# INTRODUCTION

Pregnancy defines a challenging period to the mother's bones because the building of the fetal skeleton requires a substantial transfer of calcium. This process is particularly intense during the third trimester, when fetal bones experience substantial growth and calcification. The regulatory mechanisms are still poorly understood, but it seems patent that the drainage of calcium from the mother has to bear some level of deterioration of the maternal skeleton unless compensatory mechanisms of enough potency exist. If an adequate balance is not achieved, pregnancy would define a vulnerability period from maternal bones. Osteoporosis or even fragility fractures might be conceived whether the decalcifying process is particularly intense or whether there is an osteopenic background. In fact, isolated cases of osteoporosis or fragility fractures have been described before, however the process has to be transitory, because pregnancy has not been detected as a risk factor for postmenopausal osteoporosis ***(Smith R. et al., 2011)***.

Various methods to assess the changes in bone mineral density (BMD) during pregnancy have been studied. The use of standard dual-energy X-ray absorptiometry (DXA) in pregnant women has been limited by the potential harmful

effects of radiation during pregnancy. Studies that utilize this method for assessments would obtain measurement in women before pregnancy and then repeat it in the early postpartum period, yet the actual changes during pregnancy could not be assessed. The recent development in quantitative ultrasound methods for assessment of BMD in pregnancy carries the particular advantage of being free from irradiation effects ***(Pluskiewicz W. et al., 2004)***.

Ultrasonography (USG) measurements have been found to correlate well with BMD measurements compared to conventional DXA methods in non-pregnant subjects, and could be used alone for prediction of fracture risks in postmenopausal women. Ultrasound measurements may be performed at different sites, including the tibia, os-calcis, metacarpals and phalanges. ***(Pluskiewicz W. et al., 2004)***.

Serial USG measurements across advancing gestations in pregnancy have been able to show consistent progressive BMD loss in different bone sites. The degree of BMD changes during pregnancy has been correlated with higher bone turnover as indicated by biochemical markers, as well as with maternal characteristics such as low initial BMD in early pregnancy and high body fat accumulation during pregnancy. It could also be related to calcium intake and mechanical

stress or increased bone loading during pregnancy ***(Tranquilli AL. et al., 2004)***.

Differential changes in BMD in different bone sites have also been found in different studies. In a study by Naylor, BMD increased in the arms (+2.8%) and legs (1.9%) but decreased in the pelvis (-3.2%) and lumbar spine (-4.6%), a phenomenon that was described as redistribution of bone mineral, consistent with the hypothesis that decrease in bone mass during pregnancy occurred more at trabecular bone sites, while sites consisting of cortical bone showed either no change or even a tendency towards gains in BMD. While the studies quoted in the previous section have described bone loss during and after pregnancy, such loss should be physiological and mild ***(Naylor KE. et al., 2002)***.

The risks of sustaining fractures during pregnancy due to such degree of BMD should indeed be rare, as these women are still in reproductive age groups and should still have a sufficiently high level of bone mass to prevent against atraumatic fractures. However, rarely, fractures are sustained during pregnancy in those with transient but severe loss of bone mass during their gestational period. This should be considered as a pathological process,

commonly named transient osteoporosis of pregnancy ***(Karlsson MK. et al., 2005)***.

Transient osteoporosis of pregnancy is a rare, idiopathic, self-limiting condition typically occurring in the third trimester of pregnancy. The mean gestation to occur is usually reported to be around 33 weeks, but can range from 6 weeks to term. It almost always affects a single hip although bilateral presentation or knee involvement and lumbar spine involvement have also been reported ***(Uematsu N. et al., 2005)***.

Quantitative ultrasound measurements (QUS) appear to be the most preferred method of assessment during pregnancy, as it is free of radiation exposure, and can still be utilized for monitoring in the postpartum period. There is also good supporting data of the correlation of QUS to fracture risks, or for longitudinal surveys to asses BMD changes over a time interval in different populations. Thus, one of the main objectives of the thesis would be to explore the use of QUS of the os-calcis in monitoring pregnancy BMD changes ***(Bullo M. et al., 2005)***.

The calcaneus is the most popular measurement site for several reasons. The calcaneus is 90% cancellous bone, and

because of its high surface to volume ratio has a higher metabolic turnover rate than cortical bone. The calcaneus is also readily assessable, and the mediolateral surfaces are fairly flat and parallel, thus reducing repositioning errors ***(Vogel JM. et al., 1988)***.

Quantitative ultrasound devices currently measure parameters related to either of two basic properties: bone ultrasound attenuation (BUA) and the apparent velocity of wave propagation or speed of sound (SOS). While bone quality may not be entirely reflected by bone mass or density alone, the BUA and SOS evaluations are the most readily measured and reproducible parameters using ultrasound methods of bone assessment ***(Cortet B. et al., 2004)***.

# AIM OF THE WORK

The aim of the current study is to measure directly bone mineral density using quantitative ultrasound measurement of the os calcis among first and third trimester pregnant women, so we can assess the influence of pregnancy on bone mineral density.

**Research question:**

Does pregnancy affect bone mineral density?

