing slowly develop bone size in the middle and lower tertile or by a combination of differences in the starting value and growth rates.

There is a great deal of evidence that bone traits track in their percentile location during childhood and adolescence.[7,8,11,12] However, whether tracking occurs during intrauterine growth or during the first years of life is controversial.[13–19] If traits track from early gestation, the trait variance at term will be accounted for largely by differences in the starting value, and an individual's trait location is likely to be genetically determined. If differences in growth velocity establish the trait location at term, then tracking is unlikely. We assessed whether femur length (FL) tracked during intrauterine life in a prospective study of 625 fetuses and whether maternal and fetal factors influenced

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deviation from tracking. We hypothesized that FL tracks in its percentile of origin so that the ranking of an individual's FL relative to others at the end of gestation is established early in gestation.

## Methods

Healthy women with a normal single fetus were recruited from a low-risk antenatal clinic in Bergen, Norway, during 2001 and 2003. The regional Committee of Research Ethics approved the study. All participants gave written informed consent. Inclusion criterion was certain information of a regular last menstrual period (LMP, $28 \pm 4$ days) adjusted for cycle length.[17,18] Ultrasound was not used to adjust gestational age, but if there was a discrepancy of more than 14 days between LMP dating and conventional ultrasound dating, participation was excluded. FL growth was monitored using 2D ultrasound on four to five occasions from 10 to 42 weeks of gestation in 625 women.[20]

Details of ultrasound examinations and reproducibility were published previously.[20,21] Briefly, two investigators performed the ultrasound examinations using a Philips HDI 5000 device (Seattle, WA, USA) with 2- to 5-MHz abdominal or 3- to 8-MHz vaginal scan heads or a Aloka Prosound-5000 device (Tokyo, Japan) with 2- to 5-MHz abdominal or 3- to 8-MHz vaginal scan heads. FL was obtained in a longitudinal section by placing the caliper at the ends of the diaphysis.[22] Each recorded value was a mean estimated of three measurements; the coefficient of variation (CV) was 4.8%. The reproducibility was conducted in 20 women at gestational weeks 12 to 31 for repeated examination on the same day. The intraobserver and interobserver variations were studied for FL and found to be of the same small magnitude.[20]

FL was normally distributed. We expressed variance as standard deviation $(SD)^2$ and as a CV (SD/mean $\times$ 100). FL variance was estimated based on FL measurements on five occasions in gestational weeks 10 to 19 ($n = 619$), weeks 20 to 26 ($n = 540$), weeks 27 to 33 ($n = 538$), weeks 34 to 39 ($n = 526$), and weeks 40 to 42 ($n = 105$). Tracking was assessed in 412 fetuses with four repeated measurements done on the first four occasions after the following exclusion from the original group of 625 fetuses. We excluded fetuses that had FL measurements at overlapping gestational age: first scan in week 20 ($n = 3$), second scan before week 20 or after week 26 ($n = 93$), third scan before week 27 or after week 33 ($n = 89$), or fourth scan before week 34 or after week 39 ($n = 35$), and 18 fetuses missing FL; numbers are overlapping, not additive. Fetuses with chromosomal abnormalities and bone dysplasias were excluded. We excluded neonates delivered before gestational week 37.

We used a random coefficients model to construct a conditional reference interval for FL to obtain its percentile distribution. For a fetus $i$, let $Y_{ij}$ be the FL and $T_j$ be the gestational age at scan time $j$. $Y_{ij}$ was assumed to be determined by a baseline value $\alpha_i$, a trajectory $\beta i$, and $\gamma_i$ associated with gestational age: $Y_{ij} = \alpha_i + \beta_i T_{ij} + \gamma_i T_{ij}^2 + \varepsilon_{ij}$, where $\varepsilon_{ij}$ reflects the within-fetus variability in FL. Under these assumptions, the mean FL is predicted by the equation $\mu_{ij} = \alpha_i + \beta_i T_{ij} + \gamma_i T_{ij}^2$ with variance $\sigma^2 + \sigma_\alpha^2 + \sigma_\beta^2 T^2 + \sigma_\gamma^2 T^4 + 2T\sigma_{\alpha\beta} + 2T^2\sigma_{\alpha\gamma} + 2T^3\sigma_{\beta\gamma} + \sigma_e^2$.

