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Full length article

# First assessment of bone mineral density in healthy pregnant women by means of Radiofrequency Echographic Multi Spectrometry (REMS) technology

Degennaro Valentina Anna [a], Brandi Maria Luisa [b,1], Cagninelli Greta [a,1], Casciaro Sergio [c,1], Ciardo Delia [c,1], Conversano Francesco [c,1], Di Pasquo Elvira [a,1], Gonnelli Stefano [d,1], Lombardi Fiorella Anna [c,1], Pisani Paola [c,1], Ghi Tullio [a,*]

[a] *Department of Medicine and Surgery, Obstetrics and Gynaecology Unit, University of Parma, Parma, Italy*
[b] *Department of Surgery and Translational Medicine, University of Florence, Florence, Italy*
[c] *Institute of Clinical Physiology, National Council Research, Lecce, Italy*
[d] *Department of Medicine, Surgery and Neuroscience, University of Siena, Italy*

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ABSTRACT

*Objective:* The maternal bone structure is the largest calcium reserve for the fetus during pregnancy, and this is claimed to lead to a bone mineral density (BMD) reduction in pregnant women. The primary outcome of the present work was to assess the BMD in a group of healthy pregnant women.
*Study design:* In this prospective case – control observational study, a non-consecutive group of pregnant women with uncomplicated pregnancy at or >37 weeks were enrolled at the unit of Obstetrics and Gynecology, University of Parma, from February to December 2020. The study subjects were submitted to a sonographic examination of the proximal femur with Radiofrequency Echographic Multi Spectrometry (REMS) technology to quantify the BMD of the femur. The BMD values obtained in the study group were compared with those of a control group of non-pregnant women matched for age, ethnicity and pre-pregnant body mass index (BMI).
*Results:* Overall, 78 pregnant women at 39.1 ± 1.5 weeks were assessed. Compared with non-pregnant women, the femoral BMD values measured in pregnancy using REMS were significant lower ($0.769 \pm 0.094$ g/cm$^2$ vs $0.831 \pm 0.101$ g/cm$^2$, $p = 0.0001$) with a mean BMD reduction of 8.1%.
The femoral neck BMD presented a positive correlation with the pre-pregnant BMI ($p = 0.0004$) and a negative correlation with the maternal age ($p < 0.0001$). In addition, a lower femoral neck BMD in Caucasian ethnicity compared with non-Caucasian was noted ($p < 0.0001$).
*Conclusion:* In this exploratory and proof of concept study, for the first time, a decreased BMD has been objectively demonstrated in pregnant compared with non-pregnant women by means of REMS technology. New studies are required to assess the longitudinal changes of maternal bone density throughout the pregnancy.



## Introduction

The women's bone is subjected to considerable changes throughout the pregnancy due to the concurrence of several factors [1].

While the higher levels of estrogens promote the formation of bone tissue, the fetal uptake of maternal calcium destined to skeletal development leads to maternal bone resorption.

It is estimated that about 200–300 mg of calcium every day are transferred across the placenta from the mother to the fetus [2]. On this basis, the World Health Organization (WHO) recommends an extra dietary calcium intake of 200 mg/day for pregnant women compared to non-pregnant women [3,4]. Besides, some recognized hormonal factors may concur to reduce the mineralization of maternal bone during pregnancy, such as the rise of the parathyroid hormone-related protein (PTHrP) or the progressive increase of oxytocin which activate the osteoclasts and stimulate the calcium transport to the fetus.

* Corresponding author at: Department of Medicine and Surgery, Obstetrics and Gynaecology Unit, University of Parma via Gramsci 14, 43126 Parma, Italy.
*E-mail address:* tullio.ghi@unipr.it (G. Tullio).
[1] Equal contributors in alphabetical order.

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Eventually lifestyle may have a negative impact on the bone mass during pregnancy. The physical activity or the sun exposure may be lower than usual in pregnant women, particularly in the third trimester [5].

Based on the concomitant effect of all these factors, a net reduction of women's bone mineral density (BMD) is purported to occur during pregnancy.