**Research hypothesis:**

Differences in bone mineral density suggest that pregnancy is associated with deterioration of bone mass in the mother.

**Clinical Trial Identifier:**

NCT02480023

**Study design:**

Cross sectional observational case control study.

*Chapter (1)*

# OSTEOPENIA AND PREGNANCY

**Bone**

Bone is a living tissue that is constantly being removed and replaced. Building a large bone mass early in life, can help to reduce the risk of developing osteoporosis in later life. Bone mineral density normally increases steadily from birth and approaches its peak value by early adult life, depending on the skeletal site, and remains stable for some years. The greatest increase in bone occurs prepubertal 8-12, depending on the child to the early 20's, due to the hormones that are produced around puberty. This is a very important period of bone growth, during these years, the greatest amount of bone is formed and this is known as "Peak Bone Mass" For instance Peak bone mass occurs at the proximal femur in women at about 18-20 years of age, spine 20-25 years of age and the skull may continue to gain bone mass right through the 4th and 5th decades of life ***(Kahl et al., 2005)***.

BMD is on average lower in women than in men, because women have smaller bones and smaller trabeculae. Women lose more bone on average in their lifetime than men, as they also go

through the menopause, 35-40% in men Vs 50% in women. Muscle contraction increases bone strength and immature bone responds better to the stimulus of muscle contraction than mature bone ***(Birge et al., 1993)***.

Weight bearing exercise is essential in young people, as not only can it reduce their risk of developing osteoporosis but also many other problems such as: obesity, hypertension, Type 2 diabetes, heart disease, strokes, low self esteem and depression.

Bone mass is the result of a dynamic lifetime balance between two processes: bone formation and bone resorption. Bones require normal levels of sex hormones, adequate caloric intake, particularly protein, calcium and vitamin D and regular weight bearing exercise. The rate of bone turnover is determined by hormonal and local factors, as well as systemic factors, illnesses and genetics ***(Birge et al., 1993)***.

Up to the age of 20, more bone is laid down than is lost. Following that, depending on the skeletal site, the amount of bone lost and replaced is approximately the same, between the late twenties and early forties in healthy persons. The rate of bone turnover is affected by many factors, including sex hormones such as oestrogen and testosterone, vitamin D and parathyroid hormone, and many cytokines and chemokines

including tumour necrosis factor alpha, Receptor Activator of Nuclear Factor Kappa B (RANK), RANK Ligand, and its naturally occurring decoy receptor osteoprotegerin (OPG) ***(Hofbauer et al., 2004)***.

Throughout the skeleton there are basic remodelling units where signals from a resorption pit signals through a variety of factors resulting in activation of osteoclasts and bone resorption at that site, known as a resorption pit. This results in a coupled signal to osteoblasts which form new bone. When more bone is formed there is net bone gain and vice versa as bone is lost. It is estimated that each human skeleton is remodelled in its entirety several times in the average adults living into their 8th decade ***(Khan et al., 2001)***.

In today's life style, too little or excessive exercise, combined with a low calorie diet, low intake of calcium and vitamin D and an excessive fiber content, are counter productive to achieving an adequate peak bone mass. Adolescence is also the time, when there is an increased risk of eating disorders ***(Khan et al., 2001)***.

**Bone metabolism and bone mineral density changes in pregnancy**

Normal adaptative responses during pregnancy and lactation allow for adequate delivery of minerals to the fetus or

infant, while at the same time protecting to a certain degree the maternal skeleton. The maternal skeleton remains the most important source of calcium to the fetus during pregnancy. Theoretically, bone mass may decrease in response to this transfer of calcium from the maternal skeleton, bone mineral density may also increase as a result of the rise in oestrogen levels in late pregnancy, so that overall bone mineral density may decrease, remain unchanged or increase ***(Sowers et al., 1996)***.

The full term neonate contains around 30 g calcium in it skeleton while the maternal skeleton, on the other hand, should contain around 1000 g of calcium. Thus, a theoretical magnitude of bone loss of 3-4 % is logical under the assumption that the fetal calcium requirement is completely supplied by the maternal skeleton. This calculation, however, has not taken into account the maternal calcium homeostasis and adaptation of this homeostasis during pregnancy ***(Ulrich et al., 2003).***

However, it is obvious that pregnancy and lactation do pose significant stress to maternal calcium metabolism. The long term consequences of pregnancy and lactation to bone health and the possible associations and risks of subsequent osteoporosis in menopausal age have long been debated. The relevant literature in relation to changes in bone metabolism during pregnancy will be reviewed ***(Hosking et al., 1996)***.

**Calcium metabolism in pregnancy - the role of calcitriol and parathyroid hormone**

Renal conservation of calcium has been found to be less efficient in pregnancy, while active intestinal absorption of calcium was found to increase two-fold by the end of the second trimester. Pregnancy has been classically called a 'physiologic absorptive hypercalciuric state". The increase in intestinal absorption might reflect an elevation in circulating 1, 25-dihydroxyvitamin D (calcitriol) or possibly a direct intestinal effect of oestrogen to stimulate calcium transport Increases in calcitriol concentrations from 15 – 60pg/ml in the non-pregnant state to 80-120 pg/ml in the pregnant state. The process might begin as early as the first trimester, and be achieved in late pregnancy**.** Calcitriol is produced in the placenta as well as in the kidneys in pregnancy. Though the circulating vitamin D binding protein increases, 'free' calcitriol concentrations are also elevated, as are circulating albumin-adjusted calcium or ionised calcium concentrations and urinary calcium excretion ***(Kent et al., 1993)***.

