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The relationships between blood lead levels and serum follicle stimulating hormone and luteinizing hormone in the National Health and Nutrition Examination Survey 1999–2002

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ABSTRACT

The relationships between blood lead levels and serum follicle stimulating hormone and luteinizing hormone were assessed in a nationally representative sample of women, 35–60 years old, from the National Health and Nutrition Examination Survey 1999–2002. The blood lead levels of the women ranged from 0.2 to 17.0 μg/dL. The estimated geometric mean was 1.4 μg/dL, and the estimated arithmetic mean was 1.6 μg/dL. As the blood lead level increased, the concentration of serum follicle stimulating hormone increased in post-menopausal women, women who had both ovaries removed, and pre-menopausal women. The concentration of luteinizing hormone increased as blood lead level increased in post-menopausal women and women who had both ovaries removed. The lowest concentrations of blood lead at which a relationship was detected were 0.9 μg/dL for follicle stimulating hormone and 3.2 μg/dL for luteinizing hormone. Lead may act directly or indirectly at ovarian and non-ovarian sites to increase the concentrations of follicle stimulating hormone and luteinizing hormone.

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1. Introduction

In animal studies, lead affects reproductive hormone concentrations and the ovaries, and disrupts menstrual cycles. In monkeys, oral administration of lead acetate decreased levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), and estradiol [1], and progesterone [2,3]. Lead accumulated in the ovaries of mice given intraperitoneal injections of lead nitrate [4]. Oral administration of lead acetate reduced the number of follicles in mouse ovaries [5] and the number of corpora lutea in rat ovaries [6]. Intravenous injection of lead chloride inhibited the development of the follicles of rhesus monkeys [7]. Administration of lead acetate in drinking water [8] and intravenous injection of lead chloride [7] disrupted the menstrual cycles of rhesus monkeys.

In humans, the amount of lead in blood is associated with infertility in women [9,10]. In a clinical setting, Paksy et al. [11] found that lead accumulated in ovarian follicular fluid and decreased the amount of progesterone produced by granulosa cells. Using data from the third National Health and Nutrition Examination survey (NHANES III), conducted from 1988 to 1994, Krieg [12] found that serum FSH and LH concentrations increased as the blood lead level increased in women 35–60 years old. Increases were seen in pre- and post-menopausal women, and women without ovaries.

In the present study, data from the National Health and Nutrition Examination Survey 1999–2002 (NHANES 1999–2002) are used to assess the relationships between blood lead levels and the concentrations of FSH and LH in women, 35–60 years old. The methods of NHANES 1999–2002 are similar to those of NHANES III and the NHANES 1999–2002 data afford the opportunity of replicating the NHANES III results.

2. Materials and methods

The data and documentation for NHANES 1999–2002 can be found at the NHANES website (http://www.cdc.gov/nchs/nhanes.htm). The data files for alcohol use (alq.xpt, alq_b.xpt), demographics (demo.xpt, demo_b.xpt), blood lead and serum cotinine (lab06.xpt, l06_b.xpt), serum bone alkaline phosphatase and urinary N-telopeptides (lab11.xpt, l11_b.xpt), urinary creatinine (lab16.xpt, l16_b.xpt), serum FSH and LH (lab18.xpt, l40_b.xpt), and the reproductive health questionnaire (rhq.xpt, rhq_b.xpt) were downloaded on August 11–12, 2009 and September 10, 2009. Corresponding documentation files for the data files can be found at the NHANES website as can laboratory methods manuals for the assays.

2.1. Subjects

The subjects were 1783 women who were 35–60 years old. They were examined at a mobile examination center between 1999 and 2002.

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2.2. Sampling

Beginning in 1999, NHANES has been conducted as a continuous annual survey and the survey data is released on public use data files every two years. NHANES uses a complex probability sample. Primary sampling units are usually single counties. Small counties are sometimes combined to meet a minimum population size. Clusters of households are then selected. Each person in a selected household is screened for demographic characteristics, and one or more persons per household are selected for the sample.

2.3. Blood lead

Venous blood samples were taken at mobile examination centers. Blood lead was measured by atomic absorption spectroscopy in persons 1 year and older. The lower detection limit for the blood lead measurements was 0.6 μg/dL.

2.4. Serum FSH and LH

Women 35–60 years old were eligible for FSH and LH measurements. A single blood sample was taken for each woman. In pre-menopausal women, it was taken without regard to the day of their cycle. For NHANES 1999–2000, serum FSH and LH were measured using a microparticle enzyme immunoassay. The sensitivity for FSH was 0.2 IU/L and the sensitivity for LH was 0.5 IU/L. For NHANES 2001–2002, serum FSH and LH were measured using a paramagnetic particle, chemiluminescent two-step enzyme immunoassay. The sensitivity for FSH and LH was 0.2 IU/L. Non-detectable values were assigned a value of 0.2 or 0.5 IU/L divided by the square root of 2.

Hornung and Reed [13] recommend replacing values below the limit of detection with the limit of detection divided by the square root of 2 in order to produce more accurate statistical estimates.

