Human Reproduction, Vol.26, No.10 pp. 2887–2895, 2011
Advanced Access publication on July 21, 2011 doi:10.1093/humrep/der250

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

The association between cadmium, lead and mercury blood levels and reproductive hormones among healthy, premenopausal women

L.W. Jackson [1,*], P.P. Howards [2], J. Wactawski-Wende [3], and E.F. Schisterman [4]

[1]Department of Epidemiology and Biostatistics, Case Western Reserve University School of Medicine, Cleveland, OH 44106-4945, USA [2]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA [3]Department of Social and Preventive Medicine, School of Public Health and Health Professions, University at Buffalo, Buffalo, NY 14214, USA [4]Division of Epidemiology, Statistics, and Prevention Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, MD 20852, USA

*Correspondence address. E-mail: leila.jackson@case.edu

Submitted on July 22, 2010; resubmitted on June 21, 2011; accepted on June 30, 2011

BACKGROUND: Cadmium, lead and mercury have been identified in human follicular fluid and ovarian tissue, and have been associated with adverse reproductive outcomes in epidemiologic studies; however, few studies have examined the relationship between blood metal levels and reproductive hormones.

METHODS: Among 252 premenopausal women aged 18–44 years, we examined the association between blood metal levels (cadmium, lead and mercury), cycle length, and reproductive hormones [FSH, LH, estradiol (E_2) and progesterone] measured at clinically relevant time points in the menstrual cycle. The association between metal levels (continuous) and hormone levels was assessed using linear regression with hormone levels (natural) log transformed and the results interpreted as the percentage difference in hormone level per unit increase in metal level.

RESULTS: Median (interquartile range) cadmium, lead and mercury levels were 0.30 μg/l (0.19, 0.43), 0.87 μg/dl (0.68, 1.20) and 1.10 μg/l (0.58, 2.10), respectively. Each 1 μg/l increase in cadmium levels was associated with a 21% [95% confidence interval (CI): −2.9, 49.9] increase in early follicular phase E_2 levels after adjusting for age, race/ethnicity, lead and mercury. This association decreased when restricted to never smokers (10%; 95% CI: −19.5, 51.3). Cadmium was also associated with a non-significant 9% (95% CI: −0.2, 19.9), or 2.7 day, increase in cycle length among never smokers. No associations were observed between lead or mercury and the outcomes in adjusted analyses.

CONCLUSIONS: Further evaluation of the association between cadmium, E_2 and cycle length is warranted, taking into consideration cigarette smoke and its multiple components.

Key words: cadmium, lead, mercury, estradiol, menstrual cycle

Introduction

Cadmium, lead and mercury are ubiquitous in the environment following many years of industrial use, with most adults having measurable levels of these nonessential elements in their blood. Blood lead levels in premenopausal, non-pregnant women primarily represent current exposures to lead through contaminated food or water, home renovations, lead-glazed pottery, certain health care products and/or folk remedies [Agency for Toxic Substances and Disease Registry (ATSDR), 1990], as well as bone lead stores released during bone turnover (Jackson *et al.*, 2010). Generally, adult exposures to lead are low, unless one is exposed occupationally. Cadmium exposure is primarily from cigarette smoke, both active and passive, as well as consumption of green leafy vegetables, liver and kidney meats and shellfish [Agency for Toxic Substances and Disease Registry (ATSDR), 1999]. Mercury exposure is primarily through fish and shellfish consumption

(organic), and to a lesser extent air pollution (inorganic) (Mahaffey, 2005). All three metals bioaccumulate in the body with lead primarily accumulating in bone where it has a half-life of 12–27 years (Gerhardsson et al., 1993; Bergdahl et al., 1998). Cadmium and mercury accumulate in the kidney and liver with the half-life of cadmium in the kidney being ~30 years, while the half-life of mercury is ~1–3 months (Roels et al., 1991; Jonsson et al., 1999).

All three metals have been identified in human follicular fluid (Zenzes et al., 1995; Paksy et al., 2001; Al-Saleh et al., 2008) and ovarian tissue (Varga et al., 1993), and have been associated with adverse reproductive outcomes in epidemiologic studies. While the biological mechanisms by which these metals may exert adverse reproductive effects have not been fully elucidated, toxicologic studies have provided some insights. Both lead and cadmium have been associated with altered steroidogenesis, decreased gonadotrophin binding and serum gonadotrophin levels in rats (Wiebe et al., 1988; Piasek and Laskey, 1994; Paksy et al., 1997; Priya et al., 2004; Nampoothiri and Gupta, 2006), and lead is associated with altered follicular growth and maturation in mice (Junaid et al., 1997). Cadmium has been shown to activate estrogen (ERα) and androgen receptors and inhibit the binding of estradiol (E_2) to ERα (Young et al., 1977; Garcia-Morales et al., 1994; Stoica et al., 2000; Johnson et al., 2003; Brama et al., 2007). Lead and mercury have also been shown to inhibit the binding of E_2, but are thought to have anti-estrogenic effects (Young et al., 1977; Martin et al., 2003).

The epidemiologic literature supports the hypothesized anti-estrogenic effects of lead (Selevan et al., 2003; Wu et al., 2003; Denham et al., 2005; Popovic et al., 2005; Chang et al., 2006) and the estrogenic effects of cadmium (Gerhard et al., 1998; Jackson et al., 2008), while the effects of mercury are less clear. Few studies have directly examined blood metal levels in relation to hormone levels and cycle length. While Krieg examined the association between blood lead levels, FSH and LH among premenopausal women, blood collection was not timed to particular stages of the menstrual cycle and was limited to women aged 35–60 years of age (Krieg, 2007). Studies examining blood metal levels and cycle length have been primarily cross-sectional or retrospective in design and therefore limited by self-reported recall of cycle length by women (De Rosis et al., 1985; Sikorski et al., 1987; Wang and Tian, 2004). Within the BioCycle study, 2005–2007 (see below), we examined the association between blood metal levels (cadmium, lead and mercury) and menstrual cycle function assessed by prospectively collected data on cycle length and serum hormone levels (FSH, LH, E_2 and progesterone) as measured on clinically relevant days of the menstrual cycle.

