**Pregnancy-related change in pQCT and bone biochemistry in a population with a habitually low calcium intake**

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**DISCLOSURES**

The authors report no conflicts of interest.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**ABSTRACT**

In pregnancy, changes in maternal calcium (Ca) economy occur to satisfy fetal Ca demand. It is unclear whether maternal mineral reserves facilitate these requirements and no data exist from sub-Saharan Africa. The aim was to determine skeletal changes with pQCT and bone biochemistry between early second and third trimesters. Pregnant rural Gambians aged 18-45 years (n=467) participating in a trial of antenatal nutritional supplements (ISRCTN49285450) had pQCT scans and blood collections at mean(SD) 14(3) and 31(1) weeks gestation.Outcomes were pQCT: radius/tibia 4% total vBMD, trabecular vBMD, total CSA, 33%/38% radius/tibia cortical vBMD, BMC, total CSA; biochemistry: collagen type 1 cross‐linked β‐C‐telopeptide (β‐CTX), type 1 procollagen N‐terminal (P1NP), parathyroid hormone (PTH) and 1,25(OH)2D. Independent t-tests tested whether pooled or within-group changes differed from 0. Multiple regression were performed adjusting for age. Data for change are expressed as mean[CI 2.5, 97.5]%. Radius trabecular vBMD, cortical vBMD and BMC increased by 1.15 [0.55, 1.75]%, 0.41 [0.24, 0.58]%, and 0.47 [0.25, 0.69]%. Tibia total and trabecular vBMD increased by 0.34 [0.15, 0.54]% and 0.46 [0.17, 0.74]%, while tibia cortical vBMD, BMC, and cortical CSA increased by 0.35 [0.26, 0.44]%, 0.55 [0.41, 0.68]% and 0.20 [0.09, 0.31]% respectively. CTX, PTH and 1,25(OH)2D increased by 23.0 [15.09, 29.29]%, 13.2 [8.44, 19.34]%, and 21.0 [17.67, 24.29]%, while P1NP decreased by 32.4 [-37.19, -28.17]%. No evidence of mobilization was observed in the peripheral skeleton. Resorption, while higher in late- versus early-gestation, was lower throughout pregnancy compared to non-pregnant non-lactating (NPNL) in the same community. Formation was lower in late pregnancy than in early, and below NPNL levels. This suggests a shift in the ratio of resorption to formation. Despite some evidence of change in bone metabolism, in this population, with habitually low Ca intakes, the peripheral skeleton was not mobilized as a Ca source for the fetus.

**Keywords:** ANALYSIS/QUANTIFICATION OF BONE, Bone QCT/μCT; EPIDEMIOLOGY, General population studies; AGING, BONE MODELING AND REMODELING; Biochemical markers of bone turnover

**INTRODUCTION**

During pregnancy the fetus is reliant on the mother to satisfy its nutrient requirements. As fetal Ca accretion increases across gestation from ~50 mg/day at 20 weeks to 330 mg/day at 35 weeks(1) and at birth the new-born skeleton contains 20–30 g Ca(2), it is plausible that these high demands may result in bone mineral mobilization from the maternal skeleton. In the literature there are conflicting reports concerning bone mineral mobilization during pregnancy (3,4). The small number of studies that used X-ray-based techniques to explore pregnancy-related skeletal changes typically have either obtained whole body and regional dual-energy x-ray absorptiometry (DXA) scans before and after pregnancy or have scanned the forearm during pregnancy (5-20). Both these approaches have limitations because postpartum measures are confounded by lactation and because DXA cannot distinguish between cortical and trabecular bone, which may be differentially affected by pregnancy. Peripheral Quantitative Computed Tomography (pQCT), however, allows for the measurement of compartment-specific measures of volumetric bone mineral density (vBMD) and, because it involves minimal exposure to ionizing radiation, is suitable for studies during pregnancy (21,22). Recently, in a population of predominately white Caucasian women in Cambridge, UK, we found evidence of pregnancy-related decreases in maternal vBMD at the distal tibia using both pQCT (total and trabecular vBMD) and high-resolution-pQCT (total and cortical vBMD)(22). No studies have been conducted in sub-Saharan Africa or in other low and middle income countries where poor nutritional status and micronutrient deficiencies are common and where the demands on the maternal skeleton may be greater because of repeated cycles of pregnancy and lactation. Women in rural areas of The Gambia, West Africa, have a habitually low calcium intake (approximately 350 mg/day) and often have many pregnancies with long lactation-periods during their lifetime. Therefore, the aim of the current study was to determine whether pregnancy-related skeletal changes occur in rural Gambian women using pQCT and biomarkers of bone turnover. The primary objective was to determine if total and trabecular vBMD at the radius and tibia decrease during pregnancy and whether this is modified by maternal nutritional supplementation. The secondary objective was to consider the effect of pregnancy and maternal supplementation on other skeletal pQCT measures and markers of bone turnover (BTMs).

**MATERIALS AND METHODS**

*Recruitment*

The data presented here were collected as part of a bone-focused add-on study to The Early Nutrition and Immune Development (ENID) Trial (ISRCTN49285450)(23). ENID was a longitudinal randomized controlled trial of nutritional supplementation in the rural West Kiang region of The Gambia, West Africa, designed to investigate the effects of ante-natal and infant nutritional supplementation on infant immune development. The add-on study (ENID-Bone) was approved by the Joint Gambian Government/MRC The Gambia Ethics Committee (project number SCC1126v2; add-on L2009.66). Recruitment into ENID took place in all 36 villages registered within the West Kiang Demographic Surveillance System (DSS)(24). Inclusion criteria for the study were: age 18-45 years, pregnant <20 weeks on ultrasound assessment, resident in the West Kiang DSS area. Exclusion criteria were: currently being enrolled in another MRC study, severe anemia at booking (hemoglobin (Hb) <7 g/dL). All women entering the ENID trial were invited to participate in the ENID Bone add-on study; our analyses comprise women with pQCT scans of useable quality performed on two occasions during pregnancy as part of the ENID Bone add-on. These were at an initial booking visit (booking ~14 weeks pregnancy) and at approximately 30 weeks gestation (P30) as determined by ultrasonography(23). Data included in the analyses presented here are for a subset of women who had the pair of pQCT scans performed on the same instrument in order to minimize any calibration differences between the two scanners in use in the research facility (described below). Informed consent was obtained by trained field workers with written consent obtained through either a signature or a thumb print.

*Supplement groups in the ENID Trial*

Each participant in ENID was randomized into the trial to start supplementation immediately after the booking visit, and supplementation continued until delivery. Randomization to supplement group was by an automated system (23). The 4 antenatal supplements (Table S1) were: iron-folic acid (IFA, given as per standard antenatal practice in The Gambia), multi-micronutrients (MMN), lipid-based protein-rich energy and protein (PE), and a lipid-based PE+MMN these two supplements combined (23).

*Anthropometry*

Detailed maternal anthropometry was collected at each visit. Height (cm) was obtained without footwear to the nearest 0.1 cm by wall mounted stadiometer (Seca GmbH, Hamburg, Germany). Weight (kg) was measured to the nearest 0.1 kg using a digital scale, with participants wearing light clothing without footwear (Seca GmbH, Hamburg, Germany). Limb length measurements were obtained to the nearest 0.1 cm using a tape measure: tibial length from the distal edge of the medial malleolus to the tibial plateau; ulna length from the end of the olecranon to the distal end of the ulnar styloid process.

