2017

Association of Cadmium Exposure with Bone Mineral Density in U.S. Adults

Joseph P. Taggart
Wright State University - Main Campus

Association of Cadmium Exposure with Bone Mineral Density in U.S. Adults

Joseph P. Taggart

Wright State University Boonshoft School of Medicine

Master of Public Health Program

Ramzi W. Nahhas, PhD – Committee Co-Chair

Naila Khalil, MBBS, MPH, PhD – Committee Co-Chair

Acknowledgements

Thank you Dr. Naila Khalil for encouraging me to reach beyond being a Sociologist and guiding me deeper into Public Health and Epidemiology. Thank you Dr. Ramzi W. Nahhas for your expertise in Biostatistics and instructing me on the importance of accurate data. You both have been amazing co-chairs and it has truly been my privilege and an honor to work with you. Thank you Dr. Nikki Rogers you are the key that opened the door to success on this project. Thank you Dr. Cristina Redko for your support on this project and helping me to develop a broad, global health perspective.

Table of Contents

Abstract

Background: Cadmium is a toxic heavy metal and environmental pollutant; general population exposure is through occupation, diet, and smoking. Cadmium is associated with lower bone mineral density (BMD) and higher risk of fractures in women. This association is not fully known in men.

Objective: Given the toxicity and global dispersion of cadmium, we explored its association with BMD in U.S. men and women.

Methods: Data from 914 participants from the National Health and Nutrition Examination Survey 2013-2014 was utilized. Multivariable regression analysis was used to determine the independent association between cadmium exposure and BMD controlling for age, body mass index, smoking status, income, and menopausal status × gender. The Šidák method was used to adjust *p-values* for multiple comparisons between levels of each categorical variable.

Results: In adjusted analysis a small, negative association between cadmium exposure and BMD was observed. This association was not significant overall ($p = 0.908$) nor in males ($p = 0.618$) or females ($p = 0.942$). Overall, age ($p < .001$), body mass index ($p < .001$), income ($p = .001$), race ($p < .001$) and menopausal status × gender ($p < .001$) were significantly associated with bone mineral density. Unplanned analysis controlling for creatinine did not yield a significant association between cadmium and BMD.

Conclusion: In the U.S. population, cadmium is not significantly associated with BMD. As mean urinary Cadmium level has been declining in U.S. population over 1999-2014; the decreasing levels of environmental cadmium exposure may have a role in non-significant association with BMD.

Keywords: bone mineral density, cadmium, NHANES, public health, CDC

Association of Cadmium Exposure with Bone Mineral Density in U.S. Adults

Osteoporosis, defined as low bone mineral density (BMD), is a public health concern as it may increase the risk of fractures, morbidity-mortality, and health care costs (Scimeca et al., 2017). According to 2005-2008 National Health and Nutrition Examination Surveys (NHANES) data, 9% of the U.S. population older than 50 years had osteoporosis (Looker, Borrud, Dawson-Hughes, Shepherd, & Wright, 2012).

In addition to the genetic, nutrition, lifestyle, and mechanical factors that determine bone density, environmental exposure to toxic heavy metals such as cadmium (Cd), chromium, and lead also plays a role in osteoporosis (Scimeca et al., 2017). Urinary Cd was associated with hip osteoporosis in U.S. women aged 50 years and older in NHANES data from 1988-1994, and 1999-2004 (Gallagher, Kovach, & Meliker, 2008). However, a similar association in U.S. men has not been explored. Biomonitoring in the U.S. population shows men and women have different Cd body burden as detected by urinary analysis. The Centers for Disease Control and Prevention (CDC, 2016) reports women between 50 and 70 years of age have higher peak values of both urinary and serum Cd than men of the same age.

We hypothesized that environmental exposure to Cd would be associated with low BMD among both men and women.

Purpose Statement

To determine if Cd exposure is associated with low BMD among men and women in the United States.

Literature Review

Bone Density and Metabolism

Bones are living, growing tissue primarily composed of collagen (a protein) and calcium phosphate (a mineral). Bone remodeling occurs throughout life. Old bone tissue is resolved by osteoclasts and new bone is deposited by osteocytes and osteoblasts. This delicate balance is regulated by hormones (gonadal steroids, vitamin D, calcitonin, parathyroid hormone, etc.). The combination of minerals and proteins allow bones to be both flexible and strong, to bear weight and torsion (National Institutes of Health [NIH], Osteoporosis and Related Bone Diseases National Resource Center [ORBDNRC], 2015a).

Generally, the human skeleton is composed of two types of bone: cortical and trabecular. Cortical or compact bone is the denser exterior and the primary component of long bones (e.g., femur, humerus, radius, etc.). Trabecular bone is the spongy, less dense interior and is generally found in the pelvis, heel bone, vertebrae, etc. (NIH ORBDNRC, 2015b).

BMD measurement. BMD is a measure of the mass of minerals contained within a unit volume of bone expressed as grams per centimeter squared (g/cm^2). BMD is measured to assess bone health and predict fracture risk (U.S. Department of Health and Human Services [HHS], National Institutes of Health [NIH], National Cancer Institute [NCI], n.d.). Dual-energy x-ray absorptiometry (DXA) is the most widely used method for measurement of BMD at the hip and spine.

As shown in Table 1, DXA results are reported as a t-score to diagnose osteoporosis. According to the World Health Organization (WHO, 1994), a BMD t-score of -1.0 or above is *normal* bone mineral density. A t-score between -1.0 and -2.5 designates low BMD or *osteopenia*. A t-score of -2.5 or below indicates *osteoporosis*. If osteoporotic fractures have

occurred and the t-score is below -2.5 then this is referred to as *severe osteoporosis* (WHO, 1994, p. 5-6).

Table 1

Bone Mineral Density T-score Classification with Examples

BMD	Range	Examples
Normal BMD	-1.0 and above	+0.5, 0, -1.0
Low BMD (Osteopenia)	Between -1 and -2.5	-1.1, -1.5, -2.4
Osteoporosis	-2.5 and below	-2.5, -3.0, -4.0

Note: t-score values are not limited to examples provided (World Health Organization [WHO], 1994).

BMD turnover and remodeling. The exchange of minerals in bone is similar to a 24-hour bank, deposits (osteoblasts) and withdrawals (osteoclasts) are always being processed. The preferred 'currency' in bone is calcium phosphate ($Ca_3O_8P_2$), but it is not the only mineral. When more minerals are deposited, growth occurs (gain in mass). When more withdrawals occur, the bone loses mass (NIH ORBDNRC, 2015b).

BMD and its sociodemographic correlates. BMD is associated with ethnicity, age, gender, socioeconomic status, and lifestyle factors including smoking and physical activity. Persons of African descent have significantly greater overall BMD than those of Asian, Hispanic, or Caucasian ethnicities (Norris, Micklesfield, & Pettifor, 2013). Female gender is associated with lower peak BMD. DXA measurements from quantitative computed tomography studies show that, after the third decade, women (-55%) experience more trabecular and cortical bone loss than men (-45%) (Drake & Khosla, 2013).