Materials and Methods

Study population

This research was carried out as an ancillary study to the BioCycle Study, which has been described in detail elsewhere (Wactawski-Wende et al., 2009). Briefly, the BioCycle Study was designed to examine the association between endogenous hormones and biomarkers of oxidative stress across the menstrual cycle. The study was carried out at the University at Buffalo under an Intramural Research Program contract from the 'Eunice Kennedy Shriver' National Institute of Child Health and Human Development, from 2005 to 2007.

A total of 259 healthy women were recruited from the community and enrolled for up to two menstrual cycles. Premenopausal women aged 18–44 years were eligible for the study if they had a self-reported cycle length between 21 and 35 days. Women who reported the following were not eligible: BMI < 18 or >35 kg/m^2; use of Depo-Provera, Norplant or an intrauterine device in the past 12 months; oral contraceptive or other hormonal supplements in the past 3 months; planning to attempt pregnancy in next the 3 months; actively trying to conceive in the past 6 months; currently pregnant or pregnant in the past 6 months; and breastfeeding in the past 6 months. In addition, women were excluded if they self-reported various medical conditions, medication or supplement use, or diets that may alter hormone or oxidative stress levels (Wactawski-Wende et al., 2009).

Eligible women who consented to participate in the BioCycle Study were asked to come to the study clinic eight times per cycle. The visit days were planned to capture critical windows of hormonal variability during the menstrual cycle and were approximately timed to early- and mid-follicular phase; E_2 peak; LH surge; ovulation; and early-, mid- and late-luteal phase. (For the present study, data for three time points are analyzed.) Participants were provided with and trained to use the Clearblue Easy Fertility Monitor (Inverness Medical Innovations, Inc., Waltham, MA, USA) to help identify and time visits around the LH peak and ovulation (Howards et al., 2009).

During the screening and baseline visits, information on lifestyle factors and reproductive history was collected through structured questionnaires. Dietary information was obtained using a food frequency questionnaire developed by the Nutrition Assessment Shared Resource of Fred Hutchinson Cancer Research Center (http://www.fhcrc.org/science/shared_resources/nutrition/ffq). Height and weight were measured at the baseline visit for the determination of BMI.

Exposure measurement

Whole blood was collected during the screening visit for the measurement of cadmium, lead and mercury with the median time between the screening visit blood draw and first day of the study cycle being 16 days [interquartile range (IQR): 11–31 days]. Concentrations in whole blood primarily represent recent exposure, which is hypothesized to have acute effects resulting in altered follicular growth and steroidogenesis in the subsequent cycle. Collection kits and EDTA purple top tubes were prescreened for trace metals by the Centers for Disease Control and Prevention (CDC). Samples were refrigerated until shipment to the CDC (Division of Laboratory Sciences, National Center for Environmental Health) where they were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). Limits of detections (LOD) using this method were 0.2 μg/dl for lead (0% <LOD), 0.2 μg/l for mercury (13% <LOD) and 0.3 μg/l for cadmium (27% <LOD). Values below the LOD were used as obtained by ICP-MS, without substitution in order to minimize potential measurement error (Richardson and Ciampi, 2003; Schisterman et al., 2006; Whitcomb and Schisterman, 2008).

Outcome assessment

During each cycle visit, women provided 12-h fasting blood specimens. Samples were processed according to a standardized protocol and stored at −80°C. FSH, LH and progesterone concentrations were measured by Specialty Laboratories Inc. (Valencia, CA, USA) using a solid-phase competitive chemiluminescent enzymatic immunoassay on a DPC IMMULITE 2000 analyzer (Siemens Medical Solutions Diagnostics, Deerfield, IL, USA). E_2 concentrations were determined by radioimmunoassay (Kaleida Health Center for Laboratory Medicine, Buffalo, NY, USA).

Only hormone levels from the first study cycle were used for this analysis. This was based upon the hypothesized acute effect of metals as

described above, and the potential fluctuation of cadmium and mercury levels over time caused by sporadic exposures to cigarette smoke and fish consumption. All cycles were standardized to a 28-day cycle as described by Whitcomb *et al*. (2010). FSH and E_2 levels quantified during the early follicular phase (standardized Day 2) were used as measures of ovarian reserve, and follicular growth and maturation. LH levels during the ovulatory window (standardized Day 13), and progesterone levels during the mid-luteal phase (standardized Day 22) were examined as measures of ovulation and corpus luteum function. Menstrual cycle length was determined using prospectively collected data and defined as the difference in time between the date menstrual bleeding began in the first cycle and the date menstrual bleeding began in the next cycle.

Statistical analysis

The exposure and outcome variables were not normally distributed with further analyses taking this into consideration as indicated. Median blood metal levels, hormone levels and cycle length were examined across categories of potential confounders and compared using the Kruskal–Wallis test, and the correlation between metals was examined using Pearson's correlation coefficient. We examined the association between metal levels (continuous) and hormone levels using linear regression with hormone levels (natural) log transformed. Results were back transformed and interpreted as the percentage difference in hormone level per unit increase in metal level. Cycle length was investigated as both a continuous and dichotomous outcome variable in linear (log transformed) and logistic regression models, respectively. Logistic models compared women with short cycles (<25 days) or long cycles (>35 days) to women with normal cycles (25–35 days), independently. Factors shown in the literature to be associated with the metals or the outcomes of interest, or that changed the association between metals and outcomes by more than 10%, were examined as potential confounders including age, race/ethnicity, education, marital status, income, BMI, alcohol consumption, age at menarche, gravidity and parity; all variables were entered as categorized in Table I. Only age and race/ethnicity were found to be confounders and therefore retained in the final models. Models were not adjusted for smoking status given the small number of current and former smokers; however, in sub-analyses, we restricted all models to never smokers to eliminate potential confounding or effect modification by smoking status. Models were run for each metal independently, then including all metals in the model together. Statistical significance was set at $P < 0.05$. All analyses were completed in SAS Version 9.2 software (Cary, NC, USA).

