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Endochondral Bone Growth During Early Pregnancy Compared with Pseudopregnancy in Rats

Beth M. Bowman and Scott C. Miller

Division of Radiobiology, School of Medicine University of Utah, Salt Lake City, UT

There are physiological and skeletal changes that occur during pregnancy to accommodate the increased calcium needs of late pregnancy and lactation in the rat. Endochondral bone growth is accelerated during early to midpregnancy, but the endocrine basis of this is not clear. The purpose of this study was to define the role, if any, of placental factors in changes in endochondral growth by comparing changes that occur during pregnancy with pseudopregnancy in the rat. Many hormones change during pseudopregnancy, except placental hormones (e.g., placental lactogens) because a placenta is lacking. Rates of endochondral growth were increased during pregnancy and pseudopregnancy compared to age-matched, unmated controls. There were also increases in body weight in both pregnant and pseudopregnant animals. Since the observed changes occur in both pregnant and pseudopregnant animals, this indicates that endocrine factors other than those secreted by the placenta are involved in increased growth during early pregnancy.

Key Words: Pseudopregnancy; pregnancy; endochondral growth; placental lactogen; rat.

Introduction

During early to midpregnancy, an accumulation of calcium in skeletal tissues has been observed in sheep *(1)*, rats *(2)*, and humans *(3,4)*. It has been suggested that the accumulation of calcium is a maternal adaptive response to accommodate the calcium needs of fetal skeletal mineralization during later pregnancy and milk production during lactation.

The skeletal mechanisms involved in the accumulation of calcium appear to include those that optimize the ability to store more calcium, yet preserve structural and weight bearing capabilities. In rats, endochondral bone elongation is increased during early to midpregnancy, which also increases the rate of production of new cortical and cancellous bone in the metaphyseal regions (endochondral osteogenesis), particularly in the long bones *(5)*. Concomitant with increases in endochondral osteogenesis, periosteal bone formation also increases during pregnancy *(6)*. New periosteal, cortical, and cancellous bone would permit a greater accumulation of calcium as well as increasing the structural capabilities of the skeleton to withstand increased mechanical loading associated with maternal weight gain during pregnancy. This accumulation of new bone may also serve to compensate for losses on endosteal and endocortical surfaces that occur during lactation *(6)*.

The endocrine basis of changes in endochondral growth is not clear, but could include the gonadal hormones (including estrogens, androgens, and progesterone), prolactin (PRL) and/or substances secreted by the placenta such as placental lactogens. Placental lactogens are members of the PRL and growth hormone (GH) gene family, and it is possible that the presence of a placenta is necessary for the changes in endochondral growth during early to midpregnancy. The ability of GH to effect changes in endochondral growth is well-recognized. The purpose of this study was to compare the changes in indices of endochondral growth in pregnant and pseudopregnant rats. During pseudopregnancy in rats there are many endocrine changes that are similar to a normal early pregnancy, but the animal lacks placental and decidual tissues. This study provides evidence that placental factors are not essential for changes in maternal endochondral growth during early to midpregnancy in rats.

Results

Serum progesterone (P) levels were determined to confirm the state of pseudopregnancy. Progesterone levels in the pregnant rats at the time of necropsy ranged between 80 and 130 ng/mL. The pseudopregnant rats, which at the end of the experiment were also near the end of pseudopregnancy, had P levels ranging from 24 to 63 ng/mL. Both the pregnant and pseudopregnant animals had higher levels of P than the controls, which were all below 20 ng/mL.