Women generally have smaller, thinner bones than men, increasing their risk to develop osteoporosis (NIH ORBDNRC, 2015b). Regarding socioeconomic status (SES), men with less

than a high school education have lower BMD than men with a college or graduate education. Women with an annual income less than $20,000 per year have lower BMD than women with higher income (Du, Zhao, Xu, Wu, & Deng, 2017). Smoking tobacco may be associated with an increased risk of hip fracture due to its toxic impact on BMD. In a meta-analysis from United Kingdom, it was reported that one out of every eight fractures among women could be caused by smoking. Although comparable data among men is very limited, smoking may have a similar magnitude of effect on fractures in men (Law & Hackshaw, 1997).

Cadmium

Cd is a naturally occurring element, but its abundance as an environmental pollutant is associated with being a byproduct of the industrial and agricultural processing of minerals such as zinc, lead, or copper. During smelting and electroplating (a process using electricity to alter the surface of other metals or plastics), Cd can become an oxide, chloride, and/or sulfate and exist as an air particle or vapor. Cd can be carried vast distances via environmental media, eventually settling onto land and water surfaces. The deposited Cd eventually enters the food supply either through plants or through water biota (this impacts the seafood, especially fish that are available for human consumption). According to the Agency for Toxic Substances and Disease Registry (ATSDR), exposure to Cd is a public health concern because it is carcinogenic, tumorigenic, associated with lung and kidney dysfunction, deoxyribonucleic acid (DNA) damage, and diminished bone development (U.S. Department of Health and Human Services [HHS], Public Health Service [PHS], Agency for Toxic Substances and Disease Registry [ATSDR], 2012).

Cd exposure. Inhalation is the primary route of exposure followed by ingestion (dermal contact poses little threat of exposure). The primary sources of exposure for nonsmokers are

through the consumption of leafy greens, grains, nuts, beans, and/or seeds. The highest risk, occupational exposure, is observed in workers involved with heating materials that contain Cd. Tobacco plants may gather high levels of Cd which significantly increases the exposure for tobacco smokers (doubles the Cd body burden compared to nonsmokers). Individuals that smoke tobacco or reside near zinc or lead smelting facilities have the highest exposure, followed by people that have diets high in kidney, liver, fish, or shellfish (HHS PHS ATSDR, 2012).

Cd-containing plumbing also increases exposure through drinking water. Individuals that reside near hazardous waste sites have an increased risk of exposure to foods grown in contaminated soil, contaminated water, and possible inhalation of dust (HHS PHS ATSDR, 2012).

There are little data on Cd exposure in individuals under 18 years of age. In animal studies, the young absorb more Cd than adults. This may be associated with Cd passing from the mother to the infant through breast milk; concentrations of Cd rely on the level of exposure to the mother. According to the ATSDR, it is speculated that exposure at an early age may pose long-term, adverse health effects due to the 10-year half-life of Cd. Adults may be more prone to renal toxicity if exposed to Cd during childhood. An individual's age, genetics, nutrition, and exposure to other toxic substances may be associated to a heightened response to Cd exposure (HHS PHS ATSDR, 2012). Table 2 shows data from the 1999-2012 NHANES cycles, representing body burden of Cd (CDC, 2015, p. 214-216).

Table 2

Urinary Cadmium, Creatinine Corrected

Survey Years	Geometric Mean	Median	95th Percentile	Sample Size
1999-2000	0.181	0.219	0.941	2257
2001-2002	0.199	0.212	0.919	2689
2003-2004	0.210	0.208	0.940	2543
2005-2006	0.189	0.180	0.910	2576
2007-2008	0.193	0.190	0.960	2627
2009-2010	0.191	0.190	0.960	2848
2011-2012	0.176	0.172	0.907	2502

Note: Measurements are in micrograms per gram (µg/g) of creatinine (Centers for Disease Control and Prevention [CDC], 2015).

Metabolism. A lack of iron or other nutrients may cause the body to take up larger amounts of Cd to compensate for nutrient deficits. Cd is processed by the liver and kidneys where it can remain for many years, slowly being excreted through urine and feces. Excessive amounts Cd may overload the liver and kidneys hindering the body's ability to convert Cd into a less harmful form. High levels of Cd can cause gastrointestinal (GI) issues. Low levels of exposure over long periods of time will damage the kidneys and indirectly may increase bone fragility and fractures as kidneys play an important role in calcium, phosphate, Vitamin D and protein metabolism (HHS PHS ATSDR, 2012).

Impact on human health. The U.S. Department of Health and Human Services (DHHS), International Agency for Research on Cancer (IARC), and Environmental Protection Agency (EPA) have determined that Cd is either "carcinogenic to humans" or a "probable human carcinogen" (HHS PHS ATSDR, 2012, p. 15). In a Canadian health report, it was stated that Cd

may be associated with a greater risk of cancer, kidney ailments, skeletal damage, and possible cardiovascular dysfunctions (Garner & Levallois, 2016).

Cd and BMD in epidemiological studies. In Japan, ingestion of rice contaminated by Cd was associated with decreased BMD and fractures. This was believed to be the cause of itai-itai[1] (ouch ouch) disease (Wallin et al., 2016, p. 733). According to 1988-1994 and 1999-2004 NHANES data, women are 43% more likely to have osteoporosis, as measured at the hip, when their urinary Cd levels are between 0.50 and 1.00 µg/g creatinine (Gallagher et al., 2008). The Osteoporosis in Men (MrOS) study reported that Cd exposure (low level), regardless of lifestyle, is associated with an increased risk of low BMD and non-vertebral osteoporotic fractures in Swedish men (Wallin et al., 2016).

Cd and BMD in in-vitro studies. Animal studies have shown that environmental exposure to Cd concentrations at very low levels (10-500 nM) interferes with osteoclast processes in-vitro. Higher concentrations (0.1-20 µM) affect osteoblast pathways. The "uncoupling between osteoclasts and osteoblasts would lead to a lower quality of the bone" (Engström et al., 2012, p. 1376).

Methods

Data Source and Study Sample

Data from the 2013-2014 NHANES were used for this analysis. Details pertaining to the NHANES study design and methods are publicly available through the Centers for Disease Control and Prevention (CDC) website. During the NHANES 2013-2014 cycle, 14,332 noninstitutionalized United States civilians were selected from 30 different study locations.

[1] Itai-itai or ouch ouch is a "form of Cd-induced renal osteomalacia" and a "bone disease with many fractures and severe pain" first reported after World War II (Nordberg, 2009, p. 192).

Participants were residing in any of the 50 states including D.C. Of the 10,175 that completed the interview process, 9,813 participants were also examined.

Participants provided consent and were sent to a mobile examination center (MEC) where laboratory measurements (including urine samples), physical assessments, and examinations were conducted. Random urinalysis of Cd was conducted by the Division of Laboratory Sciences, National Center for Environmental Health, CDC, Atlanta, GA. The study sample for this analysis consisted of NHANES participants between 40 and 80 years of age who had femoral neck BMD (FNBMD) measurements, urinalysis of Cd, and non-missing values for the potential confounders described below (N = 914) (Centers for Disease Control and Prevention [CDC], National Center for Health Statistics [NCHS], 2017).