However, this alleged demineralization of maternal bone during pregnancy has never been demonstrated or quantified due to lack of an appropriate method of BMD assessment which can be safely employed across gestation.

Actually, the use of dual-energy X-ray absorptiometry (DEXA), which is still widely considered as the gold standard method in determining the amount of bone loss, is limited by the potential harmful effects of radiation during pregnancy.

Recently, an innovative ultrasound-based technique has been introduced in the clinical practice for an accurate estimation of the BMD on the central reference anatomical sites such as the femoral neck or the lumbar spine. This method, known as Radiofrequency Echographic Multi Spectrometry (REMS), has been shown to be as reliable as DEXA in the diagnosis of osteoporosis among non-pregnant women [6].

The aim of the present work was to assess the BMD of the femoral neck by means of REMS technology in a group of healthy pregnant women and to compare its values with a reference non-pregnant control group.

## Materials and methods

### *Study design and study population*

This was a prospective case-control observational study conducted at the Department of Medicine and Surgery of the University of Parma between February 2020 and December 2020. A non-consecutive series of pregnant women with uncomplicated singleton pregnancy at or >37 weeks of gestation was recruited during antenatal routine visits or during routine third trimester ultrasound examinations.

For the purpose of the study, the included pregnant women were matched with a ratio 1:1 with a reference group of non-pregnant women on the basis the following characteristics: ethnicity, age and body mass index (BMI). For each considered case, the BMI matching was done using the weight and height referred by the patient before conception.

Exclusion criteria were presence of current or previous medical conditions which could potentially interfere with the bone metabolism (e.g. thyroid, liver, kidney disease etc.); walking disability; history of bone fractures or recent traumatic fractures; previous diagnosis of osteopenia or osteoporosis according to the Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases (SIOMMMS) criteria [7]; vitamin D or other drug intake during pregnancy; BMI > 40 kg/m$^2$; age < 18 years; smoking addiction or chronic consumption of drugs including steroids or anticonvulsants.

All pregnant women reported to assume regularly folic acid and multivitamins since the early stages and the 16th week of pregnancy, respectively.

Gestational age was calculated from crown-rump length measure between 11(+0) and 13(+6) weeks of gestation or head circumference if the first ultrasound scan was performed after 14 weeks.

The study protocol was approved by the Local Ethic Committee (number 32888). All the enrolled patients voluntarily entered the study after giving written informed consent.

### *Measurements*

REMS technology consists in a fully automatic algorithm implemented to calculate the same diagnostic parameters provided by a DEXA examination (BMD, T-score and Z-score) starting from an ultrasound scan of both lumbar spine and femoral neck.

With REMS approach, the unfiltered radiofrequency (RF) signals acquired during an ultrasound scan of the bone region, are automatically processed and compared with previously derived reference spectral models of healthy and osteoporotic bones in order to assess the investigated bone health status [8]. This approach exploits the maximum information from the acquired signals, that is usually discarded in a conventional ultrasound-based approach, whereas the reconstructed B-mode ultrasound images serve as a guide for the correct identification of the investigated bone [9].

The implemented simultaneous acquisition of several RF signals for each frame is integrated with ultrasound imaging, which are utilized as a guide for the identification of the region of interest (ROI), providing a solid and reliable statistical basis for subsequent spectral analyses [6].

The included women underwent a sonographic examination of the proximal femur using REMS technology. Measurements were obtained by an expert trained operator [6] using an EchoStation device (Echolight Spa, Lecce, Italy) equipped with a convex probe operating at the nominal frequency of 3.5 MHz according to a standard procedure.

More specifically, a 40-seconds software-guided ultrasound scan was performed with the ultrasound probe placed in correspondence of the head-neck axis of the femur. The ultrasound beam was placed parallel to the femur long axis, transducer focus and scan depth were appropriately set in order to have femoral neck interface in the beam focal zone and in the central part of the image [10].

Then, the software automatically analyzed the unfiltered ultrasound signals, identifying the bone interface and the ROI; processing the deriving signals and, after this automatic process, producing the diagnostic report.

### *Outcome*

The primary outcome of the study was the comparison of the BMD between pregnant and non-pregnant women matched for age, ethnicity, and pre-pregnant BMI.