2.5. Covariates

2.5.1. Bone alkaline phosphatase

Bone alkaline phosphatase was measured in persons 8 years and older. In NHANES 1999–2000 a solid-phase, monoclonal antibody immunoenzymetric assay was used to measure skeletal alkaline phosphatase in serum. The lowest reportable value was 0.7 μg/L. For NHANES 2001–2002, in 2001, the same immunoenzymetric assay was used. The lowest reportable value was 0.7 μg/L. In 2002, a one-step immunoenzymatic assay was used to measure skeletal alkaline phosphatase in serum. The lowest reportable value was 0.1 μg/L.

The 1999–2001 values were adjusted to 2002 values using a regression equation provided in the documentation for bone alkaline phosphatase. The adjustment was necessary because of the change in laboratory methods. The distributions of the sample person results were compared between the 2001 method and the 2002 method, and the two methods had statistically significantly different means. A cross-over study between the two methods was performed to develop a regression equation to convert 2001 values to 2002 equivalent values. The regression equation was:

$$\mathrm{LBDBAP} = \exp[-0.5326 + 1.1139x - 0.7963\max[0, x - 4.5151] + 0.9660\max[0, x - 4.9030]],$$

where $x = \log(\mathrm{LBXBAP})$, LBXBAP is the value in μg/L for 1999–2001 method, and LBDBAP is the value in μg/L for the 2002 method.

2.5.2. N-telopeptides

N-telopeptides were measured in persons 8 years and older. In NHANES 1999–2000, a competitive inhibition enzyme-linked solid-phase immunosorbent assay was used to measure the cross-linked N-telopeptides of type I bone collagen in urine. The lowest reportable value was 20 nM bone collagen equivalents (BCE). For NHANES 2001–2002, in 2001, the same assay was used. The lowest reportable value was 20 nM BCE. In 2002, a competitive immunoassay was used to measure N-telopeptides in urine. The lowest reportable value was also 20 nM BCE. Non-detectable values were assigned a value of 20 nM BCE divided by the square root of 2.

The 2002 values were adjusted to 1999–2001 values using regression equations. The adjustment was necessary because of the change in laboratory methods. The distributions of sample person results were compared between the 2001 method and the 2002 method. The N-telopeptide concentrations were higher in 2001. A cross-over study between the two methods was performed to develop regression equations to convert 2002 values to 1999–2001 equivalent values.

Urinary N-telopeptide concentrations were adjusted for creatinine. Urinary creatinine was measured in persons 6 years and older using a Jaffé rate reaction. The limit of detection was 1 mg/dL.

2.5.3. Cotinine

Serum cotinine was measured in persons 3 years and older by isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. No limit of detection was reported.

2.5.4. Mobile Examination Center Computer Assisted Personal Interview

Information about alcohol use and reproductive health came from items in the Mobile Examination Center (MEC) Computer Assisted Personal Interview (CAPI) questionnaire that was administered to persons, 20 (alcohol use) or 12 (reproductive health) years or older, that were examined at a MEC. The questions that were used are listed in Table 1. Positive responses were coded as '1'. 'Refused' and 'Don't know' were coded as missing. All other values were coded as '0'.

A variable called 'status' was created with six levels: post-menopausal, pregnant (pre-menopausal only), currently having a period, both ovaries removed, currently taking birth control pills (pre-menopausal only), and pre-menopausal women. Post-menopausal women were defined as women who had not had a period in the last 12 months. Women were categorized as currently having their period if they reported that they were currently having their period or that their period started within the previous 7 days. The levels of status were mutually exclusive. The 'post-menopausal' and 'pre-menopausal' categories contained women who were not included in one of the other categories.

Alcohol use was calculated from two questionnaire items, ALQ120Q and ALQ120U. Alcohol use was coded '1' if a woman reported having 12 or more alcoholic beverages in the past twelve months, and '0' if she reported having less than 12.

The variable medical conditions or treatments was coded as '1' if the response to item RHQ040 was 'medical conditions or treatments' and it was coded '0' otherwise.

Hormone pill use was calculated from three items, RHQ558, RHQ566, and RHQ574. Hormone pill use was coded as '1' if a woman answered 'yes' to any of the items and '0' if she answered 'no' to all three. Hormone patch use was calculated in the same way from two items, RHQ584 and RHQ600.

2.5.5. Demographic variables

Two demographic variables were used. The first was age at screening (RIDAGEYR). A classification variable called 'age group' was created from this variable with levels 35–39, 40–44, 45–49, 50–54, and 55–60 years old. The second was pregnancy status at the time of the MEC exam (RIDEXPRG). This variable was used in conjunction with the reproductive health questions discussed above.

2.6. Statistical analysis

The computer program SAS® (Release 9.2, SAS Institute, Inc., Cary, North Carolina) was used to analyze the survey data. Survey procedures were used that took into account the complex survey design. Two design variables were used, masked variance pseudo-PSU (SDMVPSU) and masked variance pseudo-stratum (SDMVSTRA). The full sample 4 year MEC-exam weight (WTMEC4YR) was used.