The $Z$-score for any gestational age then was determined as $z = (Y_{ij} - \mu_{ij})/\sigma$. To assess tracking, we classified the FL $Z$-score into quartiles at each scan time. We inferred that tracking occurred if an individual's FL remained within their quartile from the first through the second, third, and fourth scans. Concordance in FL quartiles between time points was assessed by the $\kappa$ coefficient. In addition, tracking was assessed including baseline FL $Z$-score in the generalized estimating equation (GEE) model. The standardized coefficient for baseline FL $Z$-score from the model was interpreted as the coefficient of tracking (or coefficient of stability). We analyzed the potential effects of maternal height, weight, age, parity, and smoking and of placental weight on FL $Z$-score using the GEE model. Significance level was set at $p < 0.05$. The SAS Software Version 9.1 (SAS Institute, Inc., Cary, NC, USA) was used for data analyses.

## Results

Characteristics of the participants are shown in Table 1. Mean FL increased 25-fold (from 2.9 to 74.6 mm) from 10 to 41 weeks of gestation, whereas SD increased only about threefold from 1 to 2.7 mm, so the CV decreased from 36% to 3.6% as gestation advanced (Fig. 1).

Baseline FL $Z$-score (weeks 10 to 19) correlated with subsequent FL $Z$-scores but more weakly as gestation advanced: $r = 0.59$ (weeks 20 to 26); $r = 0.45$ (weeks 27 to 33); and $r = 0.32$ (weeks 34 to 39; all $p < 0.001$), and the differences in $r$ values were significant ($p < 0.001$; Table 2). FL tracked within the same quartile throughout gestation in 13% of fetuses ($\kappa$ statistic = 0.12; 95% confidence interval 0.6–0.19; Fig. 2). Temporary or permanent deviation from tracking occurred in 87% of fetuses. However, of this 87%, 21% of fetuses had FL at the end of gestation in the same quartile as in early gestation, giving a total of 34% (13% plus 21%) of fetuses who began and ended in the same FL quartile; 38% deviated by one quartile, and 28% deviated by two or more quartiles by the end of gestation.

The coefficient of tracking of FL $Z$-score was 0.25, so each SD increment in baseline FL $Z$-score (weeks 10 to 19) was associated with a 0.25 SD higher FL $Z$-score at the end of gestation ($p < 0.001$; Table 3). A 1 SD higher placental weight (150 g) and

**Table 1.** Characteristics of Mothers and Neonates

| | Mean $\pm$ SD | Range |
|---|---|---|
| Mothers | | |
| Age (years) | $29.4 \pm 4.4$ | 17–45 |
| Height (cm) | $168.0 \pm 5.6$ | 152–184 |
| Weight (kg) | $67.8 \pm 12.9$ | 43–142 |
| BMI (kg/cm$^2$) | $24.0 \pm 4.3$ | 16.0–48.5 |
| Smoking (%) | 8.6 | |
| Nulliparous (%) | 43.7 | |
| Neonates | | |
| Birth length (cm) | $50.9 \pm 2.1$ | 44–58 |
| Birth weight (g) | $3737 \pm 516$ | 2200–5500 |
| Placental weight (g) | $682 \pm 150$ | 300–1330 |
| Female (%) | 47.1 | |

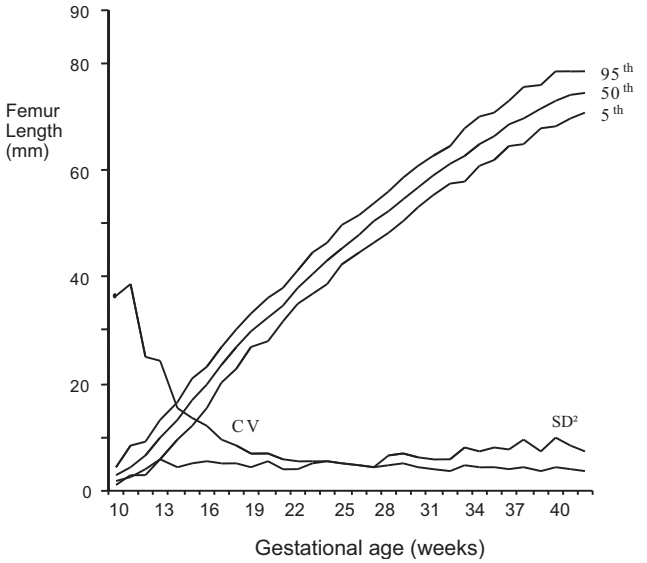


**Fig. 1.** Femur length by gestational weeks 10 to 42 presented as the 5th, 50th, and 95th percentile, variance ($SD^2$), and coefficient of variation (CV).

maternal height (5 cm) and weight (10 kg) increased the FL *Z*-score at weeks 34 to 39 by 0.15, 0.10, and 0.05 SD ($p < 0.001$, $p < 0.001$, and $p < 0.02$, respectively). Of the variance in FL *Z*-score at weeks 34 to 39, baseline FL *Z*-score accounted for 10%, placental weight 5%, maternal height 4%, and maternal weight 0.7%, with no significant effect of maternal age, smoking, or parity, leaving 80% of the variance unexplained.

