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# Cadmium, follicle-stimulating hormone, and effects on bone in women age 42–60 years, NHANES III ☆

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ABSTRACT

*Background:* Increased body burden of environmental cadmium has been associated with greater risk of decreased bone mineral density (BMD) and osteoporosis in middle-aged and older women, and an inverse relationship has been reported between follicle-stimulating hormone (FSH) and BMD in middle-aged women; however, the relationships between cadmium and FSH are uncertain, and the associations of each with bone loss have not been analyzed in a single population.

*Objectives:* The objective of this study was to evaluate the associations between creatinine-adjusted urinary cadmium (UCd) and FSH levels, and the associations between UCd and FSH with BMD and osteoporosis, in postmenopausal and perimenopausal women aged 42–60 years.

*Methods:* Data were obtained from the Third National Health Examination and Nutrition Survey, 1988–1994 (NHANES III). Outcomes evaluated were serum FSH levels, femoral bone mineral density measured by dual energy X-ray absorptiometry, and osteoporosis indicated by femoral BMD cutoffs based on the international standard. Urinary cadmium levels were analyzed for association with these outcomes, and FSH levels analyzed for association with bone effects, using multiple regression. Subset analysis was conducted by a dichotomous measure of body mass index (BMI) to proxy higher and lower adipose-synthesized estrogen effects.

*Results:* UCd was associated with increased serum FSH in perimenopausal women with high BMI ($n$=642; $\beta$=0.45; $p \leq 0.05$; $R^2$=0.35) and low BMI ($n$=408; $\beta$=0.61; $p \leq 0.01$; $R^2$=0.34). Among perimenopausal women with high BMI, BMD was inversely related to UCd ($\beta = -0.04$; $p \leq 0.05$) and FSH ($\beta = -0.03$; $p \leq 0.05$). In postmenopausal women with low BMI, an incremental increase in FSH was associated with 2.78 greater odds for osteoporosis (109 with and 706 without) (OR=2.78; 95% CI=1.43, 5.42; $p \leq 0.01$).

*Conclusion:* Long-term cadmium exposure at environmental levels is associated with increased serum FSH, and both FSH and UCd are associated with bone loss, in US women aged 42–60 years.



## 1. Introduction

Long-term environmental cadmium exposure has been associated with increased bone resorption and decreased bone mineral density (BMD) in middle-aged and older women (Akesson et al., 2006; Gallagher et al., 2008; Schutte et al., 2008). There is evidence that cadmium directly affects the bone remodeling process (Regunathan et al., 2003; Brzoska and Moniuszko-Jakoniuk, 2005; Smith et al., 2009); however, the mechanism of action by which cadmium affects bone physiology is not fully understood (Klaassen, 2008). Estrogen deficiency is well documented as a cause of bone loss among postmenopausal women (Riggs et al., 2002). More recently, an increase in follicle-stimulating hormone (FSH) in pre- and perimenopausal women has been associated with decreased BMD (Sowers et al., 2003, 2006) and increased bone resorption (Perrien et al., 2006). The chronic nature of both environmental cadmium exposure (ATSDR, 1999) and the process of bone loss (USDHHS, 2004) with aging raises questions regarding cadmium's possible effects before and after menopause. The current study focuses on the relationship between cadmium and FSH, and the effects of cadmium and FSH on BMD, in perimenopausal and postmenopausal women.

Cadmium accumulates in the human kidney, with a half-life of 20–30 years (ATSDR, 1999). Urinary cadmium increases in proportion to stored cadmium, and thus, is a biomarker for lifetime cadmium body burden in people with environmental exposures (CDC, 2005; IPCS, 1992). Presumably due to lower iron stores, women tend to have higher absorption of cadmium from

☆ No human subjects participated in this unfunded analysis of secondary data.

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the diet and higher levels of cadmium in the kidneys and urine than men (Vahter et al., 2002), which may account for some of the increased risk for bone loss and osteoporosis among women.