Descriptive statistics were calculated, and regression analyses were performed to test for and estimate the relationships between the measurements of FSH and LH, and the $\log_{10}$ of the blood lead levels adjusted for covariates. The covariates were status, age group, $\log_{10}$ serum bone alkaline phosphatase, $\log_{10}$ urine N-telopeptides, $\log_{10}$ serum cotinine, alcohol use, currently breastfeeding, hysterectomy, one ovary removed, Depo-Provera use, medical conditions or treatments, hormone pill use, and hormone patch use. Status and age group were included as classification variables. All other variables were included as continuous variables. The two-way interactions between status and blood lead, and status and the other covariates were included in the models in order to calculate separate estimates for each level of status.

In order to determine the lowest concentrations of blood lead at which a relationship could be detected, two variables for blood lead were created. If the lead value was less than or equal to a cutoff value, the first lead variable was assigned the value and the second lead variable was set to zero. If the lead value was greater than the cutoff value, the first lead variable was set to zero and the second lead variable was assigned the value. The cutoff value varied from the next to lowest value of blood lead to the highest value and was incremented in steps of 0.1. The slopes of the two lead variables were calculated at each step. The lowest blood lead value at which a relationship could be detected was defined as the cutoff value for which the slope of the first lead variable was statistically significant ($p < 0.05$) at the cutoff value and all subsequent greater values. Covariates were included in the models, and separate estimates were made for each level of status. This method has been used and described previously [12,14].

S-PLUS® (7.0 for Windows Professional Developer, Insightful Corporation, Seattle, WA) was used to make the graphs. Simple linear least squares regression was used to fit the data in the graphs.

3. Results

The serum FSH values ranged from 0.01 to 196.10 IU/L. The estimated arithmetic mean was 28.70 IU/L. The serum LH values ranged from 0.10 to 132.96 IU/L. The estimated arithmetic mean was 20.25 IU/L. The blood lead concentrations of the women whose FSH and LH were measured ranged from 0.2 to 17.0 μg/dL. The estimated geometric mean of the concentrations was 1.4 μg/dL and the estimated arithmetic mean was 1.6 μg/dL.

Table 1
Items used from the Mobile Examination Center Computer Assisted Personal Interview questionnaire.

Name	Description
ALQ120Q	In the past 12 months, how often did you drink any type of alcoholic beverage?
ALQ120U	Unit of measure (week, month, year).
RHQ030	Have you had regular periods in the past 12 months?
RHQ040	What is the reason you are not having regular periods?
RHQ050	When did you have your last period?
RHD080	Number of days since last period started.
RHQ200	Are you now breastfeeding a child?
RHD280	Have you had a hysterectomy including a partial hysterectomy, that is, surgery to remove your uterus or womb?
RHQ300	Have you had at least one of your ovaries removed (either when you had your uterus removed or at another time)?
RHQ310	Were both ovaries removed or only one?
RHD440	Are you taking birth control pills now?
RHQ520	Are you now using Depo-Provera or injectables to prevent pregnancy?
RHQ558	Are you taking pills containing estrogen only now?
RHQ566	Are you taking pills containing progestin only now?
RHQ574	Are you taking pills containing both estrogen and progestin now?
RHQ584	Are you using patches containing estrogen only now?
RHQ600	Are you using patches containing both estrogen and progestin now?

Estimated mean blood lead levels, and serum FSH and LH concentrations by level of status are shown in Table 2. Estimated means of the covariates by status are shown in Table 3. The means of alcohol use, currently breastfeeding, hysterectomy, one ovary removed, Depo-Provera use, medical conditions or treatments, hormone pill use, and hormone patch use are proportions. In both tables, the minimum and maximum values are for the sample and are not weighted population estimates.

Scatter plots of serum FSH and LH concentrations as a function of blood lead level for each level of status are shown in Figs. 1 and 2. There were increasing trends in the post-menopausal women, women with both ovaries removed, and pre-menopausal women.

Estimates of the slopes between blood lead level and serum FSH and LH concentrations adjusted for covariates are shown in Table 4. Serum FSH statistically significantly increased as blood lead level increased in the post-menopausal women, women who had both ovaries removed, and pre-menopausal women. The slope of the post-menopausal women was not statistically significantly greater than the slope of the pre-menopausal women, $p = 0.0519$. The slope of the women who had both ovaries removed was not statistically significantly greater than the slope of the pre-menopausal women, $p = 0.2184$. Serum FSH was not statistically significantly related to blood lead level in the pregnant women, women who were menstruating, women who were taking birth control pills. Serum LH statistically significantly increased as blood lead level increased in the post-menopausal women and women who had both ovaries removed. The slope for the pre-menopausal women was in the same direction as that for FSH, but it was not statistically significant. Serum LH was not statistically significantly related to blood lead level in the pregnant women, women who were menstruating, women who were taking birth control pills.

The lowest concentrations of blood lead at which a relationship could be detected are shown in Table 5. For serum FSH, the lowest concentration was 0.9 μg/dL in the group of pre-menopausal women. For LH, the lowest concentration was 3.2 μg/dL in the group of women who had both ovaries removed.