The secondary outcome of the study was the analysis of the overall effect of the following characteristics on the BMD during pregnancy: maternal age, ethnicity, parity, pre-pregnant BMI.

### *Statistical analysis*

Continuous variables were reported as average value ± standard deviation (SD), and T-test were performed to assess the statistical significance between patient groups.

Univariate regression analysis was performed to assess the correlation between femoral BMD values and patient characteristics (such as age, BMI, ethnicity and parity). For continuous variables, the linear correlation with femoral BMD was represented through scatterplots and related Pearson correlation coefficient *r*. The variables with $p < 0.1$ at univariate analysis were included in the multiple regression analysis.

The calculations were performed using MATLAB® (R2018a, MathWorks, Natick, MA), MedCalc (version 19.6, MedCalc Software Ltd, Mariakerke, Belgium) and R 3.6.1 (RStudio version 1.1.456).

## Results

Overall, 78 pregnant women at a mean gestational age of 39.1 ± 1.5 weeks (range: 37.0 to 41.4 weeks) were enrolled, with mean age of 32.9 ± 5.0 years and pre-gestational mean BMI 23.46 ± 3.89 kg/m$^2$. The control group was represented by non-pregnant women, whose age and BMI were 32.9 ± 5.2 years and 23.07 ± 2.77 kg/m$^2$, respectively.

The main demographic and anthropometric characteristics of both the study and the control group together with the densitometric parameters are shown in Table 1.

The mean femoral BMD measured in the pregnant women was significantly lower than non-pregnant controls (0.769 ± 0.094 g/cm$^2$ vs 0.831 ± 0.101 g/cm$^2$, p = 0.0001).

Considering the group of non-pregnant women as reference, the mean relative reduction of BMD in pregnant women was 8.1%.

At univariate linear regression, femoral BMD appeared positively associated with BMI (p < 0.001) and negatively with age (p = 0.040) and Caucasian ethnicity (p = 0.0001). These results were confirmed at Pearson correlation analysis, with $r$ = 0.75 (p < 0.001) between femoral BMD and BMI (Fig. 1a) and $r$ = -0.23 (p = 0.042) between femoral BMD and age (Fig. 1b). Considering ethnicity, BMD distributions in Caucasian and non-Caucasian patients are shown in Fig. 2a, whereas Fig. 2b shows the BMD distributions in nulliparous vs parous women.

At multiple regression analysis, the association between femoral BMD and patients' BMI, age and ethnicity remained statistically significant (p = 0.0004, <0.0001 and <0.0001, respectively).

The results of the univariate and multivariate analyses assessing the association between BMD at femoral neck and maternal characteristics are reported in Table 2.

**Table 1**
Demographic and anthropometric patients' characteristics. Results are reported as average value ± standard deviation, p-value are obtained using T-test.

| | Pregnant women (n, ± SD) | Non-pregnant women (n, ± SD) | p-value |
|---|---|---|---|
| Age (years) | 32.9 ± 5.0 | 32.9 ± 5.2 | 0.95 |
| Height (m) | 1.65 ± 7.2 | 1.66 ± 6.5 | 0.54 |
| Weight (kg) | 63.9 ± 11.0 | 63.1 ± 11.2 | 0.38 |
| BMI (kg/m$^2$)* | 23.46 ± 3.89 | 23.07 ± 2.77 | 0.64 |
| Ethnicity | | | |
| Caucasian | 68 | 68 | Not available |
| Non-Caucasian | 10 | 10 | |
| o African | 8 | 8 | |
| o Asian | 2 | 2 | |
| Parity | | | |
| 0 | 36 | | Not available |
| 1 | 35 | | |
| 2 | 6 | | |
| 3 | 1 | | |
| BMD (g/cm$^2$) | 0.769 ± 0.094 | 0.831 ± 0.101 | 0.0001 |
| T-score | −0.7 ± 0.9 | −0.2 ± 0.9 | 0.0001 |
| Z-score | −0.7 ± 0.8 | −0.1 ± 0.8 | <0.0001 |

*In pregnant women, body mass index (BMI) was obtained considering pregestational weight.