Body weights of the rats at the beginning of the study averaged 244 ± 10 g. The changes in body weight from d 6 to 11 of the experiment were averaged for each group (Fig. 1). During this period, the pregnant rats showed the

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Author to whom all correspondence and reprint requests should be addressed: Beth M. Bowman, Division of Radiobiology, Bldg. 586, University of Utah, Salt Lake City, UT 84112. E-mail: bbowman@msscc.med.utah.edu

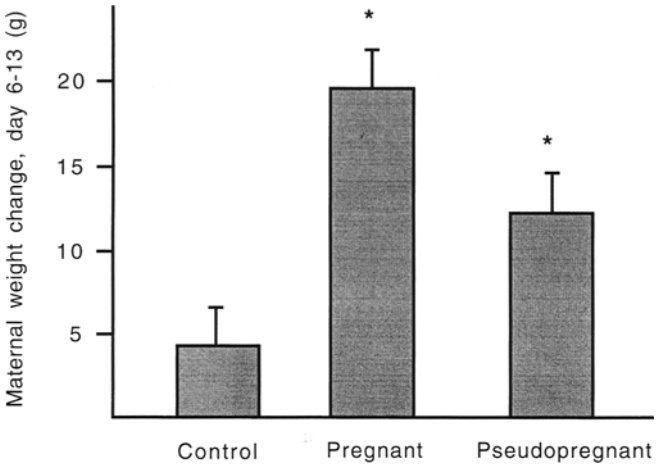

Fig. 1. Changes in weight from d 6 to 11 of pregnancy and pseudopregnancy and compared with age-matched, nonmated controls. The greatest increase in weight was observed in the pregnant animals (significantly different from both the controls and the pseudopregnant animals), but there was also a significant increase in the weight of the pseudopregnant animals when compared with controls. Data expressed in grams ± SD ($p < 0.0001$).

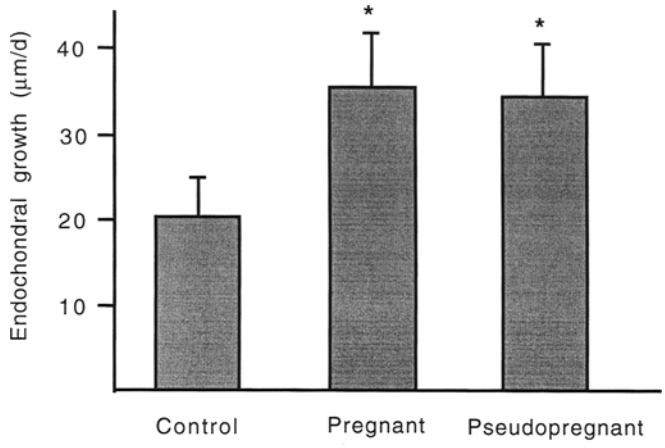

Fig. 2. Endochondral bone elongation rates measured at the distal femurs from d 6 to 12 during pregnancy, pseudopregnancy, and in age-matched, nonmated controls. There were significant increases ($p < 0.0001$) in endochondral growth in both the pregnant and pseudopregnant rats when compared with the controls. Data expressed in μm/d ± SD.

greatest increase of 20 ± 5 g, whereas the weight change in the pseudopregnant groups was 14 ± 4 g and the controls 5 ± 4 g. All groups were statistically different from each other.

The rates of endochondral bone elongation measured at the distal femoral growth plate were significantly increased in both the pregnant and pseudopregnant groups when compared to the age-matched, nonmated control group (Fig. 2). The controls had an average endochondral growth rate of 21.7 ± 3.1 μm/d, and the pregnant and pseudopregnant groups were 36.8 ± 6.1 and 34.9 ± 5.6 μm/d, respectively. The growth rates in the pregnant and pseudopregnant animals were not significantly different from each other. The fluorochrome-labeling patterns in the distal femoral metaphyseal primary spongiosa used to measure endochondral growth are illustrated in Fig. 3. The average growth plate widths in the pregnant and pseudopregnant animals were increased, but not significantly when compared with the controls (Table 1). There were no changes in the size of the hypertrophic lacunae, but the calculated rates of chondrocyte production in the growth plate were significantly increased in both the pregnant and pseudopregnant groups compared with controls.