Results

Metals were quantified for 252 of the 259 women who participated in the study. Among these women, 249 had FSH and E_2 measured during the early follicular phase, 213 had peak LH levels measured during the ovulatory window, 217 had progesterone levels measured during the mid-luteal phase and 247 had data on cycle length for the first study cycle. Overall, the study population was young with 51% of the women between the ages of 18 and 24 years (Table I). The majority of the women identified themselves as being white, non-Hispanic (59%) and 40% had a college degree or higher. Only 25% of the population reported drinking alcohol at least once a week, and 81% reported never smoking. Sixty-nine percent of women reported never being pregnant; among the 76 women reporting a previous pregnancy, 65 women reported a previous live birth. Overall, 36% of the study population was overweight or obese.

Median (IQR) cadmium, lead and mercury levels were 0.30 μg/l (0.19, 0.43), 0.87 μg/dl (0.68, 1.20) and 1.10 μg/l (0.58, 2.10), respectively (Table I). Blood lead and cadmium levels increased significantly with age (lead: $P = 0.03$; cadmium: $P = 0.02$); mercury levels did not differ significantly but were highest in the 30–34 year age group. Median metal levels differed significantly by race/ethnicity with those in the 'other' group having the highest concentrations for all three metals (lead and cadmium: $P < 0.01$; mercury: $P = 0.02$). This was primarily explained by women of Asian descent ($n = 36$) who had higher blood metal levels when compared with all non-Asian women ($n = 216$; $P < 0.01$). Blood lead levels decreased with increasing income ($P = 0.04$), but increased with smoking ($P = 0.03$) and alcohol consumption ($P = 0.01$). Blood cadmium levels differed by smoking status ($P < 0.01$) with current smokers having the highest cadmium levels, followed by former smokers, and never smokers. Non-smokers (never and former) who reported environmental tobacco smoke (ETS) exposure at baseline had similar blood cadmium levels ($n = 118$; median: 0.28 μg/l; IQR: 0.19, 0.39) when compared with non-smokers who reported no ETS exposure ($n = 97$; median: 0.32 μg/l; IQR: 0.19, 0.45). Mercury levels differed by alcohol consumption with those consuming one or more drinks per week having the highest levels when compared with those drinking less than one drink per week and never drinkers ($P = 0.02$). Mercury levels increased with increasing frequency of fish and shellfish consumption ($P < 0.01$): never (median: 0.38 μg/l; IQR: 0.16, 0.93), one to four times per month (median: 1.00 μg/l; IQR: 0.53, 1.60) and greater than four times per month (median μg/l: 1.60; IQR: 1.00, 2.65). Blood lead levels were weakly but significantly correlated with cadmium ($r = 0.15$; $P = 0.02$) and mercury levels ($r = 0.14$; $P = 0.03$), and cadmium and mercury levels were similarly correlated ($r = 0.15$; $P = 0.02$).

Follicle-stimulating hormone

The median serum FSH level as measured during the early follicular phase was 6.3 mIU/ml (IQR: 5.3, 7.8; Table II). FSH levels increased with age, gravidity and parity, and decreased with increasing BMI. In unadjusted analyses, neither lead nor mercury was associated with FSH levels (Table III). A 1 μg/l (SD: 0.3) increase in cadmium was associated with a 20% [95% confidence interval (CI): −2.9, 46.9] increase in FSH levels; however, after adjusting for age and race/ethnicity, the association was attenuated and not statistically significant.

Estradiol

The median serum E_2 level as measured during the early follicular phase was 32.0 pg/ml (IQR: 25.0–43.0; Table II). Though E_2 levels did not differ greatly by various demographic, lifestyle or reproductive factors, levels were highest among women who were 40 years of age or older, and among gravid and parous women. In unadjusted analyses, a 1 μg/l (SD: 0.3) increase in blood cadmium levels was associated with a 24% (95% CI: 1.1, 52.9) increase in E_2 levels (Table III). After adjusting for lead, mercury, race/ethnicity and age, this association decreased marginally (21%; 95% CI: −2.9, 49.9) and was no longer statistically significant. When restricted to never smokers, the association between cadmium and E_2 was weaker and imprecise in adjusted analyses (10%; 95% CI: −19.5, 51.3). Neither lead nor mercury was associated with serum E_2 levels.

Table I Median (IQR) blood metal levels stratified by demographic, lifestyle and reproductive factors, BioCycle Study, 2005–2007.