## Discussion

### Principal findings

The main finding of our study is that the measurement of BMD during pregnancy is feasible with the REMS method. By means of this technique we demonstrated that pregnant women have a significant lower BMD compared with non-pregnant ones; the BMD at femoral neck was found to be positively correlated to the pre-pregnant BMI and negatively correlated with the maternal age. In addition, we reported a lower femoral neck BMD in Caucasian women compared with non-Caucasian, whereas parity does not seem to impact femoral BMD.

### Study interpretation

During pregnancy, parathyroid hormone (PTH), PTHrP, 1,25-(OH)2-D3, calcitonin and estrogen levels are acknowledged among the key factors in the regulation of bone metabolism. Although the increased estrogen levels promote the synthesis of new bone tissue, the concurrent fall in the PTH release and its replacement by the placental-produced PTHrP facilitate the bone resorption [1,11].

These hormonal changes, together with the placental transfer of calcium as a contribute to the fetal skeletal development, may account for the decreased femoral BMD in pregnant women when compared with non-pregnant ones [1].

On the other hand, the restoration of ovarian hormone production and the concurrent drop of the PTHrP are responsible for the recovery of the BMD after pregnancy [12–14], and this seems to explain why parity does not seem to influence the maternal BMD values.

The maternal BMD increases after puberty through the years, till a peak around the age of 30, after which its level progressively decreases. Moreover, estrogens are also produced by the peripherical conversion of androstenedione in estrone in the adipose tissue [15], thus it may be plausible that the BMD loss in pregnancy in younger women with a higher BMI is more limited [16].

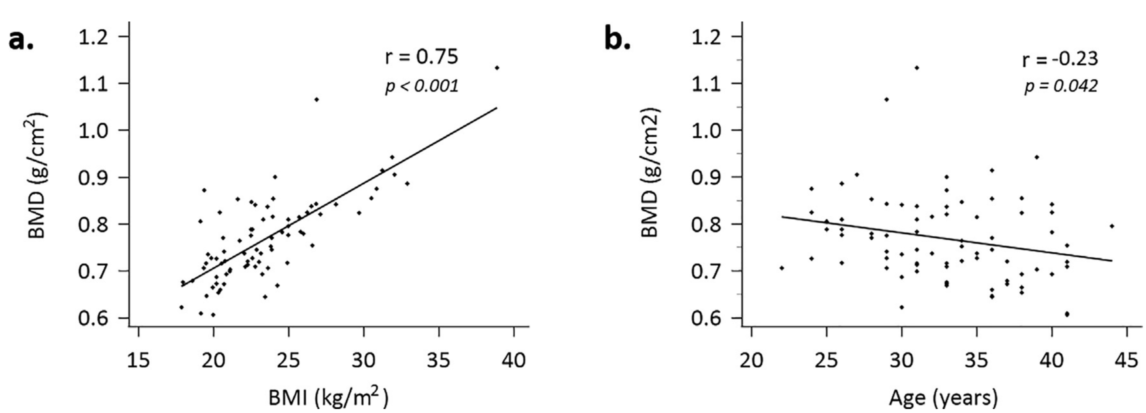


**Fig. 1.** Scatterplot representation of the correlation between femoral bone mineral density (BMD) and pre-pregnant body mass index (BMI) in panel A, and age in panel B. Pearson correlation coefficient r were also reported.