4. Discussion

In the NHANES III analysis, total bone mineral density was used as a covariate. Total bone mineral density was not measured in pregnant women. The relationships for pregnant women could not be adjusted for bone mineral density. In the NHANES 1999–2002 analysis, measurements of bone alkaline phosphatase and N-telopeptides were used instead of bone mineral density because they were measured in pregnant women and because they distinguish between bone formation and resorption. Bone alkaline phosphatase is an indicator of the activity of osteoblasts and bone formation [15]. Osteoclasts mediate the production of N-telopeptides, whose concentration is used as a measure of bone resorption [16].

Table 2
Blood lead and serum FSH and LH concentrations by level of status.

Variable	Status	*n*	*M*	SE	LCL	UCL	Min	Max
Blood lead (μg/dL)	Post-menopausal	649	1.92	0.07	1.79	2.06	0.2	17.0
	Pregnant	56	0.93	0.09	0.74	1.12	0.2	3.6
	Menstruating	231	1.44	0.07	1.31	1.58	0.2	10.6
	Both ovaries removed	180	1.72	0.10	1.51	1.93	0.2	7.7
	Birth control pills	58	1.12	0.07	0.97	1.27	0.2	4.9
	Pre-menopausal	605	1.49	0.07	1.36	1.63	0.2	13.8
Serum FSH (IU/L)	Post-menopausal	647	45.569	1.664	42.165	48.972	0.14	196.10
	Pregnant	55	0.362	0.082	0.194	0.530	0.01	5.05
	Menstruating	230	12.294	0.984	10.282	14.306	2.25	84.20
	Both ovaries removed	178	49.284	2.658	43.848	54.720	0.74	135.51
	Birth control pills	57	5.656	0.984	3.644	7.668	0.27	69.41
	Pre-menopausal	601	15.677	1.125	13.376	17.978	0.22	187.53
Serum LH (IU/L)	Post-menopausal	646	29.150	1.283	26.526	31.773	0.23	131.90
	Pregnant	56	4.444	1.468	1.442	7.447	0.14	31.22
	Menstruating	231	8.473	0.708	7.024	9.921	0.72	55.10
	Both ovaries removed	176	34.873	1.993	30.796	38.950	0.57	132.96
	Birth control pills	58	3.418	0.491	2.415	4.422	0.10	36.96
	Pre-menopausal	600	14.197	0.890	12.376	16.017	0.24	99.06

M, mean; SE, standard error; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit; Min, minimum; Max, maximum.

Table 3
Means of the covariates by level of status.