	n	%	Cadmium (μg/l)[a]			Lead (μg/dl)[b]			Mercury (μg/l)[c]		
			Median	Percentile		Median	Percentile		Median	Percentile	
				25th	75th		25th	75th		25th	75th
Total Population	252	100	0.30	0.19	0.43	0.87	0.68	1.20	1.10	0.58	2.10
Age											
18–24 years	129	51	0.28	0.17	0.36	0.82	0.66	1.10	1.10	0.45	2.20
25–29	40	16	0.32	0.18	0.47	0.90	0.67	1.40	1.25	0.74	2.60
30–34	19	8	0.34	0.22	0.48	0.84	0.56	1.20	1.30	0.58	1.50
35–39	32	13	0.29	0.20	0.36	0.93	0.67	1.25	1.15	0.77	1.80
40+	32	13	0.42	0.22	0.56	0.99	0.82	1.40	1.05	0.71	1.90
Race/ethnicity											
Non-Hispanic white	148	59	0.27	0.16	0.37	0.81	0.62	1.05	1.00	0.47	1.80
Non-Hispanic black	48	19	0.31	0.20	0.47	0.86	0.69	1.10	1.05	0.63	1.55
Other	56	22	0.36	0.27	0.53	1.15	0.81	1.60	1.65	0.80	2.75
Education											
High school degree or less	31	12	0.32	0.17	0.46	0.81	0.61	1.00	1.00	0.57	1.50
Some college or associates degree	119	47	0.28	0.17	0.38	0.81	0.65	1.10	1.00	0.44	2.30
College degree or higher	102	40	0.31	0.21	0.47	0.96	0.70	1.40	1.40	0.77	2.10
Marital status											
Married or living as married	64	25	0.31	0.20	0.45	0.89	0.69	1.20	1.00	0.75	1.75
Neither married nor living as married	188	75	0.29	0.19	0.41	0.87	0.67	1.20	1.20	0.50	2.20
Income ($n = 250$)											
<$19 999	53	21	0.32	0.25	0.47	0.98	0.73	1.20	1.10	0.61	2.20
$20 000–$39 999	60	24	0.32	0.21	0.49	0.97	0.69	1.35	1.35	0.81	2.30
$40 000–$74 999	69	28	0.30	0.18	0.40	0.82	0.67	1.10	0.99	0.58	1.80
$75 000 or more	68	27	0.28	0.15	0.38	0.81	0.65	1.05	1.15	0.44	2.35
BMI											
<18.5 kg/m^2	9	4	0.35	0.28	0.48	0.93	0.81	1.10	1.40	0.88	1.50
18.5–24.9	152	60	0.30	0.19	0.41	0.92	0.69	1.20	1.30	0.61	2.25
25.0–29.9	65	26	0.28	0.18	0.39	0.75	0.61	1.00	0.97	0.54	1.80
>29.9	26	10	0.32	0.22	0.44	0.80	0.72	1.40	0.97	0.45	1.70
Current smoking status ($n = 249$)											
Never	201	81	0.29	0.19	0.39	0.84	0.67	1.10	1.10	0.58	2.20
Former	37	15	0.31	0.19	0.45	0.97	0.81	1.30	1.00	0.63	1.80
Current	11	4	0.84	0.35	1.00	1.20	0.76	1.50	1.40	1.00	1.80
Alcohol ($n = 250$)											
Never	82	33	0.31	0.20	0.43	0.77	0.66	1.10	0.99	0.57	1.80
<1 drink per week	105	42	0.31	0.19	0.42	0.85	0.65	1.20	1.10	0.44	1.80
1 or more drinks per week	63	25	0.27	0.17	0.44	1.00	0.81	1.30	1.50	0.91	2.40
Gravidity ($n = 246$)											
No prior pregnancies	170	69	0.29	0.19	0.41	0.86	0.67	1.20	1.10	0.52	2.20
Any prior pregnancies	76	31	0.32	0.21	0.45	0.90	0.69	1.20	1.15	0.80	1.75
Parity ($n = 246$)											
Nulliparous	181	74	0.29	0.19	0.41	0.89	0.68	1.20	1.20	0.57	2.20
Parous	65	26	0.31	0.21	0.45	0.85	0.68	1.10	1.10	0.66	1.60

IQR, interquartile range.
[a]Cadmium concentrations differed significantly across categories of age, race/ethnicity and smoking.
[b]Lead concentrations differed significantly across categories of age, race/ethnicity, education, income, smoking and alcohol consumption.
[c]Mercury concentrations differed significantly across categories of race/ethnicity, education and alcohol consumption.

Luteinizing hormone

The median peak LH level as measured during the ovulatory window was 31.6 ng/ml (IQR: 18.9, 42.8). LH levels did not change greatly over categories of demographic, lifestyle or reproductive factors but was lowest among women who were 40 years of age or older. Women missing peak LH levels ($n = 39$) had slightly higher blood mercury levels (median: 1.5 μg/l; IQR: 0.7, 2.5) and lower FSH levels (median: 6.0 mIU/ml; IQR: 5.1, 7.3) when compared with women with peak LH levels ($n = 213$; median mercury: 1.0 μg/l; IQR: 0.6, 2.0 and median FSH: 6.3 mIU/ml; IQR: 5.3, 8.0). In both unadjusted and adjusted analyses, the associations between metals and peak LH levels were weak and imprecise.

Table II Median (IQR) serum hormone levels and cycle length, BioCycle Study, 2005–2007.

	Cycle stage	*n*	Median	Percentile	
				25th	75th
FSH (mIU/ml)	Early follicular phase	249	6.3	5.3	7.8
Estradiol (pg/ml)	Early follicular phase	249	32.0	25.0	43.0
LH (ng/ml)	Ovulatory window	213	31.6	18.9	42.8
Progesterone (ng/ml)	Mid-luteal phase	217	10.1	7.0	12.4
Cycle length (days)		247	28	26	31

IQR, interquartile range.

Progesterone

The median serum progesterone level as measured during the mid-luteal phase was 10.1 ng/ml (IQR: 7.0, 12.4; Table II). Progesterone levels did not differ greatly by demographic, lifestyle or reproductive factors. Though not statistically significant, current smokers had lower progesterone levels (median: 7.6 ng/ml; IQR: 5.0, 12.0) when compared with former (median: 10.4 ng/ml; IQR: 7.1, 12.1) or never smokers (10.2 ng/ml; IQR: 7.2, 12.5). White, non-Hispanic women (median: 9.5 ng/ml; IQR: 6.8, 12.1) and Black, non-Hispanic women (median 9.9 ng/ml; IQR: 6.3, 13.1) had lower progesterone levels when compared with women of 'other' race/ethnicity (median: 11.5 ng/ml, IQR: 8.9, 13.0; $P = 0.10$). Overall, women with ($n = 217$) and without ($n = 35$) progesterone levels did not differ greatly; however, FSH levels were lower among women with missing progesterone levels (median: 4.5 mIU/ml; IQR: 3.2, 5.7)

Table III Unadjusted and adjusted results for the percentage difference in serum hormone levels or cycle length per unit change in blood metal levels, BioCycle Study, 2005–2007.