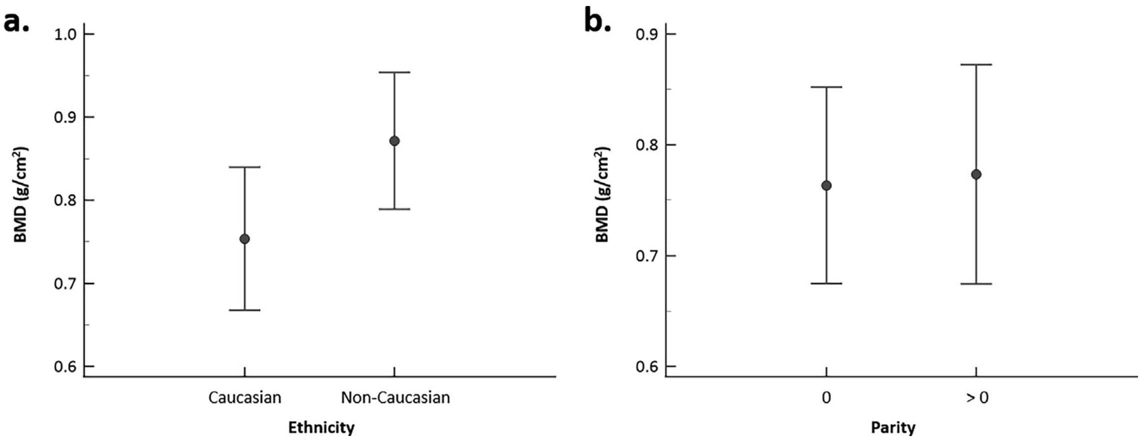


**Fig. 2.** BMD distributions in the study group of pregnant women, considering the differences between Caucasian and non-Caucasian women, in panel A, and between patients at first pregnancy (Parity = 0) or with previous parities (Parity > 0), in panel B. The central dots represent the average BMD values and the whiskers represent the standard deviation.

**Table 2**
Univariate regression showing the association between femoral BMD and patients' characteristics. Ethnicity and parity were investigated as Caucasian versus non-Caucasian and nulliparous versus non-nulliparous, respectively.

| | | Estimate | Std. Error | T-value | p-value |
|---|---|---|---|---|---|
| Univariate analysis | Age | −0.00435 | 0.00208 | −2.0943 | 0.0396 |
| | BMI* | 0.01815 | 0.00183 | 9.9250 | <0.0001 |
| | Ethnicity | 0.11777 | 0.02902 | 4.0576 | 0.0001 |
| | Parity | 0.00987 | 0.02144 | 0.4603 | 0.6470 |
| Multivariate analysis | Age | −0.00400 | 0.00109 | −3.67688 | 0.0004 |
| | BMI* | 0.01763 | 0.00141 | 12.51189 | <0.0001 |
| | Ethnicity | 0.09995 | 0.01632 | 6.12478 | <0.0001 |

* Body mass index (BMI) was obtained considering pregestational weight.

Lastly, hormonal levels, dietary vitamin D and calcium intake as well as other factors including sun exposition and lifestyle may be also related to the ethnicity whose impact on maternal BMD values and its changes during pregnancy has been shown also in this study [17].

*Previous studies*

Several studies evaluated the BMD changes throughout pregnancy and their association with maternal characteristics.

Hellmeyer et al. performed Quantitative Ultrasonometry (QUS) of the phalanges in 60 healthy, pregnant women. A significant reduction of peripheral BMD was found in each trimester of pregnancy; moreover, BMD was found to be significantly lower in the second and third trimesters compared with the first (mean amplitude dependent speed of sound measured by QUS for the first, second and third trimester were 2066.2 m/s, 2048.4 m/s and 2027.8 m/s, respectively), irrespectively from the gestational weight gain [18].

Similar results were reported in the study by Moller et al., that measured BMD and body composition in 153 women in each pregnancy trimester by using DEXA. Compared with the changes in non-pregnant women, they reported a mean BMD decrease of about 1.8% at the lumbar spine, 3.2% at total hip, 2.4% at the whole body and 4.2% at the ultra-distal forearm between measurements before conception and postpartum [19].

As regard the positive association between the BMD values and the BMI in pregnancy, our study is consistent with previous literature data. In 2019, Eroglu et al evaluated the BMD values among 93 females aged 18–40 years within 30 days after delivery by means of DEXA technology and reported a significant positive correlation between the post-partum BMI and the BMD values measured at lumbar vertebrae, femoral neck and femoral total bone ($p = 0.011$, 0.026 and 0.026, respectively) [20].

While the negative effect of the advanced age on BMD, mainly due to the drop of the estrogen levels especially after the menopause, has been widely demonstrated and discussed [21], less is known about the effects of ethnicity on the bone mineralization. Previous studies found that ethnicity influences the age at which the BMD peak is achieved [22]. More specifically, Caucasian women reach the femoral BMD peak earlier than non-Caucasian women, but at the same time, a more rapid decline in BMD following the peak in the former group has been reported [22].