Variable	Status	*n*	*M*	SE	LCL	UCL	Min	Max
Age (years)	Post-menopausal	649	50.7	0.3	50.0	51.3	35	60
	Pregnant	56	37.0	0.5	36.0	38.0	35	41
	Menstruating	231	42.7	0.4	42.0	43.5	35	60
	Both ovaries removed	180	49.3	0.7	47.9	50.7	35	60
	Birth control pills	58	41.2	0.7	39.8	42.6	35	53
	Pre-menopausal	605	43.1	0.3	42.6	43.7	35	60
Serum bone alkaline phosphatase (μg/L)	Post-menopausal	649	14.16	0.38	13.39	14.94	3.4	93.1
	Pregnant	56	8.19	0.71	6.74	9.64	3.4	25.5
	Menstruating	231	11.16	0.37	10.39	11.92	4.0	36.7
	Both ovaries removed	180	14.29	0.55	13.17	15.41	4.3	56.8
	Birth control pills	58	9.26	0.50	8.25	10.28	3.6	21.8
	Pre-menopausal	604	11.65	0.24	11.16	12.15	2.1	57.9
Urine N-telopeptides (nM BCE/mM creatinine)	Post-menopausal	633	41.048	1.759	37.450	44.645	6.89	409.50
	Pregnant	55	45.990	5.177	35.401	56.578	13.77	206.50
	Menstruating	227	39.008	7.054	24.581	53.435	8.33	935.62
	Both ovaries removed	178	37.276	1.797	33.600	40.951	3.96	150.10
	Birth control pills	58	26.102	1.623	22.783	29.421	14.51	93.98
	Pre-menopausal	600	33.328	1.118	31.041	35.615	4.95	223.75
Serum cotinine (ng/mL)	Post-menopausal	638	60.3821	4.7852	50.5953	70.1690	0.011	894.430
	Pregnant	56	3.1439	2.8439	−2.6726	8.9603	0.011	169.300
	Menstruating	228	40.8098	7.1631	26.1597	55.4599	0.011	569.900
	Both ovaries removed	178	73.6558	9.3126	54.6095	92.7021	0.011	475.000
	Birth control pills	58	7.6148	3.6375	0.1753	15.0543	0.011	211.000
	Pre-menopausal	601	48.8036	5.0773	38.4194	59.1879	0.011	989.000
Alcohol use	Post-menopausal	648	0.3218	0.0168	0.2875	0.3562	0	1
	Pregnant	56	0.3322	0.1258	0.0749	0.5895	0	1
	Menstruating	231	0.5441	0.0394	0.4636	0.6246	0	1
	Both ovaries removed	180	0.2497	0.0398	0.1683	0.3312	0	1
	Birth control pills	58	0.6032	0.0676	0.4650	0.7415	0	1
	Pre-menopausal	605	0.4732	0.0313	0.4093	0.5372	0	1
Currently breastfeeding	Post-menopausal	649	0.0000	0.0000	0.0000	0.0000	0	0
	Pregnant	56	0.0116	0.0078	−0.0044	0.0276	0	1
	Menstruating	231	0.0000	0.0000	0.0000	0.0000	0	0
	Both ovaries removed	180	0.0000	0.0000	0.0000	0.0000	0	0
	Birth control pills	58	0.0048	0.0050	−0.0054	0.0150	0	1
	Pre-menopausal	605	0.0063	0.0035	−0.0007	0.0134	0	1
Hysterectomy	Post-menopausal	649	0.3154	0.0229	0.2684	0.3623	0	1
	Pregnant	56	0.0000	0.0000	0.0000	0.0000	0	0
	Menstruating	231	0.0000	0.0000	0.0000	0.0000	0	0
	Both ovaries removed	180	0.9896	0.0104	0.9684	1.0109	0	1
	Birth control pills	58	0.0000	0.0000	0.0000	0.0000	0	0
	Pre-menopausal	605	0.0108	0.0054	−0.0002	0.0219	0	1
One ovary removed	Post-menopausal	642	0.0921	0.0154	0.0607	0.1235	0	1
	Pregnant	56	0.0000	0.0000	0.0000	0.0000	0	0
	Menstruating	230	0.0328	0.0141	0.0040	0.0617	0	1
	Both ovaries removed	180	0.0000	0.0000	0.0000	0.0000	0	0
	Birth control pills	58	0.0570	0.0405	−0.0258	0.1399	0	1
	Pre-menopausal	604	0.0348	0.0078	0.0189	0.0507	0	1
Depo-Provera use	Post-menopausal	649	0.0013	0.0013	−0.0014	0.0039	0	1
	Pregnant	56	0.0000	0.0000	0.0000	0.0000	0	0
	Menstruating	231	0.0000	0.0000	0.0000	0.0000	0	0
	Both ovaries removed	180	0.0000	0.0000	0.0000	0.0000	0	0
	Birth control pills	58	0.0000	0.0000	0.0000	0.0000	0	0
	Pre-menopausal	605	0.0120	0.0036	0.0046	0.0194	0	1
Medical conditions or treatments	Post-menopausal	649	0.0967	0.0211	0.0535	0.1398	0	1
	Pregnant	56	0.0000	0.0000	0.0000	0.0000	0	0
	Menstruating	231	0.0349	0.0132	0.0080	0.0618	0	1
	Both ovaries removed	180	0.3602	0.0544	0.2489	0.4715	0	1
	Birth control pills	58	0.0332	0.0269	−0.0218	0.0881	0	1
	Pre-menopausal	605	0.0391	0.0091	0.0206	0.0577	0	1
Hormone pill use	Post-menopausal	649	0.2505	0.0221	0.2054	0.2956	0	1
	Pregnant	56	0.0000	0.0000	0.0000	0.0000	0	0
	Menstruating	231	0.0393	0.0158	0.0069	0.0716	0	1
	Both ovaries removed	180	0.4771	0.0392	0.3969	0.5572	0	1
	Birth control pills	58	0.0000	0.0000	0.0000	0.0000	0	0
	Pre-menopausal	604	0.0742	0.0141	0.0454	0.1030	0	1

Table 3 (*Continued*)

Variable	Status	*n*	*M*	SE	LCL	UCL	Min	Max
Hormone patch use	Post-menopausal	649	0.0061	0.0039	−0.0019	0.0141	0	1
	Pregnant	56	0.0000	0.0000	0.0000	0.0000	0	0
	Menstruating	231	0.0068	0.0067	−0.0070	0.0205	0	1
	Both ovaries removed	180	0.0886	0.0302	0.0267	0.1504	0	1
	Birth control pills	58	0.0000	0.0000	0.0000	0.0000	0	0
	Pre-menopausal	605	0.0080	0.0045	−0.0012	0.0171	0	1

M, mean; SE, standard error; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit; Min, minimum; Max, maximum.

The reproductive health questionnaires in NHANES III and NHANES 1999–2002 were not identical, resulting in some different covariates being used NHANES 1999–2002. In the NHANES III analysis, NORPLANT use, an implantable contraceptive that releases the progestin levonorgestrel [17], was included as a covariate. In the NHANES 1999–2002 analysis, this covariate was replaced by Depo-Provera use, an injectable progestogen-only contraceptive [18]. In the NHANES III analysis, radiation or chemotherapy was included as a covariate. In the NHANES 1999–2002 analysis, this covariate was replaced by medical conditions or treatments. In the NHANES III analysis, vaginal cream use was included as a covariate. In NHANES 1999–2002, this covariate was not included because there were no questions regarding vaginal cream use.