While the calcium balance in pregnant women is positively maintained by these mechanisms, there is no consistent relationship found between intestinal calcium absorption and either dietary calcium intake or any index of

calcitriol ”bioavailability” in pregnant women. In contrast, although maternal calcitriol production increases with lactation, it does not achieve the same concentrations seen during pregnancy, and there is no associated increase in intestinal calcium absorption. Instead, calcium is conserved by a renal mechanism, with decreased urinary calcium excretion with lactation that may remain low for as long as six months post weaning ***(Kent et al., 1991).***

Pregnancy has previously been considered to be a hyper-parathyroid state, yet recent evidence using specific immuno-radiometric assays for the intact parathyroid hormone (PTH), have shown that PTH remained stable and within the normal range throughout pregnancy. A state of functional hyperparathyroidism may still occur, however, reflecting the increased concentrations of PTH-related protein during pregnancy, particularly in the third trimester. Sources of circulating PTH related protein in pregnancy likely include the placenta and/or the breast. Hypercalcaemia and hypercalciuria can be associated with lactation and extremely high PTH related protein concentrations. On the other hand, hypersecretion of calcitonin during pregnancy and /or lactation has been proposed to buffer the actions of PTH and PTH related protein on maternal bone ***(Bertelloni et al., 1994)***.

**Observed Physiological changes of calcium metabolism in pregnancy**

Such physiological changes were well demonstrated in the following two longitudinal studies designed to determine the effect of stages of reproduction on calcium and bone metabolism. Ten women were followed up serially when non-pregnant, non lactating, at the end of each trimester of gestation, at 3 months post-delivery lactating, and post-weaning. Fractional calcium absorption was found to be higher and concentrations of 1,25 –dihydroxyvitamin D were higher in the second and third trimesters. Total urinary calcium was higher during pregnancy and lower post-weaning. Parathyroid hormone concentrations were higher only postweaning. Markers of bone turnover, including serum tartrate resistant acid phosphatase and bone specific alkaline phosphatase, and urinary deoxypyridinoline, increased in the third trimester and during lactation. Serum procollagen I carboxypeptides increased only in the third trimester. Bone mineral density measured by single-photon absorptiometry did not differ by any period of measurement. The authors concluded that absorption and urinary excretion of calcium increased during pregnancy and lactation, but only renal changes consistent with an increase in PTH were seen post-weaning ***(Cross et al., 1995)***.

In 14 well-nourished women consuming approximately 1200mg calcium/day, measurements were made before conception, once during each trimester of pregnancy, early in lactation at 2 months postpartum, and 5 months after resumption of menses. Intestinal calcium absorption was determined from the enrichment of the first 24-hr urine sample collected after administration of stable calcium isotopes.

BMD of the total body and lumbar spine were measured by DXA and quantitative CT scan respectively. Twenty-four-hour urine samples and fasting serum samples were analyzed for calcium, calcitropic hormones and biochemical markers of bone turnover. Despite an increase in calcium intake during pregnancy, true percentage absorption of calcium increased from 32.9% to 49.9% at the second trimester and 53.8% at the third trimester. Urinary calcium increased from 4.32 mmol/day at prepregnancy to 6.21 mmol/day at the third trimester, but only minor changes in maternal bone mineral density were detected. At early lactation, dietary calcium and calcium absorption were not significantly different from the pre-pregnancy levels, but urinary calcium decreased to 1.87 mmol/day and trabecular BMD of the spine decreased significantly from 162.9 to 147.7 mg/cm3. Calcium absorption post menses increased non-significantly to 36% whereas urinary calcium decreased to 2.72 mmol/day. It was concluded that fetal calcium demand was met

by increased maternal intestinal absorption; early lactation milk calcium was provided by maternal renal calcium conservation and loss of spinal trabecular bone, this loss being gradually recovered post-menses ***(Richie et al., 1998)***.

**Clinical data on BMD changes in Pregnancy**

Under normal circumstances, the greater efficiency of intestinal calcium absorption during pregnancy meets the calcium demand. An interesting study on bone architecture compared transilieal bone biopsy samples from two groups of pregnant women in first trimester and third trimester of pregnancy. It was shown that there was a fluctuation in the cancellous bone volume in which early temporary bone loss through trabecular thinning is restored entirely through the addition of new trabeculae to produce a complex system of thinner but more numerous bars near term. How such changes in bone architecture should be interpreted in terms of bone density changes as measured by various techniques remained uncertain. There is at present no consensus as to the impact of reproductive events on bone mineral density on the ultimate BMD after pregnancy or in later life. The results from observational studies available in the literature are conflicting and controversial ***(Shahtaheri et al., 1999)***.