Cadmium also accumulates in the human ovary between 30 and 65 years of age (Varga et al., 1993). In addition to direct effects on the bone remodeling process, cadmium may also indirectly induce bone loss by altering ovarian function and disrupting hormonal homeostasis. Cellular studies and animal studies have reported FSH-induced bone resorption (Sun et al., 2006; Iqbal et al., 2006), but did not look for an association between cadmium and FSH levels. Cadmium has been shown to alter ovarian cell morphology and act as an ovarian endocrine disruptor. Paksy et al. (1997) found that cadmium altered human ovarian follicle cell morphology and suppressed progesterone production. Piasek et al. (2002) reported that in vivo cadmium exposure interfered with ovarian estradiol production in female rats, and Zhang et al. (2008) found that cadmium suppressed serum progesterone and estrogen in female rats.

Chemically induced ovarian follicle depletion has been shown to increase FSH levels in animal studies (Hooser et al., 1994), and Hoyer (2005) suggested that cadmium-induced ovarian damage might increase FSH levels by reducing 17β-estradiol, thereby disrupting negative feedback of the hypothalamic-pituitary-ovarian axis (HPOA). Cadmium-induced ovarian follicle damage might therefore contribute to decreased 17β-estradiol and increased FSH levels, hormonal changes characteristic of the menopause (Randolph et al., 2004; Riggs et al., 2002; Khosla et al., 1998, 1997), but which may also occur prior to menopause (Robertson et al., 2009). FSH levels are also regulated by inhibin, a peptide produced by ovarian granulosa cells, that inhibits FSH production (Prior, 2005). Cadmium-induced ovarian granulosa damage may decrease inhibin, thereby increasing FSH in the presence of estrogen, a hormonal change that may contribute to decreased BMD in perimenopause despite the presence of normal or elevated estrogen levels (Perrien et al., 2006).

Estrogen and FSH may act independently of one another, particularly among postmenopausal women in whom estrogen is primarily derived from nongonadal sites (Simpson, 2003). Krum and Brown (2008) acknowledged that FSH may increase bone resorption, but asserted that estrogen's protective effects on bone are attributable to its regulation of cytokines and induction of apoptosis of osteoclasts, rather than by regulation of FSH (Krum et al., 2008). If true, among postmenopausal women, it is plausible that bone demineralization due to loss of estrogen suppression of osteoclastic activity would be further enhanced by cadmium-induced decline in inhibin with increased FSH-induced bone resorption, and further augmented by cadmium's direct effects on bone.

In postmenopausal women estrogen is produced predominantly in adipose tissue. Higher BMI is associated with higher blood levels of estradiol ($R^2$=0.28) and bioavailable estradiol ($R^2$=0.36) in postmenopausal women (Mahabir et al., 2006), and the authors concluded that BMI is a useful measure for epidemiologic studies of phenomenon associated with estrogen effects among postmenopausal women. After menopause, nongonadal sites of estradiol production also include the bone osteoblasts and the breast (Simpson, 2003). Cadmium mimics the effects of estrogen in breast cancer cells (Brama et al., 2007), and Akesson et al. (2008) suggested that BMI may mask the potential estrogen-related effects of cadmium in postmenopausal women, and used the measure BMI $< 27\,kg/m^2$ as a surrogate for low estrogen.

The objective of the current study is to evaluate the associations between creatinine-adjusted urinary cadmium (UCd) and FSH, and the associations of UCd and FSH with BMD, in postmenopausal and perimenopausal women aged 42–60 years. We hypothesize that UCd is associated with increased FSH, and that both cadmium and FSH are associated with decreased femoral BMD. We also explore whether these associations are present in both perimenopausal and postmenopausal women by subset analyses of each group according to a BMI less than or greater than $27\,kg/m^2$ (BMI/(E)), as a surrogate marker of adipose-synthesized estrogen effects.