With regard to blood lead, serum FSH increased as the blood lead level increased in the post-menopausal women, women who had both ovaries removed, and the pre-menopausal women in both surveys. Serum LH increased as the blood lead level increased in the post-menopausal women and the women who had both ovaries

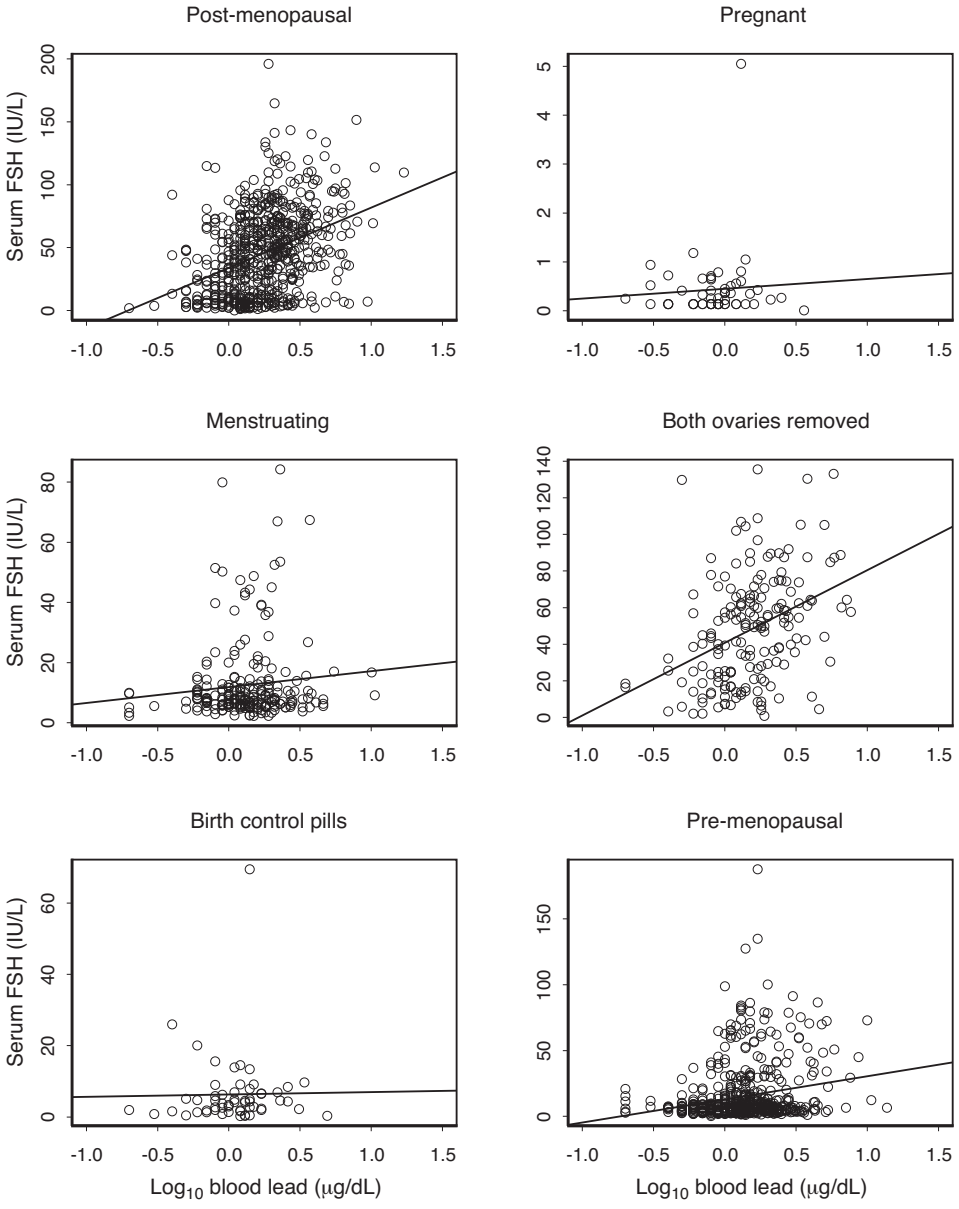

Fig. 1. Serum FSH concentration as a function of blood lead level by status.