Discussion

Pregnancy initiates high metabolic and nutritional demands on the mother. In the rat, there are also increases in somatic growth *(7)* and changes in the skeleton that include increased endochondral growth rates *(5)*, dentin appositional rates *(8)*, bone formation rates, and mineral appositional rates *(6)*. Miller et al. *(6)* suggested that increases in growth and bone mineral appositional rates during early to midpregnancy may be adaptive responses to prepare the maternal skeleton for the calcium demands associated with pregnancy and lactation.

The increased rate of endochondral bone elongation observed in the present study during pseudopregnancy was similar to that observed during a comparable period of pregnancy in age-matched rats. This increase in growth in both the pregnant and pseudopregnant rats, compared to unmated virgin controls, appears to be caused by an increase in the proliferation of growth plate chondrocytes, as the calculated rates of cartilage cell production per cartilage cell column in the growth plate were increased. There were also increases in the body weights in both the pregnant and pseudopregnant animals when compared with controls, though the increases were not as great in the pseudopregnant rats. The lack of a developing placenta in the pseudopregnant animals does not seem to effect these observed changes, which are similar to those in the true pregnancies, thus indicating that endocrine effectors other than those secreted by the placenta (e.g., placental lactogens) or decidual tissues are involved in increases in body weights and endochondral bone elongation during early to midpregnancy in the rat. These data are also consistent with other observations made during pseudopregnancy that are similar to pregnancy including increases in uterine weight, uterine blood flow, and glomerular filtration rates *(9,10)*.

The placental lactogens (PL) were considered as possible mediators of endochondral and perhaps somatotrophic growth during early pregnancy because evolutionarily they are related to the family of GH and PRL *(11–13)*. PL have been shown to have some growth-promoting effects similar to GH. For example, weight gain, epiphyseal growth, chondroitin sulfate synthesis, and DNA synthesis have been demonstrated with hPL in rat tissues, whereas oPL has been reported to increase weight gain, endochondral growth, and