	Unadjusted		Model 1[a]		Model 2[b]	
	% difference	95% CI	% difference	95% CI	% difference	95% CI
FSH (mIU/ml; $n = 249$)						
Cadmium (μg/l)	19.5	−2.9, 46.9	3.5	−15.4, 26.5	3.4	−15.7, 26.7
Lead (μg/dl)	3.9	−5.5, 14.1	−2.3	−10.9, 7.2	−2.5	−11.2, 7.0
Mercury (μg/l)	2.2	−2.3, 6.9	1.1	−3.2, 5.6	1.1	−3.3, 5.7
Estradiol (pg/ml; $n = 249$)						
Cadmium (μg/l)	24.3	1.1, 52.9	21.3	−2.1, 50.3	20.6	−2.9, 49.9
Lead (μg/dl)	5.8	−3.7, 16.3	5.6	−4.4, 16.7	4.9	−5.0, 15.9
Mercury (μg/l)	−0.1	−4.5, 4.6	0.0	−4.6, 4.8	−0.6	−5.2, 4.2
LH (ng/ml; $n = 213$)						
Cadmium (μg/l)	1.8	−26.3, 40.5	−7.7	−33.9, 28.9	−7.1	−33.6, 30.0
Lead (μg/dl)	6.3	−8.5, 23.5	1.6	−13.0, 18.6	2.5	−12.3, 19.9
Mercury (μg/l)	−1.8	−8.9, 6.0	−3.4	−10.5, 4.4	−3.4	−10.6, 4.4
Progesterone (ng/ml; $n = 217$)						
Cadmium (μg/l)	3.9	−27.8, 49.5	−4.8	−34.4, 38.4	−7.7	−36.9, 35.0
Lead (μg/dl)	10.0	−6.6, 29.5	4.7	−12.0, 24.5	4.6	−12.2, 24.6
Mercury (μg/l)	5.3	−2.8, 14.1	3.4	−5.0, 12.5	3.5	−5.0, 12.7
Cycle Length (days) ($n = 247$)						
Cadmium (μg/l)	1.0	−5.4, 7.7	2.8	−3.6, 9.6	2.7	−3.8, 9.6
Lead (μg/dl)	0.2	−2.8, 3.2	0.3	−2.7, 3.4	0.2	−2.8, 3.3
Mercury (μg/l)	0.5	−0.9, 1.9	0.3	−1.2, 1.7	0.2	−1.2, 1.6

CI, confidence interval.
[a]Models adjusted for age (18–24, 25–29, 30–34, 35–39, 40–44 years) and race/ethnicity (non-Hispanic white, non-Hispanic black, other) in linear regression.
[b]Models adjusted for cadmium, lead, mercury, age (18–24, 25–29, 30–34, 35–39, 40–44 years) and race/ethnicity (non-Hispanic white, non-Hispanic black, other) in linear regression.

when compared with women with progesterone levels (median: 6.4 mIU/ml; IQR: 5.5, 8.1; $P < 0.01$). In unadjusted analyses (Table III), lead was associated with a 10% (95% CI: −6.6, 29.5) increase in progesterone levels; however, after adjusting for cadmium, mercury, age and race/ethnicity, this association diminished (5%; 95% CI: −12.2, 24.6). Cadmium and mercury were not associated with serum progesterone levels.

Cycle length

Median cycle length was 28 days (IQR: 26, 31; Table II) with length decreasing with increasing age, gravity and parity ($P < 0.01$). No association was observed between lead and mercury concentrations and cycle length in the full study population (Table III). In adjusted analyses, a 1 μg/l (SD: 0.3) increase in cadmium was associated with a 3% (95% CI: −3.8, 9.6) or 0.8 day increase in cycle length; however, when analysis was restricted to never smokers, the association strengthened and was associated with a 9% (95% CI: −0.2, 19.9) or 2.7 day increase in cycle length. In logistic regression, a 1 μg/l (SD: 0.3) increase in cadmium was moderately associated with having a cycle length >35 days (odds ratio: 4.2; 95% CI: 0.6, 28.3) when compared with a normal cycle length (25–35 days) after adjusting for age, race/ethnicity, lead and mercury (Table IV); however, this should be interpreted cautiously given the small number of women with cycle length >35 days ($n = 17$).

Discussion

In this population of healthy, regularly menstruating women, we examined the association between blood metal levels and hormone levels as measured at clinically relevant time points during the menstrual cycle. Blood cadmium levels were positively associated with early follicular phase E_2 levels; however, this effect diminished when limiting analyses to never smokers. Cadmium was also associated with a longer cycle length though not statistically significant. No associations were observed between lead or mercury and the outcomes of interest in adjusted analyses.

Table IV Unadjusted and adjusted odds ratios and 95% CIs for the association between blood metal levels and cycle length, BioCycle Study, 2005–2007.

	Unadjusted		Adjusted	
	OR	95% CI	OR	95% CI
Short versus normal cycle[a,b]				
Cadmium	1.7	0.4, 7.1	1.4	0.3, 7.0
Lead	0.9	0.4, 2.0	0.9	0.4, 2.3
Mercury	0.7	0.4, 1.1	0.7	0.4, 1.2
Long versus normal cycle[a,b]				
Cadmium	1.9	0.5, 6.9	4.2	0.6, 28.3
Lead	0.6	0.2, 1.9	0.5	0.1, 1.9
Mercury	1.2	0.9, 1.7	1.2	0.9, 1.7

CI, confidence interval; OR, odds ratio.
[a]Models adjusted for cadmium, lead, mercury, age (18–24, 25–29, 30–34, 35–39, 40–44 years) and race/ethnicity (non-Hispanic white, non-Hispanic black, other) using logistic regression.
[b]Short cycle (<25 days; $n = 22$), normal cycle (25–35 days; $n = 208$) and long cycle (>35 days; $n = 17$).

The major sources of cadmium in the general population are through cigarette smoke (both active and passive), shellfish, kidney and liver meats, and green leafy vegetables, with levels in food directly associated with contaminated water and soil [Agency for Toxic Substances and Disease Registry (ATSDR), 1999]. Median blood cadmium levels among current smokers in our study population (0.84 μg/l) were similar to those of women aged 20–44 years in the National Health and Nutrition Examination Survey (NHANES) 1999–2006 (0.84 μg); however, never smokers in our study had somewhat higher cadmium levels when compared with women in NHANES: 0.29 versus 0.21 μg/l, respectively (Mijal and Holzman, 2010). As indicated by the increasing blood cadmium levels with age, cadmium tends to bioaccumulate in the tissues of the kidney and liver, but has been found in uterine tissue (Nasiadek et al., 2005) and follicular fluid (Zenzes et al., 1995) with levels significantly higher in smokers than non-smokers (Varga et al., 1993; Zenzes et al., 1995).