*Bone densitometry*

The research facility in The Gambia has two pQCT instruments: XCT 2000LTM & XCT 2000TM, Stratec Medizintechnik, Pforzheim, Germany. For this longitudinal study, the two scans for each individual were obtained as far as possible on the same instrument. pQCT scans of the non-dominant radius and tibia were performed with a voxel size of 0.5 mm and slice thickness of 2 mm, at the radius (at 4 and 33% of the limb length proximal to the distal endplate) and tibia (at 4 and 38% of the limb length proximal to the distal endplate). CT scan speed was 30 mm/s and scout view scan speed was 40 mm/s. pQCT scans were processed using the manufacturer’s software (Stratec XCT version 6.2): at 4% sites CALCBD analysis contour mode 1, peel mode 1 at a threshold of 180 mg/cm3 was used with trabecular bone being defined as the inner area of 45% of the total cross sectional area (CSA). At cortical sites a threshold of 710 mg/cm3 was selected in conjunction with CORTBD separation mode 1 and measures of cortical BMC, vBMD, cortical CSA and thickness derived. Total CSA was defined at proximal sites at a threshold of 280 mg/cm3. Scans were qualitatively graded by visual inspection to assess their suitability for longitudinal analysis: scans slices with excessive movement or other artefacts, and scout views that did not match longitudinally were excluded. Calibration of the XCT systems was performed on a routine basis with the manufacturer’s phantom: daily quality assurance scans and weekly quality control scans were performed throughout the study period to test scanner performance. Previous studies at our site found the inter-operator precision to be 0.3-1.8% for bone outcome measures at the tibia and 1.1-6.4% at the radius by performing two repeat scans on 30 Gambian participants. Participants were exposed to low doses of ionizing radiation, a set of radius/tibia scans had an effective dose equivalent of <3.2 μSv per visit (25), less than half background radiation levels.

*Laboratory measures*

Venous blood samples were collected into lithium heparin blood tubes after an overnight fast. Plasma was separated by centrifugation at 1800 × g for 10 min at 4°C, stored at −80°C, and subsequently transported to the MRC Elsie Widdowson Laboratory, Cambridge, UK on dry ice and stored at −80°C. Blood was processed as EDTA plasma for PTH analysis and as lithium heparin plasma for bone turnover markers (P1NP and collagen type 1 crosslinked β‐C‐telopeptide [β‐CTx]) and other analytes relating to calcium, phosphorus, and vitamin D metabolism (calcium, phosphate, magnesium, albumin, creatinine, 1, 25-dihydroxyvitamin D [1,25(OH)2D], total alkaline phosphatase [TALP]. Corrected calcium was derived as described by Payne and colleagues(26). A subset was selected for the analysis of CTx, P1NP, 1,25(OH)2D and PTH due to limited volume and the expense of the assays. The samples analyzed were selected to ensure the proportions were balanced across the supplement groups (Table S2). Urine was collected in acid-washed containers over 24 hours and kept cool in boxes with freezer packs and returned to the lab for mixing and freezing the next day. Aliquots were acidified with concentrated HCl (10 μl/ml) and stored at -20°C prior to transport to EWL and then at -80°C before analysis. The details of the assays used are given in supplementary material, as are the numbers of samples analyzed for each plasma analyte (Table S2). Urinary data from women with complete collections at booking and P30 are presented in the supplementary material (n=421, Table S3). Reference data from a separate group of non-pregnant, non-lactating (NPNL) women aged 30-45 years living in the same region of rural Gambia and obtained from assays conducted in the same laboratory are provided for context (n=136, Table S4) (A. Prentice, unpublished data, personal communication).

**STATISTICAL APPROACH**

*Post-hoc powering for pQCT analysis*

At the time of planning the analysis for this study, no comparable pQCT data were available in the literature to perform a power calculation. Instead, post-hoc powering was used, based on a previous study where changes in DXA-measured size-adjusted BMC at the lumber spine of 2.6 (SD4.2) % occurred between pre-pregnancy and 2 weeks postpartum (18). This determined that a sample size of 35 participants would be required to detect within-group change in BMD from booking to thirty weeks pregnancy of a similar magnitude with a power of 0.9, and significance of 0.05 (27). Since that time we have shown in a UK pQCT study comparing pregnant to NPNL women that significant within group differences could be observed with n = 35 (22). Thus the number of scans available from the ENID-bone study (n=467) was more than sufficient to detect changes of a similar magnitude.

*Data analyses*

All analyses were performed using R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria; <https://www.r-project.org/>) with dplyr (version 0.8.3) for data manipulation (28). Baseline characteristics are presented with data given as mean (SD), except for parity which is expressed as median [IQR]) and also in categories as n(%). Natural logarithms of all variables except age were calculated to normalize the data distributions and to describe proportional (percentage) differences between (and within) groups (29). Between-visit change in each variable was expressed as a sympercent ([difference/mean]\*100), derived by subtracting loge(booking) from loge(P30) and multiplying by 100 (29).

ANOVA with post hoc Tukey test was used to test for significant differences between the supplement groups at booking. Independent t-tests, equivalent to paired t-tests, were used to determine whether the mean between-visit change in each variable was significantly different from zero. Multiple regression was used to explore the extent of between-visit change after adjusting for maternal age at booking. Maternal age at booking (in years) was included in the models centered on the mean age of the participants. This allows the intercepts from the models to be interpreted as the between-visit change related to pregnancy and the beta-coefficient for age as the sympercent difference in change per year of maternal age. No additional adjustment for parity was made because maternal age and parity were highly correlated in this cohort (r = 0.72). There was no significant differences between women for whom this was their first pregnancy and those who had more pregnancies. We also looked at splitting the cohort on the basis of age (<30 years and >30 years) but this also had little effect. A secondary analysis to explore whether pregnancy-related change differed between supplement group and the standard care was performed by adding a categorical supplement variable to the above model, the beta-coefficients for each supplement group representing the sympercent difference in between-visit change compared to IFA.

**RESULTS**

Eight hundred and seventy five women were recruited into the main ENID Trial, of whom 811 went on to participate in the ENID Bone add-on study. A subset of women (n=564) had pQCT scans of useable quality performed on two occasions during pregnancy 116 (SD24) days apart, of those 467 had a pair of pQCT scans performed on the same instrument. Table 1 presents the descriptive data at booking for the subset of women (n=467) included in the analyses and those recruited into ENID-bone who were not included (n= 344). Women in these two subsets did not differ significantly by age, though women without longitudinal scans data were slightly heavier (p<0.01) and had a lower parity (p<0.05) (Table 1). Women in the subset presented in this paper were 14.0 (SD3.4) weeks pregnant at booking which was not substantially different to that of the main trial (13.8 (SD3.3) weeks) (30). In our sample, pregnant women gained 5.3 (3.0) kg between booking and P30.

Plasma biochemistry was available for the subset of 467 women with longitudinal pQCT scans (Table 2), though the numbers with data at each pregnancy visit differed for each analyte (Table S2). The summary data for the 4% distal and 33%/38% proximal sites at the radius/tibia, and for the plasma analytes are in Table 2 for all participants included in this analyses and stratified by supplement group in Table 3. Urine data were also available for 421 women at both visits and are summarized in Table S3. The plasma and urine data for the NPNL reference group are provided in Table S4. There were significant differences (p<0.001) between the plasma biochemistry for the pregnant women compared to these NPNL women: higher 1,25(OH)2D and lower CTX, PTH, albumin and magnesium concentrations at both Booking and P30; lower phosphate at Booking only; and higher TALP plus lower P1NP and calcium concentrations at P30 only. The 24hour urinary phosphate and creatinine outputs of the pregnant women were lower than NPNL women at both timepoints (p<0.002 and p<0.001 respectively), whereas urinary calcium output was higher at Booking (p=0.02) but lower at P30 (p=0.03). These differences remained when expressed relative to creatinine output, with the effect size reduced, except the ratio for magnesium was higher at both Booking and P30 (p<0.001). No significant differences were found at booking between the supplement groups for any characteristic, bone outcome or biochemistry measure.