Confounders

Potential confounders of the exposure-outcome relationship were: age, body mass index (BMI), race/ethnicity, gender, income, menopausal status, and smoking status. Socioeconomic and sociodemographic information (i.e., age, race/ethnicity, gender, and income) were collected by trained interviewers using the in-home NHANES Family Questionnaire via the Computer-Assisted Personal interview (CAPI) system. Race/Ethnicity was reported as Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, non-Hispanic Asian and other multi-racial. Height and weight were measured by trained health technicians assisted by a recorder using a stadiometer for height in meters (m) and a digital weight scale, either portable or built into the floor of the MEC, for weight in kilograms (kg). The equation kg/m^2 was to determine BMI (CDC NCHS, 2015a).

Data on tobacco use and smoking were collected both by self-report interviews using the CAPI system and during MEC interviews prior to the examination. Data collected included:

"history of use, age at initiation, past 30-day use, cigarette brand, sub-brand and other related details" (CDC NCHS, 2016, para. 1). Smoking status data were collected on participants that were 12 years of age or greater. For this study, smoking status was grouped into three categories: never, former, and current.

Femoral Neck BMD as Measured by DXA

The NHANES' MECs acquired femoral neck scans using "Hologic QDR-4500A fan-beam densitometers (Hologic, Inc., Bedford, Massachusetts) using software version Apex 3.2 . . . analyzed with Hologic APEX version 4.0 software" (CDC NCHS, 2015b, para. 8). Central DXA is widely accepted due to speed, ease of use, and low exposure to radiation. The femoral neck is a part of the femur bone, between the femoral head (which attaches to the pelvis) and femoral shaft. For FNBMD, the left hip was routinely scanned. Possible reasons the right hip was scanned instead of the left may include: metal objects in the left leg, or left hip replacement. Possible reasons the participant's ineligibility included: the presence of bilateral hip fractures, bilateral hip replacements, pins, weight greater than 450 pounds (DXA table limitation), or a positive urine sample for and/or self-report of pregnancy. Each respondent's scan was administered by a trained, certified radiology technologist and reviewed by the University of California, San Francisco, Department of Radiology (CDC NCHS, 2017, p. 10).

Cd Concentration Measured via Urinalysis

The participants, under no dietary or fasting restrictions, were examined in an MEC where a urine sample was collected in a sterile container and then frozen for transportation. The specimen was rejected if the volume was low or was contaminated. If rejected, a second sample was requested. Dynamic reaction cell (DRC) technology was utilized with the technique known

as *inductively coupled plasma mass spectrometry* (ICP-DRC-MS) to measure various elements within the specimen (CDC NCHS, 2017).

Statistical Analysis

The analysis was performed using Statistical Package for the Social Science (SPSS) version 24.0 (IBM Corp, Released 2016). Descriptive statistics computed for continuous variables were measures of centrality (mean or median) and dispersion (standard deviation or interquartile range). Frequency distributions (number and proportion) were computed for categorical variables (gender, smoking status, income, race, and menopausal status).

Simple linear regression was used to test the unadjusted associations between the outcome and exposure or each confounder. Cd and BMI were skewed and so were each natural log (ln) transformed for all regression analyses.

The unadjusted exposure-outcome relationship was graphically displayed using a scatterplot of FNBMD vs. ln(Cd), including the unadjusted regression line.

Multiple linear regression was used to test the associations between ln(Cd) and FNBMD after controlling for confounding by age, ln(BMI), gender, smoking status, income, race, and menopausal status. Continuous covariates (ln(Cd), age, and ln(BMI)) were centered at their median values so that the intercept could have meaningful interpretation as the mean FNBMD at the median value of each continuous covariate and the reference level of each categorical variable. Plots of standardized residuals and partial residual plots were used to check model assumptions (normality, linearity, and homoscedasticity) and the extent of outliers.

All tests were two-tailed and conducted at the $\alpha = 0.05$ level of significance. For both unadjusted and adjusted analyses, multiple comparisons of the mean outcome between levels of

categorical variables were adjusted using the Šidák method (Šidák, 1967). The official NHANES dataset variable names used are shown in Table 3.

Table 3

NHANES 2013-2014 Variable Names

Variable	NHANES Dataset Variable Name	Description of Variable
Urinary Cd	URXUCD	Specified mass of Cd per specified volume of urine
Age	RIDAGEYR	Age in years
BMI	BMXBMI	Body Mass Index (kg/m^2)
Femoral Neck BMD	DXXNKBMD	Femoral neck BMD as measured by DXA
Gender	RIAGENDR	Gender of participant
Smoking	SMQ020 and SMQ040	Smoked at least 100 cigarettes in life and smoke cigarettes now
Income	INDHHIN2	Total household income (reported as a range value in dollars)
Race	RIDRETH1	Recode of reported race and Hispanic origin information
Menopausal Status[a]	RHQ031, RHD043	Had regular periods in past 12 months and reason not having regular period

[a] Not an official NHANES category, was created for this analysis.
Note: NHANES = National Health and Nutrition Examination Survey; Cd = cadmium; BMI = Body Mass Index; BMD = bone mineral density; DXA = dual-energy x-ray absorptiometry
Source: Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS), 2017.

Results

Description of Study Population

Table 4 shows the descriptive statistics for the sample, overall (N=914) and by Cd exposure. Study participants in the Low Exposure (LE) group fell below the overall median (0.23 µg/L) and those in the High Exposure (HE) group were above the median. The mean age

of participants was 58.5 (±11.8) years, with the LE group (56.5 ±11.6) being, on average, almost 4 years younger than those in the HE group (60.4 ±11.6).

There was no gender difference between the LE and HE groups, each comprised of 51% males and 49% females. In the LE group, 33.2% were current of former smokers. In the HE group 59.9% were current or former smokers. Participants with a household income of ≥$55,000 comprised the highest proportion (50%) in the LE group. In the HE group, it was only 33.6%. Regarding race, 52% of the LE group was non-Hispanic (NH) White followed by 22.3% of participants of Hispanic descent. In the HE group, 41% were NH White followed by 25% NH Black. Regarding menopausal status, 61.6% identified as post-menopausal in the LE group and 74.6% in the HE group.

Table 4

Characteristics of Adult Study Participants in the NHANES 2013 - 2014 with DXA Measurements [a], Overall and by Cadmium Exposure

Characteristic	Overall		Low Exposure (<.23 µg/L)		High Exposure (≥ .23 µg/L)	
N (%)	914	(100%)	458	(50.1%)	456	(49.9%)
Cd, urine (µg/L), median (IQR)	0.23	(0.34)	0.11	(0.11)	0.45	(0.39)
Age (years), mean (sd)	58.5	(11.8)	56.5	(11.6)	60.4	(11.6)
BMI (kg/m^2), median (IQR)	27.8	(7.3)	28.1	(45.8)	27.4	(7.2)
FNBMD (g/cm^2), mean (sd)	0.77	(0.14)	0.79	(0.14)	0.77	(0.14)
Gender, n (%)						
Male	467	(51.1)	234	(51.1)	233	(51.1)
Female	447	(48.9)	224	(48.9)	223	(48.9)
Smoking Status, n (%)						
Never	489	(53.5)	306	(66.8)	183	(40.1)
Former	152	(16.6)	45	(9.8)	107	(23.5)
Current	273	(29.9)	107	(23.4)	166	(36.4)
Income, n (%)						
<$25,000	270	(29.5)	103	(22.5)	167	(36.6)
$25,000 to <$55,000	262	(28.7)	126	(27.5)	136	(29.8)
≥$55,000	382	(41.8)	229	(50.0)	153	(33.6)
Race, n (%)						
Hispanic	189	(20.6)	102	(22.3)	87	(19.1)
NH White	425	(46.5)	238	(52.0)	187	(41.0)
NH Black	182	(19.9)	68	(14.8)	114	(25.0)
Other	118	(12.9)	50	(10.9)	68	(14.9)
Post-Menopausal, n (%) [b]						
Yes	304	(68.0)	138	(61.6)	166	(74.4)
No	143	(32.0)	86	(38.4)	57	(25.6)

[a] Adult participants age 40 to >80 years with DXA measurements and non-missing values of all study variables; [b] Female n=447.