Eventually, other studies investigated the association between maternal parity and BMD. In a prospective study on 91 multiparous women and 31 nulliparous women, Terzi et al. found no difference between the BMD of multiparous and nulliparous women [23].

In addition, a recent metanalysis evaluated the effect of parity on the BMD. The overall effect of parity on bone mineral density was positive ($p = 0.001$). The effect appears site-specific as parity was not significantly associated with the BMD of the femoral neck ($p = 0.09$) and lumbar spine ($p = 0.17$), but parous women had significantly higher BMD of the total hip compared to nulliparous women ($p = 0.006$) [24].

*Clinical implications*

According to the WHO, osteoporosis leads to an increase in bone fragility and susceptibility to fracture. Early diagnosis is essential for a prompt and effective identification and treatment of patients at risk for osteoporotic fractures. The diagnosis of osteopenia and osteoporosis relies on the quantitative assessment of the BMD [25].

DEXA still represents the gold standard method for BMD assessment [25], but its use is not allowed in pregnancy, due to concerns regarding fetal exposure to ionizing radiations.

Several studies have demonstrated that REMS technology is as accurate and reproducible as DEXA in the assessment of BMD at femoral neck [6,26]. Recently, this approach has been validated as diagnostic tool in female patients aged 30–90 years and its diagnostic accuracy based on femoral neck BMD assessment showed a sensitivity and specificity of 90.4% and 95.5%, respectively, compared to DEXA [27]. In addition, REMS technology is considered safe for the fetus due to the use of ultrasound technology.

Based on these considerations, REMS may be suggested as the new gold standard for the evaluation of the BMD in pregnant women.

Other advantages of REMS over DEXA are in the lower costs and the availability in primary care settings without need of dedicated structures or certified operators, which allow its use in an outpatient setting.

A further feature of the adopted approach is the extreme ease of use, due to a simple and friendly acquisition procedure followed by fast and fully automated data processing, which contribute to high reproducibility rates of the examinations. All these features allow its use for extensive mass screening.

This pioneer study may be considered as a model for future researches. Further studies are needed to develop customized BMD curves during pregnancy to promptly recognize and treat women at higher risk of osteoporosis or to longitudinally assess women with known pathological conditions (rickets, anorexia, osteogenesis imperfecta or assuming corticosteroids, cyclosporin or anticoagulants).

## Strengths and limitations

This is the first study matching pregnant and non-pregnant women with the aim of evaluating maternal BMD at femoral neck by using REMS technology.

The small sample size and the lack of a longitudinal assessment of the BMD throughout the pregnancy represent the main limitations of this study. Therefore, this has to be considered as an exploratory, proof of concept study.

Moreover, most of the non-Caucasian women were African. It is well known that blacks have higher BMD than Caucasians, and also that Asians tend to have lower values [17,28]. Therefore, given the small number and the mixed composition of the non-Caucasian, the influence of ethnicity on BMD at femoral neck is still to be confirmed.

Likewise, the lack of association between parity and BMD needs to be reassessed on a larger population since in this series only 7-women have had more than one childbirths.

Furthermore, the weight and height of the enrolled patients were not measured at the time of enrollment, but simply reported by the patients themselves. Finally, the matching with non-pregnant patients was done indirectly by considering the reported pre-gestational weight of pregnant women. The BMD data of the control group were therefore obtained from the device's proprietary database in accordance with the preselected characteristics of the women.

## Conclusion

Our data shows that in pregnant women the evaluation of BMD at femoral neck by using REMS technology is feasible. In this exploratory and proof of concept study, for the first time a decreased BMD at femoral neck has been objectively demonstrated with a safe and reproducible method.

As expected, the BMD values obtained at the end of pregnancy are correlated to anthropometric and demographic maternal parameters.

In perspective, REMS method would allow both to identify pregnant women with osteopenia or osteoporosis and to monitor those with risk factors for bone loss.

New studies are required to assess the longitudinal changes of maternal BMD throughout the pregnancy.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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