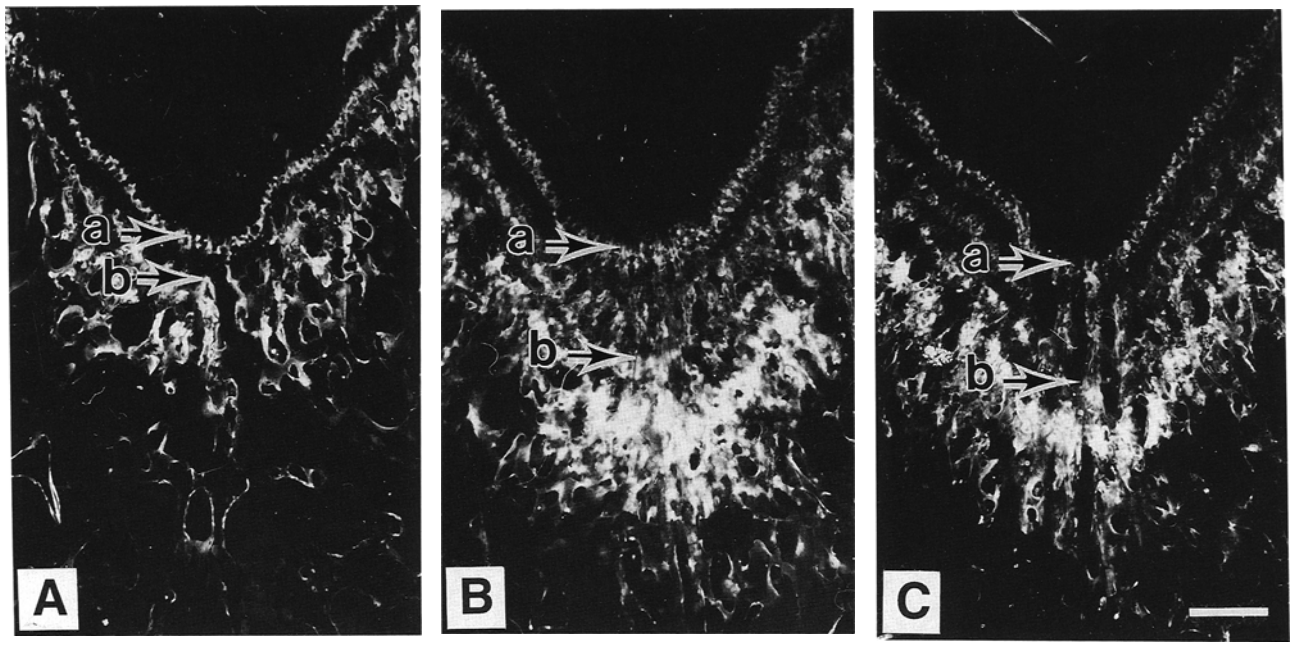

Fig. 3. Micrographs of the distal femoral metaphysis illustrating the fluorochrome bone markers in the primary spongiosa used to measure endochondral growth. Growth was measured from the top of the tetracycline label (a, arrow) to the top of the Calcein label (b, arrow). The pregnant animals **(B)** and pseudopregnant animals **(C)** had significantly greater endochondral bone growth when compared to the unmated control animals **(A)**. Bar, 200 μm.

Table 1
Growth Plate Thickness, Hypertrophic Cell Size and the Calculated Rate of Chondrocyte Production

Group	Growth plate thickness μm ± SD	Hypertrophic cell size μm ± SD	Cartilage cell production rate cells/day ± SD
Controls	127.4 ± 19.8	19.8 ± 1.3	1.11 ± 0.13
Pregnant	145.0 ± 33.6	20.6 ± 1.1	1.80 ± 0.37*
Pseudopregnant	135.0 ± 11.5	19.4 ± 1.1	1.80 ± 0.32*

Growth plate indices were measured at the distal femurs. The cartilage cell production rate was calculated from the endochondral bone elongation rates (Fig. 2) divided by the hypertrophic cell size. This measurement reflects the rate of chondrocyte production per cartilage cell column in the growth plate. The values given are the mean ± SD.

*Significantly different from controls, $p < 0.005$.

the production of insulin-like growth factor (IGF-I) *(14)*. Two rat placental lactogens, identified as rPL-I and rPL-II, are present in high concentrations in the maternal circulation during specific periods of gestation. PL-I is the midgestational PL associated with the choriovitelline placenta, the first of the two rodent placentas, which degenerates by d 14 of gestation. The presence of the rPL-I appears in the maternal circulation on d 8 of pregnancy and increases to a peak on d 12 *(15)*. However, in an earlier study, a modest, but significant increase in endochondral growth rate was observed during the first week of pregnancy in the rat *(6)*, prior to the reported appearance of rPL-I in the circulation. The second lactogen, rPL-II is found only later in pregnancy. This would support the observations made in the present study that placental hormones are not necessary for the increases in endochondral growth and some of the body weight gained during early to midpregnancy.

Likely mediators of endochondral and somatic growth during early to midpregnancy, other than the placental lactogens, are reproductive hormones. Estrogen (E) is a known suppressor of endochondral and somatic growth in situations other than pregnancy. Circulating levels of E decrease during early to midpregnancy and may be responsible for some of the changes in growth as observed in this study. For example, endochondral growth rates increase following ovariectomy in rats *(16)*, similar to those observed during pregnancy. The decreases is E, however, cannot account for some other observations made in skeletal tissues during pregnancy *(17)*.

The corpus luteum begins secreting high levels of P upon the initiation of pregnancy. The effects of P on bone are not clearly understood, however, P may increase bone formation during periods of low E, such as in the ovariectomized animal *(18,19)*. Recently it has been demonstrated that E lowers and P raises bone IGF-I mRNA, suggesting a local system for regulating bone formation *(20)*, and a possible explanation for the apparent bone-sparing effects of P. Besides the low serum levels of E and the high levels of P and prolactin during early pregnancy and pseudopregnancy, there are other endocrine factors, such as androgens and lutinizing hormone, which are increased during pseudopregnancy and pregnancy.

