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The effect of pregnancy and lactation on bone mineral density in fluoride-exposed rats

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Fluoride increases metabolic turnover of the bone in favour of bone formation. Excessive intake of fluoride may lead to pathological changes in teeth and bones: dental and skeletal fluorosis. In this study, we investigated the effect of pregnancy and lactation on bone mineral density (BMD) in fluoride-exposed rats. Female Wistar rats were given commercially available spring water with 100 ppm fluoride ($N=8$), or without addition ($N=8$) for 18 weeks. At 16 weeks of age, four female rats and one male rat were kept in a cage for 5 days; all females were successfully impregnated. BMD was measured at 16 weeks of age, on the first day postpartum, and at the end of lactation. Spinal BMD was significantly higher in fluoride-exposed rats than control ($P<0.05$), but there were no differences in femoral BMD ($P=0.670$). During pregnancy, spinal BMD and femoral BMD were not significantly changed in fluoride-exposed rats, whereas BMD of the spine was significantly decreased in the control rats ($P=0.013$), but not in the femur. During lactation, BMD was significantly decreased at the two regions compared to initial values ($P<0.05$) in both groups. This study shows that pregnancy has no effect on bone, but lactation has a decreasing effect on BMD in fluoride-exposed rats. *Toxicology and Industrial Health* 2006; **22:** 217–222.

Key words: *bone mineral density; fluorosis; lactation; pregnancy; rat*

Introduction

Fluoride is a cumulative toxic ion with the potential to increase skeletal mass (Kleerekoper, 1996). It is incorporated into bones and replaces the hydroxyl ion in the crystal lattice of apatite (Grynpas, 1990). Fluoroapatite is less soluble, more compact, and slower to undergo remodelling in bone (Gruber and Baylink, 1991). Fluoride stimulates bone cell proliferation by directly inhibiting osteoblastic acid phosphatase activity, and prolonging or enhancing the mitogenic signals of growth factors (Krishnamachari, 1986; Lau *et al*., 1989; Gruber and Baylink, 1991). Excessive intake of fluoride may lead to pathological changes in teeth and bones: dental and skeletal fluorosis (Krishnamachari, 1986). Osteosclerotic picture is evident when small doses of fluoride are ingested over a long period of time. On the other hand, a combination of fluoride excess and calcium deficiency may lead to osteopenia. Osteoporosis related fracture prevalence is increased in skeletal fluorosis (Teotia *et al*., 1998).

During pregnancy, the fetus takes calcium from the mother to develop its skeleton, which may lead to bone mineral loss in the mother's skeleton (Kovacs and Kronenberg, 1997; Ensom *et al*.,

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2002). In the third trimester, Ca is transported daily through the placenta for normal bone mineralization in the developing fetus. On the other hand, the increased estrogen levels accompanying pregnancy (Battin *et al*., 1985) might inhibit or even reverse this bone loss (Sowers *et al*., 1991).

During lactation, Ca demands are greater than during pregnancy. Calcium demand is higher in neonatal life and it is usually met with breast milk calcium (Ensom *et al*., 2002). Multiparity and low Ca intake could lead to osteopenia or even osteoporosis. Prolonged periods of amenorrhea and hypoestrogenemia are seen during lactation, which also contributes to the increased maternal bone loss (Kolthoff *et al*., 1998; Shoji *et al*., 2000; More *et al*., 2001).

Isparta is situated in an endemic fluorosis area, and the aim of this study was to investigate the effect of pregnancy and lactation on bone mineral density (BMD) in fluoride-exposed rats using dual-energy X-ray absorptiometry (DEXA). To the best of our knowledge, this is the first report of a relationship between pregnancy and lactation, and BMD in fluorosis.