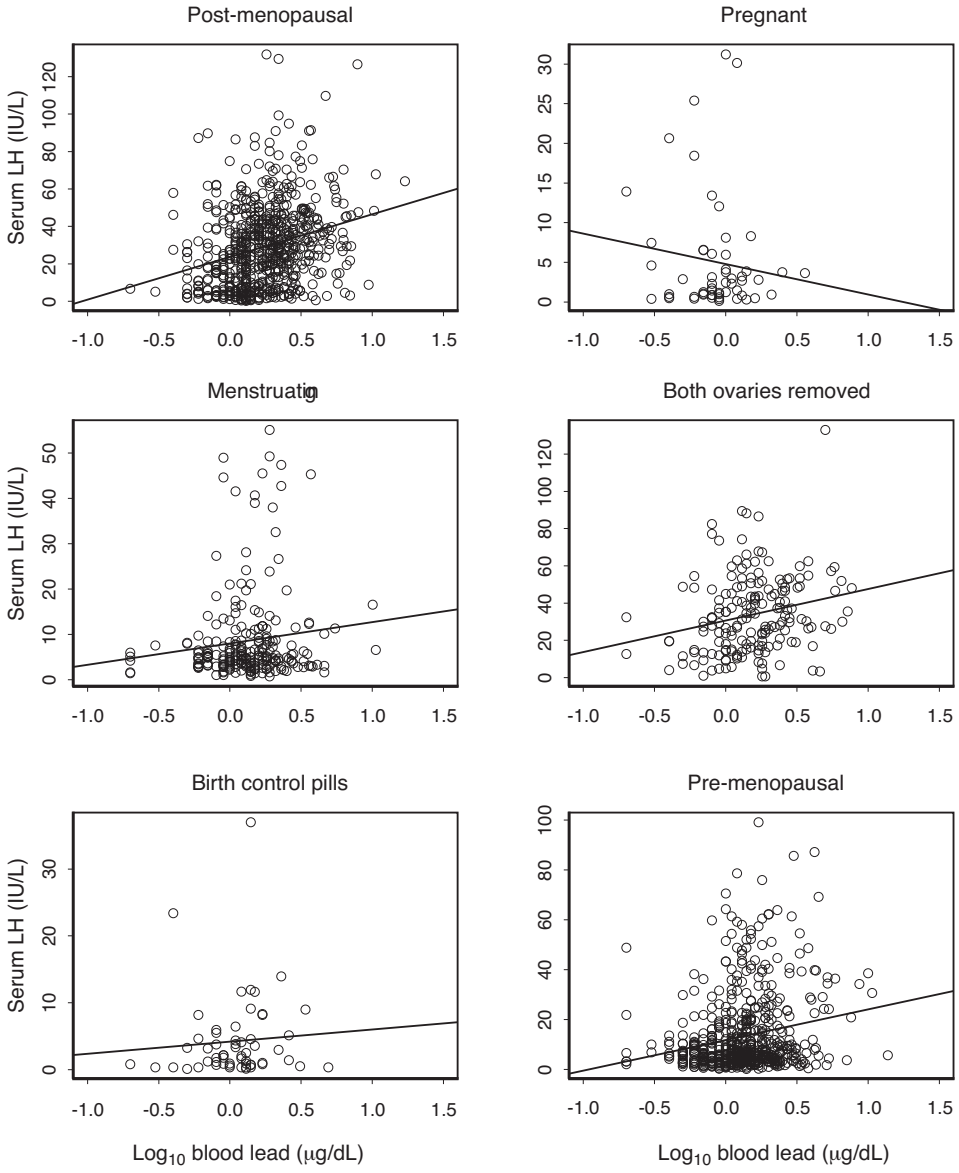

Fig. 2. Serum LH concentration as a function of blood lead level by status.

Table 4
Slopes for serum FSH and LH, and $\log_{10}$ blood lead (μg/dL) by level of status.

Variable	Status	Slope	SE	LCL	UCL	*t*	*p*
Serum FSH (IU/L)	Post-menopausal	26.38	6.35	13.39	39.38	4.15	0.0003
	Pregnant	−0.08	0.50	−1.11	0.95	−0.15	0.8783
	Menstruating	1.50	1.86	−2.29	5.30	0.81	0.4249
	Both ovaries removed	27.71	12.75	1.64	53.78	2.17	0.0380
	Birth control pills	−0.33	3.03	−6.52	5.86	−0.11	0.9133
	Pre-menopausal	11.97	4.25	3.27	20.66	2.81	0.0087
Serum LH (IU/L)	Post-menopausal	11.63	3.54	4.40	18.86	3.29	0.0026
	Pregnant	2.12	8.18	−14.62	18.86	0.26	0.7974
	Menstruating	0.87	1.50	−2.20	3.94	0.58	0.5669
	Both ovaries removed	20.59	9.02	2.14	39.04	2.28	0.0300
	Birth control pills	2.19	1.73	−1.35	5.72	1.27	0.2156
	Pre-menopausal	7.44	3.76	−0.26	15.14	1.98	0.0576

SE, standard error; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit. The slopes were adjusted for age, $\log_{10}$ serum bone alkaline phosphatase, $\log_{10}$ urine N-telopeptides, $\log_{10}$ serum cotinine, alcohol use, currently breastfeeding, hysterectomy, one ovary removed, Depo-Provera use, medical conditions or treatments, hormone pill use, and hormone patch use. For FSH, $n = 1713$; for LH, $n = 1712$; denominator DF = 29.

Table 5
Lowest blood lead concentration at which a relationship was detected by level of status.