Note: NHANES = National Health and Nutrition Examination Survey; DXA = dual-energy x-ray absorptiometry; Cd = cadmium; µg = microgram; L = liter; IQR = interquartile range; sd = standard deviation; BMI = body mass index; kg = kilogram; m = meter; FNBMD = femoral neck bone mineral density; g = gram; cm = centimeter; NH = non-Hispanic.

Univariate Regression

As shown in Table 5, ln(Cd) was negatively associated with BMD (β = -0.008; 95% CI = -0.017, 0.001), but this association was not statistically significant (p = .084). The unadjusted association between Cd and BMD is illustrated in Figure 1. The association was of similar magnitude between genders. Overall, and within each gender, age, ln(BMI), income, race, and postmenopausal status × gender (overall only) were significantly associated with BMD as measured at the femoral neck (p ≤ 009). Additionally, for males only, those that never smoked had significantly greater BMD than current smokers (D = 0.042; 95% CI = 0.008, 0.076; Šidák adjusted p = .010) and, for females, being post-menopausal was associated with significantly lower BMD (D = -0.119; 95% CI = -0.145, -0.093; p <. 001).

Every one-year difference in age was associated with .004 g/cm^2 less BMD overall (95% CI = -0.005, -0.003; p <. 001), with the age effect being twice as large in females (β = -0.006; 95% CI = -0.007, -0.005; p < .001) than in males (β = -0.003; 95% CI = -0.004, -0.001; p < .001). In the overall sample, every one-unit difference in ln(BMI) was associated with 0.277 g/cm^2 more BMD (95% CI = 0.234, 0.319; p < .001). This corresponds to a 10% difference in BMI being associated with 0.0264 g/cm^2 more BMD. Overall (p < .001) and within both males (p = .005) and females (p = .022), participants with an annual household income of <$25,000 had significantly less g/cm^2 of BMD than those in the ≥$55,000 annual household income category. Overall and by gender, NH Black participants had significantly greater BMD than each of the race categories (p ≤ .003). Post-menopausal females had 0.119 g/cm^2 less BMD than premenopausal females and 0.100 less than males (p < .001).

Table 5

Univariate Regression Coefficients (β) and Differences between Groups (D) for FNBMD (g/cm^2), Overall and by Gender

Variable	Overall			Males			Females		
	β	(95% CI)	*p-value*	β	(95% CI)	*p-value*	β	(95% CI)	*p-value*
ln(Cd), urine (μg/L)	-0.008	(-0.017, 0.001)	.084	-0.008	(-0.020, 0.005)	.238	-0.010	(0.022, 0.003)	.124
Age (years)	-0.004	(-0.005, -0.003)	<.001	-0.003	(-0.004, -0.001)	<.001	-0.006	(-0.007, -0.005)	<.001
ln(BMI) (kg/m^2)	0.277	(0.234, 0.319)	<.001	0.321	(0.254, 0.387)	<.001	0.265	(0.213, 0.318)	<.001
	D	(95% CI)	*p-value*	D	(95% CI)	*p-value*	D	(95% CI)	*p-value*
Smoking Status			.623			.014			.757
Never vs Current	0.010	(-0.016, 0.037)	.713	0.042	(0.008, 0.076)	.010	0.001	(-0.039, 0.041)	1.00
Never vs Former	0.006	(-0.026, 0.038)	.954	0.018	(-0.026, 0.061)	.692	0.014	(-0.032, 0.060)	.842
Current vs Former	-0.004	(-0.039, 0.031)	.989	-0.024	(-0.069, 0.020)	.472	0.013	(-0.041, 0.067)	.914
Income ($1,000s)			<.001			.005			.009
<25 vs 25 to <55	-0.021	(-0.051, 0.009)	.262	-0.042	(-0.083, -0.001)	.041	-0.004	(-0.045, -0.038)	.995
<25 vs ≥55	-0.047	(-0.074, -0.019)	<.001	-0.049	(-0.086, -0.012)	.005	-0.044	(-0.082. -0.005)	.022
25 to <55 vs ≥55	-0.026	(-0.053, 0.002)	.075	-0.007	(-0.044, 0.031)	.968	-0.040	(-0.078, -0.001)	.039
Race			<.001			<.001			<.001
NHW vs Hispanic	-0.037	(-0.068, -0.005)	.015	-0.032	(-0.077, 0.012)	.280	-0.043	(-0.087, 0.001)	.062
NHW vs NHB	-0.113	(-0.145, -0.081)	<.001	-0.102	(-.0146, -0.058)	<.001	-0.123	(-0.169, -0.078)	<.001
NHW vs Other	-0.008	(-0.030, -0.045)	.996	-0.002	(-0.054, 0.051)	1.00	0.015	(-0.037, 0.067)	.972
NHB vs Hispanic	0.076	(0.039, 0.114)	<.001	0.070	(0.018, 0.122)	.003	0.081	(0.028, 0.133)	<.001
NHB vs Other	0.121	(0.078, 0.164)	<.001	0.100	(0.041, 0.160)	<.001	0.138	(0.077, 0.168)	<.001
Hispanic vs Other	0.044	(0.001, 0.087)	.039	0.031	(-0.029, 0.090)	.678	0.058	(-0.001, 0.116)	.057
Post-Menopausal by Gender			<.001						
F/Post vs F/Pre	-0.119	(-0.152, -0.086)	<.001				-0.119	(-0.145, -0.093)	<.001
F/Post vs M	-0.100	(-0.124, -0.076)	<.001						
F/Pre vs M	0.019	(-0.012, 0.050)	.376						

Note: p-values for multiple comparisons between levels of each categorical variable were adjusted using the Šidák method. FNBMD = femoral neck bone mineral density; CI = confidence interval; g = gram; cm = centimeter; Cd = cadmium; μg = microgram; L = liter; IQR = interquartile range; sd = standard deviation; BMI = body mass index; kg = kilogram; m = meter; NH = non-Hispanic; NHW = non-Hispanic White; NHB = non-Hispanic Black; Other = all races not specified; F = female; Post = post-menopausal; Pre = pre-menopausal; M = male.