Materials and methods

Animals and experimental design

Sixteen female Wistar rats, 4 weeks of age, were obtained from the Animal Breeding Unit of Süleyman Demirel University School of Medicine. Animals were randomly assigned into two groups; the fluoride-exposed group ($N=8$) and the control group ($N=8$). To investigate the effect of pregnancy and lactation on BMD in continuous fluoride exposure, rats in the fluoride-exposed group were given commercially available spring water with the addition of 100 ppm fluoride and rats in the control group were given the same spring water without any addition (containing only 0.007 ppm fluoride). All animals were kept under controlled conditions, which included a $20\pm1^{\circ}$C environmental temperature and a 12:12 h light-dark cycle. Animals were fed with rodent food *ad libitum*. At 16 weeks of age, four female rats were kept in a cage with a male rat for 5 days; all females in both groups were successfully impregnated. Rats in the fluoride-exposed group continued to receive spring water with the addition of 100 ppm fluoride during pregnancy and lactation period, whereas rats in the control group received the same spring water without any addition during the same periods.

BMD was measured at the beginning (16 weeks of age), on the first day postpartum (19 weeks of age), and at the end of lactation (22 weeks of age), in both groups.

Prior to this study, the protocol was reviewed and approved by the Süleyman Demirel University Ethic Committee.

Bone mineral density assessment

All rats were scanned under ketamine (50 mg/kg) and xylazine (2 mg/kg) anaesthesia administered by intramuscular injection. BMD (g/cm) of the lumbar spine and femoral diaphysis was measured with DEXA scanner (Norland XR-46 Bone Densitometer, Norland Corp., Fort Atkinson, WI, USA) using a small animal scan software. To minimize the interobserver variations, all analyses were carried out by the same technician. The reproducibility of the measurement system was assessed by measuring one rat three times. The coefficient of variation (cv) was 1.4% for lumbar spine and 1% for femur diaphysis.

Statistical analysis

Data were analysed using the statistical package SPSS for Windows (Ref. 9.05, SPSS Inc, Chicago, IL, USA). Results were expressed as mean $\pm$ SD. A P-value of <0.05 was considered significant. Comparison between groups was assessed by Mann–Whitney U-test. Differences within the same group were tested by repeated measures of ANOVA.

Results

The mean BMD values of the spine and femur at baseline, on the first day postpartum and at the end of lactation in both fluoride-exposed and control groups are shown in Table 1.

At 16 weeks of age, although BMD of the spine in fluoride-exposed group was significantly higher than that of controls ($P=0.025$), there were no

Table 1. BMD (mean ± SD, g/cm) of the spine and femur in two groups.

	Baseline		Postpartum		End of lactation	
	Fluoride-exposed	Control	Fluoride-exposed	Control	Fluoride-exposed	Control
Spine	0.150 ± 0.01^{a}	0.130 ± 0.02	0.146 ± 0.02^{a}	0.104 ± 0.01^{b}	0.113 ± 0.01^{abc}	0.088 ± 0.01^{bc}
Femur	0.146 ± 0.01	0.143 ± 0.01	0.159 ± 0.02	0.152 ± 0.02	0.116 ± 0.01^{bc}	0.111 ± 0.02^{bc}

[a] $P < 0.05$ compared with controls.
[b] $P < 0.05$ compared with initial value for each group.
[c] $P < 0.05$ compared with postpartum value for each group.

significant femoral BMD differences between the two groups ($P = 0.670$).

During pregnancy, there was a slight decrease in spinal BMD and a slight increase in femoral BMD in the fluoride-exposed group. The changes were not found statistically significant ($P = 0.597$ and 0.116, respectively). BMD of the spine was decreased significantly ($P = 0.013$) and BMD of the femur was increased non-significantly ($P = 0.109$) in the control group. While BMD of the lumbar spine in the fluoride-exposed group was significantly higher than that of controls ($P = 0.001$), there were no significant BMD differences at the femoral diaphysis between the groups ($P = 0.286$) in the postpartum period.