Variable	Status	Blood lead (μg/dL)	Slope	SE	LCL	UCL	*t*	*p*
Serum FSH (IU/L)	Post-menopausal	1.9	26.58	12.52	0.97	52.19	2.12	0.0424
	Both ovaries removed	5.8	28.05	13.30	0.84	55.25	2.11	0.0438
	Pre-menopausal	0.9	12.59	5.77	0.78	24.40	2.18	0.0375
Serum LH (IU/L)	Post-menopausal	3.4	9.30	4.26	0.58	18.02	2.18	0.0375
	Both ovaries removed	3.2	14.70	7.13	0.12	29.29	2.06	0.0483

SE, standard error; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit. The slopes were adjusted for age, $\log_{10}$ serum bone alkaline phosphatase, $\log_{10}$ urine N-telopeptides, $\log_{10}$ serum cotinine, alcohol use, currently breastfeeding, hysterectomy, one ovary removed, Depo-Provera use, medical conditions or treatments, hormone pill use, and hormone patch use. For FSH, $n = 1713$; for LH, $n = 1712$; denominator DF = 29.

removed in both surveys. In women currently menstruating and pregnant women, there were no statistically significant relationships between blood lead level and serum FSH or LH in either survey. In NHANES III, there was a statistically significant inverse relationship between serum FSH and blood lead level in the women taking birth control pills. In NHANES 1999–2002, the slope was also negative, but the relationship was not statistically significant. A larger sample of women taking birth control pills may be necessary to get a consistent statistically significant relationship. In women taking birth control pills, there were no statistically significant relationships between blood lead level and serum LH in either survey.

In the NHANES 1999–2002 analysis, the estimated geometric and arithmetic means of the blood lead concentrations of the 35–60 year old women were 1.4 μg/dL and 1.6 μg/dL, respectively. These means were less than the geometric (2.2 μg/dL) and arithmetic (2.8 μg/dL) means from NHANES III. In the NHANES 1999–2002 analysis, the lowest concentrations of blood lead at which a relationship could be detected ranged from 0.9 to 5.8 μg/dL. In the NHANES III analysis, they ranged from 1.7 to 4.2 μg/dL.

The same increases in FSH and LH as a function of blood lead level occurred in both surveys. In addition to acting on the ovaries, lead could increase FSH and LH concentrations by acting on the adrenal gland [19], hypothalamus [20], or pituitary [21]. These tissues produce FSH and LH or regulate their concentrations. Lead could act directly on these tissues by interacting with calcium [22] or cell proteins [23]. Lead could also act indirectly by increasing concentrations of δ-aminolevulinic acid [24,25] or homocysteine [26]. Lead may act simultaneously at multiple sites to produce increases in the concentrations of FSH and LH.

Lead is found in cells in the adrenal gland [27]. Lead has been shown to affect an enzyme in the adrenal gland of rats [28], as well as increase aldosterone production [29] and the plasma the concentration of plasma corticosterone [30] in rats. No studies of lead and the adrenal hormones that may regulate FSH and LH were found.

Lead is found in the hypothalamus of rats [31,32]. In male rats, lead affects the secretion of luteinizing hormone releasing hormone [33] and the concentration of gonadotropin-releasing hormone (GnRH) messenger ribonucleic acid [34] in the hypothalamus. No studies of lead and GnRH in the hypothalamus of females were found.

Lead is found in the in the pituitary of rats [31,35]. Lead affects the fluidity of the pituitary membrane of female rats [36]. No other studies of the effects of lead on the pituitary were found.

Lead can inhibit the flow of calcium through calcium channels [37], it can mimic calcium in stimulating the release of neurotransmitters [38,39], and it can affect the amount of calcium in the mitochondria in synaptosomes [40]. Exocytosis from neuroendocrine cells [41] and from secretory cells in the anterior pituitary [42] is dependent on calcium. Lead may interact with calcium in these cells to increase the concentrations of FSH and LH in the blood. An increase in intracellular calcium is necessary for the development of an oocyte [43]. Lead may interact with calcium in the ovary and affect hormonal feedback to the hypothalamus and pituitary.

Lead can bind to calmodulin and activate it [44], it can inhibit adenylate cyclase activity [45], and it can activate protein kinase C [46]. These proteins are part of the signaling network that controls the pulsatile release of GnRH from hypothalamic cells [47] and the signaling network that is activated when GnRH binds to receptors in pituitary cells [48]. Lead may interact with one or more of these proteins to increase the concentrations of FSH and LH in the blood.

δ-Aminolevulinic acid synthetase is found in rat ovaries [49] and δ-aminolevulinic acid induces oxidative stress in Chinese hamster ovaries [50]. Aminolevulinic acid may act on the ovary and affect hormonal feedback to the hypothalamus and pituitary. In addition, δ-aminolevulinic acid has inhibitory effects at acetylcholine [51] and γ-aminobutyric acid (GABA) [52–54] synapses. Neurons that release GnRH have acetylcholine [55] and GABA [56,57] receptors. Aminolevulinic acid may act at acetylcholine or GABA synapses to increase the concentrations of FSH and LH in the blood.

Homocysteine has been found in the follicular fluid of women [58]. Women with polycystic ovary syndrome have higher homocysteine levels than women who do not [59]. Homocysteine may act on the ovary and affect hormonal feedback to the hypothalamus and pituitary. In addition, homocysteine acts as an *N*-methyl-D-aspartate agonist [60–63] and a GABA antagonist [64–69]. *N*-methyl-D-aspartate induces the release of luteinizing hormone [70] and GABA regulates hypothalamic neurons that release GnRH [71]. Homocysteine may act at *N*-methyl-D-aspartate or GABA receptors to increase the concentrations of FSH and LH in the blood.

Conflict of interest

None declared.

Disclaimers

Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

The findings and conclusions in this report are those of the author and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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