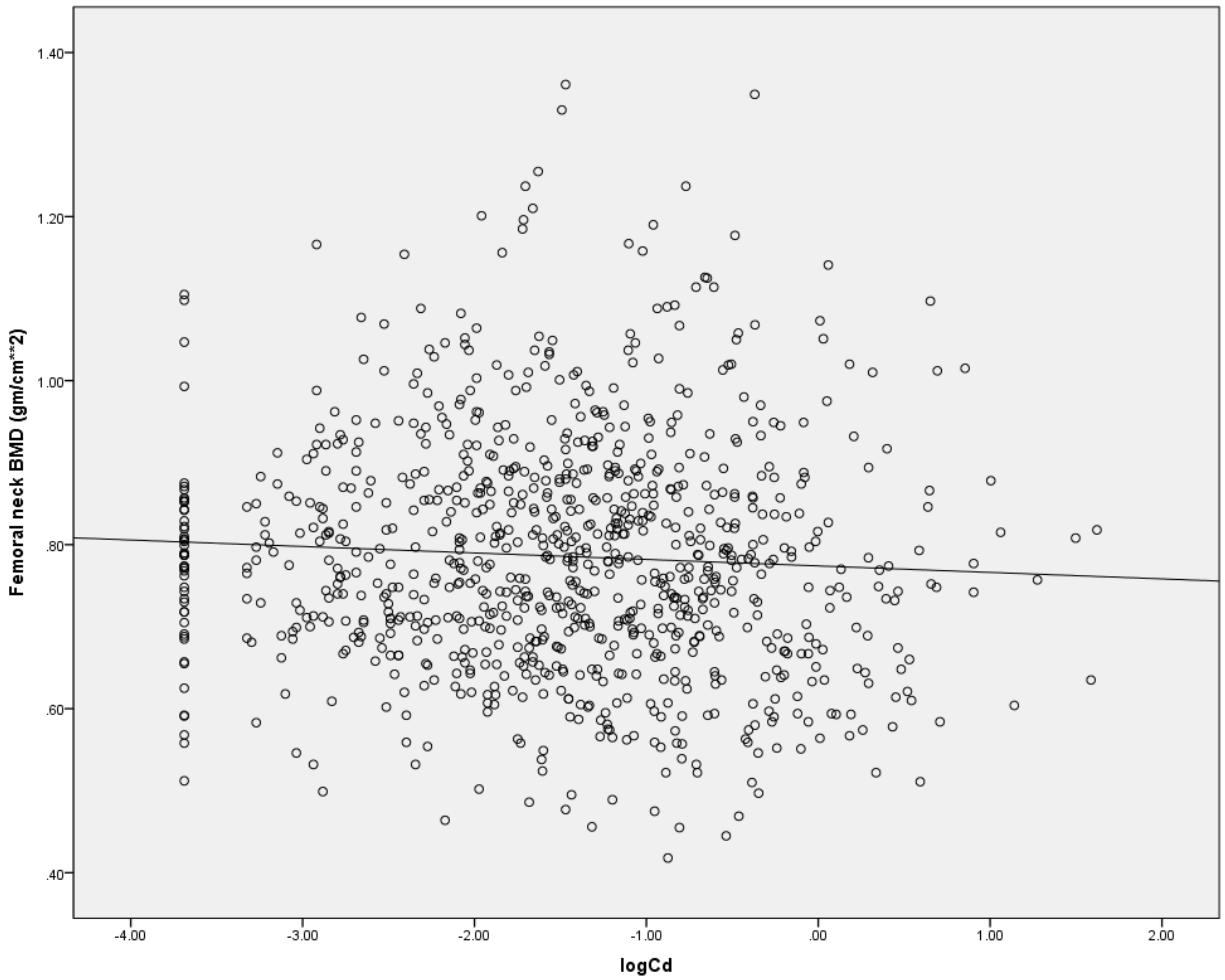


Figure 1. Unadjusted association between bone mineral density and log transformed cadmium. Source: IBM Corp, Released 2016

Multivariable Regression

As reported in Table 6, after adjusting for age, ln(BMI), smoking status, income, race, and gender x postmenopausal status, ln(Cd) was not associated with BMD, either overall (p = 0.908) or in males (p = 0.618 or females (p = 0.942). Age was significantly associated with less BMD overall and by gender ($p \leq .002$). Overall (D = -0.036; 95% CI = -0.059, -0.013; p = .001) and among males (D = -0.041; 95% CI = -0.075, -0.006; p = .016), participants with an annual household income of <$25,000 had significantly less BMD than those in the ≥$55,000 annual household income category. For female participants, those with an annual household income of

$25,000 to ≤$55,000 had significantly less BMD than those in the ≥$55,000 categories (D = -0.029; 95% CI = -0.057, -0.000; $p = .045$). NH Black participants had significantly greater BMD than other race categories ($p \leq .009$). In the model pooled over sexes, females that identified as postmenopausal had significantly lower BMD than premenopausal females (D = -0.068; 95% CI = -0.099, -0.037; $p < .001$) and males (D = -0.092; 95% CI = -0.112, -0.071; $p < .001$). In the model for females alone, postmenopausal women had 0.041 g/cm^2 less BMD than premenopausal women (95% CI = -0.068, -0.013; $p = .004$).

As shown in Figure 2, the standardized residuals were approximately normally distributed. Additionally (not shown), partial residual plots affirmed the assumptions of linearity and homoscedasticity.

Table 6

Multivariable Regression Coefficients (B) and Differences between Groups (D) for FNBMD (g/cm^2), Overall and by Gender

Variable	Overall			Males			Females		
	β	(95% CI)	*p-value*	β	(95% CI)	*p-value*	β	(95% CI)	*p-value*
Intercept	0.722	(0.689, 0.756)	<.001	0.840	(0.795, 0.886)	<.001	0.695	(0.652, 0.737)	<.001
ln(Cd), urine (µg/L) [a]	0.000	(-0.007, 0.008)	.908	0.003	(-0.009, 0.016)	.618	0.000	(-0.010, 0.009)	.942
Age (years) [a]	-0.003	(-0.004, -0.002)	<.001	-0.002	(-0.003, -0.001)	.002	-0.005	(-0.006, -0.004)	<.001
ln(BMI) (kg/m^2) [a]	0.250	(0.211, 0.288)	<.001	0.307	(0.241, 0.373)	<.001	0.209	(0.165, 0.254)	<.001
	D	(95% CI)	*p-value*	D	(95% CI)	*p-value*	D	(95% CI)	*p-value*
Smoking Status			.738			.155			.147
Never vs Current	0.006	(-0.016, 0.028)	.879	0.025	(-0.007, 0.057)	.178	-0.010	(-0.041, 0.021)	.827
Never vs Former	0.007	(-0.021, 0.035)	.909	0.004	(-0.037, 0.046)	.992	0.024	(-0.013, 0.062)	.320
Current vs Former	0.001	(-0.029, 0.030)	1.00	-0.021	(-0.062, 0.021)	.547	0.034	(-0.008, 0.076)	.150
Income ($1,000s)			<.001			.014			.035
<25 vs 25 to <55	-0.015	(-0.039, 0.009)	.346	-0.035	(-0.070, 0.001)	.065	0.005	(-0.026, 0.036)	.971
<25 vs ≥55	-0.036	(-0.059, -0.013)	.001	-0.041	(-0.075, -0.006)	.016	-0.024	(-0.054, 0.006)	.168
25 to <55 vs ≥55	-0.021	(-0.043, 0.001)	.074	-0.006	(-0.040, 0.028)	.963	-0.029	(-0.057, 0.000)	.045
Race			<.001			<.001			<.001
NHW vs Hispanic	-0.021	(-0.048, 0.006)	.213	-0.034	(-0.074, 0.007)	.152	-0.005	(-0.041, 0.030)	.999
NHW vs NHB	-0.097	(-0.124, -0.069)	<.001	-0.099	(-0.139, -0.058)	<.001	-0.094	(-0.131, -0.057)	<.001
NHW vs Other	-0.006	(-0.039, 0.026)	.997	-0.032	(-0.081, -0.018)	.433	0.018	(-0.024, 0.061)	.826
NHB vs Hispanic	0.076	(0.044, 0.108)	<.001	0.065	(0.017, 0.113)	.002	0.088	(0.047, 0.129)	<.001
NHB vs Other	0.091	(0.054, 0.128)	<.001	0.067	(0.012, 0.123)	.009	0.112	(0.064, 0.160)	<.001
Hispanic vs Other	0.015	(-0.022, 0.051)	.865	0.002	(-0.054, 0.059)	1.00	0.024	(-0.022, 0.070)	.686
Post-Menopausal by Gender			<.001						
F/Post vs F/Pre	-0.068	(-0.099, -0.037)	<.001				-0.041	(-0.068, -0.013)	.004
F/Post vs M	-0.092	(-0.112, -0.071)	<.001						
F/Pre vs M	-0.023	(-0.052, 0.005)	.136						