## Discussion

Contrary to our hypothesis, tracking in FL occurred in only a minority of fetuses; deviation from tracking was far more common. Both FL starting size and growth rates contributed to the variance in FL in late gestation, but starting size accounted for only 10% of the variance in the final FL. Maternal and placental factors contributed to deviation from tracking.

Many cross-sectional and longitudinal studies support the view that tracking occurs during childhood and adolescence.[7,8,11,12] For example, in one study of prepubertal children, tracking in bone size and mass was reported during 3 years; an individual with a large vertebral or femoral shaft cross section or higher vertebral volumetric bone mineral density or femoral cortical area retained this relative position to maturity.[11] In another study of prepubertal girls, tracking in tibial morphology occurred during 2 years, and variance was similar to that of their premenopausal mothers.[8] Thus trait variances and the percentile location of an individual's trait are established at some time before puberty.

**Table 2.** Correlation[a] Between Femur Length Across Gestation

| | Weeks 10–19 | Weeks 20–26 | Weeks 27–33 | Weeks 34–39 |
|---|---|---|---|---|
| Weeks 10–19 | | 0.62 | 0.43 | 0.17 |
| Weeks 20–26 | 0.59 | | 0.68 | 0.34 |
| Weeks 27–33 | 0.45 | 0.69 | | 0.60 |
| Weeks 34–40 | 0.32 | 0.57 | 0.69 | |

[a]Pearson correlation coefficient, FL (in mm) to the right and FL *Z*-score to the left of the diagonal; all $p < 0.001$.

Three studies suggest that trait variances are established during the first 2 years of postnatal life. Maresh reported a longitudinal study of children aged from 6 months to 6 years. Variance in diaphyseal diameter was established at 1 to 2 years of age, but deviation from tracking was common.[23] Pietilainen and colleagues reported tracking in body size from birth to late adolescence.[16] Clayton and colleagues reported that height in adulthood correlated poorly with birth length ($r = 0.3$) but strongly with length at 3 years of age ($r = 0.8$), suggesting that the percentile location of height was established at some time between birth and 3 years of age.[17]

To the best of our knowledge, no studies have assessed tracking throughout the whole of intrauterine life. Despite this, intrauterine growth is presented graphically as a continuous function, assuming that an individual's trait (eg, FL, biparietal diameter, head and abdominal circumference) track at a given velocity relative to others from early gestation and that deviation from this percentile is indicative of abnormal growth.[15] Smith and colleagues implied that tracking occurred in utero by reporting that fetuses with smaller-than-expected crown-rump length in the first trimester had doubling of risk of a low birth weight; the absolute risk was low. However, only 38 (2.9%) of 1289 smaller-than-expected fetuses in the first trimester remained small throughout gestation, and 1251 did not, suggesting the contrary—that tracking is uncommon in utero.[18] The data presented here demonstrate that deviation from tracking occurred in about 87% of fetuses. Of this 87%, 21% returned to the quartile of origin at the end of gestation, but 66% deviated by one or more quartiles above or below the quartile of origin.

The deviation was the result of changes in growth velocity throughout gestation within a fetus and between fetuses, giving rise to changes in percentile or *Z*-score ranking at any given gestational age. Therefore, documentation of a given percentile location, or slow growth, at least for FL is not necessarily indicative of disease. We confirm the work of Cole and colleagues, who reported that ultrasound measurements at 20 and 30 weeks of gestation were poor predictors of birth size; growth charts mislead by suggesting that tracking occurs.[15,19]

The causes of differences in the tempo of growth in utero are largely undefined but are partly the result of maternal, placental, and other factors.[13,24] Studies of mouse models suggest that knockouts of specific genes, for example, the placental *Igf2* gene, reduce placental and fetal growth.[24,25] One study in monochorionic and dichorionic monozygotic twins suggests that the variance in bone mass is influenced by intrauterine environmental factors.[26] Maternal factors influence fetal growth independent of the paternal/fetal factors.[13] Maternal diet and lifestyle influence newborn size.[13,15,24,27] We confirmed that maternal height and weight increased the growth of FL. Maternal "constraint" is an important determinant of birth size.[13] For example, foal birth size follows female size in experiments crossing a female Shetland pony with a male Shire horse or a