During lactation, BMDs of both lumbar spine and femoral diaphysis were significantly decreased compared to both baseline BMD values ($P = 0.001$ and 0.030, respectively) and pregnancy BMD values ($P = 0.001$ and 0.001, respectively) in fluoride-exposed group. BMDs of both lumbar spine and femoral diaphysis were significantly decreased compared to both baseline BMD values ($P = 0.001$ and 0.015, respectively) and pregnancy BMD values ($P = 0.030$ and 0.004, respectively) in the controls. BMD values of the spine in the fluoride-exposed group were found significantly higher than that of controls ($P = 0.001$). No significant BMD differences were present at the femur diaphysis between the groups ($P = 0.593$).

Figure 1 shows the percent changes in BMD of the spine and femur in the fluoride-exposed group and the control group. Between baseline and delivery, a BMD change of 7% and −2% at femur and lumbar spine, respectively, was observed in fluoride-exposed rats. In the control rats, these changes were −2% and −19%, respectively. The changes in femoral and spinal BMDs from baseline to the end of lactation were −18% and −24%, respectively in fluoride-exposed rats. These changes were −21% and −31%, respectively in the controls.

Discussion

Fluoride is a cumulative poison, which increases metabolic turnover of the bone in favour of bone formation. It has a great affinity for calcified tissues. Fluoride is derived from various sources. Drinking water is the main source of fluoride for humans. Excess fluoride primarily affects skeletal and dental tissues, leading to a condition known as fluorosis. Skeletal fluorosis is characterized by a wide range of skeletal changes, such as osteoporosis, osteopenia and osteosclerosis. These differences may be due to several factors, such as dose, duration of fluoride exposure, dietary habits or their combination. Unless there are some factors, such as poor nutrition and low calcium intake, BMD values are higher in skeletal fluorosis (Krishnamachari, 1986; De la Sota *et al*., 1997; Teotia *et al*., 1998; Czarnowski *et al*., 1999). Fluoride is incorporated into trabecular bone more readily than cortical bone (Dequeker and Declerck, 1993). In the current study, spinal BMD was found to be higher in fluoride-exposed rats than controls at the beginning. No differences were present between the groups with respect to the baseline femoral BMD values. Our results were in agreement with the literature findings.

Normal pregnancy and lactation induce a series of hormone-mediated changes in bone metabolism with different effects on maternal BMD. The low estrogen concentrations in early pregnancy may possibly explain early bone loss. The increase of insulin-like growth factor-I (IGF-I) after 28 weeks gestation may play a role in stimulating bone formation during the last trimester of pregnancy. During pregnancy, changes in lifestyle factors and

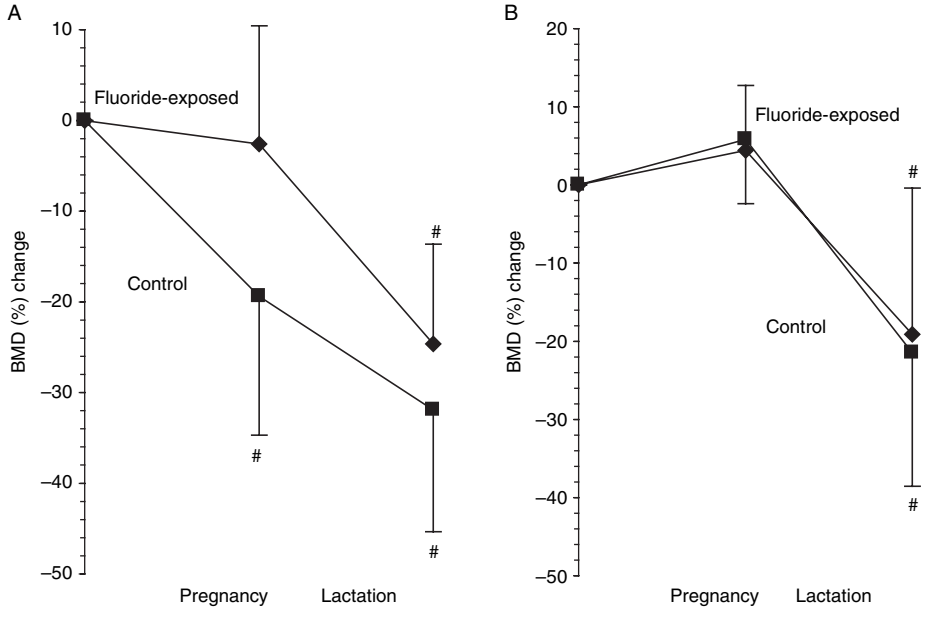

Figure 1. Percent changes in BMD of fluoride-exposed group and control group during pregnancy and lactation. #$P<0.05$ compared with previous measurement. (A) Percent changes in BMD of the spine. (B) Percent changes in BMD of the femur.