[a] centered at median.

Note: p-values for multiple comparisons between levels of each categorical variable were adjusted using the Šidák method. FNBMD = femoral neck bone mineral density; CI = confidence interval; g = gram; cm = centimeter; Cd = cadmium; µg = microgram; L = liter; IQR = interquartile range; sd = standard deviation; BMI = body mass index; kg = kilogram; m = meter; NH = non-Hispanic; NHW = non-Hispanic White; NHB = non-Hispanic Black; Other = all races not specified; F = female; Post = post-menopausal; Pre = pre-menopausal; M = male.

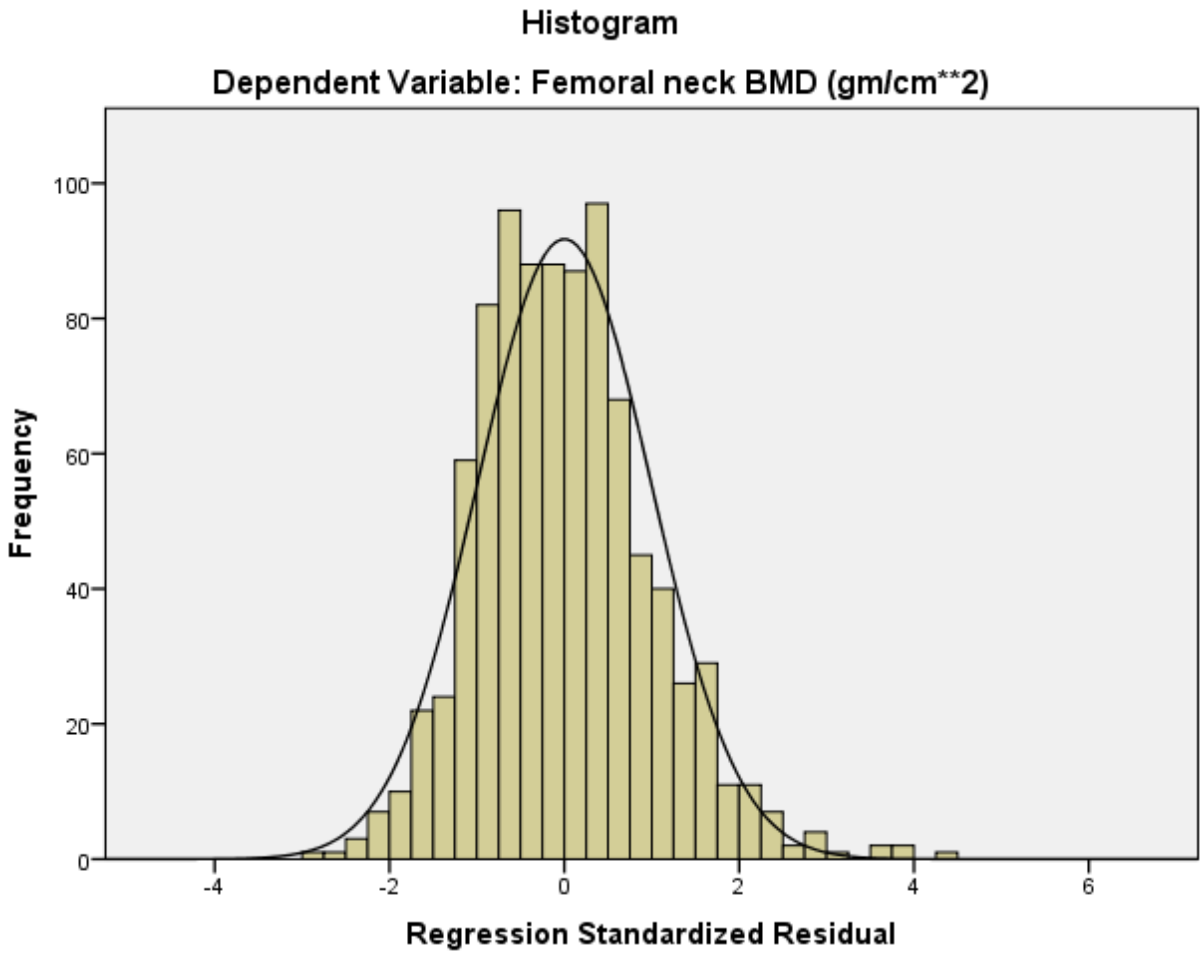


Figure 2. Standardized residuals for the multivariable regression of FNBMD (g/cm^2).[a]

[a] Pooled over sexes

Source: IBM Corp, Released 2016

Discussion

In a cross-sectional analysis of NHANES 2013-2014 data, we observed a small, non-significant, negative association between Cd exposure (as measured in urine) and BMD. This association was near zero after adjusting for confounders, both overall and by gender. The median Cd exposure was much lower in this U.S. sample (0.23 µg/L) than in earlier population studies in Sweden, Japan, and China in which greater Cd was significantly associated with lower

BMD (HHS PHS ATSDR, 2012; Wallin et al., 2016; Gallagher et al., 2008). A smaller exposure range results in less power to detect a significant effect, and it is possible that the effect only exists at higher levels of exposure.

Postmenopausal status × gender, age, and BMI were all significantly associated with BMD. Age and being a post-menopausal female were independently associated with lower BMD; BMI was independently associated with greater BMD. Greater income was associated with greater BMD confirming previous findings that income may impact BMD (HHS PHS ATSDR, 2012). NH Blacks had greater mean BMD than those of other races, parallel to observations from the literature (HHS PHS ATSDR, 2012).