While cigarette smoke has been associated with anti-estrogenic effects including a decreased risk of endometrial cancer and an earlier age at menopause (Baron et al., 1990; Parente et al., 2008; Zhou et al., 2008), cadmium, one of many contaminants in cigarettes, has been associated with estrogenic effects. Cadmium has been associated with an earlier onset of vaginal opening, increased uterine weights and enhanced mammary development in rats (Johnson et al., 2003); decreased gonadotrophin binding and altered steroidogenic enzyme activity in granulosa cells of rats (Priya et al., 2004; Nampoothiri and Gupta, 2006); menstrual cycle abnormalities among young women (Wang and Tian, 2004); endometriosis (Jackson et al., 2008); difficulties getting pregnant among married women (Wang and Tian, 2004); and preterm delivery (Laudanski et al., 1991; Fagher et al., 1993).

Based upon previous studies, it is unclear why cadmium would be associated with increased E_2 levels during the early follicular phase; however, this association may be reflective of cigarette smoke exposure, and not cadmium. In epidemiologic studies, smoking has been associated with increased E_2 levels (Windham et al., 2005), as well as shorter overall cycle length (Kato et al., 1999; Rowland et al., 2002), and shorter follicular phase length (Liu et al., 2004). Given the low prevalence of smoking in our study population, we were unable to examine effects among smokers; however, the observed association between cadmium and early follicular phase E_2 in the main analysis decreased when restricted to never smokers. This is consistent with a study of men, in which smoking was shown to be a strong confounder (and not an effect modifier), explaining an observed association between cadmium and E_2 levels in adjusted analyses (Menke et al., 2008). Alternatively, the lack of an association when excluding smokers may be due to exclusion of those individuals with the highest blood cadmium levels with there being a potential threshold effect. In regards to cycle length, the observed association between cadmium and increased cycle length became stronger when restricting analyses to never smokers, suggesting possible independent and opposing effects of cadmium and cigarette smoke on cycle length.

We did not observe any associations between blood lead or mercury levels and the outcomes of interest in adjusted analyses despite

previous studies indicating they may have endocrine disrupting effects. This may be a reflection of the low lead and mercury levels experienced by our study population, or may indicate the lack of a true association with the measured hormones or cycle length. Alternatively, the study eligibility criteria, the timing of exposure measurement, and/or the selected biomarker of exposure (whole blood) may have limited our ability to detect an association as discussed below.

Our sample size ($n = 252$) may have limited our ability to detect significant effects between metals and the outcomes of interest, should an association exist. Furthermore, given the eligibility criteria and primary objectives of the BioCycle Study, the study population was comprised of healthy, young women, which may have decreased the variability in blood metal levels and outcome measures, thereby decreasing our power to detect associations. In addition, women with conditions potentially related to higher metal levels (e.g. shorter or longer menstrual cycle lengths, medication or supplement use, ever having treatment for infertility, presence of endometriosis or fibroids, history of polycystic ovary syndrome, kidney disease, diabetes, cancer, thyroid disease, endocrine dysfunction or treatment of anemia) were excluded, which may have limited our ability to detect a threshold effect if an association were present at higher metal levels but not lower metal levels. The exclusion of women with self-reported cycle lengths <21 days or >35 days limited our analysis of cycle length with only 22 women having an observed cycle length <25 days and 17 women having a cycle >35 days. Overall, the strict eligibility criteria for the study limit our ability to generalize the results to all premenopausal women.

We had good estimates of hormone levels as measured on clinically relevant days of the menstrual cycle, and then standardized to a 28-day cycle to ensure comparisons across women were being made at the same physiologic time point in the cycle. LH and progesterone levels were missing for 15% ($n = 39$) and 14% ($n = 35$) of women in the study, respectively; however, we did not find major differences between those with and without data that would explain the observed associations. As women with missing LH levels had slightly higher mercury levels, a possible inverse association between increasing mercury levels and decreased LH levels may have been missed. We had excellent measures of blood metal levels representative of current metal exposures. The majority of participants had metal levels measured within 16 days of the study cycle when follicles are growing and exposure may result in altered steroidogenesis and cycle length in the subsequent cycle. The study may have been limited if exposure was not measured during the critical window of exposure. Animal studies suggest that prenatal or childhood exposure may represent a sensitive window of exposure for lead and cadmium as the hypothalamic–pituitary–ovarian axis is developing during this time period (Wide, 1985; Wide and D'Argy, 1986; Wiebe *et al.*, 1988; McGivern *et al.*, 1991; Ronis *et al.*, 1996, 1998; Dearth *et al.*, 2002; Johnson *et al.*, 2003). Alternatively, if cumulative metal exposure is more relevant to the outcomes of interest, metal levels should have been measured in alternative matrices, such as bone (lead), hair (mercury) or urine (cadmium).

This is one of the first studies to look at the association between metals and reproductive hormones in women. In adjusted analyses, we observed a non-significant increase in early follicular phase E_2 levels in relation to cadmium; however, this association diminished after restricting analyses to never smokers. Cadmium was also potentially associated with an increased cycle length in never smokers. These observed associations between cadmium, E_2 and cycle length need to be examined in future studies, taking into consideration cigarette smoke and its multiple components. The effects of lead and mercury on reproductive hormones deserve further study in larger and more representative populations.

Authors' roles

L.W.J.: conceived of the ancillary study, developed the associated study design, oversaw statistical analyses, interpreted results and wrote the manuscript. P.P.H.: helped to execute the parent study, completed statistical analyses and reviewed manuscript. J.W.-W.: carried out the parent study, and reviewed statistical analyses and manuscript. E.F.S.: conceived of and carried out the parent study, reviewed statistical analyses and manuscript.

Acknowledgements

We wish to thank the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention for their technical assistance in analyzing samples for the quantification of blood metal levels.

Funding

This work was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health. None of the authors have competing financial interests related to this work.

References

Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Lead*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 1990.

Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Cadmium*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 1999.

Al-Saleh I, Coskun S, Mashhour A, Shinwari N, El-Doush I, Billedo G, Jaroudi K, Al-Shahrani A, Al-Kabra M, El Din MG. Exposure to heavy metals (lead, cadmium and mercury) and its effect on the outcome of in-vitro fertilization treatment. *Int J Hyg Environ Health* 2008; **211**:560–579.

Baron JA, La VC, Levi F. The antiestrogenic effect of cigarette smoking in women. *Am J Obstet Gynecol* 1990;**162**:502–514.