*Unadjusted analysis*

Independent t-tests revealed significant between-visit changes in several pQCT bone measures (radius and tibia), all plasma biochemistry (with the exception of corrected Ca), and all urinary biochemistry except Mg (Table 2, Table S3). Small, but statistically significant, increases were seen in vBMD and related measures at distal and proximal sites of both the radius and tibia with increases in CTX, TALP, PTH, 1,25(OH)2D and phosphate concentrations and decreases in P1NP, albumin, Ca and Mg concentrations. There were also decreases in the urinary mineral and creatinine outputs between Booking and P30 (Table S3). No significant changes in bone geometry were observed at these sites, except for an increase in cortical CSA at the proximal tibia (Table 2).

Stratifying these data by maternal supplement group (Table 3), the magnitudes of the changes remained small and positive for the bone measures, with the effect sizes slightly attenuated. At the distal sites, the increases were significant at the radius for total vBMD in the MMN group and for trabecular vBMD in the PE+MMN group, while the significant increases at the tibia were for total and trabecular vBMD in the PE+MMN group. At the proximal sites, increases in cortical vBMD and BMC were significant at both sites in all groups, except at the proximal radius of the PE group (Table 3). Similarly, the changes in the biochemistry following stratification by supplement group remained mostly consistent with the overall pattern. This was with the exception of the increases in CTX, PTH and plasma phosphate, which were not significant in the PE group, nor CTX and urinary Mg and creatinine outputs within MMN. Plasma magnesium only decreased significantly in the protein-containing supplement groups and plasma phosphate did not decrease in the PE+MMN group (Table 3).

*Adjusted analyses*

After age-adjustment, significant increases were found at distal sites in radial and tibial trabecular vBMD (1.15 [0.55, 1.75]% and 0.46 [0.17, 0.74]% respectively) and in tibial total vBMD (0.34 [0.15, 0.54]%) (Figure 1). No significant changes in total CSA were observed at distal sites. At proximal sites small but significant increases in cortical outcomes were observed. Radius cortical vBMD and BMC increased by 0.35 [0.26, 0.44]% and 0.55 [0.41, 0.68]% respectively, while tibia cortical vBMD and BMC increased by 1.15 [0.55, 1.75]%, 0.41 [0.24, 0.58]%, and 0.47 [0.25, 0.69]% respectively. Only tibia cortical CSA was found to increase (0.20 [0.09, 0.31]%) (Figure 1). After age-adjustment, the magnitude and significance of between-visit changes in maternal plasma and urinary biochemistry (Figure 2, Figure S2) were broadly consistent with the unadjusted data (Table 2, Table S3). At the radius, the change in BMC was inversely associated with maternal age (-0.04 [-0.07, -0.01] %/year), i.e. the older the woman, the smaller the increase, but no change was noted in cortical vBMD (-0.02 [-0.05, 0.01] %/year). No significant age effects were found at the tibia. Older maternal age was associated with a greater increase in CTX (1.75 [ 0.57, 2.80] % /year) and a smaller increase in PTH (-1.10 [-1.95, -0.23]%/year). There were no significant associations with age among the other plasma or any of the urinary analytes.

Adjusting for supplement group revealed a modifying effect on changes in maternal bone measures and biochemistry after controlling for age. Figures 3A and 3B depict the age-adjusted differences in change over time at the radius and tibia for 3 supplement groups relative to the IFA group, Figure 4 depicts the biochemistry. The increase in radial cortical vBMD was lower for MMN and PE than for IFA by -0.54 [-1.01, -0.07]% and -0.73 [-1.20, -0.26]% respectively. The increase in cortical CSA in MMN was 0.67 [0.10, 1.25]% greater than that of the IFA group. Tibial total vBMD for PE+MMN was 0.76 [0.22, 1.30]% greater than the IFA group. In contrast, no differences in markers of bone turnover were found for any supplement group compared to IFA. While albumin decreased overall, the decrease was 3.8 [-6.92, -0.72]% and 3.7 [-6.66, -0.48]% greater in the PE and PE+MMN groups respectively compared to IFA. Decreases in Mg in the PE+MMN group were 3.2 [-5.47, -0.85]% greater than the IFA group (p<0.01), however, this was attenuated following adjustment for albumin. While there was an increase in phosphate overall, this was significantly less in the PE (-8.1 [-12.45, -3.71]%) and PE+MMN (-6.5 [-10.82, -2.11]%) groups compared to IFA. The only urinary analyte where differences were found compared to IFA was urinary phosphate creatinine ratio where the decrease in the MMN, PE, and PE+MMN groups was lower by 19.8 [4.13, 35.57]%, 16.1 [0.09, 32.19]%, and 19.4 [3.58, 35.02]% respectively.

**DISCUSSION**

This study has shown that pregnancy-related bone mineral mobilization is not evident in the appendicular skeleton of rural Gambian women between the early-second and mid-third trimesters of pregnancy. In contrast, there was evidence of mineral accretion in the cortical compartment of both the radius and tibia although the magnitude of the increases for cortical vBMD and BMC were no greater than 0.5%. There was also some limited evidence of supplement modulation in the changes seen in the bone measures. While the magnitudes of the pQCT changes were modest and their clinical or physiological significance likely to be minimal, they may still reflect alterations in maternal Ca homeorhesis during pregnancy.

To date, few comparable studies have been conducted using pQCT in pregnancy. In a study based in Cambridge (UK) we recently reported decreases in maternal vBMD at the tibia with HR-pQCT (total and cortical) and pQCT (total and trabecular) (22). The Cambridge study had a similar design to the one presented here, with identical pQCT scan sites imaged and the same protocol used at both centers, although the final visit occurred later in pregnancy (34-36 weeks gestation, mean 35.5 weeks). Two other studies have used pQCT technologies during gestation. A Swiss study, using a different pQCT modality, with slightly different scans sites, reported decreases in trabecular vBMD at the radius during pregnancy (21). In that study, no change in cortical vBMD was observed although their scan site was more distal than the 33% site used in our study and will have contained some trabecular bone. A recent pQCT study from an ethnically diverse US population reported no changes in maternal cortical bone outcomes (31).

Studies that have used DXA and its precursors to image the forearm have mostly found little evidence of mineral mobilization at the trabecular-rich distal radius (10,12,20), consistent with the findings from our Gambian pQCT study. Studies that have used DXA to scan the 33% radius (almost exclusively cortical bone) reported no evidence of mineral mobilization (10,12,18,20). However, studies of pregnancy and lactation have suggested conservation of bone mineral at cortical-rich sites of the appendicular skeleton (18). Our pQCT data also suggest conservation and possible accretion of bone mineral at cortical-rich sites in the peripheral skeleton during pregnancy.

As has been shown in previous Gambian studies (32,33), the NPNL reference group had high PTH and low urinary calcium excretion compared to typical Western values, indicative of adaptation to their low calcium intake (34). At the Booking visit (early second trimester), the pregnant women in the pQCT study had lower PTH, CTX, albumin, plasma phosphate and plasma magnesium plus higher 1,25(OH)2vitamin D and urinary calcium excretion than the NPNL reference group. As such, these findings are in line with the pregnancy-related differences expected due to metabolic changes and haemodilution(35). Unlike CTX, where decreases in the second trimester are considered to reflect haemodilution(35), there was no indication of lower P1NP concentrations at Booking, possibly suggesting a subtle shift in the balance of bone turnover in these women in favour of formation at this stage of pregnancy.