Females that identified as postmenopausal, as found in the literature, had lower BMD than pre-menopausal women or males (HHS PHS ATSDR, 2012). Results from a study on a Chinese population confirm that postmenopausal females have less BMD. In the same Chinese population, it was found that urinary Cd was not associated with less BMD (Chen et al., 2014). Previous analysis of NHANES data found that Cd exposure exacerbated any decrease in BMD associated with postmenopausal status (Gallagher et al., 2008) which is consistent with our findings (although in our study, this interaction was not statistically significant – see Study Limitations below).

When comparing results from NHANES 2013-2014 to earlier reports in 1988-1994 and 1999-2004, the mean exposure levels were significantly less for females in our study than the earlier reports (Gallagher et al., 2008). Differences in exposure levels are not fully known for males. This observation may indicate a secular decline in Cd exposure over the last three decades. Others have looked at creatinine-adjusted-Cd, which may explain differences between our findings and other studies. To check if this was the case, an unplanned analysis controlling

for (log transformed) urinary creatinine was conducted, however our conclusions did not change (no significant association between Cd exposure and BMD was found).

Study Limitations

Although DXA is considered a sensitive assessment of BMD it cannot distinguish calcium phosphate from Cd. Thus, DXA BMD measures may be upwardly biased due to not differentiating Cd (and other heavy metals) from actual bone mineral content. Our conclusion of no Cd effect on BMD could also be biased due to misspecification of the model. For example, the cadmium effect may differ by gender. However, in a secondary analysis, we found that the cadmium effect did not differ significantly between men, pre-menopausal women, and post-menopausal women. Alternatively, the cadmium effect may differ by age. Other explanations for differences between our conclusion and the conclusion of other studies include that this study reported median cadmium and analyzed ln(Cd), whereas some other studies reported mean Cd. Also, some other studies used either blood Cd or urinary Cd adjusted for creatinine clearance.

Conclusion

According to the present study, Cd exposure is not significantly associated with less BMD. Given the possible bias due to use of DXA, more sensitive instruments such as *peripheral computed tomography* (p-QCT) may provide less biased measurement of BMD and, therefore, be more effective in assessing the osteotoxic effect of Cd on bone tissue, if it actually exists. Further research is needed to examine how the Cd effect may vary with age, BMI, gender, smoking status, income, race, and menopausal status when adjusted for creatinine clearance due to the inconclusive results of unplanned analysis.

References

Centers for Disease Control and Prevention (CDC). (2015). *Fourth national report on human exposure to environmental chemicals.* Atlanta, GA: Centers for Disease Control and Prevention. Retrieved from https://www.cdc.gov/biomonitoring/pdf/fourthreport_updatedtables_feb2015.pdf

Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). (2015a). *National health and nutrition examination survey 2013-2014 data documentation, codebook, and frequencies body measures*. Retrieved from https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/BMX_H.htm

Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). (2015b). *National health and nutrition examination survey 2013-2014 data documentation, codebook, and frequencies dual-energy x-ray absorptiometry - femur.* Retrieved from https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/DXXFEM_H.htm

Centers for Disease Control and Prevention (CDC). (2016). *National biomonitoring summary program biomonitoring summary: Cadmium (Case No. 7440-43-9).* Atlanta. Retrieved from https://www.cdc.gov/biomonitoring/Cadmium_BiomonitoringSummary.html

Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). (2016). *National health and nutrition examination survey 2013-2014 data documentation, codebook, and frequencies smoking - cigarette use.* Retrieved from https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/SMQ_H.htm

Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). (2017). *National health and nutrition examination survey 2013-2014 data*. Retrieved from https://wwwn.cdc.gov/nchs/nhanes/Search/Nhanes13_14.aspx

Chen, X., Wang, K., Wang, Z., Gan, C., He, P., Liang, Y., . . . Zhu, G. (2014). Effects of lead and cadmium co-exposure on bone mineral density in a Chinese population. *Bone, 63*, 76-80. doi:10.1016/j.bone.2014.02.017

Drake, M. T., & Khosla, S. (2013). The role of sex steroids in the pathogenesis of osteoporosis. In C. J. Rosen (Ed.), *Primer on metabolic bone diseases and disorders of mineral metabolism* (pp. 367-375). Hoboken, NJ: John Wiley & Sons, Inc.

Du, Y., Zhao, L. -J., Xu, Q., Wu, K.-H., & Deng, H. -W. (2017). Socioeconomic status and bone mineral density in adults by race/ethnicity and gender: The Louisiana osteoporosis study. *Osteoporosis International, 28*(5), 1699-1709. doi:10.1007/s00198-017-3951-1

Duval, J. C. (2005). *Terrestrial radioactivity and gamma-ray exposure in the United States and Canada: U.S. Geological Survey open-file report 2005-1413.* Retrieved from http://pubs.usgs.gov/of/2005/1413

Engström, A., Michaëlsson, K., Vahter, M., Julin, B., Wolk, A., & Åkesson, A. (2012). Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. *Bone, 50*(6), 1372-1378. doi:10.1016/j.bone.2012.03.018

Gallagher, C. M., Kovach, J. S., & Meliker, J. R. (2008). Urinary cadmium and osteoporosis in U.S. women ≥ 50 years of age: NHANES 1988-1994 and 1999-2004. *Environmental Health Perspectives, 116*(10), 1338-1343. doi:10.1289/ehp.11452

Garner, R., & Levallois, P. (2016). *Cadmium levels and sources of exposure among Canadian adults.* Health Reports. Retrieved from https://www.researchgate.net/profile/Rochelle_Garner/publication/294918153_Cadmium

_levels_and_sources_of_exposure_among_Canadian_adults/links/5719170008aed8a339e6fd45/Cadmium-levels-and-sources-of-exposure-among-Canadian-adults.pdf

Harris, R. E. (2013). Epidemiology of osteoporosis. In R. E. Harris, *Epidemiology of chronic disease global perspectives* (pp. 517-535). Burlington: Jones & Bartlett Learning, LLC.

IBM Corp. (Released 2016). IBM SPSS statistics for Windows. *Version 24.0*. Armonk, NY: IBM Corp.

Law, M. R., & Hackshaw, A. K. (1997). A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: Recognition of a major effect. *BMJ, 315*(7112), 841-846. doi:10.1136/bmj.315.7112.841

Looker, A. C., Borrud, L. G., Dawson-Hughes, B., Shepherd, J. A., & Wright, N. C. (2012). *Osteoporosis or low bone mass at the femur neck or lumbar spine in older adults: United States, 2005–2008.* (NCHS data brief no. 93). Hyattsville, MD: National Center for Health Statistics. Retrieved from https://www.cdc.gov/nchs/data/databriefs/db93.pdf

National Institutes of Health (NIH), Osteoporosis and Related Bone Diseases National Resource Center (ORBDNRC). (2015a). *Bone mass measurement: What the numbers mean.* Bethesda: National Institutes of Health Publication No. 15–7877–E . Retrieved from https://www.niams.nih.gov/Health_Info/Bone/Bone_Health/bone_mass_measure.pdf

National Institutes of Health (NIH), Osteoporosis and Related Bone Diseases National Resource Center (ORBDNRC). (2015b). *What is bone?* Bethesda: National Institutes of Health Publication No. 15–7876. Retrieved from https://www.niams.nih.gov/Health_Info/Bone/Bone_Health/What_Is_Bone.pdf

Nordberg, G. F. (2009, August 1). Historical perspectives on cadmium toxicology. *Toxicology and Applied Pharmacology, 238*(3), 192-200. doi:10.1016/J.TAAP.2009.03.015

Norris, S. A., Micklesfield, L. K., & Pettifor, J. M. (2013). Ethnic differences in bone acquisition. In C. J. Rosen (Ed.), *Primer on metabolic bone diseases and disorders of mineral metabolism* (pp. 135-141). Hoboken, NJ: John Wiley & Sons, Inc.