Bergdahl IA, Stromberg U, Gerhardsson L, Schutz A, Chettle DR, Skerfving S. Lead concentrations in tibial and calcaneal bone in relation to the history of occupational lead exposure. *Scand J Work Environ Health* 1998;**24**:38–45.

Brama M, Gnessi L, Basciani S, Cerulli N, Politi L, Spera G, Mariani S, Cherubini S, d'Abusco AS, Scandurra R *et al.* Cadmium induces mitogenic signaling in breast cancer cell by an ERalpha-dependent mechanism. *Mol Cell Endocrinol* 2007;**264**:102–108.

Chang SH, Cheng BH, Lee SL, Chuang HY, Yang CY, Sung FC, Wu TN. Low blood lead concentration in association with infertility in women. *Environ Res* 2006;**101**:380–386.

De Rosis F, Anastasio SP, Selvaggi L, Beltrame A, Moriani G. Female reproductive health in two lamp factories: effects of exposure to inorganic mercury vapour and stress factors. *Br J Ind Med* 1985; **42**:488–494.

Dearth RK, Hiney JK, Srivastava V, Burdick SB, Bratton GR, Dees WL. Effects of lead (Pb) exposure during gestation and lactation on female pubertal development in the rat. *Reprod Toxicol* 2002;**16**:343–352.

Denham M, Schell LM, Deane G, Gallo MV, Ravenscroft J, DeCaprio AP. Relationship of lead, mercury, mirex, dichlorodiphenyldichloroethylene, hexachlorobenzene, and polychlorinated biphenyls to timing of menarche among Akwesasne Mohawk girls. *Pediatrics* 2005; **115**:e127–e134.

Fagher U, Laudanski T, Schutz A, Sipowicz M, Akerlund M. The relationship between cadmium and lead burdens and preterm labor. *Int J Gynaecol Obstet* 1993;**40**:109–114.

Garcia-Morales P, Saceda M, Kenney N, Kim N, Salomon DS, Gottardis MM, Solomon HB, Sholler PF, Jordan VC, Martin MB. Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. *J Biol Chem* 1994; **269**:16896–16901.

Gerhard I, Monga B, Waldbrenner A, Runnebaum B. Heavy metals and fertility. *J Toxicol Environ Health A* 1998;**54**:593–611.

Gerhardsson L, Attewell R, Chettle DR, Englyst V, Lundstrom NG, Nordberg GF, Nyhlin H, Scott MC, Todd AC. In vivo measurements of lead in bone in long-term exposed lead smelter workers. *Arch Environ Health* 1993;**48**:147–156.

Howards PP, Schisterman EF, Wactawski-Wende J, Reschke JE, Frazer AA, Hovey KM. Timing clinic visits to phases of the menstrual cycle by using a fertility monitor: the BioCycle Study. *Am J Epidemiol* 2009; **169**:105–112.

Jackson LW, Zullo MD, Goldberg JM. The association between heavy metals, endometriosis and uterine myomas among premenopausal women: National Health and Nutrition Examination Survey 1999–2002. *Hum Reprod* 2008;**23**:679–687.

Jackson LW, Cromer BA, Panneerselvam A. The association between bone turnover, micronutrient intake and blood lead levels among pre- and post-menopausal women, NHANES 1999–2002. *Environ Health Perspect* 2010;**118**:1690–1696.

Johnson MD, Kenney N, Stoica A, Hilakivi-Clarke L, Singh B, Chepko G, Clarke R, Sholler PF, Lirio AA, Foss C *et al.* Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nat Med* 2003;**9**:1081–1084.

Jonsson F, Sandborgh-Englund G, Johanson G. A compartmental model for the kinetics of mercury vapor in humans. *Toxicol Appl Pharmacol* 1999; **155**:161–168.

Junaid M, Chowdhuri DK, Narayan R, Shanker R, Saxena DK. Lead-induced changes in ovarian follicular development and maturation in mice. *J Toxicol Environ Health* 1997;**50**:31–40.

Kato I, Toniolo P, Koenig KL, Shore RE, Zeleniuch-Jacquotte A, Akhmedkhanov A, Riboli E. Epidemiologic correlates with menstrual cycle length in middle aged women. *Eur J Epidemiol* 1999; **15**:809–814.

Krieg EF Jr. The relationships between blood lead levels and serum follicle stimulating hormone and luteinizing hormone in the third National Health and Nutrition Examination Survey. *Environ Res* 2007; **104**:374–382.

Laudanski T, Sipowicz M, Modzelewski P, Bolinski J, Szamatowicz J, Razniewska G, Akerlund M. Influence of high lead and cadmium soil content on human reproductive outcome. *Int J Gynaecol Obstet* 1991; **36**:309–315.

Liu Y, Gold EB, Lasley BL, Johnson WO. Factors affecting menstrual cycle characteristics. *Am J Epidemiol* 2004;**160**:131–140.

Mahaffey KR. Mercury exposure: medical and public health issues. *Trans Am Clin Climatol Assoc* 2005;**116**:127–153.

Martin MB, Reiter R, Pham T, Avellanet YR, Camara J, Lahm M, Pentecost E, Pratap K, Gilmore BA, Divekar S *et al.* Estrogen-like activity of metals in MCF-7 breast cancer cells. *Endocrinology* 2003; **144**:2425–2436.

McGivern RF, Sokol RZ, Berman NG. Prenatal lead exposure in the rat during the third week of gestation: long-term behavioral, physiological, and anatomical effects associated with reproduction. *Toxicol Appl Pharmacol* 1991;**110**:206–215.

Menke A, Guallar E, Shiels MS, Rohrmann S, Basaria S, Rifai N, Nelson WG, Platz EA. The association of urinary cadmium with sex steroid hormone concentrations in a general population sample of US adult men. *BMC Public Health* 2008;**8**:72.

Mijal RS, Holzman CB. Blood cadmium levels in women of childbearing age vary by race/ethnicity. *Environ Res* 2010;**110**:505–512.

Nampoothiri LP, Gupta S. Simultaneous effect of lead and cadmium on granulosa cells: a cellular model for ovarian toxicity. *Reprod Toxicol* 2006;**21**:179–185.