In contrast to finding evidence of a lack of maternal bone mineral mobilization with pQCT, there were significant changes in both CTX and P1NP concentrations between the early second to third trimesters in the Gambian women. In keeping with other studies of pregnancy (6,36-38) we observed increases in CTX between early and late pregnancy, however at both Booking and P30 mean CTX concentration was lower than that seen in the local population of NPNL (Figure S3). Few studies have been published using P1NP, and these report increases during the third trimester(6,37), a pattern also reported for another bone formation marker, procollagen type 1 C propeptide (P1CP) (6,10). The physiological significance of this is difficult to interpret as increases in P1NP and P1CP in late gestation may be related to fetal production rather than maternal (6). Our findings, however, indicate that P1NP decreased into the third trimester of pregnancy in the Gambian women, suggesting a decrease in bone formation and a shift in the balance of bone turnover in favor of resorption.

The lack of evidence of mobilization from appendicular bone in the context of increased resorption (vs booking) and reduced bone formation (vs booking and NPNL) does not preclude that mineral mobilization may occur elsewhere in the maternal skeleton. However, taking the BTM data in context, and noting the reduction in urinary calcium output at P30 below that of the NPNL reference group, it appears more likely that maternal mineral reserves may be preserved (at least to 30 weeks) by reduced bone turnover and increased renal calcium conservation. It is possible that these findings represent a further manifestation of metabolic adaptation to the low calcium intake this rural Gambian population. An early study in The Gambia (39) reported elevated concentrations compared to Western values of calcitonin, a hormone that increases during gestation and may have a physiological role in protecting the skeleton during pregnancy (35). It is also possible that the apparent conservation of appendicular bone mineral, especially at the distal tibia, may relate to the fact that almost all women in this rural region are involved in physically demanding farming throughout pregnancy, and that walking, often with heavy head-loads, is the primary mode of transport (40).

Unlike many of the studies in the literature, the age range of this study cohort was very broad, with women aged between 18-45 years recruited. In addition, compared to other studies, the mean parity of these women was high and highly correlated with age. Maternal age, and by proxy parity, had little impact as a predictor of between-visit changes in bone and biochemical measures, but, interestingly, significant inverse associations with age were found for the increases in cortical BMC at the radius and PTH concentrations, plus a positive association for the increase in CTX. This indicates that the older the mother, the smaller the increases in cortical BMC and PTH but the greater the increase in CTX. This pattern suggests that being older in this cohort was associated with a greater increase in resorption between the two pregnancy visits 3-4 months apart. It is possible that this reflects age-related increases in bone resorption superimposed on those related specifically to pregnancy. This possibility would require comparison with an age-matched, non-pregnant reference group of women to have been scanned over the same time period as the pregnant women, which was not possible in our study.

*Strengths*

This study presents, to the best of our knowledge, the first use of pQCT during pregnancy in sub-Saharan Africa. In addition, it is the largest study to date anywhere to use pQCT in pregnancy. While some studies have only measured the non load-bearing forearm, we investigated changes at the load-bearing tibia in addition to the radius. By investigating changes in bone turnover markers and maternal biochemistry we were able to obtain information about possible systemic changes in maternal bone metabolism. These data complement our pQCT scans of the peripheral skeleton and may indirectly provide insights into potential changes occurring in the axial skeleton. Although we did not have an NPNL control arm in the study we were able to use NPNL reference values for plasma and urinary biochemistry from another study in this rural Gambian population.

*Limitations*

The limitations of this work include that we cannot determine whether mineral mobilization that may have occurred in the first trimester of pregnancy nor for any natural age-related changes in bone. Also, as the third trimester visit was at around 30 weeks gestation, and as there is evidence that late-pregnancy is a resorptive state, this will have limited the potential to detect changes in the appendicular skeleton that may have occurred closer to term. As we were unable to use DXA during pregnancy it is difficult to interpret what may be taking place in the axial skeleton. Another limitation is that, although we found no evidence of mineral mobilization in the appendicular skeleton using pQCT, it is conceivable that mobilization could occur without an apparent decrease in vBMD through other mechanisms that cannot be detected with single-slice pQCT, such as reorganization of bone microarchitecture (22). Women taking part in the study all received Gambian standard of care IFA supplementation, and, as such, represented the control arm for this study. We do not think it is likely that women in the IFA only could have benefited from improved nutrition compared to non-study women in the population, while these women may have received additional healthcare because of regular contact with the study team before and during the main trial. The lack of consistent findings when stratified by supplement group may suggest that maternal supplementation had little effect on calcium and bone metabolism in pregnancy but it should be noted that none of the supplements contained calcium and the supplements were not designed to specifically impact bone. Further limitations include the lack of pQCT measures in an age-related NPNL group, the difficulty of interpreting changes in plasma and urine data during a period of physiological alterations in haemodilution and glomerular filtration rate, and that the women in the study had received healthcare before and during pregnancy beyond what is typical in this community because of their involvement in the ENID trial.

**CONCLUSIONS**

These data provide an insight into maternal bone mineral metabolism during pregnancy in the appendicular skeleton of rural Gambian women. To date these are the only such densitometric data collected during pregnancy in sub-Saharan Africa. No evidence of bone mineral mobilization was found at the radius and tibia, but changes observed in BTMs between the second and third trimesters suggest a shift in the ratio of resorption to formation, though both CTX and P1NP concentrations were lower than the NPNL reference group at both visits. In contrast, small but significant increases in vBMD and BMC were observed at both trabecular- and cortical-rich sites in the radius and tibia representing both load-bearing and non load-bearing sites of the appendicular skeleton. While the magnitudes of these increases were modest in this index pregnancy, it is possible that, in populations where high parity is common, these small increases in vBMD and BMC could increase bone strength if accumulated across multiple pregnancies. Research with pQCT and DXA in populations where parity is high has previously suggested potential beneficial adaptations related to high parity(41). Further, we observed some modulation by dietary supplement group but these effects were marginal in magnitude. In conclusion, maternal bone mineral does not appear to be mobilized from the appendicular skeleton during pregnancy in this population on a low Ca diet, but there is evidence that maternal mineral reserves may be preserved through reduced bone turnover and increased renal calcium conservation.

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**Table and Figure headings**

Table 1. Descriptive statistics at booking visit of the subset of women with longitudinal pQCT scans and the larger study population. Descriptive statistics are also presented stratified by supplement group for those with longitudinal pQCT data.

Table 2. Bone outcomes, plasma biochemistry, and bone turnover makers for pregnant women with longitudinal pQCT measures at booking and P30. Between-visit differences are expressed as sympercents (∆ sym%).

Table 3. Booking values and between-visit change (∆ sym%) in bone outcomes, biochemistry, and bone turnover makers for pregnant women with longitudinal pQCT measures.

Table S1. Composition of the 4 supplements used in the ENID Trial (23)

Table S2. Counts of participants who had pQCT and bone biochemistry and bone turnover analytes stratified by group

Table S3. Urinary biochemistry for pregnant women at booking and P30. Between-visit differences are expressed as sympercents (∆ sym%).

and between-visit change

Table S4 Reference plasma and urinary biochemistry values from non-pregnant non-lactating rural Gambian women aged 30-45 years.

Figure 1. Pregnancy-related between-visit change in pQCT bone outcomes at the radius and tibia.

Figure 2. Pregnancy-related between-visit change in maternal bone turnover markers and analytes of bone biochemistry.

Figure 3A & 3B. Differences in pregnancy-related between-visit change between Iron Folate group and supplement groups in pQCT bone outcomes at the radius and tibia.