In summary, increases in endochondral growth and endochondral osteogenesis during early to midpregnancy may represent mechanisms to increase skeletal stores of calcium and perhaps improve the mechanical competence of the skeleton. Endochondral growth rates were about equally increased in pregnant and pseudopregnant rats indicating that placental and/or decidual hormones are not involved in this process. Other hormonal effectors and their relative ratios are likely involved and may include estrogens, progestins, androgens, and/or prolactins.

Materials and Methods

Animals

Female Sprague-Dawley rats, 80-d old were obtained from Simonsen Laboratories (Gilroy, CA). They were exposed to a daily photoperiod of 14 h light and provided lab rodent chow and water ad libitum. Vaginal smears were taken daily for 2–3 estrus cycles to record the length of a cycle in each animal. Animals that did not exhibit regular estrus cycles were not used. One group of females were mated at estrus and the following day considered d 1 of pregnancy if sperm were present in the vaginal smear. A second group of females were cervically stimulated at estrus with a glass rod for 30 s to induce pseudopregnancy *(21)*. If the rat was in diestrus the day following cervical stimulation, this was considered d 1 of pseudopregnancy (PSP). The control group consisted of females allowed to continue normal estrus cycles. Vaginal smears were continued daily for the duration of the experiment to insure that the pseudopregnant rats had not resumed estrus, and that the controls were maintaining a normal estrus cycle.

To assess endochondral bone elongation rates, fluorochrome markers were given to each animal. On d 6 of pregnancy and pseudopregnancy, the rats were given an intraperitoneal injection of 10 mg/kg of Calcein (fluorescien-methylene-iminodiacetic acid, Sigma Chemical Co., St. Louis, MO) and 1 d prior to necropsy, d 12 of pregnancy or pseudopregnancy, the rats were given an intraperitoneal injection of 25 mg/kg of tetracycline-HCl (Sigma). Age-matched, unmated control rats were given Calcein and tetracycline labels over a comparable time interval.

Tissue Preparation

The morning of d 13, the animals were killed and blood was collected by cardiac puncture and the serum was stored at –75°C until used for hormone assays. P was measured by radioimmunoassay with commercially available kits (Diagnostic Products, Los Angeles, CA).

The femurs were collected at necropsy, cleaned of soft tissue and fixed in 0.1*M* phosphate buffered formalin. After 24 h in fixative, the bones were dehydrated in ethanol and embedded in methyl methacrylate. Longitudinal, 8-μm thick sections of femurs were cut with a Reichert-Jung 2050 Supercut microtome. These sections were mounted unstained for observing the fluorochrome labels.

Body Weights

The animal weights were recorded on d 1 of pregnancy or pseudopregnancy, on the days of fluorochrome label injections and at necropsy. The change in body weight from d 6 to d 11 was calculated for each animal and then a mean calculated for each group. The mean change in weights for each group are expressed as g ± standard deviation (Fig. 1).

Endochondral Growth Measurements

Frontal sections of the distal femur were used to measure endochondral growth and the width of the growth plate. For endochondral growth, sections were viewed using a fluorescent microscope and the distance between the fluorochrome labels were measured with an eyepiece ocular grid at seven equidistant intervals along the epiphyseal growth plate. The average distance between the labels was divided by the time between injected fluorochrome labels and growth expressed as μm/d *(22)*. The growth plate thickness and height of the hypertrophic cell lacunae were measured in these same areas. Cartilage cell production rate was calculated by dividing the amount of longitudinal growth by the average height of the hypertrophic cell lacunae *(23)*.

Statistical Analyses

Statistical evaluation of the data used the one-factor analysis of variance (ANOVA) followed by a Dunnett's test. Values are expressed as means ± standard deviation. Significant levels of probability were <0.05.

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