## 2. Methods

The study sample data were obtained from US National Health and Nutrition Examination Survey (NHANES III) 1988–1994 survey data (CDC, 2008). NHANES is a cross-sectional, random household survey of the civilian population based on a complex probability sampling design (CDC, 2006). Bone mineral measurements were obtained by dual energy X-ray absorptiometry, conducted at the Medical Examination Center (MEC). Osteoporosis status was indicated by either femur neck BMD less than $0.56\,g/cm^2$ or total hip BMD less than $0.64\,g/cm^2$, as per the osteoporosis cutoffs reported in the NHANES III femoral bone density study conducted by Looker et al. (1997), and consistent with the international reference standard (WHO, 2004). Urinary cadmium was obtained from a single urine specimen collected at the MEC and measured by the CDC laboratory using atomic absorption spectrometry and reported in ng/mL. Urinary creatinine was obtained from the same urine specimen and measured in mg/dL. A creatinine-adjusted urinary cadmium measure (μg/g) was generated by dividing urinary cadmium (ng/mL) by urinary creatinine (mg/dL). Analyses were limited to women with CDC laboratory-measured cadmium and creatinine, with observations restricted to participants whose urinary cadmium levels were less than or equal to 20 ng/mL, in order to exclude non-environmental exposure levels (Friberg et al., 1985; Whittemore et al., 1991). As a result, no observations were excluded. A reference urinary cadmium level of $\leq 0.05$ μg/g creatinine was used for comparison with the following urinary cadmium levels: $>0.50$–$1.00$ μg/g creatinine and $>1.00$ μg/g creatinine. The current analysis was further limited to women with a minimum age of 42 years in order to include likely perimenopausal women (Sower et al., 2006) and a maximum age of 60 years as that was the age limit for serum FSH level measurement, also conducted at the MEC. Five outlier observations with FSH levels over 152 μg/dL were excluded. Serum lead levels were also obtained at the MEC. Creatinine-adjusted urinary cadmium, FSH and lead values were log transformations of the value plus 1. Pregnant or breastfeeding women were excluded. Women reported whether or not they were currently using the birth control pill or patch, using hormonal treatments, being treated for osteoporosis, were heavy smokers, i.e., currently smoked more than 20 cigarettes per day, or earned less than $20,000 annual income. Additionally, multiparity, i.e., whether a woman had delivered more than one child, was determined by self-report.

Postmenopausal was defined as per a woman's report that she had not menstruated for 12 months or longer. All others were categorized as perimenopausal. BMI less than $27\,kg/m^2$ was categorized as low body mass index/(estrogen surrogate) (low BMI/(E)) (Akesson et al., 2008), and BMI greater than or equal to $27 kg/m^2$ was categorized as high BMI/(E).

Sensitivity analysis was conducted using higher ($\geq 30\,kg/m^2$) and lower ($\leq 25\,kg/m^2$) BMI cutoff values. Observations were weighted by NHANES III MEC weights. Linear and logistic regression were conducted using SAS version 9.2, using the Taylor Linearization method for complex survey calculation of variance, with 95% confidence limits presented for statistically and marginally significant effect estimates. Multivariate models adjust for the influences of serum lead, osteoporosis treatment, race, age, smoking status, hormonal treatment, medical or surgical amenorrhea, income, multiparity, and in the perimenopausal subgroup, birth control. Multiple linear regression analysis was conducted to quantify the adjusted associations of UCd with FSH, and to quantify the adjusted associations of UCd and FSH, separately and together, with bone mineral density for peri- and postmenopausal women, with subset analyses by BMI/(E) subgroup (Models 1–4). For postmenopausal women, multiple logistic regression analysis was conducted to quantify the adjusted effects of UCd and FSH, separately and together, on the odds for osteoporosis by BMI/(E) subgroup (Models 5–7). The convergence criterion was satisfied for logistic regression analyses, and global chi square statistics were significant, thus providing no indication to question the validity of model fit for the models presented.

## 3. Results

### 3.1. Unadjusted

Table 1 presents mean values for key sample characteristics by menopausal and BMI subcategories. The highest mean femoral BMD value ($1.02\,kg/cm^2$) is found in combination with the lowest mean UCd (0.69 μg/g creatinine) and FSH (21.16 μg/dL) levels in the perimenopausal, high BMI/(E) subgroup; the lowest mean BMD ($0.61\,kg/cm^2$) is found together with the highest mean