Figure 4. Differences in pregnancy-related between-visit change between Iron Folate group and supplement groups in maternal bone turnover markers and analytes of bone biochemistry*.*

Figure S1. Plasma biochemistry and bone turnover markers for pregnant women at booking and P30, and non-pregnant non-lactating women (NPNL, aged 30-45 years).

Figure 2S. Urinary biochemistry for pregnant women at booking and P30, and non-pregnant non-lactating women (NPNL, aged 30-45 years).

Tables

Table 1. Descriptive statistics at booking visit for the subset of women with longitudinal pQCT scans and the larger study population. Descriptive statistics are also presented stratified by supplement group for those with longitudinal pQCT data.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | | | | |
|  | **Women with longitudinal pQCT** | | **Women without longitudinal pQCT** | | |
|  | n = 467 | | n = 344 | | |
| Age (years) | 29.7 (6.5) | | 29.4 (7.1) | | |
| Parity, median [IQR]\* | 4 [2,6] | | 4 [1,6]d | | |
| Nulliparous  Parity 1-4  Parity 5-6  Parity 7+ | 40 (8.7%)  203 (44.3%)  116 (23.3%)  99 (21.6%) | | 44 (13.0%)  157 (46.4%)  71 (21.0%)  66 (19.5%) | | |
| Weight at booking (kg) | 54.8 (9.2) | | 56.6 (10.6)b | | |
| Height (cm) | 161.7 (5.8) | | 162.0 (6.0) | | |
| Sitting height (cm) | 80.2 (2.9) | | 80.1 (3.8) | | |
|  | **Total n = 467** | | | | |
|  | **IFA** | **MMN** | | **PE** | **PE+MMN** |
|  | n=123 | n=119 | | n=112 | n=113 |
| Age (years) | 29.2 (6.3) | 29.7 (6.2) | | 29.6 (6.4) | 30.3 (7.0) |
| Parity, median [IQR] | 4 [2,6] | 4 [2,6] | | 4 [2,6] | 5 [3,7] |
| Nulliparous  Parity 1-4  Parity 5-6  Parity 7+ | 13 (10.7%)  50 (41.3%)  37 (30.6%)  21 (17.4%) | 10 (8.5%)  58 (49.2%)  27 (22.9%)  23 (19.5%) | | 11 (10.5%)  46 (43.8%)  25 (23.7%)  23 (21.9%) | 6 (6.7%)  29 (32.2%)  27 (30%)  28 9 (31.1%) |
| Weeks pregnant | 14.2 (3.5) | 13.8 (3.7) | | 14.0 (3.2) | 13.9 (3.3) |
| Weight at booking (kg) | 54.7 (8.7) | 54.2 (9.8) | | 55.7 (9.2) | 54.5 (9.0) |
| Weight change (booking to P30, kg) | 4.9 (2.9) | 5.5 (3.0) | | 5.4 (3.0) | 5.5 (3.1) |
| Height (cm) | 161.9 (6.3) | 161.8 (5.7) | | 162.0 (5.8) | 161.2 (5.4) |
| Sitting height (cm) | 80.3 (2.8) | 80.3 (2.9) | | 80.2 (3.2) | 80.3 (2.8) |
| All variables expressed in Mean(SD), except parity median[IQR] and n(% of total) for parity bands. Welch Two Sample t-test a = p<0.05, b = 0.01, c = 0.001. Mann-Whitney U-test d = 0.05, e = 0.01, f = 0.001. IFA = iron folate, MMN = multiple micronutrients, PE = protein energy, PE+MMN = protein energy and multiple micronutrients \*parity available for 458 women with longitudinal pQCT data and for 338 without longitudinal pQCT | | | | | |

Table 2. Bone outcomes, biochemistry, and bone turnover makers for pregnant women with longitudinal pQCT measures at booking and P30. Between-visit differences are expressed as sympercents (∆ sym%).

|  |  |  |  |
| --- | --- | --- | --- |
|  | **booking** | **P30** | **∆ sym%** |
| **4% Radius** | n=435 | | |
| Total vBMD (mg/cm3) | 319.23 (44.98) | 320.12 (45.44) | 0.26 [0.25] |
| Trabecular vBMD (mg/cm3) | 160.51 (35.63) | 162.03 (34.71) | **1.15 [0.30]c** |
| Total CSA (mm2) | 335.94 (42.09) | 337.85 (44.21) | 0.49 [0.31] |
| **33% Radius** | n=440 | | |
| Total CSA (mm2) | 119.82 (16.12) | 119.49 (16.82) | -0.34 [0.42] |
| Cortical vBMD (mg/cm3) | 1239.66 (35.17) | 1244.76 (37.32) | **0.41 [0.09]c** |
| Cortical BMC (mg/mm) | 94.05 (10.77) | 94.51 (10.99) | **0.47 [0.11]c** |
| Cortical CSA (mm2) | 75.84 (8.17) | 75.91 (8.32) | 0.06 [0.11] |
| Cortical Thickness (mm) | 2.45 (0.23) | 2.46 (0.23) | 0.38 [0.35] |
| **4% Tibia** | n=407 | | |
| Total vBMD (mg/cm3) | 281.86 (36.08) | 282.77 (35.83) | **0.34 [0.10]c** |
| Trabecular vBMD (mg/cm3) | 190.49 (31.19) | 191.26 (30.74) | **0.46 [0.14]b** |
| Total CSA (mm2) | 945.65 (112.59) | 944.46 (112.90) | -0.13 [0.13] |
| **38% Tibia** | n=409 | | |
| Total CSA (mm2) | 377.60 (47.95) | 378.28 (49.15) | 0.15 [0.14] |
| Cortical vBMD (mg/cm3) | 1230.23 (35.20) | 1234.61 (37.60) | **0.35 [0.04]c** |
| Cortical BMC (mg/mm) | 280.01 (34.20) | 281.53 (34.27) | **0.55 [0.07]c** |
| Cortical CSA (mm2) | 227.52 (26.26) | 227.94 (26.13) | **0.20 [0.06]c** |
| Cortical Thickness (mm) | 4.07 (0.41) | 4.08 (0.41) | 0.13 [0.13] |
| **Bone biochemistry and bone turnover markers\*** | | | |
| β‐CTX (ng/l, n = 293) | 230 [66, 665] | 290 [114, 685] | **23.0 [3.64]c** |
| P1NP (μg/l, n = 295) | 59.7 [29.1, 140] | 43.4 [21.3, 90.0] | **-32.4 [2.30]c** |
| PTH (ng/l, n = 283) | 27.4 [12.6, 51.9] | 31.2 [13.6, 61.6] | **13.2 [2.78]c** |
| 1,25(OH)2D (pmol/l, n = 205) | 255 [181, 361] | 314 [219, 433] | **21.0 [1.65]c** |
| TALP (U/l, n = 464) | 79.0 [44.7, 122] | 106 [67.4, 172] | **29.0 [1.53]c** |
| Albumin (g/l, n = 464) | 31.8 [25.8, 38.1] | 25.5 [22.2, 30.0] | **-21.4 [0.56]c** |
| Creatinine (µmol/l, n=464) | 36.3 [26.5. 48.9] | 36.6 [25.9, 53.2] | **1.08 [0.89]** |
| Phosphate (mmol/l, n = 464) | 1.05 [0.81, 1.34] | 1.08 [0.85, 1.34] | **2.99 [0.80]c** |
| Calcium (mmol/l, 464) | 2.25 [2.05, 2.41] | 2.12 [1.97, 2.27] | **-5.36 [0.28]c** |
| CalciumCorr (mmol/l, 464) | 2.41 [2.27, 2.53] | 2.41 [2.29, 2.51] | 0.32 [0.19] |
| Magnesium (mmol/l, 464) | 0.76 [0.66, 0.87] | 0.75 [0.65, 0.86] | **-1.75 [0.42]c** |
| All pQCT measures expressed as Mean(SD), biochemistry as GMean[5th centile, 95th centile], change between visits as ∆ Mean(SE)%. vBMD = volumetric bone mineral density, CSA = cross sectional area, BMC = bone mineral content, β‐CTX = collagen type 1 cross‐linked β‐C‐telopeptide, P1NP = type 1 procollagen N‐terminal, PTH = parathyroid hormone, 1,25(OH)2D = 1,25-dihydroxyvitamin D; TALP = total alkaline phosphatase.  CalciumCorr= corrected using the Payne equation i.e. Albumin-corrected plasma calcium (mmol/l) = measured plasma calcium mmol/l +([40-plasma albumin g/l]\*0.02). \*Reference data for a separate group of non-pregnant non-lactating women can be found in table 4S.  Between-visit change explored with Independent t-test absolute between-visit change vs zero.  a = p<0.05  b = p<0.01  c =p<0.001 | | | |