Scimeca, M., Feola, M., Romano, L., Rao, C., Gasbarra, E., Bonanno, E., . . . Tarantino, U. (2017). Heavy metals accumulation affects bone microarchitecture in osteoporotic patients. *Environmental Toxicology, 32*(4), 1333-1342. doi:10.1002/tox.22327

Šidák, Z. (1967). Rectangular confidence regions for the means of multivariate normal distributions. *Journal of the American Statistical Association, 62*(318), 626-633. doi:10.1080/01621459.1967.10482935

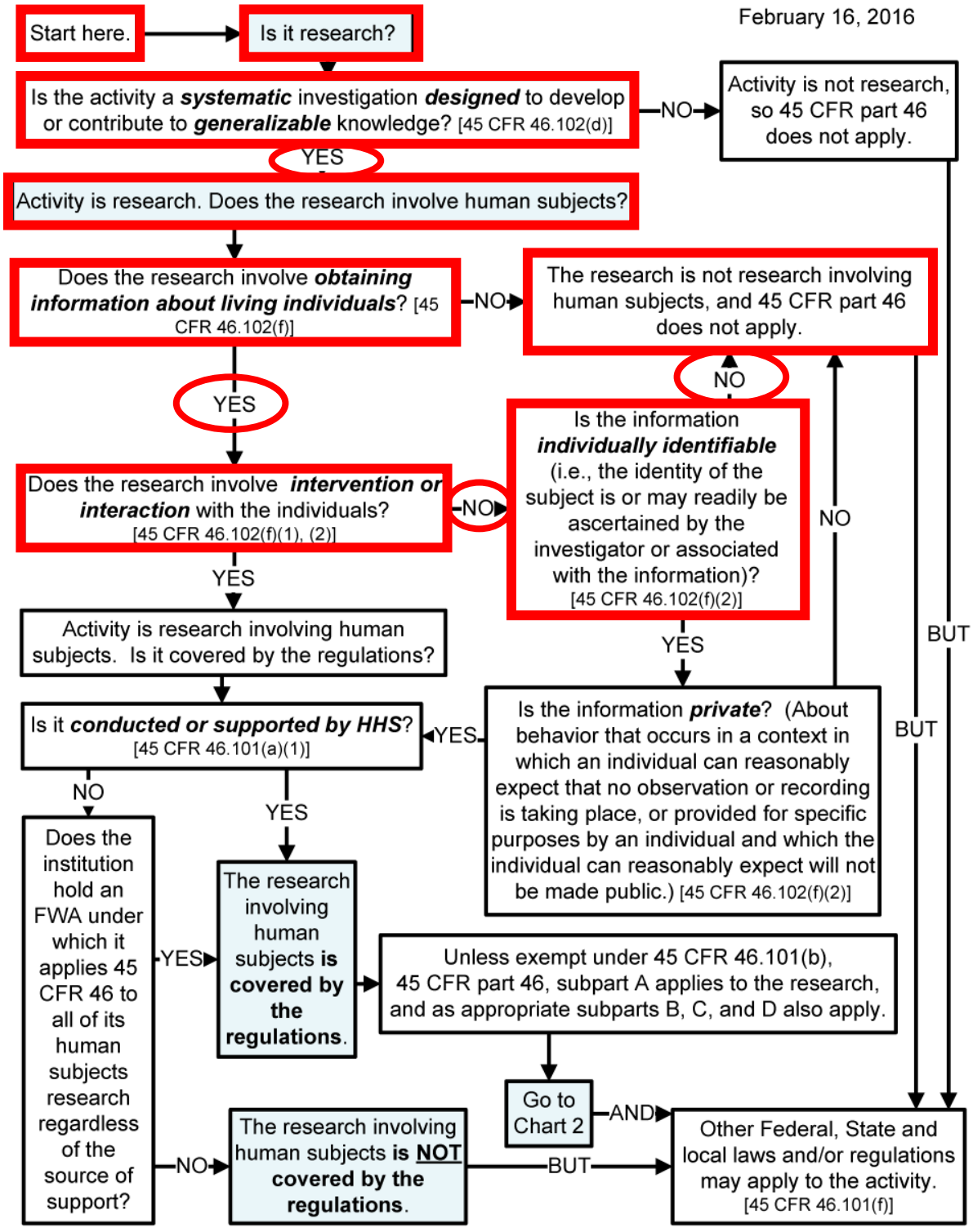
U.S. Department of Health and Human Services (HHS), National Institutes of Health (NIH), National Cancer Institute (NCI). (n.d.). *NCI dictionary of cancer terms*. Retrieved from NIH: National Cancer Institute: https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=415875

U.S. Department of Health and Human Services (HHS), Public Health Service (PHS), Agency for Toxic Substances and Disease Registry (ATSDR). (2012). *Toxicological profile for cadmium.* Atlanta, GA: Agency for Toxic Substances and Disease Registry, Division of Toxicology and Human Health Services. Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf

Wallin, M., Barregard, L., Sallsten, G., Lundh, T., Karlsson, M. K., Lorentzon, M., . . . Mellström, D. (2016). Low-level cadmium exposure is associated with decreased bone mineral density and increased risk of incident fractures in elderly men: The MrOS Sweden study. *Journal of Bone and Mineral Research, 31*(4), 732-741. doi:10.1002/JBMR.2743

World Health Organization (WHO). (1994). *Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: Report of a WHO study group [meeting held in Rome from 22 to 25 June 1992]*. Retrieved from http://apps.who.int/iris/bitstream/10665/39142/1/WHO_TRS_843_eng.pdf

Appendix A: Human Subject Regulations Decision Chart



Appendix B: List of Competencies Met in CE

Wright State Program Public Health Competencies Checklist

Assess and utilize quantitative and qualitative data.
Apply analytical reasoning and methods in data analysis to describe the health of a community.
Describe how policies, systems, and environment affect the health of populations.
Communicate public health information to lay and/or professional audiences with linguistic and cultural sensitivity.
Evaluate and interpret evidence, including strengths, limitations, and practical implications.
Demonstrate ethical standards in research, data collection and management, data analysis, and communication.

Concentration Specific Competencies Checklist

Global Health
Exhibit interpersonal skills that demonstrate willingness to collaborate, trust building abilities, and respect for other perspectives
Conduct evaluation and research related to global health
Enhance socio-cultural and political awareness
Apply systems thinking to analyze a diverse range of complex and interrelated factors shaping health at local, national, and international levels