physical activity may also influence the BMD. Reported BMD changes with pregnancy are inconsistent. BMD has been reported to increase (Naylor *et al*., 2000) or decrease (Lamke *et al*., 1977; Black *et al*., 2000) during pregnancy. Naylor *et al*. (2000) reported an increase in BMD at cortical sites and a decrease in BMD at trabecular bone sites during pregnancy. Lamke *et al*. (1977) measuring two sites at the radius, reported loss at trabecular but not cortical bone, whereas More *et al*. (2001) and Black *et al*. (2000) found a significant decrease of BMD at both the trabecular and cortical sites during pregnancy. In our study, BMD was found to be decreased at trabecular bone, but unchanged at cortical bone in non-fluoridated control rats. In fluoride-exposed rats, BMD was found decreased slightly, but non-significantly at trabecular bones. There was also a slight increase in BMD at cortical sites, probably due to the potential of fluoride to increase skeletal mass and increase estrogen levels.

Lactation is associated with low estrogen levels and an increased demand for calcium. During the same period, changes are also found in hormones other than estrogen, body weight, body composition and lifestyle factors, all with the potential to influence BMD (Cummings *et al*., 1995). For example, prolactin and parathyroid hormone-related peptide (PTHr P) have been shown to increase in the maternal circulation (Lippuner *et al*., 1996), in human breast milk and in umbilical cord blood (Khosla *et al*., 1990) during lactation. These elevations also seem to be associated with bone loss (Sowers *et al*., 1996). During lactation, BMD was decreased (Lamke *et al*., 1977; Holmberg-Martilla *et al*., 1999; Kalkwarf and Specker, 2002) and, these took place mainly at the trabecular sites (Kolthoff *et al*., 1998; More *et al*., 2001). Shoji *et al*. (2000) suggested that lactation can be a risk factor for trabecular bone loss, and calcium deficiency in the mother causes decreases in the BMD. Tojo *et al*. (1998) reported significant decreases in the trabecular bone during lactation and that lactational intensity is related to BMD. Zeni *et al*. (1999) showed that normal pregnancy appears to exert little influence on bone, whereas lactation induces a greater impact in rats. We found that BMD was significantly decreased at both trabecular and cortical sites in both fluoride-exposed and control rats. This might be due to increased calcium demand and decreased estrogen levels in lactation.

In conclusion, our experimental results indicate that the trabecular BMD in fluoride-exposed rats was higher than that of controls. Pregnancy has no effect on trabecular and cortical bones in fluoride-exposed rats. In control rats, although pregnancy appears to have a detrimental effect on trabecular

bone, it does not seem to have any influence on cortical bone. It may be explained that fluoride is incorporated into trabecular bone more readily than cortical bone. Although lactation decreases BMD at both trabecular and cortical bones in both fluoride-exposed and control rats, these decreases were less prominent in fluoride-exposed rats. This can be explained by the effects of continuous fluoride exposure on metabolic turnover of the bone in favour of bone formation. Yuan *et al*. (1991) studied the effect of fluorosis on lactation, lactotroph function and ultrastructure and found that lactation was inhibited and serum prolactin level was decreased with chronic fluorosis. They indicated that hormone release of pituitary lactotrophs is obstructed in lactating rats with fluorosis. Although we did not measure serum prolactin levels and assess the amount of milk secreted, we believe that the inhibition of lactation and low serum prolactin levels are other factors causing less BMD decrease in fluoride-exposed rats. Further studies are needed to elucidate the definite mechanisms.

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