Table 3. Bone outcomes, biochemistry, and bone turnover makers for pregnant women with longitudinal pQCT measures at booking and P30 stratified by supplement group. Between-visit differences are expressed as sympercents (∆ sym%).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **IFA** | | **MMN** | | **PE** | | **PEMNN** | |
|  | booking | ∆ sym% | booking | ∆ sym% | booking | ∆ sym% | booking | ∆ sym% |
| **4% Radius** | n=117 | | n=106 | | n=107 | | n=105 | |
| Total vBMD (mg/cm3) | 317.62 (42.48) | 0.10 [0.49] | 318.13 (45.65) | **1.19 [0.51]a** | 321.25 (45.87) | -0.28 [0.53] | 320.08 (46.64) | 0.06 [0.42] |
| Trabecular vBMD (mg/cm3) | 160.81 (35.31) | 1.12 [0.63] | 160.71 (34.07) | 0.92 [0.57] | 162.54 (36.61) | 1.12 [0.72] | 157.91 (36.84) | **1.46 [0.48]b** |
| Total CSA (mm) | 338.00 (43.33) | 0.37 [0.64] | 332.33 (37.12) | -0.49 [0.59] | 338.87 (41.97) | 1.17 [0.66] | 334.31 (45.65) | 0.93 [0.54] |
| **33% Radius** | n=116 | | n=113 | | n=109 | | n=102 | |
| Total CSA (mm) | 120.64 (16.62) | -1.62 [0.87] | 116.93 (14.35) | 0.41 [0.71] | 121.52 (16.92) | 0.09 [0.90] | 120.27 (16.36) | -0.19 [0.87] |
| Cortical vBMD (mg/cm3) | 1232.65 (32.32) | **0.76 [0.15]c** | 1243.42 (36.63) | 0.21 [0.17] | 1242.09 (36.53) | 0.02 [0.20] | 1240.88 (34.52) | **0.63 [0.17]c** |
| Cortical BMC (mg/mm) | 93.28 (11.82) | **0.57 [0.21]b** | 93.23 (10.21) | **0.67 [0.26]b** | 95.31 (11.10) | 0.06 [0.21] | 94.48 (9.72) | **0.57 [0.19]b** |
| Cortical CSA (mm2) | 75.63 (8.92) | -0.19 [0.21] | 74.96 (7.73) | **0.47 [0.24]a** | 76.70 (8.30) | 0.04 {0.18] | 76.14 (7.58) | -0.06 [0.19] |
| Cortical thickness (mm) | 2.43 (0.23) | 1.06 [0.71] | 2.46 (0.22) | 0.34 [0.61] | 2.46 (0.25) | -0.01 [0.73] | 2.46 (0.22) | 0.08 [0.72] |
| **4% Tibia** | n=106 | | n=104 | | n=93 | | n=104 | |
| Total vBMD (mg/cm3) | 281.99 (38.87) | 0.07 [0.18] | 282.67 (35.60) | 0.24 (0.21] | 285.68 (35.13) | 0.22 [0.21] | 277.49 (34.48) | **0.84 [0.20]c** |
| Trabecular vBMD (mg/cm3) | 190.56 (35.05) | 0.28 [0.30] | 191.00 (28.58) | 0.32 (0.28] | 193.06 (31.05) | 0.57 [0.31] | 187.59 (29.84) | **0.67 [0.28]a** |
| Total CSA (mm) | 948.83 (113.23) | -0.04 [0.20] | 935.78 (109.51) | -0.30 [0.24] | 946.80 (116.33) | 0.18 [0.27] | 951.25 (112.57) | -0.33 [0.32] |
| **38% Tibia** | n=105 | | n=107 | | n=93 | | n=104 | |
| Total CSA (mm) | 382.75 (52.93) | -0.29 [0.29] | 373.16 (49.67) | 0.37 [0.32] | 378.99 (43.92) | 0.37 [0.24] | 375.71 (44.33) | 0.17 [0.25] |
| Cortical vBMD (mg/cm3) | 1228.45 (32.41) | **0.30 [0.08]c** | 1231.39 (36.67) | **0.38 [0.01]c** | 1228.95 (37.76) | **0.23 [0.01]a** | 1231.98 (34.37) | **0.48 [0.09]c** |
| Cortical BMC (mg/mm) | 281.06 (37.48) | **0.51 [0.12]c** | 278.75 (36.20) | **0.68 [0.17]c** | 283.06 (32.21) | **0.38 [0.12]b** | 277.50 (30.38) | **0.60 [0.13]c** |
| Cortical CSA (mm2) | 228.77 (29.65) | 0.20 [0.10] | 226.30 (28.04) | **0.30 [0.14]a** | 230.20 (24.05) | 0.15 [0.10] | 225.12 (22.42) | 0.12 [0.10] |
| Cortical thickness (mm) | 4.06 (0.42) | 0.48 (0.29] | 4.08 (0.44) | 0.08 [0.31] | 4.12 (0.41) | -0.07 [0.21] | 4.03 (0.38) | 0.02 [0.23] |
| **Bone biochemistry and bone turnover markers\*** | | | | | | | | |
| β‐CTX (ng/l) | 252 [68, 614] | **23.5 [6.83]b** | 204 [54, 679] | **26.8 [8.29]b** | 247 [83, 596] | 13.6 [6.91] | 221 [68.7, 728] | **27.4 [7.17]a** |
| P1NP (μg/l) | 63.4 [32.8, 143] | **-32.9 [4.05]c** | 56.3 [25.8, 136] | **-28.2 [4.44]c** | 59.7 [32.1, 116] | **-34.2 [4.98]c** | 59.7 [29.4, 137] | **-34.6 [5.02]c** |
| PTH (ng/l) | 25.5 [11.4, 48.9] | **20.1 [4.57]c** | 27.7 [13.3, 55.2] | 6.47 [6.00] | 28.2 [15.0, 46.1] | 7.95 [6.63] | 27.9 [12.6, 60.3] | **17.0 [5.20]b** |
| 1,25(OH)2D (pmol/l) | 250 [174, 358] | **19.2 [2.98]c** | 260 [202, 365] | **21.8 [3.39]c** | 245 [169, 324] | **21.4 [3.47]c** | 262 [200, 365] | **21.6 [3.41]c** |
| TALP (U/l) | 77.5 [44.7, 123] | **28.6 [3.37]c** | 78.3 [44.7, 126] | **26.1 [2.97]c** | 79.8 [43.8, 123] | **31.7 [3.20]c** | 80.6 [47.5, 122] | **30.0 [2.64]c** |
| Albumin (g/l) | 31.2 [25.8, 38.5] | **-19.5 [1.14]c** | 31.5 [26.5, 37.3] | **-20.2 [1.17]c** | 31.8 [25.8, 39.3] | **-23.3 [1.02]c** | 32.1 [26.3, 38.5] | **-23.0 [1.15]c** |
| Creatinine (µmol/l) | 35.4 [27.0, 46.5] | 1.69 [1.88] | 36.3 [25.8, 48.7] | 0.22 [1.57] | 36.3 [26.6, 53.2] | 2.84 [1.74] | 37.7 [27.2, 48.8] | -0.41 [1.93] |
| Phosphate (mmol/l) | 1.02 [0.79, 1.32] | **7.5 [1.54]c** | 1.06 [0.84, 1.30] | **3.66 [1.65]a** | 1.08 [0.83, 1.38] | -0.59 [1.50] | 1.05 [0.82, 1.34] | 1.01 [1.59] |
| Calcium (mmol/l) | 2.23 [2.05, 2.41] | **-4.54 [0.71]c** | 2.25 [2.08, 2.41] | **-4.97 [0.49]c** | 2.25 [2.08, 2.43] | **-6.03 [0.45]c** | 2.25 [2.05, 2.44] | **-5.99 [0.56]c** |
| CalciumCorr (mmol/l) | 2.39 [2.28, 2.53] | 0.60 [0.47] | 2.41 [2.28, 2.53] | 0.32 [0.34] | 2.40 [2.28, 2.54] | 0.13 [0.33] | 2.40 [2.26, 2.55] | 0.19 [0.38] |
| Magnesium (mmol/l) | 0.76 [0.67, 0.85] | -0.45 [0.85] | 0.76 [0.68, 0.86] | -1.25 [0.80] | 0.76 [0.66, 0.85] | **-1.79 [0.84]a** | 0.77 [0.65, 0.89] | **-3.53 [0.85]c** |
| All pQCT measures expressed as Mean(SD), biochemistry as GMean [5th centile, 95th centile], change between visits as ∆ Mean(SE)%. vBMD = volumetric bone mineral density, CSA = cross sectional area, BMC = bone mineral content, β‐CTX = collagen type 1 cross‐linked β‐C‐telopeptide, P1NP = type 1 procollagen N‐terminal, PTH = parathyroid hormone, 1,25(OH)2D = 1,25-dihydroxyvitamin D; TALP = total alkaline phosphatase. CalciumCorr= corrected using the Payne equation i.e. Albumin-corrected plasma calcium (mmol/l) = measured plasma calcium mmol/l +([40-plasma albumin g/l]\*0.02). Between-visit change explored with Independent t-test absolute between-visit change vs zero.  a = p<0.05  b = p<0.01  c = p<0.001  \* exact numbers for each outcome by supplement group summarized in table S2 | | | | | | | | |