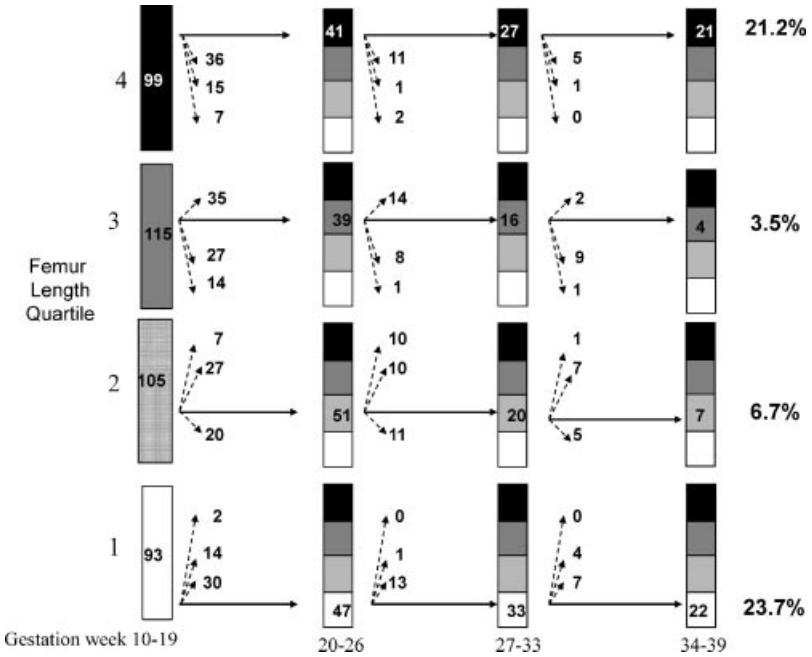


**Fig. 2.** The proportion of 412 fetuses whose FL remained within the same quartile throughout gestation was 13% ($n = 54$). Percentages shown on the right refer to the percentage tracking within a given quartile. The numbers to the right of the bars give examples of the disposition of individuals' FL from their baseline quartile location throughout gestation. The numbers are fetuses that kept their quartile (*solid line*) or deviated (*dashed line*) from quartile 1 (*white*), quartile 2 (*light gray*), quartile 3 (*dark gray*), and quartile 4 (*black*).

female Shire horse with a male Shetland pony.[28] Birth size is not necessarily "genetic." In a study of human egg donations, birth size correlated with recipient size, not donor size.[29]

A limitation of this study was that measurement error may contribute to lack of tracking, especially in the early measurements, when FL is very small. However, the ultrasound measurement of FL is a well-defined technique for assessing the length of the diaphysis of the femur and its growth, and mineralization of the diaphysis generally is good from gestational week 10 and later. Even later calculation of tracking from the second or third measurement to the fourth measurement confirmed that deviation from tracking was more common than tracking, so the idea that there is deviation from tracking is valid. In addition, the FL precision was good because the CV was below 5%.

In summary, growth-related factors regulating the attainment of peak bone strength are important because differences in structural determinants of bone strength in old age, such as bone dimensions, are almost entirely established during growth.[1–3] Rapidly growing fetuses are susceptible to environmental factors, and changes in fetal development within the normal range may have lasting effects on bone structure and long-term consequences for health and risk of disease.[4,10,13,27,30] Had there been tracking from 10 weeks such that the variance in position at term was explained by the position at 10 weeks of gestation, we would infer that the position at term was largely genetically determined. We did not find this, so we cannot infer whether genetic or environmental factors are operative in utero. This study suggests that the percentile location of a trait during gestation is highly variable, so establishment of tracking and trait ranking takes place after birth. Factors operating before birth and during the critical first 2 years of postnatal life that influence the trajectory of growth and variance remain to be established.

**Table 3.** Predictors of Femur Length (FL) *Z*-Score in Gestation Weeks 34 to 39

| Predictors[a] | Regression coefficient ± SE | *p* Value |
|---|---|---|
| Baseline FL *Z*-score | 0.254 ± 0.044 | <0.001 |
| Placental weight (150 g) | 0.145 ± 0.029 | <0.001 |
| Maternal height (5 cm) | 0.101 ± 0.028 | <0.001 |
| Maternal weight (10 kg) | 0.054 ± 0.024 | 0.02 |
| Maternal age (5 years) | 0.053 ± 0.033 | 0.11 |
| Smoking (no/yes) | −0.136 ± 0.108 | 0.21 |

[a]In general estimating equation (GEE) models.

## Disclosures

All the authors state that they have no conflicts of interest.

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