Nasiadek M, Krawczyk T, Sapota A. Tissue levels of cadmium and trace elements in patients with myoma and uterine cancer. *Hum Exp Toxicol* 2005;**24**:623–630.

Paksy K, Rajczy K, Forgacs Z, Lazar P, Bernard A, Gati I, Kaali GS. Effect of cadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells. *J Appl Toxicol* 1997;**17**:321–327.

Paksy K, Gati I, Naray M, Rajczy K. Lead accumulation in human ovarian follicular fluid, and in vitro effect of lead on progesterone production by cultured human ovarian granulosa cells. *J Toxicol Environ Health A* 2001;**62**:359–366.

Parente RC, Faerstein E, Celeste RK, Werneck GL. The relationship between smoking and age at the menopause: a systematic review. *Maturitas* 2008;**61**:287–298.

Piasek M, Laskey JW. Acute cadmium exposure and ovarian steroidogenesis in cycling and pregnant rats. *Reprod Toxicol* 1994;**8**:495–507.

Popovic M, McNeill FE, Chettle DR, Webber CE, Lee CV, Kaye WE. Impact of occupational exposure on lead levels in women. *Environ Health Perspect* 2005;**113**:478–484.

Priya PN, Pillai A, Gupta S. Effect of simultaneous exposure to lead and cadmium on gonadotropin binding and steroidogenesis on granulosa cells: an in vitro study. *Indian J Exp Biol* 2004;**42**:143–148.

Richardson DB, Ciampi A. Effects of exposure measurement error when an exposure variable is constrained by a lower limit. *Am J Epidemiol* 2003;**157**:355–363.

Roels HA, Boeckx M, Ceulemans E, Lauwerys RR. Urinary excretion of mercury after occupational exposure to mercury vapour and influence of the chelating agent meso-2,3-dimercaptosuccinic acid (DMSA). *Br J Ind Med* 1991;**48**:247–253.

Ronis MJ, Badger TM, Shema SJ, Roberson PK, Shaikh F. Reproductive toxicity and growth effects in rats exposed to lead at different periods during development. *Toxicol Appl Pharmacol* 1996;**136**:361–371.

Ronis MJ, Gandy J, Badger T. Endocrine mechanisms underlying reproductive toxicity in the developing rat chronically exposed to dietary lead. *J Toxicol Environ Health A* 1998;**54**:77–99.

Rowland AS, Baird DD, Long S, Wegienka G, Harlow SD, Alavanja M, Sandler DP. Influence of medical conditions and lifestyle factors on the menstrual cycle. *Epidemiology* 2002;**13**:668–674.

Schisterman EF, Vexler A, Whitcomb BW, Liu A. The limitations due to exposure detection limits for regression models. *Am J Epidemiol* 2006; **163**:374–383.

Selevan SG, Rice DC, Hogan KA, Euling SY, Pfahles-Hutchens A, Bethel J. Blood lead concentration and delayed puberty in girls. *N Engl J Med* 2003;**348**:1527–1536.

Sikorski R, Juszkiewicz T, Paszkowski T, Szprengier-Juszkiewicz T. Women in dental surgeries: reproductive hazards in occupational exposure to metallic mercury. *Int Arch Occup Environ Health* 1987;**59**:551–557.

Stoica A, Katzenellenbogen BS, Martin MB. Activation of estrogen receptor-alpha by the heavy metal cadmium. *Mol Endocrinol* 2000; **14**:545–553.

Varga B, Zsolnai B, Paksy K, Naray M, Ungvary G. Age dependent accumulation of cadmium in the human ovary. *Reprod Toxicol* 1993; **7**:225–228.

Wactawski-Wende J, Schisterman EF, Hovey KM, Howards PP, Browne RW, Hediger M, Liu A, Trevisan M. BioCycle study: design of the longitudinal study of the oxidative stress and hormone variation during the menstrual cycle. *Paediatr Perinat Epidemiol* 2009; **23**:171–184.

Wang X, Tian J. Health risks related to residential exposure to cadmium in Zhenhe County, China. *Arch Environ Health* 2004;**59**:324–330.

Whitcomb BW, Schisterman EF. Assays with lower detection limits: implications for epidemiological investigations. *Paediatr Perinat Epidemiol* 2008;**22**:597–602.

Whitcomb BW, Bodach SD, Mumford SL, Perkins NJ, Trevisan M, Wactawski-Wende J, Liu A, Schisterman EF. Ovarian function and cigarette smoking. *Paediatr Perinat Epidemiol* 2010;**24**:433–440.

Wide M. Lead exposure on critical days of fetal life affects fertility in the female mouse. *Teratology* 1985;**32**:375–380.

Wide M, D'Argy R. Effect of inorganic lead on the primordial germ cells in the mouse embryo. *Teratology* 1986;**34**:207–212.

Wiebe JP, Barr KJ, Buckingham KD. Effect of prenatal and neonatal exposure to lead on gonadotropin receptors and steroidogenesis in rat ovaries. *J Toxicol Environ Health* 1988;**24**:461–476.

Windham GC, Mitchell P, Anderson M, Lasley BL. Cigarette smoking and effects on hormone function in premenopausal women. *Environ Health Perspect* 2005;**113**:1285–1290.

Wu T, Buck GM, Mendola P. Blood lead levels and sexual maturation in U.S. girls: the Third National Health and Nutrition Examination Survey, 1988–1994. *Environ Health Perspect* 2003;**111**:737–741.

Young PC, Cleary RE, Ragan WD. Effect of metal ions on the binding of 17beta-estradiol to human endometrial cytosol. *Fertil Steril* 1977; **28**:459–463.

Zenzes MT, Krishnan S, Krishnan B, Zhang H, Casper RF. Cadmium accumulation in follicular fluid of women in *in vitro* fertilization-embryo transfer is higher in smokers. *Fertil Steril* 1995;**64**:599–603.

Zhou B, Yang L, Sun Q, Cong R, Gu H, Tang N, Zhu H, Wang B. Cigarette smoking and the risk of endometrial cancer: a meta-analysis. *Am J Med* 2008;**121**:501–508.