**Tables for supplementary materials**

Table S1. Composition of the 4 supplements used in the ENID Trial (23)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Nutrients** | **IFA** | **MMN** | **PE** | **PE+MMN** |
| Iron (mg) | 60 | 60 | 60 | 60 |
| Folate (μg) | 400 | 400 | 400 | 400 |
| Vitamin A (RE μg) | 0 | 1600 | 2.85 | 1600 |
| Vitamin D (IU) | 0 | 400 | 0 | 400 |
| Vitamin E (mg) | 0 | 20 | 4.2 | 20 |
| Vitamin C (mg) | 0 | 140 | 2.25 | 140 |
| Vitamin B1 (mg) | 0 | 2.8 | 0.3 | 2.8 |
| Vitamin B2 (mg) | 0 | 2.8 | 0.45 | 2.8 |
| Niacin (mg) | 0 | 36 | 1.35 | 36 |
| Vitamin B6 (mg) | 0 | 2.8 | 0.15 | 2.8 |
| Vitamin B12 (μg) | 0 | 5.2 | 0.1 | 5.2 |
| Zinc (mg) | 0 | 30 | 3.3 | 30 |
| Copper (mg) | 0 | 4 | 1.05 | 4 |
| Selenium (μg) | 0 | 130 | 6.15 | 130 |
| Iodine (μg) | 0 | 300 | 2.6 | 300 |
| Energy (kcal) | 0 | 0 | 746 | 746 |
| Protein (g) | 0 | 0 | 20.8 | 20.8 |
| Lipids (g) | 0 | 0 | 52.6 | 52.6 |
| All women received 60 mg Iron and 400 μg Folate. All women were randomised to supplementation from booking for antenatal care (<20 weeks gestation) until delivery. IFA = iron folate, MMN = multiple micronutrients, PE = protein energy, PE+MMN = protein energy multiple micronutrients | | | | |

Table S2. Counts of participants who had pQCT and bone turnover markers and other biochemical analytes stratified by group

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Bone biochemistry and bone turnover markers** | **IFA** | **MMN** | **PE** | **PE+MMN** |
| β‐CTX | 80 | 72 | 66 | 75 |
| P1NP | 80 | 73 | 66 | 76 |
| PTH | 75 | 71 | 63 | 74 |
| 1,25(OH)2D | 52 | 51 | 49 | 53 |
| TALP | 122 | 116 | 112 | 114 |
| Albumin | 122 | 116 | 112 | 114 |
| Creatinine | 122 | 116 | 112 | 114 |
| Phosphate | 122 | 116 | 112 | 114 |
| Calcium | 122 | 116 | 112 | 114 |
| Magnesium | 122 | 116 | 112 | 114 |
| β‐CTX = collagen type 1 cross‐linked β‐C‐telopeptide, P1NP = type 1 procollagen N‐terminal, PTH = parathyroid hormone, 1,25(OH)2D = 1,25-dihydroxyvitamin D, TALP = total alkaline phosphatase | | | | |

Table S3. Urinary biochemistry for pregnant women with longitudinal pQCT measures at booking and P30.

|  |  |  |  |
| --- | --- | --- | --- |
| Urinary biochemistry for women with Booking and P30 samples (n = 421) | | | |
|  | booking | P30 | ∆ sym% |
| Urinary calcium output (mmol/d) | 1.02 [0.12, 6.83] | 0.58 [0.06, 5.39] a | -57.6 [6.54] |
| Urinary phosphate output (mmol/d) | 5.87 [1.13, 29.7] | 3.92 [0.61, 23.5] a | -40.3 [5.38] |
| Urinary magnesium output (mmol/d) | 1.70 [0.38, 8.10] | 1.41 [0.28, 7.5] a | -18.7 [4.78] |
| Urinary creatinine output (mmol/d) | 4.29 [1.15, 20.5] | 3.44 [0.88, 15.8] a | -22.6 [4.45] |
| Urinary calcium creatinine ratio (mmol/mmol) | 0.239 [0.046, 0.817] | 0.168 [0.025, 0.692] a | -35.0 [5.04] |
| Urinary phosphate creatinine ratio (mmol/mmol) | 1.369 [0.631, 2.543] | 1.139 [0.494, 2.138] a | -17.7 [2.90] |
| Urinary magnesium creatinine ratio (mmol/mmol) | 0.396 [0.188, 0.777] | 0.411 [0.198, 0.800] | 3.8 [2.95] |
| Creatinine clearance rate (ml/min\*1.73m2) | 132 [59, 246] | 103 [36, 215.1] a | -22.6 [4.45] |
| All data expressed as Gmean [5th centile, 95th centile], change between visits as ∆ Mean(SE)%.  a Difference between P30 and Booking value: p<0.001 | | | |

Table S4. Reference values for non-pregnant non-lactating Gambian women aged 30-45 years

|  |  |
| --- | --- |
| Biochemistry for non-pregnant non-lactating Gambian women aged 30-45 years | |
| Plasma β‐CTX (ng/l, n=136) | 427 [155, 159] |
| Plasma P1NP (μg/l, n=136) | 63.7 [28.0, 158] |
| Plasma PTH (ng/l, n=136) | 53.1 [27.1, 113] |
| Plasma 1,25(OH)2D (pmol/l, n=136) | 200 [106, 327] |
| Plasma TALP (U/l, n=136) | 77.5 [48.6, 122] |
| Plasma albumin (g/l, n=136) | 35.4 [31.3, 40.3] |
| Plasma creatinine (µmol/l, n=136) | 62.3 [47.5, 81.0] |
| Plasma phosphate (mmol/l, n=136) | 1.10 [0.85, 1.36] |
| Plasma calcium (mmol/l, n=136) | 2.25 [2.10, 2.43] |
| Plasma calciumCorr (mmol/l, n=136) | 2.34 [2.20, 2.49] |
| Plasma magnesium (mmol/l, n=136) | 0.82 [0.72, 0.93] |
| Urinary calcium output (mmol/d, n=127) | 0.77 [0.07, 3.71] |
| Urinary phosphate output (mmol/d, n=127) | 7.27 [1.82, 18.0] |
| Urinary magnesium output (mmol/d, n=127) | 1.60 [0.41, 4.32] |
| Urinary creatinine output (mmol/d. n=127) | 5.40 [1.70, 11.1] |
| Urinary calcium creatinine ratio (mmol/mmol, n=127) | 0.140 [0.022, 0.485] |
| Urinary phosphate creatinine ratio (mmol/mmol, n=127) | 1.332 [0.698, 2.381] |
| Urinary magnesium creatinine ratio (mmol/mmol, n=127) | 0.293 [0.135, 0.620] |
| Creatinine clearance rate (ml/min\*1.73m2) | 65.6 [22.2, 134] |
| All data expressed as Gmean [5th centile, 95th centile]. ‐CTX = collagen type 1 cross‐linked β‐C‐telopeptide, P1NP = type 1 procollagen N‐terminal, PTH = parathyroid hormone, 1,25(OH)2D = 1,25-dihydroxyvitamin D, TALP = total alkaline phosphatase. CalciumCorr= corrected using the Payne equation i.e. Albumin-corrected plasma calcium (mmol/l) = measured plasma calcium mmol/l +([40-plasma albumin g/l]\*0.02). | |

Figures

Figure 1.



Beta-coefficients of percentage between-visit change (mean[CI95%]) for pQCT bone outcomes at the radius and tibia. Model adjusted for maternal age only. vBMD = volumetric bone mineral density, CSA = cross sectional area, BMC = bone mineral content. a = p<0.05, b = p<0.01, c = p<0.001

Figure 2.



Beta-coefficients of percentage between-visit change (mean[CI95%]) in maternal biochemistry and bone turnover markers. Model adjusted for maternal age only. β‐CTX = collagen type 1 cross‐linked β‐C‐telopeptide, P1NP = type 1 procollagen N‐terminal, PTH = parathyroid hormone, TALP = total alkaline phosphatase. a = p<0.05, b = p<0.01, c = p<0.001

Figure 3.



Beta-coefficients of percentage difference in change between supplement groups and IFA reference group (mean[CI95%]) for pQCT bone outcomes at the radius (A) and tibia (B). Model adjusted for maternal age and supplement group. IFA = iron folate, MMN = multi-micronutrients, PE = protein energy, PE+MMN = protein energy and multi-micronutrients, vBMD = volumetric bone mineral density, CSA = cross sectional area, BMC = bone mineral content. a = p<0.05, b = p<0.01, c = p<0.001Figure 4.



Beta-coefficients of percentage difference in change between supplement groups and IFA reference group (mean[SE]) for maternal biochemistry and bone turnover markers. Model adjusted for maternal age and supplement group. IFA = iron folate, MMN = multi-micronutrients, PE = protein energy, PE+MMN = protein energy and multi-micronutrients, β‐CTX = collagen type 1 cross‐linked β‐C‐telopeptide, P1NP = type 1 procollagen N‐terminal, PTH = parathyroid hormone, TALP = total alkaline phosphatase. a = p<0.05, b = p<0.01, c = p<0.001

Figure 1S.

Panels A-H plasma biochemistry and bone turnover markers for pregnant women at booking and P30, and non-pregnant non-lactating women (NPNL, aged 30-45 years). All data expressed as Gmean[5th, 95th]. β‐CTX = collagen type 1 cross‐linked β‐C‐telopeptide, P1NP = type 1 procollagen N‐terminal, PTH = parathyroid hormone, TALP = total alkaline phosphatase.

Figure S2

Panels A-G unirnary biochemistry for pregnant women at booking and P30, and non-pregnant non-lactating women (NPNL, aged 30-45 years). All data expressed as Gmean[G5th, G95th].

Supplementary material

Laboratory assays

Biochemical analysis was conducted at the MRC Elsie Widdowson Laboratory, Cambridge, UK, using commercially available assay kits and platforms as follows for plasma: albumin (bromocresol purple], calcium [arsenazo III], phosphate [ammonium molybdate], magnesium [xylidyl blue 1), creatinine [Jaffé] and TALP [p-nitrophenylphosphate] (Thermo Konelab 20i, Thermo Fischer Scientific, Vantaa, Finland); 1,25(OH)2D (ELISA, Immunodiagnostics Systems Ltd., Tyne & Wear, UK). Plasma intact PTH, β-CTX and P1NP were measured on the iSys platform (Immunodiagnostics Systems Ltd, Tyne and Wear, UK). Urine analysis for calcium, phosphate, magnesium and creatinine was performed using the same assays as for plasma, with appropriate dilution as necessary. The reference group data were obtained with the same sample types and assays as those of the study women,

The Konelab assays were run in duplicate, the iSys and ELISA assays in singleton. Quality assurance was achieved using low, medium and high control materials supplied by the kit manufacturers and commercial materials: for minerals, albumin and TALP: Roche serum control (Roche Diagnostic Corporation, Indianapolis, USA), Lyphochek (Bio-Rad Laboratories, Herts, UK), NEQAS Clin Chem (Birmingham, UK) and an internal plasma drift control; for PTH: NEQAS (Edinburgh, UK), for β-CTX and P1NP: NEQAS IIA EQA (Sheffield, UK); for 1,25(OH)2D International Vitamin D External Quality Assessment Scheme (DEQAS, www.deqas.org). All assays performed well and were within specification. The numbers of samples successfully collected, transported and analyzed varied depending on the analyte and timepoint, and are detailed in Table S2.

Plasma calcium was corrected for albumin concentration using the Payne equation i.e. CalciumCorr = measured plasma calcium mmol/l +([40-plasma albumin g/l]\*0.02) (mmol/l)

Plasma creatinine values measured using the Konelab Jaffé method were adjusted to produce compensated values, traceable to the international reference method (Isotope Dilution Mass Spectrometry) using the equation supplied by the manufacturer as follows: Compensated plasma creatinine = (plasma creatinine measured by Jaffé method - 26.8) x1.168 µmol/l. This was achieved by calculation post-assay for the samples from the study women, and by kit calibration for the reference samples.

Creatinine clearance rate (ml/min) was calculated to provide an estimate of glomerular filtration rate (GFR) using values from the 24h urine collection as followed: Urine volume (mmol/d)xplasma creatinine (µmol/l)/urine creatinine (mmol/l). GFR was then adjusted to a standard body surface area of 1.73m2 using the Du Bois formula (0.007184 x height (m)0.725 x weight (kg)0.425